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# PERMEABILITY OF LUNG CAPILLARIES TO MACROMOLECULES IN FOETAL AND NEW-BORN LAMBS AND SHEEP

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SUMMARY

1. The permeability of lung capillaries to macromolecules was investigated in immature and mature foetal lambs, new-born lambs and young sheep. The placental circulation of the foetal animals was maintained intact after delivery by Caesarian section. New-born lambs and sheep were mechanically ventilated. Samples of plasma and lymph that had drained from the lung via the thoracic duct were collected over a period of 1–5 hr.

2. The proteins in plasma and lymph samples were separated by fractionation on columns of Sephadex G-200. Plasma yielded three peaks of protein concentration. The $K_{av}$ value of each peak was determined, and, by calibrating the columns with known proteins, the mean radius of equivalent sphere ($a$) of the proteins in peak I was shown to be similar to that of fibrinogen $\geqslant$ 110 Å, peak II to $\gamma$-globulin $\approx$ 54 Å and peak III to albumin $\approx$ 34 Å. Lung lymph contained the same three constituent peaks as plasma but in lower concentration. In all four groups mean lymph/plasma concentration ($L/P$) ratio was significantly different for each of the three peaks, being lowest for the largest molecules (peak I) and highest for the smallest (peak III).

3. In five mature foetal lambs polydisperse polyvinylpyrrolidone labelled with $^{125}I$ ([$^{125}I$]PVP) was injected I.V. early in the experiment: count rates in fractionated samples showed for plasma a continuous decline with time after injection, and for lung lymph an increase to a maximum then a decline. Steady-state $L/P$ ratios for eleven fractions of PVP of differing molecular size ranging from 110 to 17 Å were derived by compartmental analysis. For a given molecular size PVP $L/P$ ratios were similar to protein $L/P$ values.

4. The regression of PVP $L/P$ ratio on $K_{av}$ was linear (correlation

coefficient $r = 0{\cdot}99$), and the slope of the regression of protein $L/P$ ratio on $K_{av}$ was significantly steeper for new-born lambs than for mature foetuses ($P < 0{\cdot}025$) and sheep ($P < 0{\cdot}005$), and steeper for immature foetuses than sheep ($P < 0{\cdot}01$).

5. PVP and protein $L/P$ ratios (mature foetuses) plotted against $a$ showed a sigmoid relation with agreement between the two sets of $L/P$ ratios. The goodness of fit between our experimental results and Landis & Pappenheimer's (1963) capillary pore theory (eqn. (1)) was examined: $L/P$ ratios for the larger molecules ($\geqslant 75$ Å) appeared to be too high. By recalculating ratios on the assumption that the largest molecules (110 Å) escape unrestricted from the capillary via leaks, the discrepancy disappears.

6. Values for pore radius ($r$), and pore area per unit path length ($A/\Delta x$) have been calculated for each of the four groups; $r$ ranged from 90 to 150 Å, $A/\Delta x$ from 3·3 to 0·2 cm $\times 10^3$.kg$^{-1}$. In new-born lambs the value of $r$ was significantly smaller, and $A/\Delta x$ larger than that of any other group. The inferences to be drawn from these results are discussed.

## INTRODUCTION

Measurements of the protein concentration and flow of pulmonary lymph have shown that protein molecules leak at a greater rate, per kg body weight, from the pulmonary circulation in foetal lambs than in new-born lambs or adult sheep (Humphreys, Normand, Reynolds & Strang, 1967), suggesting that there may be differences before and after birth in the net filtration pressure across the pulmonary capillaries, or in their permeability to large molecules. Such differences could be important in the pathogenesis of hyaline membrane disease in babies and lambs, since the membrane contains fibrin and other plasma proteins (Gitlin & Craig, 1956; Gajl-Peczalska, 1964). The present paper is concerned with estimating lung capillary permeability in immature and mature foetal lambs, new-born lambs, and young sheep.

We have studied the passage of macromolecules across the wall of pulmonary capillaries by comparing their relative concentration in plasma and lung lymph and have attempted to interpret our experimental findings in the light of the capillary pore theory (Landis & Pappenheimer, 1963). A general conformity with the pore theory was established in a group of mature foetal lambs in which comparisons were made between the concentration in lung lymph and plasma of the animal's endogenous proteins, and of injected polyvinylpyrrolidone (PVP) containing various sizes of molecule. Measurements in the other groups of lambs were confined to proteins which required less elaborate analysis and had greater reliability than the PVP results. From the data obtained it is possible to

characterize the permeability of the lung capillary walls in the four groups of animals in terms of a membrane containing uniform pores of defined radius and area per unit path length. A brief preliminary account of this work has been published (Boyd, Hill, Normand, Reynolds & Strang, 1968).

## METHODS

### *Experimental procedure*

Experiments were performed on fourteen foetal lambs (seven mature—gestation 140–147 days, and seven immature—gestation 120–125 days), seven spontaneously delivered new-born lambs aged 12 hr–7 days (mean 3 days) and seven young sheep aged 2–4 months. The experiments were conducted as described by Humphreys *et al.* (1967): as before, the animal's rectal temperature, heart rate, blood pressure, $P_{aO_2}$, $P_{aCO_2}$ and $pH_a$ were monitored throughout each experiment so that the physiological condition of the animal was known to be good. In foetal animals these measurements were similar to those given in Table 2 of Humphreys *et al.* (1967). The mean values for new-born lambs and sheep respectively were: heart rate (beats/min) 238, 117; B.P. (mm Hg) 63, 81; $P_{aO_2}$ (mm Hg) 90, 95; $P_{aCO_2}$ (mm Hg) 40, 37; $pH_a$ 7·35, 7·44. The thoracic duct component of pulmonary lymph was collected as described by Humphreys *et al.* (1967). Collection of lymph was started as soon as flow was established at a steady level, and was continued for 1–3 hr (mean coefficient of variation of lymph flow during experiments 11 %). Arterial blood was obtained from the carotid cannula to provide samples of plasma for comparison with the lymph samples.

In three of the mature foetal animals, as soon as lymph flow was steady, a solution containing 10–15 mg PVP labelled with $^{125}I$ ([$^{125}I$]PVP, Radiochemical Centre, Amersham) and having a total radioactivity of 200–300 $\mu$c contained in up to 5 ml. NaCl (0·9 g/100 ml.) was injected intravenously through a cannula advanced into the superior vena cava. In two further mature foetal lambs [$^{125}I$]PVP was injected soon after delivery of the lamb and before the dissection was completed. In these five animals the count rate of [$^{125}I$]PVP was followed in samples of plasma and lymph collected for a period of up to 5 hr. In all the animals the proteins in samples of lymph and plasma were analysed.

### *Analysis of proteins in lymph and plasma samples*

*Fractionation.* Samples were fractionated within 7 days on columns of Sephadex G-200 gel (Pharmacia) of internal volume $165 \pm 5$ ml. and diameter 1·6 cm, eluted at room temperature with degassed 0·5 M-NaCl buffered with phosphate to pH 6·5, the flow being controlled at a steady rate ($\pm 0{\cdot}5$ %) in the range 7·2–8·0 ml./hr. For foetuses 0·4 ml. samples were applied to the column; for new-born and older lambs, where total protein concentration was higher, 0·2 ml. samples were applied.

In gel filtration large molecules have a smaller fractional volume of the gel ($K_{av}$) available to them than smaller molecules (Laurent & Killander, 1964). In obtaining $K_{av}$ values for each elution volume ($V_e$) the void volume ($V_o$) was measured as the elution volume of the fraction before the first protein peak since Blue Dextran (Pharmacia, mol. wt. $2 \times 10^6$) was eluted in this position in each of the groups of animals studied; and the total volume of gel ($V_t$) was measured as the elution volume for free $^{125}I$, a volume which corresponded to within 5 ml. with measurements of the internal volume of the column.

