The Effect of Lung Burden on Biopersistence and Pulmonary Effects in Rats Exposed to Potassium Octatitanate Whiskers by Inhalation

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Abstract: The Effect of Lung Burden on Biopersistence and Pulmonary Effects in Rats Exposed to Potassium Octatitanate Whiskers by Inhalation: Takako OYABU, et al. Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan—The effect of lung burden on biopersistence and histopathological changes caused by potassium octatitanate whiskers (POW) which is one of the asbestos substitutes were investigated for 1-yr and 4-wk inhalation periods. In the 1-yr inhalation experiment, male Wistar rats were exposed to POW (TW) for 6 h/d, 5 d/wk under the same conditions as a previous study of POW (PT1, JFM fiber) which is made by different manufacturer. The exposure concentration was $1.9 \pm 0.7 \text{ mg/m}^3$ and the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) in the chamber were $1.6 \mu \text{m}$ and 2.9. Rats were sacrificed at 3 d and 1 yr after the inhalation experiment and TW deposits in the lungs were determined by ICP-AES. Lung burden at 3 d and 1 yr after the inhalation was $2.39 \pm 0.50 \text{ mg}$ and $1.37 \pm 0.96 \text{ mg}$, respectively, the deposition fraction was 8.1% and biological half time (BHT) was 15 months. Aggregated dust cells and mild fibrotic changes around these dust cells were observed in the exposed rat lung. These results were almost the same as those obtained in the previous 1-yr PT1 study. In the 4-wk inhalation experiment, to investigate the effect of lung burden on biopersistence and histopathological change, male Wistar rats were exposed to PT1. The exposure concentration was $102 \pm 21 \text{ mg/m}^3$, MMAD (GSD), the geometric mean length and diameter (GSD) of the PT1 in the chamber were $1.6 \mu \text{m} (3.0), 2.2 \mu \text{m} (1.8)$ and $0.33 \mu \text{m} (1.5)$, respectively. Rats were sacrificed at 3 d, 1 wk, and 1, 3, 6 and 12 months after the inhalation experiment. The lung burden of POW at 3 d after 4 wk inhalation was $1.49 \pm 0.19 \text{ mg}$, which was close to the estimated amount of overload. The BHT of the total mass (4.1 months) was not prolonged, but aggregated dust cells were observed in the subpleural region and around the bronchioles and mild fibrotic changes were observed only around the dust cells at one year after the 4-wk inhalation. It is considered that the excessive exposure which impairs the function of alveolar macrophage mediated clearance may cause the aggregation of dust cells and fibrotic changes.

Key words: Lung burden, Biopersistence, Pulmonary effect, Potassium octatitanate whisker, Inhalation

It is well known that the inhalation of asbestos in the working environment leads to pulmonary fibrosis, lung cancer and mesothelioma. With the prohibition of asbestos usage and advances in manufacturing technology, various kinds of asbestos substitutes are now being produced, but due to the fibrous nature of these substances similar to asbestos, their health effects should be investigated. One of these substitutes, potassium octatitanate whisker (POW), has two types, TW and PT1, which are made by different manufacturers. In order to examine whether the pulmonary effect of these POWs differ from maker to maker, we carried out a 1-yr inhalation study on TW and compared the results with a previous 1-yr study1) on PT1 that resulted in fibrotic changes around the dust cells (dust-containing macrophages) in the rat lung and the prolongation of...
We then examined the effect of lung burden on biopersistence, assuming that the small fibrotic foci and prolongation of the clearance found in the one-year study on PT1 inhalation\textsuperscript{1} were caused by pulmonary particle overload to the alveolar macrophages. In the case of particles, the relationship between pulmonary particle load and biological half time (BHT) was examined in detail by Morrow\textsuperscript{2} and it is known that a high pulmonary particle load causes impairment of alveolar macrophage-mediated clearance due to volumetric overload to the alveolar macrophages. In addition, when this occurs, even particles with low toxicity such as TiO\textsubscript{2} and toner also experimentally lead to the impairment of clearance and fibrotic changes and tumors\textsuperscript{3–5}. In our previous inhalation studies on three different poorly soluble asbestos substitutes, namely, glass fiber, refractory ceramic fiber and potassium octatitanate whiskers, in various concentrations and periods, we found that the higher the lung burden, the higher the fiber biopersistence became and the greater the pathological changes observed regardless of the exposure period\textsuperscript{6}. These effects appeared when the lung burden in rats was a little more than 1 mg per lung\textsuperscript{1, 6–10}. Therefore, we designed and carried out a 4-wk high concentration inhalation experiment with PT1 that approached the lung burden of 1 mg per lung. Then the relationship among the lung burden, biopersistence (BHT) and histopathological change was examined.

