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THE EFFECT OF PERFUSION RATE ON *IN VITRO* PERCUTANEOUS PENETRATION*

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ABSTRACT

This study presents data on the effect of varying dermal perfusion rates in an *in vitro* chamber on percutaneous penetration. Increasing the perfusion flow rates significantly increases the penetration rate with the compounds studied. This suggests that the data obtained in *in vitro* studies will be more meaningful when ideal flow rates are determined for such chambers and validated with *in vivo* data.

Dermal perfusion rate is rarely commented upon as a significant variable in *in vitro* experiments on percutaneous penetration. Investigators varied chamber designs; but few attempted to approximate *in vivo* dermal perfusion rates or establish what effect varying this rate had on penetration. Attempts have not been made to standardize epidermal surface conditions, dermal perfusion rates, types of perfusate, continuity of dermal perfusion flow, or stirring of perfusate. Tregear states that 10 μl/min/cm^2 is the rate beyond which increasing perfusion fails to alter pentration (1). In that paper no perfusion data were presented to validate this figure; reference was made to Feldberg and Paton's data on perfused rabbit thigh skin in 1951 (2). Review of Feldberg and Paton's experiments on perfused cat skin did not include a study of percutaneous penetration.

Data presented in this paper indicate a significant increase in penetration of the substances tested when the dermal perfusion rate is increased in the range of 2–16 μl/min/cm^2.

An appendix is included to help those who are interested in previous work done in this field.

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MATERIALS AND METHODS

Mature guinea pigs were sacrificed by intraperitoneal injection of 300 mg of pentobarbital and skinned. The pelt was pinned by the edges on a tray of wax, shaved with an Oster® animal clipper, using head #10, and carefully reshaved with head #40. The resulting skin was turned epidermal side down and repinned on a dry bed of wax. Kenwood® kerchiefs moistened with saline were laid over the subcutaneous muscle and exposed dermis to prevent desiccation while a sharp dissection was performed between the subcutaneous muscle and the dermis. Dissection was easiest over the belly and most difficult over the rump.

A thin wall brass ring 2.4 cm in diameter with smoothed edges was placed on the dermal side and a metal hose clamp was brought up and around it from beneath and tightened with a screw-driver to form a structure looking like an inverted tambourine with the dermal side forming the inside bottom of a 1.5 cm tall "cup" (Fig. 1). Scissors were used to trim away excess skin from the edges. The cup was then placed in a broom clamp attached to a rotating wheel slanted at 30° such that when the cup was filled with 2 ml of saline a portion of the dermal surface was left exposed to the air but would be washed 6 times a minute as the wheel rotated and the fluid covered and then uncovered all portions of the dermis. A Pasteur pipette was used to carefully remove all the fluid and 2 ml of fresh saline was immediately replaced in the cup when samples were taken. This method allowed a simple comparison of the effect of the frequency of changing the perfusate (and thus varving volumes of dermal perfusion).

In another method, referred to as the "drip method," a bottle of saline was hung with intravenous tubing leading to a 30 gauge hypodermic needle. Saline was allowed to drip on a cup similar to the one previously described with the exception that the skin sides were reversed. The ring was placed on the epidermal side so that the cup, when inverted, would expose the outside bottom or dermal side to saline dripped from 5 to 10 cm above it. Perfusate was allowed to run off the sides of this apparatus from which it fell to a collection funnel conducting it to a calibrated test

