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Effect of Dimethyl Sulfoxide on Permeability of Human Skin *In Vitro*

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Abstract □ A diffusion flow cell is described for the continuous monitoring of skin permeability. The technique was used to study the permeability behavior of human skin subsequent to treatment with dimethyl sulfoxide. Such treatment produced an increased penetration rate of tritiated water, which was dependent upon the time of exposure and the concentration of dimethyl sulfoxide applied. Removal of the solvent resulted in partial recovery of barrier capacity. Skin, incubated *in vitro* in growth medium containing dimethyl sulfoxide, survived only at very low concentrations. Degeneration occurred after a few days in 4.5% dimethyl sulfoxide and much sooner at higher concentrations.

Keyphrases □ Dimethyl sulfoxide—effect on growth and permeability of human skin *in vitro*, diffusion flow monitoring cell described □ Permeability—human skin *in vitro*, effect of dimethyl sulfoxide □ Diffusion flow cell—description, continuous monitoring of skin permeability *in vitro* □ Skin, human—effect of dimethyl sulfoxide on growth and permeability *in vitro*

There are numerous reports concerning the enhancement of skin permeability by dimethyl sulfoxide (I) but very little agreement regarding the extent or degree of reversibility of this effect. High concentrations of I *in vivo* caused striking increases in permeability, which were almost completely reversible upon removal (1). Measurement of *in vivo* water vapor loss found an 11- to 17-fold permeability increase subsequent to contact with I, and the effect was partially reversible (2); other results (3) indicated a 25-fold increase in penetration of hexapyronium bromide *in vivo* after exposure to 50% I, with no mention of reversibility.

In vitro work has yielded more quantitative data. A maximum of a 66-fold permeability increase was found upon exposure to I, but the degree of reversibility was variable (4). With I–water mixtures, there was no effect until 60–70% I was applied (5). A 90-fold increase observed with 100% I was not reversible. Concentrations in excess of 70% I were necessary to produce appreciable enhancement of picrate-ion penetration, and such concentrations reduced the lag time from 5 to 2 hr (6). Subsequently it was demonstrated that the effect was not reversible (7).

In the present study, the effect of varying exposure times and concentrations of I on the permeability to tritiated water was investigated. Preliminary experiments revealed a rapid recovery of a fraction of the initial barrier capacity upon removal of I. Therefore, a continuous diffusion flow cell was developed to follow the permeability behavior in a more quantitative manner.

An *in vitro* system (8) was used to study the effect of I on the growth and maturation of human epidermis and thus to relate toxicity levels to the concentrations that enhance penetration.

Table I—Permeability of Human Skin to Tritiated Water Subsequent to Storage[a] at −20°

Sample	Storage Time at −20°			
	Zero	1 Month	3 Months	6 Months
1	3.2 ± 0.1[b]	2.3 ± 0.7	4.3 ± 0.2	3.4 ± 0.4
2	3.8 ± 0.1	3.4 ± 1.0	4.3 ± 0.3	3.2 ± 0.9
3	4.1 ± 0.2	1.8 ± 0.5	4.5 ± 0.4	3.4 ± 0.9
4	3.5 ± 0.3	2.4 ± 0.6	5.9 ± 3.0	2.6 ± 0.6

[a] Permeability coefficients × 10^7 (cm/sec). [b] Errors are quoted as ±95% confidence limits.

EXPERIMENTAL

Skin Samples—Excised thigh or lower leg skin (nominally 0.38 mm thickness) was obtained from amputated limbs using a battery-operated dermatome[1]. Before excision, the skin was cleaned by swabbing with 0.5% chlorhexidine in 70% ethanol, followed by 50% ethanol.

Strips of skin were rolled in sterile gauze, moistened with Hank's balanced salt solution, and stored in screw-capped containers at −20° until required for use. Such storage did not significantly affect the permeability of the skin (Table I).

Skin for growth studies was stored at 4° until required for use. This storage was without effect upon subsequent behavior (9).

Organ Culture—Explants were incubated by the method described by Levine (8) and harvested at 2, 4, 8, 11, 17, and 22 days after 24 hr in medium labeled with thymidine-6-T[2], 1 μCi/ml.

Dimethyl sulfoxide in growth medium[3] was tested at concentrations of 0, 0.9, 4.5, and 9% I.

Histology and Autoradiography—The methods used were described previously (8).

Diffusion Cells—*Glass Diffusion Cell*—The design and use of this type of cell were described previously (9). The "initial" permeability of the skin specimen is determined by filling the dermal compartment with a phosphate buffer medium (pH 7.0) and applying to the epidermal side a known volume of the same medium containing tritiated water[4] at a concentration of 80 μCi/ml.