Materials and Methods

In this study, two types (TW and PT1) of POW produced by different companies were used. PT1 is the JFM standard reference sample from the Japan Fibrous Materials Research Association (JFMRA). The chemical formula ($K_2O \cdot 8TiO_2$) and crystalline structure of the two types are the same, as shown in Fig. 1, but the color is different as shown in Fig. 2. The geometric mean fiber diameter (geometric standard deviation, GSD) and geometric mean fiber length (GSD) of TW are 0.28 $\mu$m (1.5) and 3.4 $\mu$m (2.7), respectively, and those of PT1 are 0.35 $\mu$m (1.6) and 4.4 $\mu$m (2.7), respectively. TW is
rather shorter than PT1 and the percentages of each fiber longer than 20 μm are less than 1% and 3%, respectively.

One year inhalation of TW

The exposure apparatus has been reported in a previous paper\(^1\). TW exposure conditions are summarized in Table 1 and the set up was similar to that for the one year PT1 exposure\(^1\). Mass concentration in the chamber was measured gravimetrically at 2-d intervals by isokinetic suction of air through a glass fiber filter. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were measured three times each before and after the exposure by means of an Andersen cascade impactor (Model 3351, Kanomax, Japan) and validated no change.

Twenty-seven Kud: Wistar male rats, aged 5 wk at the beginning of the exposure, were exposed to TW for 1 year. Twenty-five control rats were exposed to clean air only. At 3 d after 1-yr inhalation, 6 exposure rats and 5 control rats were sacrificed by an intraperitoneal injection of pentobarbital. The remaining rats were sacrificed in the same way at the end of the 1-yr observation period after one year inhalation. After weighing the body and the wet organs, the TW lung burden was determined and histopathological changes were examined.

Four weeks inhalation of PT1

Exposure conditions are summarized in Table 2. The mass and fiber concentration in the chamber were measured twice a week, while the MMAD in the chamber was measured on four occasions during the exposure. The fiber concentration in the exposure chamber was measured by means of an asbestos sampler (Air Monitoring Cassette Z008BA, Zefon Analytical Association, USA). To measure the size distribution, PT1

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**Table 1.** Experimental conditions for 1 yr inhalation studies

<table>
<thead>
<tr>
<th></th>
<th>TW</th>
<th>PT1 (Yamato, 2003)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure duration</td>
<td>1 yr (6 h/d, 5 d/wk)</td>
<td>1 yr (6 h/d, 5 d/wk)</td>
</tr>
<tr>
<td>Mass exposure concentration (mean ± standard deviation, mg/m(^3))</td>
<td>1.9 ± 0.7</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>MMAD (GSD)*in chamber (μm)</td>
<td>1.6 (2.9)</td>
<td>1.7 (2.4)</td>
</tr>
<tr>
<td>Animal</td>
<td>Kud: Wistar male rats</td>
<td>Kud: Wistar male rats</td>
</tr>
<tr>
<td>Number in exposure group</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>Number in control group</td>
<td>25</td>
<td>29</td>
</tr>
</tbody>
</table>

*Mass Median Aerodynamic Diameter (Geometric Standard Deviation)

