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The exclusion of human serum albumin by human dermal collagenous fibres and within human dermis

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Preparations of human dermal collagenous fibres and slices of human dermis have been equilibrated with ^{125}I-labelled monomeric human serum albumin. The space inaccessible to the albumin in the fibres and in the dermis was determined by subtraction of the accessible space, calculated from the radioactivity of the specimen, from its total fluid. For a fibre preparation examined in detail, the fluid exclusion was independent of the concentration of either albumin or collagen. Binding of albumin to the fibres was not demonstrable. Three fibre preparations excluded albumin from 3.75 ± 0.96, 3.55 ± 0.67, and 2.05 ± 0.39 g of fluid/g of collagen ($\pm$S.D.). Slices from three specimens of dermis excluded albumin from 1.45 ± 0.08 g of fluid/g of insoluble solids or 1.57 ± 0.11 g of fluid/g of collagen ($\pm$S.D.). Thus the exclusion of albumin by dermis was much less than expected from its content of collagenous fibres. On the basis of these data and the published composition of dermis, the concentration of albumin in the accessible interstitial space was estimated to be close to that in the plasma.

The volume of fluid accessible to plasma proteins in connective tissues is limited by the exclusion properties of the components of the interstitial space (Comper & Laurent, 1978; Watson & Grodins, 1978). Although exclusion in tissues was attributed initially to hyaluronate and proteoglycans (Ogston & Phelps, 1961; Gerber & Schubert, 1964; Laurent, 1964), recent studies of collagenous fibres (Wiederhielm & Black, 1976; Meyer *et al.*, 1977; Pearce & Laurent, 1977; Bert *et al.*, 1980) have shown that they too contribute significantly to exclusion (Rutili, 1978; Fox & Wayland, 1979). The plasma protein found most abundantly in the interstitial space is serum albumin. At least 60% of the body's albumin is extravascular (Rothschild *et al.*, 1955; Berson & Yalow, 1957; Schultze & Heremans, 1966; Katz *et al.*, 1970*a*) with most interstitial albumin being found in skin and muscle: 18 and 15% of the body's total respectively (Rothschild *et al.*, 1955).

In the present work, the fluid exclusion of albumin by preparations of dermal collagenous fibres and by intact human dermis have been measured from the equilibrium distribution of radioiodinated albumin. The space in dermis inaccessible to albumin was much less than could be calculated from the specific exclusion of albumin by collagenous-fibre preparations and the collagen content of the tissues.

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Materials and methods

Measurement of radioactivity

Radioactivity was measured in polystyrene tubes (15.6 mm × 125 mm; Amersham Corp., Oakville, Ont., Canada) with an automatic well-type gamma counter (Nuclear–Chicago, Des Plaines, IL, U.S.A.) adjusted to count ^{125}I optimally. The sample size was selected to give at least 5×10^4 counts in a pre-set interval; the volume was adjusted with water to 1 ml. All samples and controls for each experiment were counted together to minimize systematic errors.

Specimens and collagenous-fibre preparations

Skin from the posterolateral thorax of cadavers was obtained at autopsy in the morgue of the Vancouver General Hospital (with the informed consent of the next-of-kin and with the approval of the Human Experimentation Committee of this University). The sources of the specimens are summarized in Table 1. After dissection of the underlying fat, subcutaneous tissue and muscle, the epidermis was removed with a Stryker dermatome. For the studies of dermis, discs 12 mm in diameter were punched from the remaining tissue with a sharp cork borer, then sliced with a modified Stadie–Riggs (1944) slicer to a thickness of 1 mm. The water content of each dermis sample was determined by freeze-drying five discs, then drying further *in vacuo* over P_2O_5

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Table 1. *Source of specimens*

	Code no.	Age (years)	Sex	Principal cause of death
Collagenous fibres	F1	46	Female	Ruptured 'berry' aneurysm
	F2	71	Male	Atherosclerotic heart disease with myocardial infarction
	F3	33	Male	Motor vehicle accident with multiple injuries
Dermis	D1	69	Male	Ruptured atherosclerotic aortic aneurysm
	D2	58	Female	Bilateral bronchopneumonia
	D3	78	Male	Spontaneous intracerebral haemorrhage

to constant weight. The loss in weight was taken as the water content of the tissue (Sweeny *et al.*, 1963). Collagenous fibres were prepared from other donors by a procedure described elsewhere (Pearce & Laurent, 1977). The fibre preparations were stored at 4°C as a slurry containing 20mg of collagen/ml of phosphate-buffered saline (Dulbecco & Vogt, 1954) containing 0.2g of NaN_3/litre.

