

DECONTAMINATION AND DETOXIFICATION OF TOXIC
CHEMICAL WARFARE AGENTS USING POLYURETHANE SPONGES

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ABSTRACT

Organophosphorus (OP) nerve agents are some of the most potent toxic agents conceived by mankind, are specific inhibitors of cholinesterase (ChE), and therefore are a serious threat to military and civilian personnel. OP nerve agents can exist as a liquid and contaminate skin, penetrate, resulting in deleterious systemic effects including a cholinergic crisis. Another serious problem that may be encountered while caring for personnel contaminated with organophosphorus chemical warfare nerve agents is the possibility that there will be cross-contamination to the medical personnel. In addition, during combat or terrorist acts, individuals might be exposed to chemical toxins before they don their protective gear. We have been developing enzyme immobilized polyurethane foams that can effectively decontaminate the skin and other such exposed surfaces of the organophosphorus toxins in a wide variety of environmental conditions. With additives, the sponges provided increased protection for guinea pigs exposed to neat soman and VX compared to currently fielded kits, and reduces methylene blue uptake in mustard-exposed animals. Sponges have several criteria to meet to be effective personal decontaminants and detoxifiers of chemical warfare agents. The sponges must be lightweight for individual use, yet be shelf-stable under environmental conditions found in the field. In this manner, the sponges would provide immediate use for either the individual or a buddy in a field.

Report Documentation Page				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 01 JUL 2003		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Decontamination And Detoxification Of Toxic Chemical Warfare Agents Using Polyurethane Sponges				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Walter Reed Army Institute of Research, Division of Biochemistry, Department of Biochemical Pharmacology 503 Robert Grant Ave, Silver Spring, MD 20910-7500, USA				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES See also ADM001523.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 10	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

INTRODUCTION

Organophosphorus (OP) nerve agents are a serious threat to military and civilian personnel. These agents are some of the most potent toxic agents and are specific inhibitors of cholinesterase (ChE). OP nerve agents can exist as a vapor and be inhaled, as a liquid they can contaminate skin, or can be ingested if food or water is contaminated. Another serious problem that may be encountered while caring for personnel contaminated with organophosphate (OP) chemical warfare nerve agents is the possibility that there will be cross-contamination to the medical personnel. In addition, during combat or terrorist acts, individuals might be exposed to chemical toxins before they don their protective gear. We have been developing enzyme immobilized polyurethane foams that can effectively decontaminate the skin and other such exposed surfaces of the organophosphate toxins in a wide variety of environmental conditions (Munnecke, 1979; Wood et al., 1982; Havens and Rase, 1993; Gordon et. al., 1999). Sponges have several criteria to meet to be effective personal decontaminating sponges and detoxifiers of chemical warfare agents. The sponges must be lightweight for individual use, yet be shelf-stable under environmental conditions found in the field. In this manner, the sponges would provide immediate use for either the individual or a buddy in a field. The importance for ready availability and rapid use of a decontamination and detoxification sponge is due to the rapid damage caused by CWAs – OPs penetrate skin in less than 5 min and mustard causes irreversible cell damage as a result of alkylation equally rapidly.

METHODS

SPONGE SYNTHESIS

Briefly, the polyurethane sponges (figure 1) were molded in a Tupperware® container to the size of a human hand, then cut for use. The final size used on the animals was approximately 7 x 3.5 x 1 cm. Details are provided in Gordon et. al., 1999.

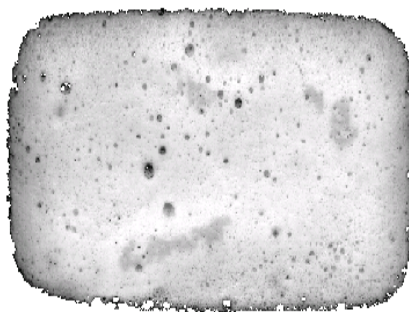


Figure 1. Sponge product.