*Protein content.* Protein concentration in each effluent fraction was measured by the method of Lowry, Rosebrough, Farr & Randall (1951) modified for use with an Autoanalyser (Gibbs & Bright, 1968). Versatol (Warner) standards were used for calibrating each set of fractions. Repeated estimations on two standard samples containing 700 and 12 $\mu$g/ml. gave values for S.D. of $\pm 12$ and $\pm 1{\cdot}4$ $\mu$g/ml. respectively (coefficient of variation 1·7 % and 11·7 %), $n$ being 11 and 7. Total protein in lymph and plasma was measured

similarly. Mean percentage recovery of protein applied to columns was 97·2 % in foetal samples (S.E. of mean ±1·1 %, $n = 30$) and 92·8 % in non-foetal samples (S.E. of mean ±1·1 %, $n = 19$).

As shown in results proteins were separated by gel filtration into three peaks. The height of each, calculated as the mean of its three highest concentrations, was shown to be a constant numerical fraction of the total protein in the peak. In each experiment an average of three plasma and three lymph samples collected over a period of 1 hr were analysed. Lymph/plasma ratios were calculated from the mean of the peak height in the lymph samples divided by the mean in the plasma samples.

*Analysis of [$^{125}I$]PVP in lymph and plasma samples*

*Count rates.* Fractionation was performed as described for proteins having first added 0·2 ml. of 30 % non-radioactive PVP (Polyvidone, May & Baker) to the 0·4 ml. sample since preliminary experiments had shown that this was necessary to ensure full quantitative recovery of large molecular fractions. The radioactivity of each fraction was measured in a well-type scintillation counter with a 5 × 5 cm NaI crystal (counting efficiency ≈ 35 %). Count rates were determined to a standard deviation of ±5 % or less and corrected to the day of the experiment (half-life of $^{125}I$ = 60 days). The total recovery of applied activity was satisfactory (mean recovery 95·0 % S.E. of mean 0·3 % in thirty-two samples). Allowance for small differences in recovery between samples was made by multiplying the count rate of each fraction by the ratio: total counts applied/sum of the counts in the fractions. The level of radioactivity per unit weight of [$^{125}I$]PVP was said to be uniform throughout its range of molecular sizes (personal communication from Dr G. F. W. Smith, Radiochemical Centre).

*Radius of equivalent sphere.* To obtain molecular radius of equivalent sphere ($a$) for a protein peak or a PVP fraction from its $K_{av}$, a calibration curve (Fig. 1) was constructed using purified proteins whose molecular radius was calculated from their known diffusion coefficient ($D_{20}$) using the Stokes–Einstein equation (Einstein, 1905, Sutherland, 1905). We determined the $K_{av}$ of the following proteins: bovine serum albumin (British Drug Houses), lactic dehydrogenase, ovalbumin, bovine γ-globulin, horse heart cytochrome $c$, bovine fibrinogen (Sigma Chemicals), and $^{125}I$ human serum albumin (Radiochemical Centre): duplicate determinations differed by less than 0·02. Our values for the last three were within 3 % of the values given by Laurent & Killander (1964) on Sephadex G-200. $D_{20}$ values were taken from Schultze & Heremans (1966) for human serum albumin and from Andrews (1965) for the other proteins.

## RESULTS

### *Proteins*

Figure 2*A* shows protein concentrations in the eluate from a column of Sephadex G-200 following the separate application of 0·4 ml. samples of lymph and plasma from a mature foetal lamb; Fig. 2*B* gives similar results from the lymph and plasma of a new-born lamb. In all samples there were three peaks of protein concentration (I, II and III). Peak I probably contains lipoproteins, macroglobulins and fibrinogen, whereas peak II is mainly γ-globulin and peak III mainly albumin (Schultze & Heremans, 1966). Peak II is large in the new-born due to the absorption of γ-globulin from colostrum (Charlwood & Tomson, 1948). Scales of $K_{av}$ and molecular radius of equivalent sphere ($a$) were applied to Fig. 2 by calibration of the gel columns with purified proteins as described in Methods. Table 1 gives

mean values for $K_{av}$ and $a$ for the protein peaks in each group of animals. The difference in $K_{av}$ and $a$ between 'foetal' and 'non-foetal' animals presumably reflect differences in plasma protein composition at different ages.

Lymph/plasma ratios ($L/P$) for peaks I, II and III were calculated from the means of the peak concentrations in the lymph and plasma samples

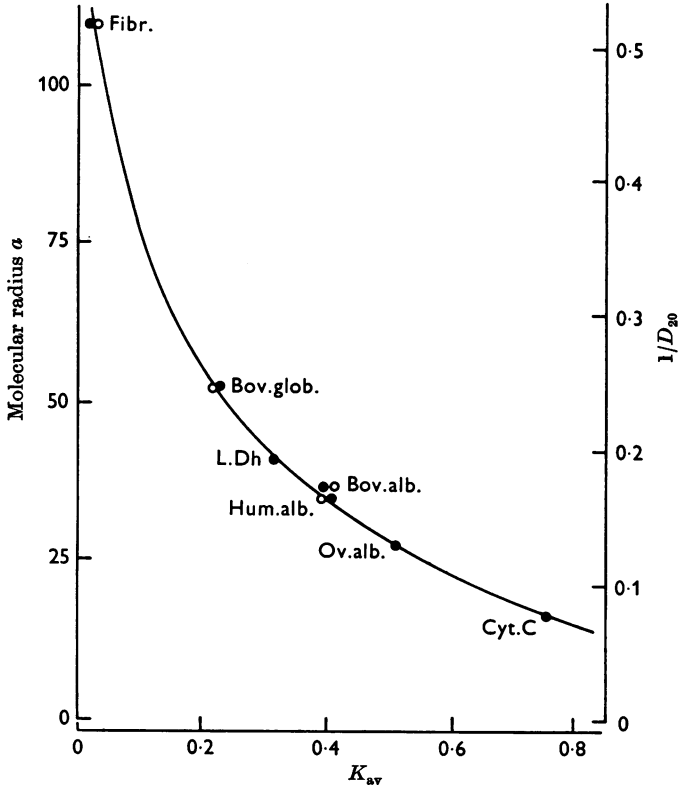


Fig. 1. Values of $K_{av}$ for purified proteins plotted against reciprocal of diffusion coefficient at 20° C ($1/D_{20}$ in $cm^{-2}$. sec $\times 10^7$) and molecular radius ($a$ in Ångströms), see text. Fibr. = bovine fibrinogen. Bov.glob. = bovine gamma globulin. L.Dh = lactic dehydrogenase. Bov.alb. = bovine serum albumin. Hum.alb. = $^{125}I$ human serum albumin. Ov.alb. = ovalbumin. Cyt. C = cytochrome *c*. ○ and ● represent duplicate determinations. Duplicates for Ov.alb. and L.Dh were identical. The line has been drawn by eye.

analysed. These ratios are given in Table 2 together with measurements of body weight, lung weight and lymph flow. The table also includes some quantities derived from the $L/P$ ratios, which are referred to below.

