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Drug-induced Histological Changes and Its Consequences on the Permeability of the Small Intestinal Mucosa.[1] II[2]

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The influence of chelating agents such as EDTA disodium salt and tetracycline hydrochloride, and surfactants such as sodium lauryl sulfate, sodium desoxycholate, sodium taurocholate, benzethonium chloride, polysorbate 80, HCO-50, and Pluronic F 68, upon the intestinal mucosa was investigated by measuring the apparent exsorption rate constant (K) of sulfaguanidine administered intravenously in the rat. Except Pluronic F 68, all the agents showed increase in K with concomitant histological change. Because of this histological change the permeability of sulfaguanidine from the blood vessel to the intestinal lumen was found to increase. Further, the value of K and its change with time are suggested to be usable as an indicator of the damaging effect of drugs on the intestinal mucosa.

Employing a scanning electron and a light microscope, we have previously shown[2] that the perfusion of a solution of sodium lauryl sulfate or tetracycline hydrochloride through the rat intestine gives rise to a remarkable histological change in the intestinal tissue. The histological change was suggested to be the primary cause of the enhancement in the absorption of drugs which are coadministered with these substances. As a part of the investigation to clarify this possibility, we have studied the variation in the permeability of the intestinal mucosa upon the histological change and the results are reported here.

In this study sulfaguanidine was chosen as a marker and the effect has been investigated of chelating agents and surfactants which are expected to influence the behavior of the intestinal tract by following the change in the apparent exsorption rate constant of sulfaguanidine. The result of the present investigation have shown that the histological changes brought about by these agents can be the cause of the increased outward permeability of the drug through the intestinal mucosa (permeation from the blood vessel to the intestinal lumen). Further, we have found that the apparent exsorption rate constant such as the one used here and its variation with time can be employed as a measure of the influence of drugs towards the intestinal tissue.

Experimental

Experimental Procedure——The experimental procedure was similar to the one reported previously.[4] Male Wistar rats weighing 130—180 g were used under urethane anesthesia following overnight fasting. The abdomen was exposed by the usual method and the bile duct was tied. Catheters were fixed at the duodenal and ileum ends. Fifteen milligrams of sulfaguanidine dissolved in N,N-dimethylacetamide was administered *via* the tail vein. The substances under study were dissolved in an isotonic pH 7.4 phosphate buffer and perfused at a rate of 4 ml/min through the intestine. The single-pass perfusion solution was

1) The work was presented at the 92nd Annual Meeting of the Pharmaceutical Society of Japan in April, 1972.
2) Part I: T. Nadai, R. Kondo, A. Tatematsu, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 1139 (1972).
3) Location: *a*) *Yagotourayama, Tempaku-cho, Tenpaku-ku, Nagoya*; *b*) *Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto.*
4) T. Nadai, K. Nishii, and A. Tatematsu, *J. Pharm. Soc. Japan*, **90**, 262 (1970).

sampled at 5 min intervals into a 20 ml volumetric flask. Approximately 0.2 g of blood sample is obtained through the tail vein into a previously weighed weighing bottle containing 0.1 ml of 3.8% sodium citrate. These samples were assayed for sulfaguanidine by the usual diazotized colorimetric method and the apparent exsorption rate constants[5] were calculated according to the previously published method.[4] As for the specimens for scanning electron microscopy the intestine was washed with normal saline, treated with glutaraldehyde and dried by the usual method. The specimens for examination under a light microscope were treated with paraffin and the sections were made by the conventional method and stained with hematoxylin-eosin.

Materials——As chelating agents, ethylenediaminetetraacetic acid (EDTA) disodium salt, tetracycline hydrochloride, and as surface active agents, sodium lauryl sulfate (SLS), sodium desoxycholate, sodium taurocholate, benzethonium chloride, HCO-50,[6] polysorbate 80, and Pluronic F 68 were chosen. Sodium lauryl sulfate, EDTA disodium salt, benzethonium chloride, and sodium desoxycholate were all of reagent grade. Sodium taurocholate was purchased from Nakarai Chemicals, Ltd. Pluronic F 68 and HCO-50 were of Pharmaceutical grade, and polysorbate 80 of J.P. grade. Sulfaguanidine (J.P. grade) was recrystallized prior to use.

