BBA 75796

A CONNECTIVE TISSUE MEMBRANE AS A MOLECULAR SIEVE

P. ELSBACH AND P. PETTIS

Department of Medicine, New York University School of Medicine, New York, N.Y. (U.S.A.)

(Received July 12th, 1971)

SUMMARY

1. The urinary bladder of the toad, from which the mucosal cells had been removed, was mounted between two chambers and used for the study of the barrier properties of such a tissue with respect to various proteins and lipoproteins.

2. The passage of [^{14}C]urea, [^{125}I]albumin, albumin labeled with attached ^{14}C-labeled fatty acid or [^{32}P]lysolecithin, γ-[^{125}I]globulin and low density lipoprotein labeled with [^{14}C]cholesterol and ^{3}H-labeled fatty acid was studied as a function of time, temperature and concentration.

3. The intact bladder is impermeable to albumin. In the absence of mucosal cells, however, albumin passes through the membrane at a mean rate of 5 % of the initial amount, per 30 min.

4. Transfer is linear with time and concentration and, is 2.8 times greater at 37° than at 6°. The rate for fatty acid or lysolecithin complexed to albumin is the same as for albumin alone, as is the passage of purified γ-globulin. Urea at equimolar concentration is transferred 4 times faster and low density lipoproteins about 10 times slower than albumin.

5. Transferates appear to be determined by diffusion through pores of limited size. Thus, the speed with which fatty acids and lysolecithin, as opposed to other lipids, are metabolized by parenchymal cells may in part be due to differences in transferates of the various lipid–protein complexes through the tissue barriers.

INTRODUCTION

A vast literature has appeared concerned with the clearance of various lipids from the circulating blood[1–3]. Different lipid species are removed from the circulation at different rates. A major portion of differences in removal rates undoubtedly must be attributed to differences in uptake and metabolism of various types of lipid by cells and tissues. Another factor that may be important in determining differences in removal and metabolism of lipids is the obligatory association of lipids with solubilizing proteins and hence the passage of various lipid–protein complexes across the barriers that are interposed between the blood and the parenchymal cell. Such barriers include the vascular wall and various elements of connective tissue. Little is known about the properties of such barriers with respect to lipids and/or lipoproteins. A few histochemical studies of the translocation of lipid from capillary to extravascular space have been carried out[4–7], but these provide limited information concerning the

joint or separate passage of lipids and protein. A systematic study of the barrier properties of, for example, connective tissue is hampered by the lack of a suitable model system that can be observed *in vitro*. BRACHET AND RASIO[8] have used rat mesentery mounted between two chambers and have examined the rates of passage of various macromolecules, such as albumin, inulin and insulin.

In the present study another connective tissue membrane was used to investigate the rates of passage of molecules of different sizes. In earlier studies of the urinary bladder of the toad (*Bufo marinus*)[9-11] we had found that after scraping off the mucosal cells of this thin structure, a tough membrane remains that can be mounted between two chambers. This membrane consists mainly of collagen fibers, a thin smooth muscle layer at the mucosal side of the bladder, a serosal layer and a few blood vessels.

This report concerns the results of experiments carried out with this preparation which indicate that it functions as a molecular sieve.

MATERIALS AND METHODS

Preparation of scraped urinary bladders

Urinary bladders of well hydrated toads (*Bufo marinus*) were removed and placed in toad Ringer's solution (pH 7.4) containing 113 mM Na^+, 117 mM Cl^-, 3.2 mM K^+, 1.0 mM Mg^{2+}, 2.0 mM Ca^{2+}, 1.2 mM PO_4^{3-}, 2.0 mM HCO_3^-, 1.0 mM SO_4^{2-} and 5.5 mM glucose[12]. The bladders were cut into two halves and the mucosal layer

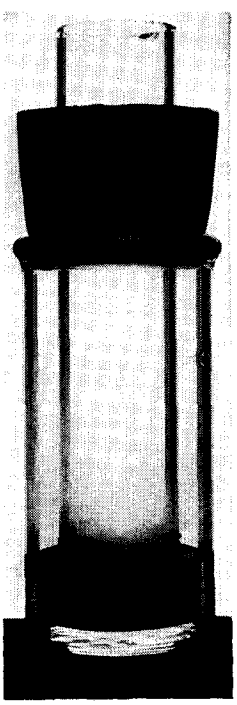

Fig. 1. Photograph of apparatus. The outside chamber measured 26 mm × 60 mm (inside diameters) and the inside cylinder 17 mm × 100 mm (inside diameters). The scraped bladder was mounted over the open bottom part of the inside cylinder (see MATERIALS AND METHODS).

was removed from the underlying structures by gently scraping with a glass microscope slide.