In the text below, the code numbers F1, F2 and F3, and D1, D2 and D3 are used to refer to the three collagenous fibre preparations and the three dermis samples respectively (Table 1).

Preparation of ^{125}I-labelled monomeric albumin

Approx. 150mg of human serum albumin (25g/100ml; lot no. 346-3; Connaught Laboratories, Toronto, Ont., Canada) was dialysed at 4°C against two changes of phosphate-buffered saline containing azide and then was applied to a 26mm × 910mm column of cross-linked allyl-dextran [Sephacryl S-200 (Superfine grade); Pharmacia (Canada) Ltd., Dorval, P.Q., Canada]. The column was eluted with the phosphate-buffered saline at 4°C; 10ml fractions were collected and their A_{280} measured. A sample from each tube in the major peak was examined by polyacrylamide-gel electrophoresis (Davis, 1964) and stained for protein by using the method of Holbrook & Leaver (1976), save that 5% (v/v) acetic acid was incorporated into the staining solution (W.-P. Chan, personal communication). Fractions containing more than a trace of dimer were discarded; those containing principally monomer were pooled. In four subsequent batches, similar pools were prepared from the corresponding tubes. The combined pools were concentrated to approx. 70mg of protein/ml by ultrafiltration at 4°C using a PM-10 membrane (Amicon Canada Ltd., Oakville, Ont., Canada). The homogeneity of the pool was checked by polyacrylamide-gel electrophoresis, as described above.

After dialysis against azide-free phosphate-buffered saline, a portion of the protein was iodinated with $Na^{125}I$ (17Ci/mg; NEN Canada Ltd., Montreal, P.Q., Canada) with lactoperoxidase/glucose oxidase beads (Enzymobeads; Bio-Rad Laboratories, Mississauga, Ont., Canada) as recommended by the manufacturer, save that 20μl (approx. 1.4mg) of the albumin monomer were used at 35°C for 30min. After centrifugation, the reaction mixture was applied to a 9mm × 600mm column of Sephacryl S-200 (Pharmacia) with azide-free phosphate-buffered saline as an eluent; 1ml fractions were collected and monitored for both A_{230} and radioactivity. The fractions showing absorbance and radioactivity were pooled, the volume was measured and the solution filtered through a 5μm-pore-size filter (Millipore Ltd., Mississauga, Ont., Canada). Bacterial growth was prevented by the addition of 0.2g of NaN_3/litre. The homogeneity of each preparation was assessed after polyacrylamide-gel electrophoresis by counting 2mm transverse slices of the gel. The specific radioactivity of the labelled monomer was 3.30×10^7c.p.m./g of albumin.

The protein content of both the labelled and unlabelled monomer was determined by a micro-Kjeldahl–Nesslerization procedure for nitrogen (King, 1951) and calculated by assuming 87.5μg of protein/μg-atom of nitrogen.

Equilibration with collagenous fibres

Approx. 1.5ml of the slurry of collagenous fibres were pipetted into each of six tared vials and the vials were centrifuged at 2000*g* for 10min at room temperature. A record was kept of the weight of all substances added to or withdrawn from each vial. After removal of as much as possible of the supernatant fluid from three of the vials, approx. 300μl of labelled albumin solution were added to each and the fibres suspended by gentle agitation. Only a portion of the supernatant was removed from the remaining three vials; a volume of labelled albumin solution equal to the volume of fluid estimated to be present in the vial was added to each and the contents were mixed as described above. In this manner, the collagenous-fibre concentration was adjusted to a desired value while maintaining the concentration of albumin relatively constant. The range of concentrations achieved was 15–100mg of collagen/g of solution. Before the addition of the albumin to the two sets of three vials, the concentration and specific radioactivity were adjusted by mixture of the stock labelled and unlabelled solu-

tions with the phosphate-buffered saline. The concentration range investigated was 10–60mg of albumin/g of supernatant fluid.

The vials were kept at 4°C for 96h then allowed to warm to room temperature before withdrawal of the fluid for counting. At the lower concentrations of collagenous fibres the supernatant fluid was withdrawn by suction after simply tilting the vial after the fibres had settled. The vials with higher fibre concentrations were centrifuged at 750*g* for 10min at room temperature to pack the fibres into a pad. After either procedure, duplicate 50μl portions of the fluid were weighed into counting vials.

