INFLUENCE OF SOLVENTS AND SURFACE ACTIVE AGENTS ON THE BARRIER FUNCTION OF THE SKIN TOWARDS SARIN

II. Increase in rate of absorption

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In a previous paper (9) a method was outlined to test the influence of various pretreatments of the skin surface of guinea-pigs on its barrier function in regard to the organophosphorus cholinesterase inhibitor, Sarin, or isopropoxy-methylphosphoryl fluoride. It was shown that the time until respiratory arrest gave a satisfactory, indirect measure of the rate of absorption of the test compound provided that the area of absorption was kept constant, that the weight of the animals was uniform, that the absorption was optimal and that evaporation was prevented. In the present paper the results of pretreatment of the skin surface with various organic solvents and surface active agents will be described.

Material and Methods

The main method used was described in detail in an earlier paper (9). A metal ring was glued to the clipped skin of the belly in groups of ten guinea-pigs. Thirty minutes later 0.5 ml of the pretreatment liquid was pipetted on to the skin area within the ring. It was allowed to remain on the area for 1, 5 and 30 minutes (for one compound 1, 5, 10, 15, 20, 30 and 60 minutes). The ring was covered with a cover-glass in order to avoid evaporation. After the pretreatment time the liquid was removed by gentle blotting with dental sorbent rolls. The area was left uncovered in order to allow free evaporation of any remaining liquid. Thirty minutes later, when the skin area always appeared to be completely dry, the animals were challenged, i.e. 25 μl of Sarin was applied to the skin surface, the ring covered and the time until respiratory arrest noted. Analytic grade of acetone, ethanol, ether, chloroform and dimethylsulfoxide were used undiluted, like destilled water which served as a control. Furthermore, a 5 per cent water solution of a commercial soap and 0.045 N water solutions of a non-ionic, a cat-ionic and an an-ionic surfactant were used. The non-ionic agent was alkylpolyglycholic ether, the cat-ionic benzethonium chloride, and the non-ionic the sodium salt of alkylethersulfate.

In some experiments the area within the ring was rinsed with 0.5 ml destilled water twice after blotting, and in some additional experiments dimethylsulfoxide, DMSO, was mixed with Sarin in various proportions. In order to get information whether there was a correlation between the increase in rate of absorption and the surface tension reducing capacity of the pretreatment liquids the spread of Sarin on the skin surface was investigated in guinea-pigs. A clipped skin area of 5 by 5 cm was pretreated as earlier (for one minute and without any ring glued to the skin). Thirty minutes later 25 μl of Sarin as a single drop was applied in the center of the skin area. Two diagonal diameters of the visible spot were measured with the aid of a pair of compasses at regular intervals, and the area of the spot was calculated as being an ideal circle. Controls showed that no increase in

Table 1. Time in minutes until respiratory arrest in guinea-pigs challenged with 25 µl Sarin applied on a skin area of 3.1 cm² following various forms of pretreatment.

<table>
<thead>
<tr>
<th>Type of pretreatment</th>
<th>Time in minutes until respiratory arrest</th>
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<tbody>
<tr>
<td></td>
<td>pretreatment time in minutes</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Destilled water</td>
<td>31.4 ± 1.4</td>
</tr>
<tr>
<td>Acetone</td>
<td>20.2 ± 1.7</td>
</tr>
<tr>
<td>Ethanol</td>
<td>19.4 ± 1.6</td>
</tr>
<tr>
<td>Ether</td>
<td>12.0 ± 1.3</td>
</tr>
<tr>
<td>Chloroform</td>
<td>10.5 ± 1.2</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>32.0 ± 1.4</td>
</tr>
<tr>
<td>Soap</td>
<td>25.1 ± 1.5</td>
</tr>
<tr>
<td>Non-ionic surfactant</td>
<td>29.8 ± 1.7</td>
</tr>
<tr>
<td>Cat-ionic surfactant</td>
<td>26.5 ± 1.4</td>
</tr>
<tr>
<td>An-ionic surfactant</td>
<td>27.1 ± 1.9</td>
</tr>
<tr>
<td>None</td>
<td>31.2 ± 1.3</td>
</tr>
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</table>

