EFFECT OF TEMPERATURE ON INTESTINAL TRANSFER AND TISSUE UPTAKE OF SULFANILAMIDE AND AMINOPROPYRON IN **VITRO**

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The effect of temperature on the transfer and the tissue uptake of sulfanilamide and aminopropyron, an aminopyrine derivative, was investigated using the everted and the non-everted sacs of rat intestine. The M (mucosa) to S (serosa) transfer of sulfanilamide was slightly faster than reverse S-to-M in the ileum at the temperatures studied. Decreased transfer and tissue uptake of sulfanilamide with decreasing temperature were observed using both ileal everted and non-everted sacs.

On the contrary, the M-to-S transfer of aminopropyron was slower than the S-to-M transfer in the ileum. The tissue uptake of aminopropyron was almost constant at any temperature in the ileal non-everted sac experiments, while a decreased transfer of aminopropyron was observed with a decrease in temperature.

Similar results, like aminopropyron in the ileum, were obtained in the experiments of aminopropyron and sulfanilamide using the colonic sacs.

It is concluded that a close relationship may exist between the directional superiority in the transfer and the temperature independency of the tissue uptake.

**Keywords** — intestinal transfer; tissue uptake; temperature; everted sac; non-everted sac; sulfanilamide; aminopropyron

**INTRODUCTION**

The intestinal membrane cannot be considered as a simple homogeneous diffusion barrier.\(^1\) Permeation proceeds by different routes and in both directions. Much information is known regarding the directional difference of the transfer\(^2\)\(^-\)\(^6\) and the factors influencing transfer.\(^6\)

Few studies have been done to evaluate the effect of temperature on the passive transfer and the tissue uptake of drugs.\(^7\) However, experiments under low temperature have been performed to justify the existence of an active transport system.\(^8\)\(^,\)\(^9\) Such an approach to the intestinal barrier function provides another basis for evaluation of factors which determine the transfer of drugs across the intestine.

Many studies have been carried out and have demonstrated the utility of intestinal sac technique for the evaluation of the *in vitro* absorb-ability\(^10\)\(^,\)\(^11\) and the absorption mechanisms of drugs and nutrients.\(^12\)\(^,\)\(^13\)

Accordingly, the present study was carried out to examine the effect of temperature on the passive directional transfer and the tissue uptake using the ileal and colonic everted (mucosa (M)-to-serosa (S)) and non-everted (S-to-M) sacs.

**MATERIALS AND METHODS**

**Materials** — Aminopropyron was supplied by Nipponshinyaku Co. Sulfanilamide of reagent grade was obtained from Nakarai Chemical Co. All other reagents used in these experiments were of the finest grade available.

**Measurement of Directional Transfer and Tissue Uptake** — Directional transfer of sulfanilamide and aminopropyron in rat intestine was measured *in vitro* according to the method of Yasuhara *et al.*\(^13\) Briefly, the intestinal evert-
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d and non-everted sacs (20 cm) were prepared from the ileum, 20 cm above the ileocecal junction. The sacs were filled with 2.5 ml of pH 6.5 isotonic buffered saline (NaH₂PO₄-Na₂HPO₄-NaCl) and were placed in 20 ml of the same buffer solution containing 0.1 mM sulfanilamide or 1 mM aminopropyrone. They were incubated with continuous shaking and aerated with 95% O₂-5% CO₂ at various temperatures (25—42 °C). After 45 min incubation, the inner solution was collected and the concentration of the transferred drug was determined after centrifugation. The M-to-S transfer was obtained from the everted sac experiments and the reverse transfer (S-to-M) was obtained from the non-everted sac experiments.

For determining the tissue uptake, the sac was rinsed, weighed and homogenized in 4 volumes of pH 6.5 isotonic buffered saline. One milliliter of 30% trichloroacetic acid was added to the homogenates and the protein was removed by centrifugation. The supernatant fluid of the homogenate was assayed for drugs. The large intestine was removed from the anus to the proximal end of the colon and the everted or the non-everted sac (10 cm) was prepared in the same way as that described for the small intestine.

The transfer and the tissue uptake were expressed as concentration of drugs (nmol/ml and nmol/g, respectively).

The concentration of a given drug in the outer solution remained essentially constant throughout the experiment due to the large volume of the outer solution.

Analytical Methods — Sulfanilamide: Sulfanilamide was diazotized following a regular procedure, coupled with 2-diethylaminoethyl-naphthylamine and extracted with isooamyl alcohol after the addition of 1 g of NaCl. The optical density of the isoamyl alcohol layer was determined spectrophotometrically at 555 nm.

Aminopropyrone: One milliliter of sample solution was alkalinized with 1 ml of 2 N NaOH and extracted with chloroform. An aliquot of the organic phase was shaken with 0.1 N HCl and the optical density of the aqueous phase was determined spectrophotometrically at 260 nm.

Statistical Analyses — Results were expressed as the mean ±S.E.M. of at least four experiments. Statistical analyses were performed using Student's t-test. Regression lines were obtained by the linear least square methods.