[1] Davol Ltd., Providence, R.I.
[2] Radiochemical Centre, Amersham, Bucks, England.
[3] Eagle's Minimal Essential Medium plus 10% fetal bovine serum.
[4] Radiochemical Centre, Amersham, Bucks, England.

Table II—Effect of Exposure to Dimethyl Sulfoxide upon Subsequent Permeability of Skin to Tritiated Water[a]

		Exposure Time, min					
		0	5	15	30	60	120
Initial (least squares)		2.56 ± 0.27	1.89 ± 0.45	2.08 ± 0.17	2.47 ± 0.72	2.24 ± 0.13	2.52 ± 0.69
Test	75 min	2.28	9.23	29.2	36.1	47.3	62.9
(individual	135 min	1.96	7.85	20.5	27.9	33.1	32.0
values)[b]	210 min	2.19	6.94	17.6	24.3	34.2	43.7
	300 min	2.18	7.13	13.4	21.1	28.3	34.0
	405 min	2.22	5.84	14.2	16.1	26.3	29.1
	525 min	2.37	5.80	10.2	15.8	22.8	32.5
Recovery (least squares)		2.55 ± 0.31	4.20 ± 0.56	9.4 ± 0.9	15.2 ± 4.3	20.2 ± 1.85	20.2 ± 2.1

[a] Permeability coefficients × 10^7 (cm/sec) ±95% confidence limits. [b] Individual values refer to calculation of an individual permeability coefficient for each sample taken during the measurement by substitution of the relevant values into the rate equation.

Samples are removed from the dermal compartment at required time intervals and replaced by buffer. The activity of the samples and a suitably diluted standard is determined in ethanolic scintillator[5] using a liquid scintillation spectrometer[6].

The cell is emptied, and all radioactivity is washed from the skin by soaking in several changes of buffer. The skin is then dried and exposed to I for the required time interval. After the solvent is removed, the "test" permeability is determined in the same way as described for initial permeability. Twenty-four hours later, "recovery" is determined, giving the skin permeability subsequent to recovery of barrier capacity. Permeability coefficients are calculated as described previously (9).

Diffusion Flow Cell—The Perspex cell is shown in Fig. 1 with a specimen of skin inserted between the two blocks and clamped into position using three nylon screws. Tubing is stainless steel (18 gauge).

The assembled apparatus is shown in Fig. 2. Buffered medium containing tritiated water (80 μCi/ml) is placed in the donor compartment and stirred. Buffered medium flows from the reservoir through the acceptor compartment in the direction shown by arrows; the geometry of this compartment ensures efficient elution of penetrant from the whole surface of the skin. The eluent passes *via* a small prefilter to a scintillation flow cell assembly[7].

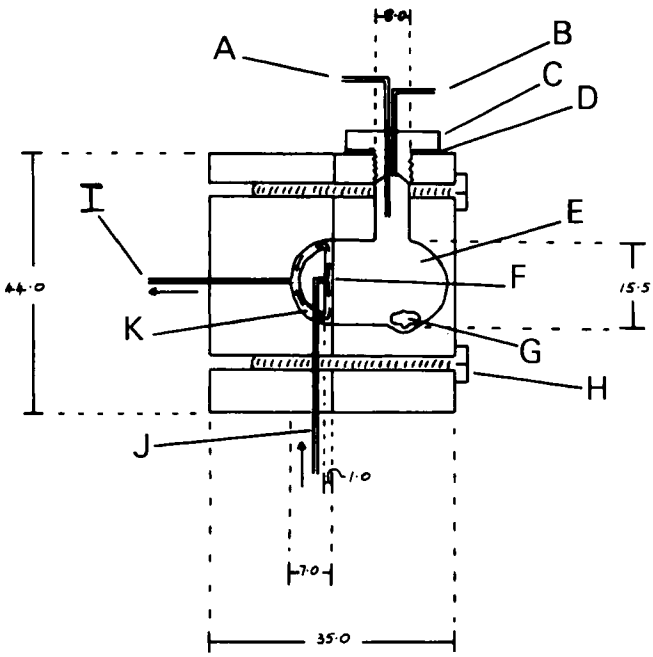


Figure 1—*Diffusion flow cell. Key: A, application tube; B, removal tube; C, donor compartment stopper; D, Neoprene rubber washer; E, donor compartment; F, skin specimen clamped between Perspex blocks; G, stirrer; H, clamping screw; I, inlet tube; J, acceptor compartment; and K, outlet tube.*

The prefilter, a glass tube packed with anthracene and plugged with glass wool at each end, removes any contaminants in the eluent which might otherwise adsorb onto the anthracene in the scintillation flow cell and affect the counting efficiency. It also prevents any air bubbles in the system from reaching the scintillation flow cell. A constant flow rate is maintained using a peristaltic pump[8].

