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Transport of Hexoses, Potassium and Neutral Amino Acids into Capillaries Isolated from Bovine Retina

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Capillaries were isolated from bovine retina by homogenization and glass bead filtration in order to study their ability to transport certain solutes. Viability of the microvessels was demonstrated by their ability to maintain linear rates of substrate oxidation for more than two hours. Hexose uptake (measured using 3-*o*-methyl-D-glucose) could be inhibited by cytochalasin B, phloretin and phlorizin, but not by 2,4-dinitrophenol or ouabain. D-Glucose, 2-deoxy-D-glucose, D-mannose, D-galactose and D-xylose inhibited 3-*o*-methyl-D-glucose uptake, while L-glucose, D-ribose and D-fructose did not. When incubated at 37°C with 5 mM-D-glucose, the microvessels contained much more free D-glucose than D-glucose metabolites. Thus, transport was not rate limiting for metabolism. L-Glucose entered capillaries more slowly than other hexoses and served as a marker for simple diffusion of sugars into the cells. $^{86}Rb^+$ was used as an indicator of K^+ transport activity. Uptake of $^{86}Rb^+$ was temperature sensitive and markedly inhibited by 1 mM ouabain, thus indicating the presence of an active K^+ transport system. Na^+-dependent amino acid transport was demonstrated using α-(methylamino)isobutyric acid as a model substrate. Capillary uptake of this neutral amino acid analogue was inhibited after abolishing the Na^+ gradient with 1 mM-ouabain. Uptake of the organic anion *p*-aminohippuric acid (PAH) was slightly greater than uptake of the extracellular marker sucrose. There was a small inhibition of PAH uptake by fluorescein and penicillin but not by probenicid. Our results indicate the presence of several transport processes in retinal capillary endothelial cells which may be important for the maintenance of homeostasis within the retina.

Key words: glucose; 3-*o*-methylglucose; cytochalasin B; phloretin; phlorizin; glucose transport stereospecificity; rubidium; α-(methylamino)-isobutyric acid; *p*-aminohippuric acid; endothelial cells; metabolism.

1. Introduction

The term "blood–retinal barrier" (BRB) is used to describe the selective exchange of certain solutes between blood and retina. This permeability barrier is thought to involve both restricted diffusion and carrier-mediated transport processes (Cunha-Vaz, 1976). Although there is considerable morphologic evidence indicating that both the capillary endothelium and the pigment epithelium of the retina are responsible for maintaining a normal BRB (Cunha-Vaz, 1976; Raviola, 1977), there is little information regarding their relative contributions. One approach to this problem is to study transport processes in preparations of pigment epithelium or retinal capillaries isolated from other cellular elements of retina. So far, there have been few investigations of this type.

The mechanism by which sugars move between the blood and retina is particularly important since D-glucose is a major metabolic substrate for the retina (Graymore, 1969) and because microangiopathy is an important component of diabetic retinopathy. Studies in vivo demonstrate that D-glucose uptake from blood to vitreous humor is stereospecific, thus implying a carrier-mediated mechanism (Dollery, Henkind and Orme, 1971). Using the isolated pigment epithelium from the frog,

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Zadunaisky and Degnan (1976) observed net movement of sugars from choroidal to apical surfaces, however, in a similar preparation, Miller and Steinberg (1976) could not detect net sugar flux. There are no studies of D-glucose transport using isolated retinal capillaries.

There is considerable evidence that neutral amino acids (Reddy, Chakrapani and Lim, 1977a), potassium (Bito, 1970, 1977; Bito and Davson, 1964) and organic anions (Cunha-Vaz and Maurice, 1967) are actively transported out of the vitreous across the blood–retinal barrier but the cellular location of these active transport systems has been difficult to determine. Based on results of experiments in vivo, it has been suggested that amino acids are transported only at the pigment epithelium (Reddy et al., 1977) while organic anions are transported at the capillary endothelium and the pigment epithelium (Cunha-Vaz and Maurice, 1967). However, it is difficult to interpret studies in which both BRB tissues are present. Direct evidence for active transport at the pigment epithelium has been presented by Miller and Steinberg (1976, 1977) and Lasansky and de Fisch (1966) who used the isolated frog pigment epithelium to demonstrate transport of ions, taurine and methionine. Recently, Hjelle, Baird-Lambert, Cardinale, Spector and Udenfriend (1978) described a non-energy dependent transport system for large neutral amino acids in isolated retinal capillaries.

