dosing are in good agreement with water flux values found by Osborne et al (1988a) with skin soaked in chilled water for 52 h. This agreement can be attributed to the cadaver skin used in the present study being significantly hydrated before being mounted in the cell. Hydrated stratum corneum should be significantly swollen. Application of the low water microemulsion causes water to be pulled from the stratum corneum into the donor liquid. During this dehydration of stratum corneum its thickness continues to decrease until an equilibrium gives rise to a "steady-state" tissue that is less hydrated and less permeable to water and glucose than at the start of the experiment. This description is supported by a calculated lag time of 0·9 h ($r^2 = 0.999$) based on the 3–6 h collection period (Fig. 4). Note that for a constant diffusion coefficient of $4{\cdot}2 \times 10^{10}$ cm^2 s, a decrease in stratum corneum thickness from 43 μm to 23 μm results in a 1·5 h difference in lag time as calculated by the $h^2/6D$ relationship.

Conclusions

Based upon results for water and glucose, it was found that topical delivery from the microemulsions studied was highly variable and extremely dependent upon composition. While each of the three microemulsions evaluated had the same ratio of surfactant to cosurfactant, differences in water content caused the in-vitro percutaneous transport of water and glucose to vary fifteenfold and greater than thirtyfold, respectively. As hypothesized, glucose transport was achievable only from microemulsions that had water in excess of that required for amphiphile hydration as indicated by flux values greater than the steady-state flux value for pure water. Thus, while microemulsions may provide topical formulations with increased physical stability and superior drug solubility/solubilization, microemulsion compositions must be carefully optimized to achieve maximum percutaneous transport.

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Letter to the Editor

Effect of heparin sodium on limulus amoebocyte lysate preparations from two manufacturers

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The limulus amoebocyte lysate (LAL) test is included in the USP XXI (1985) as a bacterial endotoxin test (BET). However, for the test to be valid for end-product evaluation of any preparation the properties of the product itself should not invalidate the endotoxin-lysate reaction (USFDA 1987).

Inhibition of the limulus reaction by the heparin preparations was first reported by Sullivan & Watson (1975), who found that the inhibition could be overcome by the addition of calcium and sodium salts. Ronneberger (1982) found inhibition of gel formation with 50 units mL^{-1} of heparin. Heparin sodium tested at 1000 units mL^{-1} also inhibited three out of five lysates from different manufacturers (Twohy et al 1983).

During a routine procedure in this laboratory to validate the LAL test for detection of pyrogens in commercially available heparin preparations, it was found that heparin produced different effects on lysate preparations from two manufacturers (Whittaker M. A. Bioproducts (Pyrogent) and Associates of Cape Cod Inc. (Pyrotell)). While levels greater than 2·5 units mL^{-1} inhibited the Pyrogent brand lysate, no inhibitory effects were observed up to 100 units mL^{-1} tested with Pyrotell brand lysate. An attempt was made to see if the inhibitory effects of heparin on Pyrogent brand lysate could be overcome by the addition of calcium and sodium salts to the endotoxin-lysate mixture in the laboratory. The results are presented.

Control Standard Endotoxin, Lot No. L02087 obtained from Whittaker M. A. Bioproducts, Strain *Escherichia coli* 055:B5 with a defined activity of 9 EU ng^{-1} in terms of US Reference Standard Endotoxin (RSE), EC-5, was added to the heparin solutions. Details of the lysate brands used, along with their batch numbers and labelled sensitivities, are shown in the footnote to Table 1.

Serial dilutions of endotoxins bracketing the sensitivity of the lysate used were prepared in sterile water for injection (SWI) containing specified concentrations of heparin sodium. Similar dilutions of endotoxins containing no heparin formed the controls for confirmation of the labelled sensitivities of the lysates. A sample of each diluted solution (0·1 mL) was distributed into 10 × 75 mm borosilicate test tubes. A portion of the reconstituted lysate (0·1 mL) was then added to each tube

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Table 1. Effect of heparin sodium on the sensitivity of limulus amoebocyte lysate preparations from two different manufacturers.

