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The diffusion of nandrolone through occluded and non-occluded human skin

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Diffusion constants have been measured for nandrolone in human cadaver skin *in vitro* under conditions which approximate to both the occluded and non-occluded topical therapeutic situation. The results suggest that occlusion affects the diffusion parameters only marginally, if at all, but appears to reduce the ability of the stratum corneum to 'irreversibly' bind a fraction of the applied dose. Studies of nandrolone diffusion in freshly excised human epidermis yield similar results to those from measurements on cadaver skin, and it has also been found that epidermal samples excised from non-involved sites of human psoriatic patients behave under these conditions in essentially the same way as normal epidermis.

In previous work (Foreman & Kelly, 1976) the diffusion of nandrolone through human abdominal cadaver skin was examined using a technique whereby the skin sample is maintained between an effectively infinite solvent sink and a suitable donor solution. Under these conditions, when water is the solvent used, or at least the major component of the solution, the skin rapidly becomes extensively hydrated, and the results obtained can only relate, at best, to the therapeutic situation of rigorous occlusion. An alternative method has however been described (Foreman, Kelly & Lukowiecki, 1977) whereby the skin sample is maintained with the dermal face in contact with a sink solution, whilst the epidermal surface is open to the ambient atmosphere, the diffusant being applied as a thin surface film to this face. Such an arrangement relates to the non-occluded, topical therapeutic situation. The occluded state may also be simulated using this approach by simply covering the skin within the diffusion cell with a water-impermeable sheet. It therefore becomes possible, in principle, to study the effects of skin hydration on the diffusion parameters of selected molecules. The method is novel, however, and by no means well proven. We have therefore used the technique to study the diffusion of nandrolone in occluded and non-occluded human cadaver skin and have compared the results with those from the more conventional method. In this way it is hoped to establish a body of data by which the value of the method may be assessed, and to extend current understanding of the nature of the occlusive effect on skin penetration. Measurements were also made on freshly excised human epidermis maintained viable, so far as possible, over a suitable culture medium in order to throw further light on the vexed question of the relevance of *in vitro* studies to the *in vivo* situation.

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MATERIALS AND METHODS

Samples of human abdominal cadaver skin were prepared as described previously and stored at −4° until used.

Sheets of freshly excised epidermis, approximately 5 × 1·5 cm were obtained from human donors by keratotome excision from the outer surface of the thigh. Eagles M.E.M. (Wellcome) was prepared immediately before use, streptomycin (250 mg) and benzylpenicillin (150 mg) were added to each 100 ml and the solution was buffered with CO_2/O_2.

The diffusion cells were as shown in Fig. 1. The fragile samples of freshly excised epidermis were supported by placing them on a glass grid mounted

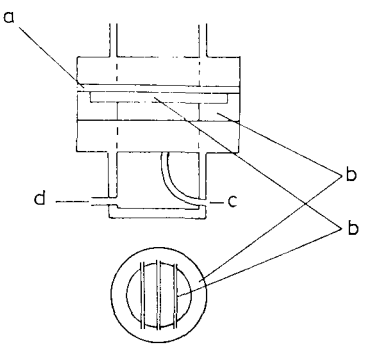

FIG. 1. Glass diffusion cells in cross-section. (a)—Skin sample. (b)—Telfon washer with recessed glass supports. (c)—Solvent in. (d)—Sink solution out.

on a Teflon washer as shown, the cell being held secure by brass clamps. The cadaver skin samples were mounted in the normal way. With the freshly excised samples the diffusion study commenced not more than 15 min after excision, the samples being stored over Eagles M.E.M. during the intervening period.

In each case, nandrolone [4-^{14}C] (sp. act. 40 mCi m mol^{-1}) was applied as a thin film to the skin surface in 0·05 mM ethanol solution. Approximately 0·05 ml was applied in each case, dispersed over the surface with a fine hair brush, and the solvent allowed to evaporate. At suitable intervals thereafter the sink solution was washed out of the cell with fresh solution, and the radioactivity present estimated by conventional scintillation counting techniques. Where necessary, occluded conditions were simulated by covering the skin with a water impermeable plastic sheet leaving a small air space between the skin and the sheets. In other respects the experimental techniques and methods of data analysis were as described by Foreman & others, (1977).

