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A microbalance technique for measuring water vapour uptake and release by stratum corneum in vitro

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Summary

An apparatus was developed for investigating water absorption and desorption patterns in stratum corneum in vitro. To validate this technique, absorption and desorption of water vapour by human abdominal stratum corneum was studied using relative humidity changes of 0–91% and 91–0%. These changes were attained by suspending the test samples in a chamber above a drying agent (Drierite) or a saturated salt solution (stationary system), or air was passed in different proportions through either water or Drierite and then recombined before contacting the stratum corneum (flow system). These two methods produced different absorption and desorption patterns and the stratum corneum samples attained 2–3-fold higher water content levels if the stationary system was used. Changes in the rate of air flow during absorption affected the results obtained, an effect not observed during desorption. Initial diffusion coefficients and rate constants for absorption and desorption were calculated. The apparatus should prove useful in fundamental studies of the hydration of the stratum corneum and in applied investigations such as treatment of the corneum with non-volatile agents, for example, moisturizers and penetration enhancers.

Introduction

Water exerts a major role in skin health and maintains the skin's soft and flexible mechanical properties. In pathological conditions such as psoriasis, ichthyosis and eczema there is an impaired barrier function which leads to dry skin (Grice et al., 1973; de Jongh, 1981). The permeability of the skin and hence the rate of drug penetration depends upon the water content of the stratum corneum (Barry, 1983). This in turn is also affected by environmental conditions such as humidity of the atmosphere, the temperature (Allenby et al., 1969), or applied cosmetic or pharmaceutical products (Aubert et al., 1985).

A wide variety of in vitro and in vivo physical procedures is available for investigating phenomena associated with the hydration of the stratum corneum (e.g. Leveque, 1983; Nilsson, 1977; Quattrone and Laden, 1976). The principle in vitro measurements are achieved by gravimetric or force-elongation studies (Wildnauer, 1971). Other methods of a more fundamental nature are designed to study the states of binding and mobility of water molecules, for example, infrared absorption at ambient or low temperatures (Gloor et al., 1980) and differential scanning calorimetry (Walkley, 1972). The authors of this present paper chose

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to compare and modify a direct method of assessing moisture uptake (gravimetric) reported by Scheuplein and Morgan (1967) and Anderson et al. (1973) and used their methods of data analysis.

The apparatus was designed so that as many variables as possible could be monitored and controlled including a permanent record of skin weight changes during absorption and desorption, air flow, air pressure, humidity and temperature control. The facility of a permanent computer printout of skin weight changes and the controlled conditions enabled experiments to be performed overnight without the attendance of an investigator.

It was felt that the flow system in which moist (or dry) air was passed across the stratum corneum samples at a controlled flow rate was akin to the in vivo situation in which the skin of the face and hands, for example, was in contact with the open air. The stationary system mimicked the situation of a skin area under a few clothes layers, surgical dressings or topical preparations and little air movement. In vivo, at the lowest cell layers the stratum corneum is in equilibrium with aqueous viable tissue and may be considered to be fully hydrated. The outermost layers are in contact with environmental relative humidity and thus a water concentration gradient exists across the skin. We are mainly interested in changing the hydrating ability of the surface layers of the stratum corneum. Thus we arrange our in vitro experimental conditions with two main aims: to use the full-thickness stratum corneum as a model of the in vivo surface layers and to expose this tissue only to the level of water activity which the skin surface would meet under normal non-tropical conditions, e.g. up to 91% relative humidity.

Materials and Methods

Apparatus

The 1 g head standard weighing compartment (C.I. Electronics, U.K. Mark 2B) (Fig. 1) consisted of two Pyrex glass chambers, on the left-hand side of which strips of dried human abdominal stratum corneum were suspended on an aluminium suspension rod. To overcome problems caused by static attraction between the rod and the glass wall of the sample tube, a glass-to-metal seal was made between the inner and outer walls of the flask. The inside of each flask was then coated with gold in a

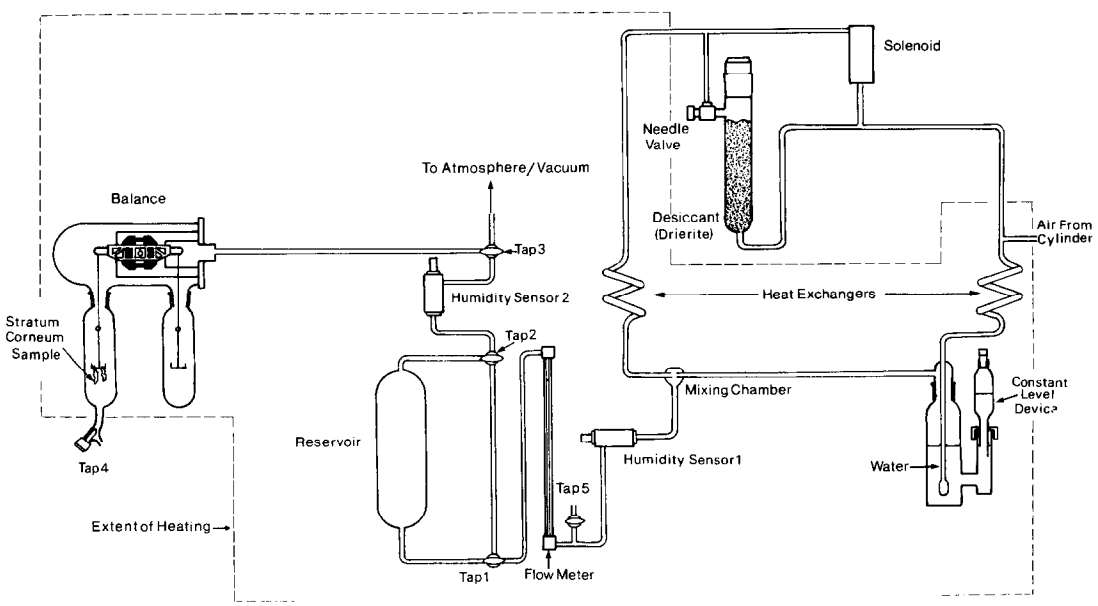

Fig. 1. Apparatus for water vapour absorption and desorption studies in stratum corneum in vitro. The dotted line indicates the equipment housed in the controlled temperature cabinet at $32 \pm 0.2°C$.

vacuum evaporation unit. Any static build-up was effectively prevented by connecting the glass-to-metal seals to a suitable earth. As a further precaution, the outside of the flasks and weighing compartment was sprayed with an antistatic spray (Baird and Tatloch, U.K.). The balance movement was also returned to the manufacturer for a P.T.F.E. coating to protect against possible corrosion.

