PROGRESS REPORT. COOPERATIVE WORK BETWEEN THE UNITED STATES PUBLIC HEALTH SERVICE AND THE METROPOLITAN LIFE INSURANCE COMPANY. FROM July 1, 1932 to December 1, 1934.

General Scope of the Work.

(a) Behavior of dusts introduced into the peritoneal cavity as foreign bodies.

(b) Behavior of dusts in the peritoneal cavity in conjunction with tubercle bacillus infection.

(c) Experimental studies of fusospirochoetal organisms of pulmonary origin.

(d) Studies of synthetic culture media for the cultivation of tubercle bacilli.

The present study of the intraperitoneal response to various dusts was begun at the Pittsburgh station of the United States Bureau of Mines on April 5, 1932. Previous work on this problem had been done intermittently at this station since 1924.

The technique then in use was the introduction of 2 cc of a sterile 10 percent suspension of 200 mesh particle-size dust in physiological saline solution into the peritoneal cavity of guinea pigs. Examination of the animals was made at the end of 14 days for the presence of peritoneal adhesions. Animals injected with limestone were used in parallel as controls. The formation of peritoneal adhesions was considered as indicative of the harmfulness of a dust. For some unknown reason the sample of limestone used as a dust control did not produce sufficient peritoneal damage to cause the formation of adhesions, yet

further study with other limestones, equally pure as far as silica and calcium carbonate content were concerned caused the formation of adhesions in the animals injected. The occurrence of adhesions in a large number of animals injected with a variety of dusts was so inconsistent that it became apparent that variations in technic must be made to further this study.

Dusts Used in Preliminary Study
(Particle size, less than 200 mesh.)
(Dose 0.2 grams.)

Diatomite	Calcite
Limestone, 5 samples	Tripoli
Granite	Flint(Quartz)
Leucite	Siderite
Dolomite	Cyanite
Pyroxene	Strontianite
Rhodochrosite	Muscovite
Albite	Willemite
Pyrophyllite	Andalusite, 2 samples
Smithsonite	Amphibile, 2 samples
Kaolinite	Witherite
Microcline	Serpentine

The dust used in this portion were all derived from pure mineral specimens. The results were all negative with regards to a relation between the formation of peritoneal adhesions and the silica content of the dusts.

Three possible variations in technic presented themselves: - (1) particle size of injected material, (2) duration of tests, and (3) size of dose.

The first variation attempted was with regards to particle size. Three groups of screened dusts were used; (1) that which passed through a 100 mesh sieve and was collected on a 200 mesh sieve; (2) that which passed through a 200 mesh sieve and was collected on a 325 mesh sieve, and (3) that which passed through a 325 mesh sieve. The duration of the tests was 14 days and the size of the dose was 0.2 grams of dust per pig. Pure quartz dust

was first used. Results showed no peritoneal adhesion formation with the 100 and 200 mesh material and adhesions in all of the animals injected with the 325 mesh material. A second series of tests with the same particle sizes, duration and dose including quartz, calcite, chert, limestone and anthracite coal was made. It was observed that the 325 mesh material behaved more constantly in the peritoneal cavity than did the coarser material.

A series of dusts was then injected, using 325 mesh material, 0.2 gram dose and 14 days duration. The dusts used were: calcite, chert, whiting, quartz, bituminous coal, 4 samples of limestone, and 2 samples of anthracite coal. The results, while more constant than when the coarser material was used, did not show peritoneal adhesions with the regularity expected. Adhesions occurred occasionally in animals injected with limestone and sometimes failed to form in animals injected with quartz. It was concluded that tests of longer duration than 14 days would give more information as to the behavior of dusts in the peritoneal cavity.

Two series of tests, one of quartz and the other of calcite were set up. The size dusts used were: 100 to 200 mesh, 200 to 325 mesh, and less than 325 mesh. Animals were killed and examined 7, 14, 56 and 90 days after injection. In addition to the formation of adhesions or their absence, another observation was made. The nodules formed by the calcite were much smaller in 90 days than those noted at the end of 7 days after injection. The nodules formed by quartz, on the other hand, were larger in 90 days than those observed in 7 days.