RESULTS AND DISCUSSION

The data for stripped cadaver skin under non-occluded and occluded conditions are quoted in Table 1a and b respectively; 25% ethanol was used as the sink solvent. The diffusion constant for stripped skin is taken to be the value appropriate to the viable epidermal and dermal layers, which are considered to comprise a single homogeneous layer (ed) so far as diffusion is concerned, and denoted D_{ed}. It can be seen that occlusion has no significant effect on D_{ed}, which is consistent with the currently held view that occlusion exerts an effect primarily on the stratum corneum.

Surprisingly, occlusion also appears not to have any effect on the measured diffusion constants for the whole skin samples, which can also be seen from these Tables. However, whole skin (ws) is not a homogeneous entity, and it is better to obtain some measure of diffusion in the stratum corneum (sc) *per se*. It was earlier argued (Foreman & Kelly, 1976) that, for the case of nandrolone, equation 1 holds true, where each resistance, R_l, is defined as L_l/D_l, L_l being the thickness of the skin sample:

$$R_{ws} = R_{sc} + R_{ed} \quad .. \quad .. \quad (1)$$

and therefore, in the context of the data of Table 1a, b,

$$\bar{R}_{sc} = \bar{R}_{ws} - \frac{\bar{L}_{ed}}{\bar{D}_{ed}} \quad .. \quad .. \quad (2)$$

For the non-occluded state, $\bar{L}_{ed} = \bar{L}_{ws} - \bar{L}_{sc}$ and it is reasonable to take $\bar{L}_{sc} = 0{\cdot}001$ cm (Scheuplein, 1972) for the subsequent calculations. In most cases, therefore $\bar{L}_{sc}$ is negligible in comparison to $\bar{L}_{ws}$. However, this is not so for the freshly excised samples reported in Table 4, which mostly comprise the epidermis alone, and $\bar{L}_{sc}$ is therefore 5–10% of the total skin thickness. To avoid possible confusion, therefore, this term is included in the calculations throughout.

The same value for $\bar{L}_{sc}$ may also be used for the estimation of $\bar{L}_{ed}$ in the occluded state, provided that $\bar{L}_{ws}$ is measured for each sample before hydration takes place. After the sample becomes hydrated the stratum corneum will swell to many times its original thickness. The thickness of the viable epidermis and dermis will not however be affected to the same degree. By taking the measurement of L_{ws} before hydration takes place, errors which may arise due to swelling of the stratum corneum are avoided, and a reasonable approximation to $\bar{L}_{ed}$ is obtained for each experiment.

In this way, using the data of Table 1a and 1b, $\bar{R}_{sc} = 3{\cdot}6 \pm 1{\cdot}6 \times 10^6$ s cm^{-1} for the non-occluded state, and similarly $\bar{R}_{sc} = 1{\cdot}5 \pm 0{\cdot}9 \times 10^6$ s cm^{-1} for the occluded state. The stratum corneum does swell appreciably when hydrated, although the time taken to attain its equilibrium thickness under the present conditions is negligible in comparison with the whole time course of the experiment. However, it is not practical at this stage to measure L_{sc} during the experimental run and it is therefore not possible to obtain values of D_{sc} under occlusive conditions by this method. It should be noted, however, that $\bar{R}_{sc}$ is not significantly affected by occlusion. Since the stratum corneum thickness is known to increase when hydrated, it follows that D_{sc} is increased by occlusion, but that, for the case of nandrolone, this is countered by a corresponding increase in the stratum corneum thickness, the measured resistance R_{sc} therefore remains the same. It is perhaps instructive to compare the present results with those obtained earlier using a modification of the more conventional 'lag-time' approach. The two sets of data are therefore collected below.

Present method, non-occluded:

$\bar{D}_{ws} = 3{\cdot}6 \pm 0{\cdot}7 \times 10^{-8}$ cm^2 s^{-1}

$\bar{R}_{sc} = 3{\cdot}6 \pm 1{\cdot}2 \times 10^6$ s cm^{-1}

$\bar{D}_{ed} = 7{\cdot}4 \pm 0{\cdot}8 \times 10^{-8}$ cm^2 s^{-1}

Table 1. *Diffusion constants (D), skin thickness (L) and resistance to diffusion (R) for nandrolone in human abdominal cadaver skin at 22 ± 2°.* Sink solvent: 25% ethanol in water. Values on each horizontal line relate to skin from a single donor.