After withdrawal of the fluid, the residue in the vial was liquefied by digestion with papain (Pearce & Mathieson, 1967); papain (32.6mg/ml; twice-crystallized; Worthington Biochemical Corp., Freehold, NJ, U.S.A.) was diluted to 1mg/ml with 0.1M-sodium acetate buffer, pH5.5, containing 5mM-EDTA and 5mM-L(+)-cysteine hydrochloride, then activated at 55°C for 30min; 10μl were added to each vial for each mg of collagen estimated to be present. After digestion overnight at 55°C, duplicate weighed samples of the digest were taken for both the assay of radioactivity and the measurement of collagen. Collagen content was determined by a micro-modification of the method of Woessner (1961) as described previously (Bert *et al.*, 1980). The samples for assay of radioactivity were chosen to give approximately the same counting rate as 50μl of the corresponding supernatant fluid. The albumin contents of the digest and the supernatant fluid were used to calculate exclusion and recovery.

Equilibration of dermis

The experiments with dermis were similar to those described above. Seven discs from each dermis were placed in separate vials, each layered with the labelled albumin solution and stored at 4°C. The approach to equilibrium was monitored in two samples by measuring the albumin concentration in the supernatant fluid. The remaining five vials were treated similarly to the fibre preparations from which the supernatant fluid was collected by settling.

Binding of albumin to fibres

About 1.5ml of a 2% (w/w) slurry of collagenous fibres were placed in each of three tared vials and centrifuged at 750*g* for 10min at room temperature. All fluids added to or withdrawn from the vial were weighed. The clear fluid above the pad was removed and 1.0ml of stock labelled albumin solution added. The mixture was agitated gently several times and allowed to equilibrate for 48h. After equilibration, each vial was re-centrifuged as before and sufficient supernatant fluid removed to lower the albumin concentration by approx. 15mg of albumin/g of fluid when replaced by an equal weight of phosphate-buffered saline. This procedure was repeated five times to yield concentrations of albumin ranging from 10–90mg/g of fluid. Duplicate weighed portions of the five successive washes and of standards were counted to determine the albumin concentration at each step. After the final dilution, collagen was determined as described above.

Results

Albumin preparations

Commercial preparations of serum albumin usually contain dimers and higher oligomers (Pedersen, 1962; Andersson, 1966; Hellsing, 1969; Janatova, 1974; Blaabjerg & Hyltoft Petersen, 1979). The concentrate purchased for these experiments appeared to contain approx. 80% monomer as judged by scanning a polyacrylamide gel after electrophoresis and staining. Most of the remainder appeared to be dimer. After purification on a column of cross-linked allyl-dextran (Sephacryl S-200, Superfine grade; Pharmacia), the dimer content was much decreased. Polyacrylamide-gel electrophoresis of the iodinated albumin showed more than 98% of the radioactivity in a single peak with the mobility of monomeric albumin (Fig. 1). This preparation was used in subsequent experiments.

The stability of serum albumin exposed to dermis was tested by equilibration of duplicate discs of

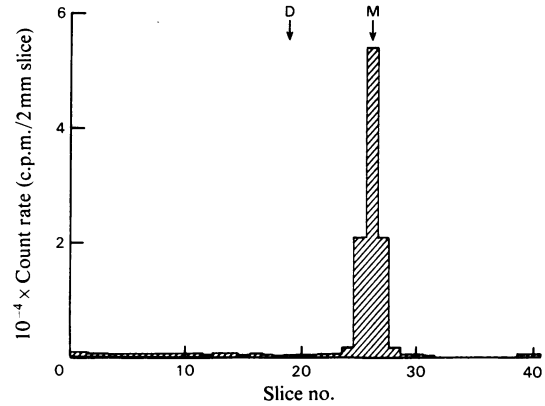

Fig. 1. *Distribution of radioactivity in a polyacrylamide gel after electrophoresis of the albumin preparation*
After electrophoresis of 1.7mg of albumin, the gel was cut into slices 2mm thick. The radioactivity of each slice was assayed. The data shown represent the running gel extending from the stacking gel (slice 1) to the tracking dye (slice 43). M and D represent the position of albumin monomer and dimer, respectively, in a gel run simultaneously and stained with Coomassie Blue.

dermis with albumin in phosphate-buffered saline for 10 days at 4°C. The supernatant fluid was examined by polyacrylamide-gel electrophoresis followed by slicing and counting the gel for radioactivity. The albumin exposed to tissue did not differ detectably from untreated albumin; further, after dialysis of the supernatant fluid, no radioactivity was found in the diffusible material. Thus the albumin probe was not degraded by the tissue.