A series of tests using 325 mesh particle-size material, 0.2 gm. dose and examination intervals of 7, 14, 30, 56, 90 and 112 days with the

following dusts was set up: quartz, chert, calcite, limestone, soapstone and bituminous coal. These tests showed the dusts produced three different types of reaction in the peritoneal tissue: (1) quartz and chert produced a cellular proliferation which caused the formation of large nodules about the dust; (2) calcite and limestone decreased in amount in the tissues as the interval between injection and autopsy increased; (3) bituminous coal and soapstone apparently remained inert, causing neither a cellular proliferation nor did they show evidence of disappearance. This varied response was constant in every animal injected with the same dust.

Inasmuch as the 325 mesh material gave such constant results, it was thought that the use of finer material might speed up the reaction. An air separater was borrowed from the Bureau of Mines and a number of dusts were separated. Particle-size determinations were made. All of these dusts had a median particle-size of less than 5 microns with the exception of soapstone which was 8 microns. Series of guinea pigs were injected with 0,2 gm. doses of the following dusts. Examinations were made in 7, 14, 30, 56, 90, 180 and 360 days after injection. Results of these tests are listed with the dusts:

Dusts giving an absorption type of reaction

Calcite	Limestone
Gypsum	Precipitated calcium carbonate

Portland cement.

Dusts giving a proliferative type of reaction

Quartz(rock crystal) Flint (normal quartz

Chert (chat)

Dusts giving an inert type of reaction

Soapstone	Anthracite coal (2 samples)
Carborundum	Bituminous coal (2 samples)
Precipitator ash	Jewelers rouge (hematite)

Metro-nite white, a mixture of calcium carbonate, magnesium carbonate and calcium magnesium silicate produced a reaction in which the first two components were absorbed and the silicate remained inert in the tissues.

A specimen of talc produced an inert reaction; only 180 and 360 day examinations were made of these two dusts.

A specimen of dust from the mixing room of a glass plant was tried but could not be used as the high percentage of soda ash was too toxic when introduced into the peritoneal cavity.

The finer sized particles, i.e., elutriated dust, produced a more rapid response than did the 325 mesh material. This was particularly apparent in the case of calcite which disappeared entirely in 180 days. However the gross reaction produced by the 325 mesh material was sufficiently differentiated to permit classifying a dust as to the type of response it produced.

Another series of 325 mesh dusts, examined later because the use of the air separator was not available, showed results comparable to those in the other series, i.e., they all could be differentiated into one of the three groups. These dusts, for the most part, were not part of the planned study but were sent in by various individuals to determine their suitability for rock dusting coal mines or other potential industrial hazards. These dusts were: -

Dusts producing an inert reaction

Atalcite, a mixture of tremolite and talc

Calfos, a crude calcium phosphate parting compound

Foundry sand, two samples, both containing sericite

Sericite, an almost pure sample

Fullers earth

Precipitator ash

Feldspar

Dusts showing a proliferative reaction

Tripoli

Silica-Sericite. This sample was 50 percent silica and 50 percent sericite.

Conclusions derived from the behavior of dusts in the peritoneal tissue

1. That dusts elicit three types of response, (a) are absorbed, (b) cause cellular proliferation, (c) remain inert in the tissues.

2. This differential foreign body reaction has always been constant, - the same dust producing the same response in all of the animals injected with that dust.

3. All of the dusts studied have behaved in a manner characteristic of one of these three groups.

4. This biological classification may be used as a basis for correlating the relative harmfulness of a dust.

5. Those dusts eliciting an absorptive reaction are known clinically

6. These dusts eliciting a proliferative reaction are known clinically to cause silicosis.

7. Most of the dusts eliciting an inert reaction are known to cause pneumoconioses of varying degrees of severity.

8. Sufficient reaction has taken place in 90 days after injection to permit differentiation of the type of reaction. This length of time seems not too long when compared with inhalation methods of examination and considering the slow development of silicosis.

