[Chem. Pharm. Bull. 23(2) 400—408 (1975)]

UDC 615.31.033.076.9

Effect of Fasting and Antineoplastic Agents on the Intestinal Absorption of Drugs in the Rats

Tetsuo Komuro, Shikifumi Kitazawa[1a] and Hitoshi Sezaki[1b]

Hospital Pharmacy of Kyoto University[1a] and Faculty of Pharmaceutical Sciences, Kyoto University[1b]

(Received August 7, 1974)

Relation between diminution of intestinal tissues brought about by fasting and/or by treatment with antineoplastic agents and intestinal absorption of drugs was investigated.

Both in fasted and antineoplastic agent pretreated rats, intestinal absorption was markedly decreased in a similar manner. In the latter case, a remarkable loss in the weight of small intestine with a little decrease in the body weight was noted. These phenomena are closely resembled the results observed in fasted rats which showed a severe atrophy in intestine rather than in body. In all cases, there exists a close relation between drug absorption and intestinal weight. The loss of the latter seemed to be partly due to a decrease in mucosal tissues, thus resulting in the reduction of surface area.

From these results, it is concluded that a reduction in the absorptive surface area of intestine seems to be one of the predominant factors which induced the apparent fall in intestinal drug absorption observed in fasted and antineoplastic agent pretreated rats under the experimental conditions.

It is well known that the epithelium of the small intestine has one of the most rapid renewal rates in body tissues. Many lines of evidences were revealed by the investigations of Leblond and Messier[2] that renewal of the epithelial lining starts with proliferations of columnar and goblet cells in the crypts and that this is followed by migrations of newly formed cells upward on the sides of the villi. As the result of releasing the old epithelial cells into the intestinal lumen at the tips of villi, the migration continues from the crypts to the tops of villi all the time. Hooper[3] reported that total turnover time of villus of normally fed rat's intestine is approximately 1.6 days in duodenum and 1.4 days in jejunum and ileum, and that these turnover rates in the intestinal epithelium are extremely rapid as compared with that of the epidermis.

In such a way the renewal of the surface of the small intestine which has an important function of absorbing many nutritional substances proceeds. Therefore, the proliferation of cells in crypts plays an important role in keeping conditions of the body normal. When the process of cellular proliferation is inhibited by some causes, it can be anticipated that the total surface area of intestinal epithelium should be reduced due to the reduction of total number of epithelial cells and villous heights. These phenomena will occur when the animal is fasted for a long time,[4] irradiated by X-ray so many times[5] and administered some antineoplastic agents.[6]

As for fasting, because of the decrease in supplying nutritions to the crypts, the rate of cellular proliferations should be decreased and this affects the balance between cellular proliferation and extrusion and thus resulted in a reduction in number of epithelial cells. It

1) Location: *a*) *Shogoin, Sakyo-ku, Kyoto*; *b*) *Yoshida, Sakyo-ku, Kyoto.*
2) C.P. Leblond and B. Messier, *Anat. Rec.*, **132**, 247 (1958).
3) C.E. Hooper, *J. Histochem. Cytochem.*, **4**, 531 (1956).
4) H.O. Brown, M.L. Levine and M. Lipkin, *Am. J. Physiol.*, **205**, 868 (1963).
5) M.J. Mattila, L.R. Holsti, V.M.K. Venho and S. Takki, *Arzneim. Forsch.*, **20**, 533 (1970).
6) R. Inaba, *Okayama Igakukai Zasshi*, **81**, 430 (1969).

would also bring about reduction of epithelial surface area which eventually leads to the reduction of the absorption of substances.

The decrease in intestinal cell populations is also recognized in the rat intestine after whole body irradiation by X-ray which produces almost complete cessation of mitosis in intestinal crypts without altering the rate of extrusion.

On the other hand, it is demonstrated that oral administration of antineoplastic agents produces the decrease in the overall epithelial cell population as a result of inhibiting not only the proliferation of tumor cells but also that of normal cells.

The present work was undertaken to clarify the relation between the reduction of the epithelial surface of the intestine caused by fasting or by treatment with antineoplastic agents and the intestinal absorption of drugs.

