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

III. Restoration of the barrier function

TORSTEN FREDRIKSSON

In the previous papers (1, 2) a method was described to test the influence of various organic solvents and surface active agents on the barrier function of the skin towards an organophosphorus cholinesterase inhibitor *iso*propoxy-methylphosphoryl fluoride (Sarin) and data were given regarding the barrier injuring effects of the different pretreatments. It was found that organic solvents in general (with the exception of dimethylsulfoxide) had a rapid injuring effect, while in the case of soap-solution and surface active agents longer pretreatment periods were necessary to produce an optimal damage.

In these experiments the animals—guinea-pigs—were challenged with the test substance 30 minutes after the pretreatment, a period of time which was not chosen at random. Thus, after the pretreatment the test area was left uncovered in order to allow free evaporation of any remaining liquid and within 30 minutes the area always appeared to be completely dry. Earlier application of the test substance could introduce a factor of uncertainty, and later application would be impractical due to the large number of experiments.

However, the rate of restoration of the barrier function after injury is of great interest and might give clues to the type of barrier injury produced by the various pretreatments. In order to study this it was decided to challenge the animals after various periods of time after the pretreatment. Two types of pretreatment liquids were selected, one organic solvent and one ionic surfactant.

Material and Methods

All experiments were run in groups of ten animals (guinea-pigs). Both sexes were used in about equal proportions. The weight of the animals were kept as constant as the stock allowed, 465–480 g, the highest standard of the mean being ± 10 g. In all 110 animals were used.

The unanaesthetized animals were fixed with the back down on small operating tables, which were adjusted so that the abdominal region was approximately horizontal. A metal ring with an inner area of 3.1 cm^2 was glued with collodion to the clipped abdominal skin. Thirty minutes later 0.5 ml of the pretreatment liquid was pipetted on the skin area within the ring. It was allowed to remain on the area for 30 minutes while the ring was covered in order to counteract evaporation. The liquid was then removed by gentle blotting with dental sorbent rolls. The area was then left uncovered in order to allow free evaporation of any remaining liquid. Thirty, 60, 120, 180 and 240 minutes later the animals were challenged, *i.e.* 25 µl of Sarin was applied on the skin surface, the ring covered and the time until respiratory arrest noted. The organic solvent used was analytical grade of ether and the surfactant 0.045 N water

Department of Dermatology, Centrallasarettet, 721 89 Västerås, Sweden.

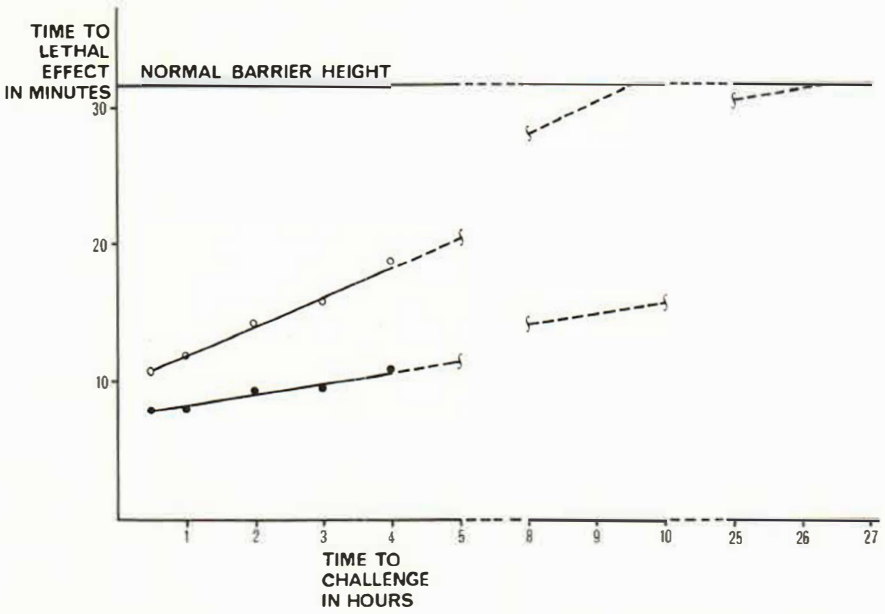

Fig. 1. Relationship between time to respiratory arrest and time to challenge with Sarin after pretreatment of the skin area with ether and an an-ionic surfactant. Filled circles denote the surfactant, unfilled ether. The broken lines show extrapolation to the normal barrier height (1, 2).

Table 1. *Time until respiratory arrest in guinea-pigs challenged with Sarin after various periods of time after pretreatment with ether and an an-ionic surfactant*

Challenge with Sarin in minutes after pretreament	Time to lethal effect in minutes	
	Ether	Surfactant
30	11.0 ± 0.9*	7.6 ± 0.4
60	12.1 ± 0.7	7.7 ± 0.3
120	14.7 ± 1.1	9.5 ± 0.7
180	16.0 ± 1.1	9.9 ± 0.9
240	18.7 ± 1.4	10.8 ± 0.8

* $\bar{x}$ s.e.m. n = 10

solution of the sodium salt of alkylether-sulfate, an an-ionic agent. Details of the method are given in the earlier papers (1, 2).

Results and Discussion

The results are summarized in Table 1 and illustrated by Fig. 1. There are relatively few points of observation, and this was due to the fact that it was not possible to keep the unanaesthetized[1] guinea-pigs restrained longer than four hours, even though this particular animal is very calm in recumbent position. If the animals were released after the pretreatment they tried to get rid of the rings with subsequent mechanical injury to the test area. Thus, this could have given more but highly uncertain points of observation, and this solution of the problem was therefore discarded.

The two curves obtained are thus uncertain, and the linear plotting could have been substituted by semilogarithmic, which from theoretical reasons seems to be more justifiable. However, the present material does not allow such differentiations. It follows that the extrapolation of the curves to the level of the normal barrier height, which is 31 to 32 minutes (1, 2), does not justify a determination of even approximate time to full restoration of the barrier

[1] The necessity of using unanaesthetized animals in experiments of this kind has been explained earlier (1).

function, which Fig. 1 might indicate. However, the experiments were not designed primarily to determine *exact* rates of regenerations but to differentiate between two possible types of barrier injury. The recovery after pretreatment with ether is evidently much more rapid than after pretreatment with the an-ionic surfactant. This indicates that the barrier injuries produced are of different type or produced at different levels of the epidermis. It is tempting to suggest that the injury to the barrier function produced by the surfactant is due to permanent denaturation of proteins and that full restoration of the barrier function is not obtained until the epidermis replaces the denaturated proteins. In the case of ether the more rapid recovery could be due to the fact that the initial injury at least partly was due to removal of the lipid surface film or due to a more superficial type of injury. One implication from the present experiments—if they are valied also in humans—is that what we regard as normal skin never is really normal, as *e.g.* Isherwood has suggested (3).

SUMMARY

The approximate rate of restoration of the injured barrier function of the skin of guinea-pigs has been investigated. The injuries were produced by ether and an anionic surfactant, and as a test of the barrier function the time until lethal effect produced by isopropoxy-methylphosphoryl-fluoride (Sarin), an organophosphorus cholinesterase inhibtor, was used. The barrier function returned more rapidly in the case of ether produced injury, while recovery was considerably slower in the case of the surfactant. It was suggested that this was due either to differences in type of injury or to injuries produced at different epidermal levels.

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