The microbalance was connected via a multiway cable to the Robal microprocessor control unit. This displayed the weight of the skin in milligrams to three decimal places. The Robal unit was also linked to a multipen recorder (Rikadenki Kogyo, Japan; R-50 series Model 83, range set at 2 mg) which produced a constant trace of the skin weight. Prior to each experiment with a new piece of skin, the microbalance was calibrated according to the manufacturer's instructions. Parts of the apparatus as shown in Fig. 1 within the dotted line were housed in a temperature-controlled cabinet at $32 \pm 0.2°C$ and the entire apparatus was located in a constant temperature room ($22 \pm 1°C$).

The cabinet was constructed of laminated chipboard lined with 1 inch thick expanded polystyrene and the windows were of double-glazed Perspex. Air was circulated and the cabinet heated by blowing air from an axial flow fan (Radiospares, U.K.) over an infrared heating lamp (Osram, U.K.; 275 W) which was operated by a proportional controller using a thermistor as a temperature detector. If during an experimental run the heating lamp failed, a second fan and lamp mounted below the first one would automatically switch on after an interval of 1 min. The interior temperature of the cabinet was measured by a thermocouple and displayed on a Digitron temperature display unit (Type 2751-K).

Compressed air from a cylinder at a pressure of 10 lb./inch^2 was passed through a miniature pressure regulator (Platton, U.K., Type MPRA) set at 7 lb./inch^2 and a Flowstat flow meter (Platton, U.K., Type FM/NAL) to compensate for any changes in air flow and pressure. Prior to entering the system the air flow was divided by a simple T-piece and part of it passed through into a desiccator tube containing Drierite (B.D.H., U.K.),

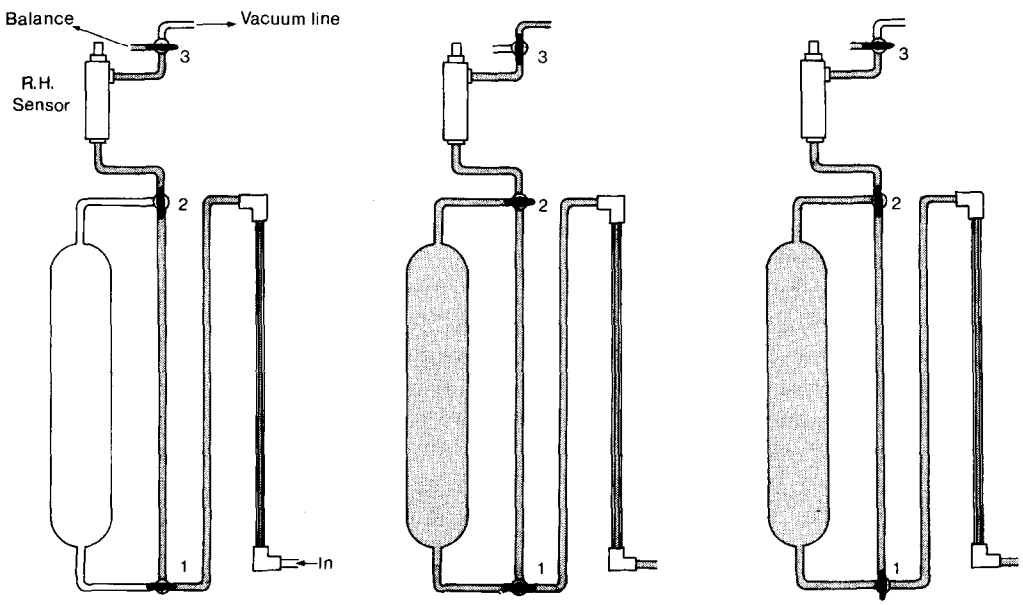

Fig. 2. Three stages in the arrangement of the taps within the flow system apparatus. Stage 1 was used during an actual absorption or desorption experimental run. Stage 2 was employed 1 h prior to an experimental run to isolate the microbalance chamber and set the rest of the apparatus at the required relative humidity. Stage 3 was used during the initiation of an experimental run. F.M. is the flow meter. (For a more detailed explanation see Materials and Method section.)

flowed out through a needle valve into a copper heat exchanger and then entered the mixing chamber. The remainder of the air passed into another copper heat exchanger before entering a water-containing Dreschell bottle with a constant level device. By bubbling through a sintered glass diffuser in the water, the air moisture level increased and the air then flowed out into the mixing chamber to combine with the dry air. By adjusting the needle valve installed in the "dry" line, a range of relative humidities could be attained.

The mixed air then passed through humidity sensor 1 (Shaw, M.M., Bradford, U.K.), and then through a flow meter marked on a height scale from 0 to 15 mm corresponding to a flow rate of 0 to 85 ml · min^{-1}, and which was set at 15 mm for all routine experiments using tap 5. The air then flowed through taps 1 and 2, through humidity sensor 2 and tap 3 and into the microbalance chamber containing the skin samples and out to air via tap 4. By closing taps 3 and 4, the skin could be maintained at a required relative humidity whilst that in the rest of the apparatus could be changed (Fig. 2). Hence by arranging the taps as shown in Stage 2 of Fig. 2, the air in the apparatus and reservoirs was set to the required moisture level (for example, 91% relative humidity), while the skin remained at 0% R.H. Stage 3 of Fig. 3 depicts the tap positions for the initiation of an experimental run. Tap 1 was adjusted to isolate the flow meter and tap 5 (Fig. 1) opened to vent to atmosphere and a vacuum line attached to the system at tap 3. Tap 3 was then turned to evacuate the balance head. After one minute tap 3 was slowly turned to isolate the vacuum and to allow air from the reservoirs to flow into the balance head. Tap 5 was then closed and tap 1 was turned to bring the flow meter back into line and allow air to flow through the system and take the pressure in the microbalance head to atmospheric. When this had occurred (the float in the flow meter began to drop), tap 4 was opened to allow flow through the system. The flow rate was then adjusted to 85 ml · min^{-1} using tap 5 and the experimental run was initiated (Stage 1, Fig. 2).

