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Effect of Inhaled Commercial Hydrous Calcium Silicate Dust on Animal Tissues

An Experimental Study

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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 poten-

tially 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 1.—Composition of Commercial Hydrous Calcium Silicate

Component Analysis	Per Cent	Compound Analysis	Per Cent (Approx.)
SiO ₂	43.3	Hydrous calcium silicate	80
Fe ₂ O ₃ , Al ₂ O ₃ , TiO ₂	3.3	Calcium carbonate	6
CaO	16.0	Magnesium silicate	15
MgO	7.3		
Na ₂ O, K ₂ O	0.3		
Ignition loss	19.7		
Total	99.7	Total	100

an atmospheric suspension of the hydrous calcium silicate product was created by the action of a paddle which rotated inside a hopper containing the material in finely divided form. The dust cloud generated in this manner floated out into the room where it was maintained for eight hours on five days of the week and for four hours on Saturdays. Some of the animals were exposed to the dust for periods as long as three years. At regular intervals during the experiment a few animals were killed, and the organs examined grossly, and microscopically to determine the nature and the extent of the tissue reaction to the calcium silicate product. The tissue was also analyzed chemically to estimate the amount of the inhaled dust that was retained in the lungs of animals exposed for definite periods of time. Only guinea pigs were used in the studies dealing with infected animals. The R₁ low-virulence strain of the tubercle bacilli was introduced intratracheally by means of the insufflation technique.

Dust counts of atmospheric samples collected in the dust room were made regularly by means of the midget impinger. The concentration of the hydrous calcium silicate aerosol to which the uninfected animals were exposed was generally within the range of 100,000,000 to 125,000,000 particles per cubic foot of air by light-field count, and the overall average was 115,000,000. In the studies on infected animals the concentration was higher, the average being 205,000,000.

EXPOSURE OF UNINFECTED ANIMALS

Three species—guinea pig, rat, and hamster—were employed in this phase of the investigation. Summaries of the findings are given in Tables 2, 3, and 4. Attention should first be drawn to the relatively high mortality rate reflected in Table 2. That these deaths were due to intercurrent epizootic infection was almost certain in the case of the guinea pigs and is emphasized the more forcefully by the fact that the animals died not only of pneumonia but also of pericarditis, peritonitis, and cervical adenitis with abscess formation. That all the hamsters which died did so within the first year may also have some bearing on the question. In the case of the rats, on the contrary, no animals died within the first year, and the rate at which animals succumbed increased with the passage of time. In view of the progressive nature of the pulmonary disease which ensued as a result of the exposure to the dust of the hydrous calcium silicate product, there is some presumptive evidence that the deaths and dust exposures bore some relation to each other in this instance.

TABLE 2.—Biological Action of Commercial Hydrous Calcium Silicate Dust
Guinea Pigs, Hamsters, and Rats Were Exposed by Inhalation to an Aerosol of Commercial Hydrous Calcium Silicate Dust Until Death
Record of Animals, Exposed to the Dust Alone, Which Died Spontaneously

Species	Cause of Death								Total	
	Pneumonia		Pericarditis		Peritonitis		Cervical Adenitis			
	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent		
	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent		
Guinea Pig.....	51*	40.9	7	5.6	12	9.6	3	2.4	73	58.5
Hamster.....	10†	80.0	..	...	..	...	..	...	10‡	80.0
Rat.....	20‡	80.0	..	...	..	...	..	...	20	80.0

* 46.5% died within first year of experiment.

† 100.0% died within first year of experiment.

‡ 10.0% died within first year of experiment.

§ Two deaths from other causes.

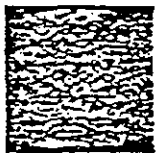


TABLE 3.—*Pulmonary Reaction to Inhaled Commercial Hydrous Calcium Silicate Dust*
Guinea Pigs Were Exposed by Inhalation Continuously to the Dust Until Death

