[Chem. Pharm. Bull. 25(2) 327—337 (1977)]

UDC 615.31.033.076.9 : 612.32-08

Effect of Fasting on Body Fluids and Intestinal Drug Absorption in Rats

Shikifumi Kitazawa and Tetsuo Komuro[1)]

Department of Pharmacy, Kyoto University Hospital[1)]

(Received July 28, 1976)

The present work was designed to make clear a mechanism of fasting-induced reduction in the *in situ* intestinal absorption of sulfanilamide. Physiological characteristics of fasting in rats were investigated with attention to homeostatic fluid transfer within body. An increase in arterial hematocrit was found to be in proportion to the duration of fasting, which indicates unphysiological conditions in internal fluid environment of body. The method was consisted of intensive monitorings of both the ratio in the transmucosal fluid movement and the arterial hematocrit of the subjected animals during the course of the intestinal perfusion experiments. There observed significant depressions in the transmucosal fluid movement and also in the fluid transfer across the capillary wall in 60-hour-fasted rats as compared with non-fasted controls. These results suggest that fasting induced a fall in both the rate and extent of water exchange between fluid compartments in body, and secondary resulted in the depression of intestinal sulfanilamide absorption.

Keywords——fasting; body fluids; intestinal drug absorption; rat; sulfanilamide; homeostatic fluid transfer; transmucosal fluid movement; hematocrit

It is well known that the gastrointestinal drug absorption is dependent on not only physicochemical properties of drug but also physiological conditions of an alimentary tract of experimental animal. Since Brodie and his co-workers presented the pH-partition hypothesis,[2)] too much attentions have been concentrated on the relation between physicochemical properties and permeability of drug through epithelial layer of the gastrointestinal tract and many lines of evidences have been accumulated. On the other hand, relatively little attentions have been focused on investigations of the physiological conditions of animal on drug absorption. However, the intestinal tract should have its own functions such as absorption of water and secretion of digestive fluids and keeping an electrolyte balance in the tract at the normal ratio to maintain in better conditions, which are recognized to contribute in keeping the whole body in healthy conditions. These functions are not always favourable for drug. Drug should pass through such physiological environments as transmucosal bidirectional fluid movement or pH in the tract in the process of absorption. These estimations easily introduced an idea that in a certain case physiological factors should play more determining effect than physicochemical characteristics of drug in the absorption.

Reflecting the idea, Fisher[3)] and other investigators[4)] have accumulated evidences suggesting a close relation in the absorption of fluid and organic molecules. Recently, one of the authors[5)] presented a method of graphical illustration of this relationship and clearly demonstrated that the transmucosal bidirectional osmotic fluid movement influences the drug absorption from rat small intestine in subtlety. However, it should be noticed that the transmucosal fluid movement are not always induced by osmotic changes of fluid in the tract but there should

1) Location: *Shogoin Kawahara-cho, Sakyo-ku, Kyoto, 606, Japan.*

2) L.S. Schanker, P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957); L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *ibid.*, **123**, 81 (1958); C.A.M. Hogben, J.D. Tocco, B.B. Brodie, and L.S. Schanker, *ibid.*, **124**, 275 (1958).

3) R.B. Fisher, *J. Physiol.*, **130**, 655 (1955).

4) *a*) H. Kunze and W. Vogt, *Naunyn-Schmiedeberg's Arch. Pharmak. exp. Path.*, **256**, 139 (1967); *b*) J.S. Fordtran, F.C. Rector, and N.W. Carter, *J. Clin. Invest.*, **47**, 884 (1968); *c*) H. Ochsenfahrt and D. Winne, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **281**, 175 (1974); *d*) *Idem, ibid.*, **281**, 197 (1974).

5) S. Kitazawa, H. Ito, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 1856 (1975).

be such a movement induced as a result of physiological functions or conditions as mentioned before, and in such a case volume of fluid moved should be dependent on the amount of the fluid contained in the whole body of the animal.

