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October 30, 1947

OWENS ILLINOIS GLASS COMPANY

TOLEDO, OHIO

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Interim Report on Animal Inhalation  
Experiments with KAYLO

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SUMMARY: The first inhalation experiment with this dust was begun in February, 1945. Due to a high incidence of fetal pneumonia, another similar experiment was started in August, 1945. Both of these experiments were designed to test the effect of the dust alone by inhalation in guinea pigs. In August, 1945, also, an experiment was begun to test the effect of inhaled dust on tuberculous infection in guinea pigs. In October, 1945, a group of white rats was placed in the dust room in order to study the effect of Kaylo inhalation in this species.

It may be said here that there is no evidence to suggest that the dust alone when inhaled into the lung produces pulmonary fibrosis in rats in an 18 month period, or in guinea pigs in a 30 month period. The results of the experiment in which dust exposure and tuberculosis were combined are inconclusive and further work should be done before a definite statement can be made as to the possible effect of the dust on tuberculous infection. All these experiments are virtually completed, although several animals in the original groups are being kept alive for more prolonged exposure.

INVESTIGATIONS:

- A. Petrographic and x-ray diffraction analyses of the dust supplied and used. Analyses were carried out initially and periodically during the course of the experiment. The composite of such analyses reveals the following:

Samples from the supply carton

Quartz <1% (approximately)  
Chrysotile and Serpentine 15 - 20%;  
largely fibrous. The fibers and  
bundles present are usually several  
millimeters long; and seldom are less  
than 300  $\mu$   
Calcite 5%

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Balance consisted of clay-like masses which gave a definite x-ray diffraction pattern not unlike a hydrous calcium silicate.

Samples collected from the atmosphere of the chamber.

Quartz 1%  
Chrysotile and Serpentine 10 - 15%;  
less fibrous material than in sample above; many fibers are shorter than 20  $\mu$ .

Calcite 10%  
Balance consisted of clay-like masses which gave a definite x-ray diffraction pattern not unlike a hydrous calcium silicate.

The analyses reveal several points of interest:

The low quartz content (1%) indicates that the amount of crystalline free silica in the dust is practically negligible as a possible cause of silicosis.

Although diatomaceous earth is one of the materials from which KAYLO is made, only a trace, probably much less than 1%, is unaltered and appears in the final product in its original form. Nearly all has been changed by the manufacturing process into another chemical compound, calcium silicate.

Most of the material consisted of clay-like masses which gave a definite x-ray pattern similar to that of a hydrous calcium silicate.

About 10 to 15% of the atmospheric dust in the experimental dust room was chrysotile or serpentine. Both of these minerals produce the same x-ray pattern. From petrographic examination of the original material, it appears that chrysotile, which is a fibrous mineral, is present in much greater amounts than the non-fibrous serpentine. In the atmospheric dust also, the fibers still predominate over the non-fibrous serpentine.

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In the original material, the fibers were present in bundles often several millimeters long; not many were shorter than 300  $\mu$ . The fibrous portion of the atmospheric sample, however, consisted mostly of shorter bundles and of fibers relatively few in number, which were generally about 10 to 40  $\mu$  in length.

B. Techniques of Investigation.

Two species of animals were introduced into the dust chamber assigned to this investigation. They included guinea pigs and white rats, animals which the Saranac Laboratory has used routinely in its studies apropos pneumoconiosis. Appropriate quantities of the original material supplied by the company were introduced into the dust generator of the chamber and pilot runs were made to ascertain the character and concentration of the air-borne dust produced. Having established the desired concentration, the animals were then introduced and throughout the course of the investigation dust counts were taken periodically.

The average concentration of the air-borne material was 122 million particles per cubic foot of air. This figure varied somewhat from month to month, ranging from a lower limit of 99 million to an upper of 122 million particles. This range is not unique for this material but has been observed also with most agents which we have employed. It results no doubt from day to day variations in atmospheric conditions which cannot be precisely controlled with our present methods. Nevertheless, we have elected to continue with such methods, believing that to do otherwise would introduce factors uncommon to actual industrial exposure.

The results of the experiments will be presented under the following headings:

- I. KAYLO inhalation alone in guinea pigs.
- II. KAYLO inhalation alone in white rats.
- III. KAYLO inhalation and simultaneous tuberculosis infection in guinea pigs.
- IV. Conclusions.

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I. KAYLO inhalation alone in guinea pigs:

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The course of this experiment through the first fourteen months was discussed in a summary written by Doctor Gardner in May, 1946. In this report he described the difficulty encountered with acute pneumonia in the guinea pigs and concluded that it was an accidental epidemic. His conclusion is supported by the fact that subsequently only four animals have died of pneumonia. This occurred, however, only after all apparently sick guinea pigs were disposed of, the apparently well pigs isolated, the dust room thoroughly cleaned, aired and disinfected, and all the cages sterilized. A third and new series of guinea pigs were introduced after control of the epidemic described. Throughout the course of the study, all animals were exposed continuously to the chamber cloud for eight hours a day, five and one-half days a week.