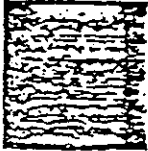
Guinea Pig No.	Exposure to Dust, Mo.	Pigmentation	Microscopic Evidence of Pneumoconiosis										Reaction in Pulmonary Lymph Nodes			
			Bronchial Cilia										Macrophage Induction			
			Lymphoid Hyperplasia	Hyperplasia	Ulceration	Distortion	Iron Distortion	Adenoma	Phagocytosis	Cellular Erythema	Adenoma	Ulceration	Enlargement	Phagocytosis	Fibrosis	Pert. adenitis
1, 2, 4, 6	2	..	+	+	+	+	+	+	+	+	+	+	+	+	+	..
10, 11, 12, 14	4	..	+	+	+	+	+	+	+	+	+	+	+	+	+	..
16, 18, 20, 24	6	..	+	+	+	+	+	+	+	+	+	+	+	+	+	..
26, 30, 34, 37	8	..	+	+	+	+	+	+	+	+	+	+	+	+	+	..
40, 42, 47, 60	10	..	+	+	+	+	+	+	+	+	+	+	+	+	+	..
	12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
	20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
	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

Animal No.	Exposure to Dust, Mo.	Reaction in Rats Exposed to the Dust	Microscopic Evidence of Pneumoconiosis										Reaction in Pulmonary Lymph Nodes			
			Bronchioles and Distortion										Macrophage Induction			
			Lymphoid Hyperplasia	Hyperplasia	Ulceration	Distortion	Iron Distortion	Adenoma	Phagocytosis	Cellular Erythema	Adenoma	Ulceration	Enlargement	Phagocytosis	Fibrosis	Pert. adenitis
701, 702, 703, 704	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
705, 706, 707, 708	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
709, 710, 711, 712	17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
713, 714, 715, 716	17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
717, 718, 719, 720	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
Reaction in Hamsters Exposed to the Dust																
1, 2	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
13, 14	9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
15, 16	17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..
17, 18	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	..

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

The three species of experimental animals reacted somewhat differently to the dust, though the difference was more one of degree than of quality. The most marked lesions were provoked in the guinea pigs, but this was probably largely due to the fact that dusting was carried on for a total of 36 months in the case of this species, while the procedure was terminated at the end of the 18th month in the case of the rats and hamsters. When cognizance is taken of this fact, it appears that, stage for stage, the latter two animal groups actually suffered greater pulmonary damage sooner than did the guinea pigs. This difference is brought out by a comparison of Tables 3 and 4, in the construction of which the same scales of values were used.

Pigmentation of the pulmonary tissue or of pulmonary lymph nodes never became prominent features in these animals. It increased diffusely as a light brown discoloration which was most marked along the anterior margins of the lungs in the guinea pigs. As it became macroscopically detectable at the 12th month of exposure only, its absence in the case of the rats or hamsters before the 18th month may have no true significance. It would seem that the pigment was almost entirely due to hemosiderin, as shown by Prussian blue staining. Pigmented konoiphores and giant cells became a prominent feature toward the terminal phase of the experiment on guinea pigs, and clusters of such cells could even be found in rat lungs where they were grouped around the smaller blood vessels.

Lymphoid hyperplasia was a prominent and persistent feature of the reaction in the guinea pigs. There was mild hyperplasia about the main bronchi in some of the rats, but this was not an impressive observation for this species. No lymphoid hyperplasia was seen in the hamsters.



The hyperplasia consisted almost entirely of lymphoid cell proliferation without any evidence of central macrophage accumulation such as may occur as a result of exposure to certain amorphous siliceous dusts. Mostly the lymph deposits remained spherical in shape and were thus well defined from sur-

rounding structures. Occasionally, however, there was some lymphoid invasion of the walls of adjacent alveoli. At a later stage macrophages tended to accumulate around these lymph foci.

In spite of this tendency toward intrapulmonary lymphoid hyperplasia, the pulmonary lymph nodes showed no consistent corresponding change. In some of the animals there was early lymphoid hyperplasia, but in the majority the nodes enlarged slightly or moderately only at the start. After the animals had been in the dust atmosphere for two years, the nodes became more consistently enlarged, and in the instances in which this occurred the cause was almost always infiltration of the medullary zones by macrophages, which no doubt migrated thence from the pulmonary tissues. The cortical follicles did not enlarge and often showed signs of atrophy. In both the rats and hamsters the reaction in the pulmonary lymph nodes was even less marked.

Undoubtedly the most prominent lesion discovered in this series of animals was present as chronic bronchiolitis and bronchial ulceration, with emphasis on the former. The disease was progressive in nature and destructive in its effects, although the tendency to epithelial desquamation, so characteristic of the reaction to quartz dust, was not present.

