Asbestos Hemolysis

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Chrysotile fibers exert a marked hemolytic activity on suspensions of washed sheep erythrocytes, but amphibole asbestos minerals such as crocidolite, amosite, tremolite, and anthophyllite possess only negligible hemolytic properties. The minimal hemolytic concentration of chrysotile asbestos is dependent on the openness of the fibers as characterized by the air permeability surface area and decreases with increasing surface area. This fact was also observed in the evaluation of hemolysis of fibrous, nonasbestiform minerals, e.g., sepiolite and nemalite. Chrysotile hemolysis can be inhibited by disodium ethylenediaminetetraacetic acid (EDTA) and by certain acidic polymers particularly by poly(styrene sulfonates, carboxymethylcellulose ether sodium salt (CMC), and the pyran-copolymer NSC 46015. These substances are not effective as inhibitors of silica (quartz) hemolysis, whereas polyvinylpyridine-N-oxide (PVNO), a very potent antagonist of silica hemolysis, interferes with chrysotile only at high concentrations. The hemolytic action of chrysotile can be markedly reduced by repeated treatment with red blood cells. The mechanism of asbestos hemolysis cannot yet be satisfactorily interpreted. There is a marked correlation of hemolytic potency with the surface area and the degree of opening of the fibers which may be influenced by the action of the antagonistic polymers. The role of the magnesium hydroxide surface characteristic for chrysotile is not yet elucidated, because magnesium hydroxide is not hemolytic, although nemalite, a natural fibrous magnesium hydroxide, and a lightly burned magnesium oxide are active hemolytic agents.

INTRODUCTION

Hemolysis of plasma-free mammalian erythrocytes by minerals and other solid materials was first described by Stalder and Stijber (1965), and by Nash et al. (1966) using crystalline and amorphous silica species. In 1967 Macnab and Harington found that certain varieties of asbestos minerals also had hemolytic properties. This work, which was later confirmed by Schlipkoter (1968), and Secchi and Rezzonico (1968), showed that chrysotile asbestos had marked hemolytic property while amosite, crocidolite, and anthophyllite had much less, if any, activity.

Although there is no known relationship between these observations and the disease-causing potential of the minerals, the method does seem to offer a comparatively simple in vitro system for the study of the effect of mineral fiber surfaces on mammalian cells. Experiments on the hemolytic activity of asbestiform and non-asbestiform fibers of different origin and with various physical properties and surface treatments were initiated by Dr. I. J. Selikoff and carried

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out in the Environmental Microbiology Laboratory. Included in these studies was a number of non-fibrous minerals, among them a series of samples of magnesium hydroxide and burned magnesium oxide. The experiments with these materials were prompted by consideration of the presence of the magnesium hydroxide layer on the surface of chrysotile fibrils. Magnesium hydroxide has been considered to be responsible for the hemolytic effect (Macnab and Hargrington, 1967).

This report contains the results of hemolytic experiments with 64 materials including natural minerals, modified minerals, and metallic oxides and studies on the inhibition and elimination of the hemolytic effect of some of the materials.

**Materials and Methods**

*List of Minerals:*

**Asbestos Fibers**

1. **Chrysotiles**

(1A) Crude fiber from Jeffrey Mine, Asbestos, Quebec, Canada, separated by hand and cut to short lengths with scissors; surface area = 3920 cm²/g.
(1B) Crude (1A) hammer-milled, one pass through micropulverizer.
(1C) Air-jet-milled 1B fiber, two passes through micronizer fluid energy mill.
(1D) -4, -18 Mesh fraction from sample 1E; surface area 19,560 cm²/g.
(1E) Sample 1A, milled 3 times through a Trost fluid energy mill (air-jet-milled). Surface area = 63,200 cm²/g.
(1F) -18 Mesh, +100 mesh fraction from sample 1E. Surface area = 63,900 cm²/g.
(1G) -100 Mesh fraction from sample 1E. Surface area = 59,420 cm²/g.
(2A, 2B, 2C) Fibers from Coalinga, California.
(3A, 3B, 3C) Fibers from Advocate Mine, Newfoundland.
(4A, 4B, 4C) Fibers from Reeves Mine, Reeves Township, Ontario, Canada.
(5A, 5B, 5C) Fibers Arizona soft.
(6A, 6B, 6C) Fibers Arizona harsh.
(7A, 7B, 7C) Fibers from Carey Mine, Province of Quebec, Canada.
(8A, 8B, 8C) Fibers from Cassiar Mine, Province of British Columbia, Canada.
(9) Chrysotile from Munro Province of Ontario, Canada (0X873).