BACK-TITRATION MONITORING OF SPONGE DECONTAMINATION OF GUINEA PIG SKIN

After the guinea pig skin was wiped with the sponge(s), each sponge was placed in a separate 50 mL capped polypropylene tube and thoroughly mixed by vortexing. Then, an aliquot was removed and placed in 1 mL tubes containing 0.05% bovine serum albumin and 50 mM potassium phosphate buffer pH 8. The samples were sequentially diluted in the same buffer. Aliquots of all the dilutions were next transferred to a 96-well microtiter plate containing acetylcholinesterase (typically, 0.055 units). After incubation of the diluted soman with the known quantity of cholinesterase, 10 μ L was removed to a second 96-well

microtiter plate and inhibition determined using a Molecular Devices Plate Reader and a modified Ellman procedure as previously described (Maxwell et. al., 1992; Caranto et. al., 1994). In this manner, the soman samples were diluted between 105 and 1011 fold, permitting quantification of the resulting inhibition of the cholinesterase (10-90% activity remaining) in at least one of the dilutions.

ANIMAL USE

The protocols for the animal experiments were approved by the U.S. Army Medical Research Institute of Chemical Defense Committee on Animal Care and Use, and research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals, and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, NRC publication 96-23, 1996 edition.

SPONGE DECONTAMINATION

The sedated and shaved guinea pigs were cutaneously exposed to neat soman on their sides. One minute after the exposure, a sponge wrapped around a pair of forceps was moved across the guinea pig's side; then the forceps were rotated 180 degrees, so that the clean surface of the sponge was pointed at the animal. Three more passes were taken from the rear towards the front. An identical procedure was used when the protocol required an additional second sponge to decontaminate the animal.

M291 DECONTAMINATION KIT

The sedated and shaved guinea pigs were cutaneously exposed to neat soman on their sides. One minute after the exposure, an M291 pad, previously removed from the M291 kit and cut it in half, was held in forceps and the guinea pig was decontaminated using five counter clockwise swipes. The second half of the pad was used to perform an additional five clockwise swipes.

HD EVALUATION

Twenty-four hours post-neat HD exposure and decontamination, animals were injected with trypan blue and then killed. The skin covering the backs of the animals was removed. In addition, skin punches were taken from each of the exposure sites (control, exposed, and decontaminated sites).

RESULTS

Molecular modeling: The result of *in situ* polymerization with enzyme is depicted in figure 2, where the enzyme becomes cross-linked to and a part of the matrix during synthesis. In this manner, the enzyme gains some of the structural integrity of the cross-linked polymer, and as described below, resistance to environmental denaturing conditions. The immobilization process is feasible because the cross-linking occurs between the prepolymer and free amino groups.

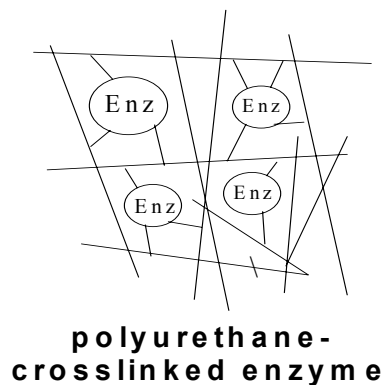


Figure 2. Schematic of immobilized enzyme.