Each half was then mounted on the fire-polished end of a glass cylinder, open at both ends, and secured in place by wrapping a long strip of parafilm over the edge of the bladder in such a fashion that a tight seal was provided. A rubber stopper surrounding the cylinder at the other end permitted insertion of the cylinder into an outer glass chamber (Fig. 1). By raising or lowering the stopper the level of the cylinder could be adjusted within the outer chamber. By such manipulation care was taken to ensure that the levels of 2 ml of inside solution and of 4 ml of outside solution were the same. The amount of tissue used for mounting was always enough to allow its periphery to extend well above the fluid level in both chambers. The addition of 2 ml of inside solution produced a slight bulging of the tissue prior to its insertion into the outside solution when it resumed its horizontal position. The latter was not the case during incubation at 6° when the tissue slackened due to relaxation of the smooth muscle fibers at the low temperature. Occasionally scraping resulted in gross leaks. Only hemibladders were used that permitted no passage of Ringer's solution after mounting, during careful inspection for several minutes.

A small hole in the wall of the outer chamber served as a vent.

Labeled materials

Human serum albumin labeled with ^{125}I (Albumotope-^{125}I) was obtained from E. R. Squibb, Inc., New Brunswick, N. J. A suitable amount of radioactive material was passed through a small 'P-B-I' ion-exchange column (Rezikit, Squibb, Inc., New Brunswick, N. J.) to remove any contaminating free ^{125}I, and diluted with bovine serum albumin (Armour Fraction V) in toad Ringer's solution, to the desired concentration.

Rabbit γ-globulin labeled with^{125}I, kindly given to us by Dr. Isaac Schenkein of the Department of Medicine, N.Y.U. School of Medicine, was radioiodinated according to modification by McConahey and Dixon[13] of the method reported by Hunter and Greenwood[14]. Purification was carried out by precipitating rabbit serum with 52 % ammonium sulfate followed by desalting on a Sephadex G-25 column. The protein eluted in the void volume was passed through a column of DEAE-cellulose, lyophilized and dissolved in 0.01 M Tris buffer (pH 7.6). The labeled rabbit γ-globulin was diluted with purified human γ-globulin (a gift from Dr. E. C. Franklin of the Department of Medicine, N.Y.U. School of Medicine).

Low density lipoproteins labeled with [4-^{14}C]cholesterol and [9,10-$^{3}H_2$]oleic acid were prepared as follows: A rat weighing about 250 g was anaesthetized with ether. A mixture consisting of 20 μC of [4-^{14}C]cholesterol and 125 μC of [9,10-$^{3}H_2$] oleic acid in 0.3 ml of ethanol and 0.5 ml of triolein was injected through a gastric polyethylene catheter. The tube was rinsed through with an additional 0.5 ml of triolein and 1.0 ml of saline. The rat was given free access to food and water. 16 h after the radioactive meal the rat was again anaesthetized and blood was taken by cardiac puncture. The plasma was obtained by centrifugation of the blood, siphoned off and brought to a density of 1.063 with KBr, divided equally between two 12-ml plastic tubes and subjected to centrifugation at 100000 $\times$ g at 4° for 18 h. At the end of this period the bottoms of the tubes were punctured and the top 3-ml layers were collected as 'low density lipoproteins'. This fraction was dialyzed overnight at 8° against normal

saline to remove the KBr. An aliquot of the dialyzed material was taken for protein determinations[15] and for determination of ^{14}C and ^{3}H radioactivity.

[^{14}C]Urea (specific activity 30 mC/mmole) was obtained from ICN – Chemical and Radioisotope Division, Irvine, Calif.

[4-^{14}C]Cholesterol (specific activity 50 mC/mmole) and [1-^{14}C]linoleic acid (specific activity 60 mC/mmole) were purchased from New England Nuclear Corp., Boston, Mass., and [9,10-$^{3}H_2$]oleic acid (specific activity 3 C/mmole) was bought from Amersham-Searle, Chicago, Ill.

[^{32}P]Lysolecithin was prepared biosynthetically as previously described[16].

In all experiments in which two different radioactive labels were used, absolute radioactivity of each isotope was determined by applying appropriate corrections for quenching and overlap. Counting of radioactivity was carried out in a Packard Liquid Scintillation counter, using toluene based BBOT (2,5-bis-2-(5-*tert.*-butylbenzoxazolyl)-thiophene; Packard Instrument Co., Downers Grove, Ill.) scintillator and Biosolv (Beckman Instrument Co., Calif.) as a solubilizing agent for aqueous solutions.

Incubation procedure

In most experiments the labeled compounds dissolved in toad Ringer's solution were introduced into the inside solution, in some experiments into the outside solution. In the usual experiment 5-μl aliquots were taken from the solution into which the radioactive material had been introduced (the inside solution) and 0.2-ml aliquots from the outside solution, at various time intervals.