*The letters A, B, C signify in this and the following materials: A = Hand cut, as in 1A; B = hammer milled, as in 1B; C = air jet milled, as in 1C.*
(11) Chrysotile B (UICC).

II. Amphibole Asbestiform Minerals
(12A, 12B, 12C) Crocidolite from Africa.
(13) Crocidolite (UICC).
(14A, 14B, 14C) Amosite from Africa.
(15) Amosite (UICC).
(16A, 16B, 16C) Anthophyllite.
(17) Anthophyllite (UICC).
(18A, 18B, 18C) Tremolite.

III. Non-asbestiform Fibrous Materials
(19A, 19B, 19C) Sepiolite.
(20A, 20B, 20C) Nemalite, an impure fibrous magnesium hydroxide.
(21) Micro-quartz. (fibrous silica) Code 16. Mean fiber diameter 0.6μ.
(22) Fibrous talc. Fibrous component is predominantly tremolite.
(23) Palygorskite, a fibrous clay from the western United States.

IV. Magnesium Hydroxide
(24) Mg(OH)₂.
(25) MgO lightly burned. Synthetic magnesite as received from Dow Chemicals.
(26) MgO medium burned. No. 25 calcined 1 hour at 1200°C.
(27) MgO dead burned. No. 25 calcined 1/2 hour at 1620°C. See also Nemalite (Section III Nos. 20A, 20B, 20C).

V. Miscellaneous Products
(29) Alcon C. Ultrafine alumina, Godfrey Cabot Co.
(30) Sorb-O-Cel. Diatomite coated with aluminum hydroxide (Source?).
(31) Activated alumina.
(32) Platy talc.

Technique of Hemolysis Experiments
The experimental technique was similar to that used by earlier investigators (Macnab and Harington, 1967; Secchi and Rezzonico, 1968). Weighed amounts of asbestos fibers and the other materials tested (12.5 mg/ml for routine tests; graded amounts from 0.625 mg/ml for titrations) were placed in test tubes with 8 ml 2% suspension of 3 times washed sheep erythrocytes in isotonic veronal buffer solution of pH 7.3–7.4. After incubation at 37°C for 2 hours a direct reading of presence and absence of hemolysis was taken, followed by short centrifugation and colorimetric determination of the optical density of the supernatant fluid in a Photovolt Lumetron Colorimeter (model No. 401) using a green filter No. 530. The experiments included as a negative control 8 ml of the
red cell suspension and one or two positive controls containing either two minimal
hemolytic doses of chrysotile (sample Nos. 1E or 1F or Nos. 10 or 11) or con-
taining 10 mg saponin. In some experiments both chrysotile and saponin controls
were used.

Complete hemolysis in the controls gave density readings of 18–20 correspond-
ing to 90–100% hemolysis. The density values of the negative controls (zero to
trace hemolysis at direct observation) varied from 0.2 to 0.8 depending on the
blood samples used, corresponding to 1–4% hemolysis. In experiments with inhib-
itors double concentrations of the antagonistic agents were made up in 4 ml buffer
solution to which the hemolytic substances in a final concentration of two minimal
hemolytic doses (MHD) and 4 ml of a 4% red cell suspension were added.