Prior to each experiment humidity sensor 2 was calibrated against a salt solution (saturated potassium nitrate for a relative humidity of 91%) and the value recorded by humidity sensor 1 was noted. Both of these sensors were connected to a digital display moisture meter (Shaw, M.M., Bradford, U.K.), which was also linked to the multipen recorder (Fig. 4). The two humidity sensors were used in order to ensure even humidity distribution within the apparatus. The humidity sensors were expensive and may have been permanently damaged if a sudden drop in temperature was to occur causing water to condense within them. To prevent this, a solenoid valve was incorporated into the system in order to flow the sensors with dry air directly from the air cylinder. This solenoid valve (Erots Pneumatics, U.K.), operated if either the back-up infrared heater or the mains power failed. Taps 1 and 2 were made from P.T.F.E. tee bore, three-way pattern (G. Springham & Co.), tap 3 was an all glass tee bore, vacuum tap (G. Springham & Co.), tap 4 was a 2 mm bore P.T.F.E. "O" type (Quickfit, U.K.), and tap 5 was a P.T.F.E. 2 mm, straight bore pattern (G. Springham & Co.).

The Robal microprocessor unit was connected to a Commodore Pet Series 2001 computer and printer which gave a print-out of the weight of the

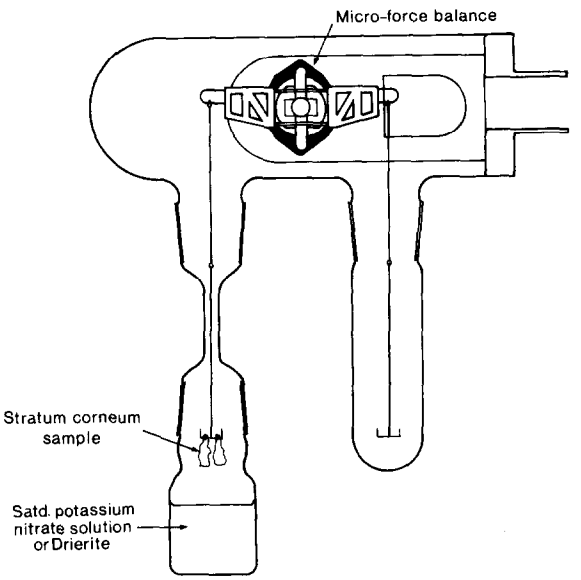

Fig. 3. Modification of the apparatus of microbalance chamber for the stationary or static experiments.

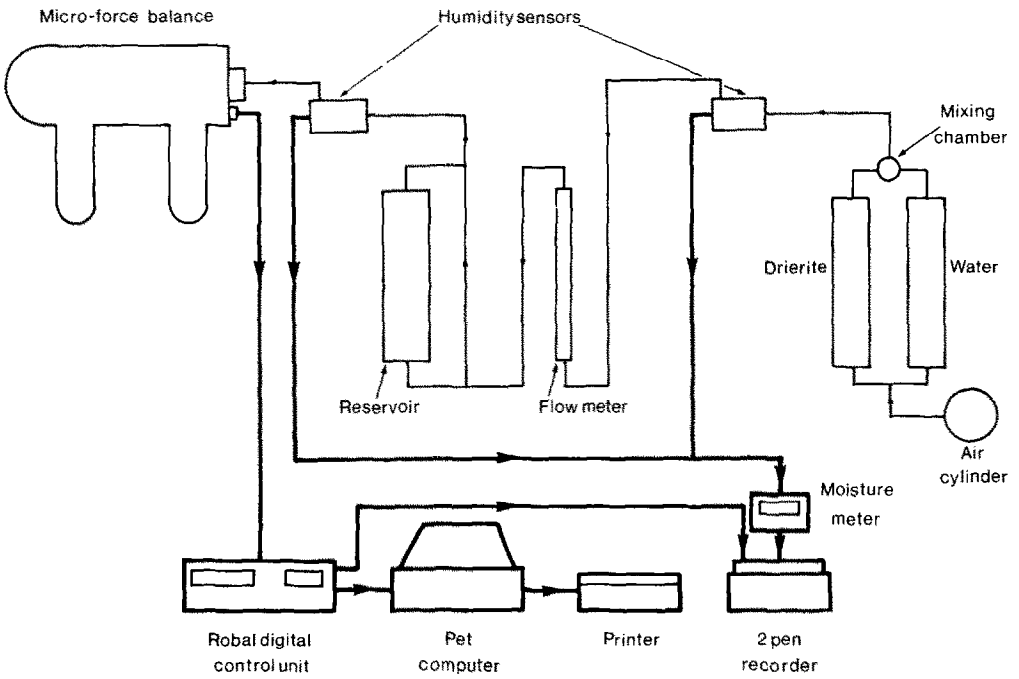

Fig. 4. Block diagram depicting apparatus arrangement for water absorption and desorption experiments, including associated computer and recording equipment.

skin sample, accumulated weight difference, accumulated percentage difference, number of the reading and the time interval between readings (Fig. 4). This program was written in such a way as to allow its user to select the time interval between the initial readings, number of initial readings, change in weight to initiate a change in time interval and an option to change any of these values. The apparatus so far described is termed the flow or dynamic system.

Changes in the air flow rate were investigated with respect to the final equilibrium moisture uptake or loss attained by the skin samples. Three pieces of stratum corneum were stabilized for 24 h at a relative humidity of 91% at a flow rate of 85 $ml \cdot min^{-1}$. Then this flow rate was decreased to 5 $ml \cdot min^{-1}$ and the weight of the water vapour absorbed was recorded at equilibrium. The flow rate was then increased and decreased in increments (5, 11, 17, 26, 50, 85 and 50, 26, 17, 11, 5 $ml \cdot min^{-1}$) and at each stage the skin water uptake or loss was allowed to reach equilibrium (2–3 h) before the weight of water absorbed or desorbed was recorded.