Measurement of albumin concentration by radioactivity

In the optimal range of the counter, the coefficient of variation of replicate samples was approx. 1%, the precision being limited by the precision of weighing and the statistical error of counting. Since the count rates were found to be linear with sample size in the range 5000–50000 c.p.m., with significant deviations above and below these limits, all measurements were made within this range. Quenching was negligible, since the recovery of $Na^{125}I$ was quantitative whether it was added to phosphate-buffered saline that had been exposed to collagenous fibres or tissue, or to papain digests of the tissue.

At very low concentrations of albumin, measurable amounts of the protein were bound both to the glass vials used for the equilibrium experiments and to the polystyrene counting tubes. The albumin concentrations in this work were chosen to eliminate from the results any measurable effect of binding. Since all the label was bound to the albumin, the measurement of radioactivity of the sample gave a precise measure of the albumin content.

Recovery of ^{125}I-labelled albumin from equilibration experiments

Recovery was determined by a comparison of the summed count rates of the supernatant fluid and the digest with the count rate calculated from the weight of stock labelled albumin added to the sample. For the fibres the average recoveries, expressed as a percentage of added material ± S.D. (no. of samples), were: F1 and F2, measured simultaneously, 98.0 ± 1.4% (9); F3, 100.6 ± 1.7% (5), and 102.9 ± 3.0% (11) in two separate experiments. For the dermis samples the recoveries were: D1 and D2, measured simultaneously, 97.4 ± 0.8% (10); D3, 101.8 ± 0.7% (5).

Determination of fluid exclusion in fibre preparations

In the absence of binding, which will be discussed below, fluid exclusion was calculated as follows:

$$V_e = [W_s - (W_a/[A])]/C \quad (1)$$

where V_e = g of fluid in the fibre pad from which albumin was excluded/g of collagen; W_s = g of total fluid; W_a = g of albumin in the fibre pad; [A] = equilibrium concentration of albumin in the supernatant fluid, g of albumin/g of fluid; and C = g of collagen. W_s was determined by subtracting the weight of collagen from the weight of the pad. All concentrations have been expressed relative to the weight of the solvent to avoid assumptions about the density of the solvent.

The fluid exclusion calculated in this manner is the weight of fluid inaccessible to albumin relative to the weight of collagen and does not include the volume occupied by the collagen itself. The specific volume of collagen, 0.66 ml/g (Elden, 1968), must be added to values of fluid exclusion to give values for total exclusion similar to those reported in previous work (Bert *et al.*, 1980).

The period of 4 days was considered ample to permit the fibres and albumin solution to reach equilibrium. Consistent values with dextrans had been obtained after 24 h (Bert *et al.*, 1980).

Methods of separating supernatant fluid from fibre preparations

The supernatant fluid could be separated from thick slurries of collagenous fibres only by centrifugation of the vial to pack the fibres, entailing a risk of trapping albumin within the pad at a concentration different from that in the supernatant fluid. This possibility could be assessed by comparison of the apparent exclusion found when the vials were centrifuged with that obtained when the fibres were allow to settle by gravity. The values obtained by the two methods did not differ significantly, although the concentrations of collagenous fibres did (Table 2).

Effects of albumin and collagen concentrations on exclusion by fibres

The experiments described in Table 2 were designed to provide albumin concentrations ranging from 10 to 60 mg/g of supernatant fluid and collagen concentrations ranging from 15 to 100 mg/g of total fluid. The data were examined to ascertain any relationship between these variables and fluid exclusion. The covariances of fluid exclusion with albumin and collagen concentrations were both negligible ($P = 0.15$ and 0.74, respectively, by F-tests).

Binding of albumin to collagenous fibres

The calculation of fluid exclusion described in eqn. (1) ignored the possibility of binding of albumin to collagenous fibres. Although the absence of binding had been tested previously in a limited way (Engvall & Ruoslahti, 1977), confirmation was important to establish the validity of our data. In the absence of binding, the concentration of albumin in the supernatant fluid for a series of dilutions can be

Table 2. *Effect of the method of separating the supernatant fluid on fluid exclusion by a collagenous-fibre preparation*
After equilibration of F3 with ^{125}I-labelled albumin in phosphate-buffered saline, the supernatant fluid was collected either after allowing the fibres to settle or after centrifugation. The albumin concentration in the supernatant fluid, the collagen concentration in the system during equilibration and the weight of phosphate-buffered saline from which the albumin was excluded were determined. The differences between the data for the two methods were examined by an analysis of variance; the probability, *P*, that the differences were attributable to chance was calculated from an F-distribution.