The bronchiolar lesions commenced early in the guinea pigs and hamsters but were delayed in the rats. In neither of the latter groups were the severe grades of obliterative bronchiolitis or bronchiolitis deformans, which characterized the guinea pig response, observed. In the rat the stress was rather on bronchiolar ulceration and peribronchiolitis.

As the dusting proceeded beyond the first year of experiment, the inflammatory reaction in the bronchioles gave way to progressive distention and distortion. This change was brought into sharp focus by the increasing cellular deposits around the air passages. Toward the end of the second year the crenated outlines of the bronchiolar lumina were thrown into sharp relief by a




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 demonstrable 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 opaque clubbed bodies could be found, but 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.




Unlike in many other dust experiments, there was no marked tendency toward subpleural congregation of the hydrous calcium silicate dust. Consequently the reaction at this site was minimal and delayed. The most conspicuous changes occurred in the rats where there were occasional subpleural cellular condensations and a recurrent tendency to the formation of interlobular adhesions. In some of the guinea pigs which outlasted three years of exposure to the dust, foci of subpleural cellular proliferation attended by giant-cell accumulation were demonstrable.

The relatively marked macrophage infiltration of the pulmonary lymph nodes contrasted sharply with the paucity of giant cells among them, with the minimal amount of interstitial fibrosis which ensued, and with the absence of asbestos bodies. Periadenitis of a mild degree became a consistent finding in a majority of animals beyond the end of the first year of dust inhalation. Deposition of fibrous strands around the lymph nodes followed successive phases of afferent lymphangiectasia and macrophage transportation along these channels, with littoral arrest of increasing numbers of these cells.

The lack of fibrosis, except at sites where asbestos bodies occur with greatest prevalence, is indeed remarkable, the more so when regard is had to the dense cellular infiltrations which ultimately ensue. One is led to the conclusion, therefore, that the hydrous calcium silicate dust alone, except for its chrysotile component, is nonfibrogenic though it provokes a cellular reaction.

EXPOSURE OF TUBERCULOUS ANIMALS



There are three types, or phases, of investigation that have been found invaluable for studying the effect of inhaled dust on the course of experimentally induced tuberculosis in animals. These three types of experiment are sometimes called the simultaneous phase, the reactivation phase, and the predisposition phase. In the simultaneous phase, normal animals are infected with attenuated tubercle bacilli of the R_1 strain by an inhalation method and then are immediately transferred to the dust room. Thus the tuber-

culous disease and the tissue reaction to the inhaled dust will develop simultaneously. In the reactivation-phase experiment, the animals, after being infected with the tubercle bacilli, are allowed to live in a normal atmosphere for several months before being exposed to dust. During this period the tuberculous lesions generally regress, and in some instances complete healing by resolution may take place. Following this interval, the exposure of the animals to some dusts has no significant effect on the usual course of the tuberculous process, and the lesions continue to regress, while exposure to other dusts may cause the tuberculous process to become active and to spread. A predisposition-phase experiment is one in which animals are exposed to dust for several months, are then infected with the tubercle bacilli, and are immediately returned to the dust room where their exposure to dust is continued. In this phase the effect of a previous dust exposure on the early course of a tuberculous process can be studied.

SIMULTANEOUS PHASE: REACTION IN GUINEA PIGS INFECTED WITH TUBERCLE BACILLI AT ONSET OF DUST INHALATION

In the first experiment in which animals were infected with tubercle bacilli at the time that their exposure to the dust was started, 25 guinea pigs were used, but an epidemic of pneumonia during the first 10 months reduced to 16 the number of animals available for study. Two of these died at fourteen months, apparently from progressive tuberculosis, and two others died from undetermined causes but not from tuberculosis. In the remaining 12 animals the course of the tuberculous disease was followed by killing 1 or 2 animals at 1, 6, 9, 12, 15, 18, and 24 months after infection. In 9 of the 25 animals the lesions were multiple, isolated, and healed, often with central calcification. In six of the remaining animals the lesions were still circumscribed but showed neither a tendency to heal nor to spread even at the end of 18 months. In six other animals there was a moderate tendency to local spread of the tuberculous process, while in the remaining