### *Polyvinylpyrrolidone*

*Fractionation.* Figure 3 shows count rates in the eluate from a column of Sephadex G-200 following duplicate applications of [$^{125}I$]PVP with

scales of fraction number and molecular radius. As shown in the figure reapplication of four of the eluate fractions to the column gave symmetrical elution patterns each with a peak very close to the position of the fraction in the first eluate. Comparison of the distribution of count rates following application of a single PVP fraction with the distribution of concentration when single proteins were applied showed a somewhat greater dispersion in the case of PVP of a degree which indicated that in

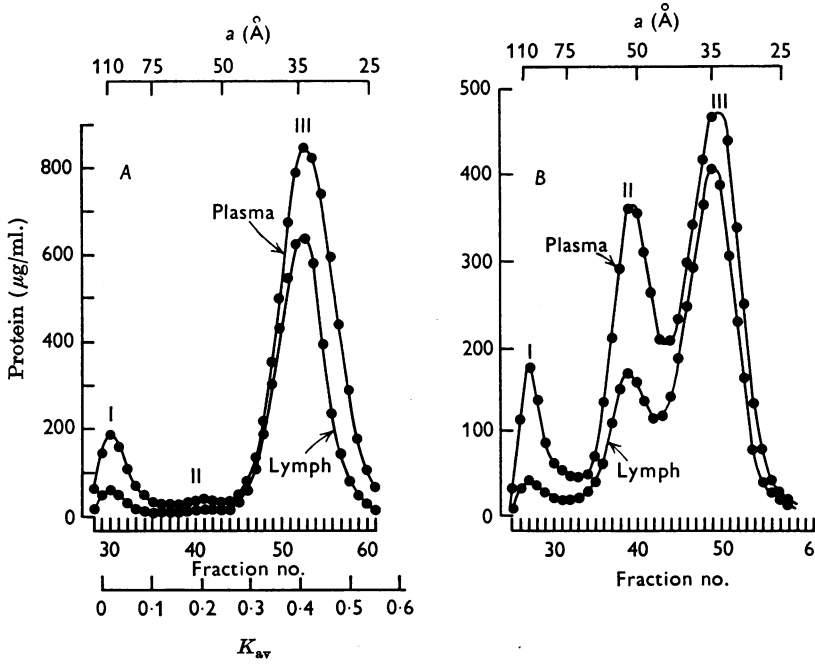


Fig. 2. Concentration of protein in effluent fractions from a column of Sephadex G-200 following separate applications of (*A*) 0·4 ml. of plasma and lymph from a mature foetal lamb and (*B*) 0·2 ml. samples from a new-born lamb. Three peaks of protein concentration are evident, indicated as I, II and III. Scales of $K_{av}$ and molecular radius, *a* (Å) are also given on the abscissae.

any one PVP fraction two thirds of the molecules had a $K_{av}$ within $\pm 0{\cdot}05$ of the mean (equivalent range in $a \approx \pm 5$ Å). We decided to examine in detail the changes in count rate with time of eleven fractions of PVP each containing molecules of a different size. These fractions (indicated as A–K in Table 3) ranged in mean *a* from 17 Å in fraction K to 110 Å in fraction A.

*Changes in count rate with time.* Figure 4 shows the total count rates in lymph and plasma following [$^{125}$I]PVP injection. Plasma counts declined following injection and after an interval of 2–3 min ascribed to transit time ($t_R$) through lymph channels, count rates increased in lymph and then

TABLE 1. Mean $K_{av}$ values with S.E. of mean and $n$ for protein peaks in samples from five immature foetal lambs, six mature foetal lambs, four new-born lambs and two sheep (see text). Values are also given for the corresponding radius of equivalent sphere, $a$ (Å) and $D_{39}$ ($cm^2 . sec^{-1} \times 10^{-7}$) (see Methods)

| | Peak I | | | | Peak II | | | | | Peak III | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $n$ | $K_{av}$ | $a$ | $D_{39}$ | $n$ | Mean $K_{av}$ | S.E. of mean | $a$ | $D_{39}$ | $n$ | Mean $K_{av}$ | S.E. of mean | $a$ | $D_{39}$ |
| Immature foetuses | 35 | 0·018 | ⩾ 110 | 3·1 | 22* | 0·203 | ±0·002 | 56 | 6·1 | 35 | 0·408 | ±0·001 | 34 | 10·1 |
| Mature foetuses | 40 | | | | 36† | 0·211 | ±0·002 | | | 40 | 0·417 | ±0·001 | | |
| New-born lambs | 22 | | | | 22 | 0·239 | ±0·001 | 51 | 6·7 | 22 | 0·423 | ±0·002 | 33 | 10·4 |
| Sheep | 15 | | | | 14‡ | 0·244 | ±0·001 | | | 15 | 0·443 | ±0·003 | | |

* In thirteen samples and † in four samples peak II too poorly defined to assign $K_{av}$.

‡ One peak lost.

Since the $a$ values for both foetal groups and for both post-foetal groups are close together a mean value is given for each.

$D_{39}$ values were obtained from the Stokes–Einstein equation (see Methods) by substituting appropriate values for $a$, viscosity of water at 39° C and T° K.

TABLE 2. Results for body weight, lung weight, lymph flow ($\dot{Q}_L$), protein $L/P$ ratios, flow through pores ($\dot{Q}_P$), protein $F/P$ ratios, pore radius ($r$), area of pore per unit flow and path length ($A/\dot{Q}\Delta x$) and area of pore per unit path length ($A/\Delta x$) (see text). The mean and S.E. of mean are given, the numbers of animals was 7 in all cases except for starred values where it was five. Values in columns 5–9 are derived from experimental values given in columns 1, 3 and 4

| | 1 Body wt. (kg) | 2 Lung wt. (g) | 3 $\dot{Q}_L$ $\left(\frac{\text{ml.}}{\text{hr.kg}}\right)$ | 4 $L/P$ ratios, peaks I | II | III | 5 $\dot{Q}_P$ $\left(\frac{\text{ml.}}{\text{hr.kg}}\right)$ | 6 $F/P$ ratios, peaks II | III | 7 $r$ Å | 8 $A/\dot{Q}\Delta x$ $\left(\frac{\text{sec} \times 10^7}{\text{cm}^2}\right)$ | 9 $A/\Delta x$ $\left(\frac{\text{cm} \times 10^3}{\text{kg}}\right)$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Immature foetuses | 3·0 ±0·3 | 108 ± 9 | 1·1 ±0·17 | 0·29 ±0·03 | 0·52* ±0·01 | 0·84 ±0·02 | 0·7 ±0·1 | 0·35* ±0·01 | 0·78 ±0·02 | 125 ±10 | 0·5 — | 1·0 — |
| Mature foetuses | 5·1 ±0·4 | 149 ±15 | 1·5 ±0·26 | 0·28 ±0·03 | 0·51 ±0·03 | 0·77 ±0·02 | 1·1 ±0·2 | 0·32 ±0·03 | 0·68 ±0·02 | 150 ±13 | 0·2 — | 0·6 — |
| New-born lambs | 5·1 ±0·3 | 124* ± 7 | 1·1 ±0·16 | 0·21 ±0·02 | 0·50 ±0·03 | 0·85 ±0·04 | 0·9 ±0·1 | 0·37 ±0·03 | 0·81 ±0·04 | 90 ±10 | 1·3 — | 3·3 — |
| Young sheep | 23·7 ±1·9 | 343 ±26 | 0·76 ±0·16 | 0·24 ±0·03 | 0·45 ±0·03 | 0·68 ±0·04 | 0·6 ±0·1 | 0·28 ±0·02 | 0·59 ±0·05 | 130 ±6 | 0·1 — | 0·2 — |

fell after reaching a maximum. Similarly in each experiment the changes in count rate with time of the eleven fractions were plotted out.