In this report, we describe some of the properties of hexose, small neutral amino acid, potassium and organic anion transport into isolated retinal capillaries. Our results suggest that transport of these solutes may occur at the microvascular component of the BRB.

2. Materials and Methods

Retinal capillaries were isolated by modification of methods previously described for use with retina (Meezan, Brendel and Carlson, 1974) and brain (Goldstein, Wolinsky, Csejtey and Diamond, 1975). Bovine eyes were obtained from a local slaughterhouse and transported to the laboratory on ice. The globes were incised and the retinae were removed and placed in an iced buffer consisting of oxygen-saturated Ringer solution with 1·2 mM-$MgCl_2$, 15 mM-*N*-2-hydroxyethylpiperazine-*N'*-2-ethanessulfonic acid (HEPES), pH 7·4, 1% fraction V bovine serum albumin and 5 mM-sodium pyruvate. The retinae were washed extensively with buffer on a 335 μm nylon mesh to remove small fragments of pigment epithelium. A 10% (w/v) homogenate was made using 5 up-and-down strokes in a Teflon and glass homogenizer (0·25 mm clearance) at 390 r/min and pelleted by centrifugation at 1000×**g** for 10 min. In order to obtain a uniform suspension of microvessels, the pellet was resuspended in a small amount of buffer and passed through a 335 μm nylon mesh. The capillaries were then separated from nuclei and other cellular debris by passing the suspension through a 1·2×1·5 cm column containing 0·25 mm glass beads. The capillaries were retained by the beads and were recovered by gentle agitation in buffer. After the beads settled, the supernatant was decanted and the capillaries were collected by centrifugation at 500×**g** for 5 min. Cell protein was determined (Lowry, Rosebrough, Farr and Randall, 1951) after overnight solubilization in 1% sodium dodecyl sulfate, using bovine serum albumin as the standard. The usual capillary yield was 0·17–0·25 mg of cell protein per retina.

The quality of each preparation was judged by its appearance under phase microscopy. Figure 1 shows a typical preparation which is largely free of contamination by non-vascular components. Occasional erythrocytes are seen trapped within the lumens of the microvessels. A few larger vessels were also seen. Contamination by erythrocytes was estimated by measuring the total hemoglobin content of the preparation using the method

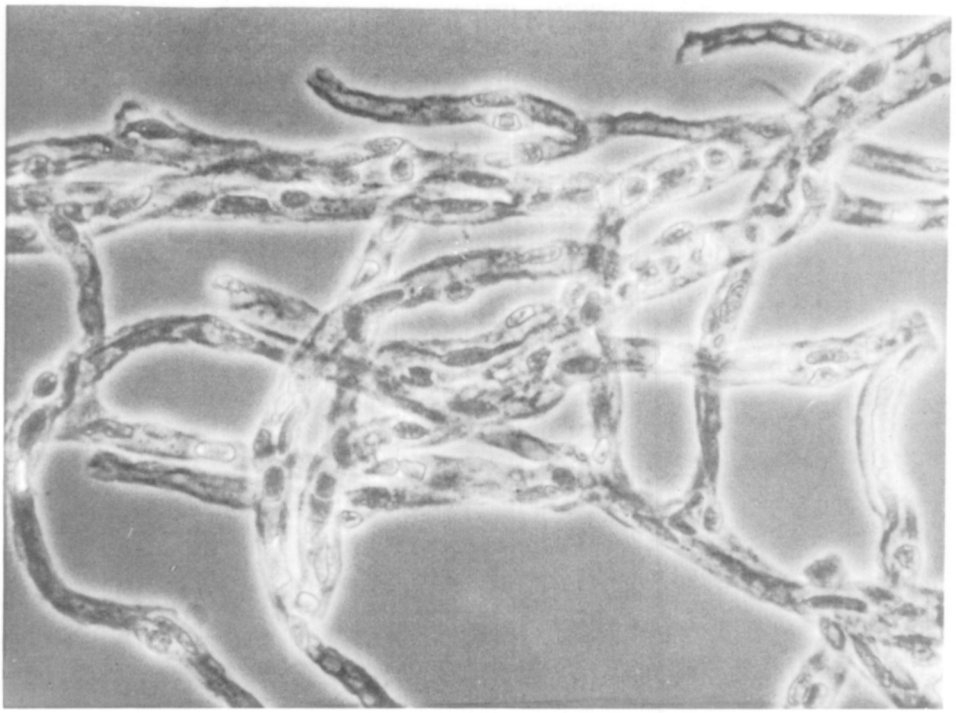

FIG. 1. Phase contrast photomicrograph of isolated retinal capillaries (×450).

