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Review Section

THE BIOLOGICAL EFFECTS OF TALC IN THE EXPERIMENTAL ANIMAL: A LITERATURE REVIEW

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Summary—A critical review of the literature dealing with the biological effects of a variety of talcs shows the mineral to be fibrogenic when administered by various routes to many species of animal. The literature also indicates clearly that the fibrotic response is a function of the dose administered and that there are levels of exposure that are tolerable. The exposures and doses used in the majority of the studies were not chosen on the basis of their relationship to occupational or cosmetic use. In none of the reported studies was there any indication of neoplasia. The literature offers the reader a good descriptive account of the pathogenesis of experimental pneumoconiosis and identifies the critical role played by the alveolar macrophage.

Introduction

The biological effects of talc have been studied extensively by a variety of *in vivo* and *in vitro* procedures but, unfortunately, most of the literature fails to identify the mineral adequately in respect of its source, particle size, particle distribution and content. The responses to varying doses administered by various routes have been described but here, too, the dose and route of administration frequently bore no relation to the usual levels of cosmetic or industrial exposure. It is unclear from the literature what portion of the research was conducted for reasons of occupational or user safety. In fact, most of the work appears to have been done to satisfy scientific curiosity.

One of the most prominent and early workers on the pulmonary effects of dust was Mavrogordato (1918), who established a hypothesis regarding the pathogenesis of various dust diseases. He concluded from his studies that dusts that create problems are dusts that accumulate. Dusts that produce a marked initial reaction are eliminated because they result in shedding of the epithelium. Those that accumulate do not.

Experimental studies

Haynes (1931) published the results of an extensive study in guinea-pigs on a variety of dusts. From these studies, he was able to construct a beautifully detailed description of the pathogenesis of experimental pneumoconiosis, a description which does not differ much from our concept today. One of the dusts studied was talc. He concluded that talc stimulated the elimination of alveolar macrophages and in no way acted as a toxic agent. In animals given a 2-hour/day dust exposure for 2 weeks, bronchial and lymphatic clearance were proceeding moderately briskly 60 weeks later with no sign of pulmonary damage. Haynes (1931) classified talc among the non-harmful groups of minerals that were found to cause no permanent damage in the lungs.

Stüber (1934) studied the cellular response of the popliteal and cervical lymph nodes of dogs to various dusts which had been characterized according to their content of free silica. The dusts were injected directly into the lymphatics at a dose of 50–100 mg, administered in a volume of 10 ml water at a pressure that did not exceed 70 mm Hg. Talc was one such dust, and it was described as containing 60% silica. Within 24 hours, the author observed giant cells crammed with mineral particles; at day 5 most of the particles were present in endothelial macrophages and by day 108 the node appeared normal but, scattered throughout, were groups of mononuclear phagocytes containing mineral particles. The author concluded that in terms of the reaction evoked, talc and other organic and inorganic dusts differed from silica in that they did not stimulate the formation of abnormal phagocytes but instead were retained exclusively in the reticular cells and endothelial phagocytes.

Fossel (1935) postulated that rocks such as kaolin, muscovite, sericite and talc, which have reached their optimal chemical stability in nature, are probably less reactive biologically than those that are less stable. He also appreciated the difficulty of studying mill workers, in whom the definition of dust exposures was impossible at that time. Accordingly, he exposed guinea-pigs to a talc dust for 1 hour/day for as long as 8 weeks. The total exposure was not defined, but the talc was identified as "Naintsch 00/v" containing 62·0% SiO_2, with 55·4% of the particles falling within the 0·06–0·13 mm range: 14·8% were less than 0·007 mm and 5% were greater than 0·06 mm. While the total number of animals exposed was small (ten), the author completed thorough histopathological studies on the lungs of a few animals and found that there was no evidence of nodulation, as in human silicosis, although numerous dust particles were deposited in the lungs. The longer the interval of time

between exposure and autopsy, the fewer inflammatory responses and the less evidence of talc.