Some substances, e.g., magnesium hydroxide or t alc, which tended to sediment
rapidly required agitation during the incubation period. This was accomplished
by placing the tubes containing the hemolytic system in a rotating drum or in a
reciprocating shaker at low speed. This procedure had no influence on the hemo-
lytic action of the standard chrysotiles which, as a rule, remained suspended in
the cell suspension.

RESULTS

General Survey of Experimental Hemolysis

The hemolytic activity of all the materials was studied qualitatively at a
standard dose of 12.5 mg/ml of washed sheep erythrocytes. The results can be
summarized as follows:

(1) All of the hammer-milled and air-jet-milled samples of chrysotile asbestos
(Nos. 1B, 1C, 2B, 2C, etc.) were hemolytic. Conversely, none of the hand-cut
crude samples was active. (Nos. 1A, 2A, 3A, etc.)

(2) None of the samples of the amphibole asbestiform minerals was substan-
tially active (Nos. 12A–18), regardless of their physical form (i.e., hand-cut
crude, hammer-milled, or air-jet-milled). The amphibole minerals include cro-
cridolite, amosite, anthophyllite, and tremolite.3

(3) Among the non-asbestiform fibrous materials (Nos. 19–23), all were active
in hemolysis tests except the hand-cut crude samples of nematicite (No. 20A) and
sepiolite (No. 19A).

(4) Magnesium hydroxide and the samples of burned magnesium oxide (Nos.
24–27) were not hemolytic with the exception of lightly burned MgO (No. 25).

(5) Of the miscellaneous materials, Alon C (No. 29) was active while the
remainder of materials in this group were not.

Quantitative Evaluation of Hemolysis

Detailed studies of the process of asbestos hemolysis and particularly exper-
iments on its inhibition required determination of the minimal hemolytic dose
covering the range of concentrations from 0.125 to 6.25 mg/ml for selected
samples. Although the minimal hemolytic dose (MHD) of the asbestiform

3Recent observations seem to indicate that hemolysis of amphibole minerals can occur
under different experimental conditions which are presently under study.
minerals was considered a concentration of the agents producing at least 50% hemolysis, the MHD was found, as a rule, to produce between 60 and 100% hemolysis. For the standard chrysotiles A and B (Nos. 10, 11) values of 3.125 mg/ml were obtained (see Table III) which is in agreement with the figure of 2.5 mg/ml given by Secchi and Rezzonico (1968). It was observed that the MHD is dependent on the physical form of the fibers as shown in Table I. The comparison of a crude (No. 1A) and an opened chrysotile fiber (No. 1E) indicates that the process of opening by air-jet milling increased the hemolytic potency more than 40-fold. The degree of opening is best characterized by the air permeability surface area. The higher the opening of the fiber, the greater the surface area. A more highly opened fiber sample will have individual fibers with smaller diameters. Because of the natural tendency of the mineral to fiberize, the opening techniques have much less influence on fiber length than on the diameter. Table II shows the decrease of the minimal hemolytic dose for the various samples correlated with their air permeability surface areas.

As mentioned earlier, hemolytic action was observed in most of the non-asbestiform fibrous materials as shown in Table III. The minimal hemolytic doses of chrysotiles are given for comparison. The moderate hemolytic action of nemalite is a surprising observation since non-fibrous Mg(OH)$_2$ did not produce hemolysis under the experimental conditions of our tests. In Table IV are given the results of the experiments with various samples of Mg(OH)$_2$ and burned samples of MgO. Only a lightly burned specimen of MgO (No. 25) exerted an appreciable hemolytic effect.

**Inhibition of Hemolysis**

The work of Macnab and Harington (1967) and Nash et al. (1966) demonstrated that hemolysis caused by asbestos as well as silica can be inhibited by several substances. For example, the presence of serum interferes with hemolysis by asbestos and by silica. Asbestos hemolysis is also inhibited by such compounds as phosphate ions and disodium ethylenediaminetetraacetic acid (EDTA). Our experiments on the inhibition of hemolysis covered EDTA and other chelating or complexing agents.