Experimental

Materials——Metoclopramide (4-amino-5-chloro-N-[2-(diethylamino)-ethyl]-o-anisamide) and Hexacol® (2-allyloxy-4-chloro-N-(2-diethylaminoethyl) benzamide hydrochloride) were kindly supplied by Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan. Mitomycin C (Mitomycin S®, Sankyo Co. Ltd., Tokyo, Japan) and N,N′,N″-triethylen triphosphoramide which is abbreviated as thio-TEPA in this paper (Tespamin®, Sumitomo Chemicals, Osaka, Japan) were obtained commercially. All other drugs and reagents used in these experiments were of analytical grade.

Animals——Male albino rats of Wistar strain weighing 130 to 170 g were used in all experiments. The rats were housed in the stainless-steel cages placed in a room maintained at 20° to 25° and had free access to water and commercial labolatory rations for a period of three or more days in order to acclimatize to laboratory conditions. All cages had wide mesh-floors to prevent coprophagy.

In case of fasting, one group of rats was deprived of food but allowed free access to water and housed in an individual cage separately to prevent devouring one another during fasting and the other group of animals was provided both food and water freely as non-fasted controls. After a given period of time, each group was used for absorption or exsorption studies.

In case of pretreatment with antineoplastic agents, rats were given a single intraperitoneal injection of mitomycin C (3 mg/kg) or thio-TEPA (17 mg/kg). They were administered 24, 48 or 72 and 12, 24 or 36 hours before experiments, respectively. Untreated rats were used as controls. Both groups were provided food and water freely.

Absorption and Exsorption Procedures——The animals were anaesthetized with "Nembutal" (5% sodium pentobarbital solution) given intraperitoneally (35 mg/kg for fasted rats and 50 mg/kg for antineoplastic agent pretreated rats and intact controls). The entire length of the small intestine, from the proximal end of the duodenum to the distal end of the ileum, was used for the absorption or exsorption experiments. In all experiments, bile ducts were ligated in order to avoid any effect of bile on the intestinal absorption or exsorption of drugs. The abdomen was opened by a mid-line incision and a glass and silicon tubing cannulae were inserted through small slits at the duodenal and ileal ends respectively to be secured by ligation with suture. As a means of cleaning the gut, physiological saline was passed gently through it untill the effuluent solution became clear. The cannulae were then connected to the perfusion apparatus[7] (perfusion apparatus model CV-1, Tokyo Precision Machine Co. Ltd., Tokyo, Japan).

Intestinal absorption was conducted by the *in situ* recirculation methods of Schanker.[8] Forty milliliters of drug solution which had been kept at 37° were recirculated through the intestine for one hour at the rate of approximately 5 ml/min. Tonicity of drug solution was maintained by physiological saline. The initial concentration of drug in the test solution was set 1 mM in all cases except thiopental (0.2 mM). Absorption of drug was determined by the method of subtracting the amount remaining in the drug solution after recirculation for one hour from the initial amount in the perfusate.

Intestinal exsorption was conducted by the *in situ* single-pass perfusion technique. Physiological saline solution without any drug was perfused at the rate of approximately 5 ml/min through the small intestine. Sulfanilamide dissolved in 5% N,N-dimethylacetamide with the concentration of 9 mg/ml was injected for two minutes into the femoral vein with dose of 30 mg/kg. After injection, samples of the perfusate were collected every 9 minutes from the ileal outflow and the volume of effuluent solution was measured by volumetric glass cylinder. Exsorption was represented as the amount of a drug transfered across the epithelial membrane from blood into gut lumen per minute.

7) T. Koizumi, T. Arita and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).
8) L.S. Schanker, P.A. Shore, B.B. Brodie and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957).

In the experiments to study the effect of body temperature of animals on transepithelial movement of drugs, body temperature was measured by using the thermometer inserted directly into the rectal lumen. In order to elevate body temperature from 37° to 40° during the course of experiments, body was warmed with an electric lamp to maintain a given temperature and at the same time, perfusate which had been kept at 37° was warmed rapidly to about 40°. With these devices, it was possible to elevate body temperature of animal within five minutes.