When the inside solution or the outside solution were to be replaced by fresh solutions, the mounted tissue was first incubated for 10 min with toad Ringer's–albumin solution on both sides, to wash off any loosely adherent radioactivity. The wash solutions were discarded. In experiments in which the effect of temperature on transfer was examined, the chambers were allowed to equilibrate at the new temperature for 15 min prior to sampling.

Incubations were carried out during shaking at approx. 150 strokes per min.

Fractionation of lipid extracts by thin-layer chromatography and counting of the radioactive fractions were performed as previously reported[16].

Calculations

To compute the transfer in nmoles from one compartment to the other, it was assumed that the specific activity of the radioactive material transferred was the same as of that introduced initially. Corrections were made for removal of the 0.2-ml aliquots, but not for removal of the 5-μl aliquots.

RESULTS

Since albumin is the carrier for free fatty acid and lysolecithin, the plasma lipid constituents that are most readily utilized by the tissues, the initial experiments served to establish the permeability of the scraped toad bladder for this serum protein. Fig. 2A shows that the passage of [^{125}I]albumin from inside solution to outside solution is linear with time and increases with increasing concentration. Fig. 2B shows that the increase in transfer at 30 min is also linear over a 100-fold concentration range. In other experiments we found that linearity of transfer in terms of time and

concentration was maintained through physiological plasma albumin concentrations (4 g/100 ml or approx. 1 mM). Introduction of the labeled albumin solution to the outside solution and sampling from the inside solution gave essentially similar results.

In contrast to the scraped bladder, the bladder with intact epithelial layer permits no detectable passage of [^{125}I]albumin from inside solution to outside solution

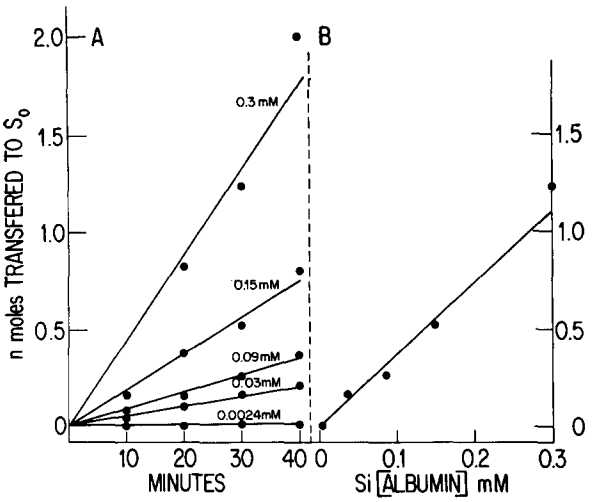

Fig. 2. Effect of time and concentration on transfer of albumin across the mounted scraped toad-bladder. Transfer was calculated from the specific activity of the albumin in the inside solution, and is given as nmoles transferred during the indicated time periods. In B, nmoles transferred at 30 min (ordinate) are plotted against albumin concentration in the inside solution (abscissa). For details, see MATERIALS AND METHODS. S_i = inside solution; S_0 = outside solution.

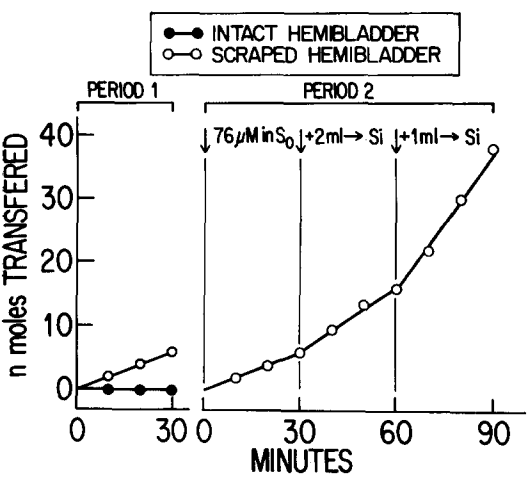

Fig. 3. Effect of hydrostatic pressure and of addition of albumin to the outside solution on transfer of [^{125}I]albumin. Labeled albumin was added to the inside solution in a concentration of 76 μM. At the beginning of Period 2 the toad Ringer's solution in the outside solution was replaced with fresh solution that contained unlabeled albumin in a concentration of 76 μM. At 30 min in Period 2 another 2 ml and at 60 min another 1 ml of labeled albumin solution (76 μM) were added to the initial 2 ml of inside solution.

(Fig. 3, first period). Addition of equimolar amounts of unlabeled albumin to the outside solution has no effect on transfer from inside solution to outside solution (Fig. 3, second period, first 30 min). Passage of [^{125}I]albumin is markedly affected by hydrostatic pressure. At 30 min during the second period 2 ml of the same labeled albumin solution were added to the inside solution, and at 60 min another 1 ml. The increase in rate and the prompt resumption of linearity with each addition is readily apparent. Dialysis of the outside solution against saline revealed that all ^{125}I is non-dialyzable after transfer through the membrane.

