Review

Occurrences and Biological Effects of Sepiolite and Palygorskite

by

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ABSTRACT

The palygorskite and sepiolite occur widely in marine and non-marine sediments and in the oceans. Geological situation of these minerals in sedimentary basins are reviewed. They are considered to be generated in lagoon, shallow lacustrine playa which are formed during the last part of drying process of Mg-rich and pH lakes or seas. Hence, these minerals are believed to be good palaeoclimatic indicators.

Since it is currently believed that fibrous minerals are potentially active to induce mesothelioma, several clay minerals were tested for their cytotoxicity *in vitro* using an established cytotoxicity assay. However, it is difficult to evaluate the cytotoxicity and hemolytic action of clay minerals for a number of reasons, because of the presence of variable amounts of impurity in clays, e.g. quartz or cristobalite. The cytotoxicity of the clay minerals *in vitro* may not accurately reflect their pathogenicity *in vivo*. Our studies of biological effects of pure palygorskite and sepiolite are reviewed to show severe biological effects of pure palygorskite and sepiolite are reviewed to show severe biological effects of these minerals to rats. With increasing temperature, their toxicity and hemolytic action were reduced. This may be attributed either to structural changes or to the diminution of their surface area.

Key Words : Sepiolite, Palygorskite, Depositional environment, Pneumoconiosis, Cytotoxicity.

INTRODUCTION

Palygorskite (or as it is referred to commercially, attapulgit) is industrially produced in larger quantities than sepiolite, with the United States being the most prominent producer. The palygorskite occurs widely in nature and the mineral is mined in Europe, Africa and India. Sepiolite is produced commercially almost exclusively in Spain. However, sepiolite and palygorskite are relatively rare in nature. These minerals have received much attention in recent works because of intensive studies of deep sea deposits and increased interests in soil mineralogy in semi-arid to arid areas

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during the past one decade. Despite this, these minerals have wide range of industrial applications and are the most interesting minerals used in industry.

Since 800 years ago, Maya Indians of Yucatan Peninsula have used palygorskite which was called "Sac lu'um" (white earth), for the production of ceramics, and have manufactured the highly prized organic pigment known as "Maya Blue" when mixed it with indigo.^{1,2)} In Spain, "Tierra del Vino", a mixture of calcite and sepiolite has been used for the purification of wine for hundreds of years, and it has been used for making curved tobacco pipes for centuries in Spain, Turkey, Hungary and Germany. At present time these minerals are currently used in more than 100 different applications. Most of the applications are similar to those of the other clays more traditionally employed. The applications are based on following three types of properties. 1) sorptive properties, 2) rheological properties, and 3) catalytic properties. The typical industrial applications are decoloring agents, absorbent granules, cat litter, carriers for insecticides and herbicides, dispersants, saline drilling muds, rubber and plastic industries, asbestos substitution and cosmetics.

On the other hand, much attention is now focussed on fibrous minerals such as asbestos, attapulgite and erionite, because they appear to have some potential for inducing neoplastic diseases. Asbestos has become one of the best known and most feared occupational and environmental hazards in the world. Exposure to asbestos has been associated with the development of pulmonary fibrosis, bronchogenic carcinoma, mesothelioma, probably gastrointestinal carcinoma.³⁾ The general population of industrialized countries is exposed to low but increasing levels of asbestos dusts. The studies from North America, Europe, and South Africa have clearly demonstrated that ferruginous (absestos) bodies, once thought to be the hallmark of exposure in asbestos workers, can be recovered from the lungs of almost everyone in general population.^{3,4)} Because a number of different fibrous dusts, including fibrous glass, aluminum silicates and silicon carbide, can form ferruginous bodies in experimental animals. Gross et al.⁵⁾ have proposed the use of the noncommittal term "ferruginous body" rather than the older term "asbestos body" when the nature of the central core of ferruginous bodies in humans is uncertain. Since many effects of asbestos are thought to be related to fiber size and shape, rather than chemical composition,⁶⁾ fibrous minerals presumably will behave in biological systems in much the same way as the asbestos minerals. Experimentally it has been demonstrated that any long thin insoluble fiber of the proper size (diameter less than $1\ \mu\text{m}$, length upward of 5 to 8 μm) may induce mesotheliomas when instilled into the pleura or peritoneum of experimental animals.⁶⁾ Fibrous minerals appear to have special carcinogenic effects which relate to size and shape, especially small size and high length to which (aspect) ratio.⁷⁾ These effects are clearly seen in both humans and experimental animals in the genesis of mesothelial tumors.