Whole skin				Stripped skin		
D_{ws} (10^{-8}cm^{2}s^{-1})	L_{ws} (cm)	R_{ws} (10^6s cm^{-1})	100 M/M_{true} (%)	D_{ed} (10^{-8}cm^{2}s^{-1})	L_{ed} (cm)	100 M/M_{true} (%)
(a) Skin open to ambient atmosphere						
2·69	0·138	5·13	29	6·19	0·140	45
3·81	0·130	3·41	67	4·58	0·127	63
1·77	0·121	6·84	12	6·50	0·129	51
2·37	0·155	6·54	12			
0·63	0·079	12·54	62			
0·81	0·086	10·62	25			
2·03	0·119	5·86	—	5·06	0·091	
5·37	0·148	2·76	—	11·30	0·117	
3·48	0·133	3·82	—	7·46	0·121	
8·10	0·143	1·77	—			
3·88	0·147	2·96	—			
7·67	0·115	1·50	—			
				8·23	0·134	
				10·55	0·143	
				6·35	0·105	
Mean values: $\bar{L}_{ws}$ = 0·126±0·006 cm $\bar{R}_{ws}$ = 5·3±1·0 × 10^6s cm^{-1} (100 M/M_{true}) = 35±9% $\bar{D}_{ws}$ = 3·6±0·7 × 10^{-8} cm^2 s^{-1}				$\bar{D}_{ed}$ = 7·4±0·8 × 10^{-8} cm^{2}s^{-1} (100 M/M_{true}) = 53±5%		
(b) Skin covered with occlusive film						
11·20	0·189	1·69	66	23·70	0·189	80
3·81	0·155	4·07	83	6·10	0·155	80
2·56	0·139	5·43	107	9·99	0·152	95
5·33	0·113	2·12	—	7·08	0·100	
5·98	0·142	2·37	82	7·05	0·155	83
7·41	0·138	1·86	83	9·16	0·146	81
4·29	0·142	3·31	62	7·25	0·128	85
Mean values: $\bar{L}_{ws}$ = 0·145±0·009 cm $\bar{R}_{ws}$ = 3·0±0·5 × 10^6 s cm^{-1} (100 M/M_{true}) = 81±7% $\bar{D}_{ws}$ = 5·8±1·1 × 10^{-8} cm^2 s^{-1}				$\bar{D}_{ed}$ = 10·0±2·3 × 10^{-8} cm^{2}s^{-1} (100 M/M_{true}) = 84±2%		

* See text for discussion of M/M_{true}.

occluded:

$\bar{D}_{ws} = 5{\cdot}8 \pm 1{\cdot}1 \times 10^{-8}$ cm^2 s^{-1}

$\bar{R}_{sc} = 1{\cdot}5 \pm 0{\cdot}9 \times 10^6$ scm^{-1}

$\bar{D}_{ed} = 10{\cdot}0 \pm 2{\cdot}3 \times 10^{-8}$ cm^2 s^{-1}

Foreman & Kelly (1976):

$\bar{D}_{ws} = 3{\cdot}9 \pm 0{\cdot}8 \times 10^{-8}$ cm^2 s^{-1}

$\bar{R}_{sc} = 3{\cdot}4 \pm 0{\cdot}4 \times 10^6$ scm^{-1}

$\bar{D}_{ed} = 29 \pm 3 \times 10^{-8}$ cm^2 s^{-1}

It seems from this comparison therefore that the $\bar{D}_{ws}$ and $\bar{R}_{sc}$ values previously observed are consistent with the present values. The value of $\bar{D}_{ed}$, however, is higher than expected and may be a consequence of the much higher degree of saturation of the skin samples with the solvent used in the earlier study. We therefore checked the possibility that occlusion does increase the diffusion constant, albeit slightly, but that the experimental scatter of the measurements in Table 1 is tending to obscure the effect. Four further skin samples from different donors were therefore taken, divided into four, and each sample studied, stripped and whole, under occluded and non-occluded conditions. The data from the experiment are given in Table 2. (These data were also included in Table 1 to obtain the overall mean values).

From Table 2, therefore, it is possible to amplify the conclusions drawn so far. For skin from each donor it does seem that occlusion increases slightly

Table 2. *Diffusion parameters for nandrolone in human abdominal cadaver skin at 22 ± 2°.* Sink solution: 25% ethanol in water. Values on each horizontal row of the Table relate to skin from a single donor.