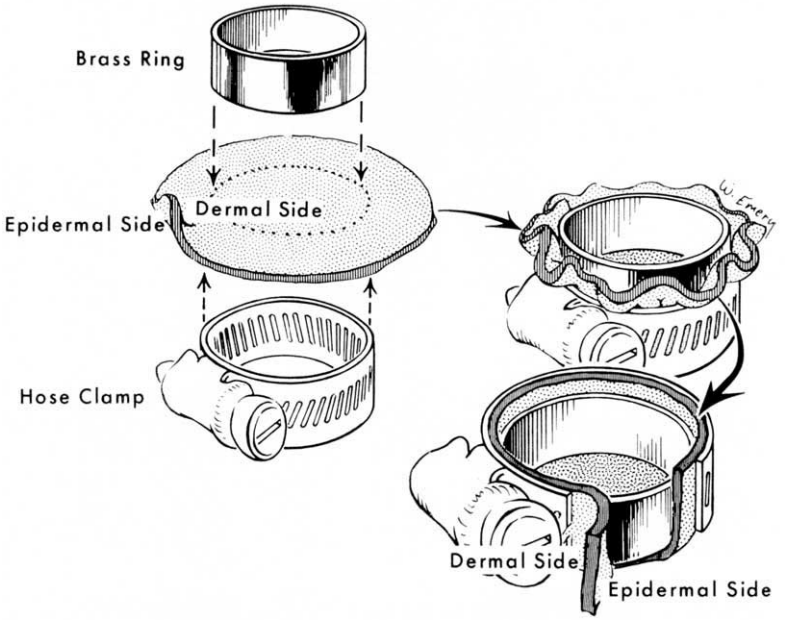

FIG. 1. Absorption cell employed in this study (see text for description)

tube for collection. Saline rate of flow was determined by raising and lowering the bottle. In this method a rate of 7–8 ml per 2 hours was used.

Application of test material to the epidermis was carried out with a 100 lambda pipette to gently and evenly spread the acetone solution containing ^{14}C-testosterone or testosterone propionate. Standards were made with the same pipette. The standard consisted of 100 μl of testosterone or testosterone propionate added to 10 ml. of methanol. One-hundred μl of this solution was added to 2 ml of saline in the Packard low potassium counting vials, and 17 ml of counting solution was added.

To determine the radioactivity of the perfusate, a 2 ml aliquot of the sample was added to 17 ml of the counting solution containing:

6% PPO (2,5-diphenyloxazole)

2:1 Baker reagent grade toluene: Filtered Rohm and Hass Triton X-100

The filtration was carried out by gravity using #1 filter paper. Radioassay was performed in a Beckman liquid scintillation system using a window of 110–300, having a counting efficiency of 56 per cent and figure of merit of 191. The figure of merit (efficiency^2/background) reached a maximum at 110–300 with a ^{14}C standard.

From the counts obtained and the volumes of recovered perfusate, percentage of sample recovery per hour was calculated by the following formula and graph:

$$\frac{\text{volume of perfusate recovered}}{\text{volume of the aliquot of perfusate}} \times \text{counts obtained from the aliquot of perfusate} = X$$

$$\frac{(x)}{\text{counts obtained from the standard}} \times \mu\text{gm of sample in the standard} \times 100 = Y$$

y = percent of applied sample recovered

RESULTS

Representative data are included in Figures 2 and 3. The perfusate was changed at intervals of from ½ to 4 hours as noted. The notation as to the "drip method" refers to a constant flow rate of 7–8 ml/2 hrs. These representative curves show the data until just prior to reaching the steady state. In most experiments sample collections continued for 4 to 8 hours after reaching a steady state.

In Figure 2, utilizing testosterone, the rate of penetration approximately doubled each time the dermal perfusate doubled (i.e. changing at 1, 2, and 4 hours). It is emphasized that this was not a continuous flow but the data may be considered roughly equivalent to 2, 4, 8, and 16 μl/min/cm^2. When a continuous flow of 2 ml/hr was employed, the penetration more than doubled. The data from testosterone penetration studies show slightly increasing penetration rates for 2 ml/hr, 1 ml/hr, and ½ ml/hr samples at 24 hours which are near their eventual steady states based on previous experiments.

