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Drug-induced Histological Changes and Its Consequences on the Permeability of the Small Intestinal Mucosa. I. EDTA, Tetracycline, and Sodiun Laurylsulfate[1)]

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Acute effect of EDTA-2Na, tetracycline-HCl, and Sodium laurylsulfate on the permeability of the rat small intestine was examined histologically using light microscope and scanning electron microscope. Specimens were obtained from the proximal small intestine after recirculation of the physiological buffer solution of the test compounds through the entire rat small intestine. It was observed that although the remarkable facilitating effect in the absorption of otherwise poorly absobable compounds reported in the literature was common, histological changes were different each other.

The factors influencing the small intestinal permeability are extremely complex. Much evidence accumulated about possible effects of drugs and pharmaceutical adjuvants in the change of permeability of the small intestinal mucosal membrane.

Windsor and Cronheim[3)] reported that heparin and sulfopolyglycine, normally very poorly absorbed from the intestinal tract, were absorbed to an appreciable extent when administered together with the chelating agent, EDTA. In addition, the absorption of mannitol, inulin, a quaternary ammonium compound, and sulfanilic acid were all markedly increased in the presence of EDTA.[4)]

A recent report shows that a number of sulfated or sulfonated surfactants are also capable of facilitating heparine absorption when administered in solution[5)] or in a certain type of emulsions.[6)]

In the studies on the effect of tetracycline on the intestinal absorption on sulfanilic acid and sulfaguanidine,[7)] we have observed the presence of interactions of tetracycline with the absorptive membrane mediated by Ca^{2+}.

These changes in the permeability, however, have largely been studied from the standpoints of drug interactions in the gut lumen, model interactions with the membrane components, and of the changes in physiological factors, and remarkably little morphological as well as histological information is available about such interactions.

In this series of work, the features of small intestinal structure in normal control rat and in drug-treated one have been examined histologically and an attempt has been made to evaluate critically the acute effect of various drugs and pharmaceutical adjuvants on the permeability of the small intestine.

1) Presented to the 91st Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April 1971.
2) Location: *a) Yagoto Tenpaku-cho, Showa-ku, Nagoya; b) Yoshidashimoadachi-cho, Sakyo-ku, Kyoto.*
3) E. Windsor and G.E. Cronheim, *Nature*, **190**, 263 (1961).
4) L.S. Schanker and J.M. Johnson, *Biochem. Pharmacol.*, **8**, 421 (1961).
5) R.H. Engel and S.J. Riggi, *J. Pharm. Sci.*, **58**, 706 (1969).
6) R.H. Engel and S.J. Riggi, *Proc. Soc. Exptl. Biol. Med.*, **130**, 879 (1969).
7) T. Nadai, K. Nishii, and A. Tatematsu, *Yakugaku Zasshi*, **90**, 262 (1970).

Experimental

Materials——Tetracycline HCl used in the present studies were from commercially available sources. All other chemicals were of analytical grade.

Perfusion——Male Wistar rats weighing 130—150 g were fasted for 18 to 24 hours before the experiment. Using pentobarbital anesthesia, the small intestine was exposed by a midline abdominal incision, and glass cannulae were inserted through small slits at the duodenal and ileal ends. Care was taken to handle the small intestine gently and to reduce surgery to a minimum in order to maintain an intact blood supply. The cannulae were secured by ligation with suture and connected to the perfusion assembly.[8] As a means of clearing the gut, perfusion fluid was then passed gently through it until the effuluent solution was clear. Twenty-five ml of the perfusion fluid was then recirculated through the intestine at a rate of 3 ml per minute.

In the case of horse-radish peroxidase preloading, twenty mg of horse-radish peroxidase was injected as a physiologic saline solution through the external jugular vein prior to the perfusion.

Histological Methods——At the end of one-hour perfusion, the lumen was gently washed with approximately 25 ml of cold physiologic saline and immediately fixed with 5% solution of glutaraldehyde (4—5°) in pH 7.4 physiologic phosphate buffer solution. The upper part of the small intestine was carefully resected and washed several times with cold pH 7.4 physiologic phosphate buffer solution (0.1 M). The tissues were dehydrated, mounted in paraffin-wax, serially sectioned at 5 μ thickness, and stained with haematoxylin-eosin prior to histological examination. In the case of scanning electron microscopy, the segments, 1 to 1.5 cm in length, were dehydrated with acetone, treated in an conventional manner, and examined in Hitachi SSM-2 and HMS-2 scanning electron microscopes.