The procedure is as follows. The apparatus is assembled as shown in Fig. 2 but omitting the diffusion flow cell. Background is determined by passing buffered medium through for long periods, so background subtraction can be carried out automatically. The activity of the donor solution is now determined by passing a suitably diluted solution through the scintillation cell. Measurement of donor activity is repeated at the end of the determinations to ensure that there has been no change in counting efficiency over the test period.

The skin is now clamped between the two Perspex blocks of the diffusion cell, using a small amount of vacuum grease around the edge of the skin to maintain an efficient seal. The acceptor compartment is filled by means of a syringe, and its volume is adjusted so that the skin is planar. The inlet and outlet are clamped until the donor compartment is filled and sealed, the fixed volume maintaining planarity of the skin when flow begins.

The donor compartment volume (V_0) is determined by weighing. The diffusion cell is then inserted into the system, and stirring of the donor compartment and flow through the acceptor compartment are commenced. The flow rate is determined by weighing the eluent collected in a known time interval. The counting interval is chosen to suit the rate of permeability change observed. Permeability values for any time interval may then be calculated from Eq. A8 (*Appendix*).

RESULTS AND DISCUSSION

Glass Diffusion Cell—Permeability was measured before exposure (initial), immediately after exposure (test), and 24 hr later (recovery). Two milliliters of I was applied to the epidermal surface of the skin, and the same time interval elapsed between removal of the solvent and the beginning of the test determination

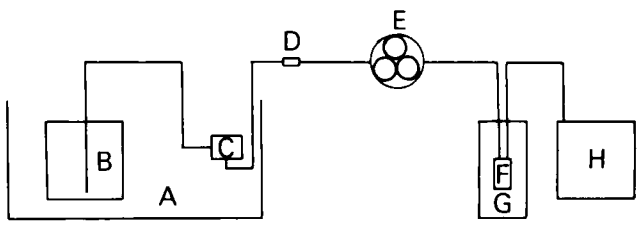


Figure 2—*Block diagram of flow cell apparatus. Key: A, thermostated water bath; B, reservoir; C, diffusion flow cell; D, anthracene prefilter; E, peristaltic pump; F, flow cell; G, scintillation counter; and H, collector.*

[5] Composed of 0.4% 2,5-diphenyloxazole and 0.04% 1,4-bis[2-(4-methyl-5-phenyloxazolyl]benzene in xylene–ethanol (68:30, v:v).
[6] Packard Tri-Carb model 3320.
[7] Packard Ltd.
[8] L.K.B. Varioperpex model 12000.