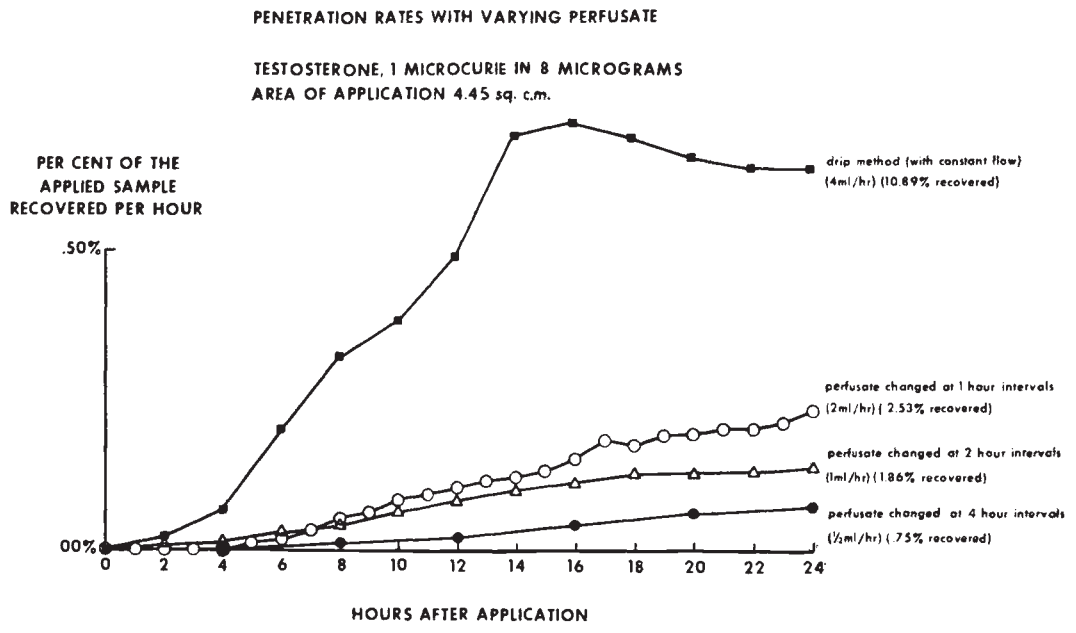

FIG. 2

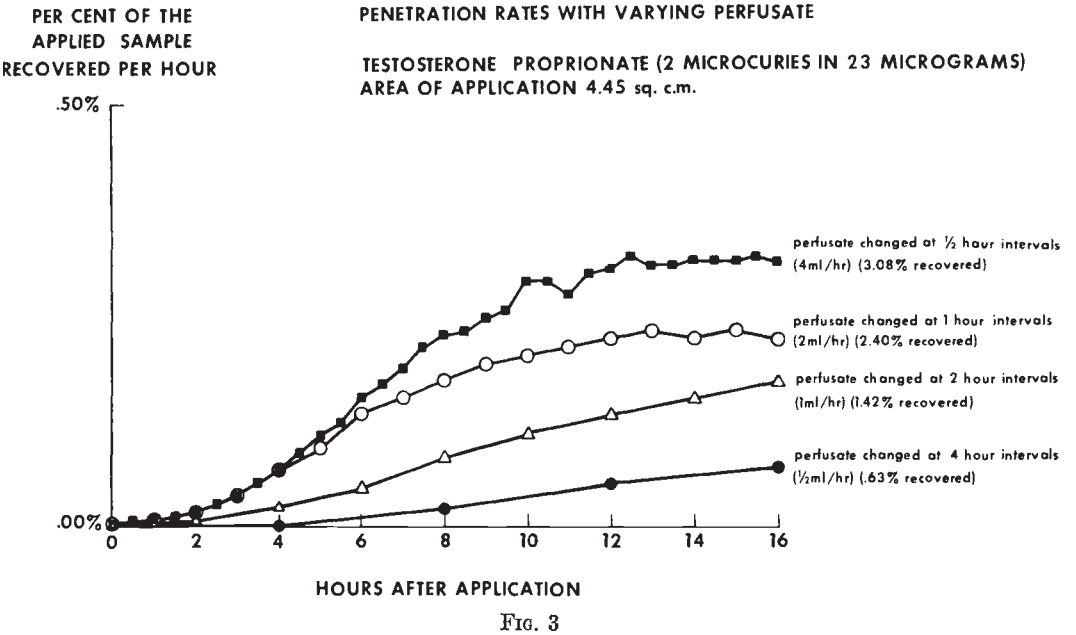

FIG. 3

Figure 3 demonstrates similar findings with testosterone propionate. Doubling the flow rate doubles the penetration rate. Testosterone propionate samples taken at 4 hour intervals reached a steady state at 16 hours and the samples changed at 2 hour intervals showed an insignificant rise in penetration rate before achieving steady state at 22 hours. The 1 and ½ hour sample changes were discontinued in their steady states at 16 hours as graphed.

During steady state, the perfusion rate continued to directly affect the recovery rate. The experiment was not carried beyond 24 hours as it was felt that the cutaneous barrier could not

be assumed to remain intact beyond this time. Time lag in reaching steady rate of penetration varied with the perfusion flow rate. This was an inverse relationship i.e., by increasing dermal perfusion rate, time lag before reaching steady state was reduced. We can not explain the mechanism involved, but suspect this phenomenon of importance in comparing *in vitro* data to *in vivo* experience where perfusion rates are high.