It is reviewed in this paper, first, to make clear the occurrences and mechanisms to generate these minerals in surface conditions, and second, to summarize biological effects of these minerals.

A. Occurrences and Depositional Environments

A.1. Latitudinal distribution and occurrences

Palygorskite has been reported in soils from many countries in the Near and Middle East, from northern and southern Africa, northern and Central America, Australia and even Eroupe. Pedogenic palygorskite is commonly associated with either : ① modern soils that are at present or have in the past been affected by rising ground water. ② soil formation that include distinct and sharp textural

transitions ; this group includes many paleosols. ③ pedogenic features, not necessarily associated at present with soils, such as calcretes, calccrusts and caliches.⁸⁾

On the other hand, sedimentary deposits containing palygorskite, or sepiolite, or both, have been described as forming or formed in normal marine waters, in marine and nonmarine hypersaline waters and fresh water lakes.⁹⁾ The most extensive occurrence of these minerals is in marine sediments with locally important occurrences of lacustrine and hypersaline origin.¹⁰⁾

In general, palygorskite and smaller amounts of sepiolite are apparently characteristic of the perimarine, coastal lagoon environment adjacent to landmasses undergoing intensive chemical weathering in Miocene Series of the southeastern U. S.,¹¹⁾ or the earlier Tertiary System of northwestern France¹²⁾ and Southern Australia.¹³⁾ Sepiolite formed in lacustrine environments in Tertiary System of Spain.¹⁴⁾ These deposits of palygorskite and sepiolite are widely distributed throughout the world in marine, transitional marine, and lacustrine depositional environments. Especially these minerals are formed in soils, lakes or shallow seas of Mediterranean under semi-arid climate.

Fig. 1. Distribution of palygorskite and sepiolite deposits based on geological time.
(Callen, 1984)¹⁵⁾

- : Cambrian - Triassic. (550-190 m.y.)
- : Upper Cretaceous - Early Eocene (80-40 m.y.)
- △ : Middle Eocene - Middle Miocene (40-20 m.y.)
- : Middle Miocene - Pliocene (10 m.y.)

Callen¹⁵⁾ reported the distribution of sepiolite and palygorskite in continental environment which is subdivided into lacustrine and pedogenic, with time and space. The latitudinal concentration of these mineral deposits is fallen between 30 and 40 degrees north or south, as shown in Fig. 1. The distribution of these minerals in arid climatic belt of the Mediterranean reveals a favorable sedimentary temperature effect on the processes leading to the formation of these minerals. Singer¹⁶⁾ concluded that palygorskites are typical of arid and semi-arid soils, and one of the few useful palaeoclimatic indicators among the clay minerals.

These arid and semi-arid climatic conditions were present during the Late Devonian and Carboniferous and Late Permian to Triassic in the northern hemisphere, and in the Early and Late Eocene, Late Oligocene and Late Neogene, and possibly the Late Cretaceous in both hemispheres.¹⁵⁾ The major periods of abundance are in the Late Cretaceous and Tertiary, irrespectively of whether they occur as terrestrial or marine deposit.

Palygorskite occurs as an important constituent of the clay fraction of continental shelf and deep sea sediments.^{17, 18)} The main period of palygorskite occurrence in several Deep Sea Drilling Project (DSDP) cores is the Late Cretaceous.^{15, 19)} In deep-sea sediments without hydrothermal activity, authigenesis of palygorskite is less easy to demonstrate and alternative hypotheses such as reworking and alteration of volcanic materials have been proposed.^{15, 20~21)}

Another occurrences of these minerals are vein type and weathering. Palygorskite veins in acidic igneous rocks were found in Israel and Sinai.²³⁾ Fibrous attapulgite-palygorskite was found to associate with calcite in hydrothermally deposited veins in Pennsylvania.²⁴⁾ Imai and Otsuka²⁵⁾ reviewed the occurrence and genesis of palygorskite and sepiolite in Japan. The palygorskites occur along fissures or faults in dolomite and is considered to have been formed by direct precipitation from an aqueous solution at earth-surface temperature. The sepiolites are considered to be of either hydrothermal or supergene origin, and to have been formed by direct crystallization from low-temperature hydrothermal solution and/or from supergene or marine water which are rich in silica, magnesium and other components.