The inhibiting action of chrysotile hemolysis by EDTA was studied using UICC (B) (No. 11). Concentrations of 2.5 mg/ml and greater inhibited the hemolysis by chrysotile 6.25 mg/ml. The hemolysis caused by the same dose of micro-quartz (No. 21) was not inhibited by EDTA 10 mg/ml. This concentration antagonized nemalite, however, at a dose of 12.5 mg/ml.

Other chelating and complexing agents, e.g., sodium sulfide, 1,10-phenanthroline, dimethylglyoxime, DPN-penicillamine, S-hydroxyquinoline, failed to inhibit either the chrysotile or the quartz hemolysis if added in final dilutions of $10^{-2}$ M or more. Moreover, these compounds were not quite indifferent for the blood cells. The marked inhibiting effect reported for polyvinylpyridine-N-oxide (PVPNO) on silica hemolysis is, according to Macnab and Harington (1967)

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*According to recent investigations of Drs. R. S. Leibling and A. M. Langer (personal communication) of the Mineralogy Section of the Environmental Sciences Laboratory, the presence of chrysotile fibers was observed in samples of nemalite.
TABLE I
TITRATION OF HEMOLYTIC ACTION OF CHRYSOTILE

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>Chrysotile</th>
<th>Dose (mg/ml)</th>
<th>Percent hemolysis (after 2 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crude (1A)</td>
<td>25</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>Crude (1A)</td>
<td>12.5</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>Crude (1A)</td>
<td>6.25</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>Air-jet-milled (1E)</td>
<td>3.125</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Air-jet-milled (1E)</td>
<td>1.25</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Air-jet-milled (1E)</td>
<td>0.625</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>Air-jet-milled (1E)</td>
<td>0.3125</td>
<td>45</td>
</tr>
<tr>
<td>8</td>
<td>Air-jet-milled (1E)</td>
<td>0.125</td>
<td>7.5</td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>-</td>
<td>1.3</td>
</tr>
</tbody>
</table>

TABLE II
CORRELATION OF HEMOLYSIS WITH SURFACE AREA OF CHRYSOTILE

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Surface area (cm²/g)</th>
<th>MHD* (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>3920</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>1D</td>
<td>19,560</td>
<td>3.125</td>
</tr>
<tr>
<td>1E</td>
<td>63,200</td>
<td>0.625</td>
</tr>
<tr>
<td>1F</td>
<td>63,900</td>
<td>0.625</td>
</tr>
<tr>
<td>1G</td>
<td>59,420</td>
<td>0.625</td>
</tr>
</tbody>
</table>

* MHD = Minimal hemolytic dose.

TABLE III
MINIMUM HEMOLYTIC CONCENTRATION OF NON-ASBESTIFORM FIBERS

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Substance</th>
<th>MHD (mg/ml)</th>
<th>Hemolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19C</td>
<td>Sipolite</td>
<td>12.5</td>
<td>75</td>
</tr>
<tr>
<td>20C</td>
<td>Nemalite</td>
<td>12.5</td>
<td>80</td>
</tr>
<tr>
<td>21</td>
<td>Micro-quartz</td>
<td>6.25</td>
<td>85</td>
</tr>
<tr>
<td>22</td>
<td>Fibrous tale</td>
<td>12.5</td>
<td>73</td>
</tr>
<tr>
<td>23</td>
<td>Palygorskite</td>
<td>12.5</td>
<td>70</td>
</tr>
<tr>
<td>10/11</td>
<td>Chrysotile (UICC)*</td>
<td>3.125</td>
<td>100</td>
</tr>
</tbody>
</table>

* Results with chrysolites A and B were identical.