Results and Discussion

In Fig. 1 is shown the variation with time of the apparent exsorption rate constant of sulfaguanidine, K (excretion into the intestinal lumen through the intestinal wall) following its intravenous administration when an isotonic pH 7.4 phosphate buffer was perfused through the intestinal lumen. During the 70 min period of the experiment the values were fairly constant. No histological changes were observed under a scanning electron or a light microscope. These observations thus indicate that the perfusion of the phosphate buffer for the period does not bring about any histological change which may affect the permeability of the intestinal mucosa.

Fig. 2 shows the variation of K with time when the perfusing buffer solution contained sodium lauryl sulfate (SLS) at various concentrations. The effect of SLS on the permeability of the intestine was so remarkable that even at a concentration of 1 mM the K value was

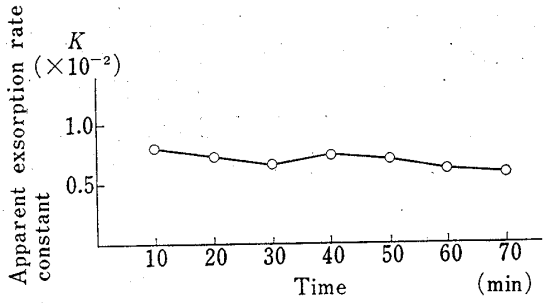

Fig. 1. Change of the Apparent Exsorption Rate Constant induced by Phosphate Buffer

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by phosphate buffer.

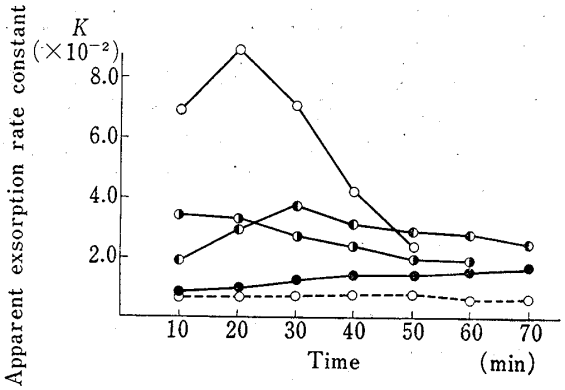

Fig. 2. Change of the Apparent Exsorption Rate Constant induced by SLS

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by SLS.
sodium laurylsulfate —○—: 34 mM, —◑—: 10 mM, —◐—: 5 mM, —●—: 1 mM, ---○---: control

5) $$K = \frac{\Delta E}{\Delta t}\frac{1}{C}$$

K: apparent exsorption rate constant

$\frac{\Delta E}{\Delta t}$: amount of sulfaguanidine excreted per minute (expressed as OD/min)

C: Plasma concentration of sulfaguanidine (expressed as OD/g plasma)

6) Polyoxyethylene derivatives of hydrogenated castor oil.

observed to increase twice as much as that of the control. At 34 mM (1%), the K value increased rapidly from the start of perfusion, but the value suddenly decreased approximately 20 min later and at 50 min after the beginning of the experiment all the animals died. In these cases, as reported previously, marked histological changes were observed. As for 5 and 10 mM SLS, at the beginning of the experiments the K values were definitely higher for 10 mM SLS. From 30 min after the beginning of the experiments, however, the K values became less for 10 mM SLS than for 5 mM. The K values were observed to increase gradually with time at 1 mM SLS.

We have then investigated whether the histological changes brought about by chemicals are reversible or not. We have studied this possibility by measuring K values in the following 3 periods. During the first 15 min following intravenous administration of sulfaguanidine the isotonic pH 7.4 phosphate buffer was perfused and for the next 15 min the same buffer containing 1% SLS was perfused followed by perfusion of the fresh buffer without SLS. The results are shown in Fig. 3. The K values were noted to rise remarkably only after about 10 min after the start of perfusion of the SLS solution. This indicates that the effect of SLS on the tissue appears in a very short time. When the perfusion of the SLS solution was discontinued the K values still continued to rise for about 10 min. Thereafter the K values decreased a little and appeared to reach a constant value.