**Table 2.** PT1 exposure conditions for 4 wk inhalation study

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<table>
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</thead>
<tbody>
<tr>
<td>Exposure period</td>
<td>4 wk (6 h/d, 5 d/wk)</td>
</tr>
<tr>
<td>Mass exposure concentration (mean ± standard deviation)</td>
<td>102 ± 21 mg/m(^3)</td>
</tr>
<tr>
<td>Fiber concentration (mean ± standard deviation)</td>
<td>2150 ± 580 f/cc</td>
</tr>
<tr>
<td>Mass median aerodynamic diameter (GSD*) in the chamber</td>
<td>1.6 μm (3.0)</td>
</tr>
<tr>
<td>Geometric mean fiber length (GSD*) in the chamber</td>
<td>2.2 μm (1.8)</td>
</tr>
<tr>
<td>Geometric mean fiber diameter (GSD*) in the chamber</td>
<td>0.33 μm (1.5)</td>
</tr>
</tbody>
</table>

*Geometric Standard Deviation
in the chamber was filtered with a 0.2 \( \mu m \)-pore sized polycarbonate filter in the above asbestos sampler. After coating platinum on the filter, SEM photomicrographs of PT1 on the filter were randomly taken and the length and diameter of PT1 were measured with NIH-Image software. The distribution of the length and diameter of PT1 in the exposure chamber is shown in Fig.3. Ninety percent of these PT1 in this experiment were shorter than 5 \( \mu m \) and not recognized as WHO designate fiber (i.e. longer than 5 \( \mu m \), thinner than 3 \( \mu m \) and with a ratio of length to diameter of over 3).

Thirty male Kud:Wistar rats, aged 8 wk at the beginning of the exposure, were exposed to PT1 for 4 wk. Five rats each were sacrificed at 3 d, 1 wk, and 1, 3, 6 and 12 months after inhalation. After weighing the body and the wet organs, the PT1 lung burden for the lower right lobe was determined and histopathological changes in the left lobes were examined. The same number of rats in the control group, which were exposed to clean air only, were sacrificed at the same points in time.

**Determination of POW in rat lung**

POW in the lung was digested with lung tissues into the element with \( \text{H}_2\text{SO}_4 \), \( (\text{NH}_4)_2\text{SO}_4 \) and \( \text{H}_2\text{O}_2 \) by microwave (mll 1200 mega, Milestone, Italy) under high temperature and high pressure conditions for 18 min and Ti amounts were determined by an Inductively Coupled Plasma - Atomic Emission Spectrometer (ICP-AES SPS1500R, SII Japan). As POW is insoluble and there is no Ti in normal tissue, all Ti element in the lungs determined by this procedure was derived from POW. The mass of POW retained in the entire lung was calculated from the Ti content (52.2%) by percentage.

**Histopathological procedures**

The rat lungs were inflated and fixed for a day with 10% buffered formalin by intratracheal infusion at 25 \( \text{cm H}_2\text{O} \) pressure and sectioned and embedded in paraffin. 3 \( \mu m \) thick paraffin sections were stained with Hematoxylin and Eosin.

**Statistical analysis**

The student’s \( t \)-test for the mean was used to analyze the statistical significance of differences. The Kaplan-Meier method was used for survival analysis to obtain more detailed information.

**Results**

**One year inhalation of TW**

Fig.4 shows the overall survival rate of the control and exposed rats during the exposure and observation periods. A slightly lower survival rate was observed in the exposed group but there was no statistically significant difference (\( p=0.106 \)).

There were no significant differences in growth curves between the exposed and control groups. There was also no significant difference in the body weights and wet organ weights when the rats were sacrificed, but small foci of aggregated TW were found on the lung surface of the exposed group when the lungs were dissected.
The amounts of TW retained in the lungs measured by chemical analysis at three days after one year of exposure as well as after the subsequent 1-yr observation period are shown in Table 3. The apparent deposition fraction and approximate biological half time are also shown. Each apparent deposition fraction in the lungs was calculated by the ratio of the measured POW content in the lungs to the estimated amount of the POW inhaled during the exposure. The estimated amount of total inhaled POW is calculated by average exposure concentration $\times$ total exposure time $\times$ respiratory volume$^{12}$. Biological half times calculated by single exponential regression were shown as the approximate BHT though the time points were very few. For comparison, the table also shows the results of 1-yr PT1 exposure. The retained amounts for both experiments at 3 d and one year after the inhalation were almost the same. The apparent deposition fractions at 3 d after the inhalation were almost the same, as were the approximate BHTs.