In 16 experiments and 26 separate observations 5.2 % ± 1.2 (mean ± S.E.) of the [^{125}I]albumin in the inside solution were transferred to the outside solution in 30 min. In several later experiments dialysis cells (Arthur H. Thomas Co., Philadelphia Pa.) were used that permitted mounting of the bladder vertically, with equal volumes of liquid on both sides of the membrane. The results of these experiments differed in no way from those obtained with the apparatus shown in Fig. 1. Variability in permeability of different scraped bladder preparations appears best explained by a varying extent of removal of the epithelial layer. This contention is borne out by electron microscopic observations of the scraped tissues that indicated patches of remaining epithelium in several preparations.

Albumin bound [1-^{14}C]linoleate or [^{32}P]lysolecithin, and [^{125}I]albumin are transferred at closely similar rates (Fig. 4). At 15 min transfer of [^{32}P]lysolecithin was somewhat less than that of the other two labeled molecular species. This appears explained by retention of ^{32}P by the tissue. Lipid extracts of the scraped bladders prepared at the end of the experiments contained appreciable quantities not only of [^{32}P]lysolecithin, but also of [^{32}P]lecithin and [^{32}P]glycerylphosphorylcholine, reflecting the marked ability of the tissue to metabolize albumin-bound lysolecithin[9,10]. By contrast little ^{125}I and ^{14}C radioactivity was found in the lipid extracts of the bladders.

Transfer of ^{14}C-labeled fatty acid is the same in the presence or absence of albumin in the outside solution. Further, no fatty acid label is released by scraped toad bladders that had previously incorporated ^{14}C-labeled fatty acid into their lipids during prolonged preincubation of the intact bladder[11]. It seems therefore that ^{14}C-labeled fatty acid and [^{32}P]lysolecithin pass through the membrane as albumin complexes.

Comparison of transfer of albumin and globulin

The transfer of albumin (mol. wt. approx. 65000) and of γ-globulin (mol. wt. approx. 160000) was compared in experiments in which the inside solution contained 0.01 mM albumin and 0.01 mM globulin, and either [^{125}I]albumin or [^{125}I]globulin (see MATERIALS AND METHODS). These experiments were conducted in three observation periods: In period 1 the inside solution of one hemibladder preparation contained [^{125}I]albumin as the label and the inside solution of the corresponding hemibladder γ-[^{125}I]globulin. At the end of Period 1, the inside solution was removed and replaced with fresh solution in which the labels were reversed. In Period 3 the inside solution was as in Period 1. Since the amounts transferred in corresponding periods were closely similar, the results were pooled and are presented as the mean ± S.E. of nmoles of albumin or globulin transferred (Fig. 5). It is evident that the rates of transfer of albumin and globulin are the same. In line with this conclusion is the

finding that passage of either [^{125}I]albumin or of [^{125}I]γ-globulin (in nmoles) was approximately half when respectively globulin or albumin were omitted from the inside solution. The effect of concentration on transfer was also the same for albumin and globulin. As in the case of albumin, addition of equimolar concentration of unlabeled globulin to the outside solution did not affect passage of [^{125}I]globulin from inside solution to outside solution.

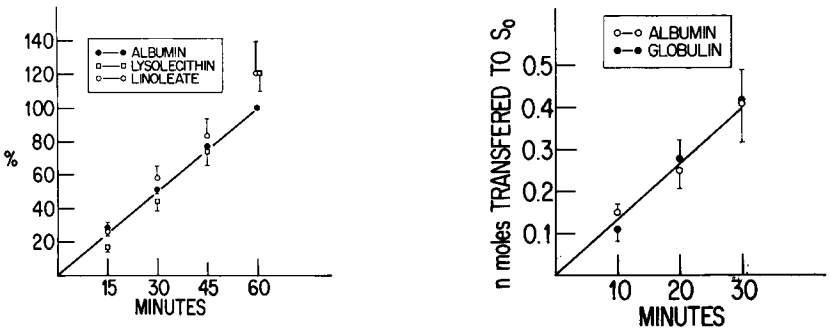

Fig. 4. Comparison of transfer of [1-^{14}C]linoleate and [^{32}P]lysolecithin complexed to albumin with transfer of [^{125}I]albumin. The concentrations of albumin, of added linoleate and lysolecithin in the inside solution were respectively 0.3, 0.125 and 0.1 mM. Complexing of linoleate and lysolecithin to albumin was achieved as previously described[16]. Transfer is expressed as percent of the 60-min value for albumin. The results are given as mean ± S.E. of observations made on four scraped bladder preparations.