Tissue Preparations——Sixty hour-fasted rat and non-fasted control were anaesthetized with Nembutal®. Portions of the proximal duodenum were immediately removed, cut open longitudinally, flattened gently on cardboard and fixed in 10% formalin solution. The tissue was embedded in paraffin wax and sectioned in the normal fashion and then about 5 μ sections were stained with haematoxylin and eosin.

Analytical Methods——Determinations of sulfanilamide and metoclopramide were carried out by a colorimetric method as follows. Aliquots of perfusion samples were acidified with 1N HCl solution, diazotized in the regular manner, coupled with 2-diethylaminoethyl-1-naphthylamine and, if necessary, extracted with isoamylalcohol and optical densities were determined by using Hitachi double beam spectrophotometer type 124. Absorption maxima in mμ were 550 for sulfanilamide and 538 for metoclopramide. For the assay of metoclopramide, the brown test tubes were adopted with a view to eliminate the influence of light. For tryptophan, 7 ml of 18.7N H_2SO_4 containing 30 mg of *p*-dimethylaminobenzaldehyde were added to a 2 ml aliquot of the drug solution. The mixture was left alone 4 hours without light under room temperature and then added 0.1 ml of 0.068% $NaNO_2$. After standing for one hour, the optical density of the resulting solution was determined at 600 mμ. The following compounds were analyzed by previously described method: Hexacol® (K. Kakemi, 1969[9]); thiopental (K. Kakemi, 1967[10]).

Results

Body Weight and Intestinal Wet Weight in the Fasted and Antineoplastic Agent Pretreated Rats

Changes in the weight of the small intestine and the whole body of fasted rats, antineoplastic agent pretreated rats and the corresponding controls are summarized graphically in Fig. 1.

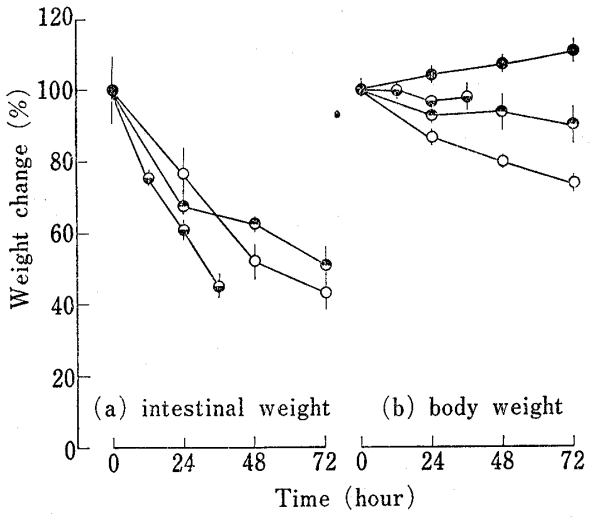

Fig. 1. Changes in the Intestinal Wet Weight and the Body Weight of Fasted and Antineoplastic Agent Pretreated Rats and Controls.

Abscissa is the duration of food deprivation for fasted rats and the length of pretreatment time elapsed prior to the experiment after injection for antineoplastic agent pretreated rats.
●: controls, ○: fasted rats, ◓: mitomycin C treated rats, ◒: thio-TEPA treated rats

During fasting, the small intestine lost weight at such a rapid rate that at the end of 72 hours of inanition more than 60% of the initial weight was lost. On the other hand, during the same periods of fasting, the total loss of the body weight was only 30% of the prefasting weight. This observation suggested that the influence of food deprivation on animals appeared more significantly in the reduction of the weight of the small intestine than in that of the whole body.

In case of rats pretreated with a single dose of mitomycin C, the extent of loss of the small intestine was about 50% at 72 hours after injection and that of the body weight was less than 10%. As for thio-TEPA, approximately 60% decrease occurred in the intestinal weight only 36 hours after an *i.p.* injection. In the body weight, a 3% decrease was at most noticed during the same period.

Comparing the above two phenomena,

9) K. Kakemi, T. Arita, R. Hori, R. Konishi, K. Nishimura, H. Matsui and T. Nishimura, *Chem. Pharm. Bull.* (Tokyo), **17**, 255 (1969).
10) K. Kakemi, T. Arita, R. Hori and R. Konishi, *Chem. Pharm. Bull.* (Tokyo), **15**, 1534 (1967).

fasting and treatment with antineoplastic agents, important differences were revealed as follows: (1) in the duration of fasting, the intestinal weight and also the body weight steadily decreased and the extent of weight loss was significantly larger in the gut than in the whole body, (2) antineoplastic agent produced a severe reduction in the intestinal weight with a little decrease in the body weight. Therefore, it was revealed that the effect of fasting is similar to that of antineoplastic agents in the points of the loss of intestinal tissues, that is to say, possible diminution of the mucosal surface area.