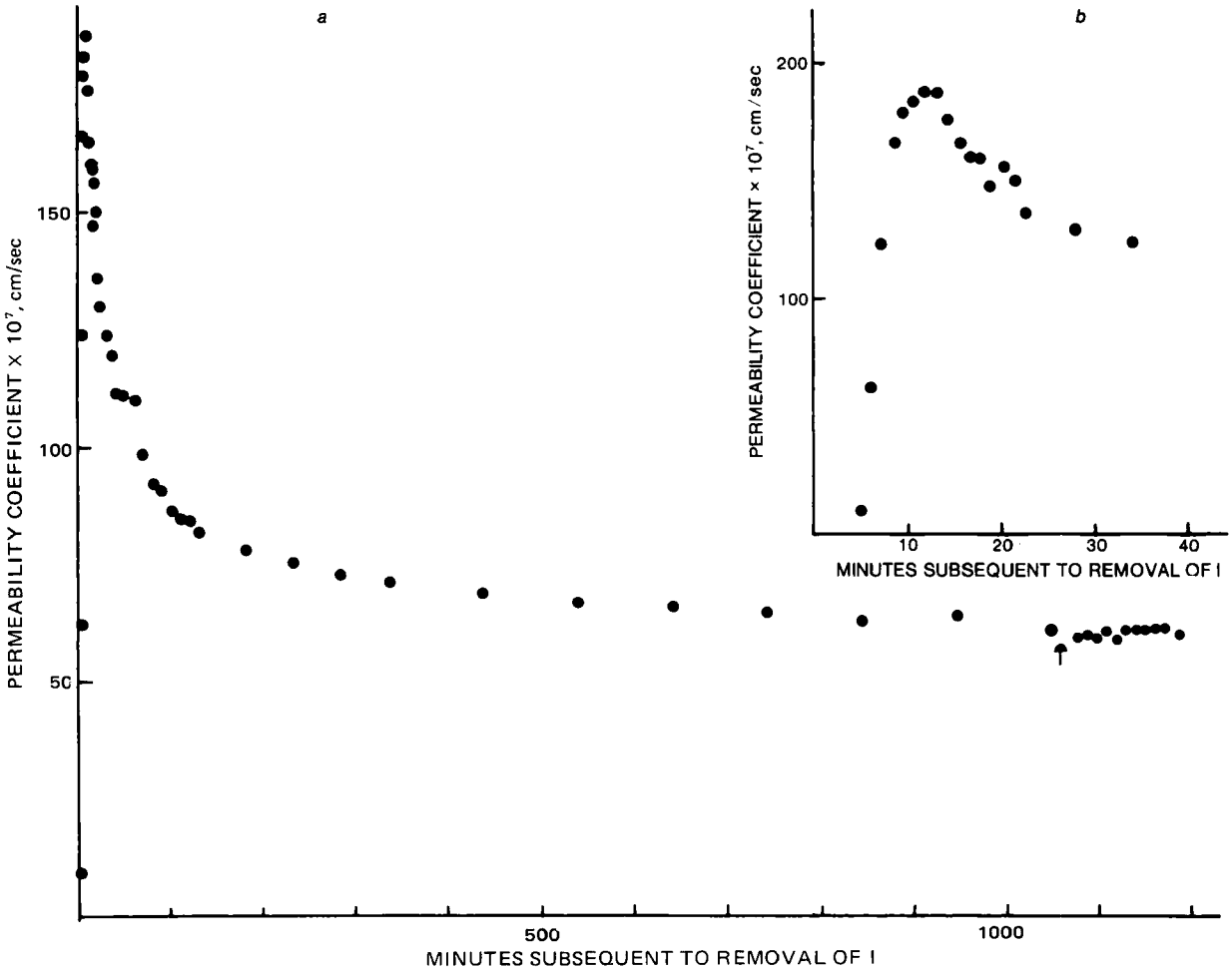


Figure 3—(a) *Permeability of human skin to tritiated water subsequent to dimethyl sulfoxide (I) treatment. Arrow refers to the replenishment of the donor solution. The final permeability value is obtained subsequent to the replenishment.* (b-*insert*) *Permeability of human skin to tritiated water subsequent to dimethyl sulfoxide treatment (early part of Fig. 3a plotted on an expanded time scale). The apparent rise in permeability to a peak value at 12 min is due to a lag phase inherent in the method of measurement. The peak permeability is given by the maximum in the plot.*

for each skin specimen. During exposure to I, no solution was in contact with the dermal surface of the skin. The results of one of two similar experiments are given in Table II.

Permeability values are usually calculated from the slope of the straight line obtained by least squares from a plot between ($t_n - t_{n-1}$) and the left side of the equation (see Eq. 14 in Ref. 9):

$$-\ln\left[\frac{C_T - (\chi/V_0)\sum_1^{n-1} C_i - C_{i(n)}(V_T/V_0)}{C_T - (\chi/V_0)\sum_1^{n-1} C_i - C_{i(n-1)}(V_T/V_0)\{1 - [\chi/(V_T - V_0)]\}}\right] = PA(t_{(n)} - t_{(n-1)})\frac{V_T}{V_0(V_T - V_0)} \quad \text{(Eq. 1)}$$

Once the steady state is established, such a plot is linear and the intercept on the ordinate (left side of Eq. 1) is close to zero.

A steady state was observed for initial and recovery permeability determinations, and the values calculated are given in Table II. But during the test determination, the steady state was not maintained, resulting in a marked curvature when counts of radioactivity were plotted against time. The linear plot according to Eq. 1 had an intercept significantly greater than that observed for initial or recovery data. This result could be due either to an initial high permeability decreasing over the test period or to the presence of residual radioactivity in the acceptor compartment of the diffusion cell at the start of the test determination. The possibility of residual radioactivity was eliminated by another experiment in which the initial permeability determination was omitted.

Results (Table III) from specimens exposed to I exhibited the large negative intercept on the ordinate of the linear plot. The individual test permeability values calculated for specimens exposed to I (Tables II and III) showed a decrease during the measurement period. An estimate of the reversibility can be obtained, since the first sample gives an approximation to the maximum value and the recovery gives the final effect, indicating approximately 50% recovery of barrier capacity subsequent to removal of the solvent. Another noticeable feature was the increase in both temporary and permanent effects with an increase in exposure time.