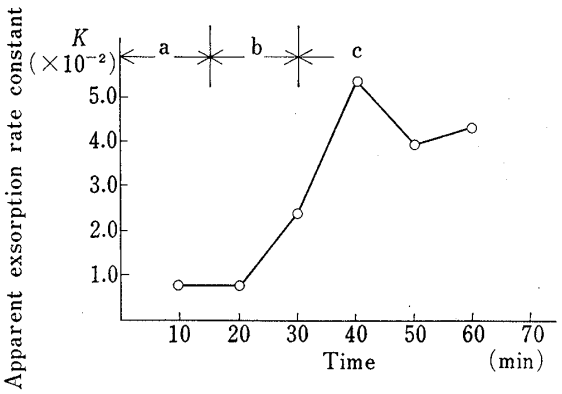

Fig. 3. Change of the Apparent Exsorption Rate Constant induced by SLS

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by SLS.
a: phosphate buffer solution
b: 1% SLS solution
c: phosphate buffer solution

Fig. 4

Fig. 4 shows a light micrograph of the small intestine when it was exposed to the SLS solution for 15 min. It can be seen that the epithelial cells are broken away indicating the beginning of histological changes by SLS. Fig. 5 shows the light micrograph of the intestine 40 min after the exchange of the SLS recirculating solution with the buffer. Many cells in villi are recognized to have undergone hyaline-like degeneration. Fig. 6 is a scanning electron micrograph of the same specimen. In this micrograph also, a significant difference was observed in comparison with that of the control.[2] From these observations the decrease in K values observed with higher SLS concentrations is not likely due to the recovery of the intestinal tissue but rather due to its extensive histological changes. Although the exact cause is not clear the following speculation may be made. The K value might have decreased because of the loss of body fluid including the blood as the result of the histological change or because of the decrease of the flow of the body fluid as the result of the contraction of the tissue in order to counteract the loss of the body fluid due to the tissue destruction.

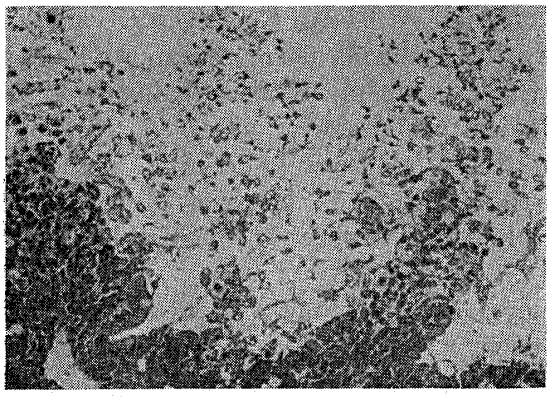

Fig. 5

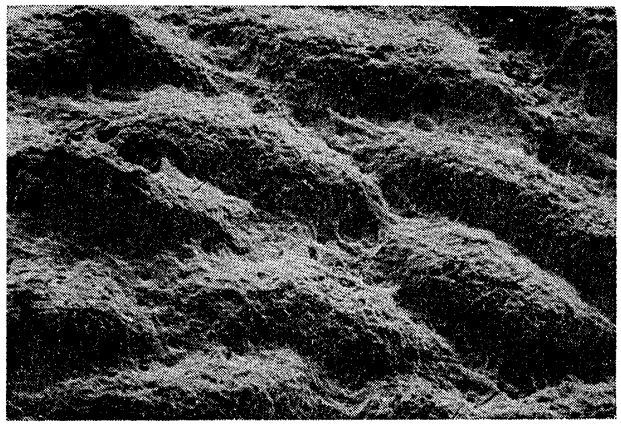

Fig. 6 100 μ

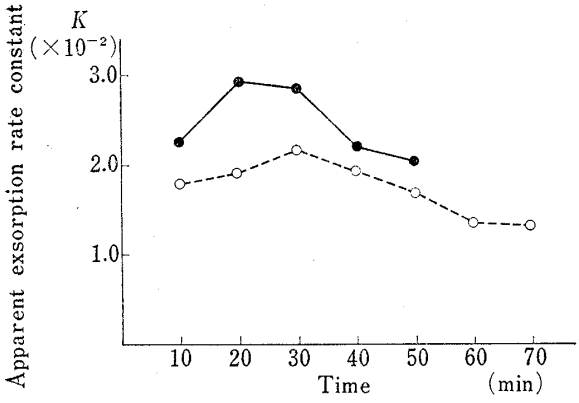

Fig. 7. Change of the Apparent Exsorption Rate Constant induced by Sodium Desoxycholate

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by sodium desoxycholate.
sodium desoxycholate —●—: 10 mM, ···○···: 5 mM

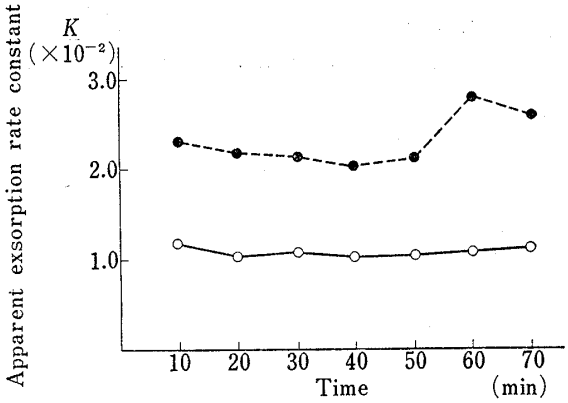

Fig. 8. Change of the Apparent Exsorption Rate Constant induced by Sodium Taurocholate

