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THE PERMEABILITY OF SKIN TO ALBUMIN, DEXTRANS AND POLYVINYL PYRROLIDONE*

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Many measurements have been made of the penetration of small molecules through skin (1, 2). Bacteria (3, 4) and colloidal sulfur particles (5) are also known to penetrate skin, but their exact penetration rate is unknown. We have measured the skin penetration of certain large molecules which are available labelled with radio-iodine, in order to determine the upper limit of molecular size which will penetrate skin readily.

METHODS

(a) *Labelled polymers*

(*i*) *Albumin.* Iodinated human serum albumin (HSA) labelled with I131 at 10 mC/g was supplied by the Radiochemical Centre, Amersham, in 0.9% NaCl + 1% benzyl alcohol. It was separated from inorganic iodide on a dextran column (Sephadex G25, Pharmacia) and stored for up to 8 days at 4°C in a concentration of 0.8-50 mg/ml. This solution was applied to the skin.

(*ii*) *Polyvinyl pyrrolidone* (PVP), labelled with I125 at 5 mC/g was supplied by the Radiochemical Centre at 12 mg/ml in a succinate buffer solution in contact with an ion-exchange resin (Deacidite FF, Permutit Co.). The solution was stored for up to 4 days at room temperature, and Amberlite IRA 400 resin (Rohn & Haas, Philadelphia) was added to it when it was applied to skin.

(*iii*) *Dextrans.* Two samples of dextran were supplied, of average molecular weight 9,400 and 153,000 respectively (Pharmacia fractions 10 and 150) labelled with I-125 at 0.1 mC/g by Dr. C. Ricketts, M.R.C. Burns Unit, Birmingham. It was stored for up to 5 days at 4°C as a 10 mg/ml aqueous solution in contact with Amberlite IRA 400 resin; the resin was renewed when the solution was placed on the skin.

(b) *Application to skin*

(*i*) *In situ.* Female albino rabbits (1.4–2 kg) were anesthetized with dial and urethane. The hair on one flank was clipped and a 7–15 cm^2 area was surrounded by a dam of silicone rubber (6). 0.4 ml of HSA-I131 solution was placed on this area and spread beneath a polyethylene cover. Blood samples were taken from the animal's ear vein at 2 hour intervals, and their I-131 content measured. After 6 hours the animal was killed and the contaminated skin was removed, using a scalpel to separate the dermis from the skin muscle. The skin muscle was then digested in HNO_3 and its I-131 content measured.

(*ii*) *Excised skin.* Clipped rabbit skin, with the skin muscle removed, was obtained from freshly-killed brown or albino rabbits. Human half-thickness (Thiersch graft) thigh skin was also obtained. Each piece of skin was placed in a small diffusion cell (area 0.3 or 0.8 cm^2), similar in design to those described by Ainsworth (7), and maintained at a temperature of 36°C. 200 $\mu l/cm^2$ of radioactive polymer solution was placed on the epidermal surface and the dermal surface was bathed in 0.9% NaCl solution containing 100 μg/ml chloramphenicol. The saline was renewed at 2 hour intervals, and its isotope content measured.

RESULTS

Skin Penetration in situ

I-131, from iodinated human serum albumin (HSA) on the flank of an anesthetized rabbit, appeared in measurable quantities in its bloodstream. The concentration of I-131 in the blood rose steadily throughout the experiment (Fig. 1). The quantity of HSA associated with this I-131 was calculated on the assumption that it distributed throughout the blood (57 ml/kg; ref. 8) but not elsewhere in the system. The systemic uptake was thus calculated to be 0.4–2.9 μg. A further, usually smaller, quantity was found in the skin muscle beneath the site of application (Table I). Taken together, 1.3–5.0 μg HSA penetrated the skin in each experiment. The average penetration rate (r) over the 6 hours' exposure was calculated from this total, and hence the permeability constant (p) obtained; $p = r/C$, where C = concentration of HSA applied (ref. 6). The units of permeability constant used here are cm/min x 10^{-6} = μcm/min and cm/min x 10^{-9} = ncm/min.