Fig. 5. Comparison of transfer of albumin and globulin. The experiments were carried out as described in the text. The results are given as nmoles of albumin or globulin transferred (mean ± S.E.), through four different mounted scraped bladders.

Comparison of transfer of albumin with that of low density lipoproteins

Rat low density lipoproteins were labeled with [4-^{14}C]cholesterol and [9,10-$^{3}H_2$]-oleate as described in MATERIALS AND METHODS. In Fig. 6 the results are shown of two experiments (carried out with two scraped hemibladders in each experiment) in which the transfer of albumin and low density lipoproteins were compared. Transfer is expressed as percent of the value for [^{125}I]albumin at 60 min of incubation at 37°. Both the low density lipoprotein-associated labeled cholesterol and oleate appear in the outside solution at detectible but much slower rates than the albumin. In each of three experiments (6 mounted hemibladders) transfer of [^{3}H]oleate was greater than that of cholesterol. Preliminary experiments further showed that [^{14}C]cholesterol and ^{3}H-labeled fatty acid labeled high density lipoproteins were transferred at rates approximately twice those observed for low density lipoproteins. Again ^{3}H radioactivity was transferred more rapidly than ^{14}C radioactivity.

Effect of temperature on transfer

In these experiments one mounted hemibladder was incubated at 37° for 40 min, then at 6° and finally again at 37° while the other hemibladder was incubated first at 6°, subsequently at 37° and finally at 6°. The transfer of albumin in four such experiments is expressed as percent of the 37° value at 40 min in Period 1 (first category) or in Period 2 (second category) (Fig. 7). In may be seen that the temperature effect on the transfer of albumin was very reproducible. The average ratio of transfer

at 37° and at 6° was 2.77 ± 0.07 (mean ± S.E.). At the lower temperature the mounted hemibladder relaxed considerably, thus tightening of the tissue at 6° cannot explain the slower transfer.

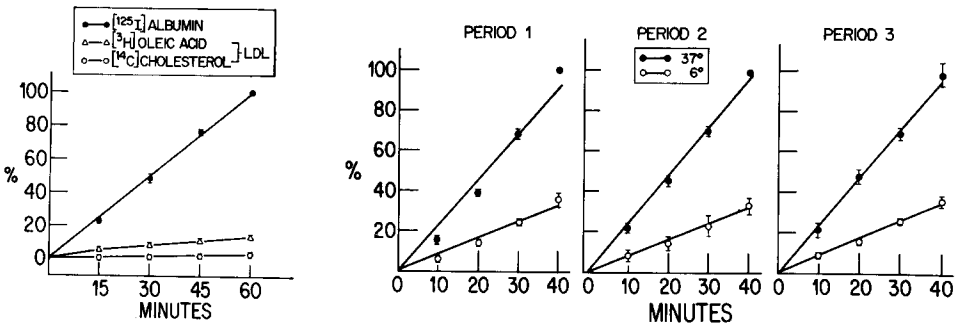

Fig. 6. Comparison of transfer of albumin and low density lipoproteins. The inside solution contained 600 nmoles of albumin in 2 ml and approx. 10 nmoles of low density lipoproteins (if it is assumed that each low density lipoprotein molecule contains 2 units of B protein with a molecular weight of 400000[3]). Each 10 nmoles of low density lipoprotein contained 40000 disint./min of ^{14}C- and 52000 disint./min of ^{3}H radioactivity. Of the total [4-^{14}C]cholesterol radioactivity of the isolated low density lipoproteins, 40% was in cholesterol esters. The distribution of [^{3}H]-oleic acid radioactivity, as determined by thin-layer chromatography in petroleum ether–diethyl ether–glacial acetic acid (80:20:1, by vol.), was as follows: triglycerides 76%, phospholipids 12.5%, free fatty acids 7% and diglycerides 4.5%. This distribution was the same in lipid extracts of the inside solution after incubation for 3 h. The amount of radioactivity in the outside solution was insufficient for accurate determination of distribution among the various fractions. Grossly the distribution was the same as that of the low density lipoproteins in the inside solution. Lipids of the scraped bladders were extracted at the end of the experiments. The extracts contained almost no radioactivity. Transfer of [^{125}I]albumin and of ^{14}C and ^{3}H low density lipoprotein (LDL) radioactivity is expressed as percent of the 60-min value for albumin.

Fig. 7. Effect of temperature on transfer on [^{125}I]albumin. For experimental details see text and MATERIALS AND METHODS. The results are those of four experiments and are given as mean ± S.E. The albumin concentration of the inside solution was 0.3 mM.

TABLE I

COMPARISON OF EFFECT OF TEMPERATURE ON TRANSFER OF [^{125}I]ALBUMIN AND [^{14}C]UREA

The experiments were carried out as those depicted in Fig. 7, except that the lower temperature was 10° instead of 6°. The concentration of albumin and of urea in the inside solution was 0.3 mM.