Thus far, a total of thirty-nine pigs have been killed in order to follow the course of the pulmonary reaction resulting from the deposition of inhaled dust in the lung. The period elected for study ranged from two to thirty months. Over this period no definite gross changes occurred in the lungs. The tracheobronchial lymph nodes have enlarged slightly, but remained soft. Microscopic study of the lungs reveal gradually increasing numbers of dust-containing macrophages. These inactive "dust cells" are found characteristically in clumps within groups of adjacent air spaces. The intervening alveolar walls are only slightly thickened by accumulated lymphocytes, but there is no undue proliferation of tissue histiocytes and connective tissue. Fibrosis is not present. Occasional multinucleated giant cells are seen. In addition, there are a very few elongated segmented brown structure which take an iron stain and are clearly asbestosis bodies. Microscopic study of the tracheobronchial lymph nodes reveals clusters of inactive "dust cells" similar to those demonstrated by the lung. There is no apparent over development of connective tissue, and again fibrosis is conspicuous by its absence.

Results of the chemical analyses carried out on the lungs of the exposed pigs sacrificed at the various periods are recorded in the following table:

TABLE I.

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CHEMICAL ANALYSIS OF GUINEA PIG LUNGS

| Animal<br>Number | Time<br>Exp. | Dry Matter |                       |                       |          | Ash                   |                       |          |
|------------------|--------------|------------|-----------------------|-----------------------|----------|-----------------------|-----------------------|----------|
|                  |              | Ash<br>%   | SiO <sub>2</sub><br>% | H <sub>2</sub> O<br>% | CaO<br>% | SiO <sub>2</sub><br>% | H <sub>2</sub> O<br>% | CaO<br>% |
| 2                | 2 mo.        | 4.42       | 0.24                  | 0.07                  | 0.08     | 5.57                  | 1.59                  | 1.25     |
| 6                | 2 mo.        | 4.94       | .20                   | .03                   | .06      | 4.06                  | 1.62                  | 1.23     |
| 10               | 4 mo.        | 4.47       | .22                   | .09                   | .09      | 4.07                  | 1.99                  | 1.09     |
| 11               | 4 mo.        | 4.42       | .19                   | .03                   | .03      | 4.42                  | 1.89                  | 1.39     |
| 16               | 6 mo.        | 4.13       | .25                   | .03                   | .03      | 5.33                  | 2.00                  | 2.00     |
| 18               | 6 mo.        | 3.74       | .20                   | .03                   | .10      | 5.33                  | 2.13                  | 2.63     |
| 23               | 8 mo.        | 4.59       | .31                   | .03                   | .13      | 6.84                  | 1.77                  | 2.75     |
| 34               | 8 mo.        | 4.34       | .28                   | .11                   | .11      | 5.36                  | 2.43                  | 2.43     |
| 43               | 10 mo.       | 4.50       | .46                   | .21                   | .14      | 10.12                 | 4.30                  | 3.04     |
| 56               | 10 mo.       | 4.61       | .41                   | .14                   | .11      | 8.14                  | 3.02                  | 2.20     |
| 45               | 12 mo.       | 4.06       | .53                   | .22                   | .14      | 10.80                 | 4.65                  | 2.50     |
| 46               | 12 mo.       | 4.99       | .51                   | .03                   | .16      | 10.25                 | 1.60                  | 3.27     |
| 67               | 18 mo.       | 4.03       | .59                   | .26                   | .09      | 12.27                 | 5.31                  | 1.83     |
| 68               | 18 mo.       | 4.90       | .56                   | .32                   | .10      | 17.65                 | 6.56                  | 2.06     |
| 105              | 21 mo.       | 5.12       | .64                   | .11                   | .19      | 12.54                 | 2.03                  | 3.68     |
| 108              | 21 mo.       | 5.23       | .54                   | .08                   | .15      | 10.30                 | 1.45                  | 2.90     |
| 49               | 24 mo.       | 5.02       | .66                   | .06                   | .13      | 12.70                 | 1.20                  | 2.54     |
| 50               | 24 mo.       | 4.71       | .60                   | .08                   | .14      | 12.52                 | 1.72                  | 2.92     |

TABLE I

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In view of the observations recorded above, certain pertinent comments appear to be warranted. The microscopic studies of the lungs and tracheobronchial lymph nodes make it obvious that dust was suspended in the atmosphere of the chamber and that portions of that dust were inhaled and gained access into the lungs of the animals exposed. This is further substantiated by the chemical analyses of the lungs which show a gradually increasing amount of the various mineral components of the dust used in direct proportion to the exposure times. In the case of  $\text{SiO}_2$ , for example, the pigs sacrificed after ten months exposure revealed a two to three-fold increase. The  $\text{H}_2\text{O}$  and  $\text{CaO}$  content also increased but the increments are inconsistent and less manifest.