Diffusion Flow Cell—Skin specimens were mounted on the flow cell and exposed to the required treatment. Then the solvent was removed, and the permeability of the skin was monitored con-

Table III—Permeability Coefficients x 10^7 (cm/sec) Subsequent to Dimethyl Sulfoxide Exposure

		Exposure Time, hr			
		16	16	0	0
Test (individual values)[a]	70 min	130	133	1.49	2.88
	120 min	104	122	1.82	2.66
	180 min	103	93	1.81	3.30
	250 min	91	83	1.92	3.02
	330 min	69	111	2.00	2.76
	420 min	71	84	2.19	4.11
Intercept x 10^4		−757.4	−484.1	+8.0	+16.1

[a] Individual values refer to calculation of an individual permeability coefficient for each sample taken during the measurement by substitution of the relevant values into the rate equation.

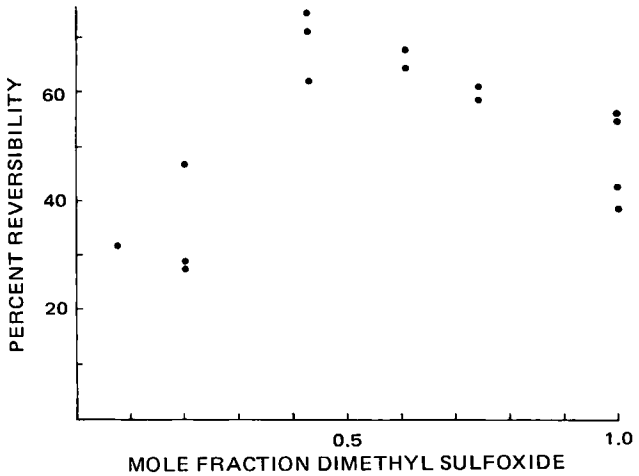


Figure 4—*Effect of dimethyl sulfoxide concentration upon reversibility of permeability enhancement (calculated from data in Table V).*

tinuously for 16–20 hr, *i.e.*, until a constant value was observed. The type of behavior exhibited is shown in Fig. 3*a*. Figure 3*b* (insert) shows the first portion of Fig. 3*a* plotted on an expanded time scale. The maximum permeability observed is shown by the peak in the plot of permeability coefficient against time. Permeability then decreased to a final steady value.

Replacement of the donor solution was carried out (indicated by the arrow in Fig. 3*a*) to avoid any possibility of its depletion. Final permeability values were obtained subsequent to the replacement. To ensure that the behavior observed for treated skin specimens was due to barrier recovery, a measurement was carried out using dialysis tubing. A steady permeability, fivefold greater than that observed for treated skin specimens, was obtained, thus confirming the validity of the technique.

The "maximum" and "final" permeability values for a series of pretreatment times are given in Table IV. A significant increase in permeability was observed after 30 min of exposure, the maximum change being attained after 24 hr.

By using exposure conditions of 24 hr in contact with stirred solvent at room temperature, the effect of a range of concentrations was studied. The results obtained using skin from two different individuals are given in Table V. The concentration of dimethyl sulfoxide is expressed as mole fraction. The generally used "volume percent" artificially introduces a nonlinear element, since water is 55.5 *M* and I is only 14 *M*. The relationship between the final permeability coefficient and the mole fraction of dimethyl sulfoxide is nonlinear and possibly correlates with removal of one or more components of the stratum corneum[9]. The relationship between maximum permeability coefficient and concentration of I, although much less curved, is again nonlinear.

The permeability increase subsequent to treatment of the skin was consistently partially reversible upon removal of the solvent. The extent of reversibility varied with the concentration of I applied (Fig. 4). The curve in Fig. 4 passes through a maximum at mole fraction = 0.5, *i.e.*, a 1:1 molar ratio of dimethyl sulfoxide to water. The reduced reversibility observed at higher concentrations of I may have been due to more efficient extraction of barrier components.

The results suggest possible reasons for the widely varying reports concerning the effect of dimethyl sulfoxide upon skin.

1. The 24-hr period required to produce the maximum effect is considerably greater than that employed by most investigators, and the conditions vary.

2. Since the recovery of barrier is an extremely rapid process, conventional permeability measurement techniques may not detect a considerable portion of the effect.

3. Since water is 55.5 *M* and dimethyl sulfoxide is 14 *M*, contamination of the applied solvent by water may result in an appreciable reduction in the mole fraction of I present and hence a reduced effect upon permeability. This factor is particularly relevant if aqueous medium is in contact with the dermal surface of the skin when the dimethyl sulfoxide is applied to the epidermis.

[9] Unpublished data.