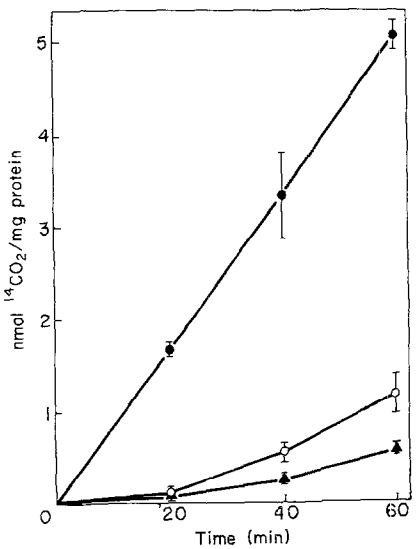

FIG. 2. Oxidative metabolism by isolated retinal vessels. 0·44 mg of capillary protein were incubated at 37°C in a total volume of 3 ml of buffer. The usual preparative buffer was used except that 1 mM glucose was substituted for pyruvate and fatty acid free bovine serum albumin was used. Each incubation contained 2 μCi of ^{14}C-labeled substrate and the flasks were flushed with 100% O_2. $^{14}CO_2$ was trapped on filter papers saturated with 10% KOH. The reaction was terminated by the addition of 1 ml of 1 N-H_2SO_4. The filters were removed and radioactivity determined in a liquid scintillation counter. ●, 1 mM [U-^{14}C]D-glucose; ○, 0·15 mM [U-^{14}C]palmitate; ▲, [3-^{14}C]D-β-hydroxybutyrate in 0·5 mM D,L-β-hydroxybutyrate. Data are the average of three determinations±S.D.

of Hunter, Grove-Rasmussen and Soutter (1950). The resulting 27 μg hemoglobin/mg total protein is equivalent to an intra-erythrocyte volume of 0·08 μl/mg protein.

The ability of isolated microvessels to oxidize glucose, palmitate, or β-hydroxybutyrate was measured by incubation at 37°C in the presence of ^{14}C-labeled substrates (Goldstein, 1979). The $^{14}CO_2$ was trapped on filter papers saturated with 10% KOH. The isolated capillaries maintained a linear rate of D-glucose oxidation for at least 1 hr (Fig. 2). After a brief delay, the oxidation of palmitate and of β-hydroxybutyrate was also linear with time. These results confirm the metabolic integrity of isolated retinal capillaries (Meezan et al., 1974) and demonstrate their ability to utilize several different energy substrates normally present in the blood.

The method for measurement of sugar transport using glass fiber filters was identical to that described previously (Betz, Csejtey and Goldstein, 1979). When indicated, [^{3}H]D-glucose and its ^{3}H-labeled products were separated by ion exchange chromatography (Lorenzo, 1976). As indicated in the figure legends, some experiments were performed at 25°C instead of 37°C in order to obtain a slower and more easily observable rate of transport. In experiments with $^{86}Rb^+$ 0·8 μm microporous cellulosic filters (Amicon, Lexington, MA) were used instead of glass fiber filters to decrease filter blanks.

The following materials were obtained from New England Nuclear Corporation (Boston, MA): [6-^{3}H]D-glucose, [U-^{14}C]D-glucose, [methyl-^{3}H]3-*o*-methyl-D-glucose, [U-^{14}C]-palmitic acid, [3-^{14}C]D-β-hydroxybutyric acid, [1-^{3}H]L-glucose, [1-^{14}C]α-(methylamino)isobutyric acid, [glycyl-1-^{14}C]*p*-aminohippuric acid, [U-^{14}C]sucrose, and $^{86}Rb^+Cl$. Phloretin was purchased from K & K Laboratories (Plainview, New York) and insulin from Eli Lilly and Company (Indianapolis, Indiana). All other chemicals were obtained from Sigma Chemical Company (St. Louis, Missouri).