The silica component of the mineral dust is of such low concentration that a significant pulmonary reaction would hardly be expected in consequence of its presence in the lung. This proved to be the case even in those animals exposed for a period of twenty-four months and revealing an average total of 12.61%  $\text{SiO}_2$  of the lung ash. The character of the tissue response at thirty months is comparable and it is anticipated that the  $\text{SiO}_2$  content will not be appreciably different.

Undoubtedly some of the dust cells constituting the mild cellular reaction observed may be in consequence of the serpentine components of the dust deposited in the lung. This, however, cannot be verified since serpentine is a relatively benign dust and fails to produce reaction other than mere dust laden macrophages. This interpretation is in accord with Doctor Gardner's observation that the silicate serpentine is a biologically inert substance producing at best a mild reaction consisting of small widely scattered local collections of dust laden phagocytes and an occasional giant cell without other cellular reaction and without fibrosis.

The presence of a rare curious body (asbestosis body) indicates that some of the air-borne fibrous component of the chrysotile gained access to the lung. However, the absence of cellular reaction or fibrosis about the terminal bronchioles would indicate that the inhaled quantity of such fibers was of extremely low intensity and insufficient to produce permanent damage. This view is supported by the petrographic and x-ray diffraction studies which disclosed that a major amount of the chrysotile component of the dust consisted of structures too large for dispersion into the breathing atmosphere and for easy access into the lung.

In consequence of the experimental studies with guinea pigs to determine the biological activity of KAYLO, it may be tentatively concluded that KAYLO alone fails to produce significant pulmonary damage when inhaled into the lung.

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II. RAYLO inhalation alone in white rats:

This species was used because the rat is known to develop fibrosis from quartz inhalation more rapidly than other animals. Forty rats were used in this experiment and, as indicated by Doctor Gardner in his report in May, 1946, no difficulty with early development of pneumonia was experienced. In fact, during the first thirteen months of the experiment no spontaneous deaths occurred. From thirteen to eighteen months, about 40% of the animals died with terminal bronchiolitis and lobular pneumonia. This is of no significance, however, because it is well known that laboratory rats beyond the age of one year are subject to this type of lesion.

The results of exposure to RAYLO were followed by killing four rats at each of the following periods: six, nine, twelve, fifteen and eighteen months. The duration of the experiment was eighteen months and all animals have been killed.

The reaction to the dust throughout the experiment has consisted of focal collections in the pulmonary alveoli of macrophages containing particles and spicules of dust. As the duration of exposure increased, the only observed change was an increase in the number of such foci. Similar dust filled macrophages were seen in the tracheobronchial lymph nodes. In no animal was there any tissue reaction of the sort seen in silicosis or asbestosis.

Chemical analyses of the lungs of these rats show that the  $SiO_2$  reached the average level of 16.6% of the lung ash within nine months and subsequently diminished. This is difficult to explain, since in the same period the percentage of  $SiO_2$  in guinea pigs' lungs exposed simultaneously (see Table I) was increasing.

The table of these results follows:

TABLE II.

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KAYLO INHALATION

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SILICA DETERMINATIONS OF LUNG TISSUE IN RATS ONLY

| Animal No. | Group | Ash in Dry<br>Tissue<br>% | SiO <sub>2</sub> in Dry<br>Tissue<br>% | SiO <sub>2</sub> in<br>Ash<br>% | Duration of<br>Exposure |
|------------|-------|---------------------------|----------------------------------------|---------------------------------|-------------------------|
| 1012-202   | IV    | 4.8                       | 0.663                                  | 14.0                            | 6 months                |
| 1012-201   | IV    | 4.6                       | 0.735                                  | 16.1                            | 6 months                |
| 1012-205   | IV    | 4.6                       | 0.85                                   | 18.3                            | 9 months                |
| 1012-206   | IV    | 3.8                       | 0.523                                  | 13.9                            | 9 months                |
| 1012-203   | IV    | 4.2                       | 0.55                                   | 13.2                            | 12 months               |
| 1012-210   | IV    | 4.4                       | 0.655                                  | 15.0                            | 12 months               |
| 1012-215   | IV    | 4.2                       | 0.443                                  | 10.5                            | 15 months               |
| 1012-223   | IV    | 4.2                       | 0.562                                  | 13.3                            | 15 months               |
| 1012-225   | IV    | 3.1                       | 0.45                                   | 13.7                            | 18 months               |
| 1012-234   | IV    | 3.9                       | 0.44                                   | 11.3                            | 18 months               |

TABLE II

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In commenting upon Part II of the investigation using white rats as the experimental animals, reference is made to the comments recorded under Part I, which apply.

