Abstract—Rat isolated small intestine was perfused at a fixed flow rate through the superior mesenteric artery with whole rat blood recycled from a devised oxygenator-reservoir. As indicated by perfusion pressure, tissue glucose and oxygen consumption, and histological studies, the perfused intestine remained in a viable state over the perfusion period of 2 hr. Rapid absorption of glucose from the intestinal tract was observed after the intraduodenal injection. When single doses of acetaminophen were injected into the duodenal lumen or poured over the perfused intestine, the absorption was rapid and dose-dependent. Shortly after single intraduodenal injections of salicylamide, salicylamide in free and conjugated forms (sulfate and glucuronide) appeared in the circulating blood. These results indicate that the vascularly perfused intestinal preparation has wide applications in biochemical experimental fields.

Numerous investigations have been concerned with attempts to develop useful preparations for evaluating functions of the intestine. Most of these studies for uptake, metabolism and transport of substrates by intestinal epithelial cells have been performed on isolated preparations immersed in physiological salt solution, e.g., intestinal rings, sheets of mucosa and serosa, and isolated villi (1-3). Although these preparations provided pertinent information leading to investigations of biochemical problems related to absorption and metabolism of drugs, there were drawbacks. Kavin et al. (4) and Windmueller et al. (5) devised an isolated intestinal preparation of the rat sustained by vascular perfusion with washed bovine red blood cells suspended in an “artificial plasma” and with rat whole blood, respectively, then carried out studies on viability. Their preparations appeared to be particularly suitable for examining entry of drugs from the intestinal tract into the blood or lymph vessels. However, most of the studies pertaining to drug absorption and metabolism have not been carried out in such preparations, and to our knowledge there is only one study, recently reported, which dealt with absorption of antipyrine, salicylic acid and urea (6).

Thus, we examined absorption and metabolism of several drugs in the intestine, using the rat isolated small intestinal preparation sustained by perfusing the mesenteric vasculatures with whole rat blood recycled from a devised oxygenator-reservoir.

MATERIALS AND METHODS

Male Sprague-Dawley rats (130-150 g) were deprived of food overnight before the
experiment but water was allowed ad libitum.

**Perfusion procedures:** The animals were anesthetized with sodium pentobarbital (65 mg/kg i.p.) and abdomen was opened by a midline incision. Both ends of the small intestine were ligated and cut off, proximally at the junction between the pylorus and the duodenum and distally at the ileum about 10 cm above the caecum. A polyethylene cannula was inserted into the ileum at the distal end to allow for free flow of the intestinal juice (unless otherwise stated). The celiac artery was tied and cut off near its origin from the aorta. The superior mesenteric artery and the portal vein were separated from the surrounding tissues. The gastroduodenal and the splenic veins were tied and cut off. After the injection of heparin sodium (1000 U/kg) into the femoral vein, a polyethylene cannula (ID 1.6 mm, OD 2.0 mm) was inserted into the portal vein. A metal cannula (ID 0.4 mm, OD 0.6 mm) was then inserted into the superior mesenteric artery. The mesenteric and portal cannulae of the isolated intestine were connected to the perfusion circuit after the circuit had been filled with about 30 ml of whole blood freshly obtained from heparinized rats, and the small intestine was perfused at a fixed flow rate by means of a peristaltic pump (Mitsumi Science, SJ-1210). When the perfusing blood entered the superior mesenteric artery, the temperature was about 37°C. Output of the pump was precalibrated and rechecked at the end of the experiment. Mean perfusion pressure was measured with a pressure transducer (Nihon Kohden, MPU-0.5). The perfused small intestine was then

![Diagram of the perfusion system](image)

**Fig. 1.** Diagram of the perfusion system. Blood from the portal vein (P.V.) was returned to the reservoir portion of an oxygenator-reservoir primed by about 15 ml blood and then led to the oxygenator portion (50 cm high) at a fixed flow rate of 7.6 ml/min by means of a pump. The oxygenated blood (about 3.8 ml/min) was delivered via a different pump into the superior mesenteric artery (S.M.A.) W, circulating warm water. B.S., blood sampling.
removed from the rat body and transferred to an acrylic plastic box. Venous blood from the portal vein was returned to the reservoir portion of a glass oxygenator-reservoir by gravity. Blood pooled in the reservoir portion was led into the oxygenator portion by another pump (Mitsumi Science, SJ-1210). The blood flowed down, through that oxygenator portion to the reservoir portion, during passage of which the blood was equilibrated with 98% O₂ and 2% CO₂. The time required to make the preparation was about 20 min. The experimental set-up is illustrated schematically in Fig. 1. The perfusion procedures and apparatus were the same as described previously (7), except that a devised glass oxygenator-reservoir was used instead of a donor rat.