Asbestos Studies

During the past three years several attempts to obtain an asbestos dust suitable for study met with failure. The mineral itself could not be reduced to dust by ordinary laboratory grinding methods. The dust obtained from plants could not be passed through a 325 mesh sieve because it matted. Water separation was impossible because the dust coagulated and occasionally showed colloidal properties. An air separator was not available. Of a number of samples of asbestos obtained through the Metropolitan Life Insurance Company, three were selected because of their small particle-size and that they made rather uniform suspensions in physiological saline solution. These three were representative of the different kinds of asbestos. They were: (1) Canadian chrysotile, (2) African crocidolite, and (3) Amosite. The sterilized material was injected as received without further attempt to separate into finer size groups.

Series 1.- Groups of guinea pigs were injected with 0.2 gm. doses of the above three dusts. Animals from each group were killed and examined 14, 30, 56, and 90 days after injection. Quite a number of the animals injected with the Canadian asbestos and amosite died in the first week. Autopsy showed a peculiar bright red appearance of the blood and of some organs, especially the lungs, which were markedly congested. It was assumed that there might be some soluble toxic substance in the dust. The animals that remained alive for the duration of the test showed an inert type of reaction in every instance with each of the three dusts.

Series 2. - A second group of animals were injected with 0.1 gm. doses of the same three dusts. The number of deaths in the first week were materially decreased. These tests have passed the 56 day period and have shown identical results with those of the first series.

Inasmuch as by this technic of examination, asbestos falls into the inert group of dusts it seems that the importance of this biological classification in relation to the clinical picture of pneumoconiosis is strengthened. As the histological picture of silicosis is a nodular fibrosis and that of asbestosis is a diffuse fibrosis, some correlation between the inert group of dusts and clinical pathology of the various pneumoconioses may be made.

Colloidal silica.

A series of animals injected intraperitoneally with 0.2 gm. of colloidal silica in physiological saline solution showed two interesting points: (1) the material is relatively toxic in itself causing the death of a number of animals in the first few days following injection; (2) the silica forms glistening [illegible] nodules and frequent peritoneal adhesions. These

nodules disappeared in 90 days and in 245 days only peritoneal adhesions, such as the omentum to the anterior abdominal wall, showed any evidence of the presence of any damaging material having been in the peritoneal cavity. Experiments with colloidal silica were not continued as such a study would have been too much of a deviation from the main problem.

Studies using variable quantities of dusts.

Early in this work four groups of animals were injected with 325 mesh particle size chert, calcite and two samples of limestone. The doses of these doses were 0.1, 0.033 and 0.011 gm. and the purpose was to determine whether the quantity of dust had any relation to the formation of peritoneal adhesions. The duration of the tests were 14 days. Adhesions were found in one of the limestone injected animals in the 0.1 gm. dose group. Simultaneously with the completion of this series the observation was made that dusts of different character produced different types of nodules so this line of approach was not continued.

Recently a second series of tests was begun to determine if the tissues could assimulate a definite quantity of dust. Air-separated quartz and calcite in doses of 0.1, 0.05 and 0.01 gms. were introduced intraperitoneally into guinea pigs. Animals were killed and examined 14, 30 and 60 days after injection. In 60 days practically all of the calcite of the 0.01 gm. dose series had disappeared. With both dusts, the greater portion of the dust, and often the entire amount, was found in the omentum. In 60 days the 0.01 gm. doses of flint showed larger nodules in the omentum than were noted in 30 days with the same dose.

This line of investigation, using smaller doses of quartz, is being continued and will be completed this year.

Proposed future study:-

Minimal doses of dust. It is desired to learn what is the largest amount of silica that can be eliminated by the tissues in a given time without causing gross damage, i.e., visible dust nodules. The portion of this study now in progress involves quartz alone. It is desired to extend this study to include some of the dusts of the inert response groups as many of these dusts are definitely known to cause pneumoconiosis.