Gardner (1938), like other investigators, recognized the difficulty of studying responses to dust in the human population and resorted to an experimental model to satisfy his scientific curiosity. His interests were not to delineate the hazards associated with occupational or user exposure, but mainly to describe the biological response to various mineral dusts. He postulated that connective tissue would respond to particle stimuli regardless of its location within the body, and therefore injected various minerals intravenously into rabbits as 1 and 10% suspensions in saline, while guinea-pigs received 200-mg doses into the abdominal cavity. Gardner (1938) made the first attempt to quantitate the response and offered a logical descriptive response profile which varied from ± to 8+. The response to talc was graded as 2+ which, in this investigation, meant that the cells directly adjacent to the mineral had been irritated and that there was slight evidence of inflammation. The author concluded that "this change has shown no tendency to progress in two years".

Policard (1939–1940) was sufficiently curious about the potential biological effects of inhaled talc to expose rats to an extremely intense dusting with industrial-grade foliated talc (for 3 hours/day for 12 days with a maximum observation period of 32 days). While the particle size was described as less than 5 μm, the concentration of dust in the atmosphere was not known. The author concluded that acute effects of exposure to a high atmosphere of talc could be irritating to the upper respiratory tract and probably accounted for the dyspnoea that he observed in his experimental animals. In contrast to silicate, talc did not appear to damage the nucleus and mummification of the cell was never observed. From histological observations, elimination of talc particles through the lymphatic system did not appear to be very extensive. The particles were trapped in alveolar macrophages and the mineral seemed to be harmless to these cells.

Schulz & Williams (1942) were the first to recognize that differences in the chemical composition of 'talc' may account for the differences in the responses observed. They selected seven commercial 'talcs' with widely different mineralogical compositions and injected guinea-pigs with these sterilized talcs, administering in each case 200 mg in the form of 5% saline suspensions. Animals were killed at intervals ranging from 10 days to 15 months. The major impurities identified were serpentine, carbonate, quartz and dolomite. Grossly, the animals were in good health and all the organs appeared normal, but nodules were observed on the ventral parietal surface of the peritoneum. The lesions became more dense and firm with time. Histological studies confirmed the observations of previous workers that the mineral was mostly phagocytosed by macrophages and giant cells. There was no indication of the proliferation that occurred in silicotic nodules. Interestingly, the carbonate and serpentine components decreased as the experiment progressed and the authors concluded that such talcs were to be preferred from the health aspect inasmuch as smaller amounts of material were permanently stored.

Schepers & Durkan (1955), prompted by the occurrence of a series of deaths from pulmonary disease following exposure to talc mining in upper New York State, initiated a series of animal studies to probe the specific effects of some of the talc contaminants (tremolite, anthophyllite, quartz, serpentine and dolomite). Both the intravenous and intratracheal routes were used in rabbits, rats and guinea-pigs. The size of the dust particles was 3 μm or less for the intravenous studies, while particles and fibres measuring 20–50 μm were used for the intratracheal study. One set of intratracheal experiments was designed to mimic the industrial exposure in New York State. Interestingly, the results of these experiments confirmed that the basic reaction to these dusts was the engorgement of particles by alveolar macrophages and their subsequent immobilization in the lungs, from which they were ultimately extracted or returned to the parenchyma. The degree to which fibrosis occurred depended upon the length of the fibre rather than upon its chemical composition. Particles of 3 μm and less evoked no fibrogenic response, whereas tremolite and anthophyllite fibres of 20–50 μm produced extensive fibrosis. Unfortunately, the investigators did not have talc in a long fibrous form. However, the talc that was used did not lead to collagen deposition. The bronchiolar lesions produced by pure quartz were less marked when talc was added. This protective effect of talc may be explained on the supposition that the koniophores* were effectively immobilized by previous exposure to talc, thus removing the potential impact of quartz.