(A) Ratio: (transfer at 37°)/(transfer at 10°)			
[^{14}C]urea		*[^{125}I]albumin*	
Hemibladder 1	*Hemibladder 2*	*Hemibladder 1*	*Hemibladder 2*
2.1 ± 0.15 (4)	2.1 ± 0.15 (4)	2.3 ± 0.1 (4)	2.25 ± 0.15 (4)

(B) Ratio: ([^{14}C]urea percent transferred)/([^{125}I]albumin percent transferred)			
Hemibladder 1		*Hemibladder 2*	
37°	*10°*	*37°*	*10°*
3.65 ± 0.1 (6)	3.9 ± 0.3 (3)	5.2 ± 0.3 (4)	5.9 ± 0.6 (7)

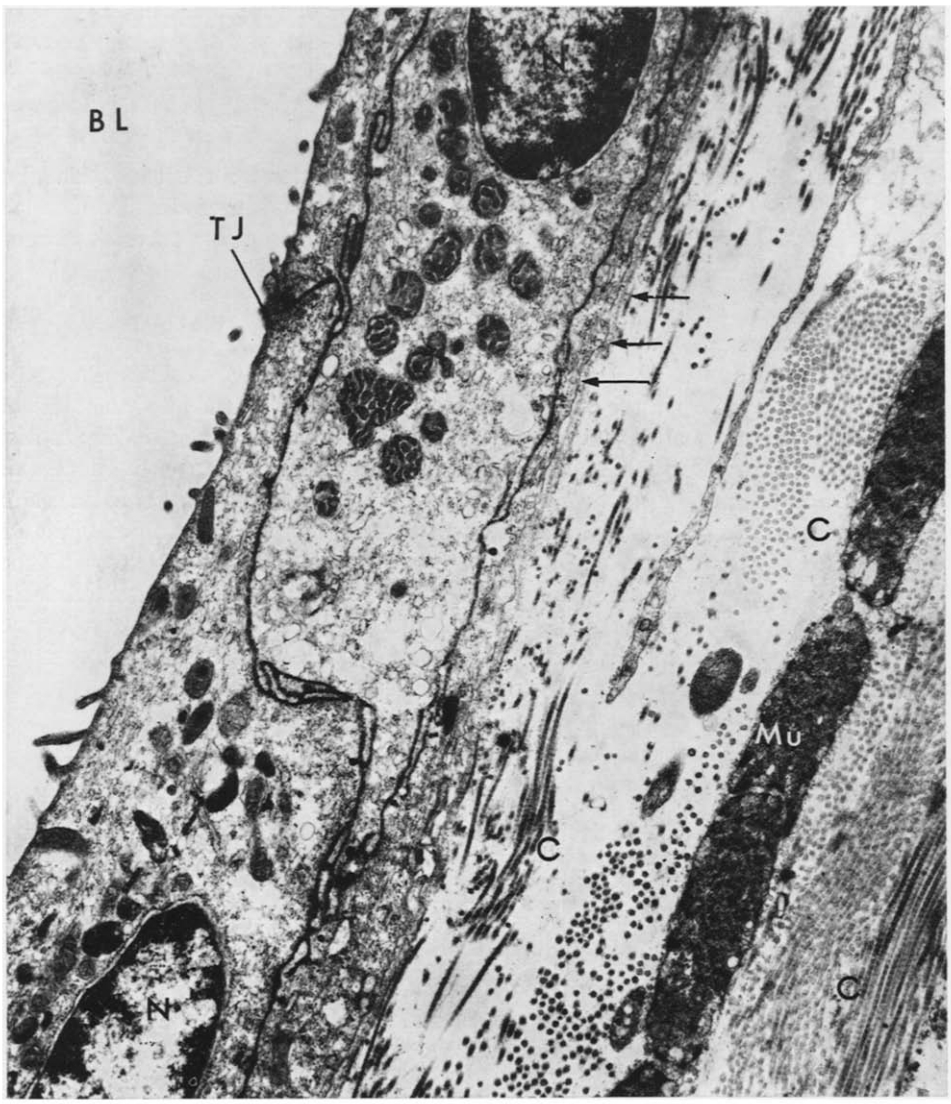

Fig. 8. Detail of thin section of toadbladder. Peroxidase (4 mg) was introduced into the outside solution (facing the serosal surface). The presence of peroxidase was demonstrated as described by KARNOVSKY[25]. Although vesicles are abundant at the mucosal side of the basement membrane (arrows), these do not appear to contain peroxidase. The enzyme is concentrated in intercellular channels (black) and is prevented from reaching the mucosal surface by a tight junction (TJ). BL, bladder lumen; N, nucleus of mucosal cell; C, collagen; Mu, muscle. Magnification × 12000.