Mixed dusts. Combinations of such dusts as limestone and quartz in varying proportion should be made to determine if the presence of one has any influence on the response elicited by the other. This would be particularly interesting with large quantities of limestone and small quantities of quartz.

Intraperitoneal response to pure inorganic silicates. It is desired to substitute chemically pure inorganic silicates in place of dusts in the intraperitoneal injection tests to determine, (1) if silica compounds will alone produce cellular responses similar to that produced by pure SiO_2, (2) whether their toxicity is dependent on their solubility, ionization or toxic ions such as Barium and potassium. Studies using colloidal SiO_2 crystalline quartz should be made in parallel with the above. A series of 8 pure silicates are now on hand for this work.

Asbestos. Further work on asbestos should be done. Laboratory methods for separating the material into particle-size groups and for removing the talc present with the material should be developed. This might be possible by air separation. Microscopic studies of the lesions produced by asbestos

should be studied for the purpose of determining the part the fibers play in the formation of the fibrosis and if possible the origin of the asbestos bodies and the mode in which they form.

Studies with dust followed by tubercle bacillus H-37.

Four groups of guinea pigs were injected intraperitoneally with 0.2 gm. doses of quartz, calcite, chert and bituminous coal (324 mesh particle size). On the 104th day after injection, a sufficiently long time to permit the formation of a well defined dust reaction, 0.1 mg. of an 18 day culture of tubercle bacillus strain H37 was introduced into the peritoneal cavity. Animals injected with dust and tubercle bacilli alone were used as controls. In all of the animals injected with dust and tubercle bacilli, the tuberculous involvement was greater than in the controls with tubercle bacilli alone.

A second series of tests in which the above named dusts remained in the peritoneal cavity for 128 days previous to an injection of 0.1 mg. dose of tubercle bacillus H37. Twenty-one days after injection of the tubercle bacilli the animals were killed and examined. The results were identical with those found in the first series.

As Tubercle Bacillus strain H 37 was found to be more virulent than was desired, cultures of strain R1, a less virulent organism, were obtained for use in the remaining portions of this study. A series of guinea pigs injected with various doses of this strain showed that the least grossly visible involvement in three weeks was given by a 0.1 mg. dose. This dosage was used in the following studies.

Dust and Tubercle Bacillus R1 introduced at the same time

Three groups of animals were injected intraperitoneally with 0.1 gm. doses of air separated calcite, quartz and anthracite coal and 0.1 mg. of a 20-day culture of tubercle bacillus R1. at the same time. Controls with dust and tubercle bacilli alone were injected simultaneously with the tests. Animals were killed and examined 21 and 42 days after injection. The results of these experiments were similar to those where the infection followed the exposure to the dust. All of the animals treated with dust and tubercle bacilli showed more tuberculous involvement than was found in the tubercle bacilli control animals. Quartz showed the most marked involvement, anthracite coal to a lesser degree and calcite least of all, but still more marked than the controls. In the case of anthracite coal, small fine tubercles appeared as clear raised nodules in the diffuse areas of dust on the anterior abdominal wall. Tuberculous involvement was, of course, more general in all of the animals but this point is mentioned here for comparison with a later series of experiments.

The above tests were checked repeated using calcite and quartz only. The results obtained were identical.

Tubercle Bacillus R1 infection followed by injections of dusts

Three groups of animals were inoculated intraperitoneally with 0.1 mg. of tubercle bacillus R1. Twenty-one days later, a sufficient time for development of visible tubercles, 0.1 gm. of calcite, quartz and anthracite coal was injected into respective groups of pigs. Dust and tubercle bacilli treated control animals of similar lengths of exposure were maintained. The animals were killed and examined 21 days after injection of the dusts.