The count rates of the fractions showed changes of the type shown for four examples (B, D, G and J) in Fig. 5. The rate of decrease in plasma counts was systematically faster for small molecules than for large ones;

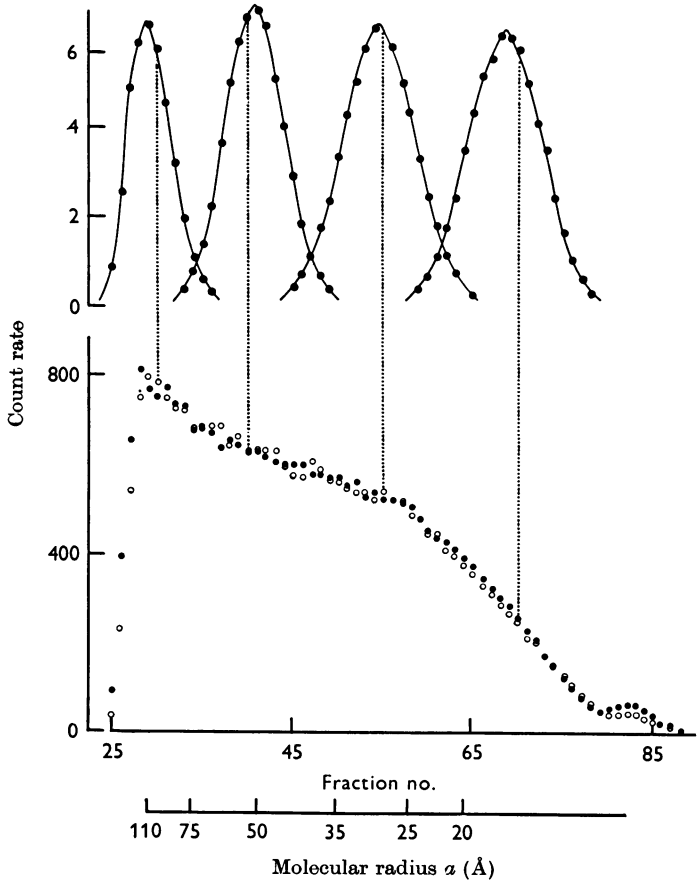


Fig. 3. Count rates (per fraction and 0·01 min) of [$^{125}$I]PVP in eluate fractions from a column of Sephadex G-200. The lower part shows counts obtained from two consecutive applications (○ and ●) of 0·5 ml. of a solution of [$^{125}$I]PVP which contained approximately 70 $\mu$c $^{125}$I per ml. The upper part shows the distribution of count rates obtained after consecutive applications of 0·05 ml. of four fractions (30, 40, 55, 70) taken from eluate ○. An interrupted line has been erected vertically above each fraction to show its relation to the peak obtained after re-application. The radioactivity concentration was highest in fraction 30, lowest in fraction 70; to give similar peak heights in the upper part of the figure, count rates for the four peaks were multiplied by the following factors: fraction (30) × 0·3, (40) × 0·4, (50) × 0·5, and (70) × 1·0. A scale of molecular radius (Å) is also given on the abscissae.

while the smaller the molecules the more rapid was the rise in lymph count rates and the shorter the time before reaching a maximum. The pattern of the fall in plasma and rise in lymph count rates, particularly the rise in lymph above plasma counts (see fractions G and J in Fig. 5), suggested the washout of one compartment (the interstitial space) from another (the plasma) in which the counts were falling exponentially (cf. Solomon, 1960).

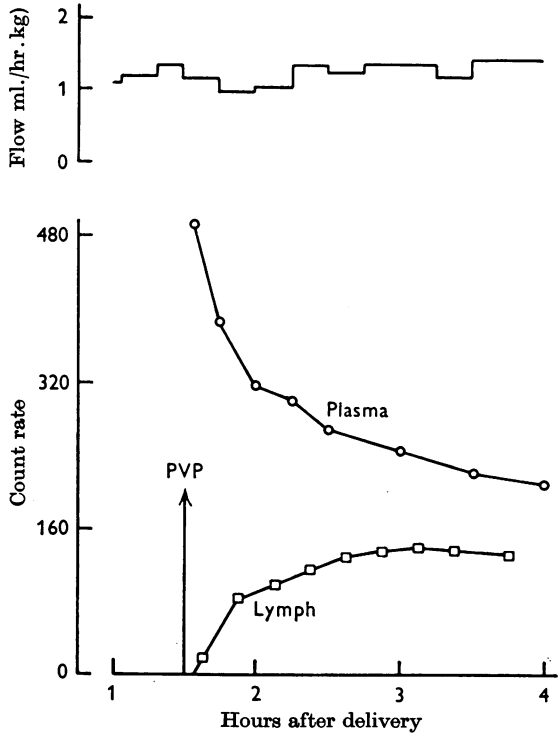


Fig. 4. Total count rates (per 0·01 min and 0·02 ml.) in lymph (□) and plasma (○) samples following I.V. injection of [$^{125}$I]PVP (at arrow) against time. The lymph measurement is shown at the mid-point of the period over which it was collected. Thoracic duct component of lung lymph flow (ml. per hour and kg body wt.) is shown above. Transit time ($t_R$) was estimated as the interval between [$^{125}$I]PVP injection and the point of intersection on the time scale of a straight line drawn through the first two lymph count rates.

*Finding steady-state PVP L/P ratios.* It was possible to account for the way in which count rates changed with time by an analysis based on an open two-compartment model in which plasma is separated from the interstitial fluid space by a porous capillary membrane. As a result of this analysis, which is described in the Appendix, we were able to arrive at estimates of steady-state PVP $L/P$ ratio for each of the fractions by two independent methods. The simpler was to obtain estimates graphically from a plot of

plasma and lymph (corrected for transit time) count rates against time, the $L/P$ ratio when the lymph count reaches a maximum (and the rate of change of lymph concentration is zero) being equivalent to the steady-state ratio. The other method involved fitting suitable constants to an inte-

