Acta derm.-venereol. 49: 484–489, 1969

PERCUTANEOUS ABSORPTION OF SOMAN AND TABUN, TWO ORGANOPHOSPHORUS CHOLINESTERASE INHIBITORS

TORSTEN FREDRIKSSON

Among the organophosphorus cholinesterase inhibitors considered as chemical warfare agents Soman (pinacoloxy-methylphosphoryl fluoride) and Tabun (dimethylamido-ethoxy-phophoryl cyanide) are of great current interest. However, previous experimental studies on the percutaneous absorption of these two compounds have been very scarce, in spite of the fact that this route of administration is regarded to be most important. There are few quantitative data available but *Holmstedt* (7) demonstrated that rabbits died within 20 minutes after application on the skin of 10 to 15 mg per kg body weight of Tabun. The lethal dose was found to lie between 1.5 and 3.0 mg per kg body weight. However, the experimental animals were depilated by means of strontium sulfide, a treatment which is very likely to change the barrier function of the skin towards absorption. Loomis and Salafsky (8) have found the lethal dermal dose of Soman in mice to be 0.78 mg per 100 g body weight.

Dose-response comparisons following percutaneous absorption and intravenous infusion have in recent years been extensively used in order to calculate *in vivo* rates of percutaneous absorption in experimental animals, see *e.g.* Fredriksson (1, 2, 3), Griesemer *et al.* (6), Shellenberger *et al.* (11) and Nabb *et al.* (10). This is a sensitive and accurate method to determine the amounts of a compound reaching the blood stream from a dermal deposit, provided that a suitable parameter can be obtained and that the rate of intravenous infusion is kept constant. This dose-response technique has mainly been used for determination of percutaneous absorption of various organophosphorus cholinesterase inhibitors, such as isopropoxy-methylphosphoryl fluoride (Sarin), paraoxon, parathion, bidrin, phosdrin and vapona (DDVP).

In the present paper such a method is used in order to determine the rate of percutaneous absorption of Soman and Tabun. The cat was used as experimental animal, and as parameter was chosen the time until cardiac arrest. This has in other investigations been shown to be reliable in dose-response studies with organophosphorus cholinesterase inhibitors, Fredriksson (2).

Methods

In the main series of experiments twenty-four cats, weighing between 2.8 and 3.2 kg, were used. They were anesthetized with sodium pentobarbital, 30 mg per kg body weight, administered intraperitoneally. In all the animals the trachea and one carotid artery were cannulated. Respiration was recorded as pressure differences in the tracheal cannula by means of a pressure transducer, and blood pressure was recorded from the carotid artery by means of a Statham electromanometer. A Grass model 5 polygraph was used to record these parameters.

In the animals given intravenous infusion a polyethylene catheter was introduced in

Department of Dermatology, Centrallasarettet, 721 89 Västerås, Sweden, and the Research Institute of National Defence, Department 1, Sundbyberg 4, Sweden.

the left femoral vein to a length of about 10 cm. It was connected to an infusion pump, which allowed a constant speed of injection. Tabun and Soman were dissolved in 0.9 per cent saline immediately before the experiment.

In the animals used for percutaneous absorption an area on the lateral aspect of the left thigh was clipped and aluminium rings of various diameters were fixed to the skin with the aid of collodion. The glue was allowed to dry for 30 minutes before the actual experiment started. Undiluted Tabun and Soman was evenly distributed on the area within the rings with the aid of micropipettes. After application the cylinder was immediately sealed by means of a cover-glass and a lubricant.

When Soman was applied on an area of 3.1 cm^2 the animals died after about 15 minutes, and then a concentration was titrated which, when given as an intravenos infusion, produced cardiac arrest in about the same period of time. In the case of Tabun cardiac arrest was considerably delayed when the same area was used. In the main series of experiments it was therefore decided to use a larger area, namely 4.5 cm^2. Cardiac arrest occurred then after about 50 minutes, and the corresponding concentration for intravenous infusion was titrated. The amounts of toxic material applied on the skin were well above those that result in optimal absorption (3).