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 R₁ Strain

Type of Experiment	Cause of Death									
	Exposed Animals, No.	Pneumonia		Pericarditis		Peritonitis		Other Causes		Total
		Deaths, No.	Per Cent	Deaths, No.	Per Cent	Deaths, No.	Per Cent	Deaths, No.	Per Cent	
Simultaneous phase	20	8	40.0	3	15.0	1	5.0	2	10.0	14
Reactivation phase	25	2	8.0	1	4.0	..	..	1	4.0	4
Predisposition phase	20	8	40.0	..	..	..	..	..	..	8
Control: Group A	24	4	16.7	..	..	..	..	..	..	4
Group B	25	3	12.0	1	4.0	2	8.0	..	..	6

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 breeders during the experiment, as were also four other animals in the experiment, which had failed to show extensive disease. Owing to the element of uncertainty introduced by this factor of breeding, and also because of the high mortality from pneumonia and to verify the suggestive evidence of stimulating action of the inhaled dust upon tuberculosis, the experiment was repeated.

TABLE 6.—Course of Tuberculosis Induced in Guinea Pigs by Infection with Tubercle Bacilli of the R₁ Strain
Control Study: Infection Only; No Dust Exposure

Guinea Pig No.	Survival After Infection, Days	Fate	Pulmonary Lesions					Reaction in Pulmonary Lymph Nodes		
			Advanced Tubercles, No.	Spreading Tuberculosis	Casation	Calcification	Pneumitis	Enlargement	Tubercle Formation	Dissemination
62	45	Died	2	..	..	..	..	+	..	..
67	60	Killed	6	..	+	..	..	+	+	T+
68	..	Killed	15	..	+	..	..	+	+	T+
69	..	Killed	7	..	..	..	..	+	..	..
60	120	Killed	4	..	..	..	..	+	..	T+
63	..	Killed	3	..	..	..	..	+	..	..
64	180	Killed	6	..	..	..	+	+	..	..
73	190	Died	..	+	..	..	..	+	..	T+
77	200	Died	2	..	..	..	..	+	..	..
65	..	Killed	4	..	..	+	..	+	..	..
66	240	Killed	11	..	..	+	..	+	..	..
86	250	Died	2	..	..	..	..	+	..	..
61	..	Killed	0	..	..	..	..	+	..	..
74	300	Killed	4	..	..	..	..	+	..	..
83	315	Died	1	..	..	+	..	+	..	..
80	..	Killed	7	..	..	..	..	+	..	..
90	350	Killed	16	..	..	+	..	+	..	..
67	..	Killed	4	..	..	..	..	+	..	..
68	420	Killed	5	..	..	..	..	+	..	..
69	..	Killed	3	..	..	..	..	+	..	..
70	430	Killed	1	..	..	..	..	+	..	..
71	..	Killed	3	..	..	..	..	+	..	..
72	480	Killed	3	..	..	..	..	+	..	..
75	..	Killed	2	..	..	..	..	+	..	..
76	540	Killed	2	..	..	..	..	+	..	..
77	..	Killed	3	..	..	..	..	+	..	..
78	..	Killed	6	..	..	..	..	+	..	..
79	..	Killed	2	..	..	..	..	+	..	..
80	..	Killed	6	..	..	..	..	+	..	..
81	720	Killed	6	..	..	..	..	+	..	..
82	..	Killed	2	..	..	..	..	+	..	..
83	..	Killed	4	..	..	..	..	+	..	..
84	..	Killed	3	..	..	..	..	+	..	..
85	..	Killed	0	..	..	..	..	+	..	..

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

TABLE 7.—Influence of Inhaled Commercial Hydrous Calcium Silicate Dust on the Course of Experimentally Induced Tuberculosis in Guinea Pigs
Simultaneous Phase: Animals Received Their Tuberculous Infection and Started Their Period of Dust Exposure Simultaneously

