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The Use of a Perfluorochemical Emulsion as a Vascular Perfusate in Drug Absorption

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Abstract—In-vitro simultaneous luminal and vascular perfusion using the perfluorochemical emulsion, FC-43 emulsion, as a vascular perfusate, was examined for drug absorption in rat jejunum. The intestinal membrane in this system was found to retain its normal barrier functions for drug transport, as evident from the following: (i) stable absorption clearance of tritiated water and salicylic acid at steady-state, (ii) agreement of this clearance with that by in-situ single-pass luminal perfusion, (iii) active transport of D-glucose and its inhibition by phloridzin and (iv) normal glutamate pyruvate transaminase activity in the intestinal mucosa. FC-43 emulsion gave a more normal absorption site blood flow than the usual vascular perfusate containing erythrocytes and albumin in Krebs-Henseleit bicarbonate buffer solution. Consequently, this emulsion was useful for examining the contribution of blood flow resistance toward drug absorption. The rate-limiting steps of absorption of tritiated water, antipyrine and salicylic acid were examined by perfusion using FC-43 emulsion. The absorption of tritiated water was almost completely blood flow-limited and its absorption clearance may possibly be an approximated absorption site blood flow. The contribution of blood flow resistance to total resistance for antipyrine absorption exceeded that for salicylic acid absorption.

In drug intestinal absorption, there are the two rate-limiting steps of epithelial membrane permeation and wash-out by absorption site blood flow. The former includes diffusion in the unstirred layer adjoining the epithelium followed by intracellular or intercellular permeation. The latter is a wash-out step through the capillary wall by absorption site blood flow; the wall is not a permeation barrier but the blood flow is a rate-limiting factor.

Absorption site blood flow is usually measured by microsphere (Dregelid et al 1986), hydrogen gas clearance (Ashley & Cheung 1984) or a laser-Doppler method (Ahn et al 1985). These methods, however, are not always adequate for measuring blood flow during drug absorption. Mailman (1981) has proposed that the absorption clearance of tritiated water may serve as an approximate value for absorption site blood flow since this absorption is almost completely blood flow-limited. By the method of tritiated water clearance, absorption site blood flow can be measured during drug absorption by adding tritiated water to a luminal drug solution.

Simultaneous luminal and vascular perfusion can be used to examine the rate-limiting steps of both membrane permeation and blood flow in drug absorption. By this method, the intestine is isolated and perfused vascularly from the mesenteric artery to the portal vein and thus it has the following advantages: (i) the net amount of drug immediately after permeation of the membrane can be measured directly and (ii) the vascular perfusion rate and perfusate component can be changed to suit the experimental objective. The fresh blood of other rats (Windmueller et al 1970; Ochsenfahrt 1979), or Krebs-Henseleit bicarbonate buffer solution containing albumin and erythrocytes with oxygen transport capacity is generally used as the vascular perfusate in rats (Kavin et al 1967; Nicholls et al 1983; Pang et al 1986). However, this perfusion system is attended with certain technical drawbacks, i.e. erythrocytes often form clumps that interrupt blood flow and haemolysis frequently occurs during vascular perfusion.

A perfluorochemical emulsion has been used as an oxygen-transporting agent in animal studies relating to physiology, biology, biochemistry, chemotherapy and metabolism because of its ability to take up oxygen in addition to its chemical and biological inertness. It can also be kept in a stable emulsified form for long-term storage.

Our purpose in the present study is to examine the use of perfluorotributylamine (FC-43) emulsified with Pluronic F-68, give FC-43 emulsion, as a vascular perfusate. This emulsion is an excellent oxygen carrying agent since it can dissolve about 20 times as much oxygen as can water; the oxygen solubilities in the emulsion and water are, respectively, 41 and 2·3 vol % at 37°C and 760 mmHg (Yokoyama et al 1975). Although it has been used for organ perfusion of isolated kidney, liver and heart (Yokoyama et al 1983; Skibba et al 1985; Segel et al 1987), its use as a vascular perfusate for absorption is examined here in detail for the first time. The first aim was to determine if intestinal membrane barrier functions were maintained during the perfusion of the emulsion, therefore, it was tested for its active transport capacity for D-glucose. Also, the activity of glutamate pyruvate transaminase in the epithelial membrane was assessed and drug absorption clearance by in-vitro luminal and vascular perfusion compared with that by in-situ luminal perfusion. Secondly, the appropriateness of tritiated water clearance as an approximated value of absorption site blood flow was examined, and the rate limiting steps of antipyrine and salicylic acid absorption were also compared.