The pH and glucose concentrations in the perfusing blood were maintained at about 7.4 and at 1–1.5 mg/ml, respectively, by continuous infusion into the reservoir portion of the oxygenator-reservoir of a solution of NaHCO₃ (238 mM) and glucose (111 mM) at 1.7 ml/hr.

Histological examination: At the conclusion of control experiments, some of the perfused intestinal preparations were removed, sectioned and fixed in 10% neutral formalin solution. The fixed tissues were embedded in paraffin and stained with hematoxylin and eosin for histological examination.

For enzyme histochemical observations, small pieces of fresh tissue specimens were fixed in 4% formal-calcium solutions (pH 7.2) and cut at 6 μ thickness in a cryostat. The sections were stained for alkaline phosphatase by a modified Burstone's naphthol alkaline phosphatase method (8) and compared with those of non-perfused (control) rat intestine.

Blood gas contents and oxygen consumption: Tissue oxygen consumption (VO₂) was estimated from the arteriovenous (A-V) oxygen difference, and expressed in ml of oxygen consumed per gram of the dry weight (DW) of the perfused intestine per min: 

\[ \text{VO}_2 (\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}) = (\text{CAO}_2 - \text{CVO}_2) \times \text{mesenteric blood flow (ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}). \]

The oxygen content (CO₂) was determined as follows: 

\[ \text{CO}_2 = 0.003 \times \text{PO}_2 + 1.34 \times \frac{\text{Hb \times SaO}_2 \%}{100}. \]

Hemoglobin (Hb) concentration (g/100ml) and the percent oxygen saturation (SaO₂) were determined with a hemoximeter (Radiometer, OSM2, Copenhagen, Denmark). Measurements of PO₂, PCO₂ and pH in blood samples were made using the blood gas analyzing system (Radiometer, BMS3-MK2, Copenhagen, Denmark).

Determinations of glucose and lactate: Glucose and lactate concentrations were measured with a spectrophotometer (Beckman, ACTA® CIII) using Blood Sugar-GOD-Period-Test and Lactate-UV-Test (Boehringer Mannheim), respectively.

Determination of Evans Blue: At the end of experiments, the perfused intestine* was removed, and homogenized in 50 ml of distilled water. To the water extract, distilled water was added to bring the total volume to 600 ml. The water extract solution and plasma samples were subjected to colorimetry at 620 nm with a spectrophotometer (Hitachi, Model 124).

* The ileum end was ligated, since the intestinal transit of water soluble substances such as Evans Blue was rapid.
Determination of acetaminophen: According to the procedures described previously by Kendal et al. (9), the acetaminophen concentrations in samples were determined colorimetrically under standardized conditions at 25°C with a spectrophotometer (430 nm) (Hitachi, Model 124). Plasma (0.5 ml) was added to a stopped centrifuge tube containing 2 ml 10% trichloroacetic acid. After thorough mixing, the tube was centrifuged briefly and the clear supernatant decanted into a test tube containing 1 ml 6 N hydrochloric acid and 2 ml 10% sodium nitrite. After allowing the contents to stand for 2 min, 2 ml of 15% sulphamic acid was added carefully, followed by 5 ml 10% sodium hydroxide.

Plasma samples extracted with tetrahydrofuran were spotted on silica Gel F-254 plate (E. Merck) developed with CHCl₃/MeOH/NH₄OH (50:10:1), and detected under ultra violet light at 254 nm.