DISCUSSION

These data demonstrate that the rate of penetration in an *in vitro* chamber is related to the rate of dermal perfusion. We submit that the rising concentration of penetrant in the perfusate of *in vitro* chambers limits the rate of percutaneous penetration. In the physiologic situation, penetrant concentration in the dermal capillaries is insignificant, hence chamber work should strive to duplicate this with frequent perfusate changes or preferably a continuous trickle at the same rate as the normal cutaneous perfusion.

We performed similar experiments with other compounds of varying solubilities including testosterone acetate, salicylic acid, benzoic acid, hydrocortisone, butter yellow, hexachlorophene, urea and thiourea; always the same dependence on perfusion rate occurred. These compounds span a wide range of solubility characteristics. Hence, any experiment in which perfusion rates are not strictly controlled hour by hour and from sample to sample may have artifact introduced into its results. We do not feel that the dependence of penetration *in vitro* on perfusion rate precludes the validity of *in vitro* studies. It does suggest the necessity for obtaining background data on perfusion rates that may yield results comparable to the *in vivo* situation.

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APPENDIX I

Summary of *In Vitro* Penetration Studies

Author	Skin	Area cm^2	Perfusate	Amt. of perfusate	Constant flow	Stirred	Epidermal surface
Ainsworth, M.	Rabbit	3.5 at max.	Ringer's	5 ml./hr. at max.	yes	yes, teflon bar	dry with one drop penetrant
Blank, I. H. (A.)	Human	3.0	.85% NaCl	2–3 ml./hr.	yes	no	wet with penetrant reservoir
Blank, I. H. (B.)	Human	3.0	.85% NaCl	2–3 ml.	no	yes	wet with penetrant reservoir
Cronin, E. and Stoughton, R. B.	Human	23.8	.9% NaCl	7 ml.	no	no	dry with one drop penetrant
Elfbaum, S. G. and Laden, K.	Guinea Pig	3.0	Buffer	15 ml.	no	no	wet with penetrant reservoir
Fritsch, W. C. and Stoughton, R. B.	Human	.78	.9% NaCl and .9% benzyl alcohol	10 ml.	no	no	dry with one drop penetrant
Garby, L. and Linderholm, H.	Frog	7.1	Ringer's	8 ml.	yes	no	wet with penetrant reservoir
Linderholm, H.	Frog	7.1	Ringer's	8 ml.	yes	no	wet with penetrant reservoir
Macey, R. I. and Meyers, S.	Frog	2.5	Ringer's with SO_4 & Mannitol	5 ml.	no	no	wet with penetrant reservoir
Mali, J. W. H., Van Kooten, W. J. and Van Neer, F. C. J.	Human	7.1	HOH	400 ml.	no	no	wet with penetrant reservoir
Mali, J. W. H., Van Kooten, W. J., Van Neer, F. C. J. and Spruit, D.	Human	8	1% NaCl	50 ml./2 days	no	no	wet with penetrant reservoir
Marzulli, F. N.	Human	.2	.9% NaCl	10–20 ml./hr.	yes	no	dry with one drop penetrant
Marzulli, F. N., Callahan, J. F. and Brown, D. W.	Human	.2	.9% NaCl	10–20 ml./hr.	yes	no	dry with one drop penetrant
Munro, D. D. and Stoughton, R. B.	Human	.78	.9% NaCl and .9% benzyl alcohol	10 ml.	no	no	dry with one drop penetrant
Scheuplein, R. J.	Human	not given	not given	not given	no	yes, teflon bar	wet with penetrant reservoir
Scheuplein, R. J. and Blank, I. H.	Human	not given	not given	not given	no	yes, teflon bar	wet with penetrant reservoir
Sweeney, T. M., Downes, A. M. and Matoltsy, A. G.	Hairless mice	.71	.9% NaCl	not given	yes	no	wet with penetrant reservoir

APPENDIX I—*Continued*

Author	Skin	Area cm^2	Perfusate	Amt. of perfusate	Constant flow	Stirred	Epidermal surface
Tregear, R. T.	Rabbit	not given	Ringer's	at least 10 μl/ cm^2/ min.	yes	not given	dry with one drop penetrant
Tregear, R. T.	Rabbit & human	3.5(?)	.9% NaCl	not given	yes	no	wet with penetrant reservoir
Treherne, J. E.	Rabbit	not given	Ringer's	8 ml	no	yes	wet with penetrant reservoir
Van Kooten, W. J. and Mali, J. W. H.	Human				no	no	wet with penetrant reservoir

This summarizes the pertinent experimental variables.