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HYDROLYSIS OF SOMAN AND TABUN (TWO ORGANOPHOSPHORUS CHOLINESTERASE INHIBITORS) IN CUTANEOUS TISSUES

TORSTEN FREDRIKSSON

In a preceeding paper (4), the percutaneous absorption of two very toxic organophosphorus cholinesterase inhibitors was studied. Since the two compounds, pinacoloxy-methyl-phosphoryl fluoride (Soman) and dimethyl-amido-ethoxy-phosphoryl cyanide (Tabun) may be inactivated by enzymic hydrolysis, a cutaneously absorbed inhibitor may be partly inactivated before it reaches the blood-stream. In earlier papers (1, 2) it has been demonstrated that another warfare agent, isopropoxy-methyl-phophoryl fluoride (Sarin) and two analogues were hydrolyzed by guinea pig skin and paraoxon by skin from man, cat and rabbit. These reactions were shown to be enzymic in nature, the enzyme belonging to the group of phosphorylphosphatases. Experiments *in vivo* furthermore suggest that up to 90 per cent of such topically applied compounds may be inactivated before they were absorbed by the subepidermal capillary net (1, 3). When considering the percutaneous absorption of organophosphorus compounds and their toxicity by the dermal route this mechanism of detoxification may thus be of the utmost importance. The aim of the present experiments was to investigate the possible occurrence of such inactivating processes also with regard to Soman and Tabun, since their percutaneous absorption was studied in a series of experiments (4).

Material and Methods

An automatic recording titrator (5) was used. This apparatus allows studies on hydrolysis reactions to be performed at a constant pH and at a constant temperature, in this case pH 7.5 and 37.0°C. The acid residues formed were neutralized by a 0.098 M solution of NaOH. The volume of NaOH consumed was recorded as a function of time, and from the curves obtained the rate constants of the reactions were calculated. These rate constants were then used to calculate the half-life of the compounds under the different experimental conditions.

Skin from guinea-pigs were treated as described earlier (2) and suspended in 0.1 M KCl. The organophosphates were also dissolved in 0.1 M KCl, and immediately before the recording started 10 ml of these solutions were added to the tissue suspension so that the final volume was 40 ml and the final concentrations of the substrates were $3.3 \cdot 10^{-3}$ M. The amount of skin was 1.0 g (wet weight) per 40 ml KCl. Skin suspension was heated at 90°C for 25 minutes in order to obtain inactivation of enzymes possibly present. The spontaneous hydrolysis of the compounds was also studied at the same pH and temperature, the concentration also being $3.3 \cdot 10^{-3}$ M. For each point 4 experiments were run.

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

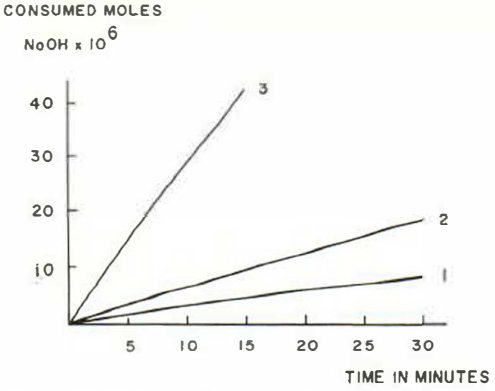

Fig. 1. Hydrolysis of $3.3 \cdot 10^{-3}$ M Soman at pH 7.5 and 37.0°C as obtained from the recorder. *Curve 1:* spontaneous hydrolysis. *Curve 2:* hydrolysis in the presence of heated skin suspension (1.0 g tissue). *Curve 3:* hydrolysis in the presence of untreated skin suspension (1.0 g tissue).

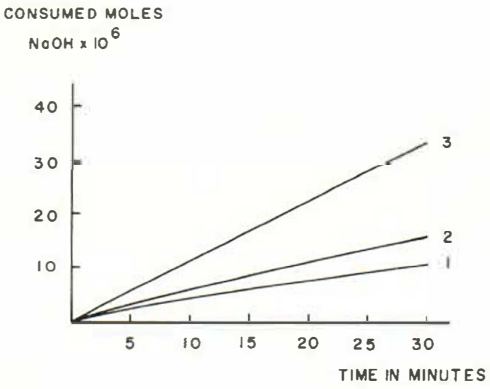

Fig. 2. Hydrolysis curves of $3.3 \cdot 10^{-3}$ M Tabun at pH 7.5 and 37.0°C as obtained from the recorder. Curves 1, 2 and 3 have the same meaning as in Fig. 1.

Results and Discussion

The results are summarized in Figs. 1 and 2 and in Table 1. At the pH and temperature chosen the spontaneous hydrolysis of both Soman and Tabun is comparatively slow. Under identical conditions the half-life for Sarin is about 230 minutes (1). In the presence of heated skin suspension the hydrolysis of both compounds is only slightly increased, while the hydrolysis is considerably increased by untreated skin suspension, particularly in the case of Soman. The hydrolysis is in both cases a continuous reaction, which is considerably decreased by heating of the skin suspension, indicating an enzymic process. Under the same conditions the half-life of Sarin in the presence of untreated skin is about 125 minutes, thus comparable with Tabun, while Soman splitting enzymic activity is considerably higher.

Table 1. *Hydrolysis of Soman and Tabun expressed as half life in minutes. The compounds were dissolved in 0.1 M KCl to a final concentration of $3.3 \cdot 10^{-3}$ M. The amount of skin was 1.0 g per 40 ml reaction solution*

Compound	$t_{1/2}$ in minutets at pH 7.5 and 37.0° C	
	Spontaneous	Skin suspension
Soman	460	45
	450	50
	465	50
	470	45
Tabun	400	130
	410	130
	400	125
	410	135

The fairly rapid hydrolysis of Soman should result in a relative decrease in the cutaneous toxicity of this compound as compared with Tabun and Sarin. Percutaneous absorption of the latter two compounds may thus be a particulary hazardous portal of entry, other conditions being equal, such as inherent systemic toxicity and rate of absorption per unit area and time. Under any circumstances the possible inactivation during epidermal passage must be considered in discussion of toxicity of organophosphorus cholinesterase inhibitors.

SUMMARY

The ability of skin from guinea pig to hydrolyze pinacoloxy-methyl-phosphory fluoride (Soman) and dimethyl-amido-ethoxy-phosphoryl cyanide (Tabun) has been investigated with the aid of an automatic recording titrator. It was shown that skin from this species containes enzymes capable of hydrolyzing both compounds; the one splitting Soman being particularly active.

The results have been discussed with regard to the percutaneous absorption and toxicity of these two compounds.

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