3. Results

Hexose transport and phosphorylation

3-*o*-Methyl-D-glucose (3MG) was used as a model substrate for characterization of the D-glucose transport system. This sugar is nonmetabolizable (Czaky and Wilson, 1956) and has been used to study hexose transport by isolated brain capillaries (Betz et al., 1979). Figure 3 shows a time course for the uptake of 5 mM-3MG and its inhibition by 0·05 mM-cytochalasin B. The nontransported sugar L-glucose was used to measure the rate of simple diffusion into the preparation. Cytochalasin B is an inhibitor of D-glucose transport in several other cell systems including isolated brain capillaries (Betz et al., 1979). In isolated retinal capillaries it inhibited 3MG transport (Fig. 3) and reduced the initial uptake to nearly that of the diffusion marker. Contamination by erythrocytes did not significantly affect our results since the water space with which 3MG equilibrated (4·3 μl/mg protein) is much greater than the volume within the erythrocytes trapped in our preparation (0·08 μl/mg protein).

The data in Table I show that phloretin and phlorizin were also effective inhibitors of sugar transport into isolated retinal microvessels. Phloretin was more effective than phlorizin, while cytochalasin B was the most potent inhibitor tested. We previously described an identical pattern for inhibition of sugar transport into isolated brain capillaries (Betz et al., 1979) and a similar pattern was observed at the blood–brain barrier in vivo (Betz, Drewes and Gilboe, 1975; Drewes, Horton, Betz and Gilboe, 1977). In contrast, Na^+-dependent sugar transport in intestine and kidney is more sensitive to phlorizin than phloretin (Alvarado, 1967; Diedrich, 1966) and not inhibited by cytochalasin B (Hopfer, Sigrist-Nelson, Ammann and Murer, 1976). Further evidence that sugar transport into retinal capillaries is not energy or Na^+-

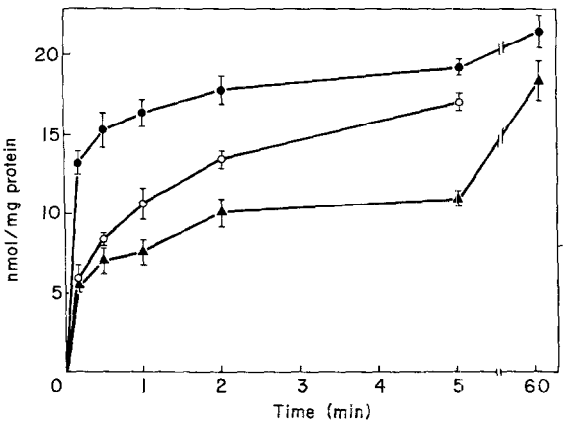

FIG. 3. Uptake at 25°C of 5 mM[^{3}H]3-*o*-methyl-D-glucose (●), 5 mM[^{3}H]-3-*o*-methyl-D-glucose in presence of 0·05 mM cytochalasin B (○), and 5 mM[^{3}H]L-glucose (▲). Data shown are averages of three determinations±S.D.

TABLE I

Effect of glucose transport inhibitors on 3-O-methyl-D-glucose uptake

Inhibition	Concentration (mM)	Uptake (nmol/mg/30 sec)	Percent Control
Cytochalasin B	0·05	1·68±0·68	15
Phloretin	0·50	5·05±0·87	46
Phlorizin	0·50	6·69±1·11	61
Control	—	10·88±0·90	100

Uptake of [^{3}H]3MG was determined after a 30 sec incubation at 25°C in the presence of 5 mM 3MG and the stated concentration of inhibitor. The data have been corrected for diffusion by using uptake of [^{3}H]L-glucose determined in simultaneous incubations with 5 mM-L-glucose (5·66 nmol/mg protein/30 sec±0·58 S.D.). Values are the averages of 4 determinations±S.D.