Excised skin penetration

I-131 from HSA penetrated skin at an approximately constant rate during 20 hours of contact between the HSA solution and the skin

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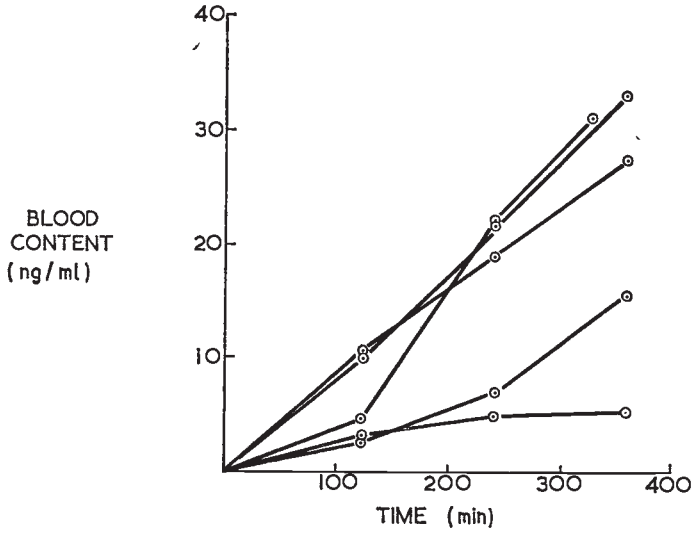

FIG. 1. Penetration of HSA-I131 into rabbit blood from a 40 mg/ml solution on 10 cm² of flank skin.

(Fig. 2). It also penetrated human skin in a similar manner. The penetration rate was extremely slow. It was unaffected by the addition of further unlabelled HSA to the radioactive solution *i.e.* Fick's law was obeyed.

After 20 hr a small fraction of the I-131 on the skin surface had become detached from the HSA, according to Sephadex and electrophoretic separations (vertical paper electrophoresis, using 2.5 V/cm for 16 hours across Whatman 3 MM paper soaked in barbitone buffer). Electrophoresis of the saline from the dermal surface showed that 50% of the penetrant I-131 was still attached to the HSA *i.e.* the actual penetration rate of HSA was 0.5 −1 × that calculated on the assumption that all the I-131, during penetration of the epidermis, remained bound to the HSA. The permeability constant of rabbit skin calculated on this assumption was, on average, approx. 3 × that found *in situ* although the difference was not significant (Table II). HSA penetrated human skin more slowly.

I-125 from polyvinyl pyrrolidone (PVP) penetrated rabbit skin at an even slower rate than HSA. This rate remained constant within one experiment (Fig. 2), but throughout the experimental series there was a consistent increase of rate, as if the iodide content of the radioactive solution were rising, despite the ion-exchange resin. Only the first few experiments were therefore accepted.

TABLE I

Penetration of rabbit skin in situ by iodinated human serum albumin from a 23–40 mg/ml solution on 7–15 cm² of a rabbit's flank for 6 hours

Expt.	Blood content* μg HSA	Skin muscle content μg HSA
1	2.35	2.61
2	0.45	0.83
3	2.18	0.58
4	2.85	0.64
5	1.31	0.34

* Calculated from the measured blood concentration of I-131, and an assumed distribution of 57 ml/kg body weight (8).

A similar phenomenon occurred with the iodinated dextrans, and only the first few experiments were accepted. The penetration rate of the smaller dextran was the fastest rate of any of the polymers tested (Table II).

DISCUSSION

The apparent penetration rates of these polymers are much less than those of smaller molecules, or of ions (1, 2, 6). Dissociation from a polymer of a small proportion of the radioactive iodine in the source solution on the skin would provide a source of iodide which might penetrate as fast as, or faster than, the polymer-bound iodine. HSA on the skin did