With regard to the histopathological changes, many foci of aggregated dust cells and TW were found in the alveoli, the subpleural resolution, the lymph nodes and around the bronchiole in the lungs of rats exposed to TW, and alveolar wall thickness around TW was observed (Fig. 5) in all exposed rats, corresponding to Yamato et al’s findings$^{11}$. There were one epidermoid cyst in the exposed group and one squamous cell carcinoma in the control.

Table 3. Comparison of the results for TW and PT1 inhalation for 1 yr

<table>
<thead>
<tr>
<th></th>
<th>TW (this work)</th>
<th>PT1 (Yamato, 2003)$^{13}$</th>
</tr>
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<tbody>
<tr>
<td>3 d after the inhalation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retained amounts (mg)</td>
<td>2.39 ± 0.50</td>
<td>2.36 ± 0.72</td>
</tr>
<tr>
<td>Apparent deposition fraction (%)</td>
<td>8.1</td>
<td>7.2</td>
</tr>
<tr>
<td>1 yr after the inhalation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retained amounts (mg)</td>
<td>1.37 ± 0.96</td>
<td>1.36 ± 0.55</td>
</tr>
<tr>
<td>Approximate biological half time (BHT, months)</td>
<td>15</td>
<td>16</td>
</tr>
</tbody>
</table>

Fig. 5. Histopathological features of lungs exposed to TW for 1 yr observation after the exposure.

The amounts of TW retained in the lungs measured by chemical analysis at three days after one year of exposure as well as after the subsequent 1-yr observation period are shown in Table 3. The apparent deposition fraction and approximate biological half time are also shown. Each apparent deposition fraction in the lungs was calculated by the ratio of the measured POW content in the lungs to the estimated amount of the POW inhaled during the exposure. The estimated amount of total inhaled POW is calculated by average exposure concentration $\times$ total exposure time $\times$ respiratory volume$^{12}$. Biological half times calculated by single exponential regression were shown as the approximate BHT though the time points were very few. For comparison, the table also shows the results of 1-yr PT1 exposure. The retained amounts for both experiments at 3 d and one year after the inhalation were almost the same. The apparent deposition fractions at 3 d after the inhalation were almost the same, as were the approximate BHTs.

With regard to the histopathological changes, many foci of aggregated dust cells and TW were found in the alveoli, the subpleural resolution, the lymph nodes and around the bronchiole in the lungs of rats exposed to TW, and alveolar wall thickness around TW was observed (Fig. 5) in all exposed rats, corresponding to Yamato et al’s findings$^{11}$. There were one epidermoid cyst in the exposed group and one squamous cell carcinoma in the control.

Four weeks inhalation of PT1

There were no significant differences statistically in body and wet organ weights between the exposed and the control groups except higher lung weight in the exposed group at 3 d after the inhalation.

Fig. 6 shows the temporal change in the amount of PT1 deposited in the lungs. The amount of PT1 in the lungs at three days after exposure was 1.49 ± 0.19 mg, which is close to the estimated amount for overload obtained from our previous studies$^{14-10}$. PT1 amounts in the lung decreased exponentially with the clearance time and the calculated biological half time (BHT) was 4.1 months.

Histopathological photographs of the lungs at 3 d and 12 months after the exposure are shown in Fig. 7. Although at 3 d after the exposure many small macrophages that had phagocytized PT1 were found everywhere in the alveoli (Fig. 7 A, B), at 12 months after the exposure almost none were found, and long PT1 were found singly in the interstitium. Nevertheless, aggregated dust cells were found around the bronchioles
Fig. 6. Temporal change in deposited amounts of PT1 after 4-wk inhalation.