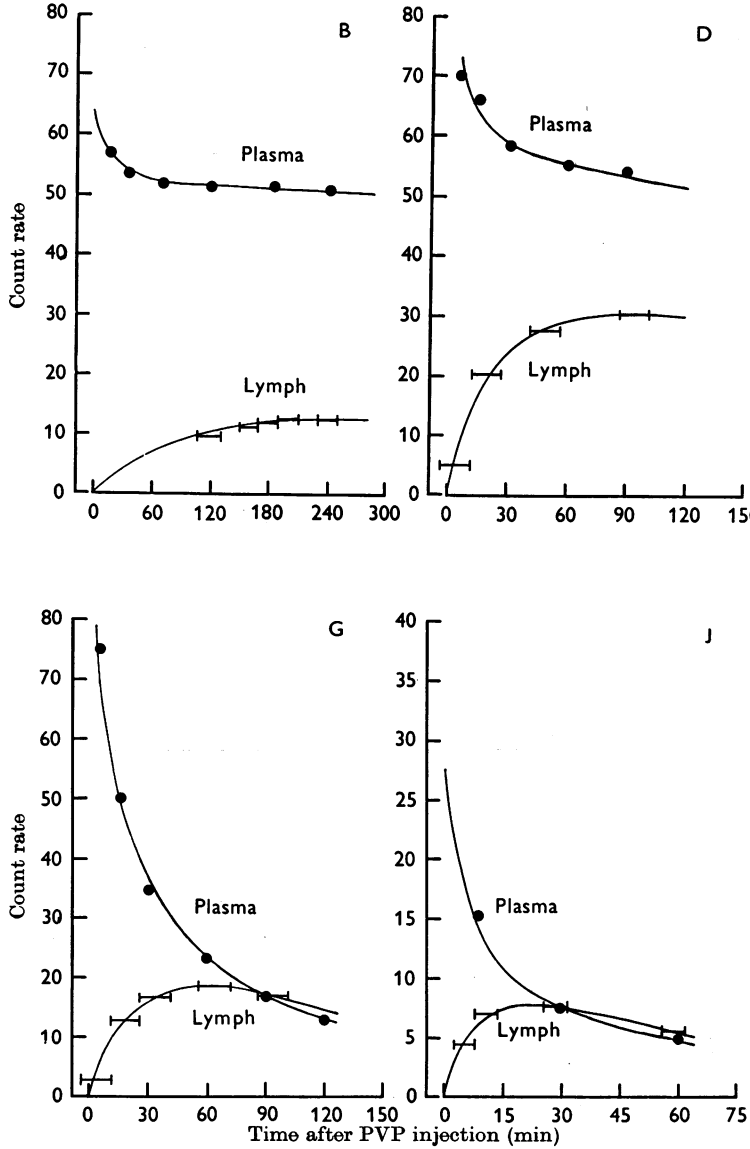


Fig. 5. Count rates (per fraction and 0·01 min) of fractions B, D, G and J in plasma (●) and lymph (horizontal bars) against time after [$^{125}$I]PVP injection. The continuous lines were calculated from eqns. (2$A$) and (3$A$) as described in the Appendix. Mean molecular radius of fractions (Å): B = 89, D = 58, G = 34, J = 21.

TABLE 3. Values for $K_{av}$, $a$ (Å), $D_{39}$ ($cm^2.sec^{-1} \times 10^{-7}$) for eleven fractions of PVP, together with mean $L/P$ ratios, S.E. of mean and no. of animals, and $F/P$ ratios (see text)

| Fraction | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $K_{av}$ | 0·018 | 0·062 | 0·107 | 0·195 | 0·284 | 0·373 | 0·412 | 0·462 | 0·550 | 0·639 | 0·728 |
| $a$ | 110 | 89 | 75 | 58 | 46 | 38 | 34 | 31 | 25 | 21 | 17 |
| $D_{39}$ | 3·1 | 3·8 | 4·6 | 5·9 | 7·4 | 9·0 | 10·1 | 11·0 | 13·7 | 16·3 | 20·1 |
| $L/P$ ratio | 0·21, 0·20 * | 0·26, 0·24 * | 0·29, 0·28 * | 0·43 | 0·54 | 0·63 | 0·68 | 0·70 | 0·80 | 0·88 | 1·00 |
| S.E. of mean | — | — | — | 0·07 | 0·06 | 0·06 | 0·06 | 0·06 | 0·03 | 0·04 | 0·01 |
| $n$ | 2 | 2 | 2 | 3 | 5 | 4 | 4 | 3 | 3 | 3 | 3 |
| $F/P$ ratio | 0·0 | 0·05 | 0·10 | 0·28 | 0·42 | 0·53 | 0·59 | 0·62 | 0·75 | 0·85 | 1·00 |

* Individual values.

grated solution of the differential equation appropriate to the model (see Appendix eqns. (1A) (2A) and (3A)) and then deriving the steady-state $L/P$ ratio mathematically. Figure 5 shows lines calculated for plasma and lymph (from eqns. (2A) and (3A) respectively) for four fractions, together with experimentally determined count rates. The good fit between the experimental points and the lines of the equation illustrates the appropriateness of the model, and the equation. Both methods of analysis were applied to each set of plasma and lymph counts, and the two estimates of steady-state PVP $L/P$ ratio thus obtained were in close agreement. A mean value from the two methods was used to obtain the results for all fractions given in Table 3. Because it took a long time ($\approx$ 4 hr) for large molecular weight fractions to reach a maximum in lymph, it was not possible to obtain lymph count maxima for the largest molecules in the three animals that were given [$^{125}$I]PVP at the end of the dissection. In the two animals in which PVP was given before the end of dissection, peaks for the large fractions were obtained, but only at the expense of missing the lymph count maxima for the smaller fractions. PVP $L/P$ ratios can be compared with measurements of the protein $L/P$ ratios which derive from continuously steady-state conditions.

*$L/P$ ratios related to molecular size and $K_{av}$*

Tables 2 and 3 show that the $L/P$ ratios of protein and PVP molecules increase as molecular radius decreases, and as $K_{av}$ increases. It will be seen later (Fig. 6) that $L/P$ ratio is not a simple linear function of molecular size, however PVP $L/P$ ratios are linearly related to $K_{av}$ (the regression equation of PVP $L/P$ ratio on $K_{av}$ fitted to data of Table 3 by least squares (eleven pairs) is $L/P = (1{\cdot}11 \times K_{av}) + 0{\cdot}20$; $r = 0{\cdot}99$, coefficient of variation of residual deviation = 3·5% of the mean). We therefore calculated regression lines similarly for protein $L/P$ ratio on $K_{av}$ in the four groups of animals. The equations are given in order of decreasing steepness of slope

New-born lambs $L/P = 1{\cdot}56K_{av} + 0{\cdot}17$ ($r = 0{\cdot}94$ $n = 21$),
Immature foetuses $L/P = 1{\cdot}43K_{av} + 0{\cdot}25$ ($r = 0{\cdot}98$ $n = 19$),
Mature foetuses $L/P = 1{\cdot}22K_{av} + 0{\cdot}25$ ($r = 0{\cdot}96$ $n = 21$),
Sheep $L/P = 1{\cdot}04K_{av} + 0{\cdot}21$ ($r = 0{\cdot}90$ $n = 21$).

The slope was significantly steeper for new-born lambs than for mature foetuses ($P < 0{\cdot}025$) and sheep ($P < 0{\cdot}005$); and steeper for immature foetuses than sheep ($P < 0{\cdot}01$). There are also differences in the $L/P$ values of peak III between the groups; peak III $L/P$ is lower in sheep than in new-born lambs ($P < 0{\cdot}01$) or immature foetal lambs ($P < 0{\cdot}005$), and lower in mature than in immature foetuses ($P < 0{\cdot}02$).

*Application of capillary pore theory to protein and PVP L/P ratios in mature foetal lambs*

The systematic decrease in protein and PVP $L/P$ ratios with increasing molecular radius suggests that molecules with $a$ values between 17 and 110 Å undergo 'sieving' in crossing lung capillary walls. Capillary pore theory as stated by Landis & Pappenheimer (1963) offers a way of expressing permeability in terms of ideal capillary walls containing uniform pores. According to this theory, the movement of molecules from plasma to interstitial liquid takes place through pores by hydrodynamic flow and by molecular diffusion and the steady-state interstitial fluid/plasma ratio of a molecule $s$ is given by

$$\frac{C_{Ix}}{C_{Px}} = \frac{1 + \frac{D_s}{\dot{Q}} \frac{A}{\Delta x}}{\frac{1}{R} + \frac{D_s}{\dot{Q}} \frac{A}{\Delta x}} \tag{1}$$

(where $\dot{Q}$ = outward flow of liquid from capillary; $D_s$ = free diffusion coefficient of $s$ at body temperature; $A$ = pore area; $\Delta x$ = length of pores; $C_{Ix}$ = steady-state interstitial fluid concentration of $s$; $C_{Px}$ = steady-state plasma concentration of $s$; $R$ = a coefficient describing the restriction to the movement of $s$ by diffusion and hydrodynamic flow through pores.) Equation (1) is similar to that derived initially by Pappenheimer (1953). The equation as stated here is the same as eqn. 7.15 of Landis & Pappenheimer (1963), except that we have represented the complex function of $a/r$ ($a$ = molecular radius and $r$ = pore radius), that they use to describe the restriction to movement of $s$ relative to that of water, by $1/R$ (see Appendix eqn. (4A) where the explicit expression is reproduced). Since in five mature foetal lambs we have, for each PVP fraction and protein peak, values for $L/P$, $D_s$ and $a$, we can see how well the observed data fit with the predictions of eqn. (1). Figure 6 shows that when the $L/P$ ratios are plotted against molecular radius there is a good agreement between PVP and protein results, but no one line of given pore radius will fit all the points. The discrepancy between eqn. (1) and the data appears to lie with the largest molecular fractions (A, B and C), since their $L/P$ ratios tend to a plateau of about 0·2, whereas the equation predicts that they should tend to zero. Thus the pore theory does not fit with the observed $L/P$ ratios of the largest molecules.