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by sodium taurocholate.
sodium taurocholate ---●---: 10 mM, —○—: 5 mM

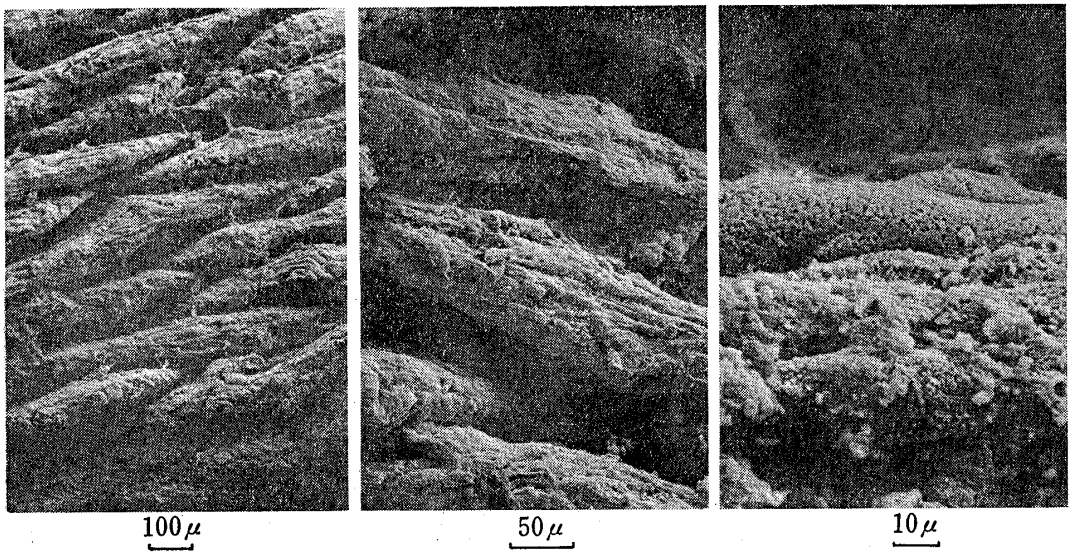

Fig. 9

Fig. 7 and 8 show the results when isotonic pH 7.4 buffer solutions containing various amount of sodium desoxycholate (SDC) and sodium taurocholate (STC), respectively, were perfused through the intestine. In either case, high K values were observed from the beginning of the perfusion. In the case of 10 mM SDC, all animals were dead at approximately 50 min after the beginning of the experiments. In Fig. 9 are shown the scanning electron micrographs of the intestine at 50 min perfusion of 10 mM SDC. Unlike the micrographs reported in the previous paper, remarkable change was observed in these micrographs.

Fig. 10 shows the effect of a cationic surface active agent, benzethonium chloride, on K. Markedly high K values were observed with 10 min perfusion. Thereafter the values decreased with time as was the case with SLS. The reason for the decline is considered to be the same as for SLS. The effect of benzethonium chloride on the intestine is thus assumed to be very remarkable and sudden. In Fig. 11 are shown scanning electron micrographs of the intestine when a test solution containing 5 mM benzethonium chloride was recirculated for 70 min. They show significant alteration on the surface of the intestine. In the case of benzethonium chloride (5 mM and 10 mM), the K values were independent of the concentration. The following two speculations can be made for this observation. Because of the good tissue permeability of benzethonium chloride it is absorbed immediately from the surface and influences the inner layer of the tissue. As the results, the drug can be diffused easier and the amounts of the drug present at the local site become independent of the drug concentration employed. Another possibility is that either the blood flow or lymph flow becomes rate determining because of a marked change in tissue permeability.