Lysate used	Heparin sodium (units mL^{-1})	Endotoxin (EU mL^{-1})					
		0·50	0·25	0·12	0·06	0·03	0·015
Pyrogent[1]	100·0	–	–	–	–	–	ND
	50·0	–	–	–	–	–	ND
	25·0	–	–	–	–	–	ND
	10·0	–	–	–	–	–	ND
	5·0	–	–	–	–	–	ND
	2·5	+	+	+	–	–	ND
	0·0	+	+	+	–	–	ND
Pyrotell[2]	100·0	+	+	+	+	–	–
	50·0	+	+	+	+	–	–
	25·0	+	+	+	+	–	–
	10·0	+	+	+	+	–	–
	5·0	+	+	+	+	–	–
	2·5	+	+	+	+	–	–
	0·0	+	+	+	+	–	–

[1] Pyrogent brand lysate, batch number 8L0950, with labelled sensitivity of 0·12 EU mL^{-1} was used. [2] Pyrotell brand lysate, batch number 36-92-538 with labelled sensitivity of 0·06 EU mL^{-1} was used. ND = Not Determined.

with gentle mixing and the contents incubated at 37°C for 60 ± 2 min after which the tubes were examined for formation of gel. Those with a firm gel that adhered to the wall after inversion through 180° were scored as positive (+) and those with no gel or a slightly opaque solution flowing freely after inversion were scored as negative (−). Inhibition or facilitation of the reaction was determined by the decrease or increase in the sensitivity of the lysate compared with the control series. At least four replicate end-point assays were performed for each concentration of heparin and controls.

Results presented in Table 1 show that heparin in sodium produced different effects on lysates from two different manufacturers; while levels greater than 2·5 units mL^{-1} inhibited the reaction with Pyrogent brand lysate, no inhibitory effects were observed with Pyrotell brand lysate up to 100 units mL^{-1} tested. In an extension of the study it was found that when Pyrogent brand lysate was reconstituted in sterile water for injection containing 0·05 M $CaCl_2$ and 0·154 M NaCl the inhibitory effects of heparin were overcome, allowing the heparin solutions to be tested up to 3000 units mL^{-1} by both lysates (Table 2).

Variability of the product inhibitory effects with lysates from different manufacturers emphasises the necessity of validation of the LAL test and therefore tests for validity for each product have to be carried out against each type of lysate used. For pyrogen testing of Heparin preparations Pyrotell lysate appears to be the better choice. Associates of Cape Cod have confirmed that the modifications suggested by Sullivan & Watson (1975) for overcoming the inhibitory effects of heparin have been incorporated in the present formulation of Pyrotell lysate (personal communication 1988). However, results of the present study show that lysates other than Pyrotell can also be used by simple modification of the technique on the basis of the observations of Sullivan & Watson (1975). We have demonstrated this with Pyrogent lysate. When Pyrogent lysate was reconstituted with SWI containing 0·05 M $CaCl_2$ and 0·154 M NaCl the inhibitory effects of heparin were completely overcome allowing heparin to be tested at 1000-fold greater concentrations.

The mechanism of action of the inhibitory effects of heparin on limulus amoebocyte lysate and the role of calcium and sodium salts, in the removal of such effects has not been elucidated. The results presented, however, do suggest one simple method of overcoming these inhibitory effects of heparin on some lysate preparations.

Table 2. Maximum non-inhibitory concentrations of heparin in relation to lysates from different manufacturers.

Lysate used	Non-inhibitory concn (units mL^{-1})
Pyrotell	3000·00
Pyrogent	2·50
Pyrogent + 0·05 M $CaCl_2$ + 0·154 M NaCl	3000·00

Concentrations of 4000, 5000 and 6000 units mL^{-1} of heparin inhibited both Pyrotell and modified Pyrogent brand lysates.

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