Materials and Methods

Materials

The chemicals used and the sources from which they were

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obtained are: tritiated water from Amersham International, UK; [^{14}C]antipyrine, [^{14}C]salicylic acid and [^{3}H]glucose from New England Nuclear, Boston, MA, USA; bovine serum albumin (fraction V), phloridzin and fluoroisothiocyanate dextran (FITC-dextran, mol. wt 40 000) from Sigma Chemical Co., St Louis, MO, USA; noradrenaline from Wako Pure Chemical Industries, Osaka, Japan. FC-43 emulsion was kindly supplied by Green Cross Co. (Osaka, Japan). All other chemicals were of analytical grade.

Animal surgery

Male Wistar rats (260–330 g) were fasted overnight. The jejunal segment for vascular perfusion was isolated according to Windmueller et al (1970) and Ochsenfahrt (1979). Briefly, under anaesthesia with urethane (4·5 mL kg^{-1} i.p., 2·5% solution), the superior mesenteric artery was freed from the surrounding mesentery. The mesenteric vessels distal to the experimental segment were ligated. The proximal cannula of glass tubing (i.d. 2·5 mm, o.d. 3·5 mm) was placed in the jejunum 5 cm from the Treitz ligament, and the glass distal cannula was inserted to 15 cm from the proximal cannula. The contents of the segment were washed out via the cannulae with saline previously warmed to 37°C. The superior mesenteric artery was then ligated at its origin from the aorta and cannulated with a polyethylene tube (PE-50). For venous outflow, the portal vein was ligated at the proximal site to the liver followed by insertion of a glass cannula (i.d. 1 mm, o.d. 2 mm). Immediately after completing the vascular cannulation, single-pass perfusion (0·5–3 mL min^{-1}, Periata Mini-Pump, SJ1211, Atto Co. Ltd, Tokyo, Japan) of FC-43 emulsion previously equilibrated with 95% O_2–5% CO_2 at 37°C was started from the mesenteric artery to the portal vein. The rats were killed by severing the inferior aorta and the vascularly perfused segment was isolated. The segment was suspended in a serosal bath containing 150 mL of Krebs-Henseleit bicarbonate buffer (mM) NaCl 18, KCl 4·74, KH_2PO_4 1·18, $MgSO_4$ $7H_20$ 1·18, $CaCl_2 \cdot 2H_2O$ 1·27 and $NaHCO_3$ 24·88, pH 7·4. Luminal single-pass perfusion of the drug solution was started, as described below.

Luminal perfusion

The luminal perfusate consisted of Krebs-Henseleit bicarbonate buffer solution (pH 7·4) containing 20 mM glucose and 0·005% FITC-dextran as a non-absorbable marker. For drug absorption by passive diffusion, sodium salicylate or antipyrine (final 2 mM) with [^{14}C]salicylic acid or [^{14}C]antipyrine (final 0·01 μCi mL^{-1}), respectively, was added to the perfusate followed by equilibration with 95% O_2–5% CO_2 at 37°C. For tritiated water absorption, tritiated water (final 0·1 μCi mL^{-1}) was added to the perfusate separately or with the drug. The osmotic pressure of the perfusate was adjusted to 280 mOsm kg^{-1} with NaCl (Vogel Osmometer, type OM-801, Giesen, Germany). The active transport of D-glucose and its inhibition by phloridzin were examined at 20 mM glucose with and without 0·1 mg mL^{-1} phloridzin in the luminal perfusate. For transamination in the intestinal membrane, the transport of glutamic acid and its conversion to alanine were examined in the outflow from the portal vein during the luminal perfusion of 10 mM sodium glutamate. Luminal single-pass perfusion was performed at a flow rate of 1 mL min^{-1} (Periata Mini-Pump) in all cases. The luminal and venous perfusates were each collected at 10 min intervals for 40 min.