The intestine was fixed, serially sectioned under freezing, and stained with N-N′-diaminobenzidine in the case of horse-radish peroxidase pre-loading.

Result

Photomicrographs of nornal control rat intestinal mucosa are shown in Fig. 1—4. Fig. 1 shows a light micrograph of lower magnification of the specimen obtained from the proximal small intestine after one-hour reciruclation on pH 6.0 physiologic phosphate buffer solution through the intestine. Mucosal integrity was essentially maintained and morphological changes in villous structure were absent as viewed with light microscope. Similar light micrograph was obtained after one-hour perfusion of pH 8.0 physiologic buffer solution (Fig. 2).

Fig. 3 and Fig. 4 show scanning electron micrographs of the normal control mucosal surface of the segments used in Fig. 1 and Fig. 2 respectively. The corrugated surfaces are normal in both cases. Fig. 1—4 rule out the possibility of any damage or morphological change to the absorptive surface of the small intestine caused by one-hour perfusion of the isotonic buffer solutions.

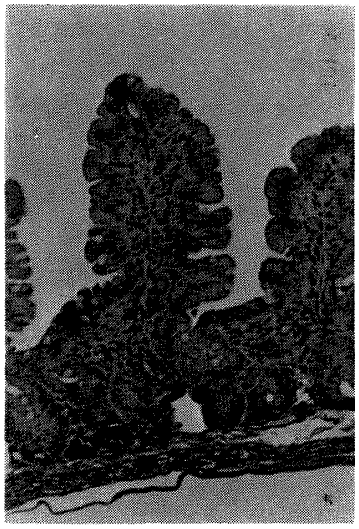

Fig. 1
×150

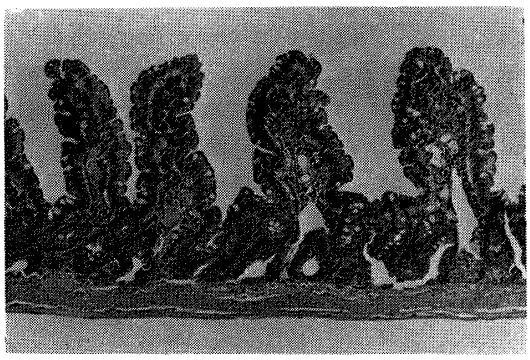

Fig. 2
×75

8) K. Kakemi, T. Arita, H. Sezaki, and I. Sugimoto, *Yakugaku Zasshi*, **84**, 1210 (1964).

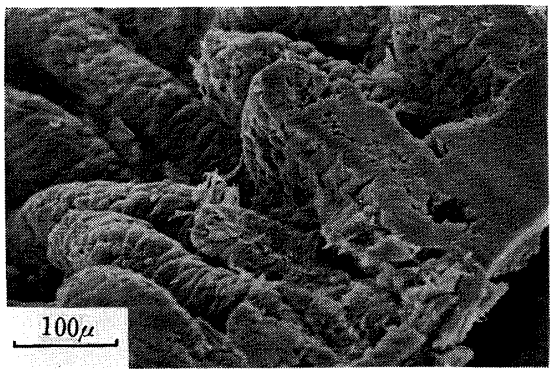

Fig. 3

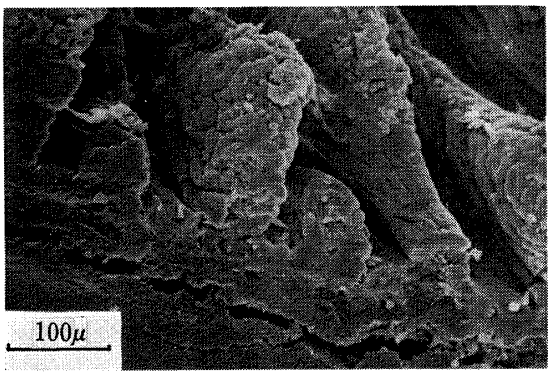

Fig. 4

Effect of EDTA, a permeability changing chelating agent, is clearly demonstrated in Fig. 5—7. Light micrograph obtained from the segments after the perfusion of 25 mM EDTA-2Na physiologic phosphate buffer solution is shown in Fig. 5. Animals could not bear one-hour perfusion and the segments was obtained immediately after their death, about 45 minutes after the beginning of perfusion. As is evident from the figure, EDTA produced significant alterations. Surface epithelial cells are broken away from thc basement membrane. Despite such marked change, basal cells of crypt appeared almost similar to the normal ones.