**III. KAYLO inhalation and simultaneous tuberculous infection in guinea pigs.**

This experiment was carried out by the use of only one of the three methods which have been found valuable in determining the effect of dust on the course of tuberculosis. In this case the animals were infected with tubercle bacilli by inhalation and immediately placed in the dust room. Thus, we are testing the effect of the early accumulation of dust on the early course of tuberculosis. The other two methods available consist in exposing the animals to either the dust or the infection alone for several months, subsequently exposing them to the other agent. We may then study the effect of accumulated dust on the early course of tuberculosis, or the effect of dust on healing tuberculous lesions.

In the experiment which has been completed twenty-five animals were used. Of these, five died within ten months of the pneumonia which has been a problem throughout all the experiments with this dust. Four others were killed because they were in poor condition, and it was hoped to cut down the incidence of pneumonia by removing them from the room. These four also showed some evidence of pneumonia.

Of the remaining sixteen animals, eleven were killed in order to follow the course of the infection. One was killed at one month and two were killed at six, nine, twelve, fifteen and eighteen months. Two died at fourteen months, apparently from progressive tuberculosis; these will be discussed further below. Two animals died, one at thirteen and one at seventeen months, of undetermined causes, but not from tuberculosis.

Of the animals killed to follow the course of the infection, the lesions were isolated, encapsulated and regressing in all except one of the two killed at fifteen months. In this animal, lesions were widespread throughout the lung, but were associated with the production of fibrosis, indicating a tendency to heal.

The interpretation of the results of this experiment is complicated by the accessory factor that some of the animals were used for breeding during the experiment. This was done in an attempt to develop a strain of animals resistant to the infection which was producing the frequent pneumonias.

A review of the records shows that each of the two animals which died of progressing tuberculous infection had been used as breeders. In addition, the animal which was killed at fifteen months and showed widespread lesions had also been used for breeding.

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Four other animals used for breeding during the same period did not show the extensive disease seen in the animals just described.

Thus, it seems hazardous to attempt to attribute the spreading of the tuberculous infection entirely either to the effect of the dust or to that of breeding.

#### IV. Conclusions:

1. It seems clear from the results presented in parts I and II above that KAYLO acts as an inert dust when inhaled by guinea pigs and rats; that is, it is phagocytized by macrophages, but does not injure them or produce fibrosis or anything suggestive of either silicosis or asbestosis. This indicates that the dust will probably be harmless on inhalation in moderate amounts over prolonged periods by normal human beings.

2. The results observed in the experiment concerning the influence of the dust on tuberculous infection are equivocal. Although it is our feeling that this dust exerts little or no adverse influence on tuberculosis, the possibility that it played a part in spreading the infection cannot be excluded.

3. In view of this possibility, further studies concerning this aspect of the problem should be carried out. It is imperative that the original experiment with infection and simultaneous dust exposure be repeated in its entirety. One further study with a small series of guinea pigs is now in progress. It is designed to reveal the influence of the dust on partially healed tuberculosis. However, it is now felt that this experiment should be carried out on a larger scale by increasing the number of animals. Should this expanded investigation be pursued, it might be identified as follows:

- a. Repeat experiment to determine the biological activity of KAYLO when inhaled into the lung harboring a tuberculous infection, which otherwise would regress and heal; viz.: Does KAYLO stimulate the disease?
- b. Repeat experiment to determine the biological activity of KAYLO when inhaled into the lung harboring a regressing or quiescent tuberculous focus; viz.: Does KAYLO re-activate a focus of infection?
- c. Introduce a new experiment to determine the biological activity of KAYLO inhaled into the lung upon infection; viz.: Does KAYLO predispose the lung to tuberculous infection?

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4. In addition, it appears important to explore the biological activity of hydrous calcium silicate either by inhalation or at least pilot-wise by injection methods should it be impossible to generate a sufficient cloud of atmospheric dust because of the character of the material.

5. These recommended investigations can be carried out simultaneously and completed in a period of eighteen months to two years. It is anticipated that these studies would remove the doubt raised by the results of the original experiments herein reported and would enable us to conclude finally that KAYLO is inert not only in regard to the production of pulmonary injury, but also in relation to associated tuberculosis.

Arthur J. Vorwald, M.D.  
Director of Research  
The Edward L. Trudeau Foundation

AJV:LB

cc. Mr. T. Lehman 12-3-47  
rjs

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