TABLE I. Effect of Fasting and Mitomycin C Pretreatment on the Intestinal Absorption

Drug	% absorbed		
	Controls	Fasted[a)]	Mitomycin C treated[b)]
Sulfanilamide	65.2±2.6	42.6±4.5	42.4±12.9
Metoclopramide	37.5±2.4	25.7±4.6	15.6±4.6
Hexacol®	27.3±1.6	20.6±2.2	13.7±1.9
Thiopental	73.8±3.9	62.8±5.1	56.6±5.8
Tryptophan	59.2±5.6	43.7±8.4	30.5±6.8

a) fasted for 60 hours
b) Absorption experiments were conducted 48 hours after a single *i.p.* injection of mitomycin C.

Effect of Fasting and Antineoplastic Agents on the Intestinal Absorption

Table I presents the effect of fasting and pretreatment with mitomycin C on the intestinal absorption of drugs. As is evident from Table I, intestinal absorption of all drugs was significantly decreased under the experimental conditions, though there was some difference in the extent of percentage reduction among drugs. Figure 2 shows that to what extent absorption of sulfanilamide was affected by the duration of fasting and the length of pretreatment time elapsed prior to the experiments after injection of antineoplastic agents. It is obvious from Fig. 2 that the prolonged fast produced more remarkable diminution in the intestinal absorption and that antineoplastic agents caused the progressive reduction of absorption in proportion to the length of pretreatment time. In case of thio-TEPA, its absorption inhibiting action is evidently stronger than that of mitomycin C.

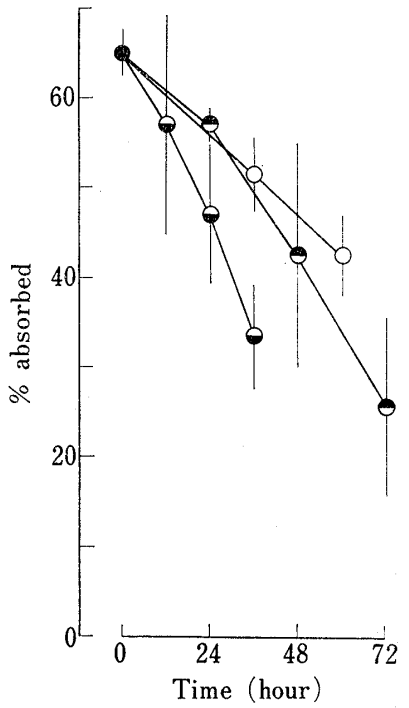

Fig. 2. Effect of Fasting, Mitomycin C or Thio-TEPA on the Intestinal Absorption of Sulfanilamide

Abscissa is the duration of food deprivation for the fasted rats and the length of pretreatment time elapsed prior to the experiments after injection for antineoplastic agent pretreated rats.
○: fasted rats, ◓: mitomycin C treated rats, ◒: thio-TEPA treated rats ●: control

Relation between the Weight of Intestinal Tissues and Absorption

As can be speculated that the loss of intestinal wet weight occurrs with the decrease of mucosal weight and thus causes the reduction of absorptive surface area of intestine, a close relation between the drug absorption and the intestinal wet weight seems to exist. Figure 3 shows that intestinal absorption of thiopental was reduced with the decrease of the weight of the intestines obtained from fed and fasted rats and that there was a close relation between the two (correlation coefficient:

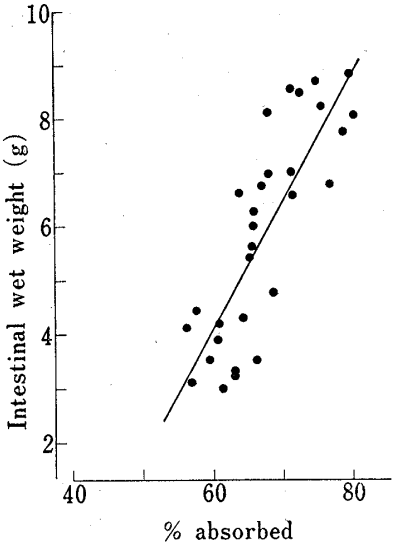

Fig. 3. Relationship between Intestinal Absorption of Thiopental and Intestinal Wet Weight Obtained from the Fasted and Fed Rats

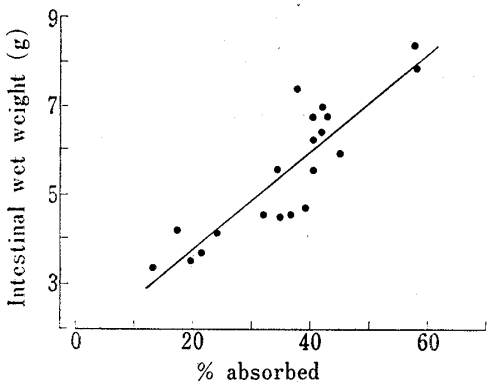

Fig. 4. Relationship between Intestinal Absorption of Sulfanilamide and Intestinal Wet Weight from the Mitomycin C Treated Rats and Controls

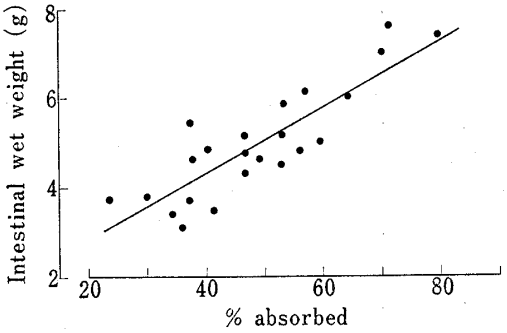

Fig. 5. Relationship between Intestinal Absorption of Sulfanilamide and Intestinal Wet Weight Obtained from the Thio-TEPA Treated Rats and Controls

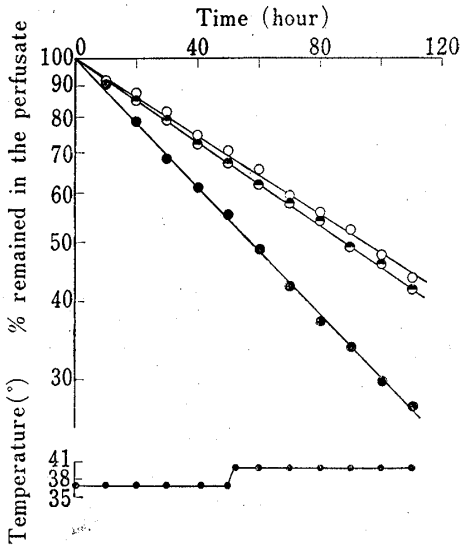

Fig. 6. Effect of Temperature on the Intestinal Absorption of Sulfanilamide

fasted rats: food deprivation for 60 hours, pretreated rats: Absorption experiments were conducted 48 hours after injection of mitomycin C.
●: control, ○: fasted rat,
◓: mitomycin C treated rat

r=0.83). On the other hand, Fig. 4 shows the relation between the sulfanilamide absorption and the weight of the intestines obtained from the mitomycin C pretreated rats (correlation coefficient: r=0.88). Figure 5 shows the result of the corresponding experiment with thio-TEPA. It is evident from Fig. 5 that the experiment follows a similar pattern to that with mitomycin C (correlation coefficient: r=0.86). These three figures demonstrate clearly that there is a significant correlation between the degree of intestinal atrophy, as indicated by the decreased weight of the intestine, and intestinal absorption of drugs.