In one series of experiments, comprising an additional 10 cats weighing between 2.8 and 3.2 kg, Soman was applied on different areas and the time until cardiac arrest was noted. A corresponding experiment with Tabun was not possible due to the fact that lethal outcome was not reached in reasonable time when smaller areas were used.

Results and Discussion

The main results are summarized in Tables 1 and 2 and illustrated by Figs. 1–4. Characteristic cholinergic signs, such as salivation, fasciculations and miosis, were noted in all the animals. The symptomatology and the sequence in which the various cholinergic signs developed were essentially the same as following percutaneous and intravenous administration of Sarin (1). Effects on respiration and blood pressure also followed the same pattern, as could be expected, and in the case of Tabun the effects were comparable with those noted by Holmstedt (7). Following percutaneous application there were also local fibrillations in muscles underlying the area of application, probably caused by diffusion of the inhibitors through the subcutaneous tissues into the muscles, McNamara *et al.* (9). The area of application did not show any sign of irritation in any of the experimental animals.

Following intravenous infusion Soman is extremely toxic. The total dose producing lethal effect was not more than about 12.5 μg in cats weighing around 3 kg, *i.e.* approximately 4 μg per kg body weight. This value was calculated from results obtained when the intravenous infusion continued until complete cardiac arrest, but the infusion could probably have been discontinued several minutes earlier with lethal outcome in the same period of time. The toxicity given here is thus at least not overestimated. The corresponding value for Tabun is about 60 μg, and for Sarin 65 μg per kg body weight (1). The figures are not directly comparable since lethal effect was not produced after the same periods of time (this was not feasible in the present series of experiments due to the comparatively "low" percutaneous toxicity of Tabun), since such factors as detoxification mechanisms might interfere. However, the relative toxicity of Soman must be considered as extremely high following this mode of administration.

From the figures in Tables 1 and 2 the rate of percutaneous absorption can easily be calculated. The absorption rate for Soman is about 0.27 $\mu g \cdot min.^{-1} \cdot cm^{-2}$ and for Tabun 0.78 $\mu g \cdot min.^{-1} \cdot {}^{-2}$. These figures denote only the absorption of active, toxic inhibitor into the blood stream from the topical deposit, and the disappearance rate through the epidermis and the dermis might be considerably higher, since part of the inhibitors might be detoxified during their diffusion through the skin. It has thus

Table 1. *Time until cardiac arrest in anaesthetized cats following intravenous infusion and percutaneous absorption of Soman (pinacoloxy-methyl-phosphoryl fluoride)*

Form of administration	Animal number	Dosage	Time until cardiac arrest in minutes
Intravenous infusion	1	0.84 $\mu g \cdot min.^{-1}$	14
	2		14
	3		15
	4		15
	5		16
	6		17
Percutaneous absorption	7	50 μl on 3.1 cm^2	14
	8		14
	9		14
	10		16
	11		16
	12		17

Table 2. *Time until cardiac arrest in anaesthetized cats following intravenous infusion and percutaneous absorption of Tabun (dimethyl-amido-ethoxy-phosphoryl cyanide)*

Form of administration	Animal number	Dosage	Time until cardiac arrest in minutes
Intravenous infusion	1	3.5 $\mu g \cdot min.^{-1}$	42
	2		48
	3		50
	4		50
	5		54
	6		55
Percutaneous absorption	7	109 μl on 4.5 cm^2	40
	8		47
	9		50
	10		51
	11		51
	12		58