Determinations of salicylamide and its conjugates: The procedures were previously described by Levy and Matsuzawa (10). The mixture containing 0.2 ml of blood, 0.2 ml of H₂O, 1.0 ml of 0.5 M phosphate buffer (pH 7.4) and 5.0 ml of ethylene dichloride was shaken for 10 min and centrifuged for 5 min at 3,000 rpm. Three ml of the lower layer (organic solvent phase) was then extracted with 5 ml of ferric nitrate reagent (freshly prepared by diluting 5 ml of ferric nitrate stock solution (1% Fe(NO₃)₃ in 0.07 N HNO₃) to 9 ml with distilled water. The developed color was measured at 530 nm against a water blank.

Salicylamide sulfate and glucuronide in the blood were determined as the free salicylamide obtained after hydrolysis with arylsulfatase and β-glucuronidase*, respectively: The mixture containing 0.2 ml of blood, 0.2 ml of H₂O, 0.5 ml of 0.2 M acetate buffer (pH 5.0) and 0.2 ml of β-glucuronidase or 0.02 ml of arylsulfatase (Boehringer Mannheim) was incubated at 37°C for 48 hr.

Statistical analysis: Values in the text are means±S.E. Student's t-test was used for statistical analyses and P values under 0.05 were considered significant.

RESULTS

1. Control observations

Mechanical parameters, and tissue glucose and oxygen consumption: Immediately after the superior mesenteric artery was cannulated, the in situ preparation of the small intestine was perfused with a fixed flow rate of about 2.5 ml/min. Shortly after the perfused intestine was transferred from the rat body to an acrylic plastic box, the flow rate was adjusted to give a mean perfusion pressure at about 100 mmHg and, thereafter, remained almost constant throughout the experiment. The perfusion pressure gradually fell and reached a steady-state level (80-90 mmHg) within 20 min of the onset of perfusion. The pH and glucose level of perfusing blood were maintained constant at about 7.4 and 1-1.5 mg/ml, respectively (see Methods) (Fig. 2). A progressive rise of lactate concentration in the blood occurred. Main mechanical parameters and tissue oxygen consumption are shown in Table 1 and

* β-Glucuronidase solution containing about 10,000 Fishman units/ml was prepared from bovine liver by the Fishman-Bernfeld method (11) and the specific activity was approximately 100,000 Fishman units/mg of protein.
tissue glucose utilization in Table 2. Peristalsis of the intestine occurred in most preparations even 2 hr after the onset of perfusion.

**FIG. 2.** Blood glucose and lactate concentrations in the perfusing blood. Arterial and venous blood were sampled at the same time. Vertical bars represent means ± S.E. In most experiments, a solution containing NaHCO₃ (238 mM) and glucose (111 mM) was continuously infused at 1.7 ml/hr into the reservoir portion of an oxygenator-reservoir. ●—●, arterial glucose with infusion (N=5); ▲—▲, venous lactate with infusion (N=5); ●—●, arterial glucose without infusion (N=2); ▲—▲, venous lactate without infusion (N=2). As a recirculation technique was used, similar patterns were observed in arterial and venous concentrations of glucose or lactate.

**TABLE 1.** Main mechanical parameters and oxygen consumption in the isolated perfused intestine

|            | 30 min       | 90 min       |
|------------|--------------|--------------|
|            | Arterial     | Venous       | Arterial     | Venous       |
| Perfusion pressure, mmHg | 90.0±4.7     | 97.6±5.0     |
| Flow rate, ml/min           | 3.8±0.1      |              |
| PO₂, mmHg                  | 365.7±33.8   | 73.4±2.83    | 331.8±40.0   | 80.2±3.8**   |
| PCO₂, mmHg                 | 26.9±0.9     | 29.3±0.8     | 27.5±1.1     | 31.0±1.6     |
| Hemoglobin, g/100 ml.blood | 13.5±0.2     | 13.9±0.2     | 14.7±0.2**   | 14.8±0.2***  |
| pH                       | 7.469±0.012  | 7.435±0.010  | 7.463±0.011  | 7.422±0.013  |
| Oxygen saturation, %       | 100.7±0.4    | 84.4±1.0     | 100.4±0.2    | 86.6±0.5*    |
| Oxygen consumption ml/min. g⁻¹ DW | 0.2012±0.0120 | 0.1887±0.0092 |

Arterial and venous blood were concomitantly examined for determination of tissue oxygen consumption. Each value is mean ± S.E. from 5 observations of 5 preparations. *P<0.02, **P<0.01, ***P<0.001 compared with the corresponding values measured 30 min after start of perfusion. Dry weight (DW) of the perfused intestine, 0.64±0.01 g (N=5).

tissue glucose utilization in Table 2. Peristalsis of the intestine occurred in most preparations even 2 hr after the onset of perfusion.