RESULTS

Effect of Temperature on the Intestinal Transfer and the Tissue Uptake of Sulfanilamide in the Ileum

Experiments were carried out using the everted and the non-everted sacs at various temperatures (25—42 °C). Figure 1 shows that the relation between the logarithm of the ileal tissue uptake or the ileal transfer of sulfanilamide and the reciprocal of absolute temperature, for 45 min incubation, is approximated by a straight line in the everted and the non-everted sac experiments. The transfer in the everted (M-to-S)

![Graph](image-url)

**FIG. 1. Plot of the Logarithm of the Tissue Uptake or the Transfer of Sulfanilamide against the Reciprocal of the Absolute Temperature in the Ileum**

The intestinal sac (20 cm) was incubated in pH 6.5 buffered saline containing 0.1 mM of sulfanilamide for 45 min at various temperatures (25—42 °C). ○, tissue uptake in the everted; ●, tissue uptake in the non-everted; △, transfer in the everted; ▲, transfer in the non-everted. a) p< 0.02. b) p< 0.01. r: The coefficient of the correlation.
was larger than that in the non-everted (S-to-M) at the temperature studied. Figure 2 shows the plot of the 45-min-tissue uptake of sulfanilamide against the transfer at various temperatures. A good correlation was obtained between the tissue uptake and the bi-directional transfer of sulfanilamide in the ileum, with the slopes of the regression lines being 1.1 and 1.4 for the everted and non-everted sacs, respectively.

*Effect of Temperature on the Intestinal Transfer and the Tissue Uptake of Aminopropyron in the Ileum*

Figure 3 is a similar Arhenius type plot between the tissue uptake or the transfer of aminopropyron and the reciprocal of the absolute temperature. As was observed for sulfanilamide, there was a linear relationship between the two parameters except for the tissue uptake using non-everted sacs. In the non-everted sac experiments, a decreased transfer of aminopropyron was noted with a decrease in temperature. However, no significant changes were observed in the tissue uptake at the indicated temperatures. The M-to-S transfer was slower than the S-to-M transfer at the temperatures studied.

Figure 4 shows the relationship between the tissue uptake and the transfer of aminopropyron in the ileum at various temperatures. In the everted sac experiment, the relationship is de-

**FIG. 2. Relationship between the Tissue Uptake and the Transfer of Sulfanilamide in the Ileum**

The intestinal sac (20 cm) was incubated in pH 6.5 buffered saline containing 0.1 mM of sulfanilamide for 45 min at various temperatures (25–42 °C). Vertical bars indicate ± S.E.M. ○, for the everted; ●, for the non-everted. r: The coefficient of the correlation.

**FIG. 3. Plot of the Logarithm of the Tissue Uptake or the Transfer of Aminopropyron against the Reciprocal of the Absolute Temperature in the Ileum**

The intestinal sac (20 cm) was incubated in pH 6.5 buffered saline containing 1 mM of aminopropyron for 45 min at various temperatures (25–42 °C). ○, tissue uptake in the everted; ●, tissue uptake in the non-everted; △, transfer in the everted; ▲, transfer in the non-everted. a) p < 0.01. b) p < 0.001. r: The coefficient of the correlation.

**FIG. 4. Relationship between the Tissue Uptake and the Transfer of Aminopropyron in the Ileum**

The intestinal sac (20 cm) was incubated in pH 6.5 buffered saline containing 1 mM of aminopropyron for 45 min at various temperatures (25–42 °C). Vertical bars indicate ± S.E.M. ○, for the everted; ●, for the non-everted. r: The coefficient of the correlation.
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scribed by only a single regression line with a slope of 0.5. In contrast, the tissue uptake of amionpropyron, using the non-everted sacs, was almost constant (about 350 nmol/g) irrespective of the temperature.

Effect of Temperature on the Intestinal Transfer and the Tissue Uptake of Sulfanilamide and Ami

npropyron in the Colon

Bimodal effect of temperature on the transfer

![Graph showing relationship between tissue uptake of sulfanilamide and temperature](image)

**FIG. 5. Relationship between the Tissue Uptake and the Transfer of Sulfanilamide in theColon**

The intestinal sac (10 cm) was incubated in pH 6.5 buffered saline containing 0.1 mM of sulfana

lilamide for 45 min at various temperatures (25–42 °C). Vertical bars indicate ± S.E.M. ○, for the everted; ●, for the non-everted. r: The coefficient of the correlation.

and the tissue uptake was further examined with respect to regional difference. Figures 5 and 6 illustrate relationship between the tissue uptake and the transfer of sulfanilamide and aminopropyron, respectively. Contrary to the ileal experiments of sulfanilamide, the colonic tissue uptake of both drugs in the non-everted sac experiments was almost constant at the temperatures studied in a manner similar to the ileal experiments of aminopropyron.