Forty-two day animals are now on test. Results showed that the tuberculous involvement was greater in the animals subsequently treated with the dusts than was found in the controls inoculated with tubercle bacilli alone. The most involvement was found with quartz, anthracite coal showed a lesser amount and calcite the least. In the case of anthracite coal, zones of fine dust particles collected about the small tubercles on the anterior abdominal wall, and the dispersed dust was not generally diffuse as noted in previous tests where the dust had been injected previously.

Proposed future studies.

Dust in combination with tubercle bacilli. Further studies with varying time intervals between the exposure of dust and infection should be made. The most important of these studies in view of the results found in the three experiments reported here, is to determine if there is an interval after exposure to dust, particularly silica, in which the animal will not show any more tuberculous involvement than would be found in the control animals. Animals injected with dusts would be infected with tubercle bacilli at some interval longer than 128 days after the injection of the dust. (Experiments involving 128 days showed a greater susceptibility to tuberculosis). Other routes of infection, such as intravenously, should be used in parallel with intraperitoneal infection. A microscopic study of all tissues from these experiments should be made.

Microscopic study

An extensive microscopic study is now being made of the nodules formed by the dusts reported in the first two publications resulting from this study. [illegible] the entire process will be studied histologically this

To date all of the proliferative and most of the absorptive type of dusts have been studied. The remainder will be completed this year.

It has been noted in the preliminary study that the fine dust particles, less than 5 microns, of the proliferative series elicit an early fibroblastic reaction followed in from 30 to 65 days with a macrophage increase which later undergoes retrograde changes and ultimate fibrosis, fatty degeneration and calcification. The larger particle material, - that passed through a 325 mesh screen - shows an initial macrophage proliferation, i.e., a typical foreign body reaction, which later becomes fibrosed and calcified. While grossly the picture presented by the quartz of two different size groups is the same, the microscopic picture, especially in the early stages after injection is decidedly different. The microscopic study promises to give some idea as to the possible prognosis of dust pathology and elimination. This study will be completed this year.

Proposed future study

Microscopic studies. Because of the variation in the early picture of silica dust when 325 mesh and 5 micron particles are used and attempt should be made to determine why the coarser material presents the microscopic appearance of a typical foreign body reaction whereas the finer material shows such an abundant proliferation of fibroblasts. As far as using the gross reaction as a test this phenomenon is not important but with the application of the information derived from these intraperitoneal studies to clinical silicosis, it presents possibilities that may be applied to the prognosis of silicotics. Various particle size groups of dusts should be used. Tissue

cultures may have to be resorted to in order to determine at just what period the formation of macrophages begins. An easier way would be to introduce the dust into the amniotic sac of developing chic embryos and observe after varying stages of incubation. Such observations may permit the shortening of the test we use to determine the harmfulness of a dust from 90 days to a few days. The information obtained regarding the action of dusts on individual cells might also give a clue as to the various types of fibrous tissue formation in the pneumoconioses.

Intratracheal injections. At this time it would be desirable to correlate the reactions produced by dust in the peritoneal cavity with those elicited in the lungs. This study would be particularly helpful in gaining further information about the group of dusts classed as inert in our previous papers. It is quite likely that the mechanism causing fibrosis is different with these dusts compared to that of silica.

Bacteriologic Investigations

1. Fusospirochaetal Organisms

During the routine examination of miners of the Tristate Zinc and Lead District of north-eastern Oklahoma at the Bureau of Mines Cooperative Clinic in Picher it was observed that a relatively large number of the men had chronic putrid bronchiectasis and lung abscess, and that in many of these acute fusospirochaetal organisms could be demonstrated in the sputum. In

consideration of the work of D. T. Smith who had shown experimentally that a symbiotic group of these organisms was capable of causing fusospirochaetal lung disease, it was decided to investigate that possibility and to determine the type of symbiosis that might be responsible for these conditions.

In order to obtain representative pathologic material for the experimental work a number of miners with chronic putrid bronchiectasis and lung abscess were visited throughout the district and sputum was carefully collected, emulsified and injected into the groin of guinea pigs.