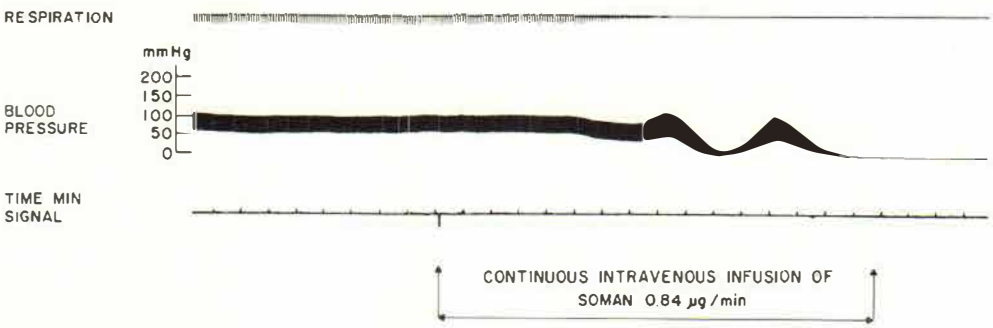

Fig. 1. Effects of Soman on respiration and blood pressure following continuous intravenous infusion. Cat, weighing 2.8 kg anaesthetized with sodium pentobarbital.

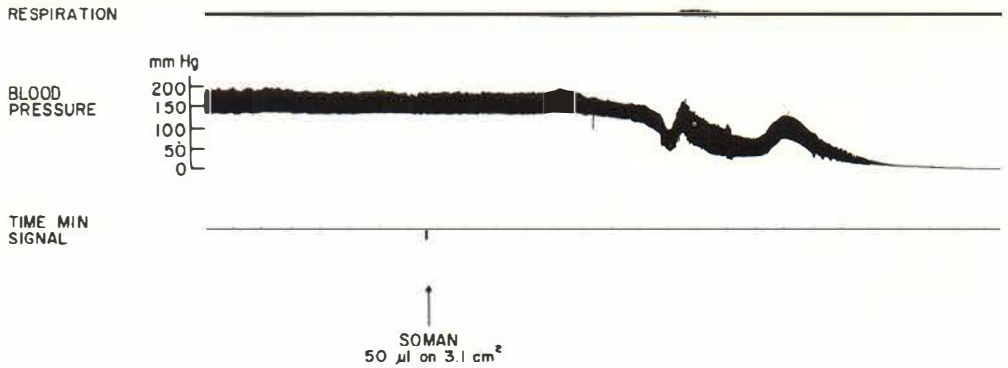

Fig. 2. Effects of Soman on respiration and blood pressure following percutaneous absorption. Cat, weighing 3.0 kg, anaesthetized with sodium pentobarbital.

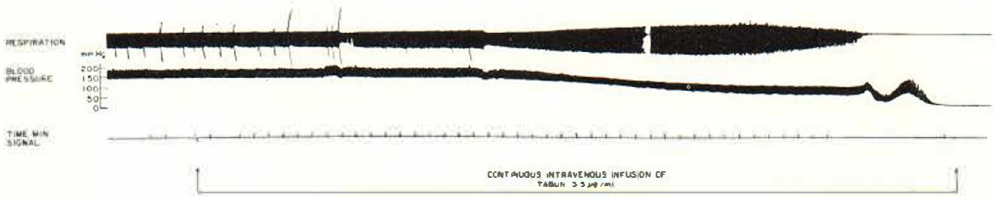

Fig. 3. Effects of Tabun on respiration and blood pressure following continuous intravenous infusion. Cat, weighing 2.9 kg, anaesthetized with sodium pentobarbital.

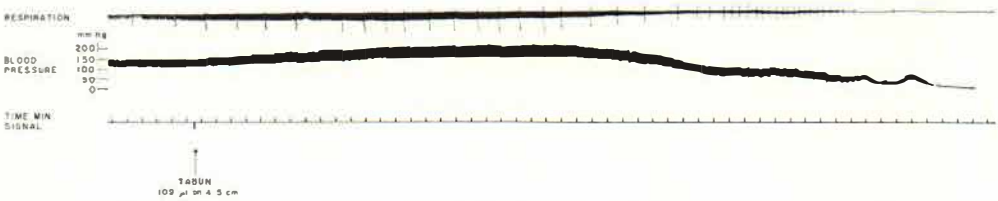

Fig. 4. Effects of Tabun on respiration and blood pressure following percutaneous absorption. Cat, weighing 3.0 kg, anaesthetized with sodium pentobarbital.