Wise (1955) compared the reaction of nervous tissue to talc and to starch glove powders. The author did not describe the type or form of talc used but identified the starch as BIOSORB®. His observations, based on applying 75 mg of each powder to the brain, spinal cord and peripheral nerves, revealed that talc produced more scarring than starch. Although the total quantity of powder applied was not in excess of that which could be recovered from a pair of surgical gloves, the method of application did not attempt to mimic the distribution that might occur in actual use; 75 mg would be likely to produce a much greater response when placed at a specific site than if it were dispersed, as might be the case during a surgical procedure.

Lüchtrath & Schmidt (1959), in an excellent and well-documented treatise, emphasized again the importance of defining the mineralogical composition of talc before attempting to develop a causal relationship to lung disease. The authors questioned the value of additional studies in man as a means of advancing our knowledge on talc but were quick to point out that experimental studies have failed to clarify the problem because of the variable composition of dusts used in animal experiments. Their studies were designed, therefore, to compare the pulmonary response in rats to a sample of pure talc containing no quartz and only traces of chlorite, to two samples containing from 1 to 3% quartz plus some chlorite and to a fourth sample containing 8–10% quartz, partly in the form of cristobalite. The latter sample

*It is believed that the authors were referring to macrophages.

was fired in the course of normal manufacturing and was subsequently ground. The test minerals were administered to rats by the intratracheal route at a dose of 50 mg in 1-ml-water, but this resulted in extensive dyspnoea and a high death rate as a consequence of bronchial occlusion, so the dose was reduced to offset the acute response. One wonders why investigators who were so meticulous in describing the shortcomings and pitfalls of previous studies erred in the direction of a poor protocol by using a method of administration that did not attempt to simulate occupational or cosmetic exposure. The rats tested by Lüchtrath & Schmidt (1959) developed an acute inflammatory effusion which "assumed pneumonia proportions" but, as the investigators stated, "it is not surprising if one considers the large amount of dust penetrating into the lungs". Following the acute phase, it became possible to discriminate between the reactions to the different forms of talc. It was concluded that the aspiration of pure talc dusts resulted in the development of small nodules consisting of histiocytic storage cells but no major fibrotic reaction was noted. In contrast to this, the dust containing free silica resulted in marked pulmonary fibrosis. On the basis of these animal experiments, Lüchtrath & Schmidt (1959) expressed the opinion that one would not be justified in condemning pure silica-free talc as having a fibrogenic effect on the lung. The authors did recommend, however, that appropriate protective measures be taken to minimize unnecessary industrial exposure.

Kuchling (1961) introduced talc into the peritoneal cavity of five different species of birds, suspending 1·5 g undefined talc in 10 ml saline and injecting volumes of 2·0–0·5 ml, depending upon the size of the bird. While there were minor differences in the responses, it appeared that these were less pronounced than the differences reported in mammalian species. The study added very little to a better understanding of the biological effects of talc.

In 1962, two groups published papers relating to the peritoneal responses of talc. Blümel, Pizá & Zischka-Konorsa (1962) were interested primarily in the response to a starch glove powder and used talc as a reference material. They described neither the strain of rat used, nor the mineral content, source or dose of talc administered. Zullig (1962) also administered talc intraperitoneally and subcutaneously to rats, but he, too, failed to disclose the composition of his talc or its source, when comparing the responses to talc and to starch injected intraperitoneally in a 2-ml dose of a 1% aqueous suspension. Both investigating groups discovered that the tissue response to talc was greater than it was to starch.

A poorly understood and probably nonspecific effect of talc was described by Nishimura, Rosenheim & Klein (1963) following the subcutaneous administration of a large (1-g) dose of talc of USP grade to mice. This dose was equivalent to 33 g/kg body weight or 1·650 kg talc administered to a 70-kg (150-lb) human adult. A transient depression of hepatic catalase was observed, a response that has also been observed in animals with actively growing malignancies. The response was more pronounced in males and probably related to the injury imposed by introducing a large quantity of mineral into the tissue. Although a humoral factor was considered to be a possible cause of the enzyme depression, the investigators suggested inhibition of protein synthesis as an alternative explanation.