TABLE IV
MINIMAL HEMOLYTIC CONCENTRATION OF Mg(OH)₂ AND HEATED MgO

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Substance</th>
<th>Heated</th>
<th>MHD (mg/ml)</th>
<th>Hemolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Mg(OH)₂</td>
<td>—</td>
<td>12.5</td>
<td>25*</td>
</tr>
<tr>
<td>25</td>
<td>MgO</td>
<td>Lightly</td>
<td>1.25</td>
<td>95</td>
</tr>
<tr>
<td>26</td>
<td>MgO</td>
<td>1200°C</td>
<td>12.5</td>
<td>15</td>
</tr>
<tr>
<td>27</td>
<td>MgO</td>
<td>1620°C</td>
<td>12.5</td>
<td>18</td>
</tr>
</tbody>
</table>

* Average of repeated tests with four different samples.
much less pronounced in case of asbestos fibers. These observations were confirmed in our experiments, and prompted studies with other polymers. The various water soluble polymers were: Methylcellulose (MC); carboxymethylcellulose ether sodium salt (CMC); polyvinylpyridine-N-oxide (PVPNO); polystyrene sulfonate, low mol. weight (CRS774); polystyrene sulfonate, high mol. weight (CRS773); pyran-copolymer (NSC 46015) [pyran-3,4-dicarboxylic anhydride, tetrahydromethyl-6-(tetrahydro-2,5-dioxo-3-furyl)polymer; mol. weight 475,000].

It was observed that most of these polymers can exert a very marked inhibiting action on asbestos hemolysis. As shown in Table V, CMC, CRS 773, CRS 774, and particularly NSC-46015 antagonized the hemolytic effect of chrysotile, both of moderately active products (chrysotile B, No. 11) and of the more potent Jeffrey (No. 1F). Methylcellulose and PVPNO were substantially less effective inhibitors. The latter exhibited, however, its marked antagonistic effect in silica hemolysis in experiments with micro-quartz (Table VI). Comparative values of

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mg/ml)</th>
<th>Chrysotile B</th>
<th>Jeffrey</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC</td>
<td>1.0</td>
<td>3.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>30.0</td>
<td>2.75a</td>
</tr>
<tr>
<td></td>
<td>0.0625</td>
<td>98.0</td>
<td>2.75a</td>
</tr>
<tr>
<td>MC</td>
<td>5.0</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>CRS 773</td>
<td>1.0</td>
<td>18</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>26.5</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>CRS 774</td>
<td>1.0</td>
<td>4.0</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7.5</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>24.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>NSC-46015</td>
<td>0.25</td>
<td>4.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>7.5</td>
<td>2.75a</td>
</tr>
<tr>
<td></td>
<td>0.0625</td>
<td>21.5</td>
<td>3.75a</td>
</tr>
<tr>
<td></td>
<td>0.03125</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>PVPNO</td>
<td>10.0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>+ +</td>
<td></td>
</tr>
</tbody>
</table>

a Chrysotile B (No. 11): 0.25 mg/ml; Chrysotile Jeffrey (No. 1F): 1.25 mg/ml.
b The chrysotile doses were 2.5 mg/ml.
c Direct reading.
d The doses of the inhibitors were 0.1 and 0.05 mg/ml, respectively.

MC and CMC were commercial products. The authors gratefully acknowledge the help of Dr. S. A. Schepartz, Associate Scientific Director and Chief Cancer Chemotherapy National Service Center and Dr. H. B. Wood, Jr., Chief, Drug Development Branch CCNSC, in supplying the pyran-copolymer NSC 46015. We also thank Dr. W. E. Smith (Fairleigh Dickinson University, Madison, N. J.) for a sample of PVPNO and Mr. J. Stewart (Esso Research and Engineering Co.) for the polystyrenes CRS 773 and 774.
the minimal inhibiting concentrations of the various polymers in experiments with chrysotiles and silica are summarized in Table VII.

The findings seem to suggest that there exist two different groups of antagonists, one of them consisting of CMC, the polystyrene sulfonates, and the anionic pyran-copolymer NSC 46015 interfering with asbestos hemolysis, whereas MC and particularly PVPNO inhibit quartz-fiber hemolysis. The fibrous magnesium hydroxide, nemalite, occupies an intermediate position owing to the presence of chrysotile fibers. Complete titrations of the inhibitors are not available, but the data presented in Table VIII seems to indicate that members of both groups of antihemolytic polymers decrease the hemolysis of this mineral fiber.