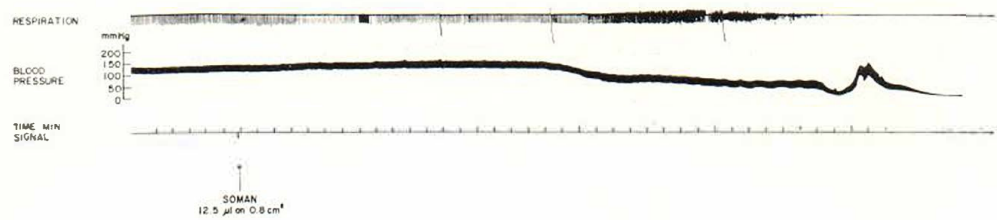

Fig. 5. Effects of Soman on respiration and blood pressure following percutaneous absorption. 12.5 µl Soman was applied on 0.8 cm². Cat, weighing 3.2 kg, anaesthetized with sodium pentobarbital.

Table 3. *Influence of the size of the area of absorption on the time until cardiac arrest in anaesthetized cats following application of Soman*

Area in cm^2	Dose in μl	Cat weight in kg	Time until cardiac arrest in minutes
0.8	12.5	2.9	40
0.8	12.5	3.2	42
1.6	25	3.0	22
1.6	25	3.0	28
3.1*	50	2.8–3.2	14–17
4.5	73	2.9	12
4.5	73	2.9	12
6.2	100	3.0	11
6.2	100	2.8	11
9.0	145	2.8	9
9.0	145	3.1	10

* Six animals from Table 1.

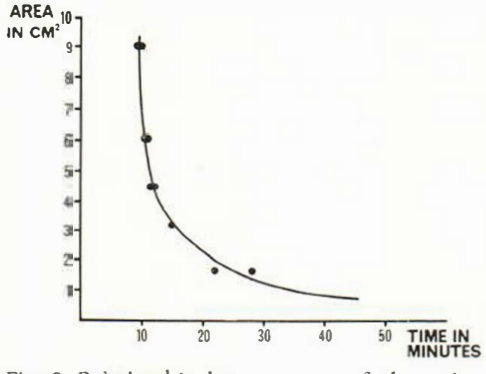

Fig. 6. Relationship between area of absorption of Soman and time until cardiac arrest in anaesthetized cats. The point at 3.2 cm^2 and 15 minutes represents the mean of 6 experiments, taken from Table 1.

been shown that skin from guinea-pigs contains enzymes capable of hydrolyzing both Soman and Tabun (4). Such enzymes, belonging to the group of phosphorylphosphatases, do not seem to be species specific, and cat skin has been shown to contain such an enzyme by Fredriksson *et al.* (5). The enzyme splitting Soman seems to be particularly active, at least in the guinea-pig, and dermal absorption might thus be a relatively less toxic way of administration of this compound as compared with Tabun and Sarin. Actual proof for this theory cannot be obtained since inherent toxicity differs and rates of dermal absorption vary. However, the rate of absorption of toxic material into the blood stream for Soman is only 1.5 $m\mu M \cdot min.^{-1} \cdot cm^{-2}$ as compared with 4.8 $m\mu M \cdot min.^{-1} \cdot cm^{-2}$ for Tabun and 10.5 $m\mu M \cdot min.^{-1} \cdot cm^{-2}$ for Sarin (Fredriksson, 1), and this might be due to differences in enzymic break-down rather than differences in rate of true dermal absorption, since the physico-chemical properties of these compounds are in many ways similar.