Changes in the Intestinal Permeability

It is known that intestinal drug absorption is affected not only by changes in the intestinal structure but also by changes in the rate of mesenteric blood flow. It has been well

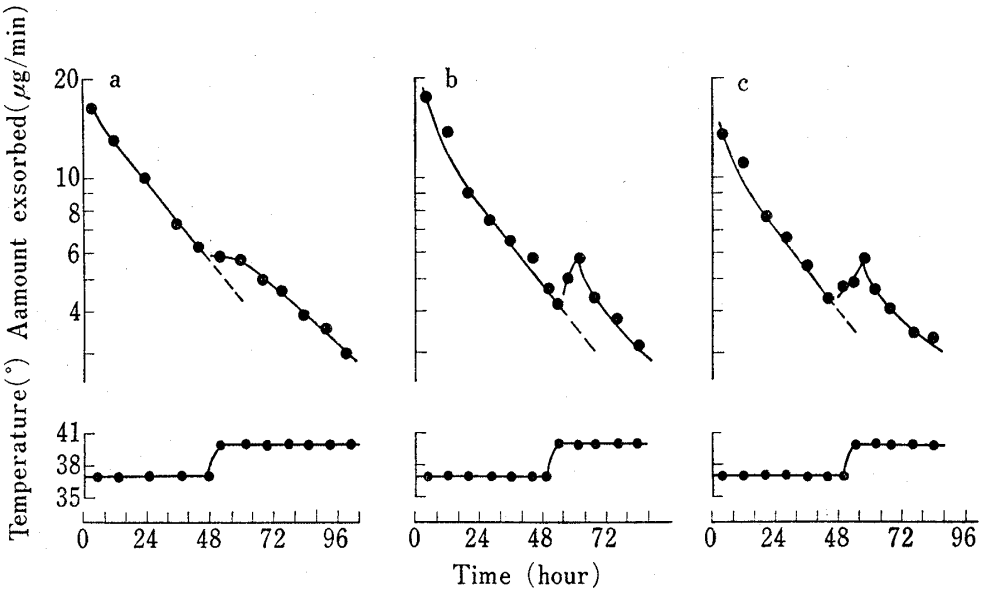

Fig. 7. Effect of Temperature on the Exsorption of Sulfanilamide

fasted rat: food deprivation for 60 hours
pretreated rat: Exsorption experiment was conducted 48 hours after injection of mitomycin C.
(a): control, (b): fasted rat, (c): mitomycin C treated rat

documented that body temperature is one of the indications of blood flow. Figure 6 shows the effect of body temperature on the drug absorption in the small intestine. Although the body temperature was increased from 37° to 40°, there brought about no difference in the absorption of sulfanilamide from lumen to blood in fasted and mitomycin C treated rats and also in the controls.

On the contrary, transepithelial movement of this drug from blood to lumen was clearly sensitive to the increased body temperature, as shown in Fig. 7. It appeared that the rate of exsorption of sulfanilamide in the fasted and mitomycin C treated rats was more rapidly and more highly increased than those in the controls.

From the facts that the modes of the permeability increase in the fasted and mitomycin C treated rats resembled each other, similar mechanism may exist between the two different treatments.

Discussion

Numerous physicochemical, pharmaceutical and physiological factors affect the gastrointestinal absorption of drugs. Changes in absorption, whether intended or unintended, are the sequences of alterations in one or more of these several determinants. During fasting, numerous physiological and biochemical events have been reported to occur, and some of which could possibly be involved in the changes of the drug absorption patterns. Our results indicate that extent of drug absorption in rats decreased for several drugs when the animals were fasted for periods beyond 24 hours. These results suggested that some non-specific phenomenon was occurring during prolonged fasting which affected absorption about equally for each drug.

It is well known that the development and growth of the body as a whole is affected by fasting and that the small intestine is more susceptible to the influence of prolonged fasting than the other organs. In one of the earliest works on the experimental fasting in animals, Sun[11] reported that 48 to 72 hours of fasting produced distortion on intestinal epithelium and

11) T.P. Sun, *Anat. Rec.*, 34, 341 (1927).