In Japan, these minerals do not occur in sedimentary deposits of economic importance. However, economical profitable deposits of palygorskite and sepiolite have been found in marine, transitional marine and lacustrine sedimentary sequences, occurring interbedded with dolomite, limestones, opaline silica and phosphates throughout the world. At present, the two most important deposits of these minerals are in the Georgia and Florida (U.S.A) for palygorskite, and in Madrid (Spain) for sepiolite. The principal deposits of commercial significance are located ① near Las Vegas, Nevada²⁶⁾; ② in Amboseli, Kenya^{27, 28)}; ③ in Hunan Province, China²⁹⁾; and ④ Eskisehir Turkey.^{30, 31)} Other deposits, such as those in Masai district, Tanzania; Grant County, New Mexico; and El Bur, Somalia; have been exploited intermittently, but, in general, have had relatively low outputs.

A.2. Depositional environments

All the deposits regardless as to whether marine or nonmarine on present landmasses are dominated by dolomites, limestones, fine or sometimes coarse clastics, and often associated with evaporites, or phosphates, and chert. According to the literature, there are two mechanisms to generate these minerals in surface conditions. ① by neoformation,^{8, 12, 32~35)} or ② by transformation of another clay, such as illite¹⁴⁾ or smectite.⁸⁾

Recent studies of palygorskite from Morocco³⁴⁾ and sepiolite from Turkey³⁵⁾ demonstrated that

these minerals were deposited by direct precipitation from Si-supersaturated lake water under alkaline and saline conditions. The Formation Rouge in Morocco is composed of detrital materials such as clay, dolomite and traces of celestite and quartz, from the erosion of Jurassic to Cretaceous marine deposits. From field observation, this area was subjected to an arid climate. Such a climate leads to concentration of solutions and consequently an increase in pH and salinity. In this concentrated solution and alkaline environment, dolomite was favourable to precipitation. After precipitation of authigenic dolomite, the waters are impoverished in Mg and Ca and become Si-rich. Palygorskite formation needs high pH values and high Si and Mg activities.¹¹⁾ In the formation Rouge, SEM observation show that palygorskite fibers cover authigenic dolomite crystals. They formed after dolomite when the solution was slightly impoverished in Mg and enriched in other elements. Chemical arguments show that palygorskite in the basin (high salinity, high pH, release of Al, Si, Mg by dolomite and detrital illites) were favourable for the formation of palygorskite.

On the other hand, Weaver and Beck¹¹⁾ suggested in their detailed studies of these mineral deposits of the Georgia-Florida that the palygorskite was formed by transformation of smectite. The reaction can be combined with the alteration of dolomite or Mg-calcite to give pure calcite coexistent with palygorskite. This result is favoured by the lower $p\text{CO}_2$ to be expected in the environment of a desiccating shallow lagoon of backish water adjacent to a land mass undergoing intensive weathering.

B. Biological Effects

B.1. Retention of attapulgite in human body

The entries of minerals dusts into the human body are following two ways such as ① the respiratory tract and ② the gastrointestinal tract. Bignon et al.³⁶⁾ reported the attapulgite in biological samples from two patients exposed to attapulgite, one by inhalation and the other one by ingestion. Attapulgite fibers, at concentration of 42,000/ $\text{m}\ell$, were estimated in the lung washing fluid of a patient suffering from lung fibrosis who had been exposed for three years during the processing of attapulgite material. This result demonstrates that attapulgite fibers are present in the alveolar spaces, and may be one of the major etiologic agents in this pneumoconiosis case. Attapulgite fibers, at a concentration of 30,000/ $\text{m}\ell$, were also found in a urine sample from a patient who had ingested 6-9 g/day of an attapulgite drug for six months. This concentration was at least 100 times higher than those measured by Cook and Olson³⁷⁾ in the urine samples from 14 Minnesota residents exposed by ingestion to Lake Superior water contaminated by amphibole fibers.³⁸⁾ These facts reveal that attapulgite and asbestos fibers pass through the human gastrointestinal mucosa.