Method	No.	Albumin (mg/g of free fluid)	Collagen (mg/g of total fluid)	Fluid exclusion (g of fluid/g of collagen)
Settling	7	30.9 ± 12.3	20.6 ± 1.8	2.17 ± 0.50
Centrifugation	9	32.8 ± 13.6	56.1 ± 19.1	1.95 ± 0.27
P		0.76	$<10^{-3}$	0.26

Table 3. *Fluid exclusion of albumin by collagenous fibres*
Replicate portions of each fibre preparation were equilibrated with ^{125}I-labelled albumin in phosphate-buffered saline and the supernatant fluid was collected after settling. The *P*-values were calculated by analysis of variance as described in the legend to Table 2. The data for F3 are the average of those listed in Table 2; thus nine of these fluid-exclusion values were obtained by analysis of supernatant fluid after separation by centrifugation.

Preparation no.	No.	Albumin (mg/g of free fluid)	Collagen (mg/g of total fluid)	Fluid exclusion (g of fluid/g of collagen)
F1	5	23.68 ± 1.58	36.0 ± 4.6	3.75 ± 0.96
F2	5	23.90 ± 0.74	36.6 ± 4.4	3.55 ± 0.67
F3	16	31.96 ± 12.68	40.6 ± 22.9	2.05 ± 0.39
P		0.16	0.85	$<10^{-3}$

calculated as described by Bert *et al.* (1980) and compared with the observed value. The calculated and the observed albumin concentrations obtained during three dilution experiments are plotted in Fig. 2. The line representing equal concentrations fitted the data closely. Significant binding of albumin to the collagenous fibres would have been indicated by an observed albumin concentration below this line, particularly at the lower concentrations. Thus, over the range of albumin concentration used, 10–90 mg/g of supernatant fluid, no evidence for binding was seen.

Comparison of fibre preparations

Collagenous fibres were prepared from the skins of three mature donors (Table 1). The fluid exclusion was measured for each preparation (Table 3). The value found for F3 was lower than that found for F1 or F2 ($P < 10^{-3}$ by F-test for both comparisons); the values for F1 and F2 were similar ($P = 0.71$ by F-test).

Comparison of dermis samples

Fluid exclusion of albumin was measured by using replicate slices 12 mm in diameter and 1 mm thick cut from the reticular layer of three separate specimens of dermis. The donors were chosen to minimize any effects of chronic illness (Table 1). The time required to reach equilibrium was assessed by using two discs from each specimen; after 10 and 14

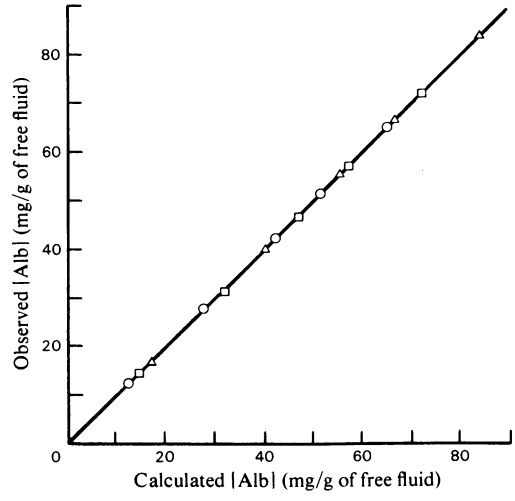

Fig. 2. *Test for the binding of albumin to collagenous fibres*
In three separate vials (○, □, △), albumin was equilibrated with F3. After removal of a portion of the supernatant fluid, phosphate-buffered saline was added and the system was again allowed to reach equilibrium. At each stage of dilution, the observed concentration of albumin, [Alb], in the supernatant fluid was compared with the value calculated from dilution. The line bisecting the angle between the axes represents the equivalence of observed and calculated values.

Table 4. *Fluid exclusion of albumin by human dermis*

Five discs of each human dermis were equilibrated with ^{125}I-labelled albumin in phosphate-buffered saline for 14 days at 4°C. The tissue and supernatant fluid were analysed to obtain the following data, as described in detail in the text. The water content of each tissue was determined separately using five additional discs (one was lost for D1).