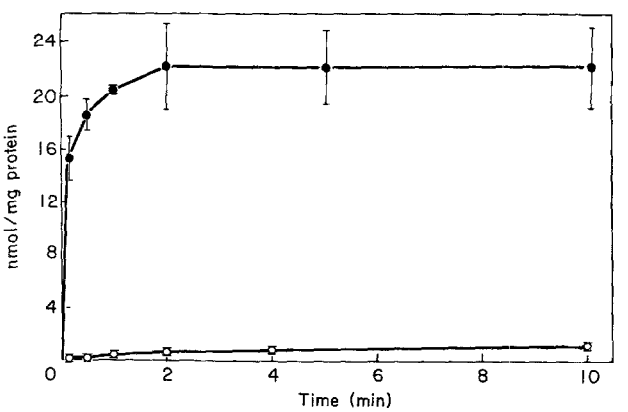

FIG. 4. Uptake and metabolism of 5 mM[^{3}H]D-glucose at 37°C. After incubation, intracellular radioactivity was separated into [^{3}H]D-glucose (●) and [^{3}H]glucose metabolites (○) by ion-exchange chromatography. Data shown are averages of three determinations±S.D.

TABLE II

Effect of possible modifiers of sugar transport on 3-O-methyl-D-glucose uptake

Compound	Concentration	Uptake (nmol/mg/30 sec)	Percent Control
Ouabain	1·0 mM	6·26±1·29	90
2,4-Dinitrophenol	0·5 mM	6·70±1·44	97
Insulin	0·1 U/ml	6·34±0·98	91
Control	—	6·94±0·83	100

Isolated capillaries were preincubated for 30 min at 37°C in the presence of the stated concentration of compound. Uptake of [^{3}H]3MG was then determined after a 30 sec incubation at 25°C in the presence of 5 mM-3MG. The data have been corrected for diffusion by using uptake of [^{3}H]L-glucose determined in simultaneous incubations with 5 mM L-glucose (2·74 nmol/mg protein/30 sec±0·22 S.D.). Values are the averages of 4 determinations±S.D.

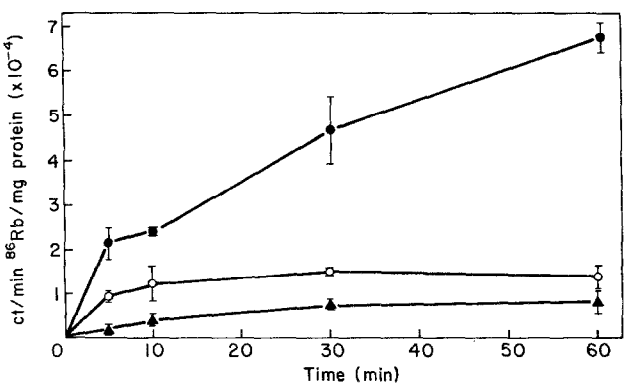

FIG. 5. Temperature dependent uptake of ^{86}Rb and inhibition by ouabain. Capillaries were incubated with 1·0×10^7 (ct/min)/ml of ^{86}Rb (SA = 82·3 μCi/μmol) at (●) 37°C, (○) 37°C and 1 mM ouabain or (▲) 4°C. Data shown are averages of three determinations±S.D.

dependent is shown in Table II. There was no inhibition of 3MG uptake after a 30 min preincubation in the presence of 1 mM-ouabain which eliminates the Na^+ gradient or 0·5 mM-2,4-dinitrophenol which depletes the supply of ATP. Sugar transport was not stimulated by insulin (Table II).

It should be noted that values for glucose uptake under control conditions do vary from one preparation to another. However, within any individual preparation, the control uptake was constant and reproducible over the 1–2 hr that were required to complete the experiment. Therefore, data presented in each figure or table were obtained using a single capillary preparation, but the pattern of results could be reproduced on different preparations.

The stereospecificity of 3MG uptake was studied by measuring the uptake of 5 mM-3MG in the presence of 100 mM concentrations of various glucose analogues. Table III shows that D-glucose, 2-deoxy-D-glucose, 3-*o*-methyl-D-glucose, D-mannose, D-galactose and D-xylose were effective inhibitors of 3MG uptake, while L-glucose, D-ribose and D-fructose were not. These results are nearly identical to those obtained in our previous study using isolated rat brain capillaries (Betz et al., 1979). Furthermore, the data justify use of 3MG as a measure of D-glucose transport since 3MG

uptake was markedly inhibited by D-glucose. Similarly, the lack of inhibition by L glucose supports use of this compound as a diffusion marker.

The relationship between the uptake of D-glucose and its subsequent metabolism is shown in Fig. 4. In the presence of 5 mM-D-glucose and at 37°C transport was more rapid than metabolism and there was a large pool of free D-glucose within the cells. This relationship was also found in isolated brain capillaries (Betz et al., 1979) and would be required for efficient trans-endothelial movement of D-glucose from blood to retina or brain.