Certain surface treatments of chrysotile asbestos were studied for their ability to inhibit hemolysis. The treatments were applied to air-jet-milled samples of chrysotile from the Jeffrey (No. 1C) and Advocate (No. 3C) mines. The details of the treatments are as follows:

1. Heat treatment: heated for 15 minutes at 540°C.
2. Acetic acid treatment: heated for 1 hour in 1 N acetic acid and washed with distilled water.

The minimal inhibiting concentrations of the various polymers in experiments with chrysotiles and silica are summarized in Table VII.

Table VIII seems to indicate that members of both groups of antihemolytic polymers decrease the hemolysis of this mineral fiber.

**Table VII**

**Inhibition of Fiber Hemolysis by Polymers**

<table>
<thead>
<tr>
<th>Polymer</th>
<th>MIC of the Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylcellulose</td>
<td>&gt; 5</td>
</tr>
<tr>
<td>Carboxymethylcellulose</td>
<td>0.0025 - 0.125</td>
</tr>
<tr>
<td>Polystyrene CRS 774</td>
<td>0.5</td>
</tr>
<tr>
<td>Polystyrene CRS 773</td>
<td>0.5</td>
</tr>
<tr>
<td>Pyran copolymer NSC 46015</td>
<td>0.0025</td>
</tr>
<tr>
<td>Polyvinylpyridine-N-oxide</td>
<td>&lt; 0.006</td>
</tr>
</tbody>
</table>

*Chrysotile B (UICC); Sample No. 11, 3.125 mg/ml; Micro-quartz; Sample No. 21, 6.25 mg/ml.

*MIC = minimal inhibiting concentration.

*Hemolysis of some other chrysotile fibers was inhibited by 0.03 mg/ml.
ASBESTOS HEMOLYSIS

TABLE VIII
Inhibition of Nemalite Hemolysis by Polymers

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>5 mg/ml</td>
</tr>
<tr>
<td>CMC</td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>CRS 773</td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>CRS 774</td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>XCS 46015</td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>PVPNO</td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>No inhibitor</td>
<td>—</td>
</tr>
</tbody>
</table>

Percent hemolysis

47.5  4.75  2.75  2.75  11.5  60.0

Nemalite (No. 20C) concentration: 12.5 mg/ml.

(3) Maleic acid treatment: fiber refluxed for 1 hour in 2% solution of maleic anhydride in methyl-ethyl-ketone.

(4) Trimethylchlorosilane treatment: fiber refluxed for 1 hour in 1% trimethylchlorosilane solution in benzene.

(5) Gelatin treatment: fiber treated for 16 hours at room temperature in a 0.25% gelatin solution.

(6) Tamol SN treatment: Tamol SN (a sodium salt of condensed naphthalene sulfonic acid, sold by Rohm and Haas Company) sorbed on the fiber.

(7) Sodium silicate—Tamol SN treatment: Fiber treated with sodium silicate (N brand Na. silicate, sold by Philadelphia Quartz Company) and Tamol SN.

The hemolytic potency of the treated fibers was tested using the dose of 12.5 mg/ml. The results from this series of experiments indicate that treatment with acetic acid reduced slightly the hemolytic titer of the Advocate fibers from 100 to 60% hemolysis. Gelatin exerted a marked influence on the action of the same fibers, reducing hemolysis to 4.9%, but there was no substantial effect on Jeffrey fibers (72% hemolysis). Tamol SN treatment reduced, however, the hemolytic titer of both Advocate and Jeffrey fibers to 45 and 49.5%, respectively. The other procedures did not change the hemolytic potency.