Occluded				Non-occluded			
D_{ws} ($10^{-8}cm^2s^{-1}$)	100 M/M_{true} (%)	D_{ed} ($10^{-8}cm^2s^{-1}$)	100 M/M_{true} (%)	D_{ws} ($10^{-8}cm^2s^{-1}$)	100 M/M_{true} (%)	D_{ed} ($10^{-8}cm^2s^{-1}$)	100 M/M_{true} (%)
5·33	—	7·08	—	2·03	—	5·06	—
5·98	82	7·05	83	2·69	29	6·19	45
7·41	83	9·16	81	3·81	67	4·58	63
4·29	62	7·25	85	1·77	12	6·50	51

the diffusion constant for both the viable epidermis and dermis, and whole skin, and by implication, therefore, reduces the resistance to diffusion of the stratum corneum.

However, there is a considerable body of evidence to show that occluding the skin greatly enhances the penetration of most topically applied materials. The evidence from the present studies, on the other hand, seems to suggest that occlusion does not markedly affect the diffusion constant for nandrolone, which implies that some factor, other than the measured diffusion constant, is responsible for this phenomenon. Nandrolone may be a special case but further work in these laboratories on a variety of steroidal and non-steroidal molecules suggest the result may be more general.

The basis for the experimental approach used here has been described by Foreman & others (1977) and it was there stated that the method allows the diffusion constant D to be determined, and also an estimate of the dose of material applied to the skin, here denoted M. This quantity is, in principle, the quantity of diffusing material which would penetrate to the sink if the experiment were allowed to continue for an indefinite period of time. In some of the experiments reported here, the true dose of material applied to the skin was determined by digesting the skin sample and measuring directly the material remaining in the skin, and adding this to the total which had penetrated to the sink during the experiment. This quantity is denoted M_{true}. In Tables 1 and 2 the value of M/M_{true} is quoted as a percentage for those cases where this ratio was determined. A number of points become apparent. Firstly, for whole skin at least, the value of M/M_{true} is always less than 100%, the effect being illustrated in Fig. 2. Further, from Table 1 it seems that the value of M/M_{true} increases if the sample is occluded, whether for whole or stripped skin, and that the lowest value obtains for non-occluded whole skin. The problem is increased by the variable results observed from sample to sample, but the general trend seems clear, particularly from a consideration of Table 2.

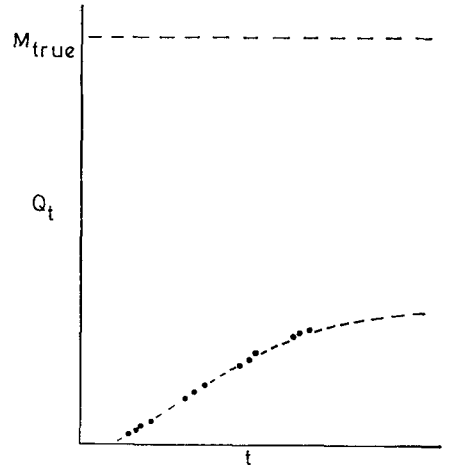

FIG. 2. Plot of nandrolone diffusion through whole cadaver skin under non-occluded conditions. Theoretical curve is shown as a dashed line tending to a limit well below the dose applied.

It seems, therefore, that the diffusional behaviour of nandrolone in cadaver skin may be defined by two parameters, the diffusion constant, D, and the fraction of the dose which is involved in the diffusion process, M/M_{true}, and that it is the latter quantity which is most affected by occlusion. Why only a fraction of the dose appears to diffuse through the skin is not clear. It seems possible that the stratum corneum is 'irreversibly' binding part of the applied dose, although the viable epidermis and dermis also seem to retain part of the dose. Certainly such an explanation is consistent with current understanding of the role of the stratum corneum, and in particular the so-called 'reservoir effect' (Vickers, 1972).

It could also be that the observation is an artifact of the experiment, possibly due to neglect of surface phenomena when the material is applied to the skin, although it is not easy to explain the effect of occlusion or stripping in these terms.

The next stage of this present study involved the measurement of the diffusion of nandrolone through freshly excised human epidermis, maintained viable so far as possible over buffered (CO_2/O_2) Eagles M.E.M. in order to investigate the relevance of *in vitro* studies to the *in vivo* state. As a first stage, therefore, a series of measurements was made for cadaver skin using Eagles M.E.M. as the sink solution, to ensure that changing the sink from 25% ethanol to Eagles M.E.M. did not significantly affect the results obtained. The results of this study are shown in Table 3. Comparison with Table 1a shows generally good agreement with the exception of M/M_{true} for the stripped skin samples.