Fig. 6 is a light micrograph obtained after the pre-loading of horse-radish peroxidase. Intestinal capillaries, stained dark brown, are exposed to the luminal border of the membrane and there is a distrinct possibility that the capillary walls were in direct contact with the perfusing solution and that blood capillaries of the intestinal mucosa were lesioned and opened in some part. Fig. 7 is a scanning electron micrograph showing the detail of the surface of the same segment. The lesions of the villi are noted and occasionally un-identified sphere-shaped substances are seen on the surface.

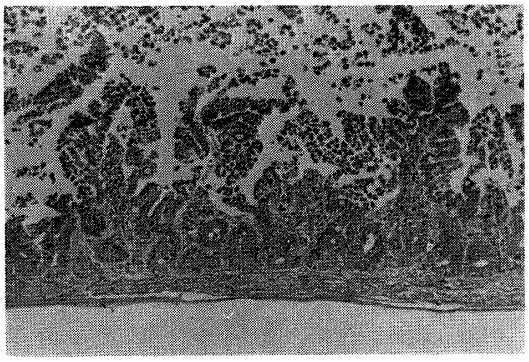

Fig. 5
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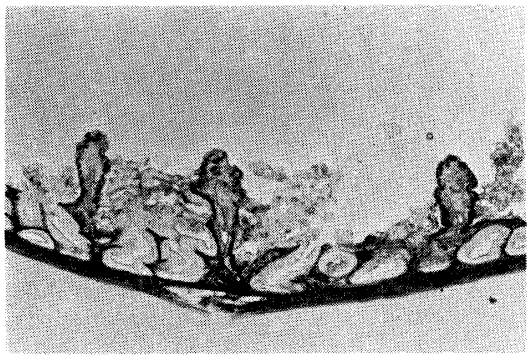

Fig. 6
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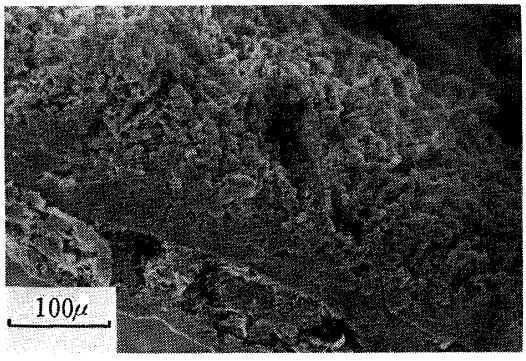

Fig. 7

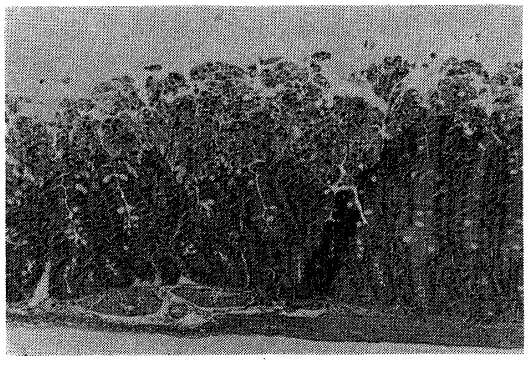

Fig. 8
×75

The changes induced by tetracycline at pH 8.0 are presented in Fig. 8—11. In our previous paper,[7] it was found that tetracycline induced change in the permeability to poorly absorbable substances was more remarkable at this pH where the drug is supposed to have greater affinity toward calcium ion and higher lipophilicity.[9] Fig. 8 is a light micrograph showing the surface of the small intestine after exposure to 3000 μg/ml of tetracycline in pH 8.0 physiologic phosphate buffer solution. Mild abnormality of separation of epithelium from lamina propria with slight shedding of epithelial cells from villous tips are noted. Slight edema and vacuoles were occasionally present. These morphological changes were observed in the crypts of Lieberkühn. Higher magnification of the same segments is shown in Fig. 9. Abnormality in the desquamated epithelial cells was not observed.