Elimination of the Hemolytic Activity of Chrysotiles

In these experiments, weighed samples (generally 100 mg) of the asbestos fibers were exposed to various procedures in an attempt to eliminate the hemolytic action. It was found that washing with 0.9% saline had no influence on the hemolytic effect. The tests were carried out with the chrysotile varieties Carey (No. 7C) and Cassiar (No. 8C) in the active, air-jet-milled form. After eight washings with volumes of 40 ml saline, hemolysis of 100% was unchanged. Also, washing with EDTA did not eliminate the hemolytic action of the fibers. If 100 mg of UICC chrysotile B (No. 11) were treated with 200 mg EDTA in 20 ml water for 1 hour at 37°C, the hemolytic property (100% hemolysis) was unchanged after removal of the EDTA by centrifuging and by three washings with a total of 120 ml saline or veronal buffer. The washwaters were not hemolytic.

Polymer CMC, on the other hand, inactivated UICC chrysotile B irreversibly. In this experiment 100 mg of the material was exposed to 160 mg CMC in 8 ml veronal buffer for 1 hour at 37°C. After three washings with 40 ml water and
The chrysotile was completely nonhemolytic, whereas a control sample, exposed only to veronal buffer showed the expected full hemolytic effect. It was, however, not possible to prevent hemolysis by keeping 1 ml of packed washed red cells in 10 ml of a 0.4% CMC solution in veronal buffer 37°C for 1 hour. A 2% suspension made of these cells after two washings responded with complete hemolysis to 12.5 mg/ml chrysotile B, although the presence of some CMC could still be noticed on the cells. Also Nash et al. (1966) mention the absence of the inhibiting effect of PVPNO on silica, if the red cells were treated with the polymer.

Repeated treatment of chrysotile with red cells and washing of the asbestos fibers between the steps of exposure to blood did eliminate the hemolytic activity. This experiment consisted of a series of hemolytic tests with eight different air-jet-milled chrysotile varieties (see Table IX), using the conventional dose of 12.5 mg/ml and the 2% red cell suspension. Following hemolysis, the centrifuged sediment of asbestos fibers and blood residues was washed twice and occasionally three times with 40 ml saline until the fibers were free of blood and hemoglobin. The sediments were generally kept in the refrigerator overnight and then retreated with blood suspension. This process was carried out with three to four successive exposures, until a marked reduction of the hemolytic titer was observed.

Table IX shows that the different varieties of chrysotile differ by their response to this treatment. Some fibers, e.g., Jeffrey, Reeves, Arizona, Cassiar, can show substantial reduction of their hemolytic property after one treatment with blood and are practically no longer hemolytic. In other instances, two or three exposures to the blood suspension are necessary, e.g., in the case of chrysotile A or Carey fibers.

**DISCUSSION**

The comparatively simple demonstration of hemolysis by chrysotile asbestos and other materials sheds some light on the mechanisms involved, but does not answer all the questions which might be asked concerning this phenomenon. One hypothesis for the mechanisms of hemolysis assumes that the hemolytic
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Agent adsorbs the lipoproteins and the proteins of the erythrocyte membrane. Secchi and Rezzonico (1968) postulated that the erythrocyte membrane components are absorbed by chrysotile. On the other hand, Macnab and Harington (1967) assume that the hemolytic activity of chrysotile is due to the magnesium hydroxide surface which can be "covered up" or modified by chelating adsorbents such as EDTA and phosphates.

Both the above hypotheses are compatible in the sense that they attribute the hemolytic activity to the surface of the fibers. In the first case, it is assumed that the chrysotile surface removes essential components from the cell membrane, and in the second case it is the chemical nature of the surface which is involved.

The results of this study, particularly those given in Table II, do indeed show that the hemolytic activity of chrysotile is related to the surface area or degree of opening of the fibers. Whether this is a relationship which involves the size of the fibers or the surface area exposed cannot be answered at this time. Electromicroscopic study of the fibers may shed light on this question.