* ± s.e.m. n=10

Table 2. The spread of 25 µl Sarin on the guinea-pig skin surface following various forms of pretreatments.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Area in mm² ± s.e.m. n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>544 ± 25</td>
</tr>
<tr>
<td>Destilled water</td>
<td>551 ± 29</td>
</tr>
<tr>
<td>Ethanol</td>
<td>600 ± 43</td>
</tr>
<tr>
<td>Ether</td>
<td>613 ± 52</td>
</tr>
<tr>
<td>Chloroform</td>
<td>583 ± 54</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>610 ± 31</td>
</tr>
<tr>
<td>Soap</td>
<td>750 ± 28</td>
</tr>
<tr>
<td>Non-ionic surfactant</td>
<td>824 ± 63</td>
</tr>
<tr>
<td>Cat-ionic surfactant</td>
<td>808 ± 58</td>
</tr>
<tr>
<td>An-ionic surfactant</td>
<td>925 ± 76</td>
</tr>
</tbody>
</table>

accuracy could be obtained by addition of a fluorescent material or measuring the skin resistance according to Blank and Finesinger (2) outlining the area and then measure it as above. Controls also showed that the calculation of the spot as being a perfect circle did not give any significant deviations from planimetric measurements.

All experiments were run in groups of ten animals. Both sexes were used in about equal proportions. The weight of the animals was kept fairly constant, 465-480 g, the highest standard error of the mean being ± 12 g. In all 540 guinea-pigs were used.

Results and Discussion

The results are summarized in Tables 1-3 and illustrated by Fig. 1. As follows from table 1 pretreatment with destilled water alone has little or no influence on the absorption, indicating also that the blotting does not produce any mechanical injury on the epidermal barrier. With the exception of dimethylsulfoxide, DMSO, all other forms of pretreatment produced an evident decrease in the survival time of the animals when the pretreatment period was 30 minutes. In the case of the two shorter pretreatment periods the organic solvents, still with the exception of DMSO, have about the same effect, while soap and the three surface active agents were considerably less active. The non-ionic surfactant was chosen for a more detailed study of the time factor involved, the results of which are illustrated in Fig. 1. From this it is evident that the barrier destroying activity appears relatively slowly, i.e. prolonged contact is necessary in order to reach optimal effect, and the asymptote for optimal effect is reached after 25 to 30 minutes. Two consecutive rinses of the treated area with destilled water (0.5 ml each time) immediately after the blotting had no influence at all.

Table 3. Time in minutes until respiratory arrest in guinea-pigs challenged with Sarin mixed with various proportions of dimethyl-sulfoxide (DMSO) and applied on a skin area of 3.1 cm²

<table>
<thead>
<tr>
<th>Amounts of Sarin/DMSO (µl)</th>
<th>Time until respiratory arrest in minutes ± s.e.m. n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>50/0</td>
<td>32.0 ± 1.1</td>
</tr>
<tr>
<td>50/50</td>
<td>10.3 ± 0.6</td>
</tr>
<tr>
<td>35/65</td>
<td>10.7 ± 0.4</td>
</tr>
<tr>
<td>35/15</td>
<td>18.4 ± 0.9</td>
</tr>
<tr>
<td>45/5</td>
<td>25.1 ± 1.2</td>
</tr>
<tr>
<td>15/35</td>
<td>17.4 ± 0.8</td>
</tr>
<tr>
<td>5/45</td>
<td>32.5 ± 1.5</td>
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on the survival time. This indicates that 
the effect on the barrier is not readily 
reversible, which is in accordance with re-
sults obtained by Blohm (6, 7) indicating 
protein denaturating effects of surfactants.