Vascular perfusion

As blood perfusate, we used 20 w/v% FC-43 (perfluorotributylamine) emulsion (specific weight 1·148), composed of (w/v%) NaCl 0·60, KCl 0·034, $MgCl_2$ 0·020, $CaCl_2$ 0·028, $NaHCO_3$ 0·210, glucose 0·180, Pluronic F-68 2·56 and hydroxyethyl starch 3·0. Before perfusing this emulsion, noradrenaline (final 0·12 μM) was added to it and the mixture was equilibrated with 95% O_2–5% CO_2 at 37°C. The osmolarity of the emulsion was 290–300 mOsm kg^{-1} and the pH, 7·4–7·6. Also, to examine the usefulness of the emulsion system as the vascular perfusate, the emulsion was exchanged for Krebs-Henseleit bicarbonate buffer solution supplemented with washed bovine erythrocytes (20% haematrocrit) and 3% bovine serum albumin while supplying 95% O_2–5% CO_2.

In-situ single-pass luminal perfusion

The surgery and experimental techniques of absorption were the same as those described by Hirasawa et al (1984). The jejunal lumen (15 cm) was perfused at a single-pass rate of 1 mL min^{-1} (Perista Mini-Pump). Samples were obtained in the manner described above.

Analytical method

Liquid scintillation counting was used to analyse tritiated water, [^{14}C]antipyrine, [^{14}C]salicylic acid and [^{3}H]glucose in luminal, vascular and serosal solutions. Luminal and vascular perfusate samples were centrifuged at 500 *g* for 5 min and at 15000 *g* for 30 min, respectively, and to 1 mL of each of supernatants were added 10 mL of Triton X-100-toluene scintillater (DPO 12 g, POPOP 0·3 g, toluene 2 L and Triton X-100 1 L). The FITC-dextran concentration was determined fluorometrically at 495 nm for the excitation and at 515 nm for the emission after diluting the supernatant of the luminal perfusate by 26 times with Krebs-Henseleit bicarbonate buffer. Glutamic acid and alanine concentrations were determined with an amino acid analyser (Hitachi 835, Tokyo, Japan). In the fluorometric determination and amino acid measurement, interference by FC-43 emulsion did not occur.

Data analysis

For absorption clearance, both disappearance clearance from the luminal solution and appearance clearance into the outflow of the vascular side were calculated at the steady state. The former was obtained from the luminal drug or tritiated water concentration using equation 1 (Hirasawa et al 1984).

Disappearance clearance =

$$\frac{Q_{in}\,C_{in}-Q_{out}\,C_{out}}{C_{in}} \times \frac{1}{L} \qquad (1)$$

where Q_{in} and Q_{out} are the luminal perfusion rates at the inlet and the outlet of the segment, respectively. Similarly C_{in} and C_{out} are the drug and tritiated water concentrations in the luminal perfusate at the inlet and outlet of the segment,

respectively. L is length of the segment measured on completion of the experiment. Since the non-absorbable FITC-dextran concentration in the luminal perfusate did not change significantly in this study, net water absorption was neglected and thus Q_{out} was regarded as equal to Q_{in} in equation 1.

Also, appearance clearance was determined from the blood concentrations of the drug and tritiated water in the vascular perfusate by equation 2.

Appearance clearance =

$$\frac{Q_{blood}\, C_{blood}}{C_{in}} \times \frac{1}{L} \quad (2)$$

where Q_{blood} and C_{blood} are the vascular outflow rate and concentration of the drug or tritiated water in the vascular perfusate, respectively. Q_{blood} was obtained from the weight of the collected vascular perfusate.

The rate-limiting step, consisting of membrane permeation and absorption site blood flow, was analyzed by equation 3 (Mailman 1981).

