Permeability of Excised Human Keratin to Lipid-Soluble Substances II

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Data are presented which compare the rate of penetration through keratin tissue of 2-hydroxystearic acid and three mixtures composed mainly of lipid-soluble esters, with oleyl alcohol. The hydroxy acid which was strongly adsorbed by keratin had a very slow rate of penetration. The ester mixture with the lowest acid number showed rapid penetration. The mathematical interpretation of the data indicated that the transport occurred by a diffusional process.

A PREVIOUS study has shown that certain lipid-soluble substances were able to penetrate sections of excised human callous tissue (1). It was also observed that substances such as linolenic and linoleic acids which were adsorbed by human keratin from *n*-heptane solutions manifested relatively slow permeability rates in sections of this tissue. This paper is a continuation of that study, and contains permeability data on a hydroxy fatty acid and three different lipid-soluble mixtures containing esters as the primary component.

Since it can be shown that the distribution coefficient (2) can affect the rate of penetration, the influence of this factor was also investigated.

EXPERIMENTAL

Procedure.—The apparatus, including the diffusion cells and the method employed to follow the permeability rate of the various lipid-soluble substances in human keratin tissue, were essentially the same as described in a previous paper (1). The preparation of the keratin tissue sections and the assay for oleyl alcohol were also the same.

The 2-hydroxystearic acid concentration in each side of the diffusion cell was determined by titrating with a standard solution of 0.001 *N* sodium hydroxide in *n*-butanol with a microburet under an atmosphere of nitrogen using *p*-naphtholbenzein as the indicator. The initial concentration of the acid was 1.0×10^{-6} moles per ml. Use of this low concentration was necessitated due to the low solubility of the acid in *n*-heptane. The reference penetrant, oleyl alcohol, was used in the same concentration in this case.

The lipid-soluble mixtures (liquid lanolin Nos. 1, 2, and 3), consisting mainly of esters (3, 4), were assayed as previously described (1) by the hydroxylamine test using 2 ml. of the *n*-heptane solution and 3 ml. of ether. The concentration of the mixtures and the oleyl alcohol in this case was based on mg./ml. (from 5.26 to 6.29 mg./ml.) rather than on a molar basis. This, of course, was necessary since these penetrants were composed mainly of a mixture of esters rather than a pure component. Naturally, minor concentrations of other components such as acids and alcohols were also present in the mixtures.

Distribution coefficients were determined according to the following method: Five grams of powdered (200 mesh) ether-extracted keratin tissue was placed in a glass-stoppered vessel; a measured volume (100 ml.) of a *n*-heptane solution of the lipid-soluble test substance was then added, and the mixture was continuously agitated and allowed to reach equilibrium at 25°. Samples of the clear *n*-heptane solution were subsequently removed and assayed according to the described procedure for each agent.

Acid numbers were determined for each lipid-soluble mixture according to the U.S.P. XVI procedure.

RESULTS AND DISCUSSION

The results of the permeability studies again showed a wide variation in the permeability coefficients for the callous tissue from different individuals to a specific penetrant. Therefore, as previously explained, each penetrant and oleyl alcohol was tested against alternate layers from the same piece of tissue (1). The permeability coefficients, K, are average values and were calculated according to the equation (5),

$$\log (C_o - 2C_b) = (-2K/2.303)t + \log C_o \quad \text{(Eq. 1)}$$

where C_o is the initial concentration of solution to which the keratin barrier was exposed and C_b is the concentration at the time, t, on the other side of the barrier in the diffusion cell.

TABLE I.—RATIO OF THE PERMEABILITY COEFFICIENTS OF VARIOUS TEST PENETRANTS TO THAT OF THE REFERENCE MATERIAL, OLEYL ALCOHOL

Test Penetrant	$\frac{K \text{ (test)}}{K \text{ (oleyl)}}$
Cholesteryl acetate	0.942
Cholesteryl oleate	0.298
Oleic acid	0.476
Linolenic acid	0.157
Linoleic acid	0.039
2-Hydroxystearic acid	0.028
Liquid lanolin No. 1	1.235
Liquid lanolin No. 2	2.029
Liquid lanolin No. 3	0.767

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Since biological variation makes it impossible to directly compare permeability constants of various penetrants tested on different tissue sections, oleyl alcohol was again used as a reference. By calculating the ratio of the permeability coefficient for the test penetrant to that obtained for oleyl alcohol on the alternate tissue layers, the values obtained for each agent were reduced to a common basis. In Table I the ratios for various agents investigated previously (1) and those included in this study are shown.