The values for transfer determined at 37° and at 6° were used to calculate the activation energy for transfer of albumin, employing the Arrhenius equation. The value obtained was 5.5 kcal/mole which is close to the values reported for the diffusion of tritiated water through the scraped bladder[17].

In other, similar, experiments the effect of temperature on transfer of albumin and urea, present in the inside solution at equimolar concentration (0.3 mM) was

compared. At both temperatures urea passed through the membrane at a faster rate. As shown in Table 1A, the ratio of transfer during 40-min observation periods at 37° and at 10° was the same for urea and for albumin, which implies that the ratio: ([^{14}C] urea percentage transferred)/([^{125}I]albumin percentage transferred) must also be the same at the two temperatures. This was indeed the case even though the two hemibladders showed somewhat different relative permeabilities for the two molecular species (Table IB). The activation energies calculated for transfer of albumin through the two scraped hemibladders were 5.0 and 5.5 kcal/mole and for transfer of urea 4.7 and 4.5.

Electron microscopic examination of vesicles in the toad bladder

Recently MASUR *et al.*[16] demonstrated peroxidase positive vesicles along the basal and lateral portions of the toadbladder epithelial cells. Treatment of the bladder with oxytocin produced an increase in the number of peroxidase-stained vesicles and the authors proposed that these vesicles might serve a transport function. In view of this report and the possibility that part of the transfer of the labeled substances in our experiments, was due to vesicular transport, thin sections of unscraped bladders were prepared after exposure to peroxidase, to determine whether vesicles could be observed across the tissue. Peroxidase staining was only apparent intercellularly (Fig. 8). Vesicles seen in the submucosal tissue did not contain peroxidase under the conditions of these experiments. It should be mentioned in this context that no polystyrene particles (0.3 μm in diameter) traversed the scraped bladder.

DISCUSSION

The transfer of large molecules such as albumin, globulin and low density lipoproteins through the heterogeneous layers of the scraped bladder seems *a priori* most reasonably ascribed to passage through interstices of the tissue. The results support this view. The transfer rates for urea were from 3.5 to 6 times faster than for albumin and for density lipoprotein roughly 10 times slower than for albumin, suggesting that molecular size is a major determinant of the rate of translocation. The observation that albumin and globulin were transferred at the same rate might seem at first glance to militate against this conclusion. However, despite a 2.5-fold difderence in molecular weight, it could be predicted on the basis of parameters related to molecular size, such as partial specific volume, diffusion coefficient and fractional ratio[19], which are closely similar for the two molecules, that the rate of transfer of albumin and globulin would be indistinguishable if molecular size determined the rate of passage. Albumin transfer was linear with time during the periods of observation and did not reach saturation with increasing concentration up to that of normal serum. Further, both the passage of albumin and of urea differed less than 3-fold between 6° and 37°. These findings and the low activation energy (approx. 5 kcal/mole) for transfer of albumin (and urea) suggested simple diffusion through aqueous channels as the mechanism of transfer.

We considered the remote possibility that pinocytosis and vesicular transport (refs. 20, 21) could account for some of the observed transfer, in view of the preliminary report of MASUR *et al.*[18], that described the existence of apparently extracellular

vesicles in the toadbladder under and between the mucosal cells. These vesicles stained for peroxidase when the intact bladder was exposed to this enzyme from the serosal (but not the mucosal) side. The virtual absence of peroxidase staining vesicles between serosa and mucosa in our studies implies that the horse radish peroxidase (mol. wt. about 40000) must have penetrated the tissue from the serosal surface prior to its appearance at the interfaces of the mucosal cells. Further, the disparate rates of transfer of the different molecular species clearly indicates that vesicular transport cannot possibly account for all passage. Even a small contribution by vesicular transport to translocation is unlikely, since linearity of transfer of all four molecules seems to exclude a significant role for two or more mechanisms operating at appreciably different rates. Thus we consider our findings consistent with the concept that diffusion of the molecular species examined through interstices of limited size is the mode of passage through the tissue. Essentially similar conclusions were arrived at by BRACHET AND RASIO[8] who examined the passage of insulin across isolated rat mesentery.

While caution must be exercised in attempting to see an analogy between the capillary wall as a barrier to the passage of plasma constituents on the one hand and the membrane model used in these studies on the other, certain resemblances in permeability properties may be observed. Physiological and morphological evidence indicates that the capillary wall behaves as a semipermeable structure containing pores which permit the restricted diffusion of protein and lipoprotein molecules[22,23]. A mass of data has accumulated indicating that the largest lipid-carrying particles in the circulation, the chylomicra, do not pass through continuous endothelium to any significant extent, and that hydrolysis of the chylomicron triglyceride must take place to permit passage of the constituent fatty acids and their utilization by the tissues[7,23,24]. Some passage of both high and low density lipoproteins through the capillary wall has been inferred from studies *in vivo*[23]. There is little doubt that albumin passes at a rapid rate through normal capillaries in many organs. To what measure this is sufficient to account for the exceedingly rapid disappearance of intravenously injected ^{14}C-free fatty acids has not been settled.