The left-hand side chamber of the weighing compartment could be replaced by a Pyrex glass attachment and a container (Fig. 3) holding either a saturated solution of potassium nitrate (B.D.H., U.K.) to yield 91% or Drierite (referred to as '0%' R.H.). By closing tap 3, the air in the weighing compartment was at the required moisture level and an experimental run could be initiated. This modified apparatus was termed the stationary system.

At the beginning of each experiment with either the flow (F) or the stationary (S) systems, the stratum corneum samples were allowed to equilibrate at 0% relative humidity (R.H.) for at least 24 h. Then a 0–91% R.H. absorption run was begun and terminated after 24 h. This period was chosen since by this time the flow system had reached equilibrium; however, the stationary system skin moisture content was still increasing. Anderson et al. (1973) reported that this moisture content from a stationary system would rise steadily for a total of about 14 days. It was felt that this

would entail unnecessarily long experimental times; therefore the 24 h maximum experimental time was chosen. At this point the desorption run 91–0% R.H. was started and again the apparatus was left to record for the next 24 h. The computer was programmed to record the skin weight parameters every minute for the first 40 min and longer if the sample weight change was greater than 0.01 mg and if less, the computer would record every 10 min.

Preparation of stratum corneum samples

Stratum corneum membranes were prepared by the heat separation method of Kligman and Christophers (1963). Frozen (−20°C) human cadaver skin obtained post-mortem was thawed for 15–30 min and then most of the subcutaneous fat was scraped off using a razor blade. The skin specimens were placed in water at 60°C for 45 s, blotted dry and then the stratum corneum was gently peeled off. This was placed epidermal side down in a Petri dish containing 0.0001% w/v trypsin (Bovine Pancreas Type III, Sigma Co., U.S.A.) and 0.5% w/v sodium bicarbonate (B.D.H., U.K.) in distilled water, and incubated at 37°C overnight. Any excess epidermis still adhering to the stratum corneum samples was gently removed using forceps. Then the samples were placed on a thin wire mesh sprayed with P.T.F.E. and blotted with moistened cotton wool. They were cut into suitable pieces and laid on a wire mesh in a dessicator.

Data treatment

Calculation of diffusion coefficients

The absorption process involves the entrance and dissolution of water molecules at the stratum corneum interface and then their subsequent diffusion into the deeper regions of the stratum corneum. The interfacial process is relatively fast and the diffusion through the stratum corneum controls the rate at which the water molecules accumulate within the tissue. The value of a diffusion coefficient gives information about how fast water molecules, for example, are diffusing through the stratum corneum sample with time. Net diffusion or flux is measured as the amount passing at right angles to a point or barrier with time. The flux (J) is defined by Fick's first law of diffusion (Fick, 1855):

$$J = -D\frac{dc}{dx} \tag{1}$$

where D is the diffusion coefficient for a substance in a medium, at a particular temperature and pressure, with units of length^2 · time^{-1}. To simplify equations, D is taken as a constant under the assumption that the solute molecules have no influence on each other's movement, otherwise absolute concentrations would appear in Fick's law as well as the concentration gradient. dc/dx is the change in concentration c of the diffusing substance across a distance x. The negative sign occurs since the diffusion is taking place in the opposite direction to the concentration gradient. An alternative formulation is:

$$\frac{dc}{dt} = D\frac{d^2c}{dx^2} \tag{2}$$

This is termed Fick's second law of diffusion and shows that the rate of diffusion is dependent on the rate of change of the penetrant concentration gradient across a barrier (stratum corneum in this case). In general, D should increase as the water molecules enter the membrane and increase its water content and hence reduce membrane resistance. We arranged experimental conditions, and approximated equations in such a way that D values were assumed constant (see below). D can be determined experimentally from the rate of water vapour absorption and desorption by the stratum corneum. Taking the distance across the stratum corneum membrane in terms of x and letting x at the barrier surface be $\pm\ell$ and when the concentration at each of these surfaces remains constant and equal to one another, with the initial membrane concentration equal to zero then Fick's second law may be solved to find the short term values of fractional uptake (Crank, 1975):

$$\frac{M_t}{M_\infty} = 2\left(\frac{Dt}{\ell^2}\right)^{1/2}\left(\pi^{-1/2} + 2\sum_{n=1}^{\infty}(-1)^n \text{ierfc}\frac{n\ell}{\sqrt{Dt}}\right) \tag{3}$$

where M_t = amount diffused into the skin at time t; M_∞ = amount diffused into the skin at infinite time (in these experiments taken as 24 h); n = an integer from 1 to infinity.

Calculation of initial diffusion coefficient (D_i)

It is possible to deduce an average diffusion coefficient value from Eqn. 3 using the initial slope of an absorpton curve when M_t/M_∞ is plotted against the square-root of time. Thus, in the very early stages of sorption or desorption, for a constant diffusion coefficient D_i and a sheet of thickness ℓ:

$$\frac{M_t}{M_\infty} = \frac{4}{\pi^{1/2}}\left(\frac{D_i t}{\ell^2}\right)^{1/2} \tag{4}$$

If the initial slope of the curve is given by:

$$m = \frac{dM_t}{dt^{1/2}} \tag{5}$$

then by substituting Eqn. 5 into Eqn. 4:

$$D_i = \frac{\pi \ell^2}{16}\frac{m^2}{M_\infty^2} \tag{6}$$

From this equation D_i may be calculated since m and M_∞ are determined experimentally and ℓ (stratum corneum thickness) can be determined experimentally or taken from the literature. For D_i values reported in this paper estimates of ℓ were taken from those reported by Blank et al. (1984) – 10 μm for stratum corneum at 0% R.H. and 15 μm for that kept at 91% R.H.