$^{86}Rb^+$ uptake

We used $^{86}Rb^+$ to study K^+ transport into retinal capillaries. $^{86}Rb^+$ has essentially identical transport properties in mammalian cells as K^+ (Vaughan and Cook, 1972) but has a longer half-life than radioactive potassium isotopes. Figure 5 shows the time course for uptake of a tracer concentration of $^{86}Rb^+$ in the presence of 3 mM-K^+. This process was nearly eliminated at 4°C and was markedly inhibited by 1 mM-ouabain. Ouabain sensitive uptake of K^+ (or Rb^+) is an energy dependent process

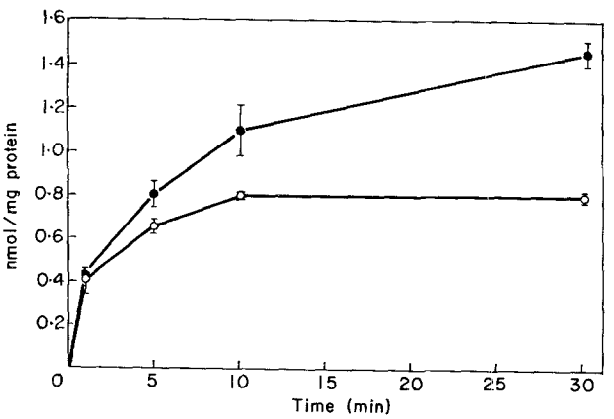

FIG. 6. Uptake of α-(methylamino)isobutyric acid and inhibition by ouabain. Capillaries were pre-incubated for 30 min at 37°C with (○) or without (●) 1 mM ouabain. Uptake of 0·29 mM[^{14}C]-α(methyl-amino)-isobutyric acid was then determined. Data shown are averages of three determinations±S.D.

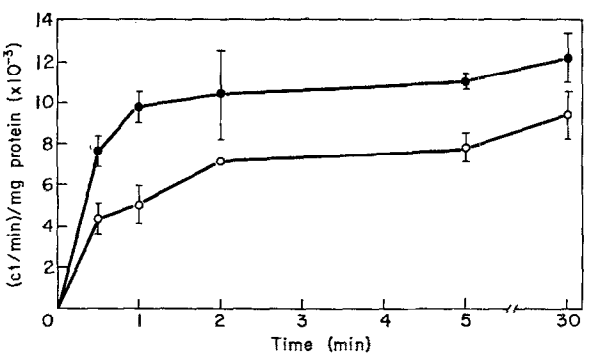

FIG. 7. Uptake of *p*-aminohippuric acid and sucrose. Capillaries were incubated at 37°C with $4{\cdot}5 \times 10^6$ (ct/min)/ml of (●) $4{\cdot}5 \times 10^{-5}$ M[^{14}C]-PAH or (○) $3{\cdot}7 \times 10^{-6}$ M[^{14}C]-sucrose and uptake determined. Data shown are averages of three determinations±S.D.

TABLE III

Inhibition of 3-O-methyl-D-glucose uptake by glucose analogues

Added sugar	Uptake (nmol/mg/30 sec)	Percent Control
D-Glucose	2·53±0·73	38
2-Deoxy-D-glucose	3·31±1·07	50
3-*o*-Methyl-D-glucose	3·39±0·65	51
D-Mannose	3·86±0·65	58
D-Galactose	4·31±1·31	64
D-Xylose	5·37±1·04	81
L-Glucose	6·67±1·07	100
D-Ribose	6·69±0·67	101
D-Fructose	7·19±0·68	108
Control	6·64±0·94	100

Uptake of [^{3}H]3MG was determined after a 30 sec incubation at 25°C in the presence of 5 mM-3MG and 100 mM inhibiting sugar. The control sample contained 100 mM mannitol to correct for osmotic effects. The data have been corrected for diffusion by using uptake of [^{3}H]L-glucose in the presence of 5 mM L-glucose and 100 mM mannitol (7·00 nmol/mg/30 sec±0·60 S.D.). Values are the averages of 4 determinations±S.D.

mediated via Na^+, K^+-ATPase which exchanges intracellular Na^+ for extracellular K^+. Thus a Na^+ gradient from outside to inside the cell is created and this gradient can be eliminated by ouabain. This property of ouabain was exploited in the following experiment.