*Effect of leaks on L/P ratios.* Grötte (1956) also found that lymph/plasma ratios of Dextran fractions of large molecular weight between 140,000 and 300,000 (the latter having a quoted $a$ of 116 Å) were almost uniform and he suggested that in addition to pores there existed a small

number of leaks, which were so large as to offer no restriction to the passage of the largest molecules. We have applied this idea to the present data and assumed that all molecules of the size of PVP fraction A or protein peak I ($a \geqslant 110$ Å) pass with unfiltered plasma through large leaks and have calculated new ratios (filtrate/plasma, $F/P$) for the filtrate formed through pores. Thus

$$\dot{Q}_P = \dot{Q}_L\left[1-\left(\frac{L}{P}\right)_A\right] \tag{2}$$

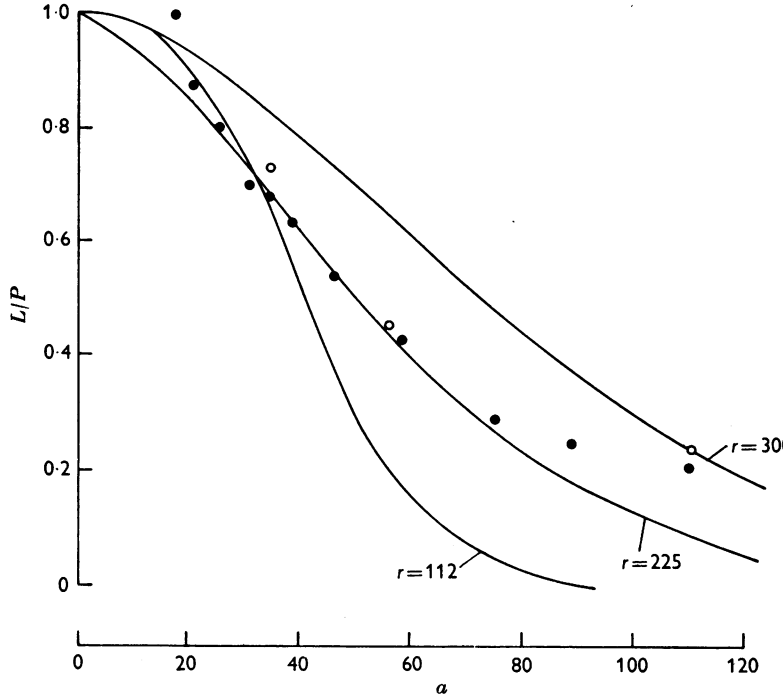


Fig. 6. Mean $L/P$ ratios of PVP fractions and protein peaks plotted against molecular radius ($a$, in Ångströms) PVP ●, protein ○ (five mature foetal lambs). The lines were calculated from eqn. (1); those for pore radii of 112 and 225 Å were made to pass through $L/P = 0{\cdot}68$ and $a = 34$ Å, and that for a pore radius of 300 Å was made to pass through $L/P = 0{\cdot}24$ and $a = 110$ Å.

(where $\dot{Q}_P$ = flow through pores; $\dot{Q}_L$ = lymph flow; and $(L/P)_A = L/P$ of fraction A) and

$$\left(\frac{F}{P}\right)_s = \frac{(L/P)_s-(L/P)_A}{1-(L/P)_A} \tag{3}$$

(where $(L/P)_s$ and $(F/P)_s$ refer to the ratios for a molecule $s$).

Figure 7 shows the $F/P$ ratios related to $a$; there is now a good fit between the points and a line calculated from eqn. (1) for a pore radius of 150 Å.

*Estimates of pore dimensions from protein L/P ratios in foetal and new-born lambs and sheep*

Having established the similarity between PVP and protein $L/P$ ratios and the general conformity of the data with the pore theory (once allowance had been made for leaks) in five mature foetal lambs, we calculated pore dimensions from the protein $L/P$ data for all the groups of lambs; these ratios have the advantage of referring to existing steady-state conditions.

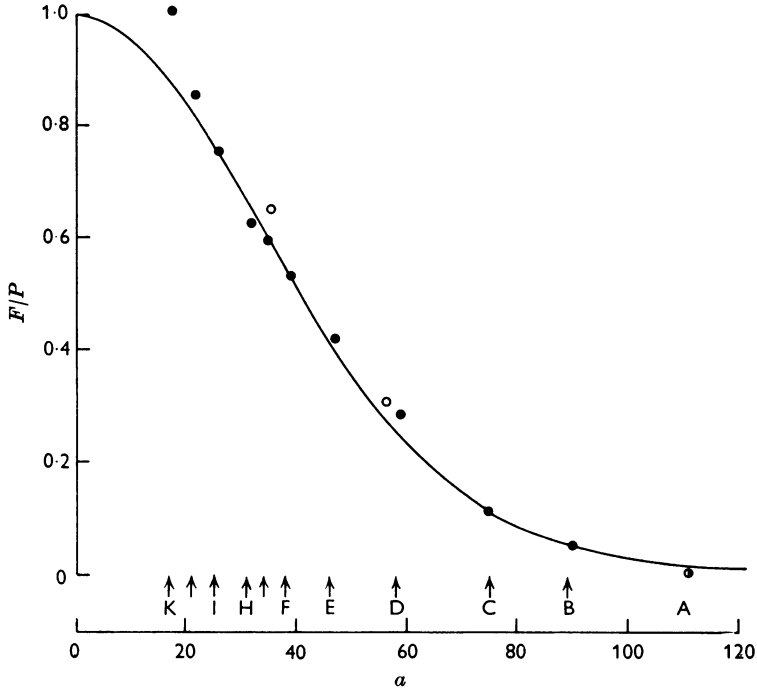


Fig. 7. Mean $F/P$ ratios of PVP fractions and protein peaks plotted against molecular radius ($a$ in Å): PVP ●, protein ○ (five mature foetal lambs). The $F/P$ ratios were calculated from eqn. (3), (see text). The line was calculated from eqn. (1) for a pore radius ($r$) of 150 Å and made to pass through $F/P = 0{\cdot}6$, and $a = 34$ Å. The positions of fractions A to K are shown.

Protein $L/P$ ratios were available for seven animals in each group; the points in Fig. 8 represent mean $F/P$ ratios for each peak plotted against molecular radius $a$.