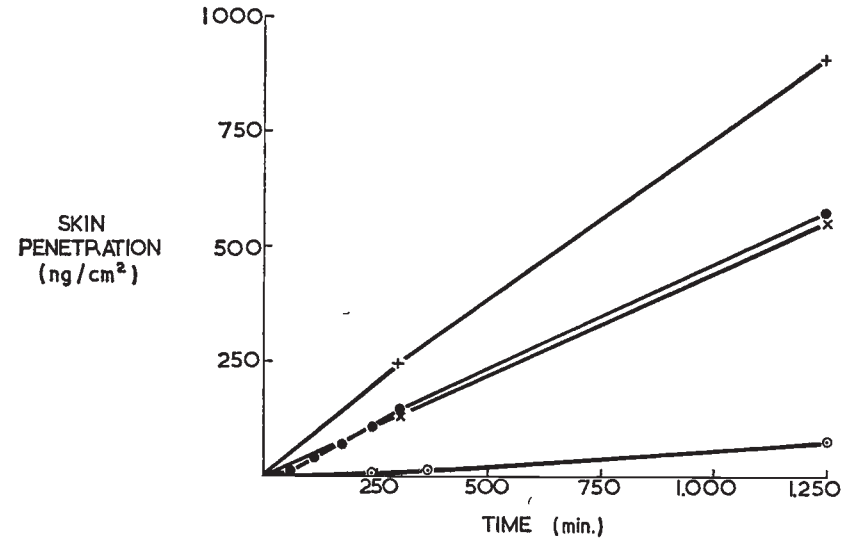

FIG. 2. Penetration of excised rabbit skin by 7 mg/ml HSA (●), 12 mg/ml PVP (⊙), 10 mg/ml dextran 10 (+) and 10 mg/ml dextran 150 (×).

TABLE II

Permeability of skin to polymers, calculated from their penetration of excised skin

The mean of each set of observations and its standard error are given, with the number of experiments in brackets.

Molecule	Contact time (hr)	Permeability constant (ncm/min.)
(a) *Rabbit skin*		
Human serum albumin	24	63 ± 27 (8)
Human serum albumin (in situ)	6	23 ± 5 (5)
Polyvinyl pyrrolidone	6	6 ± 2 (5)
Dextran fraction 10	24	108 ± 53 (6)
Dextran fraction 150	24	40 ± 17 (6)
(b) *Human skin*		
Human serum albumin	24	24 ± 2 (4)
Polyvinyl pyrrolidone	6	3 (1)

decompose slightly, and half of the I-131 which penetrated from it was inorganic iodide. There is no direct evidence of decomposition of the dextran or the PVP, but the increasing rate obtained during each experimental series indicates that the polymeric iodide dissociated over the course of days. The apparent permeability constants quoted here, small though they are, are therefore maxima: the true permeability of skin to HSA is likely to be at least half this, since half of the penetrant I-131 was still attached to the polymer, but the true permeability to PVP or dextran might be a great deal less than its apparent value.

With this proviso, one may compare the present results with the skin penetration of smaller molecules. Many small covalent aqueous solutes have permeability constants through rabbit skin in the region of 20 μcm/min (1, 2), which is 200–3,000 × the values quoted in Table II. Part of this ratio is explicable simply by the slower motion of the large molecules; at a given temperature $pM^{1/2}$ = constant (1) in a solution or in a structure whose pores are large relative to the diffusing molecule. The molecular weight of the rapidly penetrating molecules is $\leq$ 300, that of the polymers 10,000–150,000. Taking average values of 150 and 80,000, respectively, the ratio of the square roots of the molecular weights is 23. This leaves a factor of 8–150 × of the original permeability constant ratio, to be accounted for in some other way.

If one may regard the "barrier layer" of the skin as a porous system, then this large ratio shows that most of the pores within it are too small to accommodate the polymers. In Pappenheimer's terminology (9) the "available area" for diffusion is much smaller for the polymers than for the small molecules, in Kedem and Katchalsky's terminology (10) the "frictional coefficient between solute and membrane" is much greater.

The present data are inadequate to decide

between theories of epidermal porosity; they show simply that these polymers are too large to go easily through the system. For a finer analysis one requires an intermediate range of molecular sizes, with a very firmly attached radioactive label.

SUMMARY

1. The penetration of polymers through excised rabbit and human skin, and rabbit skin *in situ*, has been measured. Radio-iodinated human serum albumin (HSA), polyvinylpyrrolidone (PVP) and dextran were used.

2. All the polymers penetrated excised skin very slowly: PVP penetrated slowest, and a small-M.W. dextran the fastest.

3. HSA penetrated rabbit skin *in situ* at a slightly slower rate than excised rabbit skin.

4. The results are interpreted in terms of permeability constants, and compared with similar data for smaller molecules.

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