Table IV—Effect of Exposure Time

Time of Exposure to Dimethyl Sulfoxide[a]	Peak Permeability Coefficient[b], (cm/sec) × 10^7	Final Permeability Coefficient, (cm/sec) × 10^7
30 min	55	28.0
60 min	71	35.0
60 min	67	45.0
12 hr	143.5	75.0
12 hr	121.5	63.0
12 hr	135.0	65.0
24 hr (stirred)	250	183.0
12 hr (stirred)[c]	247	180.5

[a] Concentration of I applied was 100%. [b] Calculated from peak value observed on graph of permeability *versus* time subsequent to exposure. [c] Reapplication of I to same skin specimen that was used to study 24-hr effect.

Table V—Effect of Dimethyl Sulfoxide (I) Concentration

Mole Fraction of I in Treatment Solution	Peak Permeability Coefficient, (cm/sec) × 10^7		Final Permeability Coefficient, (cm/sec) × 10^7	
	(a)[a]	(b)[a]	(a)	(b)
0.0	—	—	7.3	—
0.076	(i)[b] 14.3	15.8	9.8	10.2
	(ii)[b]	14.8	—	9.95
0.202	(i) 19.1	39.3	13.6	11.0
	(ii) 25.0	39.4	13.3	14.1
	(iii) 28.6	—	20.8	—
0.431	(i) 84	91.0	32.0	25.2
	(ii) 136	—	35.0	—
	(iii) 120.5	—	35.5	—
0.608	(i) 185.0	91.0	60.0	33.7
	(ii) 182.0	—	65.5	—
0.744	(i) 205.0	200	86.0	70.0
	(ii) 241.0	193	94.0	73.0
1.00	(i) 312.0	248	143.0	180
	(ii) 332.0	—	205.0	—
	(iii) 252.0	—	155.0	—
	(iv) 312.0	—	137.0	—
	(v) 295.0	—	170.0	—

[a] (a) and (b) refer to experiments with skin from different individuals. [b] (i), (ii), *etc.*, refer to experiments using skin specimens from the same limb.

Scheuplein and Ross (4) reported enhancement of reversibility when the tissue was rinsed with 1-butanol between exposure to I and water contact. The exact role of the butanol was, however, uncertain. A decrease in permeability subsequent to exposure of human skin to 1-butanol was observed[9], and it was associated with extensive dehydration of the epidermis.

Claims of complete reversibility also have been made. These claims usually stem from *in vivo* work; the situation is complicated by active regeneration of the barrier, which has been shown to progress rapidly *in vivo* subsequent to injury.

The relationship between maximum permeability and dimethyl sulfoxide concentration was considerably less curved than data reported previously. A similar type of relationship was observed by Elfbaum and Laden (10) while studying the effect of I concentration upon swelling of hair fibers. The solvent also produced a swelling of globular proteins. In both cases, the swelling was reversible upon removal of the solvent, and a similar mechanism could possibly account for the barrier recovery observed in human stratum corneum.

Growth of Human Skin *In Vitro*—After 4 days of incubation in the absence of I, control explants of skin showed some thickening and vacuolization (Fig. 5*a*). By 17 days (Fig. 5*b*), further alteration of the normal growth pattern was observed. At this stage, there were two parakeratotic layers and below them were rapidly proliferating cells. This pattern of growth is generally found in organ culture of skin (8). Autoradiograms of the regenerating tis-