Absorption clearance =

$$\frac{1}{(1/P_m)+(1/ASBF)} \quad (3)$$

where P_m and ASBF are membrane permeability clearance and absorption site blood flow, respectively. Using the appearance clearance of tritiated water as ASBF, P_m was obtained from the non-linear squares fit (program MULTI (Yamaoka et al 1981)). Appearance clearance into the vascular outflow was used primarily as the absorption clearance since the appearance clearance agreed with the disappearance clearance from luminal perfusate, as will be described later.

Statistical analysis

Levels of statistical significance were assessed using Student's *t*-test. Significant differences were judged as P values less than 0·05.

Results

Absorption clearance of tritiated water and salicylic acid by in-vitro luminal and vascular perfusion and in-situ luminal perfusion

The absorption clearance of tritiated water and salicylic acid for the in-vitro simultaneous luminal and vascular perfusion of FC-43 emulsion at a flow rate of 1 mL min^{-1} is shown in Fig. 1. For both compounds, the appearance clearance into the vascular outflow had a lag time of 10 min but appearance and disappearance from the lumen was constant for 30 min after the lag time. Also, there was agreement between appearance and disappearance clearances.

The absorption clearance of tritiated water and salicylic acid by in-vitro vascular perfusion agreed well with that by in-situ luminal single-pass perfusion (Figs 1, 2). Disappearance clearance by luminal perfusate was about 11 and 5 μL min^{-1} cm^{-1} for tritiated water and salicylic acid, respectively.

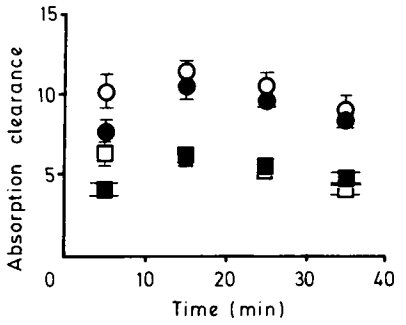

FIG. 1. Absorption clearance (μL min^{-1} cm^{-1}) (○, ●) of tritiated water and salicylic acid (□, ■) by in-vitro simultaneous luminal and vascular perfusion. Each value represents the mean ± s.e. of 5 experiments. For small s.e., the bar is included in the symbols. Open symbol, disappearance clearance from the luminal perfusate; Closed symbol, appearance clearance into the vascular perfusate.

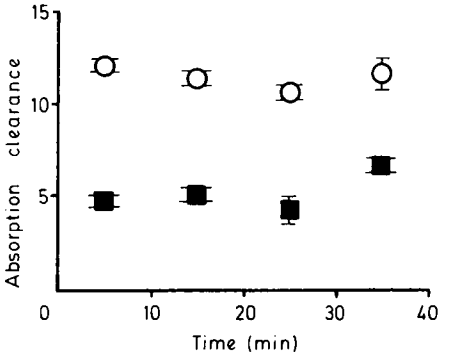

FIG. 2. Disappearance clearance (μL min^{-1} cm^{-1}) from the luminal perfusate of tritiated water and salicylic acid by in-situ single-pass luminal perfusion. Each value represents the mean ± s.e. of four experiments. ○, tritiated water; ■, salicylic acid.

Appearance clearance of tritiated water and salicylic acid for FC-43 emulsion and Krebs-Henseleit bicarbonate buffer solution containing erythrocytes and albumin as vascular perfusates

Fig. 3 shows the effects of two vascular perfusate systems on the absorption clearance of tritiated water and salicylic acid, i.e. FC-43 emulsion and Krebs-Henseleit bicarbonate buffer with erythrocytes and albumin (erythrocytes-albumin system). The absorption clearance in both perfusate systems was measured for 20 min after the lag time of 10 min. Clearance in the erythrocytes-albumin system was less than that in the emulsion system.

Active transport of D-*glucose in in-vitro and in-situ perfusion*

Fig. 4 shows the inhibition of phloridzin toward the active transport of D-glucose by in-vitro luminal and vascular perfusion and by in-situ luminal perfusion. The disappearance clearance of D-glucose from the lumen tended to be less in-situ than in-vitro, but not significantly so. The inhibitory effects of phloridzin were evident to the same degree in both methods.