Guinea Pig No.	Exposure to Dust, Days	Fate	Pulmonary Lesions				Dissemination			
			Arrested Tubercles, No.	Spreading Tuberculosis	Cavitation	Pneumia	Pulmonary Lymph Nodes		Liver	Spleen
							Enlargement	Tuberculous		
1	40	Died	2	..	..	+	++	..	..	..
1	40	Killed	6	+	..	..	+	+	..	..
2	40	Died	10	+	..	..	+	+	..	..
6	111	Died	2	++	..	..	+	..	N+	..
3	120	Killed	6	..	..	..	+	..	..	..
3	120	Killed	6	..	..	..	+	+	..	..
24	155	Died	..	+++	..	..	+	++	N+	N—
25	160	Died	..	++	+	..	+	+	..	..
10	174	Died	4	..	..	+++	++	..	..	..
7	180	Killed	..	..	..	+++	++	+	..	..
8	180	Killed	7	..	..	+	++	..	..	..
20	186	Died	1	..	..	+++	..	..	..	..
14	204	Died	2	..	..	+++	++	..	..	..
22	220	Died	..	..	..	+++	..	..	..	..
9	240	Killed	7	+	..	..	++	+	..	..
13	240	Killed	5	+	..	..	++	+	..	..
26	255	Died	..	+++	..	..	+	+	T+	F—
11	265	Killed	..	+++	..	..	+	+	T+	F—
12	265	Killed	9	..	..	..	++	++	..	..
17	415	Died	..	..	..	..	++	+	..	..
20	420	Died	..	..	..	+++	..	..	..	..
15	480	Killed	3	+++	..	+++	..	..	..	..
16	480	Killed	5	..	..	+++	+	+	..	..
27	517	Died	..	..	..	+++	+	+	..	..
28	580	Died	..	++	..	+++	+	+	..	..
18	680	Died	..	++	..	..	+	+	..	..
21	680	Died	2	..	..	++	+	..	..	..
19	720	Killed	2	..	..	..	++	..	..	..
23	720	Killed	5	..	..	++	++	..	..	..
29	720	Killed	..	++	..	..	++	+	..	..

Symbols: +, slight reaction; ++, moderate reaction; +++, marked reaction; F, fibrosis; N, necrosis; T, tuberculosis.

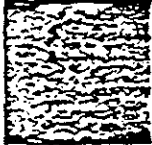
When conducted a second time, 30 guinea pigs, instead of 25, were used in this simultaneous infection experiment. The animals were killed in pairs for study at 2, 4, 6, 8, 12, 15, and 24 months after infection. Again the incidence of pneumonia was unusually high, six of the animals dying from that cause during the first 8 months of exposure and two more during the following 16 months. An additional three died of pericarditis, one of peritonitis, and two more of other causes (Table 5). This left a balance of 16 animals which were killed as planned.

The results are assembled in Table 7 which should be compared with the control study recorded in Table 6, in connection with which the same culture batch of tubercle

bacilli was used but the animals were not exposed to dust.

As may be seen in Table 5, the mortality rate among the control group was but 11.7% as against the high rate of 46.6% found in the animals which were caused to inhale dust. As these experiments were conducted simultaneously and in the same laboratory except that the animals receiving dust exposure were even better protected from outside contacts, the different death rates may have some significance after all.

The numbers of tubercles which developed in the control series did not differ materially from those which were discovered in the animals exposed to dust after infection. In one control animal there was a marked spread which caused the death of the guinea

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 reac-

tion, 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 8.—Influence of Inhaled Commercial Hydrous Calcium Silicate Dust on the Course of Experimentally Induced Tuberculosis in Guinea Pigs
Reactivation Phase: Animals, After Receiving Their Tuberculous Infection Were Allowed to Live in a Normal Environment for a Period of Two to Six Months Before They Were Exposed to Dust