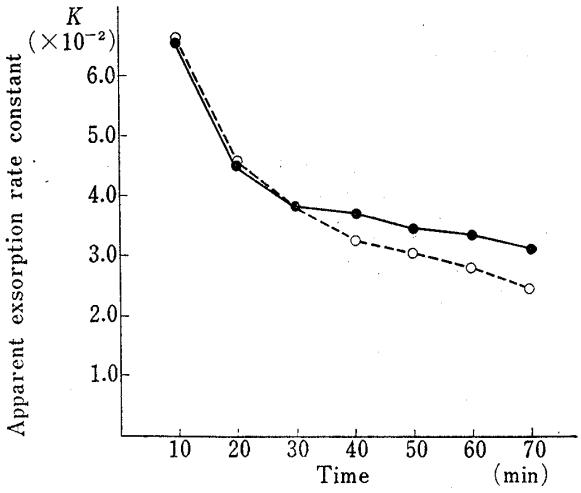

Fig. 10. Change of the Apparent Exsorption Rate Constant induced by Benzethonium Chloride

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by benzethonium chloride.
benzethonium chloride —●—: 10 mM, ---○---: 5 mM

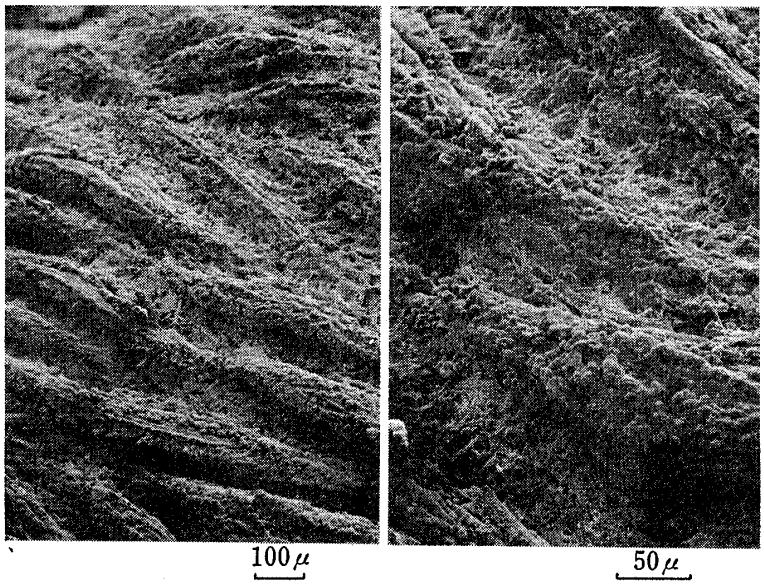

Fig. 11

Fig. 12, 13 and 14 show the K values determined for nonionic surfactants, *i.e.*polysorbate 80, HCO-50, and Pluronic F 68, respectively. These surfactants gave similar exsorption patterns. As for Pluronic F 68, in particular, the exsorption and histological results were as for the control. In Fig. 15 is shown a scanning electron micrograph of the surface of the intestine upon exposure to 1% HCO-50 solution for 70 min. Many broken-away epithelial cells can be seen indicating there was some change in the intestine.

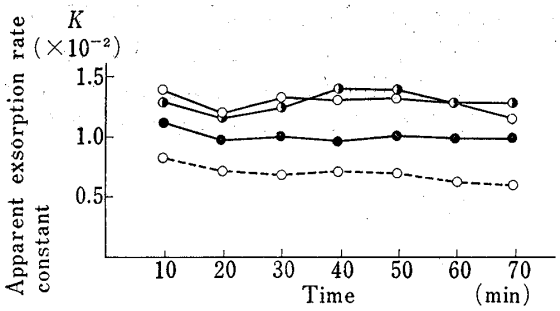

Fig. 12. Change of the Apparent Exsorption Rate Constant induced by Polysorbate 80

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by Polysorbate 80.
Tween 80 —○—: 2%, —◑—: 1%, —●—: 0.5%, ---○---: control

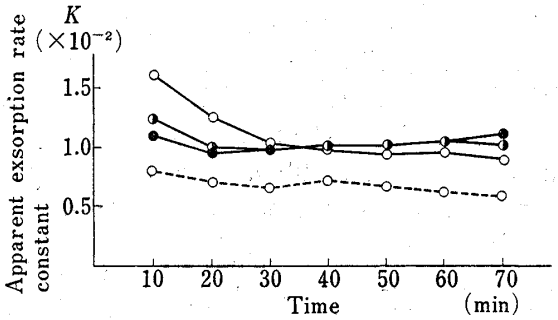

Fig. 13. Change of the Apparent Exsorption Rate Constant induced by HCO-50

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by HCO-50.
HCO-50 —○—: 2%, —◑—: 1%, —●—: 0.5%, ---○---: control