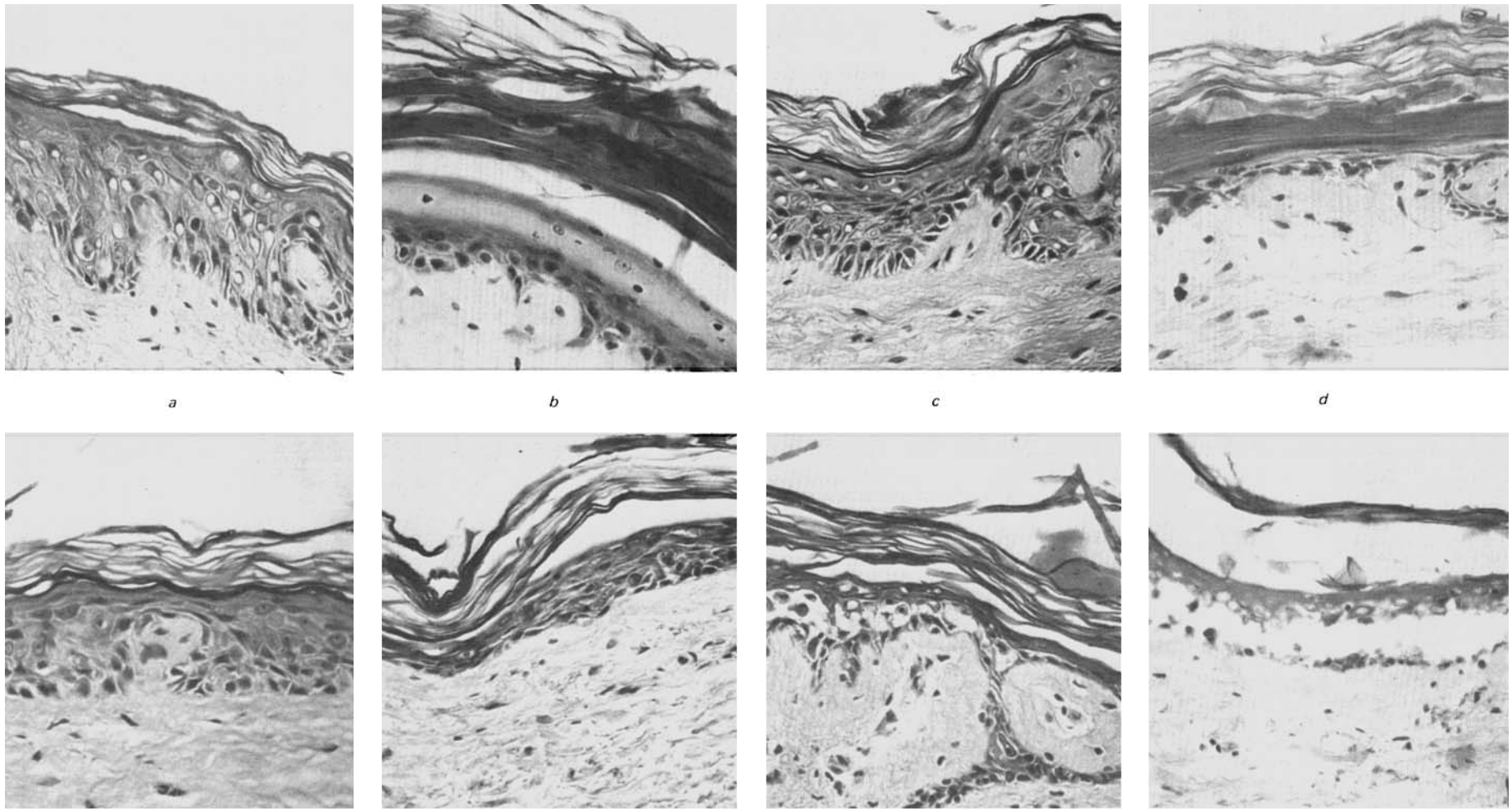


Figure 5—*Photographs of sections of skin showing the effect of dimethyl sulfoxide (I) on* in vitro *growth (×250, stained with hematoxylin and eosin).* (a) *and* (b)*: No I in the growth medium. Time of incubation was 4 days in* (a) *and 17 days in* (b)*. Vacuolization and the thickening of the epidermis can be observed in* (a)*. In* (b)*, parakeratotic layers have appeared and below them are proliferating cells.* (c) *and* (d)*: 0.9% I. Time of incubation was 4 days in* (c) *and 17 days in* (d)*. Growth of skin was similar to the control.* (e) *and* (f)*: 4.5% I. Time of incubation was 4 days in* (e) *and 11 days in* (f)*. There was no thickening at 4 days. Some flattening of the cells existed at 11 days, but no maturation was observed.* (g) *and* (h)*: 9% I. Time of incubation was 4 days in* (g) *and 17 days in* (h)*. Degeneration of the tissue was seen at 4 days, and complete disintegration was observed at 17 days.*

sue showed highly labeled cells subsequent to incubation in medium containing radioactively-labeled thymidine.

In the presence of 0.9% I, growth *in vitro* proceeded in a similar fashion to that in its absence. At 4 days, the organization of the malpighian layers was better in the presence of I (Fig. 5*c*) than in the control. At 17 days (Fig. 5*d*), the thick, parakeratotic layers indicated that growth and maturation had occurred.

Specimens in 0.9% I showed less epiboly (migration of cells around the explant) than the controls without I. In the first few days of cultivation, the stratum corneum of skin in 0.9% I appeared looser in structure, but this difference was not noticeable at longer incubation times.

DNA synthesis was evident throughout the incubation period in 0.9% I. At 11 and 17 days, autoradiograms showed more labeled cells than in the controls without I. The increase may have been due to an effect on the cell membranes resulting in an enhanced diffusion of thymidine. In addition, the thicker epiboly present in the control explants could have reduced the rate of diffusion. Both factors probably contributed to the difference observed in the degree of labeling. Another interpretation is that the presence of I resulted in alteration of the cell cycle.