A number of these animals developed typical fusospirochaetal abscesses of the groin which were characterized by large necrotic lesions containing greenish-gray, extremely foul-smelling pus which was teeming with several types of treponemata, spirochaetes, spirilla, fusi-form basilli, streptococci and vibrios. From these animals 6 were selected, three of which had been injected with material from lung abscess and three from chronic putrid bronchiectasis in miners with silicosis. Tuberculosis had been ruled out by clinical, roentgen and bacteriologic examination.

The actual experimental work was begun at the Bureau of Mines Experiment Station in Pittsburgh, Pa., after withdrawal of the Bureau of Mines from the cooperative clinic in Pieher.

The fusospirochaetal organisms which are involved in the causation of the morbid conditions under consideration are strict anaerobes and it was necessary to devise appropriate culture methods which would be applicable for the entire group of anaerobes of the upper respiratory system.

After prolonged experimentation the following organisms were isolated in pure culture and were available for animal experimentation: Treponema

microdentium, T. mucosum, T. macrodentium, several types of fusiform bacilli, T. buccale, spirillum sputigenum, anaerobic streptococci, vibrio viridans and a large fusiform bacillus growing in symbiosis with a treponema resembling that of Vincent.

The organisms were studied and classified according to their morphologic, cultural and immunologic characteristics.

Guinea pigs and rabbits were used for the animal experimental work.

In order to determine the pathogenicity of the individual organisms isolated, suspensions in saline of the various microbes were prepared and standardized to 100 million bacteria per cc. 1 cc. of these suspensions was injected into the groin of a series of guineapigs. The results showed that the treponemata, the fusiform bacilli and the spirilla were non-pathogenic. The streptococci produced slight transitory inflammation, the vibrios slight induration. This observation led to the supposition that the streptococcus and the vibrio might initiate the infection by preparing the exposed tissues for the invasion of the other organisms. To test this theory each of these species was combined with the other individual types and inoculated into the groin of guinea pigs. The results showed that more severe reactions were obtained from the combinations containing T. microdentium, T. mucosum, Bacillus fusiformis type I, sub type 2 and type II of Varney than with the combinations of the remaining anaerobes.

It had been previously observed that the injection of the combined streptococcus and vibrio caused more severe reactions than when each type was injected separately. In consequence the two types were combined with the individual other types and injected into the groin of a new set of guinea pigs. In this series, too, the reaction was more severe with T. microdentium,

T. mucosum and the two types of fusiform bacilli than with the remaining organisms and it became evident that the harmful symbiotic group was composed of the anaerobic streptococcus, the vibrio, T. microdentium, T. mucosum and the fusiform bacilli.

A combination of all these organisms was now injected into the groin of guinea pigs and typical fusospirochaetal abscesses, comparable with those occurring in man, resulted.

Further experimentation showed, by elimination, that the harmful symbiotic association was between an anaerobic streptococcus, a vibrio, T. microdentium, T. mucosum, Bacillus fusiformis Type I, sub-type 2 and Type II of Varney, and that T. microdentium and T. mucosum could be substituted for one another. This was also true for the fusiform bacilli, T. macrodentium, T. buccale, sp. sputigenum and the large fusiform bacillus growing in association with the treponema resembling that of Vincent do not enter in the harmful combination.

In addition to the above experiments all possible combinations of the ten types of anaerobes under consideration were investigated experimentally, but only the above described types were capable of causing fusospirochaetal lesions.

After determination of the microbic association responsible for the causation of fusospirochaetal lesions preliminary experiments in rabbits were undertaken.

Two sets of 12 rabbits were inoculated, intratracheally, one with .25 cc. of pus, diluted to 1 cc. with saline, from fusospirochaetal abscesses in guinea pigs, and the other with 1 cc. of a suspension in saline of pure

cultures of the symbiotic group, representing 100 million organisms.