Calculation of the half-value diffusion coefficient ($D_{1/2}$)

An appropriate solution of the diffusion equation applicable to our experimental arrangement is (Crank, 1975):

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2} \times \exp\left(-D(2n+1)^2\pi^2\frac{t}{\ell^2}\right) \tag{7}$$

Here the uptake of water vapour is considered to be a diffusion process controlled by a constant diffusion coefficient D. An assumption is also made that immediately the stratum corneum is placed in the vapour, the concentration at each surface attains a value corresponding to the equilibrium uptake for the vapour pressure existing, and remains constant afterwards. The stratum corneum is also considered to be free of vapour initially.

The value of t/ℓ^2 for which $M_t/M_\infty = 1/2$, which can for convenience be written as $(t/\ell^2)_{1/2}$ is given by:

$$\left(\frac{t}{\ell^2}\right)_{1/2} = -\frac{1}{\pi^2 D_{1/2}} \cdot \ln\left(\frac{\pi^2}{16} - \frac{1}{9}\left(\frac{\pi^2}{16}\right)^9\right) \tag{8}$$

approximately, the error being about 0.0001%. Thus:

$$D_{1/2} = \frac{0.049}{(t/\ell^2)_{1/2}} \tag{9}$$

and hence values of $D_{1/2}$ may be calculated since t and ℓ are known.

Calculation of rate constants (Anderson et al., 1973)

Water absorption process

If the weight of water absorbed per unit weight of dry tissue (W) is plotted against time (t) in hours and for the stationary system the linear portion of this curve is extrapolated back to the weight axis to produce a value termed W_B, then two rate constants may be calculated, K_0^H and K_1^H (Fig. 5). The water absorption process appears to take place in two stages: (a) an initial stage that is approximately first-order, described by Eqn. 10:

$$\frac{dW}{dt} = K_1^H(W_B - W) \tag{10}$$

$$(0 < W < W_B)$$

and (b) a zero-order stage, described by:

$$\frac{dW}{dt} = K_0^H \tag{11}$$

K_1^H and K_0^H are the first- and zero-order absorption rate constants, respectively, and W_B is the weight of water absorbed by the first-order stage. The rate constant K_0^H is determined from the slope of the final linear portion ($W > W_B$) of the W vs t plot.

Water desorption process

The desorption process is not a simple reversal of the absorption stage. It can be described by pseudo-first-order kinetics:

$$\frac{dW}{dt} = -K_1^D W \tag{12}$$

where K_1^D is calculated from the slope of a ln W vs t plot. The values of K_1^D change during an experimental desorption run and in this paper only the initial K_1^D values were calculated.

Parameters recorded or calculated

(1) Initial stratum corneum sample weight (mg).
(2) Weight of water vapour absorbed or desorbed after 24 h (mg), M_∞ divided by mg of dry stratum corneum (W).
(3) Slope m ($mg \cdot h^{-1/2}$) of the plot of the uptake or loss of water vapour by stratum corneum samples against the square root of time, and the correlation coefficient of this line.
(4) Initial diffusion coefficients D_i ($cm^2 \cdot h^{-1}$) and $D_{1/2}$ ($cm^2 \cdot h^{-1}$).
(5) Values of W_B (mg) and the rate constants K_1^H, K_0^H and K_1^D (min^{-1}).

Results and Discussion

An interesting observation which emerged from these experiments was that significant differences could be seen with absorption and desorption patterns between the stationary (S) and flow (F) systems (Fig. 4). At 24 h in the S system the water vapour was still being absorbed giving K_0^H of 0.74×10^{-4} min^{-1} (Table 1), whilst in the F system the skin had already reached equilibrium water content and K_0^H was zero. This pattern was observed in every experiment performed: in some runs the flow M_∞ value was reached 15–20 h from the start of the experiment. Anderson et al. (1973) reported a similar absorption/desorption pattern with their S system and found that equilibrium water content values were reached only after 14 days. We performed a few experiments for 3–4 days to check whether our M_∞ values would also increase steadily over this period. The results from this study were in agreement with those of Anderson et al. (1973) since K_0^H values were found to be the same after the 3–4 day period for a stratum corneum sample. For approximately 100 experimental runs values of K_0^H were $0.08–1.94 \times 10^{-4}$ min^{-1}. Anderson reported a range of $0.90–3.27 \times 10^{-4}$ min^{-1} for 10 stratum corneum samples (95% R.H. and 30°C), and a W value of 0.47 ± 0.15 mg water per mg dry tissue. Our values tended to be slightly lower 0.35 ± 0.16 mg water per mg dry tissue but we were working at a lower R.H. The values for K_1^D (desorption) were in agreement with Anderson et al. These authors reported values of 0.02–0.075 min^{-1}, ours being 0.06–0.26 min^{-1} for 100 runs.

No significant difference was observed between the S and F system initial D_i absorption values. For example, D_i (F) was $1.15 \pm 0.81 \times 10^{-7}$ cm^2 h^{-1} ($n = 4$) and D_i (S) was $0.55 \pm 0.24 \times 10^{-7}$ $cm^2 \cdot h^{-1}$ ($n = 4$); $P > 0.05$ using Student's *t*-test for significance testing. If, however, the desorption D_i values of the same skin samples were studied, it could be seen that significant difference ($P = 0.01$) occurs between D_i values for F and S (D_i (F) $5.23 \pm 2.77 \times 10^{-7}$ $cm^2 \cdot h^{-1}$ ($n = 4$) and D_i (S) $11.27 \pm 4.6 \times 10^{-7}$ $cm^2 \cdot h^{-1}$). This difference may be accounted for by the fact that in the S system the final water content M_∞ was higher than in the F system (for example, 0.252 mg (S) and 0.065 mg (F); values taken from the four samples analyzed above). When the stratum corneum sample then came into contact with dry air at 0% R.H., the excess water was lost more readily from the S sample. The range of water diffusion coefficients quoted in the literature have been found to be higher than the ones reported here. Blank et al. (1984) obtained coefficients ranging from 9.0 to 34.45×10^{-7} $cm^2 \cdot h^{-1}$ in three subjects and relative humidities of 46–93% (30 to 31°C). Scheuplein (1967, 1975) reported values of 3.6×10^{-7} $cm^2 \cdot h^{-1}$ for unhydrated stratum corneum rising to

TABLE 1

EXAMPLE RESULTS OBTAINED FOR ONE PIECE OF STRATUM CORNEUM (MALE, AGED 74)

The initial sample weight was recorded (mg), mg of water vapour absorbed or desorbed per mg of dry tissue weight (W), M_∞ (mg), slope m of the graph of water absorbed or desorbed against square-root of time and the correlation coefficient (C.C.), D_i the initial diffusion coefficient, $D_{1/2}$ the diffusion coefficient at $M_t/M_\infty = \frac{1}{2}$, and rate constants. K_1^H, K_0^H (absorption) and K_1^D (desorption).