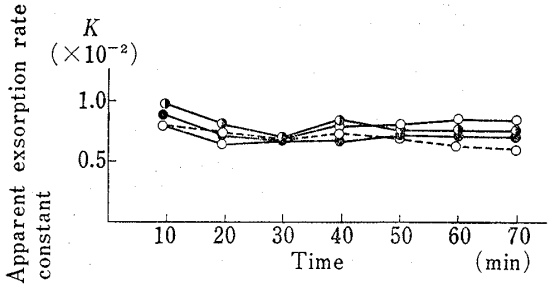

Fig. 14. Change of the Apparent Exsorption Rate Constant induced by Pluronic F 68

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by Pluronic F 68.
Pluronic F 68 —○—: 2%, —◑—: 1%, —●—: 0.5%, ---○---: control

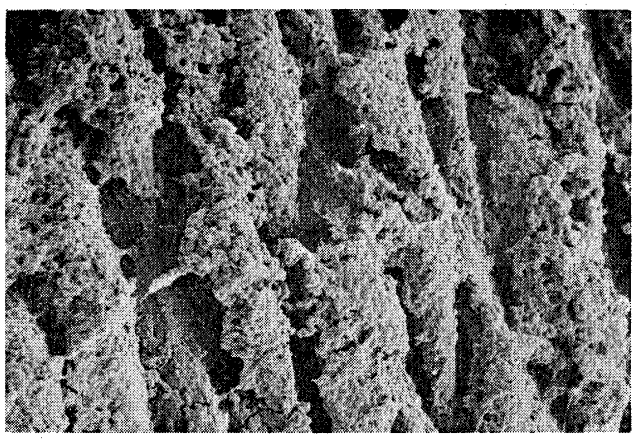

Fig. 15

The results of investigation of the effect of the concentration of EDTA were presented in Fig. 16. The K values were noted to increase with increase in the concentration of EDTA in the perfusion medium, particularly so with 25 mM. At this concentration the animals were dead before 40 min perfusion and bleeding was noted during the perfusion. A marked change in the micrographs has also been observed in this case as reported previously.[2)]

As was the case with SLS, for the first 15 min the buffer was perfused, and for the next 15 min 25 mM EDTA in the buffer followed by the buffer again. Fig. 17 shows the change in K with this treatment. It can be seen from the figure that the K values began to rise 10 min after the introduction of the EDTA solution and rose quite steeply even after the change of the perfusion medium back to the buffer. However, the value began to decline after 60 min.

Fig. 18 is a scanning electron micrograph of the intestine upon 10 min exposure to the EDTA solution. It clearly shows a change at the outermost layer of the mucosa. Fig. 19 is a light micrograph obtained 25 min after the exchange of the perfusion solution with the buffer. This similarly shows the loss of the surface cells.

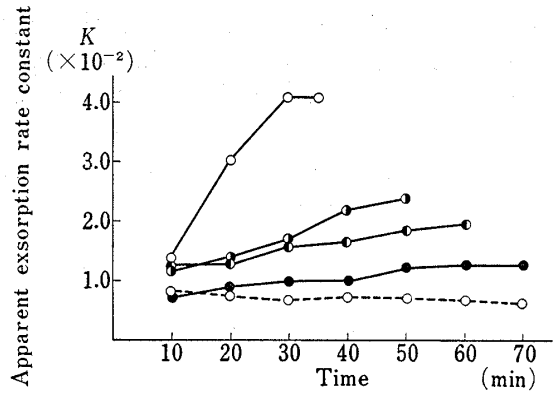

Fig. 16. Change of the Apparent Exsorption Rate Constant induced by EDTA-2Na

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by EDTA-2Na.
EDTA-2Na —○—: 25 mM, —◑—: 15 mM, —◐—: 10 mM, —●—: 5 mM, ---○---: control

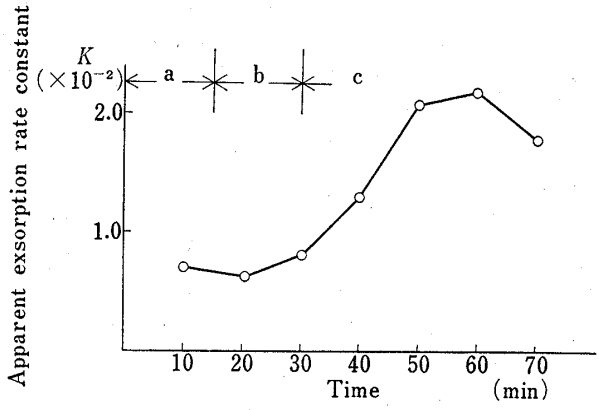

Fig. 17. Change of the Apparent Exsorption Rate Constant induced by EDTA-2Na

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by EDTA-2Na.
a: phosphate buffer solution
b: 25 mM EDTA-2Na solution
c: phosphate buffer solution