Obviously, those substances in the table having the larger values have greater permeability coefficients as a result of their more rapid penetration rates in keratin tissue. It can also be observed, as previously reported (1), that some substances which are strongly adsorbed by keratin have low penetration rates. Thus, 2-hydroxystearic acid was the most strongly adsorbed of all the substances tested (6) and had the lowest permeability rate.

In the case of the various liquid lanolin preparations tested, these, too, appear to follow the same pattern. The liquid lanolin No. 1 sample had an acid number of 1.69. The No. 2 liquid lanolin sample was characterized by its low hydroxyl content and an acid number of 1.17, whereas the No. 3 liquid lanolin had an acid number of 6.79. Since wool fat contains both unsaturated and 2-hydroxy acids (7), it is reasonable to expect that the No. 2 liquid lanolin would have the greatest, and No. 3 the smallest, permeability coefficient of these three mixtures.

Although the relative position of each of the three mixtures is no doubt correct, an additional theoretical point should be mentioned. It was necessary, for reasons already given, to follow the change in concentration of the lanolin mixtures on a weight per unit volume basis and thus, the Beer's plot which was employed to follow the change in concentration with time was constructed on a similar basis. However, the material diffusing through the keratin membrane could differ from the composition of the mixture. Since the intensity of the color produced in the assay is directly proportional to the ester concentration, the results could be misleading. For example, if the material diffusing through the membrane contained a higher concentration of esters or a larger fraction of the lower molecular weight esters than an equivalent weight of the entire mixture, the absorbance would be greater. This would then show an apparent fast rate of penetration for the mixture.

In the derivation of the previous equation, the permeability coefficient is shown to be equal to several constants

$$K = \frac{AD\,(DC)}{VL} \qquad \text{(Eq. 2)}$$

where K is the permeability coefficient, A is the cross-sectional area, D is the diffusion coefficient, DC is the distribution coefficient, V is the volume, and L is the length of the diffusional pathway. Thus, if the quantity, AD/VL is constant, the permeability coefficient should vary directly with the distribution coefficient. In Table II, the apparent distribution coefficients between powdered keratin and n-heptane for several lipid-soluble substances are shown.

In the case where the penetrating molecule is soluble in a liquid or semisolid medium in contact with a similar medium it can be shown that the permeability coefficient is directly proportional to the distribution coefficient (2). However, in the case where a liquid is in contact with a solid and the solute is strongly adsorbed by the solid, such as in the case of the powdered keratin-n-heptane systems employed in this study, the above relationship was not found to exist.

Microscopic examination of the sections of callous tissue showed them to be free of orifices through which the penetrant could stream (8). Also, the two sides of the permeability cell did not reach equilibrium even after being agitated for 10 days. Thus, the experiment and the mathematical interpretation of the data indicated that a true diffusional process occurred. This lends support to the theory that transepidermal penetration, at least through the stratum corneum, can occur.

TABLE II.—DISTRIBUTION COEFFICIENTS OF VARIOUS AGENTS BETWEEN POWDERED EXCISED HUMAN KERATIN AND NORMAL HEPTANE

Test Agent	Distribution Coefficient
Oleyl alcohol	0.40
Cholesteryl acetate	0.63
Cholesteryl oleate	0.64
Oleic acid	1.72
Linolenic acid	1.19
Linoleic acid	0.58
2-Hydroxystearic acid	36.00
Liquid lanolin No. 1	0.53

SUMMARY

The penetration rates in excised human callous tissue of 2-hydroxystearic acid and three liquid lanolin preparations were determined.

The 2-hydroxystearic acid which was strongly adsorbed by human keratin showed the lowest permeability coefficient of all the substances tested.

The acid numbers of the three liquid lanolin preparations were also determined. The liquid lanolin characterized by the lowest acid number and low hydroxyl content showed the most rapid penetration rate. The preparation having the highest acid number showed the slowest penetration rate of the three mixtures.

The apparent distribution coefficients for several lipid-soluble substances in a powdered keratin-n-heptane system were determined.

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