Transamination from glutamic acid to alanine

The cumulative permeation amount of glutamic acid from the luminal to vascular perfusate during 40 min and the

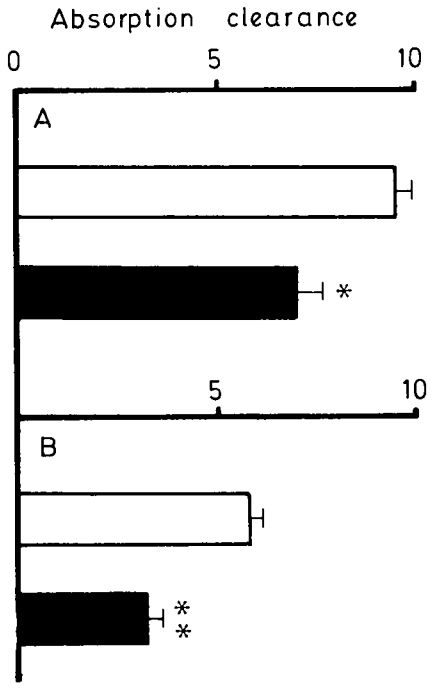

FIG. 3. Effects of the vascular perfusate composition on the absorption clearance (μL min^{-1} cm^{-1}) of tritiated water (A) and salicylic acid (B). Each value represents the appearance clearance measured for 20 min after the lag time of 10 min and is the mean $\pm$ s.e. of four experiments. □ FC-43 emulsion, ■ erythrocytes-albumin. $^{**}P < 0.01$ versus FC-43 emulsion; $^{*}0.01 < P < 0.05$ versus FC-43 emulsion.

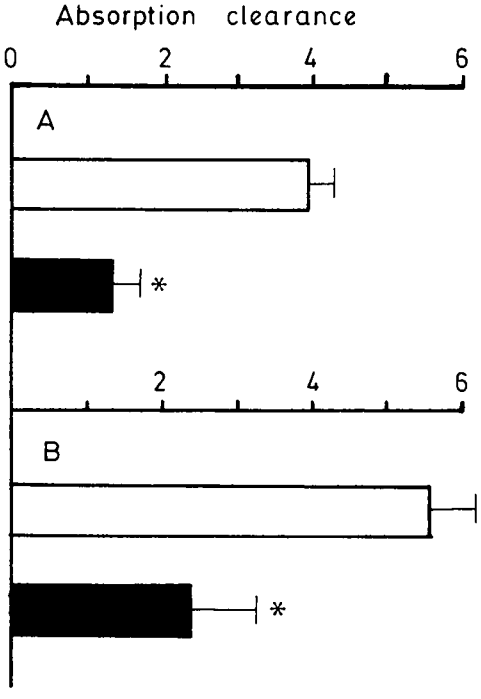

FIG. 4. Effects of phloridzin on D-glucose absorption in in-situ (A) and in-vitro (B) experiments. Each value represents the mean $\pm$ s.e. of disappearance clearance (μL min^{-1} cm^{-1}) from more than three experiments, which was measured for 20 min after the lag time of 10 min. □ control, ■ control + phloridzin. $^{*}0.01 < P < 0.05$ versus control.

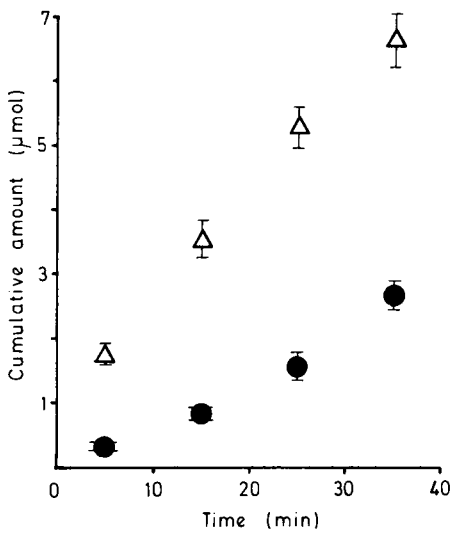

FIG. 5. Cumulative amounts of (△) glutamic acid and (●) alanine in the vascular perfusate from the luminal perfusate in in-vitro simultaneous luminal and vascular perfusion. Each value represents the mean $\pm$ s.e. of four experiments.