In animal experiments, a day or an overnight deprivation of foodstuffs, that is fasting, is often imposed to the animal prior to the experiments without careful considerations only to obtain reproducible results and, moreover, fasting is often employed in clinical medicine by physicians to patients as one of the therapeutic devices. However, it is also to be noticed that fasting may cause a decrease in water intake.

As a matter of fact, the result obtained in this study showed that water intake of the animal apparently decreased during the course of the fasting. Taking the result into account, the amount of water contained in the fasted animal might decrease compared to the non-fasted control animal, and the transmucosal fluid inflow in the direction of lumen to blood might increase in the fasted animal to compensate the lack of water content in the animal. The increase in fluid absorption in the gastrointestinal tract may accompany with an increase in drug absorption. Contrary to our expectations, in the course of drug absorption studies in the fasted rats, it was found that over 24-hour-fasting produced a depression in drug absorption and the transmucosal fluid inflow was observed depressed as compared to the results obtained from non-fasted rats.

These findings in our laboratories encouraged us in advance to more detailed study to elucidate a homeostatic fluid transfer in the body of fasted animal.

Experimental

Drug and Chemicals——Sulfanilamide and other chemicals used in the present experiment were analytical grade and were obtained from a commercial source (Nakarai Chem. Co. Ltd., Kyoto, Japan) and used without further purifications.

Animal——Male albino rats of Wistar strain were used in all the experiments. The weight of the animals was about 160 g when they were purchased. The rats were housed in stainless-steel cages in an animal room[6] maintained at $23\pm2°$ and free access to tap water and a commercial solid food for laboratory rat (Oriental Yeast Industry Co., Ltd., Tokyo, Japan) for periods of at least three days before the experiments in order to acclimatize to laboratory conditions. All cages had wide mesh floors to prevent coprophagy.

Rats of about 170 g in body weight were randomly separated into two groups, one group was deprived of food for a given period but allowed free access to tap water and housed in an individual cage to prevent devouring one another during the fasting period, and the other group of animals, also housed in an individual cage, provided both food and tap water freely. The former group was nominated the fasted group and the latter was called the control group, each group being composed of more than 4 rats. To place all the fasted group at an uniform condition, fasting was started usually in the evening of about 8: 00 p.m. The fasting was continued for up to 120 hours and during the periods of this treatment daily variations in body weight, water intake, hematocrit and sodium output were measured with both of the fasted and the control groups. Unless otherwise stated, 60-hour-fasted rats were used as "the fasted rats" in the present work, since daily water intake and urinary sodium output appeared to become in a steady state after 60-hour-fasting. Mean weight of rats was about 170 g in the control group and the fasted group was about 130 g.

Measurement of Water Intake——A tap water-containing vessel furnished to each cage was weighed using a balance. After 24 hours an amount of water consumed by a rat was obtained by subtracting the residual weight from that of the initial. In the present study, water intake per day was represented as a total amount (g) of water consumed in 24 hours through the day and night.

Measurement of Hematocrit——Blood samples obtained in a capillary tubing from tail vein under non-anaesthetization were employed to measure hematocrit. Detailed procedures will be mentioned below.

Measurement of Sodium Output——Every 24-hour-urine samples were collected before and during periods of fasting and urinary sodium analysis was made by flame photometric method with Hitachi Flame Photometer Model 205. No detectable sodium was found in the drinking tap water supplied to animals.

Intestinal Absorption——According to the recirculating perfusion method which was devised by Schanker, *et al.*,[2] an amount of the drug disappeared from the perfusate was regarded as the amount absorbed from the perfused intestine. In all of the present experiments, the entire length of small intestine from the proximal end of the duodenum to the distal end of the ileum was used for the perfusion.

6) S. Kitazawa and T. Komuro, *Igaku no Ayumi*, **100**, 521 (1977).