In a study designed to demonstrate the irritant action of an aluminosilicate mineral (spodumene), the integument of rabbits was dusted daily with the mineral for 90 days, after which the condition of the skin was compared with that in two other groups of rabbits similarly treated with rock crystal (SiO_2) and talc (Grigor'ev, 1963). The author reported no indication of irritation in any of the animals treated with rock crystal or talc.

Some of the literature on talc contains the results of studies which appeared to be designed to generate basic biological data with seemingly little relevance to occupational hazard or cosmetic use. Three such papers were published by Eger & DaCanalis (1964), Kaltenbach, Radeke, Nishimura & Siddiqui (1966) and Darcy (1966).

Eger & DaCanalis (1964) went to great lengths to introduce talc and asbestos into the portal circulation and later into the splenic vein as suspensions in carboxymethylcellulose. Using the latter technique, they were successful in depositing up to 140 mg/100 g body weight of each mineral into the spleen, from where it was gradually released to the liver. It was possible, using this dose, to block the portal vein and its branches completely, with hepatic necrosis occurring in the survivors. The authors' additional conclusions about differences in degrees of necrosis produced by the three minerals hardly seem warranted when, for example in the case of talc, they had only eight surviving rats to examine at five different times, the longest period being 10 days. They failed to mention the number of surviving animals in the groups treated with the other two minerals, both of which groups were followed for much longer periods of time.

Kaltenbach *et al.* (1966), in an extension of the work previously reported by Nishimura *et al.* (1963), showed that in addition to hepatic catalase depression, there was also a concomitant increase in the incorporation of leucine. Neither observation was of clinical significance and both probably occurred in response to the tissue destruction following the administration of massive doses of talc. Unfortunately, the investigators did not use another mineral at a comparable dose as a control, but it was believed that the catalase depression was nonspecific.

In a somewhat related study, Darcy (1966) reported on the enhancement of glycoprotein synthesis following various forms of tissue damage. Tissue necrosis was produced by the subcutaneous injection of turpentine. Subsequent injections of turpentine or talc at approximately 400 mg/kg resulted in an increase in serum globulin. The author concluded that the effect was not substance-specific but was probably mediated through direct physical damage to the tissues. Further studies of a similar nature were reported by Gordon & Koj (1968).

Rakowski (1964) was of the opinion that except for the work of two Soviet and two American authors, the literature was devoid of studies dealing with the morphological alterations occurring in talc pneumoconiosis. But as described earlier, five papers were published on this subject prior to that of Rakowski

(1964), whose study offered little, if any, new information and, in fact, has been subject to criticism. He failed to describe the talc or give its source, and his method of exposure consisted of scattering the powder by means of a rubber balloon into specifically constructed cages—a somewhat primitive procedure for the early 1960s. For this reason, the author was able to describe only the duration of exposure (2, 4, 6, 8 and 12 months) and had no knowledge of the daily or total dose. The reaction to talc was compared with that to Axotox dust, a product containing 95% talc plus DDT. Both materials produced in the early phases a diffuse inflammation in the parenchyma which later became focal. The response to Axotox was more intense than that to talc. Scarring with collagen deposition did not occur until month 10 or 12.

In a series of experiments directed toward a better understanding of the physiopathology of cobalt-induced epilepsy in the rat, talcum powder was used by Payan (1967) along with other materials. The mineral was packed into a pellet measuring 1 × 2 mm and implanted into the frontal cortex of the animals; 8 days later the convulsive threshold of the animals was checked, and on day 33 they were killed and the brains were fixed in Bouin's solution for histopathological study. The convulsion incidence was not elevated following the talc exposure and the author reported minimal or no reactions to talc, graphite and alumina cream. No mention was made of the source of the talc.

Using an *in vitro* technique to measure the lytic effects of various minerals, MacNab & Harington (1967) added 50 mg of the mineral to a 2% suspension of washed sheep erythrocytes in buffered (pH 7·4) isotonic saline. Significant haemolytic activity was associated with chrysotile, serpentine and all forms of silica tested. The remaining powders, including talc, were either completely inactive or only weakly lytic.