B.2. Pneumoconiosis

Since the man has exploited the rocks and minerals, they present in his environment and, in many cases. The risk of lung disease associated with this exploitation has been recognized for many years. Extensive studies have been made on the effects of minerals as quartz and asbestos in the pathogenesis of pneumoconiosis in man. Hayashi and Kajita³⁹⁾ have reported that the relationship between the concentration of dominant intrapulmonary mineral dusts and total dusts in pneumoconiotic lungs. The concentration of total lung dust decreases with the decrease of dominant intrapulmonary dust, enumerating in order, clay workers, coal miners and hematite workers, and miners of metal mines. The pattern of interdependence of both concentrations is almost linear. The decrease of the two concentrations seems to coincide with the paucity of quartz content of the dust to which the workers

were exposed. The quasi-linear relationship between the two concentrations suggests that lower the quartz percentage in the lung dust, the higher the contents of total lung dust required to produce significant fibrosis. Although quartz is the most harmful mineral, less harmful dust may cause pneumoconiosis when a large quantity is deposited and retained in the lung. The reports of pneumoconiosis due to clay mineral dust inhalation relatively rare. Associations have been described for kaolin,⁴⁰⁻⁴²⁾ montmorillonite,^{43, 44)} talc,^{45, 46)} sericite,⁴⁷⁾ and illite.⁴⁸⁾ The fibrous clays such as attalugite and sepiolite, if they contain long fibers, induce mesothelioma.⁴⁹⁾

B.3. Inorganic particles in lungs in general population

Early in the 1960s, the problem of asbestos bodies was disseminated from the occupational area into the general environment. The ferruginous bodies were to be regularly found at autopsy in many cities of the world. The dangers of exposure to asbestos and its potential for inducing pulmonary fibrosis and malignancies are well documented.⁵⁰⁾ The detection of the cores of ferruginous bodies from the lungs of the asbestos workers or the general population became a recognized necessity for histological diagnosis of asbestosis or asbestos-related diseases. However, many particulates in human lungs are too small to be detected with the optical microscope. Great progress has been made in the sampling method such as the fiberoptic transbronchial method and the analytical method utilizing analytical electron microscope. In order to obtain the maximum information on the lung dust. The analytical electron microscope which consists of transmission electron microscope and dispersive spectrometry, can be obtained morphological, crystal structural and chemical information on submicron individual particulates in the tissue obtained on biopsy or autopsy without destroying them⁵¹⁻⁵³⁾ and the environmental samples.^{54, 55)}

The incidence of ferruginous bodies and uncoated inorganic fibers in the lungs was investigated in 50 autopsies of adults who died in the period from July, 1970 to August, 1973 in Tokyo by Kajita and Hayashi.⁵⁶⁾ Ferruginous bodies were present in 38 out of 50 autopsies and uncoated inorganic fibers were present in 44 out of 50 cases. The incidences of both ferruginous bodies and uncoated inorganic fibers of male are higher than that of female, and also the incidence of both fibers in aged lungs shows higher than those of younger lungs. They considered that this high incidence of both fibers in the lungs of city dwellers is an indicator to asbestos contamination in the general living environment. Roggli et al.⁵⁷⁾ has determined counts of asbestos in the range from 0 to 20 per gram of wet lung tissue in adults with nonoccupational exposure to asbestos and no evidence of asbestos-related diseases. Churg and Warnock⁵⁸⁾ and have proposed that the upper limit of exposure in the general population is 100 asbestos bodies/g of wet lung tissue.

Recently Arenas-Huerta⁵⁹⁾ investigated ferruginous bodies (FE) in lungs of 270 cases over years of age in Mexico. The numbers of ferruginous bodies per gram of dry tissue increased over the years : 4.2 FB/g in cases from 1975 to 42.5 FB/g in cases from 1988. They suggested that environmental exposures to particles in Mexico tended to be higher each year according to the median counts found 1975 to 1988. Ferruginous bodies as an indicator to document inorganic particles exposure.