To obtain lines of graphical best fit from eqn. (1) to the observed $F/P$ ratios, for each pore radius ($r$) a value for the term $A/\dot{Q}\Delta x$ was first calculated from the $F/P$ of peak III, and by inserting this value into eqn (1) a line for any value of $r$ could be drawn passing through $F/P$ III; in

practice estimates of $r$ could be made to the nearest 5 Å (Fig. 8). Since the transmembrane flow is that through pores ($\dot{Q}_P$), a value for $A/\Delta x$ can also be obtained; the results are given in Table 2. Individual values for $F/P$ and $r$ in each group were normally distributed, and a $t$ test showed that

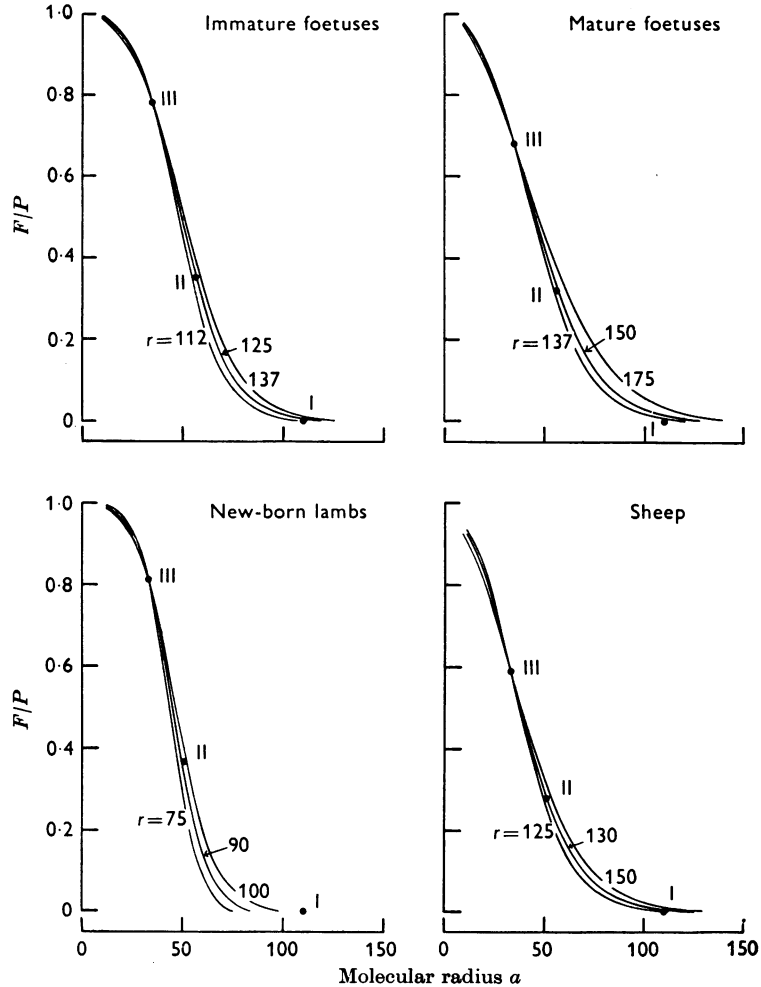


Fig. 8. Points are mean protein $F/P$ ratios of peaks I, II and III for the four groups of animals plotted against molecular radius ($a$ in Ångströms). The lines were calculated from eqn. (1) for various pore radii ($r$ in Ångströms) as indicated, each made to pass through the $F/P$ of peak III.

mean pore radius $r$ in the new-born lambs (90 Å) was significantly smaller than the mean of any other group (150–125 Å), $P < 0{\cdot}05$. Individual values for $A/\dot{Q}\Delta x$ and $A/\Delta x$ show a wide scatter and skewed distribution,

which results from the form of eqn. (1): differences in $A/\dot{Q}\Delta x$ and $A/\Delta x$ between groups were tested by the ranking method of White (Snedecor, 1957); the values for new-born lambs were significantly larger than for mature foetuses ($P < 0{\cdot}05$) or sheep ($P < 0{\cdot}01$). However, the values $A/\dot{Q}\Delta x$ and $A/\Delta x$ calculated from the mean $F/P$ ratio for the group should give the best estimate for the group and it is these that are given in Table 2, which shows that $A/\Delta x$ in new-born lambs was nearly six times larger than in mature foetal lambs, and about 16 times larger than in sheep.

## DISCUSSION

*Lymph and interstitial fluid.* We have assumed, as did Starling (1912) and Drinker & Yoffey (1941), that lymph and interstitial fluid have essentially the same composition. Estimates of average protein concentration in the interstitial fluid of man from protein isotope dilution measurements (Myant, 1951; Sterling, 1951) and of protein in interfibre fluid from guinea-pig muscle (Creese, D'Silva & Shaw, 1962), both give values similar to those in lymph. However, Landis & Pappenheimer (1963) consider that the protein concentration of interstitial fluid varies from low at the arterial end of a capillary where filtration occurs, to high at the venous end where liquid is reabsorbed. Pappenheimer & Soto-Rivera (1948) calculate that the diffusion of proteins is slow enough to allow for incomplete mixing in interstitial fluid, but Wiederhielm (1968) has pointed out that the assumed differences in protein concentration would set up osmotic pressure gradients leading to rapid mixing by convection.

In our use of eqn. (1) we assumed that filtration takes place in one direction only from capillary to interstitial space, whereas Landis & Pappenheimer (1963) suggest that up to 80% of capillary filtrate is reabsorbed from the venous end of capillaries. If we allow for a flow of liquid in both directions we can re-write eqn. (1) as follows (cf. Renkin, 1964; Winne, 1965)

$$\frac{C_{Ix}}{C_{Px}} = \frac{\dot{Q}_1 + D_s . A/\Delta x}{\frac{\dot{Q}_2}{R} + (\dot{Q}_1 - \dot{Q}_2) + D_s \cdot \frac{A}{\Delta x}}$$

(where $\dot{Q}_1$ = total outward flow through pores; $\dot{Q}_2$ = flow to lymph; $\dot{Q}_1 - \dot{Q}_2$ = flow from interstitial fluid to capillary). We have calculated the effect of varying $\dot{Q}_1$ on our estimates of pore dimensions and find that if $\dot{Q}_1/\dot{Q}_2 = 5$ (i.e. 80% reabsorption) we would have overestimated $r$ by only about 10% and $A/\Delta x$ by about 25%.

*Capillary pores and leaks.* It is unlikely that capillary walls are penetrated by perfectly uniform cylindrical openings, but we find Solomon's

(1968) notion of 'equivalent pores' a useful way of characterizing permeability. The electron microscope studies of Schneeberger-Keeley & Karnovsky (1968) provide morphological evidence for the existence of pores at cell junctions in lung capillaries. In addition, gaps over 1000 Å in radius between the endothelial cells of venules have been shown in electron micrographs of muscle (Majno & Palade, 1961; Majno, Palade & Schoefl, 1961). The number of leaks of 1000 Å radius which would be needed to account for the assumed partition of flow through pores and leaks in our experiments (from eqn. (2)) would be less than one per 8000 pores. Physiological evidence for the existence of two distinct populations of opening, differing in size, in capillaries and small vessels of frog's mesentery has been produced by Landis (1964) from experiments in which the escape of two dyes, one highly diffusible, the other bound to albumin so that the molecules were large, was followed cinematographically.

Our estimates of pore radius in the capillaries of lung are substantially larger than those given by Landis & Pappenheimer (1963) for hind limb capillaries (35–45 Å). Lung lymph has a protein concentration of 2·7–5·7 g/100 ml. (Humphreys *et al.* 1967), compared with $\approx$ 1·7 g/100 ml. in lymph from a limb (Yoffey & Courtice, 1956), so the large pore radius is not surprising. The presence of substantial concentrations of peak II protein ($a \geqslant 50$ Å) in lung lymph by itself implies a pore radius larger than Landis & Pappenheimer's estimate.