Light micrograph obtained after horse-radish peroxidase pre-loading shwon in Fig. 10 clearly demonstrates that blood vessels of the intestinal mucosa are more readily accessible to the luminal contents. The depth of mucosa became smaller and epithelial structure loosened by tetracycline treatment. Scanning photomicrograph shown in Fig. 11 can demonstrate the three-dimensional change of the intestinal surface by tetracycline in detail. To establish whether the permeability enhancement caused by tetracycline *in situ* is reversible, physiologic saline was perfused for one hour after one-hour perfusion of 3000 μg/ml of tetracycline solution, and the tissues were examined. As is evident from Fig. 12, no specific change was observed any more although slight shedding of epithelial cells from villous tips were still present. That the interaction of tetracycline with membrane is reversible indicates that integrity of intracellular metabolism was retained and excludes extensive tissue disruption as the explanation of the absorption enhancing effects observed in the previous report form this laboratory.[7]

Effect of tetracycline was also examined at pH 6.0. There was a moderate degree of submucosal swelling only, which coincides with our previous finding that the intestinal absorp-

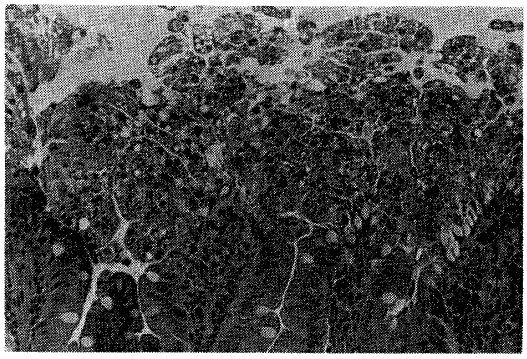

Fig. 9
×150

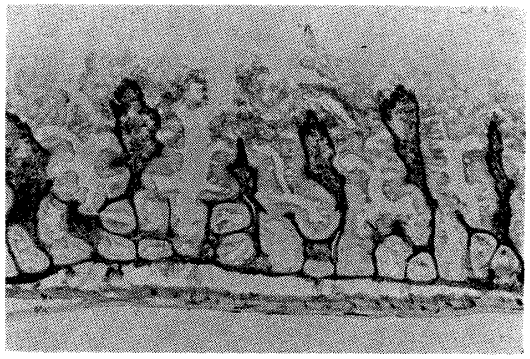

Fig. 10
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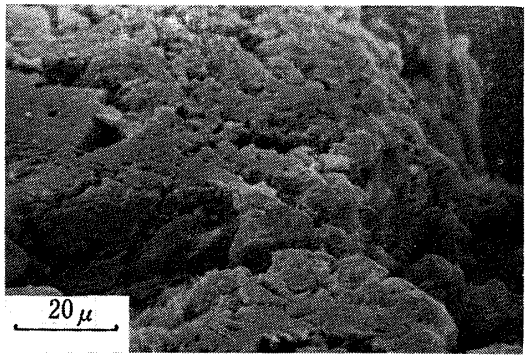

Fig. 11

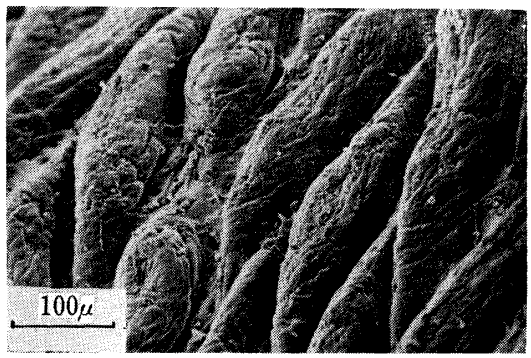

Fig. 12

9) K. Kakemi, H. Sezaki, T. Nadai, and Y. Ogata, *Chem. Pharm. Bull.* (Tokyo), **16**, 2200 (1968).

tion of poorly absorbable drugs in the presence of tetracycline at this pH was very slight and slow acting in comparison with the ones at pH 8.0.

An increase in the permeability of intestinal tissues has been observed *in vitro* and *in vivo* after exposure to anionic surfactant, sodium laurylsulfate.[8] Such permeability increase is well reflected upon the light micrographs shown in Fig. 13 and Fig. 14. One-hour perfusion of higher concentration (1%) of sodium laurylsulfate in pH 7.4 physiologic phosphate buffer solution caused great damage to the mucosal surface. Separation of epithelium from lamina propria, destruction of blood vessels in lamina propria, and hemorrahage were present. Height of villi are low in comparison with the control ones and in some part, prominent eruption and destruction due probably to the degeneration of epithelial cells were noted. Scanning photomicrographs presented in Fig. 15 and Fig. 16 show the strands of surface and depth of corrugations respectively.

