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THE PHYSIOLOGIC RESPONSE OF PLEURAL SURFACES TO IMPLANTED DUSTS*

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LATELY several inquiries have sought to establish the physiologic responses of various body tissues to divers dusts in different particle size ranges. As a consequence, there is at hand extensive experimental information dealing with the action of many specific dusts and mixed dusts, some of which have been applied in studies under conditions simulating the exposures experienced by workers in dusty trades. Among others, Mavrogordato, Gardner, and Gye, respectively, have studied the action of dusts in the experimental animal by the inhalation method. In this procedure, it appeared necessary that artificial dusting be continued over a long period, such as from two to five years. Obviously, the time factor hampers inquiries carried out in this manner.

As early as 1924, Sayers,[1] in an effort to reduce the time period required for the appraisal of the properties of unknown dusts, began experiments to determine the action of divers dusts injected into the peritoneal cavity of guinea pigs. He concluded that living animal tissue in any portion of the body tends to respond in essentially the same manner to any dust, but that the action for every individual dust was specific. Selecting the peritoneal cavity as the most suitable point of application, he observed that, in addition to the foreign body reaction provoked by all dusts, highly different changes resulted from the presence of specific dusts. Thus, quartz leads to extensive proliferation of fibrous tissue, but this was not elicited by limestone or coal. In reports based upon continuation of these experiments, Sayers and his associates[2-4] described a technique for the determination of the fibrogenic properties of unknown dusts following deposition within the peritoneal cavity. In addition to the usual foreign body reaction, three general types of specific reaction were described, namely, the proliferative, the absorptive, and the inert. Within ninety days the type of reaction provoked may be determined, and often changes provoked within thirty days may provide proper conclusions.

These three reactions in more detail were found to be:

The proliferative response: characterized by the production of nodules that progressively increased in size. These nodules, when occurring in clumps, fused together and formed a large single mass. Numerous capillaries were present on the surface and throughout the nodules. The appearance was that of cellular proliferation. It was their premise that this pathology was appar-

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ently due to the chemical irritation supplied by the solution of silica in tissue. In ninety days the nodules become more firm, contracted, and fibrous in appearance.

The inert reaction obtained when the injected dust was neither absorbed nor initiated a cellular proliferation. The nodules become more flattened and spreading with time. Fine particles of dust were usually carried over rather extensive areas by phagocytes.

The absorptive response showed the formation of irregular distinct nodules which were often clumped. These nodules became progressively smaller in size as the interval between injection and examination increased, until the original dust eventually disappeared leaving a small area of fine particles at the site of the nodules. In time these also disappeared without the formation of scar tissue.

Thus a reaction was produced in terms of weeks instead of years as was the case in dusting chamber inhalation experiments. The results of these intraperitoneal injection experiments, especially in connection with siliceous dusts, are in conformity with what would be expected from a knowledge of the clinical picture of the actions of these dusts in the pulmonary tissues of human beings.

McCord and his associates,[5-8] in a large series of experiments, supported findings of Miller and Sayers and added two modified types of responses, namely: (1) *a mixed response,* produced by mixed dusts containing some free silica characterized by a reaction which was predominantly inert, but which had a few typical silicotic nodules scattered throughout the tissue, and (2) *a miliary proliferative response,* produced by dusts that possessed more irritant properties than those exhibiting the uncomplicated proliferative reaction. When such dusts were introduced, it appeared that their irritant component incited such marked movement of the organs that the dusts were widely distributed throughout the peritoneal cavity. Wherever particles of this type of dust were deposited, a proliferative response was obtained.

This virtual biologic assay has yielded an abundance of helpful information regarding the harmful effects to be expected from a variety of dusts.

The effect on tissues elsewhere in the body following the introduction of dusts forms the basis for a number of publications. Kettle[9] studied the responses of subcutaneous tissues, and Kettle and Hilton[10] introduced dusts directly into the lungs through the trachea. Their conclusion from this latter study was that the reaction of the lung to dust introduced in this method is essentially the same as to dust introduced by the inhalation method and may advantageously replace the dusting chamber for many purposes for which it has hitherto been considered necessary. Policard[11, 12] used the cornea and conjunctiva in his studies. Drinker and his associates[13] and Stuber[14] have injected sericite and industrial dusts containing free silica into the lymphatics of dogs. It was their opinion that the cellular response of the draining lymph nodes might be developed into a rapid means of determining the toxicity of certain dusts.

It is not known that any of the aforementioned reports embrace observations with reference to the pleural cavity as a site for the experimental implantation of dusts. The creation of typical characteristic reactions at this site would serve a good purpose, offsetting the unfavorable criticism that the responses obtained from such other tissues as the peritoneum, subcutaneous areas, etc., might not reflect the change produced in pulmonary tissues. Therefore, it has been the objective of this present work to make inquiry as to the responses of pleural tissues in the presence of implanted dusts.