From the series of experiments with Soman where the area of absorption was varied the results are summarized in Table 3 and illustrated by Figs. 5 and 6. The number of observations are few, but if the various areas of absorption are plotted as ordinates against the time until cardiac arrest, as shown in Fig. 6, the curve obtained approximates a hyperpolic function in the same way as shown earlier (3). The persent findings thus give support to the theory outlined in that paper regarding the gradual increase in lag time (from beginning absorption from the topical deposit until effective concentrations in blood and tissues are reached) with decreasing area of absorption.

SUMMARY

The rates of percutaneous absorption of Soman (pinacoloxy-methyl-phophoryl fluoride) and Tabun (dimethyl-amido-ethoxy-phosphoryl cyanide) have been determined by dose-response comparison following this mode of administration and intravenous infusion in the cat. Time until cardiac arrest was chosen as a parameter. The rate of absorption for Soman was found to be about 0.27 $\mu g \cdot min.^{-1} \cdot cm^{-2}$ (1.5 $m\mu M \cdot min.^{-1} \cdot cm^{-2}$) and for Tabun 0.78 (4.8 $m\mu M \cdot min.^{-1} \cdot cm^{-2}$). These rates represent the absorption of active material into the blood stream, and the disappearance rates from the topical deposits might be considerably higher since the compounds may be subject to enzymic hydrolysis during the passage through the skin.

In one series of experiments the area of absorption of Soman was varied, and the

relationship between area of absorption and time until cardiac arrest was investigated.

REFERENCES

1. Fredriksson, T.: Studies on the percutaneous absorption of Sarin and two allied organo-phosphorus cholinesterase inhibitors. *Acta derm.-venereol. 38*, suppl. 41, 1969.
2. Fredriksson, T.: Studies on the percutaneous absorption of parathion and paraoxon. V. Rate of absorption of paraoxon. *J. Invest. Derm. 38:* 233, 1962.
3. Fredriksson, T.: Influence of solvents and surface active agents on the barrier function of the skin towards Sarin. I. Development of method. *Acta derm.-venereol. 43:* 91, 1963.
4. Fredriksson, T.: Hydrolysis of Soman and Tabun in cutaneous tissues. *Acta derm.-venereol. 49:* 490, 1969.
5. Fredriksson, T., Farrior, W. L., and Witter, R. F.: Studies on the percutaneous absorption of parathion and paraoxon. I. Hydrolysis and metabolism within the skin. *Acta derm.-venereol. 41:* 335, 1961.
6. Griesemer, R. D., Blank, J. H. and Gould, E.: The penetration of an anticholinesterase agent (Sarin) into the skin. III. A method of studying the rate of penetration into the skin of the living rabbit. *J. Invest. Derm. 31:* 255, 1958.
7. Holmstedt, B.: Synthesis and pharmacology of dimethyl-amido-ethoxy-phosphorylcyanide (Tabun) together with a description of some allied anticholinesterase compounds containing the N-P bond. *Acta Physiol. Scand.* 25, suppl. 90, 1951.
8. Loomis, T. A. and Salafsky, B.: Antidotal Action of Pyridinium Oximes in Anticholinesterase Poising; Comparative Effects of Soman, Sarin and Neostigmine on Neuromuscular Function. *Toxicol. Appl. Pharmacol. 5:* 685, 1963.
9. McNamara, B. P., Murtha, E. F., Edberg, L. J., Bergener, A. D., and Wills, J. H.: TEPP and skeletal muscle fasciculation. *Federation Proc. 12:* 350, 1953.
10. Nabb, D. P., Stein, W. J. and Hayes, W. J.: Rate of skin absorption of parathion and paraoxon. *Arch. Environ. Health 12:* 501, 1966.
11. Shellenberger, T. E., Newell, G. W., Okamoto, S. S., and Sarros, A.: Response of rabbit whole-blood chloinesterase *in vivo* after continuous intravenous infusion and percutaneous application of dimethyl organophosphate inhibitor. *Biochem. Pharmacol. 14:* 943, 1965.

Received for publication Febr. 3, 1969.