**DISCUSSION**

It is well-known that lowering the temperature results in a marked decrease in the active transport and the uptake of various substances *in vitro.* Low temperature is also known to depress the passive transport to some extent. When the incubation temperature is lowered, the mobility of drug molecules might be reduced and the membrane function and fluidity related to the permeability of the mucosal and the serosal layers might also be changed. As shown in Table I (data at 37 °C), the sulfanilamide transfer from M-to-S was slightly larger than the transfer from S-to-M in the ileum. In the case of aminopropyron, the S-to-M transfer was almost twice as fast as that of the converse process in the ileum. It is reasonable that the bi-directional transfer as well as the tissue uptake of sulfanilamide was reduced in the ileal experiments with the decrease in temperature (Figs. 1 and 2).

In the case of aminopropyron, a similar reduction was observed in the two-way transfer, while the tissue uptake was almost constant and independent of temperature in the non-everted sac experiments (Figs. 3 and 4). Such tendency held in the colon where the transfer ratios for sulfanilamide and aminopropyron are 0.81 and 0.52 in Table I, respectively.

A close relationship seems to exist between the directional superiority in the transfer and the constancy of the tissue uptake. It is noteworthy that under the conditions where the M-to-S transfer was slower than the S-to-M (*i.e.* the ratio in Table I is smaller than unity), the tissue uptake became almost constant in the non-

![Graph showing relationship between tissue uptake of amionpropyron and temperature](image)

**FIG. 6. Relationship between the Tissue Uptake and the Transfer of Aminopropyron in the Colon**

The intestinal sac (10 cm) was incubated in pH 6.5 buffered saline containing 1 mM of aminoprop-

pyron for 45 min at various temperatures (25–42 °C). Vertical bars indicate ± S.E.M. ○, for the everted; ●, for the non-everted. r: The coefficient of the correlation.
everted sac experiments and was independent of temperature.

The intestinal mucosa plays an important role in the digestive and absorptive function of the gut. The mucosa has a lining of epithelium, specialized according to site and function. In con-

**TABLE I. Transfer Rate of Sulfanilamide and Aminopropyron at 37 °C through the Intestinal Sac in Vitro**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Region of intestine</th>
<th>Transfer (nmol/ml for 45 min)</th>
<th>Ratio (M-to-S/S-to-M)</th>
<th>p &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfanilamide</td>
<td>Ileum</td>
<td>34.9±1.7</td>
<td>30.6±1.1</td>
<td>1.14</td>
</tr>
<tr>
<td>(0.1 mM)</td>
<td>Colon</td>
<td>9.8±0.6</td>
<td>12.1±0.8</td>
<td>0.81</td>
</tr>
<tr>
<td>Aminopropyron</td>
<td>Ileum</td>
<td>109.7±3.1</td>
<td>188.6±9.4</td>
<td>0.58</td>
</tr>
<tr>
<td>(1 mM)</td>
<td>Colon</td>
<td>39.8±4.6</td>
<td>77.0±7.2</td>
<td>0.52</td>
</tr>
</tbody>
</table>

The intestinal sac was incubated in pH 6.5 buffered saline containing 0.1 mM of sulfanilamide or 1 mM of aminopropyron for 45 min at 37 °C. Results are expressed as the mean ± S.E.M. of at least four experiments.

**FIG. 7. Schema of Relationship between Temperature and the Tissue Uptake and the Transfer of Aminopropyron or Sulfanilamide in the Colon**

The size of circles indicate the proportion of the tissue uptake or the transfer of aminopropyron or sulfanilamide in the colon using the everted (upper) or the non-everted (lower) sacs.
Temperature Independency of Drug Uptake

Contrast, the intestinal serosa is a thin external connective tissue layer lined by a single layer of flattened mesothelial cells. The serosal layer has itself a marked absorptive capacity, as shown by the rapid uptake of intraperitoneally administered medication and by the therapeutic effectiveness of peritoneal dialysis in nephrology. 141

Moreover when the non-everted sacs were incubated in the drug solution, drug molecules could readily enter the intestinal blood vessels from the serosal side because the vessels were torn off at the mesenteric border, and the serosal surface is porous. 142 Aminopropion, which is positively charged at pH 6.5, and sulfanilamide may be more permeable across the serosal layer than across the mucosa in the colon due to their poor absorbability. Moreover, low temperature may depress the transport of these drugs across the mucosal barrier more than across the serosal barrier. These differences between the two barriers might result in the temperature independency of the tissue uptake of aminopropion or sulfanilamide in the colon.

In the everted sac experiments (upper part of Fig. 7), low temperatures may markedly depress the initial transport from the mucosal medium to the tissue (the tissue uptake) and continuously reduce the transport from the tissue to the serosal fluid. On the contrary, in the non-everted sac experiments (lower part of Fig. 7), low temperature may depress to some extent the transport from the serosal medium to the tissue (the tissue uptake) and markedly reduce the transport from the tissue to the mucosal fluid across the mucosal barrier.

It appears, therefore, that the tissue uptake for 45 min is apparently constant and independent of temperature. However, additional studies are needed to clarify the detailed mechanisms of the temperature independency of the tissue uptake and the directional superiority of transfer.

REFERENCES