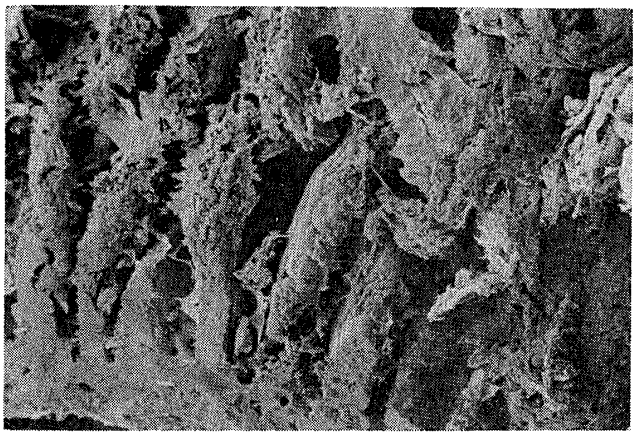

Fig. 18

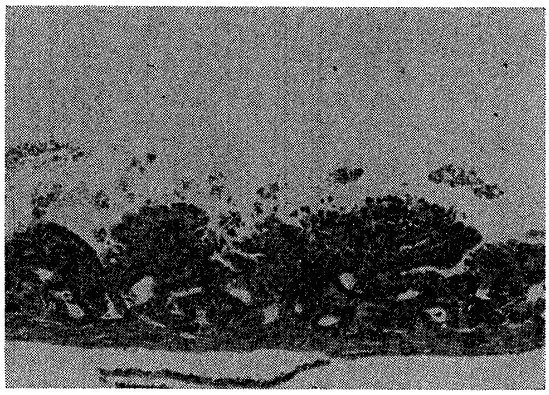

Fig. 19

In Fig. 20 are shown the effect of tetracycline hydrochloride (TC) on K values. The K values increased with increase in TC concentration. This phenomenon was observed to appear early with higher concentrations, whereas with lower concentration the tendency was observable only toward the end of the experiment. This observation agrees well with the observation previously made at pH 8.0 and confirms that the same tendency also prevails at pH 7.4.

Particularly with 2 mM, the K values increased only 50 min after the beginning of perfusion. This may indicate that some of the absorbed TC is accumulated in the tissue and only when a certain drug concentration is attained, the K value increases. Namely, corresponding histological changes may be considered to take place. When the concentration of TC was further increased, however, the increase in K values stopped at around 60 min after the beginning of perfusion, and for each TC concentration there appears to be a maximum K value. The tissue, however, is likely to suffer damage in proportion to the concentration.

As with other drugs studied so far, in Fig. 21 is shown the variation of K values with the change of the perfusion medium as indicated. The K values were observed to increase 5 min after the perfusion of the TC solution and K reached a maximum value 10 min after the exchange of the TC solution with the buffer solution and declined gradually thereafter.

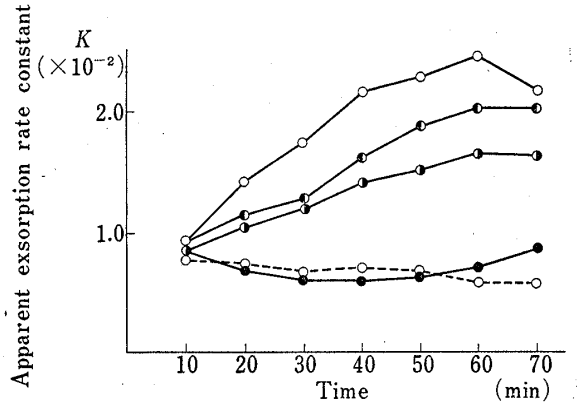

Fig. 20. Change of the Apparent Exsorption Rate Constant induced by Tetracycline

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by tetracycline.
tetracycline —○—: 6.2 mM, —◐—: 5.0 mM,
—◑—: 4.2 mM, —●—: 2.0 mM,
---○---: control