Run no.	Absorption (A) or desorption (D)	R.H. (%)	Initial skin weight (mg)	mg H_2O per mg dry tissue (W)	M_∞ (mg)	Slope m ($mg \cdot h^{-1/2}$) and C.C.	$D_i \times 10^7$ ($cm^2 \cdot h^{-1}$)	$D_{1/2} \times 10^7$ ($cm^2 \cdot h^{-1}$)	$K_1^H \times 10^4$ (min^{-1})	$K_0^H \times 10^4$ (min^{-1})	$K_1^D \times 10^4$ (min^{-1})
1	A	0–91 (S)	10.624	0.121	1.281	0.8697 (0.9973)	0.91	0.66	0.029	0.740	–
2	D	91–0 (S)	11.905	0.112	1.185	1.944 (0.9987)	11.8	5.52	–	–	0.174
3	A	0–91 (F)	10.693	0.065	0.694	0.7526 (0.9903)	2.31	1.76	0.028	0	–
4	D	91–0 (F)	11.385	0.062	0.660	0.7299 (0.9980)	5.41	2.59	–	–	0.095

36×10^{-7} cm$^2 \cdot$ h^{-1} for hydrated (water contact) samples (25°C). All authors reported large sample-to-sample variation. Scheuplein and Morgan (1967) also agreed with our observation that desorption occurs faster than absorption. Possibly our D_i values were lower since we were using skin taken from people over the age of 60, and this type of skin is known to have a low content of natural moisturizing factors which aid water binding within the stratum corneum (Quattrone and Laden, 1976). In addition, Anderson et al. (1973) have suggested that stratum corneum samples appear to fall into two groups with respect to the amount of bound water held by the tissue. These types depend on the age of the donor, with the lowest amount of bound water existing in the oldest skin and thus leading to the lower diffusion coefficients.

For 100 runs $D_{1/2}$ was always lower than D_i (absorption) which at first may suggest that it was becoming more difficult for the water molecules to diffuse through the stratum corneum at the time when $M_t/M_\infty = 1/2$, compared to the first few minutes of absorption. This is unlikely to be true as greater hydration levels should lead to faster diffusion rates; it is probably unsafe to compare D_i to $D_{1/2}$ directly because of the different assumptions and approximations made in the relevant equations. In each case, the $D_{1/2}$ value (absorption) for S was lower than for F (for example, $D_{1/2}$ was 0.66×10^{-7} and 1.76×10^{-7} cm$^2 \cdot$ h^{-1} for S and F, respectively). The probable reason for this was that time t in Eqn. 9 was read off from graphs of W vs time (Fig. 5) against $M_\infty/2$ values which were higher for S than for F. Thus care should be taken in comparing $D_{1/2(\text{stationary})}$ with $D_{1/2(\text{flow})}$.

In order to calculate D_i, a graph of mg water absorbed or desorbed was plotted against the square root of time (Fig. 6). This should give a straight line with correlation coefficients approaching one. In practice, with the F system it was found that the initial few points on this graph tended to curve and had to be ignored, i.e. a lag period was noted. In order to reduce this lag the reservoir and tap system was included in the apparatus. With this modification the incoming air would only have to displace the volume within the microbalance chamber instead of the entire apparatus and a volume of air at the correct R.H. was available for 'dumping' into the head. Lag times were reduced to a few minutes with the reservoirs in action. This was not a problem with the stationary system.

Anderson et al. (1973) reported that desorption rates (K_1^D) did not vary with air flow rates of 0–185 ml $\cdot$ min^{-1}. We therefore investigated changes in M_∞ with respect to air flow rates during absorption and desorption for 3 stratum corneum samples. At 91% R.H. changes in air flow rate from 5 to 85 ml $\cdot$ min^{-1} increased M_∞, for example, from 0.737 to 0.861 mg for sample 1

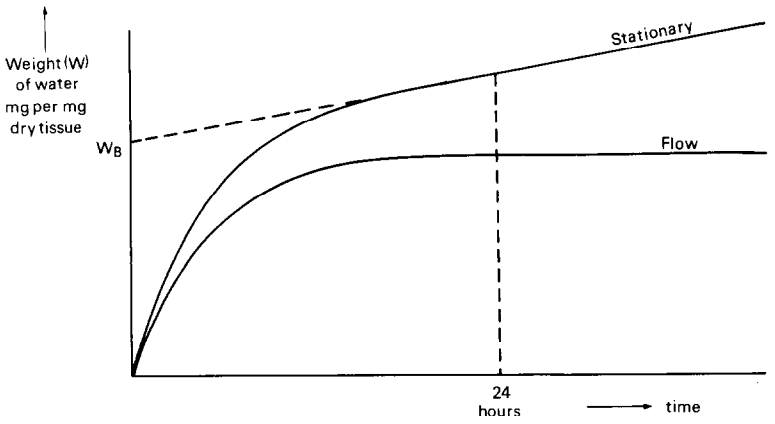

Fig. 5. Example plot of weight of water vapour adsorbed per mg of dry tissue (W) against time in hours. M_∞ values were taken at 24 h for both stationary (for which W_B could be determined) and for the flow systems.