Fig. 7. Histopathological features of rat lungs after 4 wk inhalation of PT1. (A)(B): Many small macrophages phagocytized PT1 in alveolar region at 3 d after the inhalation. (C): Mild fibrotic changes around the dust cells around the bronchiole at 12 months after the inhalation. No PT1 aggregates in alveolar region. (D): Mild fibrotic changes around the dust cells in the subpleural region at 12 months after the inhalation. No PT1 aggregates in alveolar region.
(Fig. 7C) and in the pleural region (Fig. 7D). Mild fibrotic changes were observed only around these dust cells.

Discussion

To compare the biopersistence and health effects of TW and PT1 due to long term (1 yr) exposure, we conducted a 1-yr TW inhalation study as summarized in Table 1. The results for TW and PT1 (Table 3 and Fig. 5) were almost the same. From this, the health effects of long-term exposure to POW with the same chemical composition, crystalline structure and aerodynamic diameter were found to be almost the same.

To investigate the effects of lung burden of POW on BHT and histopathological changes, we conducted a 4-wk, high-concentration inhalation exposure experiment. The conditions of the experiment were set so as to obtain a relation pattern and lung burden close to that of the estimated overload of alveolar macrophages. The amount

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**Table 4. Summary of the experimental conditions and results of POW inhalation**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>1 (this work)</th>
<th>2 (this work)</th>
<th>3 (this work)</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td>Experimental conditions</td>
<td>TW</td>
<td>PT1</td>
<td>PT1</td>
<td>TW</td>
<td>PT1</td>
<td>TW</td>
</tr>
<tr>
<td>Exposure duration</td>
<td>1 yr</td>
<td>1 yr</td>
<td>4 wk</td>
<td>4 wk</td>
<td>4 wk</td>
<td>4 wk</td>
</tr>
<tr>
<td>Fibers in the chamber</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMAD(GSD) (µm)</td>
<td>1.6 (2.9)</td>
<td>1.7 (2.4)</td>
<td>1.6 (3.0)</td>
<td>2.2 (2.0)</td>
<td>1.9 (2.2)</td>
<td>0.9 (3.1)</td>
</tr>
<tr>
<td>Geometric mean fiber length (GSD) (µm)</td>
<td>ND*</td>
<td>3.4 (2.7)</td>
<td>2.2 (1.8)</td>
<td>5.6 (2.3)</td>
<td>3.4 (2.7)</td>
<td>ND*</td>
</tr>
<tr>
<td>Geometric mean fiber diameter (GSD) (µm)</td>
<td>ND*</td>
<td>0.44 (1.4)</td>
<td>0.33 (1.5)</td>
<td>0.39 (1.5)</td>
<td>0.44 (1.5)</td>
<td>ND*</td>
</tr>
<tr>
<td>Exposure concentration (mg/m³)</td>
<td>1.9 ± 0.7</td>
<td>2.2 ± 0.7</td>
<td>102 ± 21</td>
<td>1.7 ± 0.6</td>
<td>1.1 ± 0.3</td>
<td>6.4 ± 2.2</td>
</tr>
</tbody>
</table>

**Results**

- Deposited amounts just after the inhalation (mg/rat): 2.39, 2.36, 1.49, 0.050, 0.12, 0.64
- Retained amounts at 1 yr after the inhalation (mg/rat): 1.37, 1.36, 0.22, 0.013, 0.025, 0.042
- Biological half time (months): 15, 16, 4.1, 6.5, 5.4, 3.5

**Histopathological changes**

- Dust cells – alveolar region: +, +, –, –, ND*, ND*
- – pleural and bronchiole: +, +, +, –, ND*, ND*
- Fibrotic change around the dust cells: +, +, +, –, ND*, ND*

* ND: not determined  **after 15 months
of PT1 deposits at three days after inhalation was 1.49 ± 0.19 mg, similar to the estimated amount of overload in our previous studies. In this experiment, BHT was 4.1 months and no delay of clearance was observed. Fig. 8 summarizes the relation between lung burden and the BHT of POW in this study in addition to our previous inhalation studies1, 6–8, 13–15).