The only reported study dealing with talcum powder in the chinchilla was that of Trautwein & Helmboldt (1967). These authors attempted to prove the hypothesis that chronic interstitial pneumonia in the chinchilla may be a prerequisite for the development of adenomatosis following administration of talc intratracheally as a 2% suspension in saline. The talc, described as pure and obtained from Fisher Scientific Company, Fairlawn, NJ, was administered to each animal in a volume of 2·0 ml. One group of chinchillas received injections at 1, 20, 50, 70 and 90 days and was killed 11 months after the last injection. A second group received a single injection and the animals were killed 24, 48, 72 and 120 hours and 1, 2, 3, 4, 5, 6, 17 and 28 weeks after injection. Animals in a third group were injected weekly for 9 weeks and survivors were killed 1, 2 and 3·5 months after the last injection. Single or multiple administration evoked essentially the same response. The mineral caused chronic irritation of the bronchiolar and alveolar cells and the eventual development of focal adenomatoid changes. The authors were quick to point out that in man and domestic animals, spontaneous pulmonary adenomatosis is often associated with chronic pneumonia caused by viruses, bacteria and other agents. It can be produced in guinea-pigs by Mycobacteria and in rabbits by non-carcinogenic substances such as bacterial toxins, vaccinia virus and HCl. In this study, talc served as a chronic and sistent irritant and as such created a situati was conducive to the development of adenoma

In a study designed to measure the impact of tonitis on small-intestine motility, Lill (1967) was able to find inflammatory changes in the peritor of guinea-pigs either macroscopically or microsc cally following the injection of 3·0 ml of a 0 aqueous talc suspension.

Gross, de Treville, Cralley, Granquist & Punds (1970b) studied in rats the pulmonary response fibrous dusts of diverse composition, including tren litic talc with a high or low nickel content and average fibre diameter of 0·3 or 0·1 μm respectivel All the dusts were suspended in water at levels to to 25 mg/ml. Except for four rats that were killed days after the first injection, all animals were allowe to live out their lives. The response to talc was on of proliferative inflammatory foci followed by considerable shrinkage of the lesions and subsequent con version of the argyrophilic stroma into dense collagen Gross *et al.* (1970b) made a distinction between "inert dusts" and "reactive dusts" on the basis of lesion reversibility, maintenance of the anatomical integrity of the air space and lack of significant collagenization, all of which are associated with inert dusts. The authors classified talc as a "reactive" material, but in none of the animals was there indication of neoplastic change. In a second paper, Gross, deTreville & Cralley (1970a) reported on studies relating to the carcinogenic effects of asbestos dust. They found that 35% of 72 rats exposed to an average of 86 mg chrysotile/m^3 and surviving for 16 months developed neoplasms in the lungs, but hamsters and guinea-pigs similarly exposed did not. The dust contained significant levels of cobalt and nickel as a consequence of the wearing of the hammer-mill used in its generation. To explore the possible connection between these facts, trace metals were added to synthetic chrysotile and these were injected intratracheally into rats. Other groups of rats were similarly injected with talc having a naturally high and naturally low nickel content. All of the animals were allowed to live out their lives and none developed any tumours.

The first real attempt at mineral definition came from Bethge-Iwańska (1971), who strongly emphasized the need for standardization of the mineralogical nomenclature pertaining to talc. She studied two different grades of talc by exposing rats to an aerosol of dust that varied from 30 to 346 mg/m^3. The dust was blown into the chamber six times a day at 1-hour intervals over a period of 9 months. The exposures were intended to simulate the conditions of exposure encountered by employees in the rubber industry. The two talcs used were described as technical grade and pharmaceutical grade. The technical grade contained a smaller percentage of small particles and a greater percentage of large particles than the pharmaceutical grade. This author discovered, as did others before her, that pulmonary exposure to large doses of talc resulted in a syndrome of nonspecific, chronic inflammatory changes, which initially were of the cellular-reaction type and later became fibrotic in character, resulting in a thickening of the alveolar walls and atrophy of the anatomical components of the respiratory tract and leading to emphysema. However, she