Na^+-dependent amino acid uptake

Transport of neutral amino acids into mammalian cells occurs via two distinct carrier systems (Christensen, 1973). One is Na^+ and energy dependent and prefers short-chain amino acids (A system), and the other is Na^+ and energy independent and prefers long-chain amino acids (L system). Hjelle et al. (1978) have previously described Na^+-independent neutral amino acid transport in isolated retinal microvessels but could not demonstrate Na^+-dependent uptake. We studied uptake of the non-metabolizable amino acid analog, α-(methylamino)isobutyric acid (αMeAIB) which is transported exclusively by the A system and has no affinity for the L system. Figure 6 shows a time course for uptake of αMeAIB into retinal capillaries. As expected for a Na^+ gradient dependent system, this uptake is inhibited by preincubation with ouabain. Thus, our results demonstrate the presence of an A system for neutral amino acid transport into isolated retinal capillaries. This transport system is capable of moving amino acids against a concentration gradient as long as a Na^+ gradient exists in the opposite direction.

Uptake of p-*aminohippuric acid (PAH)*

Active transport of organic anions such as fluorescein (Cunha-Vaz and Maurice, 1967) and iodopyracet (Forbes and Becker, 1960) from retina to blood has been described. This process is inhibited by probenicid, an inhibitor of active PAH transport in the kidney, choroid plexus and ciliary body (Barany, 1973). Therefore, we

TABLE IV

Effect of inhibitors on p-*aminohippuric acid uptake*

Inhibitor	Concentration (mM)	Uptake (10^3 ct/min/mg)	Percent Control
Control		13·0±2·7	100
p-Aminohippuric acid	10·0	12·4±3·3	95
Probenicid	0·5	13·2±1·7	102
Fluorescein	1·0	10·3±1·4*	80
Penicillin G	10·0	9·3±1·4†	72

Uptake was determined after 1 min incubation at 37°C in the presence of 0·045 mM[^{14}C]PAH [4·8×10^6 (ct/min)/ml] and the stated concentration of inhibitor. Values are the averages of four determinations±S.D. Results were compared to control using the Student's *t*-test.

* $P < 0.05$

† $P < 0.01$

used PAH as a marker for organic anion transport into retinal capillaries. In Fig. 7 the rate of PAH uptake is compared to the rate of uptake of an extracellular marker, sucrose. PAH enters the microvessels more readily than sucrose and this may indicate the presence of a carrier-mediated transport system. However, the affinity of PAH for this transport system is apparently very low, since neither high concentrations of PAH nor probenicid significantly decreased uptake of [^{14}C]PAH (Table IV). There is a small but significant inhibition of PAH uptake by fluorescein and penicillin G. Hjelle et al. (1978) also found no effect of probenicid on PAH uptake by isolated retinal capillaries.

4. Discussion

The term "blood–retinal barrier" is used to describe the limited retinal uptake of certain solutes from the plasma. However, some solutes, such as D-glucose (Dollery, Henkind and Orme, 1971) can easily enter the retina from the blood, while amino acids (Reddy et al., 1977) and organic anions (Cunha-Vaz and Maurice, 1967) appear to be actively excreted into the plasma across the BRB. Thus, the BRB should be considered to include both the limited permeability for some solutes and the carrier-mediated transport of others. The function of the BRB is to maintain a constant fluid and chemical environment in the retina.

Anatomically, both the capillary endothelial cells and the pigment epithelial cells contribute to the BRB (Cunha-Vaz, 1976). From in vivo studies it is often difficult to determine which component of the BRB contributes which function. One approach to this problem is to study solute transport processes in isolated tissues.

The isolated pigment epithelium from amphibians has been used to study trans-epithelial movements of ions (Lasansky and De Fisch, 1966; Steinberg and Miller, 1973; Miller and Steinberg, 1977), certain amino acids (Miller and Steinberg, 1976), and sugars (Miller and Steinberg, 1976; Zadunaisky and Degnan, 1976). Two contradictory reports on sugar fluxes have appeared. Zadunaisky and Degnan (1976) described unidirectional movement of 3MG from the choroidal to apical surface. This

flux was inhibited by phlorizin and found only when there was a low electrical resistance across the isolated tissue. In contrast, Miller and Steinberg (1976) could find no net flux of 3MG and unidirectional fluxes were not saturable nor inhibitable by phlorizin. Similarly, ouabain inhibitable ion transport at the frog pigment epithelium has been observed by some investigators (Miller and Steinberg, 1977) but not others (Lasansky and de Fisch, 1966). Thus, it is not clear whether transport of sugars or potassium occurs at the pigment epithelium.