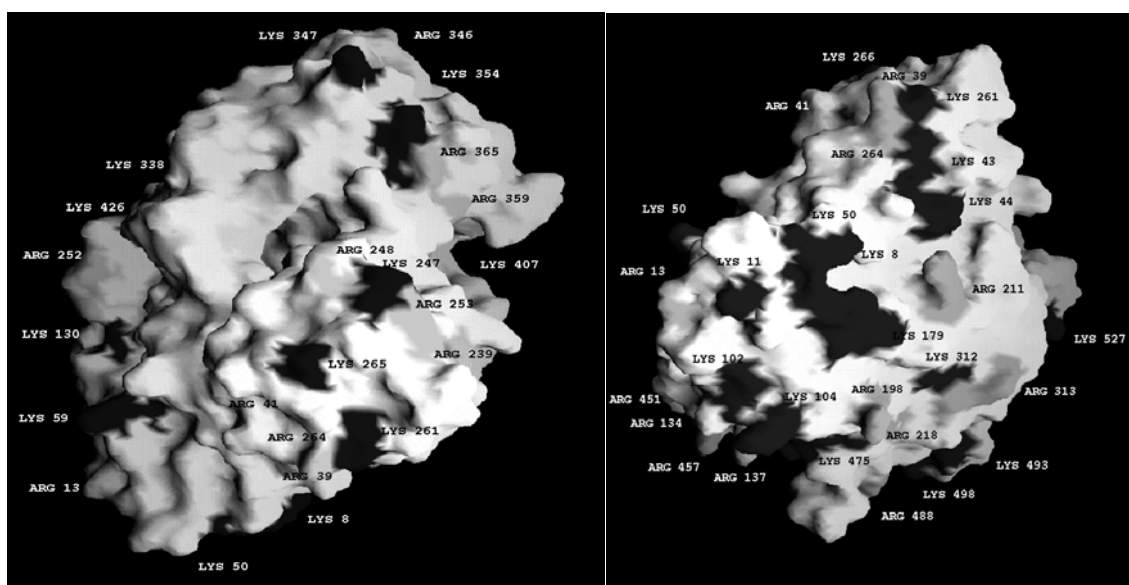


Figure 3 shows the lysine residues on the surface of horse butyrylcholinesterase (dark color) and mouse acetylcholine. The active site gorge of the enzyme is shaded in the center, through which the substrate, OP, and oxime, for reactivation, must be able to penetrate. The lysine residues do not block the active site, so that the enzyme remains functional.

Figure 3. Left, Horse butyrylcholinesterase; right, back of the enzyme. The shaded area in the center of the left figure represents the active site gorge. The dark areas are surface lysine groups to which the polymer forms a covalent bond.

Oximes enhance the capacity of AChE sponges to detoxify OPs: To increase the OP/enzyme stoichiometry, polyurethane immobilized enzyme was combined with oximes such as HI-6 so that the catalytic activity of OP-inhibited AChE (or BChE) is rapidly and continuously restored before irreversible aging of the enzyme-OP complex can occur. Thus, the OP is continuously detoxified. We combined these components in a porous form of the polyurethane foam formed *in situ* from water-miscible hydrophilic urethane prepolymers and the enzymes. Thus, we envision a reusable immobilized enzyme sponge of cholinesterases and oximes for OP decontamination. In fact, we demonstrated that the OPs diisopropyl-fluorophosphate (DPF) or MEPQ (7-(methyl-ethoxyphosphinyloxy)-1-methylquinolinium iodide) inhibited the activity of ChE-sponges, as was observed for non-immobilized ChE in solution. The oxime HI-6 restored activity of the AChE-sponge until the molar concentration of MEPQ reached approximately 1000 times that of the cholinesterase active site. However, the AChE-sponge could be recycled many times by rinsing the sponge with HI-6 in the absence of OP. In this case, most of the original ChE activity could then be restored to the sponge. Therefore, the bioscavenger approach can be used externally: the sponge would soak up organophosphate decontaminating the OP contaminated skin. Then the ChE sponge and oxime would detoxify the OP in the sponge. We have found that the ability of the immobilized enzymes and oximes such as HI-6 or TMB4 to detoxify the DFP (Figure 4) was dependent upon the efficiency of the sponge to decontaminate particular surfaces.

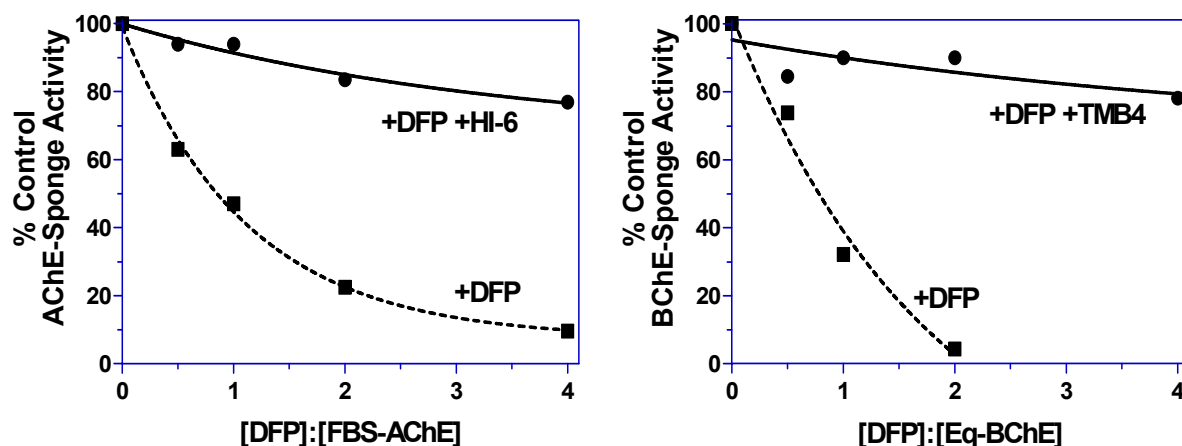


Figure 4. *Left;* Reactivation of DFP inhibited AChE immobilized enzyme by HI-6. *Right;* Reactivation of DFP inhibited BChE immobilized enzyme by the oxime TMB-4.