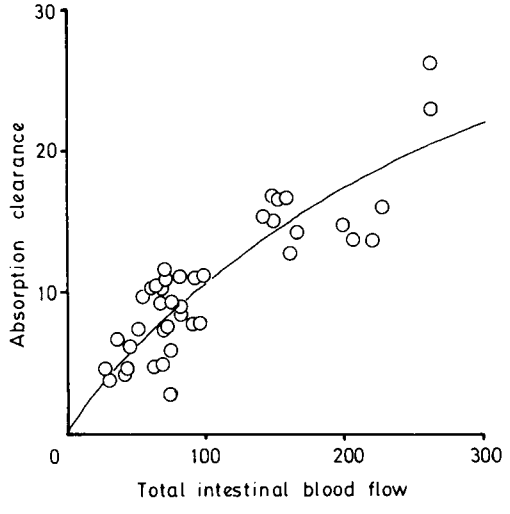

FIG. 6. Relationship between absorption clearance (μL min^{-1} cm^{-1}) of tritiated water and intestinal total blood flow (μL min^{-1} cm^{-1}) (vascular perfusion rate). Each symbol represents the appearance clearance into the vascular perfusate which was measured for 20 min after the lag time of 10 min in an individual rat. The line was fitted to eqn 3 by the non-linear least squares method (program MULTI, Yamaoka et al 1981), assuming absorption site blood flow to be the product of a constant and total blood flow. The constant and membrane permeability clearance (P_m) values were calculated as 0·14 and 47·7 μL min^{-1} cm^{-1}, respectively.

appearance amount of alanine into the vascular perfusate, produced by the transamination of glutamic acid in intestinal mucosal cells, are indicated in Fig. 5. The latter was three times the former.

Relationship between tritiated water clearance and total intestinal blood flow

The dependence of tritiated water clearance on the vascular perfusion rate, i.e. total intestinal blood flow, is shown in Fig. 6 when the perfusion rate of FC-43 emulsion used as a

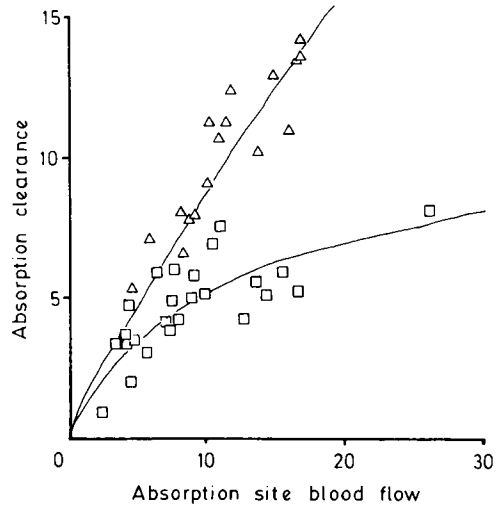

FIG. 7. Relationship between absorption clearance (μL min^{-1} cm^{-1}) of antipyrine and salicylic acid and absorption site blood flow (μL min^{-1} cm^{-1} tritiated water clearance). Each symbol represents the appearance clearance into the vascular perfusate from an individual rat, which was obtained in the same manner as that in Fig. 6. The line was fitted according to eqn 3.

vascular perfusate was changed from 0·5 to 3 mL min^{-1} (30 to 280 μL min^{-1} cm^{-1} intestine). Since disappearance clearance agreed with that of appearance, as shown in Fig. 1, the latter was used as the absorption clearance in Fig. 6. Clearance increased almost linearly with blood flow. In particular, tritiated water absorption at less than 100 μL min^{-1} cm^{-1} was regarded as almost completely blood flow-limited.