The introduction of materials intrapleurally is rather common, especially in the use of oils in connection with pneumothorax. In the field of inorganic materials, Higgins and Lemon[15] injected finely divided particulate graphite into the pleural cavity, using dogs as the experimental animal. During the first few hours after injection, a black, serous, cellular exudate was found in which polymorphonuclear leucocytes were predominant, while lymphocytes and monocytes were present in a small per cent only. However, after seventy-two hours, the picture was reversed with the mononuclear cells present in great abundance and polymorphonuclear cells in lesser abundance. The mediastinum and pulmonary ligaments were the most actively absorptive structures of the thorax, whereas the pericardial, diaphragmatic, and parietal pleura were relatively free of pigment. The lungs invariably were free of pigment, only the lymphatic system being involved in removing cells and free graphite from the mediastinum. Polymorphonuclear cells disintegrated without any further transformation. Merklen, Waitz, and Kabaker[16] injected India ink and trypan blue into the pleural cavity and found that in general they were phagocytized by vacuolate and endothelial cells and monocytes. Arnaud, Chadourne and Weiller[17] injected 10 per cent creosote in alcohol as an irrigation measure in tuberculosis and produced a strong fibrinous reaction. Desbaillet[18] dealt with the reaction of the pleura of the guinea pig to tar and to olive oil. Evolving tissue modifications showed that the pleura hypertrophied, acquired an apical brush, and devoured foreign particles. There occurred proliferation and embryonic tissue formation with verrucosities, villosities, or more often fungoid masses with a contorted surface. After absorption, the pulmonary parenchyma showed simple hypertrophy of alveolar epithelium and proliferation with an adenomatous aspect. Tar emulsion was split by the pleura, the oil being absorbed and the tar caught up by tissue proliferation or devoured by large mononuclears. Bratiano[19] injected animal fats into the pleural cavity. Matsuda,[20] in studies of absorption and elimination by serous membranes, found that the absorption of organic pigments, acid and basic, showed the peritoneum most active, the pleura less active, and the pericardium least active. Brodsky[21] studied the rate of absorption of potassium ferrocyanide from the pleural cavity of dogs and showed that the resorption of the crystalline substance occurs through the lymphatics to the mediastinal lymph nodes and thence into the blood stream without passing through the lung tissue. Bethune[22] believes that selective pleural adhesions, without fluid formation, can be produced as a preliminary to surgical operations by using a fine commercial talc powder which is blown into the pleural cavity, thus dusting the exterior of the lung.

THE PRESENT WORK

In the present series of experiments 50 rabbits were used. They received varying doses from 1 to 4 c.c. of a dust suspension (1 c.c. of suspension equals 0.1 gm. dust). Ten received amorphous silica;* ten, white rouge;† ten, calcium carbonate;‡ and ten received magnetite.§ The remainder were not injected and used as control animals. The majority of animals were submitted to autopsy thirty days after injection. Twenty animals were autopsied, as a control measure, at periods of fifteen, forty-five, sixty and ninety days. The injected material containing as much as 0.4 gm. of dust did not result in any apparent discomfort to the animals either immediately after injection or for the duration of the experiment. The weights of the animals over the experimental period showed normal gains and infection or injury produced no loss of animals.

Preparation of the Dusts.—Four dusts, shown by intraperitoneal injections into guinea pigs[4, 8] to be representative of the three main types of reactions elicited by dusts in animal bodies, were chosen for this experiment: white rouge and amorphous silica as proliferative dusts, magnetite as an inert dust, and calcium carbonate as an absorptive dust.

The dusts were injected in the form of suspensions, prepared by placing 10 gm. of dust in 100 c.c. of isotonic saline solution and shaking thoroughly.

Technique of the Injection.—No preliminary preparation of the animal was found necessary. The rabbit was stretched out by his front and rear legs on a table with the side to be injected uppermost. A 5 c.c. hypodermic syringe with a glass plunger and an 18 gauge needle were used to inject the suspensions. With particles of the size used, little difficulty was experienced due to locking of the syringe.

The pleural cavity was entered by introducing the needle through the skin in the fourth intercostal space about 3 cm. lateral to the sternum, directing the needle downward and slightly backward and carefully inserting the point until it barely penetrated the parietal pleura and entered the free pleural space. When certain that the needle was in the free space and not in the deep muscles or chest organs, the suspension of dust was injected. As a precaution against the possibility of entering the lung tissue, it was found advantageous to introduce a few cubic centimeters of air into the pleural space before injecting the material, thereby producing a partial pneumothorax.