Property measured — Dermis no. ...	D1	D2	D3
Weight (g)	0.1294 ± 0.0154	0.1270 ± 0.0084	0.1337 ± 0.0118
Water (g/g fresh wt.)	0.6601 ± 0.0049	0.6241 ± 0.0070	0.6429 ± 0.0116
Collagen (g/g fresh wt.)	0.3036 ± 0.0068	0.3351 ± 0.0057	0.2906 ± 0.0075
Collagen + water (g/g fresh wt.)	0.9636 ± 0.0068	0.9592 ± 0.0057	0.9335 ± 0.0075
Collagen (g/g dry wt.)	0.893 ± 0.020	0.891 ± 0.014	0.814 ± 0.021
Swelling ratio (g of swollen tissue/g fresh wt.)	2.30 ± 0.20	1.89 ± 0.04	2.25 ± 0.17
Albumin (mg/g of free fluid)	34.36 ± 0.27	47.70 ± 0.54	35.22 ± 0.46
Fluid in swollen tissue accessible to albumin (g/g fresh wt.)	1.529 ± 0.215	1.001 ± 0.048	1.438 ± 0.197
Insoluble solids (g/g fresh wt.)	0.3177 ± 0.0056	0.3543 ± 0.0076	0.3352 ± 0.0120
Collagen (g/g of insoluble solids)	0.955 ± 0.021	0.946 ± 0.016	0.867 ± 0.022
Swollen tissue fluid (g/g fresh wt.)	1.982 ± 0.204	1.534 ± 0.044	1.918 ± 0.169
Fluid inaccessible to albumin			
(g/g fresh wt.)	0.452 ± 0.027	0.533 ± 0.012	0.475 ± 0.032
(g/g of insoluble solids)	1.424 ± 0.084	1.505 ± 0.034	1.481 ± 0.095
(g/g of collagen)	1.492 ± 0.105	1.592 ± 0.020	1.638 ± 0.135

days' exposure of the discs to ^{125}I-labelled albumin, samples of the supernatant fluid were assayed for radioactivity. In each vial, the albumin concentrations after the two intervals were the same within the experimental error. Thus although 10 days were sufficient to attain equilibrium, for convenience all measurements were made after 14 days.

The discs weighed about 130mg (Table 4). The water contents differed between the three donors (Table 4; $P<0.01$ by analysis of variance). The collagen content of each specimen, measured by hydroxyproline analysis, differed between the specimens (Table 4; $P<0.001$ by analysis of variance). The sum of the average water content and the individual collagen contents of the specimens represented 93–97% of the weight of the specimen or, expressed differently, the collagen represented 81–89% of the dry weight [(1.000 − water content) in g/g fresh wt.; Table 4]. Both these values differed for the three specimens ($P<10^{-3}$ by analysis of variance of both the water plus collagen and the collagen per g dry wt.).

During equilibration with the solution of albumin, the slices of dermis swelled to an average between 1.9 to 2.3 times their initial weight (Table 4). The amount of swelling differed between the three specimens ($P\sim0.002$ by analysis of variance) and was related inversely to the collagen content of the specimen. Since the three specimens of dermis differed in composition, the albumin concentrations in the supernatant fluid surrounding the tissues at equilibrium also differed for the three donors ($P<10^{-4}$ by analysis of variance).

The concentration of albumin in the supernatant fluid and the content of albumin in the tissue, both measured by assay of radioactivity, were used to calculate the weight of the swollen tissue accessible to albumin (Table 4), as described in the numerator of eqn. (1). The tissue has been considered to contain three compartments: (1) a fluid space accessible to albumin, (2) a fluid space inaccessible to albumin and (3) the insoluble solids. The dry weight represented closely the latter compartment, but contained, in addition, at least two components that belonged to the fluid compartments. The first of these, namely the total solids of the plasma ultrafiltrate, has a relatively consistent value of 17.2 mg/g of water (Wolf, 1966). The weight of this component was calculated from the water content of the tissue. The second fluid component included in the dry weight was the interstitial plasma protein, which has been estimated to weigh 10.9 ± 2.9 mg/g fresh wt. (Pearce & Grimmer, 1970). The weights of these two components have been subtracted from the dry weight to obtain the weight of the insoluble solids of the tissue (Table 4). Since the correction to the dry weight amounted to about 6%, the uncertainty of the values of ultrafiltrable solids and protein contributed little to the uncertainty of the weight of the insoluble solids. Other components of the fluid compartments known to be included in the dry weight, such as hyaluronate (Pearce & Grimmer, 1972), were considered to be negligible. The collagen content of the tissue represented 86–96% of the insoluble solids (Table 4); the values differed significantly between the three tissues, that for D3 being less than those for D1 and D2 ($P<0.001$ by analysis of variance). The dermal elastin and a portion of the dermatan sulphate-containing proteoglycan comprise the balance of the insoluble solids (Pearce & Grimmer, 1972; J. M. Mathieson & R. H. Pearce, unpublished work).