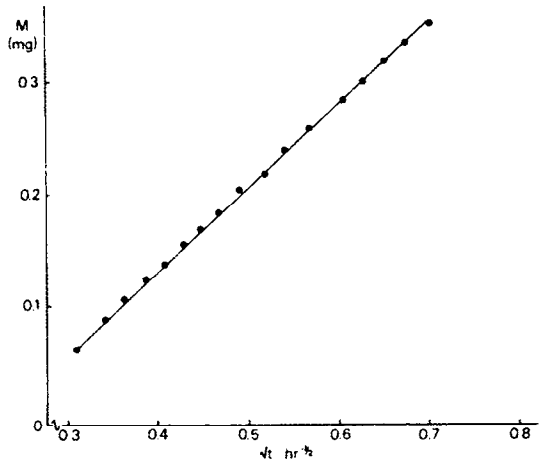

Fig. 6. A plot of uptake of water vapour in mg (M) for run number 3 (Table 1) against the square-root of time in hours. The correlation coefficient was 0.9903. From the slopes of these plots values of D_i for each run could be calculated.

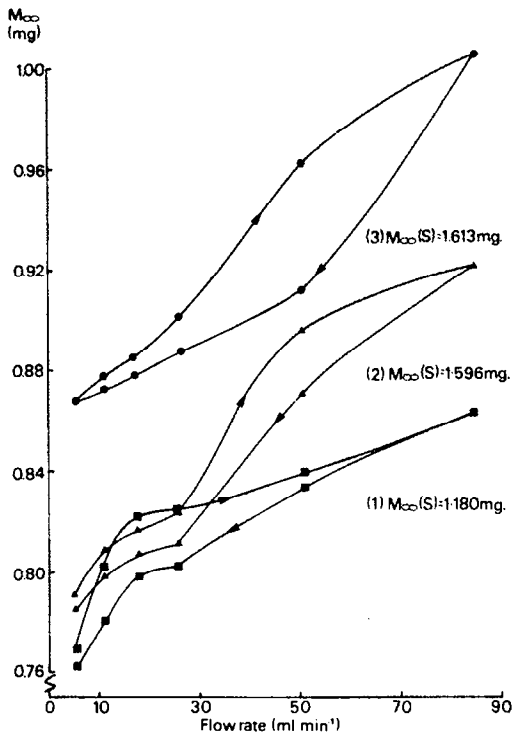

Fig. 7. A plot of the final weight of water vapour absorbed (M_∞) in mg against the flow rate of incoming air ($ml \cdot min^{-1}$) for three stratum corneum samples tested. Sample 1, female aged 81; sample 2, male aged 74; sample 3, male aged 62. The values of M_∞ are plotted during the increase and then decrease of flow rate (5–85 and then 85–5 $ml \cdot min^{-1}$). The stationary (S) values of M_∞ attained by the stratum corneum samples are also shown.

(Fig. 7). The variation between stratum corneum samples is typical of skin variation in general. The M_∞ value was higher when air flow rates were increased showing a hysteresis effect. The stationary values were significantly higher ($P < 0.001$) (1.180 mg for Sample 1), and yet from the graph we would expect this value to be nearer the M_∞ for a flow rate of 5 $ml \cdot min^{-1}$, since the stationary system can be looked upon as being equivalent to an air flow rate of 0 $ml \cdot min^{-1}$.

Variations in air flow rates during desorption (using three stratum corneum samples and the same rate changes) had no influence on the final M_∞ attained. However, stationary values were significantly higher ($P < 0.001$). Hysteresis in the absorption of water by proteins is usually attributed to the formation of microscopic pores, cracks, or capillaries in the protein–water system (Scheuplein, 1975). The system, at a given hydrating activity, will reach equilibrium at different water contents, depending upon whether this hydrating activity is reached from higher or lower values (filling or emptying of the capillaries).

Another apparatus modification made was to include a constant level device with the Dreschell water bottle. This aided the investigation since all experiments were conducted overnight without the attendance of the investigators, and hence there were little chance of the water being used up and the R.H. falling as a result during absorption experiments. Drierite was also found to be a more satisfactory drying agent than silica gel which was used initially.

At relative humidities between 0% and 80%, Anderson et al. (1973) reported that the 1- and 14-day hydration values (W) were the same; at relative humidities 80–95% the 1-day values were less than the 14-day values, and at a relative humidity of 95%, the 14-day value was approximately 10 times higher than the 1-day value. These investigators were not sure whether their samples had reached equilibrium even after this 14-day exposure. They did not employ longer test times because of sample deterioration. These differences in W, for example, were 0.53 mg water/mg dry tissue at 94% relative humidity and 7.46 mg water/mg dry stratum corneum at 95%. These data provided the reason why in the present inves-

tigation a relative humidity of 91% was chosen since the region in which rapid increases in W occur (94–95%) was thus avoided. An additional reason was that Anderson et al. (1973) quoted 1-day, i.e. 24 hour values, for their W and rate values and their data could therefore be used for comparison.

Anderson et al. (1973) included results from their thermodynamic studies (infrared and NMR) together with data from water absorption and desorption using a stationary system. The data supported their model for stratum corneum hydration. They proposed that there are at least three types of water within the hydrated stratum corneum. The first species is present to the extent of about 0.5 mg water/mg dry tissue, but for older tissue this may be nearer 0.3 mg/mg dry tissue. This "strongly bound" water is probably associated with the polar groups of the keratin side-chains in the corneocytes together with some lipid-bound water. Infrared analysis suggests that this water constitutes two fractions: primary hydration of ionic groups and the associated secondary hydration. Hydration kinetic data as a function of relative humidity indicated that the amount of this initially absorbed "bound" water increases as the total water content rises (i.e. as the water activity of the environment increases). These authors' data for desorption using disrupted tissue samples suggests that this water is mainly intracellular.

The second main water species is present from the 0.5 mg water/mg dry tissue level up to 2–12 mg water/mg dry stratum corneum and is associated with higher relative humidity levels. This is more like bulk liquid water in its hydrogen bonding capabilities and the hydration from this species is associated with zero-order kinetics with respect to time, but again depends upon the activity of the hydrating environment. Both hydration and dehydration rates for this species are lower than those observed for the first-order.