Characteristics of polyurethane immobilized enzymes: The longevity of sponges composed of immobilized cholinesterases is more than three years at room temperature (not shown). The immobilized enzymes are also very stable in aqueous environments. One significant difference and advantage the immobilized enzymes have compared to the soluble cholinesterases is that immobilized enzymes do not dissociate (leach) from the sponge. Therefore, the immobilized enzymes can be left in the liquid or other environments. For instance, the AChE activity in the immobilized sponge was stable for more than 60 days in continuous immersion in aqueous samples including Allegheny River fresh water or brackish water (figure 5). Since the results were identical for autoclaved and untreated water, the immobilized enzymes were also resistant to microbial induced proteolytic degradation. Also note that the same sponge was assayed multiple times over many days, so it is evident that the immobilization process confers dramatic stability to covalently coupled AChE, and that the enzymes do not leach from the polyurethane matrix.

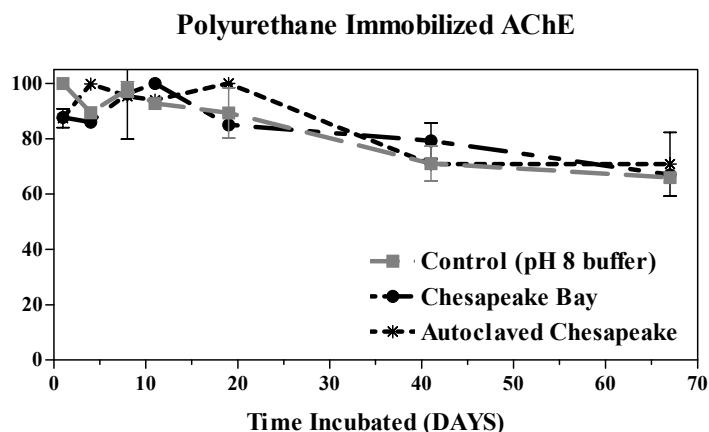


Figure 5. Polyurethane immobilized AChE is stable in a wide variety of environmental conditions and repeated use (each line represents repetitive sampling of the enzymatic activity of the sponge for more than 65 days).

Protective Ratios of the Sponge: While we were unable to modify the prepolymer since currently there is no formulation of prepolymer with an increased hydrophobic nature that might be expected to absorb the OP more effectively, we utilized several additives that provided additional ability to remove soman from the skin, protecting guinea pigs about four to five-fold better than the M291 kit. In addition, sponges were synthesized so that activated carbon would be incorporated into the polymer matrix. The addition of carbon did not interfere with the immobilization of ChEs (not shown). Sponges containing the oxime 2-PAM, or HI-6 also showed increased protection to soman skin toxicity compared to the M291 kit. Next, we evaluated the combination of oximes with the additive tetraglyme. In this decontamination treatment of GD contaminated guinea pigs using sponges containing 2-PAM and tetraglyme, the protective ratio of the sponges over the M291 kit increased to more than 7-fold, and the LD₅₀ of soman increased to 135 mg/kg (Table 1). This compares to LD₅₀ values of 9.9 and 18 mg/kg for untreated animals (not decontaminated) and the M291 kit, respectively. This combination is also effective against VX contaminated guinea pigs: 2-PAM and tetraglyme sponges yielded a protective ratio of approximately 31 for VX (Table 1), and tetraglyme and HI-6 sponges yielded a protective ratio of more than 100.