**Histological appearance:** Under the light microscope, the villous pattern of the perfused intestine appeared normal, 2 hr after the start of perfusion. There were no marked changes in the epithelium, although slight edematous changes were often apparent in the lamina propria (Fig. 3). The nuclear and cell membranes also appeared intact. The localization of alkaline phosphatase activity within the epithelium of the perfused intestine (Fig. 4)
showed patterns similar to those seen in the non-perfused intestine (not shown).

**Lack of absorption of Evans Blue from the intestinal tract:** Experiments were performed on six preparations. A single dose of 20 mg of Evans Blue was injected in a volume of 0.2 ml into the lumen, at the origin of duodenum. The arterial and venous blood were sampled at the same time, and the plasma concentrations of Evans Blue at 5, 10, 30 and 60 min after the injection were determined. No detectable amounts of Evans Blue were found in the plasma. One hour after the administration of Evans Blue, the perfused intesti-
tte was removed, homogenized and the residues of Evans Blue were determined. About 100% (100.8 ± 4.4%, N=6) of the administered dose was calculated as the recovery rate.

**Transition of barium sulfate (BaSO₄) in the intestinal tract:** Experiments were carried out on the perfused and non-perfused intestinal preparations. An injection of 0.2 ml of 20% BaSO₄ solution was given over 10 sec into the lumen, at the origin of duodenum. Under direct vision, the obvious advancement of the white BaSO₄ was observed in the intestinal tract. One hour after the administration, the transit distance of BaSO₄ from the origin of the duodenum was measured and expressed as a transit rate in percent to the length of the perfused or non-perfused entire small intestine. The transit rate of BaSO₄ in the perfused or non-perfused intestine was as follows: Perfused, 25.2 ± 3.2%, N=4; non-perfused, 41.8 ± 3.6%, N=5. Thus, the transit rate was relatively low in the perfused intestine.

2. **Absorption of glucose from the intestinal tract**

A single injection of 10 mg of glucose was made in a volume of 0.1 ml by direct puncture, using a syringe, at the origin of the duodenum. At 10 and 30 min after the administration, the perfused intestine was removed and the lumen was washed out with 5 ml of warm saline solution. Only a negligible amount of residue of glucose was detected in the solution (Fig. 5).

![Fig. 5. Absorption of glucose from the intestinal tract. The residues of glucose in the intestinal lumen were expressed as percentages of the administered dose (10 mg). Vertical bars represent means ± S.E. The number of experiments is given in parentheses.](image)

3. **Absorption of acetaminophen from the intestinal tract**

A single injection of acetaminophen, 30 or 90 mg suspended in 2% arabic gum solution, was given in a volume of 0.4 ml by direct puncture, using a syringe, at the origin of duodenum. The arterial and venous blood were sampled at the same time, and the absorption of acetaminophen at variable intervals over one hour was determined from the differences between the concentrations in arterial and venous plasma (Fig. 6A). Five minutes after the administration of acetaminophen, the absorption curve showed a peak and thereafter fell rapidly. The absorption of acetaminophen was dose-dependent. One hour after the administration, the intestinal lumen was washed out and the residues of acetaminophen were detected in about 12% of the administered dose.

The absorption of acetaminophen was also investigated through another route: After 0.4 ml of 2% arabic gum solution containing 30 or 90 mg of acetaminophen had been poured
over the perfused intestine, the arterial and venous blood were sampled at the same time and the amount of acetaminophen absorbed was determined at variable intervals over one hour (Fig. 6B). The absorption curve showed a peak 5 min after the application and, thereafter, tended toward a plateau.

Five minutes after the application of acetaminophen, blood samples were collected and analyzed by thin-layer chromatography. As shown in Fig. 7, the positive spot having the same Rf value (0.36) as authentic acetaminophen was observed, even when acetaminophen had been administered through two different routes, indicating that the acetaminophen was absorbed from the intestinal tract and transferred into the blood.

