A METHOD FOR THE PRESERVATION OF ESTERASE ACTIVITY IN HAIRLESS MOUSE SKIN

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Transdermal drug delivery involves the continuous administration of therapeutic molecules through the skin. It has the advantage of maintaining relatively constant drug plasma levels and improving patient compliance. Since most drugs do not penetrate intact skin in therapeutic amounts, there may be a need for either using penetration enhancers and/or making pro-drugs which are more lipid soluble and penetrate the skin more readily. The pro-drug is then subsequently metabolised and activated within the skin tissue or at a distant site such as the liver. Metabolic activity within the skin spans a broad range of oxidative, reductive, hydrolytic and conjugative reactions which make the skin a source of extrahepatic metabolism of many topically applied drugs. (Hadgraft 1985). The activity and specificity of the various enzyme systems within human skin has not been investigated as thoroughly as those in the liver. The preparation and estimation of enzyme activity is not always feasible on the same day that fresh tissue becomes available. This investigation set out to find a method of storing freshly obtained skin and assessing enzyme activities at a later date.

Male hairless mice were sacrificed, the dorsal skin was excised and subcutaneous fat removed. Cell cytosol and microsomal fractions were prepared from either fresh skin or skin that had been frozen in liquid nitrogen and stored at -70°C. Esterase activity was estimated using p-nitrophenol acetate (Spencer 1975) in fractions that were freshly prepared and that had also been stored at -15°C. The results in Table 1 indicate that there was was no significant differences in specific and total activities between freshly

TABLE 1. PNPA esterase activity in hairless mouse skin preparations (mean+/-s.d.)

Prèparation / Storage temperature	Specific activity nmole/mg protein/min		Total activity µmol/g tissue/min	
	microsomes	cell cytosol	microsomes	cell cytosol
Fresh tissue	730 +/-20	270 +/-9	1969 +/-55	3829 +/-137
Subcellular fractions at-15°C	764 +/-31	272 +/-9	2060 +/-84	3966 +/-138
Whole skin at -70°C	899 +/-42**	416 +/-20**	2830 +/-133**	6368 +-283**

** Highly significant ($p<0.01$, Unpaired 't' test)

prepared subcellular fractions and those stored at -15°C, whereas those fractions prepared from whole skin stored at -70°C were significantly higher. The results also show that specific activity (nmoles/mg protein/ min) was greater in the microsomal fraction but 69% of total activity (µmol/g tissue/min) was in the cell cytosol.

These results indicate that it may be feasible to freeze human skin at the time of removal, store it at -70°C and estimate enzyme activities at a later date.

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