Anderson claims that this "free" water is not strongly associated with the stratum corneum constituents, but is physically restricted in hydration and dehydration by some barrier. The dehydration rate constant K_1^D was not only a function of the water content of the sample but also of the initial water content before dehydration.

From our data and from the model postulated by Anderson et al. (1973), it seems that this "free" water species may be essentially absent in the F system. We postulate that as hydration swells the stratum corneum membrane, intercellular channels containing liquid water form, the water then slowly diffusing into the corneocytes. In the F system the passage of air prevents these channels filling with water and only the "bound" type of water develops. This may therefore account for the plateau effect seen in the F system (Fig. 5). However, our water content levels were lower than those reported by Anderson et al. (1973) who used stratum corneum samples on average from younger patients (we used skin from patients aged 60 years and older). We particularly chose older skin since it is known to lack natural moisturizing factors and lipids and one of our main objectives is to use this technique to examine the effect of moisturizers. The ability of stratum corneum to retain liquid-like water either as a surface film or within the intercellular spaces has been shown to depend upon the presence of skin lipids (Foreman, 1976; Foreman et al., 1979); these authors also postulated the existence of three types of water within the hydrated human stratum corneum.

An alternative hypothesis is that a surface layer of water develops under stationary conditions possibly trapped in surface fissures of desquamating cells but the flow arrangements prevents this layer forming. The situation is further complicated by the possible influence of stationary layers and the effects of mass transport phenomenon hinted at by, for example, the data shown in Fig. 7. Further work is required to elucidate the mechanism of the difference in water uptake behaviour shown by the flow and stationary systems.

Conclusions

(1) An apparatus was developed for the study of water vapour absorption and desorption in stratum corneum in vitro. The apparatus could be used under stationary conditions mimicking covered areas of the body in vivo, or a flow regime representing exposed regions of the skin.

(2) Experiments may be performed from room temperature upwards controlled to within $\pm 0.2°C$. In this study 32°C was chosen.

(3) The apparatus is automatic and may be left unattended for long periods during which results are recorded on the computer.

(4) Stratum corneum samples may be tested under controlled conditions, treated with, for example, moisturizers, and then re-tested under the same conditions.

(5) Values for rate constants, water vapour sorbed and desorbed after 24 h, initial diffusion coefficients D_i and $D_{1/2}$ half-value coefficients may be calculated.

(6) One skin specimen may be used having the additional benefit of no inter-specimen variation, for absorption and desorption for both stationary and flow systems.

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References

Allenby, A.C., Fletcher, J., Schock, C. and Tees, T.F.S., The effect of heat, pH and organic solvents on the electrical impedance and permeability of excised human skin. Br. J. Dermatol., 81 (1969) 31–39.

Anderson, R.L., Cassidy, J.M., Hansen, J.R. and Yellin, W., Hydration of stratum corneum. Biopolymers, 12 (1973) 2789–2802.

Aubert, L., Anthione, P., De Rigal, J. and Leveque, J.L., An in vivo assessment of the biomedical properties of human skin modifications under the influence of cosmetic products. Int. J. Cosmet. Sci., 7 (1985) 51–59.

Barry, B.W., Dermatological Formulation. Percutaneous Absorption, Dekker, New York and Basel, 1983.

Blank, I.H., Moloney, J., Emslie, A.G., Simon, I. and Apt, C., The diffusion of water across the stratum corneum as a function of its water content. J. Invest. Dermatol., 82 (1984) 188–194

Crank, J., The Mathematics of Diffusion, 2nd edn., Oxford Univ. Press (Clarendon), 1975, pp. 48, 238–239, 244–245.

Fick, A., Uber diffusion. Poggendorffs, Ann., 94 (1855) 59–86.

Foreman, M.I., A proton magnetic resonance study of water in human stratum corneum. Biochem. Biophys. Acta, 437 (1976) 599–603.

Foreman, M.I., Bladon, P. and Pelling, P., Proton NMR studies of human stratum corneum. Bioeng. Skin, 2 (1979) 48–58.

Gloor, M., Willebrandt, V., Thomer, G. and Kupferschmid, W., Water content of the horny layer and skin surface lipids. Arch. Dermatol. Res., 268 (1980) 221–223.

Grice, K., Sattar, H. and Baker, H., Urea and retinoic acid in ichthyosis and their effect on transepidermal water loss and water holding capacity. Acta Dermatol. Vener., 53 (1973) 114–118.

Jongh de, G.J., Porosity of human skin in vivo assessed via water loss, CO_2 loss and electrical impedance for healthy volunteers, atopic and psoriatic patients. Curr. Probl. Dermatol., 9 (1981) 83–101.

Kligman, A.M. and Christophers, E., Preparation of isolated sheets of human stratum corneum. Arch. Dermatol., 88 (1963) 702–705.

Leveque, J., Physical methods for skin investigation. Int. J. Dermatol., 22 (1983) 268–275

Nilsson, G.G., Measurement of water exchange through skin. Med. Biol. Eng. Comput., 15 (1977) 209–218.

Quattrone, A.J. and Laden, K., Physical techniques for assessing skin moisturization. J. Soc. Cosmet. Chem., 27 (1976) 607–623.

Scheuplein, R.J., Mechanism of percutaneous absorption II. Transient diffusion and the relative importance of various routes of skin penetration. J. Invest. Dermatol., 48 (1967) 79–88.

Scheuplein, R.J., The permeability of the skin to gases. Annual Report U.S. Army Research Office, June (1970), pp. 1–32.

Scheuplein, R.J., Sorption and retention of substances in the surface layers of the skin. Final Technical Report U.S. Army Research Office DAAC 19-72-G-0014, February (1975) pp. 1–38.

Scheuplein, R.J. and Morgan, L.J., Bound water in keratin membranes measured by a microbalance technique. Nature (London), 214 (1967) 456–458.

Walkley, K., Bound water in stratum corneum measured by differential scanning calorimetry. J. Invest. Dermatol., 59 (1972) 225–227.

Wildnauer, R.G., Bothwell, J. and Doublass, A., Stratum corneum biomechanical properties. Influence of relative humidities on normal and extracted human stratum corneum. J. Invest. Dermatol., 56 (1971) 72–78.