*Amorphous silica—an air floated cryptocrystalline material, specific gravity 2.6, 100 per cent through 600 mesh. Contains 98.47 per cent free silica (SiO_2). Purchased from Fredrick B. Stevens Co., Detroit. Size distribution of particles—25 per cent under 1 micron, 75 per cent under 2 microns, 95 per cent under 5 microns, 99 per cent under 10 microns.

†White rouge—air floated from "Silica #00" mined in Southern Illinois. Contains 99 per cent free silica (SiO_2), 100 per cent through 600 mesh. Purchased from Terminal Sales Corp., Detroit. Size distribution of particles—15 per cent under 1 micron, 62 per cent under 2 microns, 86 per cent under 5 microns, 96 per cent under 10 microns.

‡Calcium carbonate—a Mallinckrodt analytical reagent, more than 99 per cent calcium carbonate, mechanically screened through a 325 mesh screen. Size distribution of particles—10 per cent under 1 micron, 20 per cent under 2 microns, 46 per cent under 5 microns, 77 per cent under 10 microns.

§Magnetite or black iron oxide (ferrous-ferric)—a Mallinckrodt reagent mechanically screened through a 325 mesh screen. Size distribution of particles—25 per cent under 1 micron, 55 per cent under 2 microns, 75 per cent under 5 microns, 87 per cent under 10 microns.

Gross Pathology.—White rouge and amorphous silica, composed almost completely of free silica, produced identical gross reactions in the pleural cavity of the rabbit. The siliceous material was scattered throughout the cavity, although the larger deposits were found at the site of injection on the chest wall, and on the pericardium. Numerous small deposits were found on the lungs and on the mediastinal pleura overlying the vena cava. Nodules were rarely found on the diaphragm, which is worthy of note since in intraperitoneal injections the diaphragm is one of the tissues more frequently affected. This picture of the distribution of dust in the pleural space was true for black iron oxide and calcium carbonate as well as for the silica dusts.

White rouge and amorphous silica (Fig. 1) produced nodules that were comparatively large in size and showed a strong tendency to fuse with those adjacent. Deposits as large as 2 cm. in diameter, adhering to the pleura and

Fig. 1.—Free silica (white rouge). Large irregular masses on pleural surface of chest wall characteristic of a proliferative reaction.

pericardium, were commonly seen after thirty days. Numerous capillaries were present on the surfaces and throughout the nodules, giving the appearance of cellular proliferation. In almost every case, thirty or more days after injection, numerous adhesions between chest wall and lung, chest wall and pericardium, pericardium and lung, and lung and diaphragm were observed.

Calcium carbonate produced a reaction in the rabbit chest that has been designated as absorptive. Forty-five days after injection the material had completely disappeared from the body and to all appearances had left the chest uninjured. After thirty days, two of the rabbits showed a single, small, round, hard, white deposit of calcium carbonate loosely adhering to the pleural surfaces by one or two fine strands of fatty connective tissue. Capillaries were not present in or on the deposits and there were no evidences of cellular proliferation.

Magnetite (Fig. 2) tended to aggregate into one large flat smooth glistening deposit, either at the site of injection or on the pericardium. Occasional small deposits were seen on the visceral pleura. Ninety days after injection the quantity of magnetite in the pleural cavity approximated the amount injected. No cellular proliferation was observed. Thus, since neither a diminution of material nor cellular proliferation was in evidence, the reaction was termed inert.

Whenever animals were dispatched during early days after the injection of any dust, there were observable the usual and expected evidences of a foreign body reaction. Prior to the expiration of thirty days, the evidences of the initial foreign body reaction had completely subsided.

Fig. 2.—Black iron oxide (magnetite). Masses of pigmented material on pericardial pleura. An inert reaction.

Microscopic Pathology.—White rouge (Fig. 3). Microscopically the tissue response is the proliferative reaction characteristic of free silica. There are large masses of cells having the appearance of macrophages in which can be seen particulate foreign material. In many areas the nuclei are more or less distinctly grouped in rosettes around collections of particles of foreign material. In many of these nuclear groups the boundaries between the nuclei are indistinct or not visible, giving these masses the appearance of aggregation giant cells. In a few areas two types of change are observed in the nuclear rosettes. One change consists of an intensification of staining, an increase in opacity of the cytoplasmic material, and a decrease in cytoplasm relative to the amount of nuclear material present. The other change observed consists of an increase in the cytoplasm in proportion to the amount of the nuclear material with the cytoplasm becoming paler. Here the nuclei are small and are placed in the extreme periphery of the cell mass or aggregate. In a few cell groups where these cell processes are most marked, a nuclear fragmentation occurs. These changes seem to represent progressive stages of degeneration. Fibroblasts and young fibrous tissue cells are present in varying numbers in different sections of the material and also in different portions of the same section.