Isolated retinal capillaries have been used in studies of substrate oxidation (Meezan et al., 1974), amino acid flux (Hjelle et al., 1978) and basement membrane composition (Meezan, Hjelle and Brendel, 1975; Carlson, Brendel, Hjelle and Meezan, 1978). The preparation is metabolically active and thus appears to be an appropriate model for studies of the microvascular component of the BRB. However, it must be emphasized that these studies can only observe transport into the capillary cells and not across the capillary membranes, i.e. from lumen to the opposite side of the cell.

In the present report, we demonstrated the presence of hexose, neutral amino acid, and potassium transport systems in retinal microvessels. We have shown that hexose transport into these capillaries occurs by a stereospecific carrier-mediated transport system which can be inhibited by certain specific sugar transport inhibitors. Hexose transport in capillaries is not energy or Na^+-dependent nor is it stimulated by high doses of insulin. Thus, this transport system is identical to the facilitated-diffusion type of D-glucose transport system which is present in the blood–brain barrier (Betz, Gilboe and Drewes, 1976) and in isolated brain capillaries (Betz et al., 1979). D-Glucose transport is very rapid compared to its metabolism in retinal capillaries and therefore the overall result is to promote equilibration of retinal D-glucose with the blood D-glucose. Thus, endothelial cell metabolism would not normally impede the transport of D-glucose across the capillary wall into the retina.

We have also demonstrated the presence of ouabain-inhibitable $^{86}Rb^+(K^+)$ and Na^+ gradient dependent neutral amino acid transport systems. Although these transport systems are certainly not unique to endothelial cells, they may be particularly important as mechanisms for maintaining low K^+ and amino acid concentrations in the retina (Bito, 1970; 1977; Reddy et al., 1977). This could occur if K^+ and amino acids were transported from retina to blood against a concentration gradient. It is now apparent that brain capillary endothelial cells have a polar distribution of these transport systems between their two plasma membrane surfaces (Betz and Goldstein, 1978; Betz, Firth and Goldstein, 1980). Thus transport systems for K^+ and A system amino acids are not present in the luminal plasma membrane but are present in the antiluminal membranes. In contrast, hexose and L system amino acid transport probably occur at both the luminal and antiluminal membranes. Polarity is maintained by the tight junctions which prevent intermixing of membrane proteins from the two sides of the cell (Staehelin and Hull, 1978). Furthermore, polarity permits brain capillaries to move solutes against transcellular concentration gradients. By analogy, the retinal capillary is probably also polar for K^+ and A system amino acid transport but not for glucose and L system amino acid transport. This arrangement should facilitate uptake of those nutrients which are required for normal retinal metabolism while allowing for pumping of selected solutes against a concentration gradient from retina to plasma. The action of these active transport systems should contribute to maintenance of a fixed concentration of ions and other solutes in the retina which otherwise might interfere with neural function. We emphasize that this model of the retinal capillary is speculative. Although compatible with in vivo

transport data and histochemical studies using brain capillaries (Betz et al., 1980), it should be confirmed by histochemical studies using retinal capillaries.

The presence of active transport pumps in retinal capillaries suggests that this tissue has a high level of metabolic activity. This special property may contribute to the vulnerability of retinal capillaries to damage in the many disorders that produce retinal microangiopathy. A breakdown of the microvascular component of the BRB is one of the earliest detectable abnormalities in many retinal diseases including diabetic (Cunha-Vaz, Faria de Abreu, Campos and Figo, 1975), hypertensive (Garner, Ashton, Tripathi, Kohner, Bulpitt and Dollery, 1975), venous obstructive (Cunha-Vaz, 1966), ischemic (Cunha-Vaz, 1966; Cunha-Vaz and Shakib, 1967), and hyperviscosity (Wise, Dollery and Henkind, 1971) retinopathies. The causes for this breakdown are largely unknown. We feel that an important first step in understanding the pathogenesis of these disorders is to obtain more information about the normal properties of the retinal microvasculature.

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