Asbestos is not the only mineral found in lungs of city dwellers. Berry et al.⁵³⁾ and LeBouffant⁶⁰⁾ identified quartz, micas, clays, aluminum silicates such as sillimanite, spinel, gypsum, and a variety of other minerals in patients with and without occupational dust exposure. Churg⁶¹⁾ determined that total pulmonary nonasbestos mineral content in 20 patients who had no occupational dust exposure. The mean number of nonasbestos fibers, 106×10^3 /g wet lung, was almost identical to the

mean number of asbestos fibers, $102 \times 10^3/\text{g}$. Thirteen different species of nonasbestos minerals were detected, of which five (apatite, gypsum, talc, silica and attapulgite) accounted for more than half the fibers. Apatite constitute 18 % of total nonasbestos fibers and talc account for 16 % of the total. Both apatite and talc constitute more than one-thirds of the total fibers. All other forms accounted for less than 8 % each. Apatite, talc, attapulgite, gypsum and silica constitute 54 % of the total fibers. Silica was found in every lung, talc in 19 of 20 lungs, and attapulgite in 12 of 20 lungs. Although the source of these numerous and varied mineral fibers is uncertain, possible origins of some of the fibers can be speculated. The two patients who have a lot of attapulgite fibers in their lungs, one lived in insulated home and another one worked in furniture store. Atmospheric contamination would at least account for the presence of minerals such as silica in every lung examined by Churg.⁽⁶¹⁾ The fact that the numbers of different mineral species in the lung do not correlate with each other implies that each entered the atmosphere from a different source.

Toyama⁽⁶²⁾ reported that the higher incidence of fibrotic lung was found in polluted area than in nonpolluted area in his study on the pulmonary fibrosis in autopsy material from sudden deaths in Tokyo. It is very interesting what kind of air pollutant is responsible for the development of lung fibrosis of this type. The particulate pollutants in winter air of Tokyo (Jan. 22 - Feb. 18, 1964) consist of quartz, feldspar, cristobalite, mica, kaolin minerals which are the mineral assemblage of Kanto loam constituting earth surface of the outskirts of Tokyo. The highest concentration of quartz in the air during our survey was $0.034 \text{ mg}/\text{m}^3$ and mean concentration of 3,4-benzopyrene in Tokyo air was $15.1 \text{ } \mu\text{g}/1000 \text{ m}^3$.⁽⁶³⁾ These amounts seem to be too small to produce lung fibrosis. However, Ray et al.⁽⁶⁴⁾ pointed out the very important role of small amount of quartz coexisting with large amount of coal dust in the development of experimental lung fibrosis.

B.4. Cytotoxicity, hemolysis, and acute effect on injection

There is substantial evidence that silica particles are engulfed by alveolar and other macrophages, and that the primary dust lesion in animals is injury to phagocytic cells which have taken up the dust. Sakabe et al.⁽⁶⁵⁾ found a correspondence between the cytotoxicity *in vitro* and fibrogenic potency *in vivo*. Koshi et al.^(66, 67) and Hayashi et al.⁽⁶⁸⁾ reported that biological effects of sepiolite and palygorskite were determined as the toxic action on macrophage, represented by the change of acid phosphatase activity, lactic production, and hemolytic action to red blood cells. Few reports have appeared in the literature concerning the cytotoxicity of clay minerals, and a number of these clay have included ill-defined sample in experiments on other minerals such as quartz. Koshi et al.⁽⁶⁹⁾ reported that the toxic doses of various minerals of 0.5 to 2 μm in particle size were estimated by tetrazolium reducing capacity of cultured monocytes as shown in Table 1. The toxic doses was expressed as the amount of mineral necessary to depress by 50 % TTC reducing capacity of control. Therefore, the smaller the toxic doses, the more the decrease of reducing capacity. As for silica minerals, toxic doses were under 100 μg , while those of sericite from Hitachi was from 100 to 200 μg , and opal, pyrophyllite, talc, sericite from Yoji and Kibuchi-clay were from 200 to 400 μg . Both sericite specimens and Kibuchi-clay contained about 10 % quartz. It was very interesting that pure pyrophyllite, talc and sericite in which no impurity was found by X-ray analysis, were relatively toxic. Koshi et al.⁽⁶⁶⁾ reported that chrysotile, sepiolite and palygorskite did not show the marked inhibition on TTC reducing capacity, because the added serum due to electron transfer in dehydrogenase-TTC system. The range of cytotoxicities reported using the TTC reduction activity test on macrophages was wide,