Guinea Pig. No.	Exposure to Dust, Days	Fate	Pulmonary Lesions					Dissemination			
			Arrested Tubercles, No.	Spread, log. Tuberculous, No.	Cases, No.	Fibrosis	Pneumonia	Pulmonary Lymph Nodes		Liver	Spleen
								Enlargement	Tuberculous		
Exposure to Dust Was Started Two Months After Infection											
47	9	Died	6	+	+	+	+	+	+	+	+
51	21	Died	..	..	..	..	..	+	..	..	..
54	40	Died	2	..	..	..	+	+	..	..	..
52	80	Killed	5	..	..	..	..	++	+	..	..
52	80	Killed	5	..	..	..	..	++	+	..	..
55	120	Killed	3	..	..	..	..	+	+	..	..
50	120	Killed	4	..	..	..	+	+	+	..	..
57	240	Killed	7	..	..	..	..	++	+	..	..
58	240	Killed	2	..	..	..	+++	+	+	..	..
53	265	Killed	5	..	..	..	..	++	..	..	..
59	265	Killed	19	++	..	+	..	++	+	..	..
40	265	Killed	9	++	..	++	..	++	+	..	..
Exposure to Dust Was Started Four Months After Infection											
45	120	Killed	2	..	..	..	++	++	+	..	..
44	120	Killed	2	..	..	..	..	++	+	..	..
42	240	Killed	2	..	..	..	..	++	..	..	..
42	240	Killed	5	..	..	..	+++	++	+	..	..
43	265	Killed	2	..	..	..	..	++	+	..	..
44	265	Killed	2	..	..	..	..	++	..	..	..
Exposure to Dust Was Started Six Months After Infection											
49	117	Died	4	..	..	..	++	+	..	..	..
50	120	Killed	3	+	..	+	++	++	+	..	..
52	120	Killed	7	+	..	+	..	++	+	..	T+
53	240	Killed	1	..	..	..	+++	++	+	..	..
54	240	Killed	9	..	..	..	..	++	..	..	..
56	265	Killed	..	..	..	..	+	++	..	..	..
56	265	Killed	5	..	+	++	..	++	..	..	..

Symbols: +, slight; ++, moderate; +++, advanced; T, tuberculous.

PREDISPOSITION PHASE: REACTION IN GUINEA PIGS INFECTED WITH TUBERCLE BACILLI THREE MONTHS AFTER THEIR EXPOSURE TO DUST WAS STARTED

An experiment of this type is a severe test, because a dust that is only very slightly toxic may produce tissue changes which, though minor in character, may be sufficient to alter profoundly the development of a fresh tuberculous infection. In this experiment, which was designed to study the effect of the dust accumulated in the lung upon a newly developing tuberculous disease, 30 guinea pigs were exposed to the dust for three months and then were infected with attenuated tubercle bacilli. The dust exposure was immediately resumed and carried on for another 21 months.

The over-all death rate in this series of animals was relatively low (Table 5). It may

be of some significance that the majority of the eight animals that died from pneumonia did so during the latter half of the dusting phase. It is also significant perhaps that local and diffuse spreads of the tuberculous process had occurred most commonly in these animals prior to their terminal fatal illness.

The prevalence of such spreads in this series of guinea pigs does indeed appear to be significant (Table 9). Six animals showed marked local or diffuse extension of the disease, and in seven more this spread was of moderate severity. In an additional 9 cases there was slight spread of the process, so that a total of 22 animals reacted unfavorably. Cavitation occurred in 2 instances, and pleural extension in 10 of the 30 guinea pigs. One animal died from a pneumonic tuberculous process.

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 Infected with Tubercle Bacilli of the R₁ 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		Liver	Spleen
							Enlargement	Tuberculosis		
107	0	Killed	..	..	..	..	..	..	..	..
8	0	Killed	..	..	..	..	..	..	..	..
9	0	Killed	..	..	..	..	..	..	..	..
10	0	Killed	..	..	..	..	..	..	..	..
11	0	Killed	..	..	..	..	..	..	..	..
91	80	Died	..	..	..	..	++	+	..	..
92	80	Killed	17	+	..	..	..	..	..	..
93	80	Killed	27	+	..	..	++	+	..	..
94	120	Killed	16	+	..	..	++	+	..	..
95	120	Killed	14	+	..	..	++	+	..	..
96	180	Killed	7	++	..	..	++	+	..	..
97	180	Killed	11	+	..	..	++	+	..	..
114	210	Died	6	+++	+++	++	+	..	..	..
106	230	Died	8	++	..	+++	+	..	..	..
112	240	Killed	6	..	..	..	+	+	..	..
113	240	Killed	20	+	..	..	++	+	..	..
98	300	Killed	21	++	..	++	++	+	..	..
100	300	Killed	3	++	..	++	++	+	..	T ₊
115	345	Died	..	+++	..	++	+	..	..	..
99	365	Killed	2	++	..	..	++	+	..	..
100	365	Killed	4	..	..	..	+++	+	..	..
116	400	Died	..	+++	++	+	+	+	..	..
117	425	Died	..	+++	..	+	++	+	..	..
103	450	Died	..	++	..	..	+	..	..	..
114	465	Died	..	+++	..	+	++	+	..	..
118	480	Killed	..	+++	..	..	+	+	..	..
120	480	Killed	..	++	..	++	+	+	..	..
101	520	Killed	4	+	..	..	++	..	..	..
102	530	Killed	5	+	..	..	++	+	..	..
110	530	Killed	3	+	..	++	+	+	..	..