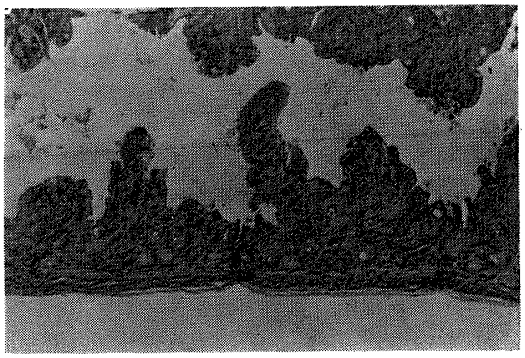

Fig. 13
×75

Fig. 14
×300

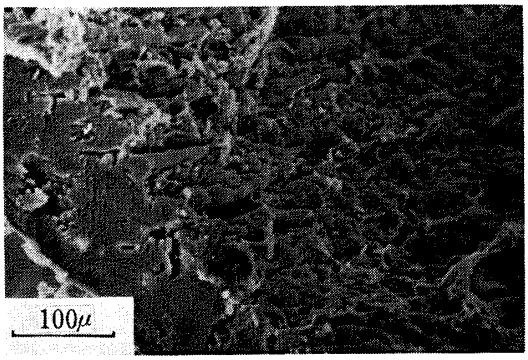

Fig. 15

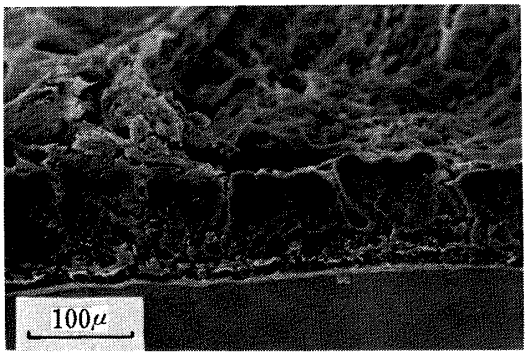

Fig. 16

Discussion

The facilitating effects of various drugs and pharmaceutical adjuvants on the transport of a wide variety of passively absorbed compounds from solutions are now well-documented phenomena. Suggestions regarding the mechanism by which these absorption enhancing effects include depletion of calcium from the intestinal epithelium, interactions of membrane lipids and other components. Most studies, however, have concentrated on the change in the apparent permeability or the transfer and so far few studies of the intestinal absorption trying to correlate such absorption characteristics with the morphological or histological changes of the intestine has been reported.

In this study we have utilized light microscopy and scanning electron microscopy to examine any change in mucosal structure caused by drugs and commonly used pharmaceutical adjuvants. Remarkable absorption enhancing effect on the poorly absorbable compounds by the three of thr agents used are common but histological changes were different each other.

In the case of EDTA, although marked separation of epithelial cells was observed, histological changes were absent as viewed with the light microscope. The role of calcium in maintaining the integrity of the intestinal membrane was affected by the chelating agent and the removal of calcium from disclosed sites in or on the intestinal mucosa caused such marked shedding of epithelial cells.

In the case of tetracycline, Ca^{2+} depletion in the membrane is not so remarkable since ternary interaction among tetracycline, calcium, and membrane lipoprotein outweighs the former. This interaction was observed in our previous experiment[9] in which tetracycline binding to the small intestinal epithelial cell fraction was studied. Tetracycline binding was higher at pH 8.0 than pH 6.0 and the binding was greatly accelerated by the presence of Ca^{2+}. Tetracycline deprived calcium of the membrane but interacts with the membrane proteins resulting in the dilation of intercellular spaces or the formation of vacuoles. Changes in the interacting properties such as the shift of pH or the decrease of the drug concentration in the binding sites readily reversed the effect of tetracycline. Permeability change caused by the interaction of tetracycline with membrane seems to be reversible and gradually diminishes as the drug is absorbed to a deeper compartment or the pH shifts to the level where little interaction is taken place.

In the case of sodium laurylsulfate, pronounced changes in the gross appearance of the mucosal surface is invariably accompanied by the changes in permeability. Morphological changes were associated conceivably with the solubilization of the lipid components of the membrane such as lipoproteins.

If the lesions caused by the drugs and pharmaceutical adjuvants are severe, however, permeability of compounds which, under normal physiological conditions, penetrate the intestinal membrane very slowly or not to an appreciable quantities, would be influenced. Permeating molecules have first to transude across the capillary wall, which, in the small intestine, comprises a continuous epithelium perforated by intracellular fenestra. In some conditions where mucosal anatomy and physiology are distorted absorption as well as exudation may be increased. Possible physiological as well as pathologica implications should be considered.

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