Table 1. The toxic doses of various mineral dust (The particle size of 0.5 to 2 μm) (Koshi et al., 1961).⁶⁹⁾

under 50 μg	50 to 100 μg	100 to 200 μg	200 to 400 μg	400 to 700 μg	700 to 1,000 μg	above 1,000 μg
tridymite	NaOH lesched quartz	sericite (Hitachi)	opal	topaz	olivine	microcline
	quartz(ground for 2 minutes)		pyrophyllite	fluorite	asbestos*	chlorite
	fused silica		talc	tourmaline		diopside
	cristobalite		sericite(Yoji)	cyanite		obsidian
			Kibushi-clay	diatomaceous earth		albite
				sericite (Nasu)		beryl
				halloysite		pyrite
				sphalerite		NaOH leached obsidian
						muscovite
						anthracite

* The particle size of asbestos was less than 5 μm

suggesting that this method is unsuitable to estimate cytotoxicity of some sort of clay minerals such as sepiolite and palygorskite. In this paper, biological effects are expressed as cytotoxicity using lactic acid production, and hemolytic action and acute effect on injection of sepiolite.

B.4.1. Cytotoxicity using lactic acid production

Toxic effect of sepiolite and palygorskite was expressed as percentage of the amount of lactic pro-

Fig. 2 (A). The effect of mineral dust on lactic acid production. (Koshi et al., 1968).⁶⁶⁾

Fig. 2 (B). Hemolytic action of mineral dust. (Koshi et al., 1968).⁶⁶⁾

duction of the cells to that of a control. Therefore, stronger toxic action of the specimens was shown by a smaller value of this percentage. Lactic acid production was remarkably inhibited in the cells added with sepiolite, palygorskite and chrysotiles as shown in Fig. 2 (A). The results are very similar

Fig. 3. Relation between structural changes of sepiolite and palygorskite on heat treatment and their biological effects. (Hayashi et al., 1969).⁸⁸⁾

S : α -sepiolite ; P : palygorskite.

(A) hemolysis ; (B) lactic acid production of the cells ; (C) phase detected by X-rays (ENS : enstatite ; S II : sepiolite II ; S I : sepiolite I ; CRI : cristoballite ; ENS : enstatite ; AMO : amorphous phase ; P II : palygorskite II ; P I : palygorskite I ; full and open circles denote major and minor constituents, respectively)

Fig. 4. Body weight of rats injected with sepiolite and antigorite. (Koshi et al., 1968)⁶⁶⁾
 + : died.

to the effect to acid phosphatase activity of the cell.⁶⁶⁾ Toxicity of heated sepiolite decreased with increasing temperature, as shown in Fig. 3. Between 610-860°C, the toxicity was very weak compared with the original sepiolite, and it was reduced further at 1250°C. There was no noticeable difference in toxicity between unheated and heated palygorskite below 610°C. The toxicity was remarkably decreased above 1000°C. Although cristobalite was detected at 1250°C by X-rays, the lactic acid yield was not affected.⁶⁸⁾

B.4.2. Hemolysis

Hemolytic action of these minerals was expressed as the ratio of the amount of hemoglobin in the supernatant of the blood cell-mineral suspension to that of the control. Therefore, the stronger hemolytic action of the mineral was shown by a larger value of this ratio. Sepiolite and palygorskite produced a marked hemolysis, while asbestos dusts showed no hemolytic effect as shown in Fig. 2 (B).⁶⁶⁾ With increasing temperature hemolytic action of sepiolite decreased, and above 600°C it was same as the control, as shown in Fig. 3. In the natural state palygorskite showed strong hemolytic action. Between 210 and 800°C this action decreased, showing a similar tendency to the lactic acid production. At 1000°C and 1250°C, it was nearly the same as that of the control.⁶⁸⁾ The hemolytic properties of palygorskite resemble those of cell toxicity.