Symbols: +, slight or indipient 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 25 guinea pigs, as the predisposition-phase experiment was started at a later stage than the preceding studies so that a fresh R₁ culture had to be employed. The results ob-

tained 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

in the lung at this stage when the tissue reaction suddenly blossomed forth into the full-blown disease process. Comparable results have been obtained in The Saranac Laboratory in inhalation experiments with other dusts.

The total amount of silica which accumulated in the lung was about a third of that which may be demonstrated in the pulmonary tissue of guinea pigs exposed to quartz dust for a comparable period.

COMMENT

The first point which may be considered is that the commercial hydrous calcium sili-

As the chrysotile dust is capable of provoking fibrosis in guinea pigs, it may also in the case of the present study have been the cause of the focal fibrogenic response, so that the hydrous calcium silicate component may have to be exonerated. It seems likely that the giant-cell reaction was largely an effect provoked by the latter component. Attention should be directed to the lack of obvious necrosis in these cells. In this respect the dust differs quite markedly from quartz dust and silica fume. Indeed, necrosis was seldom a feature in this experimentally induced disease even in the presence of tuberculosis. It is possible, therefore, that al-

TABLE 10.—Analysis of the Lungs of Guinea Pigs Exposed to Commercial Hydrous Calcium Silicate Dust

Period of Exposure, Mo.	Ash, Per Cent of Desiccated Lung	Mineral Components of Desiccated Lung			Mineral Components of Lung Ash		
		SiO ₂ , Per Cent	CaO, Per Cent	MgO, Per Cent	SiO ₂ , Per Cent	CaO, Per Cent	MgO, Per Cent
2	4.88	0.22	0.07	0.07	4.31	1.34	1.60
4	4.45	0.20	0.06	0.06	4.63	1.34	1.64
6	4.44	0.22	0.09	0.06	5.26	1.34	2.09
8	4.96	0.25	0.12	0.06	6.86	1.46	2.10
10	4.47	0.43	0.14	0.16	9.27	1.62	2.86
12	4.92	0.52	0.15	0.15	10.52	2.06	2.13
15	4.86	0.72	0.10	0.23	10.46	1.96	4.93
21	5.16	0.59	0.17	0.10	11.42	2.29	1.74
24	4.86	0.63	0.13	0.07	12.81	1.76	1.66
27	4.96	0.64	0.13	0.17	12.74	2.44	2.42
30	4.57	1.11	0.14	0.06	19.91	2.42	0.92
33	5.54	0.76	0.12	0.19	14.06	2.26	2.51
36	5.47	0.86	0.14	0.16	12.49	2.22	2.94

cate product, studied in the experiments just described, really provoked two underlying pathological processes ascribable, respectively, to the calcium silicate and to the chrysotile components. The terminal syndrome of peribronchiolar atelectasis, fibrosis, and adenomatoid change was, in fact, of the same kind as that which has been repeatedly produced in The Saranac Laboratory by means of inhaled asbestos dust. The lesion of experimental asbestosis has somewhat more fibrosis to it and shows less of a cellular reaction and the giant cells seen in the present case are less commonly observed. In character with the asbestos lesion is the absence, in the present experiments, of fibrosis of the pulmonary lymph nodes in which no asbestos bodies could be found either.

though the hydrous calcium silicate dust stimulated the proliferation or local accumulation of cells it did not kill these cells. This point is well illustrated in the case of the bronchial epithelium, which proliferated as part of the process of chronic bronchiolar inflammation but did not readily necrose or become desquamated as in animals exposed to quartz dust. In many of the worst seeming adenomatoid or cystic lesions, the bronchial epithelium even retained its ciliated epithelial surface.

The evolution of the adenomatoid reaction is clearly revealed to comprise cryptic distention of the bronchioles, secondary papillomatous ingrowths into these distended lumina, and peripheral epithelialization of atelectatic alveoli.

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 R₁ 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.