The animal was anaesthetized with intraperitoneal administration of sodium pentobarbital, 35 to 50 mg/kg of body weight, and placed on an operation board spinely. The abdomen was opened by a mid-line incision to expose both ends of the small intestine. Small incisions were made in the antimesenteric borders at each end and glass tubings connected to sillicon tubings were inserted through the openings and tied in place, care being taken not to interfere any blood flow. Bile ducts were ligated to avoid any flow of fluid into the intestinal lumen during the absorption experiments. The small intestine was first washed gently by a single perfusion of 0.9% saline solution which was maintained at 37° until the effluent became clear. The volume of the washings was suppressed to minimum not to change both the physiological conditions of the intestine and water content in the experimental animal. The cannulae were then connected to a perfusion pump (Perfusion Apparatus, Tokyo-Seiki Co. Ltd., Tokyo, Japan).

The perfusate was recirculated from duodenum to ileum at a rate of 5 ml/min with 40 ml of the perfusion solution reservoired in a graduated cylinder of 50 ml volume which was kept in a water bath at 37°. Unless otherwise stated, the perfusion solutions employed in the experiments contained one millimole of sulfanilamide and an adequate concentration of sodium chloride. Osmotic pressures of the perfusion solution were adjusted by increasing or decreasing the concentration of sodium chloride to obtain hypertonic, isotonic and hypotonic solutions, which were ascertained with osmometer (Freezing Point Osmometer Model 3D, Advanced Instruments INC, U.S.A.).

Absorption of sulfanilamide from intestine was determined by subtracting the amount remaining in the perfusate after the one hour recirculation from the initial amount of that in the perfusion solution. Analytical method of sulfanilamide in the perfusate was followed by the method described previously by the authors.[7] Transmucosal fluid movement was estimated by measuring the volume change of the perfusate during the perfusion study and a ratio of the transmucosal fluid movement was obtained following the method of Kitazawa[5] by using the initial volume of the perfusate and that of the final, which were measured by the graduated cylinder.

Similar absorption experiments were also conducted under such a condition of ligature of renal arteries and veins of bilateral kidneys with sutures to prevent urinary loss of the fluid in the animal body. Details of the method were mentioned below.

To avoid the fall of body temperature during the perfusion experiments, an electric lamp was placed over the animal and the rectal temperature was kept at 37° by adjusting the distance between the lamp and the animal.

Hematocrit——This term was abbreviated as "Ht" in this report. A polyethylene catheter of a 0.5 mm outside diameter (ATOM Polyethylene Tube for Infusion, Atom Co. Ltd., Tokyo, Japan) was inserted into a femoral artery and about 50 μl of blood was obtained in a glass capillary through the catheter. Blood sampling was made every five minutes during the course of the absorption experiments. To prevent blood coagulation through such a long period of the sampling procedures, a small amount of sodium heparin solution (40 unit/0.04 ml/100 g of body weight) was injected intravenously to all animals before the absorption experiments. One end of the capillary was sealed with clay and the other end was closed by fusion and then the capillary was centrifuged for five minutes at 12000 rpm with a centrifuge for Ht measurement (Hemato KH 120, Kubota Manufacturing Industry Co. Ltd., Osaka, Japan). Plasma and erythrocytes were separated each other and Ht, or percentage of volume of the packed erythrocytes to that of whole blood was measured.

Blocking of Renal Function——Renal arteries and veins of bilateral kidneys were tightly ligated with sutures in order to block a blood supply to kidneys where a main loss of fluid from the body is functioning. This treatment was conducted just before starting the absorption experiment. During the period of the experiment, that is, at least for one hour any change in cardiac pace and congestion of blood flow was not observed.

Results

Fasting and Physiological Changes in Rats

The fasted rats lost about 33% of their initial weights after the 96-hour-fasting and external appearances began to change on the second day of the deprivation to be lean with a coarse coat of fur. This indicates that the body weight decreased at an average rate of 14 g per day under the condition employed in this study. The effect of fasting on water intake, Ht and urinary sodium excretion were measured and the results are summarized in Fig. 1. There observed a rapid fall of water intake on the first day of the fasting, and thereafter the amount of water consumed became rather constant of about 12 g per day. On the other hand, the urinary sodium excretion decreased with duration of the fasting and seemed to be plateau after the period of the fasting reached up to 60 hours. Although an accurate volume of urine

7) T. Komuro, S. Kitazawa, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 400 (1975).