Of the animals in set I (pus) 8 died of fusospirochaetal pneumonia, 3 developed subcutaneous phlegmonous abscesses (probably as a result of extravasation of the inoculum into the tissues of the neck) and one died of fusospirochaetal abscess of the lung with empyema.

Of the second set (pure cultures) 6 died of fusospirochaetal pneumonia, 5 revived under combined arsenic and tartar-emetic treatment and one developed a fusospirochaetal lung abscess with empyema.

The rabbit experiments were interrupted when the experimental laboratory was transferred from Pittsburgh to Washington, on August 1, 1930, and are incomplete.

II. Bacillus Tuberculosis

Influence of industrial dusts upon the growth of the tubercle bacillus.

It has been reported from various sources that silica enhances the growth of the tubercle bacillus, that coal dust retards the growth and that calcite exerts an inhibitory influence. In order to test these assertions the following bacteriologic investigations were made:

Potato slants were prepared and steamed in heavy suspensions of quartz, chert (Fisher chat), bituminous coal and calcite. The dusts were suspended in Long's synthetic solution medium devised for the growth of the tubercle bacillus. Plain potato slants served as controls. This treatment produced a fine coating of dust upon the slants, the finer particles penetrating the rather large pores of the potato. After sterilization the slants were seeded with 5 loopfuls of a suspension in saline of the human

strain H-37 which had been propagated upon glycerin agar at the National Institute of Health. The slants were incubated at 37.5 centigrade and inspected for growth at regular intervals. A total of 316 tubes were examined for growth efficiency in the presence of siliceous and non-siliceous dusts. The results are shown in the following protocol:

	First discernible growth in days	Maximum growth in days
Quartz	8	16
Chert(Picher chat)	8	17
Bituminous coal	10	----
Controls	12	17

In the presence of quartz and chert (96 percent SiO_2) growth appeared much earlier than on bituminous coal and on the control slants. Maximum growth (colonies do not increase in number and size in the horizontal plane) is reached on the 17th to 18th day.

In the case of bituminous coal growth appears much later and is very scant. The colonies increase in size slowly and never reach the size and topography as those grown in the presence of silica.

In the controls initial growth is much delayed, not as luxurious as in the presence of silica, but after 14 days the growth gains speed and maximum growth is attained in 17 days.

In the presence of calcite human strain H-37 does not grow on potato slants, however, the organisms remain viable and, when transplanted upon plain potato, will gradually resume proliferation.

From these experiments it may be concluded that (a) in the presence of silica the initial, latent period of growth is considerably shortened; (b) in the presence of bituminous coal the growth is retarded and finally checked; (c) in the presence of calcite growth is entirely inhibited, but the organisms remain viable and will resume proliferation upon transplantation on favorable culture media.

The above experiments were carried out in the presence of excessive amounts of dust and quantitative ideas may therefore not be derived from them. In order to gain such quantitative information it was decided to study tubercle bacillus growth upon a synthetic medium to which known amounts of silica could be added. Long's synthetic solution was chosen and stiffened with 1.5 percent of Bacto-agar (Bacto agar contains traces of SiO_2, insufficient to interfere with the quantitative work) and the silica was added in the form of e. p. sodium silicate. The resulting medium is crystal clear and the finest growth can be detected without difficulty.

The initial experiments were made with the medium containing .4 percent of sodium silicate. Plain Long agar and Corper's egg yolk medium were used as contrast. The results appear below:

	First discernible growth in days	Maximum growth in days
Si-Long agar	6	12
Plain Long agar	9	----
Egg yolk medium	4	9

Here too, accelerated growth was obtained in the presence of silica while only scanty growth occurred in the controls. Corper's egg yolk medium proved much superior in growth qualities.

Quantitative experiments with smaller amounts of sodium silicate (.01, .05, .1, .2, and .3 percent) showed only a slight increase of the efficiency of the medium. Most favorable results were obtained with the .4 percent concentration. Larger amounts of sodium silicate can not be used in this synthetic medium because it would bring the alkalinity of the medium beyond pH 7.2 which is the optimum reaction for tubercle bacillus growth.