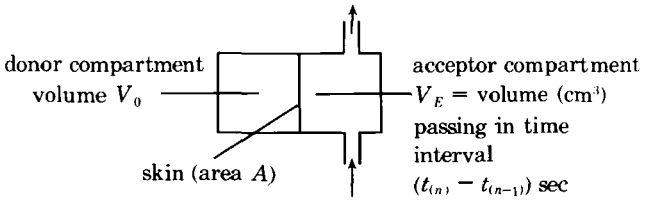
In contrast, higher concentrations of I (4.5%) resulted in an absence of thickening of the epidermis at 4 days (Fig. 5*e*) and no subsequent maturation (Fig. 5*f*). In growth medium containing 9% I, the epidermis showed considerable degeneration at 4 days (Fig. 5*g*) and complete disintegration at 17 days (Fig. 5*h*).

In either 4.5 or 9% I, there was no epiboly at any time during 22 days of incubation. Malpighian cells began to degenerate after 2 days, and disintegration was more noticeable in 9% I. No maturation into a parakeratotic layer was observed; after 4 days, no DNA synthesis could be detected by autoradiography.

It would thus appear that 0.9% I in a growth medium did not prevent growth and maturation of epidermis *in vitro* over the 22-day period of the culture studies. Higher concentrations, however, of 4.5 and 9% I cannot support *in vitro* growth, as judged by histology and DNA synthesis.

APPENDIX

The derivation of the rate equation applied to flow cell data was as follows:



From Fick's law:

$$\frac{d}{dt} V_E C_E = PAC_0(t) \qquad \text{(Eq. A1)}$$

where P is the permeability coefficient, C_E is the concentration of penetrant in the eluent (cpm), and $C_0(t)$ is the concentration of donor solution at time t (cpm).

Application of the law of mass conservation gives:

$$C_0(t) = C_0(0) - \frac{V_E}{V_0} C_E(t) \qquad \text{(Eq. A2)}$$

where $C_0(0)$ is the concentration of penetrant in donor solution at time 0, and $C_E(t)$ is the concentration of penetrant in eluent at time t. Substituting from Eq. A2 into Eq. A1 gives:

$$\frac{d}{dt} V_E C_E = PA \left[C_0(0) - \frac{V_E}{V_0} C_E(t) \right] \qquad \text{(Eq. A3)}$$

Integrating from $C_{E(n-1)}$ to $C_{E(n)}$ and from $t_{(n-1)}$ to $t_{(n)}$ gives:

$$-\ln \left[\frac{C_0(0) - \frac{1}{V_0}\left(\sum_1^{n-1} V_E C_E\right) - \frac{1}{V_0}[(V_E C_E)_n]}{C_0(0) - \frac{1}{V_0}\left(\sum_1^{n-1} V_E C_E\right)} \right] = \frac{P_{(n)} A [t_{(n)} - t_{(n-1)}]}{V_0} \qquad \text{(Eq. A4)}$$

The left side is of the form $-\ln(1 - x)$ where:

$$x = \frac{\frac{1}{V_0}(V_E C_E)_{(n)}}{C_0(0) - \frac{1}{V_0}\left(\sum_1^{n-1} V_E C_E\right)} \qquad \text{(Eq. A5)}$$

Then, provided $x \ll 1$[10], Eq. A4 becomes:

$$\frac{(V_E C_E)_{(n)}}{C_0(0) - \frac{1}{V_0}\left(\sum_1^{n-1} V_E C_E\right)} = P_{(n)} A [t_{(n)} - t_{(n-1)}] \qquad \text{(Eq. A6)}$$

Hence:

$$P_{(n)} = \frac{(V_E C_E)_{(n)}}{\left[C_0(0) - \frac{1}{V_0}\left(\sum_1^{n-1} V_E C_E\right) \right] A [t_{(n)} - t_{(n-1)}]} \qquad \text{(Eq. A7)}$$

As written in Eq. A7, V_E represents the volume flowing through the flow cell in time period $(t_{(n)} - t_{(n-1)})$. The instrument has a noncounting interval of 10.5 sec between counting periods, and the volume flowing in this time interval must be included in the summation term. Thus, if $(t_{(n)} - t_{(n-1)})$ is expressed in seconds, Eq. A7 becomes:

$$P_{(n)} = \frac{(V_E C_E)_{(n)}}{\left[C_0(0) - \frac{1}{V_0} \sum_1^{n-1} \left(\frac{(t_{(n)} - t_{(n-1)} + 10.5)}{t_{(n)} - t_{(n-1)}} \right) V_E C_E \right] A [t_{(n)} - t_{(n-1)}]} \qquad \text{(Eq. A8)}$$

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[10] In view of this approximation, it is necessary to ensure that the donor solution does not become excessively depleted and periodic replacement of the donor solution is carried out during the experiment.