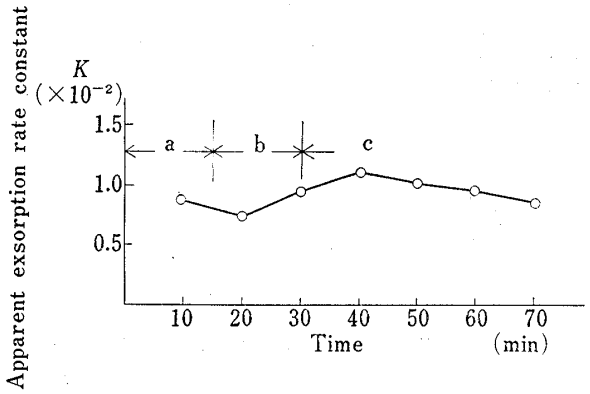

Fig. 21. Change of the Apparent Exsorption Rate Constant induced by Tetracycline

Intravenously injected sulfaguanidine was used as a marker for the permeability change of the gut induced by tetracycline.
a: phosphate buffer solution
b: 5 mM tetracycline solution
c: phosphate buffer solution

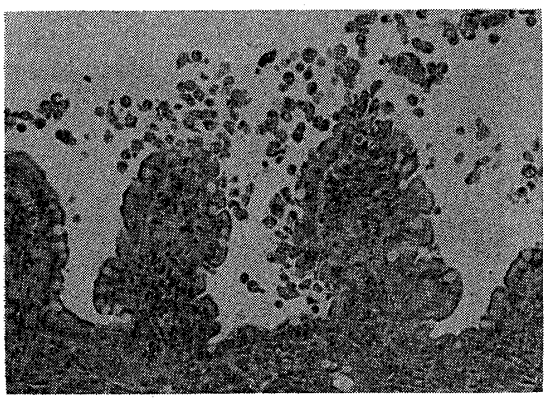

Fig. 22

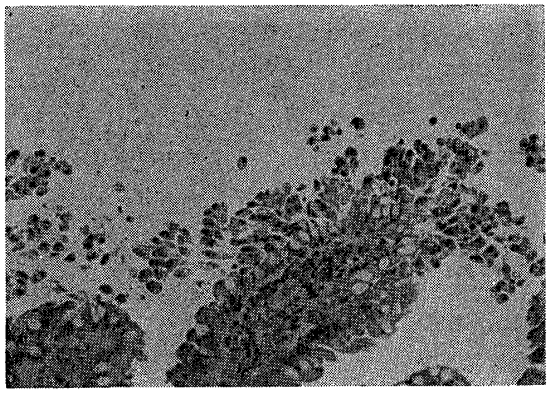

Fig. 23

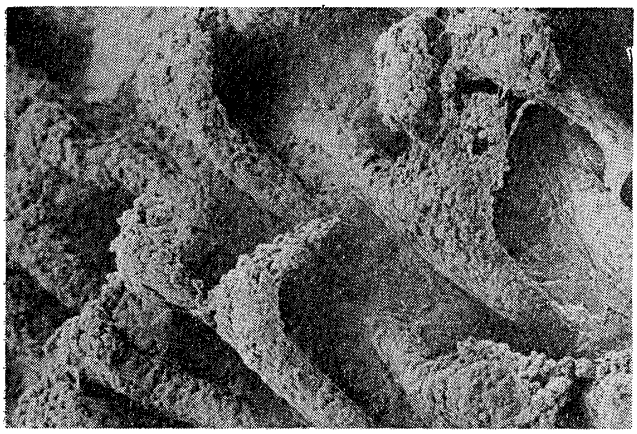

Fig. 24

Fig. 22 is a light micrograph of the tissue when the K values began to increase and shows the disintegration of the epithelial cells. Fig. 23 which corresponds to a similar micrograph at the maximum K value shows a damage even greater than that shown in Fig. 22. The scanning electron micrograph of the tissue at the maximum K value is shown in Fig. 24. The scanning electron micrograph of the intestine through which the buffer solution was perfused for 40 min following the TC solution and where the K value has declined shows, as presented in the previous report,[2] the desquamation of the epithelial cells from the tips of villi which are seriously damaged, and that the remaining tissue seemed to be recovering. Therefore, the histological change brought about by TC may be considered to be reversible in nature and the tissue may speedily recover upon the removal of TC.

In all cases except Pluronic F 68 there was a significant rise in the perfusate levels of sulfaguanidine, an intravenously administered marker substance, with single-pass perfusion of the solution containing a variety of chelating agents and surface active substances.

Blood-to-lumen flow technique used in the present investigation excludes any interaction between the marker and test substances and seems to be even more sensitive and useful indicator of drug-induced permeability as well as histological change of the small intestinal membrane.