The weight of the insoluble solids has been assumed not to change as the tissue swelled. Subtraction of this weight from the total weight of swollen tissue gives the weight of the swollen tissue fluid at equilibrium (Table 4). The albumin space in the tissue increased directly with the swollen tissue fluid for the five specimens from each donor (see Fig. 3; $P<0.001$ for D1 and D3, and $P\sim0.006$ for D2, that factors other than regression on the albumin space accounted for the variance of the swollen tissue fluid). The slopes were 1.044 ± 0.072, 1.055 ± 0.155 and 1.165 ± 0.053 g of albumin-containing fluid/g of tissue fluid for D1, D2 and D3 respectively. The probabilities that these values differed from 1.000 were 0.29, 0.37 and 0.03, respectively, by one-tailed t-distribution.

The space in dermis inaccessible to albumin was calculated by subtraction of the space accessible to albumin from the swollen tissue fluid. Since the space accessible to albumin increased directly with the tissue fluid, the inaccessible space was independent of swelling. The weight of the inaccessible fluid differed among the three specimens ($P<0.001$ by analysis of variance), but was proportional to the weights of the non-fluid solids and collagen of the specimen (a statistical assessment of regression is inappropriate for three points). Expression of the inaccessible space relative to either the insoluble solids or collagen gave data that did not differ for the three specimens ($P=0.17$ and 0.10, respectively, by analyses of variance), yielding average values ($\pm$S.D.) of 1.449 ± 0.082 g of fluid/g of insoluble solids and 1.57 ± 0.11 g of fluid/g of collagen respectively. Since the fluid in the space inaccessible to albumin is essentially phosphate-buffered saline with a density $\pm$ S.D. (no.) 1.0061 ± 0.0001 (3) at room temperature, the volume, in ml, of fluids inaccessible to the proteins is close to the values relative to weight given above.

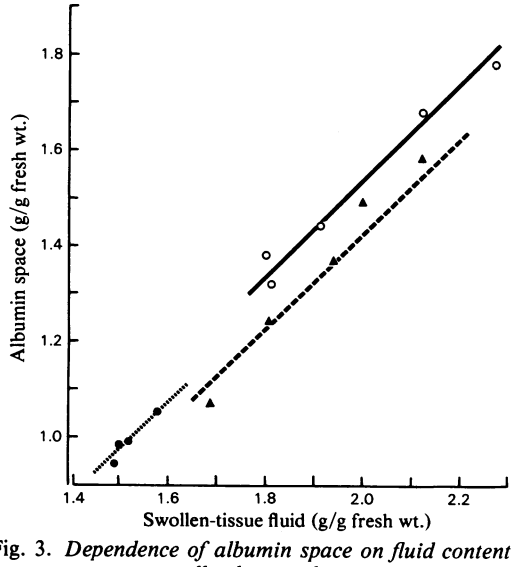

Fig. 3. *Dependence of albumin space on fluid content of swollen human dermis*

The space accessible to human serum albumin and the total tissue fluid after equilibration for 14 days at 4°C in phosphate-buffered saline are plotted for D1 (○), D2 (●) and D3 (▲). Lines with a slope of 1.000 have been drawn through the mean values for each tissue. The line for D3 has been displaced downward by 0.1 unit to separate it from the other lines.

Discussion

The fluid exclusion by two fibre preparations was similar; that of a third was significantly lower. The result obtained for F3 has been compared with those obtained with this preparation by using a series of dextran probes (Bert *et al.*, 1980). The calculated fluid exclusion ($\pm$standard error of estimate) of a dextran of Stokes' radius 3.55 nm, equivalent to albumin, was 2.80 ± 0.29 ml/g of collagen; the observed fluid exclusion ($\pm$S.D.) for albumin was 2.05 ± 0.39 g/g of collagen (Table 3). The difference is greater than would be anticiptated from the uncertainty of the data. The diameters of the bundles within the fibre preparations were quite variable (J. M. Mathieson & R. H. Pearce, unpublished work) and consistent samples were difficult to obtain because of the tendency of the fibres to settle quickly. For this reason, the discrepancy between the exclusion for albumin and for a dextran of similar size is not surprising.