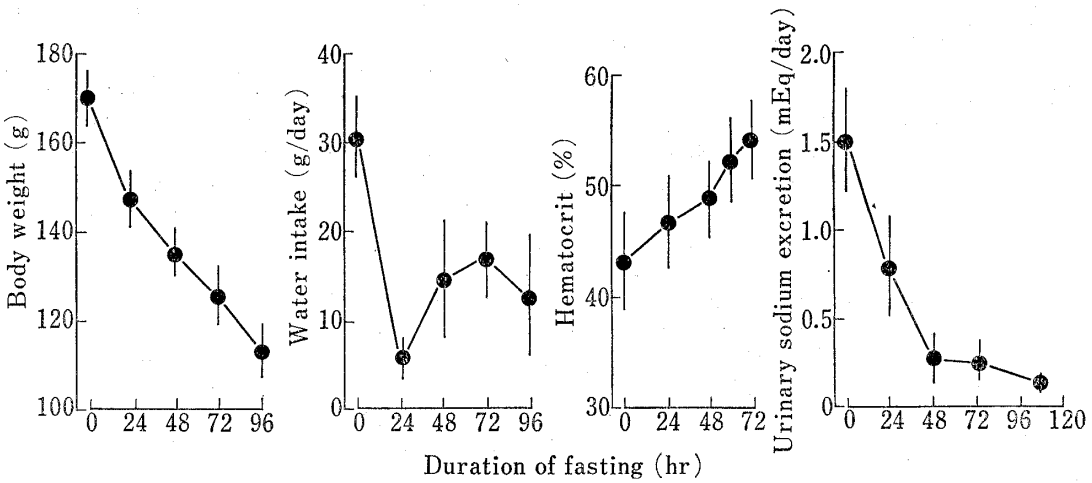

Fig. 1. Changes in the Physiological Parameters of Rats during Fasting

Points and vertical bars are means and standard deviations of values in 6 rats

excreted during the fasting was not able to be measured, as the results of observations the daily volume of urine of the fasted animal was apparently more decreased than that of the non-fasted rats. These evidences might allow to presume that the content of water stored in the fasted rat was more reduced than that of the non-fasted one. Another evidence supporting the presumption was found in the arterial Ht of the animal. The Ht increased consistently with the duration of the fasting. However, the value was not reached to a plateau as observed both in water intake and sodium excretion as shown in Fig. 1.

Intestinal Absorption of Sulfanilamide and Transmucosal Fluid Movement

Relations of intestinal absorption of sulfanilamide to transmucosal fluid movement were investigated following the method developed in our laboratory. The results are illustrated in Fig. 2. Based on the fact that apparently a straight regression line between per cent absorption of the drug and the ratio of fluid movement might be derived from these results obtained with perfusion solution having different tonicities, the mode of the intestinal drug absorption in the fasted rats was found to be essentially similar to that of the control animals.

However, the regression line of the fasted rats was apparently shifted to the left hand side of that of the control, and the intestinal absorption of the drug in the fasted rats could be concluded to be decreased as had been observed by other investigators. However, more detailed comparisons of these results revealed that the shift was not so simple. As indicated by arrows in Fig. 2, all of these shifts were clearly directed to 1.0 in the ratio of fluid movement where the inflow and the outflow across the epithelium are apparently not observed during the course of perfusion experiment. This finding suggested that transmucosal fluid movements of both inflow and outflow were restricted in the fasted animal.