Table 2. Results of the injection with sepiolite intraperitoneally. (Koshi et al., 1970).⁷⁰⁾

Injected amounts & heated temperature	Average of body weight of 3 rats			Histological changes one year after injection	
	Before injection	8 days after injection	Difference	Changes of peritoneal tissue	Fibrosis
10 mg, unheated	24.6 g	24.5 g	-0.1 g	◎	□
20 mg unheated	27.0	27.0	0.0	◎	□
30 mg unheated	28.0	25.9	-2.1	◎	○
45 mg unheated	27.0	24.0	-3.0	●	◎
10 mg 250°C	24.5	28.8	+4.3	○	□
10 mg 450°C	25.7	29.7	+4.0	▽~△	□
10 mg 610°C	24.2	32.8	+8.0	▽	△
10 mg 730°C	24.4	31.7	+7.3	▽~△	△
10 mg 860°C	23.8	30.3	+6.5	△~□	△
control	24.8	31.8	+7.0	▽	▽

Note : ▽ : None. △ : Adhesion of slight degree.

□ : Adhesion only located on several areas.

○ : Thickening and adhesion, moderate.

◎ : Thickening and adhesion, extensive.

● : Thickening and adhesion, severe.

B.4.3. Intratracheal injection of sepiolite.

In order to mark clear the acute effect of sepiolite and antigorite, they were introduced into the lung of rats intratracheally. Sepiolite showed a very high cytotoxicity and strong hemolytic action, and antigorite was most inert. Rat was injected intratracheally with 1 ml of saline containing 40 mg of mineral. Three rats were used for each mineral. In addition to these treatments, another three rats received sepiolite suspension with 1 % polyvinyl pyridine-N-oxide. After injection rats were weighted every day. As shown in Fig. 4, among three rats injected with sepiolite, two died within 24 hours and one within 48 hours. In the group which received sepiolite and PVPNO one died within 24 hours after injection, but two survived rats did not gain weight for two weeks after injection. There was no lethal case in animals injected with antigorite, and their weights followed the curve for normal weight gaining after remission for two days.⁶⁶⁾

B.4.4 Intraperitoneal injection of sepiolite.

Sepiolite, unheated or heated, of various amounts (from 10 to 45 mg) suspended in 1 ml of saline solution were injected into the peritoneal cavity of rats, every three of them being treated with the same condition. Table 2 shows the comparison of body weight of rats, before and 8 days after injection, and pathohistological findings of peritoneum one year after injection.⁷⁰⁾ The loss of body weight

was observed in the rats injected with sepiolite of the amount of 30 mg and 45 mg. On one year after injection, fibrosis of peritoneum and adhesion among intraperitoneal organs were remarkable in the rats injected with unheated sepiolite. Granulomatous nodules formed around sepiolite fibers were observed on the tissue of peritonea and mesenteries. Only slighter were found in the rats injected with 10 mg sepiolite heated by the temperature of 250°C. The intraperitoneal thickening and adhesive changes were scarcely noticed in the rats injected with sepiolite heated by the temperature above 450°C.

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Summary

Palygorskite and sepiolite occur in continental deposits in following three modes. ① in soils, especially of the caliche or calcrete type, ② in lacustrine closed basin in deposits, and ③ in the alteration of igneous rocks. Their origin has been attributed to direct crystallization, transformation of smectite and to alteration of volcanic ash.

Since their sorptive and colloidal properties, these clays have numerous commercial applications. At the same time, these fibrous minerals is a startling example of a material at once useful, because of their physical and chemical properties, and potential activity to induce mesothelioma. The diseases due to inhalation of only clay minerals, especially palygorskite and sepiolite, appear to be relatively rare. We had studied biological effects of the pure specimens of both minerals. The rise of acid phosphatase activity and hemolysis were provoked by sepiolite and palygorskite. Furthermore, intratracheal and/or intraperitoneal injection of sepiolite was very harmful to rat.

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