Compared to albumin and associated free fatty acid and lysolecithin, low density lipoprotein or high density lipoprotein traversed the scraped bladder at a far slower rate, in line with the above observations *in vivo*. It is not clear why the transfer rate of ^{3}H (fatty acid) was appreciably faster than that of ^{14}C (cholesterol). We have found no evidence of significant contamination of the lipoprotein with ^{3}H-labeled free fatty acid–albumin complexes, of release of free fatty acid from labeled esterified lipid due to toadbladder lipase activity[11], nor of retention by the tissue of [^{14}C]cholesterol relative to ^{3}H-radioactivity. We favor therefore the possibility of heterogeneity of the lipoprotein fraction. It would be of interest to establish whether such heterogeneity develops during passage of an initially homogeneous fraction, or whether this membrane preparation permits distinction of subfractions in the lipoprotein material obtained by conventional techniques of ultracentrifugal separation. Investigation of these possibilities requires much more highly radioactive lipoproteins labeled not only in the lipid portion but also in the protein moiety, thus allowing more detailed analysis of the material transferred through the bladder. Such studies can continue to employ the scraped toadbladder as a useful model.

ACKNOWLEDGEMENTS

This study was supported by a grant of the New York Heart Association.

P.E. is a Career Scientist of the Health Research Council of the City of New York, Contract I-379.

We are indebted to Dr. Dorothy Zucker-Franklin of the Department of Medicine, New York University School of Medicine, who carried out the electron microscopic studies.

REFERENCES

1 D. S. FREDERICKSON AND R. S. GORDON, JR., *Physiol. Rev.*, 38 (1958) 585.
2 V. P. DOLE AND J. T. HAMLIN III, *Physiol. Rev.*, 42 (1962) 674.
3 D. S. FREDERICKSON, R. I. LEVY AND R. S. LEES, *New Engl. J. Med.*, 276 (1967) 32.
4 F. C. COURTICE AND D. G. GARLICK, *Quart. J. Exp. Physiol.*, 47 (1962) 221.
5 V. T. MARCHESI AND R. J. BARRNETT, *J. Cell Biol.*, 17 (1963) 547.
6 E. R. SUTER AND G. MAJNO, *J. Cell Biol.*, 27 (1965) 163.
7 G. J. SCHOEFL AND J. E. FRENCH, *Proc. R. Soc. London, Ser. B*, 169 (1968) 153.
8 E. BRACHET AND E. RASIO, *Biochim. Biophys. Acta*, 183 (1969) 162.
9 A. A. ROSENBLOOM AND P. ELSBACH, *Am. J. Physiol.*, 214 (1968) 161.
10 A. A. ROSENBLOOM AND P. ELSBACH, *Biochim. Biophys. Acta*, 164 (1968) 72.
11 A. A. ROSENBLOOM AND P. ELSBACH, *J. Lipid Res.*, 10 (1969) 406.
12 P. J. BENTLEY, *J. Endocrinol.*, 17 (1958) 201.
13 P. J. MCCONAHEY AND F. J. DIXON, *Int. Arch. Allergy Appl. Immunol.*, 29 (1966) 185.
14 W. M. HUNTER AND F. C. GREENWOOD, *Nature*, 194 (1962) 495.
15 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
16 P. ELSBACH, *J. Clin. Invest.*, 47 (1968) 2217.
17 R. M. HAYES, N. FRANKI AND R. SOBERMAN, *J. Clin. Invest.*, 50 (1971) 1016.
18 S. K. MASUR, E. HOLTZMAN AND P. EGGENA, *J. Cell Biol.*, 43 (1969) 86a.
19 H. A. SOBER, *Handbook of Biochemistry*, The Chemical Rubber Co., Cleveland, 1960.
20 G. E. PALADE, *J. Appl. Physics*, 24 (1953) 1424.
21 G. E. PALADE, *Anat. Rec.*, 136 (1960) 254.
22 J. R. PAPPENHEIMER, *Physiol. Rev.*, 33 (1953) 387.
23 J. E. FRENCH, B. MORRIS AND D. S. ROBINSON, *Br. Med. Bull.*, 14 (1958) 234.
24 M. B. ENSER, F. KUNZ, J. BORENSZTAJN, L. H. OPIE AND D. S. ROBINSON, *Biochem. J.*, 104 (1967) 306.
25 V. M. J. KARNOVSKY, *J. Cell Biol.*, 35 (1967) 213.