Magnetite (Fig. 4). Microscopically the tissue response is the so-called inert reaction. Here large amounts of deeply pigmented particulate material appear in the sections. Most of this occurs intracellularly and has the ap-

Fig. 3.—Free silica (white rouge). Photomicrograph (high power). Section from nodule shown in Fig. 1. A proliferative lesion with giant cell formation and particulate foreign material in surrounding cells.

pearance of being phagocytized in such large quantities that the details of the phagocytic cells are obscured. A delicate connective tissue meshwork with a few fine capillaries supports the phagocytic cells. There are also present a few large angular masses of foreign material and around these are typical foreign body giant cells.

Calcium carbonate. Microscopically no characteristic changes are apparent.

Multiple Injections in the Same Animal.—It was carefully noted that in no case was there a spread of material from the injected side of the chest to either the opposite pleural space or to the peritoneal cavity. This suggested, in the interest of economy, the utilization of one animal for the testing of three dusts simultaneously, injections being made into each pleural cavity and into the peritoneal cavity.

A second way in which the three body cavities of one animal may be employed is to use a single dust but to make the three injections at such intervals that at autopsy it will be possible to observe the resultant pathology fifteen, thirty, and sixty days after injection.

In this study ten of the experimental animals were submitted to the implantation of dusts into both pleural spaces, and in one case dust was introduced into all three of the sites suggested above. In no case did the animals show any ill effects from the multiple injections.

X-Rays.—X-rays were made of the chests of five rabbits to determine whether the characteristic proliferative nodule produced by free silica could be distinguished from nonproliferative nodules by this means. Two of the five rabbits had received white rouge, two amorphous silica, and one magnetite.

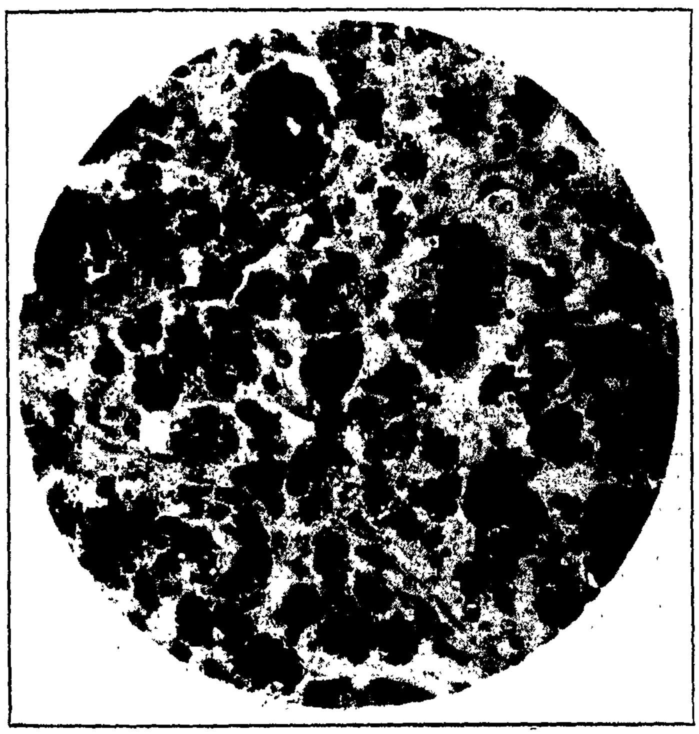

Fig. 4.—Black iron oxide (magnetite). Photomicrograph (high power). Section from pigmented mass shown in Fig. 2. An inert reaction.

Definite shadows could be seen on the x-ray plates of the magnetite rabbit and on one of the white rouge rabbits. The location of these shadows was subsequently correlated with the autopsy findings, but it was concluded that although the nodules could be readily seen on the x-ray plate, they were of no diagnostic significance.

SUMMARY

Forty rabbits were subjected to intrapleural implantation of dust and, with ten additional rabbits as controls, were autopsied after fifteen, thirty, forty-five, sixty and ninety days.

Typical inert, absorptive, and proliferative reactions were produced by the pleura in response to various dusts.

These responses were essentially the same as produced in serous cavities elsewhere in the body by other workers.

The injection of a dust or several dusts into both pleural cavities and into the peritoneal cavity of the same animal was found to be a practical procedure resulting in the saving of animals and time.

The pleural cavity, due to its close proximity to lung tissue, proved to be a suitable location for the study of the physiologic response of serous surfaces to implanted dusts.

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