It has been reported that DMSO in-
creases the rate of percutaneous absorption 
for a number of substances (for references 
see 13). Since in the present investigation 
no such effect could be demonstrated when 
the skin area was pretreated, some addi-
tional experiments were run. In these Sarin 
and DMSO were mixed in various propor-
tions, applied on the skin surface of guinea-
pigs as in the other experiments and the 
time until lethal effect determined. These 
results are summarized in Table 3. From 
this it is evident that DMSO increases the 
rate of absorption of Sarin considerably 
when they are mixed together. Lethal ef­
fect of 5 µl Sarin without any addition of 
DMSO, applied on the same area of skin 
in guinea-pigs is seen after 90.2 ± 5 min­
utes (9). In the present experiments the 
same effect was obtained in 32.5 minutes 
when 5 µl Sarin was mixed with 45 µl 
DMSO. The fact that 50 µl Sarin produces 
lethal effect at the same time as 25 µl 
when mixed with equivalent volumes of 
DMSO is only a confirmation of an earlier 
investigation, showing that optimal absorp­
tion is reached with 25 µl of Sarin applied 

on an area of 3.1 cm² (9). Reduced amounts 
of both Sarin and DMSO result, as expect­
ed, in a delayed respiratory arrest.

Since there may be a correlation between 
the surface tension reducing activity of the 
various pretreatment liquids and their ab­
sorption promoting activity, e.g. by such a 
mechanism as facilitating penetration into 
follicles, the spread of Sarin on pretreated 
skin was determined. Already on non-
treated skin Sarin spreads rapidly, and the 
maximum area is reached already after 1-2 
minutes. The spread was always measured 
2 minutes after application, since evapora­
tion may disturb the measurements if they 
are further postponed. The results appear 
in Table 2, from which it is evident that 
there is no such correlation as mentioned 
above. Other possible mechanisms involved 
in the increase in absorption produced by 
the various pretreatments are, of course, 
removal of the lipid surface film and direct 
barrier destroying effects. Blank and Sha-
piro (4) have e.g. showed that surfactants 
and soap solution remove some of the 
amino acid content of the skin surface. To 
what extent such mechanisms are involved 
is, however, impossible to judge from the 
present experiments.

Washing the skin with ether or petro-
leum ether has little effect on the perme­
ability of skin to salicylates (14), surface ac-
tive agents (3, 5) or alkylphosphate (11). On the other hand, prolonged treatment of skin with acetone, alcohol or hexane will considerably increase the permeability of skin to water (1, 12). Without questioning the accuracy of the various methods used in the above cited papers, it should be pointed out that the present method could be extremely sensitive, at least in the meaning that already a very minute injury to the barrier function of the skin will be readily recorded. This is due to the fact that the skin surface is loaded with a very high dose of toxic material. The LD₅₀ of Sarin in the guinea-pig following percutaneous application is thus 5.6 mg per kg bodyweight (8) with an area of absorption restricted to 0.4 cm². In the present experiments 27 mg are applied to an area of 3.16 cm² in animals weighing about 470 mg. The amount per area unit is about the same (the animals in the LD₅₀-determinations were of approximately the same weight), and thus there are at least 20 LD₅₀-doses applied in the present experiments. With this excess a very minute barrier damage may result in a rapid death of the animal.

Sarin is rapidly hydrolyzed by alkali (10). In at least the case of the soap solution, which had a pH of 10.5, it could be of some importance that the skin surface was more alkaline than in normal animals, i.e. some Sarin is inactivated and the effective dose reduced. However, this is not very likely considering the large dose of Sarin and the fact that the absorption of a toxic dose is very rapid. This assumption was supported by the fact that several rinses with saline after pretreatment with soap solution did not change the results.

**SUMMARY**

The influence of pretreatment of the skin of guinea-pigs with organic solvents and surface active agents on the barrier function towards an organophosphorus cholinesterase inhibitor, Sarin, has been investigated. As a measure of the rate of absorption of the challenging substance the time until respiratory arrest was chosen. Distilled water, used as a control, had no significant effect, while acetone, ethanol, ether and chloroform increased the rate of absorption already following short pretreatment periods. In the case of soap solution and non-ionic, cat-ionic and an-ionic surfactants longer pretreatment was necessary for an optimal effect on the barrier function of the skin. Pretreatment with dimethylsulfoxide had no effect, but when mixed with Sarin in various proportions the rate of absorption of the challenging substance was increased. The spread of Sarin on pretreated skin was also investigated as an indirect measure of the surface tension reducing capacity, and there was no correlation between this and the induced increase in absorption. The results have been discussed with regard to mechanisms which may explain the decrease in barrier function of skin following the various pretreatments.

**REFERENCES**


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