Other investigations have found fluid exclusion by fibres and tissues to be independent of both probe and fibre concentrations. By using dextran probes, Pearce & Laurent (1977) reported a proportional relationship between excluded volume and weight of collagenous fibres over a range of 30–100 mg of collagen/g of total fluid. This result implies that the excluded volume expressed relative to the weight of collagen is constant. Bert *et al.* (1980) found that volume exclusion by collagenous fibres was independent of dextran concentration for probes of Stokes' radius greater than 3.92 nm. Previous investigations of volume exclusion in tissues have not reported tissue composition (e.g., Rutili, 1978); thus interpretation of such data relative to the exclusion properties of the tissue constituents is not possible.

For dermis the weight of fluid inaccessible to albumin depended on the insoluble solids of the tissue (Table 4); these comprised almost entirely collagen. Although the fluid exclusions of hyaluronate and of proteoglycans are about 20-fold higher than that of collagen (Ogston & Phelps, 1961; Gerber & Schubert, 1964), the weight of collagen in dermis is more than 300 times that of

hyaluronate plus proteoglycans (Pearce & Grimmer, 1970, 1972). These and other non-collagenous constituents of dermis may have contributed to the space inaccessible to albumin. If so, the excluded space expressed relative to the weight of collagen would exceed the true value attributable to collagen by an amount corresponding to the contribution of these constituents. In our opinion, such a contribution is likely to be negligible and can be assessed only by a detailed analysis of tissue structure. Although beyond the scope of the present work, such studies needed. Whatever the contribution of these other constituents may be, the fluid exclusion attributable to collagen in dermis remains much less than that found for the preparations of collagenous fibres. A simple explanation for this difference would be the loss in the fibre preparations of the compact organization of the dermal collagen apparent in previous work (Pearce & Grimmer, 1972). As illustrated by Fig. 4, the fluid exclusion exerted by a diffuse array of small fibres would be greater than that of a compact structure containing an equal weight of large fibres; the breakdown of the organization of the tissue fibres would result in increased exclusion.

Published data permit an approximate calculation of the albumin concentration in its accessible space in dermis. The typical composition of 1 g of fresh dermis is: 650 mg of water, 300 mg of collagen, 10 mg of elastin, 1 mg of glycosaminoglycan and 11 mg of plasma proteins (Table 4; Pearce & Grimmer, 1972). The extravascular albumin content of human skin has been reported to be equivalent to 250–300 mg of plasma/g fresh wt. by ^{131}I-labelled albumin tracer studies of surgical specimens and post-mortem skin (Rothschild *et al.*, 1955) and 250 mg of plasma/g by immunochemical methods applied to surgical specimens (Katz *et al.*, 1970*b*). Values up to 400 mg of plasma/g have been reported (see review by Schultze & Heremans, 1966). For dermis, the average value of fluid exclusion (Table 4) was 1.45 g of fluid/g of insoluble solids. The average insoluble-solid content of the dermis is the dry weight, 0.35 g, corrected for the soluble solids, 0.03 g, or 0.32 g/g fresh wt. Thus 0.32×1.45 or 0.46 g of fluid/g will be inaccessible to albumin. Therefore, of the 0.68 g of fluid present/g of dermis, only 0.68–0.46 or 0.22 g is available to albumin. Hence the concentration of albumin in this space should approximate or exceed that in plasma.

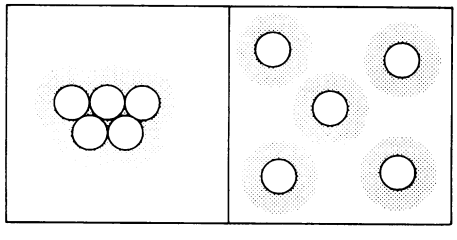

Fig. 4. *Effect of the packing density of collagenous fibres on exclusion*

The stippled area represents the fluid exclusion by collagenous fibres (open circles). The fluid exclusion by the closely packed fibres (left) is less than by the same number of fibres loosely dispersed (right).

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