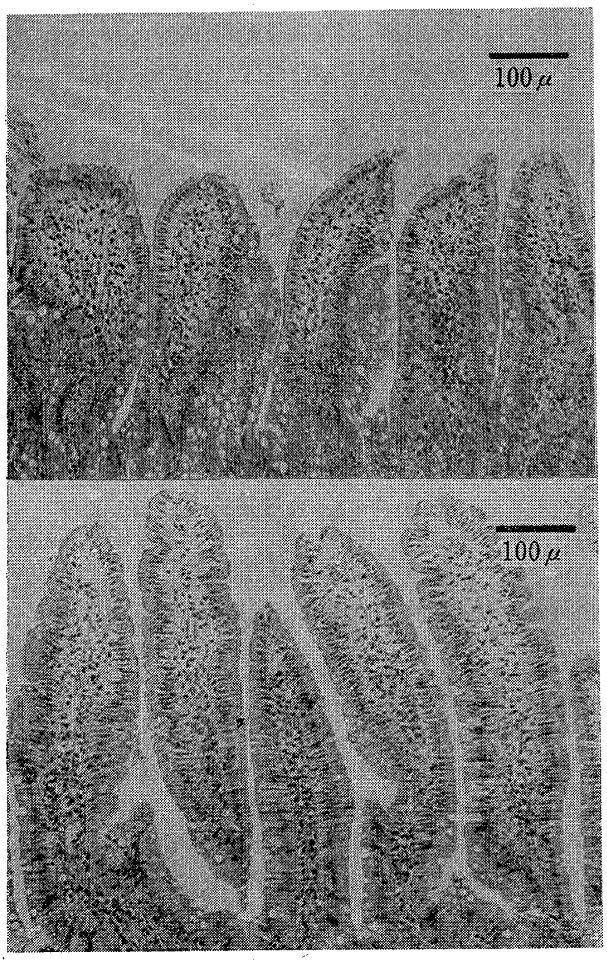

Fig. 8. Light Photomicrographs of Mucosa of the Fasted Rat and Non-fasted Control

Figure 8-A and 8-B are light photomicrographs of a 5μ section of the rat duodenum that has been stained with haematoxylin and eosin. Both photographs were taken at the same power (×33). Figure 8-A depicts the entire mucosa of the fasted rat and Fig. 8-B, that of the non-fasted control. Epithelial surface of each villus is in good condition both in fasted and non-fasted rats.

loss of villous structure in the mouse. Recently, Steiner, *et al.*[12] also reported that during fasting, the weight loss of the small intestine was unproportional to that of the body as was observed in the present experiments.

In considering the effect of a decrease in intestinal weight produced by fasting on its absorptive capacity, it is necessary to consider which part of the intestine contributes most to the weight loss.

Hooper and Blair[13] showed the lengthening of the synthetic and mitotic phases of the generation cycle, which in turn results in an increase in total cycle time and a decrease in the rate of cell production. Therefore, if the intestinal weight loss by fasting is due primarily to a reduction in mucosal tissues, it might be said that fasting produces the destruction of the tips of intestinal villi and shortening of heights of villi, thus resulting in the reduction of surface area which constitutes the first line of absorptive process. This was confirmed by the photomicrograph shown in Fig. 8. Although no gross histological change in the mucosa of both cases can be seen, each villous height of the fasted rat was by far diminished.

This view was further substantiated by the antineoplastic agent pretreatment experiments. Mitomycin C is a specific inhibitor of desoxyribonucleic acid (DNA) synthesis and acts rapidly and irreversibly. Consequently, when mitomycin C is administered to rats, it acts on the rapid DNA synthesizing sites. As the intestinal epithelium is one of the sites where DNA is most rapidly synthesized in the body, it would not be unreasonable to speculate that mitomycin C might inhibit cell renewal in the intestinal epithelial cells, thus resulting in the loss of cell population and the reduction of surface area. As shown in Fig. 2. similar reduction of the absorption was observed in the antineoplastic agent pretreated rats as in the fasted ones. Therefore, it can be suggested that mitomycin C might cause the decrease of the surface area of the small intestine.

As for the relation between fasting and antineoplastic agent treatment, Inaba[6] investigated the variations in the size of epithelial cell population of the intestinal mucosa of mouse under various conditions and reported as follows. (1) In mouse fasted for five days, the number of all epithelial cells isolated from the mucosa was gradually decreased, reaching to a minimum of about 1/6 of the normal levels. (2) In mouse given a single injection of mitomycin C in a

12) M. Steiner, H.R. Bourges, L.S. Freedman and S.J. Gray, *Am. J. Physiol.*, **215**, 75 (1968).
13) C.S. Hooper and M. Blair, *Exptl. Cell Res.*, **14**, 175 (1958).

dose of 5 mg/kg, the epithelial cell populations were rapidly diminished, reaching to a value of about 1/10 of the normal levels only two days after its injection. (3) Thio-TEPA reduced the epithelial cell population more remarkably than mitomycin C.