4. Absorption of salicylamide from the intestinal tract

A single injection of salicylamide, 100 mg suspended in 2% arabic gum solution, was
given in a volume of 0.4 ml by direct puncture, using a syringe, at the origin of duodenum. The arterial and venous blood were concomitantly sampled, and the absorption of salicylamide at variable intervals over one hour was determined from the differences between the concentrations in arterial and venous blood (Fig. 8). Ten minutes after the administration of salicylamide, the absorption curves showed a peak and the conjugates (salicylamide sulfate and glucuronide) as well as free salicylamide appeared in the blood. One hour after the administration, the intestinal lumen was washed out and about 30 mg (34.4 ± 4.4 mg, N = 5) of the residues of salicylamide were detected.

DISCUSSION

Viability of the vascularly perfused intestine of the rat for periods up to 2 hr was evidenced by oxygen consumption, glucose utilization, and gross and microscopic appearance of tissues. According to Windmueller et al. (5), the vascularly perfused rat isolated intestine could be maintained viably for at least 5 hr by adding norepinephrine and glucocorticoid to the vascular perfusate. However, we prefer not to use any medicaments, since such compounds often affect the absorption processes of drugs. Thus, we examined the validity and usefulness of the vascularly perfused intestine in biochemical experimental fields using four drugs with different characteristics.

When Evans Blue solution was intraduodenally administered, almost 100% of the administered dose was recovered from the intestinal tract, one hour after the application. This is in agreement with the fact that Evans Blue is little absorbed from the intestinal tract. Additionally, this provides evidence that the cell membrane of the perfused intestine was intact.

Glucose is absorbed through an active transport mechanism from the intestinal tract (3, 4). In our study, 10 min after the injection of glucose into the intestinal lumen, only a trace amount of glucose was detected in the lumen, indicating the active transport of glucose from the intestinal lumen to blood or lymph vessels.

Acetaminophen and salicylamide, analgesic antipyretic drugs, are only slightly soluble in water. These drugs were suspended in 2% arabic gum solution and administered into the duodenum of the perfused intestine. Since BaSO₄ as well as arabic gum is little absorbed
from the normal gastrointestinal tract, the intestinal transit function using such agents can be well estimated. The transit rate of BaSO₄ injected into the lumen, at the origin of the duodenum, was about 25% per hour in the isolated perfused intestine and about 40% per hour in the intact non-perfused one. The relatively low transit rate of BaSO₄ in the perfused intestine may be explained in part by the fact that the perfused intestine was isolated and free from the influence of the central nervous system.

Acetaminophen is transferred through a passive transport process (12) and therefore was chosen as a tool for the absorption experiment. When acetaminophen suspended in 2% arabic gum solution was injected directly into the duodenum or poured (corresponding to intraperitoneal administration in the intact animal) over the perfused intestine, a dose-dependent absorption was observed rapidly after the application. When acetaminophen was orally ingested by humans, it was rapidly absorbed from the gastrointestinal tract and overdosages lead to hepatic damage (13–16). Based on a study on humans, Levy (17) suggested that acetaminophen administered perorally would be little metabolized during absorption from the gastrointestinal tract and such was verified in our present study when we administered the drug directly into the intestinal lumen or poured it over the perfused intestine, as evidence of the compound was found in the portal vein, in an unaltered form. Acetaminophen is metabolized by enzymes in liver microsomes and produces a hepatotoxic metabolite, N-hydroxy-acetaminophen (18–20).

Salicylamide administered perorally to humans is eliminated mainly by biotransformation to the ester sulfate and the ether glucuronide (10). Shortly after we administered salicylamide directly into the duodenal lumen of the perfused intestine, the drug appeared in the blood in free and conjugate (sulfate and glucuronide) forms, thus indicating that the compound was partly metabolized in the intestinal mucosa during the transport from the lumen into the blood.

The present results taken together indicate that the rat isolated intestinal preparation sustained by vascular perfusion can provide a valid estimation of the absorption and metabolism of drugs: The vascularly perfused intestine should thus be particularly suitable for studies of drug metabolism in the intestinal wall, and to investigate the transport of drugs from the intestinal lumen to blood and lymph vessels.

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