Table 4 summarizes the experimental conditions and the results of the lung burden, BHT and the histopathological change in POW inhalation studies. No.5 and No.6 were conducted in order to obtain BHT and no histopathological examinations were performed. In the experiments on large lung burden in one year inhalation of POW (No.1 and No.2), local accumulations of POW aggregates were found in the alveoli and subpleural lesion and the lymph nodes and around the bronchioles at three days after the exposure period and 12 months later. These POW aggregates are considered to be cells that have phagocytized excessive POW. It is suggested by Morrow21 that the clearance function begins to be impaired when 6% of the alveolar macrophage volume is filled with phagocytized particles and it completely ceases when the volume is over 60%. In these one year inhalation experiments, as the density of the aggregated POW in almost all the dust cells was very high, their mobility was impaired and they remained in the lungs for a long time and this fact results in the prolongation of BHT. In comparison, in cases where lung burden was 0.05 mg (No.4), the histopathological microphotographs of the lungs were almost identical to the control group as shown in Fig. 9. Almost all the retained POW were scattered and no dust cells were observed, therefore the clearance function was not impaired and there was no prolongation of BHT. We believe that was due to the low amount of POW retained in the lungs, which was about 0.050 mg at three days after exposure and only 0.013 mg 12 months later.

In the high-concentration 4-wk exposure experiment conducted in this study, lung burden was close to the estimated amount for overload, that is 1.5 mg. BHT of the total POW amount was 4.1 months and no delay in clearance was observed. The biopersistence (BHT) of fibers deposited in the lungs depends on two main mechanisms: 1) physicochemical processes such as the dissolution in lung fluids and break down and 2) physiological processes such as mechanical clearance mediated by alveolar macrophages. As POW is insoluble16), the clearance of most POW is therefore thought to be performed by macrophages. In experiments No.4 and No.5, Yamato et al. found long fibers retained in the lung for a long time8) but in this experiment (No.3), the results of which are shown in Table 4, the geometric mean length of POW in the exposure chamber was 2.2 µm, which is shorter than those in No.4 and No.5. In addition, because none of the PT1 was longer than 20 µm, as shown in Fig. 3, we recognize that this was why clearance by macrophages proceeded smoothly and there was no prolongation of BHT. Nevertheless, POW scattered in the lungs just after the exposure became aggregated dust cells observed in the subpleural lesion and around the bronchioles at one year after the exposure. These aggregated POWs also had a high density (Fig.7 (C), (D)) like those in the one year inhalation study (Fig.5), therefore, their mobility was expected to be impaired and these aggregated POW were thought to remain in the lung for a long time. Although prolongation of total mass BHT was not observed in the study with the 1.5 mg lung burden, local accumulation of POW aggregates considered to be pre-indicators were observed after 12 months. Therefore, we speculate that the 1.5 mg lung burden is near the threshold for the prolongation of total mass BHT and the critical lung burden exists between 1.5 mg and 2.4 mg.

As for the histopathological change, as the mild fibrotic
Changes were observed only around the dust cells in both this high-concentration 4-wk inhalation experiment and the one year inhalation experiment, it is considered that the dust cells which phagocytize excessive POW have the potential to cause histopathological changes and these changes occurred before the prolongation of total mass BHT by exposure to POW.

Conclusion

There were no significant difference in the lung burden, biopersistence (BHT) and histopathological changes between two types (TW and PT1) of potassium octatitanate whiskers (POW) after the 1-yr inhalation study.

The BHT of total mass was not prolonged in the 4-wk inhalation study in which the lung burden of POW was close to the amount of the estimated overload, but aggregated dust cells were observed in the subpleural region and around the bronchioles, and the mild fibrotic changes were observed only around the dust cells at one year after the inhalation. It is considered that the excessive POW exposure which impairs the function of alveolar macrophage mediated clearance may cause the aggregation of dust cells and fibrotic changes.

Acknowledgments: We thank Ms S. Kuramoto for technical assistance with the experiment. This study was supported in part by a Grant-in-Aid for scientific research (KAKENHI No.13670401) from the Japan Society for the Promotion of Science.

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