Table 1. Protective ratio of the M291 kit and sponge with additives

Additives to Sponge	GD		VX	
	LD ₅₀	PR	LD ₅₀	PR
	(mg/kg)		(mg/kg)	
HFE	55	5.6	-	-
2-PAM (oxime)	76	7.7	-	-
HI-6 (oxime)	79	8	-	-
Tetraglyme	88	8.9	-	-
2-PAM + Tetraglyme	137	13.8	3.37	24.9
HI-6 + Tetraglyme	156	15.7	15.7	112
Reference Values	-	1.8	-	-
M291 Decon Kit	17.7	-	0.14	-
OP alone	9.9	-	0.14	-

Detoxification of mustard (HD) by the sponge: The sponge was used to wipe guinea pig skin contaminated with neat mustard. The following day, the animals were injected with trypan blue. Those areas representing vesicant injury take up the dye. As observed in figure 6, the neat HD exposed tissue (positive) has a significant dye uptake, while the area decontaminated by the sponge has only a slight uptake of the dye (decon). The control (control) has no dye uptake.

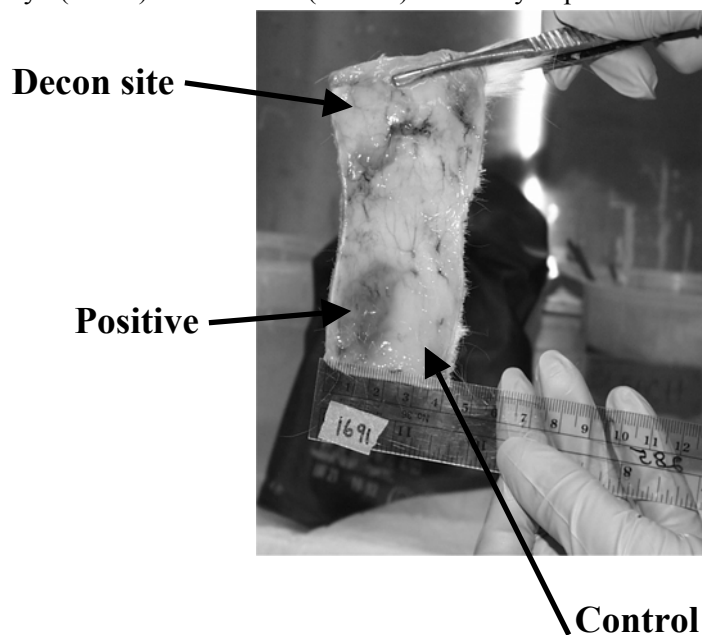


Figure 6. HD contaminated guinea pig skin decontaminated with the sponge (Decon site) showed significantly less dye uptake than the HD contaminated site which was not Decon (positive).

In addition, the amount of HD taken up and removed by the sponge was measured over time (figure 7). While neat mustard remained after 15 minutes in water, the corresponding amounts are destroyed in the matrix of the sponge and additives. Histopathology of the HD exposed skin specimens after 24h demonstrated microvesicles, coagulation at the dermal interface, and in the most severe cases, dermal coagulation (figure 8). Overall, sponge decontamination of the HD exposed area exhibited characteristics associated with reduced exposure. Thus, these sponges could reduce the damage that HD produced.

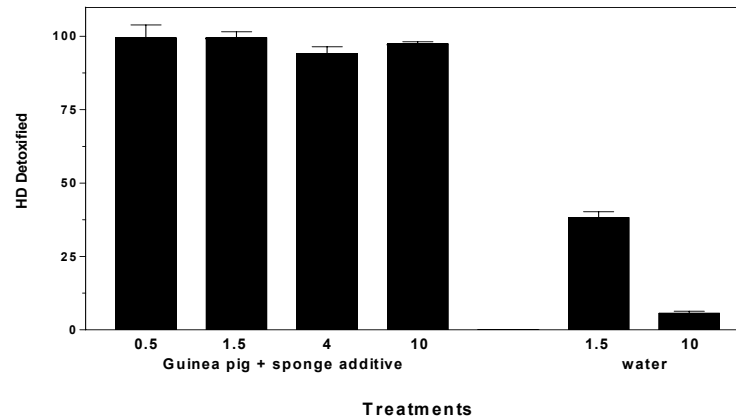


Figure 7. Guinea pigs exposed to neat HD were decontaminated with the sponge. HD in water was still detectible, while that in the sponge was detoxified.