The fact that the amphibole asbestiform minerals are not active in this type of test suggests that the chemical nature of the surface is the most important factor. The exposed surface of chrysotile is essentially magnesium hydroxide while the surface of the amphiboles is more like that of silica. However, the fact that micro-quartz (silica) fibers, silica, and a variety of other materials are hemolytically active eliminates a simple chemical relationship. In this particular vein, one area which deserves further investigation is the activity of the various forms of magnesium hydroxide and magnesium oxide. In this group only the milled nemalites (natural fibrous magnesium hydroxide) and lightly burned magnesium oxide were active hemolytic agents.

The magnesium oxide samples (Nos. 25-27) were prepared in the laboratory by heating magnesium oxide to various temperatures (see Table IV). It is known that the time of heating and the temperature influence the particle size of the final product (longer heating and higher temperatures yield larger sizes). It may be that there is a critical size range for hemolytic activity.

The inhibition of hemolysis by the various materials covered in this study indicates that those materials which inhibit asbestos hemolysis are those which are relatively strongly adsorbed by chrysotile. For example, the polymers which have acidic groups in their structures (CMC, CRS 773, CRS 774, NSC-46015) will all be adsorbed by chrysotile. The polymers PVPNO and MC which do not contain acidic groups will not be adsorbed as readily. The elimination of the hemolytic activity by repeated contact with erythrocytes could also be related to the adsorption of one or more of the cell components on the fibers. It should be pointed out, too, that many acidic polymers when adsorbed on asbestos have a tendency to flocculate the fibers in spite of agitation during the incubation period. This phenomenon could reduce the number of free fibers available in the suspension and also the total surface area available for contact with the erythrocytes.

The only link between hemolytic activity and fibrosis in animal studies is the work of Schlipkötter and Brockhaus (1961) who have shown that PVPNO inhibits
the in vivo fibrotic action of silica, but not that of asbestos (Schlipkötter, 1968; Klosterkötter, 1968). This same compound is also a strong inhibitor for the in vitro hemolysis of silica, whereas asbestos hemolysis was only inhibited by high concentrations. It seems necessary to continue this work in vivo using chrysotile asbestos and polymers with high antihemolytic activity against asbestos. NSC-46015 seems to be in small doses suitable for this purpose since its in vivo action as an interferon stimulator and antineoplastic agent is now well known (Regelson, 1968). Also the one or the other of the various types and grades of CMC should be investigated. EDTA may be less suitable on account of its nephrotoxic side effects (Doolan et al., 1967). These suggested studies refer to the possibility of a systemic treatment with suitable polymers. Prevention of fibrosis by impregnation of asbestos fibers with polymers should also include water-insoluble substances. This seems desirable because in the case of silica the esterification of Si—OH groups produced only a temporary elimination of the SiO₂ tissue damage (Strecker, 1960). The question of length of fibers which appears to be essential in the studies of fibrosis has not yet been included in our work on hemolysis.

SUMMARY

The data in the literature regarding the in vitro hemolysis by chrysotile and the absence of marked hemolytic properties in other asbestos types have been confirmed. Quantification of chrysotile dosage showed correlation of fiber openness with degree of hemolysis; this fact was also observed in the evaluation of hemolysis of fibrous nonasbestiform minerals (sepiolite, nemalite). Magnesium hydroxide was not hemolytic, but nemalite, an impure fibrous magnesium hydroxide showed a moderate degree of hemolytic action. Lightly heated MgO was markedly hemolytic, but lost this property by calcination at higher temperatures. Inhibition of chrysotile hemolysis by EDTA was confirmed; other chelating agents were not antagonistic. A series of polymers showed marked antihemolytic properties, particularly carboxymethylcellulose ether (CMC) and a pyran-copolymer (NSC-46015). Inhibition of chrysotile hemolysis does not parallel silica (quartz) hemolysis and vice versa. A characteristic example is the high activity of polyvinylpyridine-N-oxide (PVPNO), limited to silica. The hemolysis of chrysotile cannot be reduced by washing with saline or exposure to EDTA, but it can be reduced by repeated exposure to red cells. The hypothetical mechanisms of asbestos hemolysis and the correlation with fibrosis are discussed.

REFERENCES


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