Relationship between the absorption clearance of antipyrine and salicylic acid and absorption site blood flow

The appearance clearance of antipyrine and salicylic acid was determined in the presence of tritiated water. Fig. 7 shows the dependence of the absorption of both drugs on absorption site blood flow, obtained as tritiated water clearance, assuming the tritiated water to be absorbed completely in a blood flow-limited manner. The clearance of antipyrine exceeded that of salicylic acid, increasing linearly over a wide range of absorption site blood flow. That of salicylic acid indicated linear increase at less than 10 μL min^{-1} cm^{-1} of absorption site blood flow and gradually reached a plateau.

Table 1. Membrane permeability clearance (P_m) and blood flow resistance for the absorption of antipyrine and salicylic acid

	P_m[a] (μL min^{-1} cm^{-1})	Blood flow resistance[b] to total absorption resistance (%)
Antipyrine	78·2	89·3
Salicylic acid	10·8	53·7

[a] Calculated with equation 3.
[b] Calculated with [(1/absorption site blood flow)/{(1/P_m)+(1/absorption site blood flow)}] × 100 (%), where the normal value reported by Mailman (1981), 200 μL min^{-1} g^{-1}, was used as the absorption site blood flow after being converted to the value per cm, 9·3 μL min^{-1} cm^{-1}.

Membrane permeability clearance (P_m) obtained from the non-linear squares fit and the contribution of blood flow resistance to total resistance toward drug absorption at normal absorption site blood flow (Mailman 1981) are given in Table 1. The P_m value of antipyrine was about seven times that of salicylic acid, and also blood flow resistance was greater with antipyrine than salicylic acid.

Discussion

The perfluorochemical emulsion, FC-43 emulsion, used gave stable absorption clearance for tritiated water and salicylic acid (Figs 1, 2). These values and the inhibitory effects of phloridzin on the active transport of D-glucose (Fig. 4) were consistent with the results by in-situ luminal perfusion. The result that most of glutamic acid was converted to alanine during its absorption (Fig. 5) reflected well the characteristics in glutamate pyruvate transaminase reaction which Parsons & Volman-Mitchell (1974) reported in the intestinal transport of glutamate in the rat. Further, villi structures on the intestinal mucosa observed by a light microscope were maintained after perfusion (unpublished observations). It is thus evident that barrier functions for membrane transport are maintained in vascular perfusion with FC-43 emulsion and this emulsion is shown to be useful for drug absorption.

A linear increase in tritiated water clearance in the region of normal intestinal blood flow (50–100 μL min^{-1} cm^{-1}) indicated almost complete blood flow-limited absorption of tritiated water (Fig. 6). It follows that tritiated water clearance should be used as an approximated value of absorption site blood flow, as proposed by Mailman (1981).

Lowering of the absorption clearance of tritiated water and salicylic acid in the erythrocytes-albumin system obtained by the comparison with that in FC-43 emulsion (Fig. 3) may possibly be due to the decrease in absorption site blood flow. Erythrocytes sizes is 5–7 μm but 90% of the emulsion particles were less than 0·2 μm in diameter (Yokoyama et al 1974). Accordingly, the decrease in absorption site blood flow in the erythrocytes albumin system may possibly be due to larger particle size.

The relationship between the absorption clearance of antipyrine and blood flow (Fig. 7) and membrane permeability clearance (Table 1) indicated that antipyrine absorption is almost totally blood flow limited, whereas salicylic acid absorption is controlled evenly by blood flow and membrane permeation.

Ochsenfahrt & Winne (1974a, b) also analysed the absorption rate-limiting steps of antipyrine and salicylic acid by infusing fresh blood into the jugular vein of rats at three different rates and indirectly changing the intestinal total blood flow. The present findings were in agreement with the earlier results that blood flow resistance to the absorption of antipyrine exceeds that of salicylic acid.

In summary, the simultaneous luminal and vascular perfusion using FC-43 emulsion as a vascular perfusate was found to be a convenient and efficient means for comparing the contribution of the resistance of membrane permeability and blood flow toward drug absorption. For drug absorption controlled by blood flow, FC-43 emulsion as a vascular perfusate was superior to Krebs-Henseleit buffer solution containing erythrocytes and albumin since absorption site

blood flow in the former corresponds more to the in-situ value than that in the latter.

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