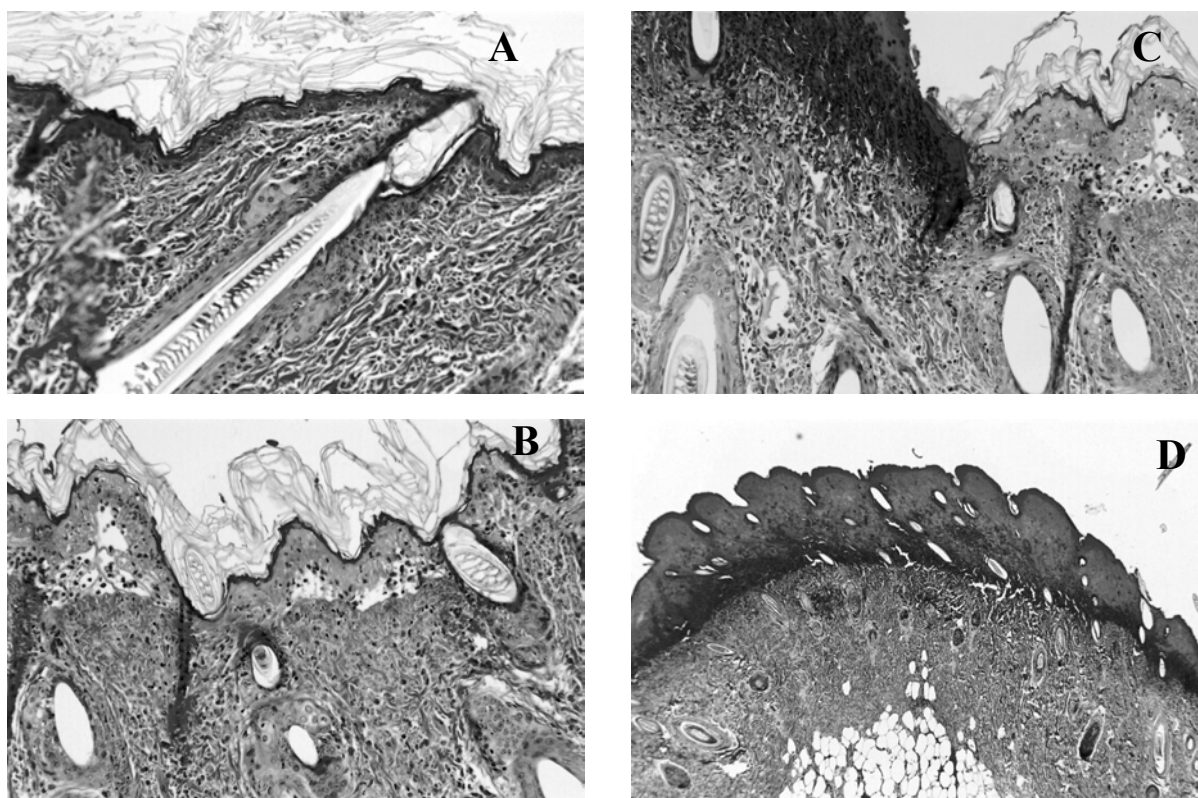


Figure 8. Normal guinea pig skin sections (A); guinea pig skin 24h after being exposed to neat HD where microvesicles can be observed (B), coagulation at the dermal interface (C), and severe coagulation (D) (Braue et.al., 1992).

CONCLUSIONS

These results demonstrate that while there are perceived limitations of the bioscavenger approach for external decontamination, the fact remains that the immobilized enzymes retain activity and stability in relevant environments, can be rapidly synthesized in a homogenous manner, and are reusable when the sponges contain cholinesterases in the presence of oximes after inhibition with the DFP or MEPQ. Another advantage of cholinesterases over OP hydrolases is that the cholinesterases are not limited in specificity to current or future OPs. Furthermore, the sponge is capable of limiting the damage induced by other chemical warfare agents, notably vesicants. Thus, we envision a reusable immobilized enzyme sponge of cholinesterases and oximes for OP and HD detoxification and decontamination.

ACKNOWLEDGEMENTS

We thank Dr. Robert Kaiser, Entropic Systems, MA, for the generous samples of HFE derivatives, and Dr. Steven Sikorski, University of Iowa, for the cholinesterase models.

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