*Changes in capillary pore dimensions and net filtration pressure at birth and afterwards.* On the basis that hydrodynamic flow through a porous membrane is determined by Poiseuille's law (Pappenheimer, 1953) we have calculated for the four groups of animals, the net filtration pressure required to drive the observed flow of lymph through capillary walls with the pore dimensions given in Table 2. The values are approximately: immature foetuses 5 mm Hg, mature foetuses 12 mm Hg, new-born lambs 4 mm Hg and sheep 26 mm Hg.

At birth pulmonary vasodilatation takes place and consequently capillaries, that had previously been unperfused, open up; this presumably explains why $A/\Delta x$ is larger in new-born lambs than in mature foetuses. Pulmonary artery pressure is high in the foetus and falls at birth (Dawes, Mott, Widdicombe & Wyatt, 1953), and a possible explanation for the high computed filtration pressure in the foetus is that before birth the relatively few capillaries that are being perfused are exposed to the high pulmonary artery pressure; this would also explain the high pulmonary lymph flow of the foetal lamb (Humphreys *et al.* 1967). The suggestion made by Shirley, Wolfram, Wassermann & Mayerson (1957) that pores can be stretched by a high capillary pressure offers an explanation for our finding that the value for pore radius is higher in mature foetuses than in new-born

lambs. The latter, which have the lowest computed filtration pressure of all the groups, also have the smallest pore radius.

In the sheep computed pore area per unit length is small and filtration pressure high. Since in the adult pulmonary artery pressure is normally lower than plasma protein osmotic pressure, lymph is likely to be formed only in the most dependent part of the lung where capillary pressure is high due to gravity. The vertical height of the lung of supine sheep is about 30 cm so that the computed value of 26 mm Hg for filtration pressure appears to be a reasonable estimate for the lowest parts of these lungs. The small lymph flow per kg body weight from adult lungs (Humphreys *et al.* 1967) presumably reflects the small portion of lung from which lymph is formed.

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## APPENDIX

*Analysis of PVP count rates in lymph and plasma.* To explain changes in count rates with time we have considered a two compartment model in which a porous capillary wall separates the plasma compartment from the interstitial fluid space, the latter being drained by a lymphatic channel. Macromolecules enter the interstitial space from plasma by diffusion and hydrodynamic flow of solution and they leave by diffusion back into plasma and by flow away in lymph. Complete and instantaneous mixing of solutes entering interstitial fluid is assumed. Concentration in lymph ($C_L$) and interstitial fluid ($C_I$) are considered to be identical, but there is a time lag ($t_R$) between the formation of interstitial fluid and its appearance as lymph at the collecting tube, i.e. $C_I$ at time $t = C_L$ at time $t + t_R$. In the equations given below $C_L$ corrected for $t_R$ has been used in place of $C_I$.

At any given time the rate of change in the concentration of a molecule $s$ in interstitial fluid is given by

$$\frac{\mathrm{d}.C_L}{\mathrm{d}t} = \frac{\dot{G}}{V}.C_P - \frac{\dot{E}}{V}.C_L., \qquad (1\,\mathrm{A})$$

where $C_P$ = plasma concentration. $V$ = volume of interstitial fluid, $\dot{G}$ = rate per unit of plasma concentration at which $s$ enters interstitial fluid and $\dot{E}$ = rate per unit of interstitial fluid concentration at which $s$ leaves interstitial fluid.

To analyse the results in terms of the capillary pore theory, it is necessary to find $C_L/C_P$ ratios which correspond to steady-state conditions.

$C_L$ passes through a maximum, and at this time $\mathrm{d}.C_L/\mathrm{d}t = 0$: from eqn. (1 A) this corresponds to the steady-state condition, which applies, for example, to the animal's own plasma proteins, where $C_P$ and $C_L$ are constant.

It is possible to obtain steady-state values of $C_L/C_P$ graphically either from the lymph and plasma count rates at the peak of the lymph count rate, or by solving eqn. (1 A) for $\dot{G}/V$ and $\dot{E}/V$, and hence $\dot{G}/\dot{E}$, from the slope of the curve of $C_L$ at two different times. The latter method was also used to test the appropriateness of eqn. (1 A) by its ability to predict $C_P$ from $C_L$. Values of $C_P$ calculated in this way were very close to experimental values.

An integrated solution of eqn. (1 A) can also be obtained. Plasma count rates fell in a manner which could be represented as the sum of two exponential terms, and for the smaller molecules these approached a constant value, thus

$$C_P = C_{P1_0}\,\mathrm{e}^{-k_1 t} + C_{P2_0}\,\mathrm{e}^{-k_2 t} + C_{Pz}, \qquad (2\text{ A})$$

where $C_{P1_0}$ and $C_{P2_0}$ are the initial concentrations of the plasma exponential terms which have slopes $k_1$ and $k_2$; $C_{Pz}$ is a constant plasma concentration to which the count rates approximate. Under these circumstances the integrated solution of eqn. (1 A) (cf. eqn. (15) in Solomon, 1960) is

$$C_L = \frac{C_{Lx}}{C_{Px}}\left[C_{P1_0}\cdot\frac{\dot{E}/V}{\frac{\dot{E}}{V}-k_1}\,(\mathrm{e}^{-k_1 t}-\mathrm{e}^{-\dot{E}t/V}) + C_{P2_0}\cdot\frac{\dot{E}/V}{\frac{\dot{E}}{V}-k_2}\,(\mathrm{e}^{-k_2 t}-\mathrm{e}^{-\dot{E}t/V}) + C_{Pz}(1-\mathrm{e}^{-\dot{E}t/V})\right], \qquad (3\text{ A})$$

where $C_L$ = lymph concentration at time $t+t_R$, $C_{Lx}/C_{Px}$ = steady-state lymph/plasma ratios of the solute. Values of $C_{P1_0}$, $C_{P2_0}$, $k_1$, $k_2$ and $C_{Pz}$ were obtained graphically from a plot of log $C_P$ on time. For every fraction, experimental values for lymph count rates at 15 min intervals were substituted in eqn. (3 A) for $C_L$ and $t$, together with an arbitrarily chosen value of $\dot{E}/V$, until, by successive approximation of $\dot{E}/V$, a solution for $C_L/f(t) = C_{Lx}/C_{Px}$ was found that remained constant to within $\pm 5\%$ for all pairs of values for $C_L$ and $t$ ($f(t)$ here represents the function in the square brackets in eqn. (3 A)). As shown in Fig. 6, lines for plasma and lymph count rates against time calculated according to eqns. (2 A) and (3 A) respectively closely approximate to observed count rates.

*Definition of* $1/R$ *(see eqn.* (1))

$$\frac{1}{R} = \frac{\left[2\left(1-\frac{a_w}{r}\right)^2 - \left(1-\frac{a_w}{r}\right)^4\right]\left[1-2{\cdot}10\left(\frac{a_w}{r}\right)+2{\cdot}09\left(\frac{a_w}{r}\right)^3-0{\cdot}95\left(\frac{a_w}{r}\right)^5\right]}{\left[2\left(1-\frac{a_s}{r}\right)^2 - \left(1-\frac{a_s}{r}\right)^4\right]\left[1-2{\cdot}10\left(\frac{a_s}{r}\right)+2{\cdot}09\left(\frac{a_s}{r}\right)^3-0{\cdot}95\left(\frac{a_s}{r}\right)^5\right]}, \quad (4\,A)$$

where: $a_w$ = molecular radius of water in Å (we used 1·97 Å); $a_s$ = molecular radius of $s$ in Å; $r$ = pore radius in Å.

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