Freshly excised human epidermis was therefore taken from the outer thigh of volunteer donors, transferred rapidly to a solution of Eagles M.E.M. and the diffusion study begun as soon as possible after excision. Samples of epidermis from non-involved sites of persons suffering from psoriasis were also included in the study, in order to determine whether or not any impairment of the barrier function of such skin sites could be detected. Only a limited number of samples were available, these were studied under non-occluded conditions. It was not possible to study stripped samples in this instance. The data obtained are quoted in Table 4.

Table 3. *Diffusion parameters for nandrolone in human abdominal cadaver skin at 22 ± 2°.* Sink solution: buffered Eagles M.E.M. Non-occluded conditions. Data on each horizontal line of the Table relates to skin from a single donor.

Whole skin				Stripped skin		
D_{ws} ($10^{-8}cm^2s^{-1}$)	L_{ws} (cm)	R_{ws} ($10^6 s\ cm^{-1}$)	100 M/M_{true} (%)	D_{ed} ($10^{-8}cm^2s^{-1}$)	L_{ed} (cm)	100 M/M_{true} (%)
5·89	0·100	1·72	—			
4·16	0·155	3·69	—			
3·87	0·138	3·54	38			
1·70	0·088	5·18	35			
2·88	0·159	5·52	39	8·62	0·176	76
0·83	0·080	9·64	61	5·71	0·087	87
0·61	0·087	14·26	49	7·89	0·092	87

Mean values: $\bar{L}_{ws} = 0{\cdot}115 \pm 0{\cdot}013$ cm
$\bar{R}_{ws} = 6{\cdot}2 \pm 1{\cdot}6 \times 10^6$ s cm^{-1}
$(100\ M/M_{true})_{ws} = 44 \pm 5\%$
$\bar{D}_{ws} = 2{\cdot}9 \pm 0{\cdot}7 \times 10^{-8}\ cm^2s^{-1}$
$\bar{D}_{ed} = 7{\cdot}4 \pm 0{\cdot}9 \times 10^{-8}\ cm^2s^{-1}$
$(100\ M/M_{true})_{ed} = 83 \pm 4\%$
$\bar{R}_{sc} = 4{\cdot}7 \pm 0{\cdot}8 \times 10^6$ s cm^{-1} *

* $\bar{R}_{sc}$ calculated using equation 1 and the method described earlier in the text.

Table 4. *Diffusion parameters for nandrolone in freshly excised human epidermis* (outer thigh) at 22 ± 2°. Sink solution: buffered Eagles M.E.M.

Source	D_{ws} ($10^{-9}cm^2s^{-1}$)	L_{ws} (cm)	R_{ws} ($10^6 s\ cm^{-1}$)	100 M/M_{true} (%)	$R_{ws\ calc}$ ($10^6 s\ cm^{-1}$)
(a) Normal					
M	3·22	0·011	3·42	40	2·0 ± 0·4
F	3·65	0·021	5·75	22	2·2 ± 0·4
(b) Uninvolved psoriatic					
M	4·00	0·021	5·25	39	2·2 ± 0·4
M	1·20	0·015	12·50	10	2·1 ± 0·4
M	5·85	0·019	3·25	24	2·1 ± 0·4

M—male, F—female.

In general, the diffusion constants are lower than previously observed. This is largely because keratotome excision removes the epidermis alone, with virtually no dermis attached, as is evident from the quoted figures for L_{ws}. The stratum corneum therefore comprises a greater fraction of the thickness of the skin sample, and the apparent diffusion constant is thereby decreased. It is possible to compensate for this effect to some degree by using the values of Table 1a to calculate the resistance to diffusion of each epidermal sample, using equation 1, these figures being quoted in the final column of Table 4. In each case, the observed resistance to diffusion is higher than predicted from the results of the cadaver skin studies, but not markedly so, and, bearing in mind the inherent approximations used, the variability amongst the samples and the fact that it was necessary to compare samples from different areas (thigh and abdomen), the agreement seems reasonable. There is also no very obvious difference between the behaviour of the non-involved psoriatic epidermis and the normal samples.

The skin samples in this last experiment could not be considered viable throughout the whole period of the experiment (about 72 h). In no case studied, however, was there any indication, judged from the diffusion curves, of a progressive change which might reflect the loss of viability of the sample. Other studies, to be reported elsewhere, also suggest that metabolism of nandrolone by freshly excised human epidermis is relatively slight. It is therefore concluded that, for nandrolone at least, diffusion studies of cadaver skin *in vitro* are a good indicator of the *in vivo* situation for normal skin.

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