From these results, it would seem rational to assume that mitomycin C has the same apparent effects on the epithelial cells of intestine as fasting, although a multitude of physiological and biochemical changes occurred during fasting and antineoplastic agent treatment.

Thio-TEPA seems to have the same effects as mitomycin C and fasting with respect to the inhibition of the cell proliferation of intestinal epithelium. Although the results presented here may be interpreted, phenomenologically at least, in terms of the reduction of the effective surface area, there is other possibility that decreased rate of absorption was caused by the reduced intestinal blood flow.

With regard to the relation between fasting and intestinal drug absorption, Levin, *et al.*[14] assumed that decrease in absorption of passively absorbed substances in fasted rats would be due to non-specific factors such as a decrease in surface area or a reduction in blood flow to the intestine.

In this connection, Diamond, *et al.*[15] reported suggestively that prolonged inanition, particularly exceeding 20 to 25 hours, might inhibit the intestinal drug absorption process by virtue of an induced diminution in intestinal blood circulation.

In view of the absorptive capacity of the intestine, the blood flow in the mucosa and particularly in the villi is of vital importance since the blood constitutes the major transport vehicle for absorbed materials. In fasted rats, it is reasonable to speculate that the decreased rate of intestinal drug absorption was, in part, the consequence of reduced intestinal blood circulation. A little reduction in blood flow, however, will not probably produce any significant change in the transport process of passively absorbed materials, since the intestinal mucosa is seemed to be somewhat overperfused in relation to its nutritional demands.

This was demonstrated by the drug exsorption which is more sensitive to the mesenteric blood flow than absorption. As shown in Fig. 6, the increase in mesenteric blood flow caused by elevation of the body temperature did not produce any change in the rate of intestinal absorption of sulfanilamide both in fasted and fed animals. On the other hand, exsorption is more rapidly and more largely increased in fasted rat than in fed one in proportion to the increased body temperature, as shown in Fig. 7. These results may indicate that prolonged fasting could probably interfere with the process of intestinal clearance by means of an induced decrease in intestinal blood flow. However when the exsorption rate constants which reflect the permeability characteristics of drug from blood to intestinal lumen were measured in an individual fasted rat before and after the increase of body temperature from 37° to 40°, the

Table II. Exsorption Rate Constants of Sulfanilamide and Metoclopramide in the 60 hour-fasted or Fed Rats

		k_{ex}	$(k_{ex})_f/(k_{ex})_c$
Sulfanilamide	control	0.410±0.024	0.608
	fasted	0.236±0.041	
Metoclopramide	control	2.45±0.62	0.567
	fasted	1.49±0.30	

k_{ex}: exsorption rate constant described as (amount exsorbed into gut lumen per minute)÷(blood concentration); (ml)
$(k_{ex})_f$, $(k_{ex})_c$: exsorption rate constant in the fasted and fed rats, respectively

14) R.J. Levin, H. Newey and D.H. Smith, *J. Physiol.*, **177**, 58 (1965).
15) L. Diamond, J.T. Doluisio and W.G. Crouthamel, *Europ. J. Pharmacol.*, **11**, 109 (1970).

rate constant at 37° was 0.222 for sulfanilamide and 1.28 for metoclopramide, and at 40°, 0.316 and 1.92, respectively. As is evident from Table II, these rate constants at 40° fall about in between the two values obtained from fed and fasted rats at 37°. This means that the transmucosal movement of drug is significantly affected by the reduction of mucosal surface area, though the reduction of mesenteric blood flow may well be the contributable cause of the decrease of the intestinal absorption in fasted rats.

Although the data reported here do confirm that the intestinal drug absorption is decreased mainly by a reduction in the effective surface area, the *in vivo* actions, whether fasting or antineoplastic agent treatment, on the structure and the function of the cells are rather complex in nature. It seems likely that alterations of the retarded function of cells are first recognized, followed by tissue injury such as the loss of weight of the intestinal tissues. Thus the disturbance of cellular function affects secondary the drug absorption from intestine.