Another group of cultures on synthetic long medium containing c.p. silicic acid were seeded with H-37. The pH was adjusted with sodium carbonate to 7.2. A slightly earlier growth was observed on this medium, but after a few days the growth upon sodium silicate medium had attained the same proportions and from then on proceeded more rapidly.

A comparison of the growth efficiency of the synthetic sodium silicate medium just described with that of Corper's egg yolk medium in 137 clinical cases, of which 13 were positive for tuberculosis, revealed 11 positives for the egg yolk medium and only 8 for the sodium silicate medium. To account for this fact it was reasoned that egg yolk contains a considerable amount of lecithin and that the addition of this substance might improve the synthetic medium and render it as efficient as the egg yolk medium.

To this end pure lecithin, prepared from egg, was added to the synthetic medium in graded amounts and its growth qualities compared with egg yolk medium. H-37 was used. The results were as follows:

	Maximum growth in days
Egg yolk medium:-	
Si-Long agar	12
" " plus .5% lecithin	12
" " " 1.0% "	12
" " " 2.0% "	11

The addition to the synthetic sodium silicate medium of pure lecithin seems to enhance its growth factors definitely though slightly, but it is evident that much larger amounts than 2.0 percent will have to be added. Experiments to that effect are now under way.

Pathological examinations of human material

In the past three years, 16 specimens of lung tissue, some as prepared slides and some as whole lungs, were received and examined pathologically. Reports were made to the persons submitting the material. It was endeavored to make these reports as complete and attractive as possible to encourage physicians to submit such material for examination. The reports included, when possible, a clinical and industrial history, autopsy report, gross and microscopic report of the tissue submitted, pictures of the lungs, prints of X-ray plates, and chemical analysis of the lung tissue. A gross specimen mounted in a celluloid container and duplicate microscopic slides were sometimes sent with the report. Such material has been very helpful in studying the pathological picture of pneumoconioses and the trouble taken in preparing the reports has been well worth while.

Laboratory equipment

When this work was started at the Bureau of Mines it was necessary to equip a laboratory suitable for this work. About a month was spent in this and preliminary reading and experiments. When the health work of the Bureau of Mines ceased, this work was transferred to the Public Health Service and moved to Washington. Some of the equipment used in Pittsburgh was brought to Washington which enabled the work to go on uninterrupted. In Washington the physical equipment of the laboratory has constantly improved. By dividing ~~partitioning~~ a large room, we now have a well lighted and ventilated animal room containing 70 cages in good condition, a bacteriological and pathological laboratory with all necessary equipment and a small shop which has enabled us to make innumerable pieces of useful and necessary furniture and equipment. Recent acquisitions of new equipment from the Public Health Service aided the work materially.

Personnel Active on this problem.

J. W. Miller, pathologist

F. C. Proske, bacteriologist and medical technician

H. F. Brubach, technical aide

Ray Stoll, laboratory aide and mechanic

Due to savings resulting from salvage of equipment soon after transfer to Washington, it was possible to hire Mr. Stoll to take care of the animals. He has handled this very satisfactorily and in addition had taken care of all of the shop work that has been required. Practically all of the Metropolitan Life Insurance appropriation is now used for salaries.

Cooperative aid from the Public Health Service

F. W. Coldman, chemist in the Office of Industrial Hygiene and Sanitation has performed a number of the dust analyses of the materials used in our work.

Dr. D. W. Armstrong, physicist, has been working on the determination of silica by spectrographic methods. A satisfactory qualitative method has been developed. A quantitative method is now being studied. Results indicate that it will be eventually successful. Such a method of determining minute quantities of silica in body fluids will be of much value in studies on silica metabolism.

Dr. J. M. DallaValle has aided on numerous occasions in measuring dust particles and in the construction of an air separator for use in obtaining fine dust for study.