Taking the results mentioned above into account, that is, because of a decrease of water content in the fasted animals, it seemed to be reasonable that in a condition of intestinal perfusion with hypertonic solution, the volume of fluid to be outflowed was more reduced in the fasted rats as compared with the control. Contrary to our expectations in the fasted rats, transmucosal fluid inflow was not increased in the case of intestinal perfusion with hypotonic solutions, while more inflow was observed in the control animals. Such a peculiar and unexplainable phenomenon occurred in both of the perfusions with isotonic and hypotonic solutions. Two possibilities were considered to explain these findings. The first was an inhibitory function of epithelial layers of the small intestine induced by the drug which existed in all the perfusion solutions. To make sure the possibility, solutions contained only sodium chloride in different concentrations were perfused. The results are depicted in Fig. 3. As is evident from Fig. 3, the slope of the regression line obtained from the fasted animals was lesser, which in-

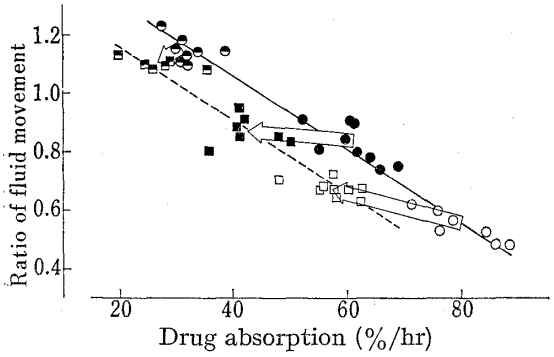

Fig. 2. Intestinal Absorption of Sulfanilamide and Transmucosal Fluid Movement in the 60-Hour-fasted and Control Rats

concentration of saline solution (%)

	1.35	0.9	0.45
control rats	◒	●	○
fasted rats	◨	■	□

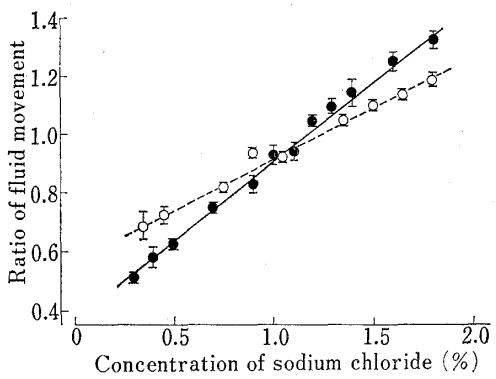

Fig. 3. Net Fluid Movement through Small Intestine in the 60-Hour-fasted and Control Rats

Points and vertical bars are means and standard deviations of values in 4 rats.
●: control rats, ○: fasted rats

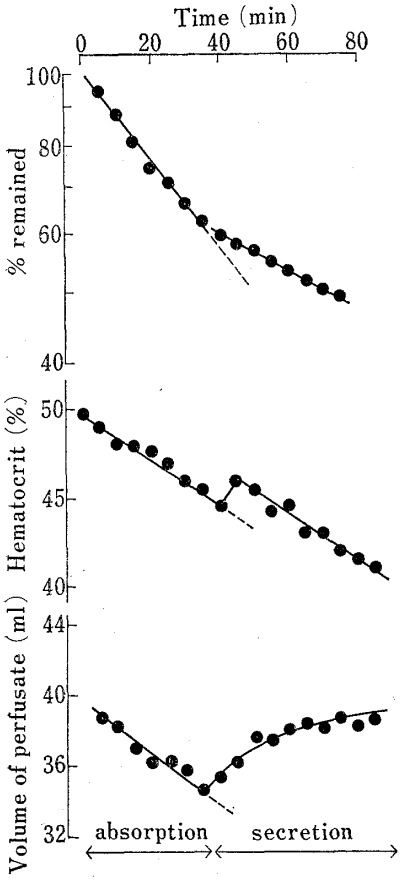

Fig. 4. Effect of Perfusate's Tonicity on Intestinal Absorption of Sulfanilamide, Femoral Arterial Hematocrit and Perfusate Volume in the Control Rat

A single addition of 1.5 ml of 15% saline solution into the perfusate was made 35 minutes after the beginning of the perfusion experiment.

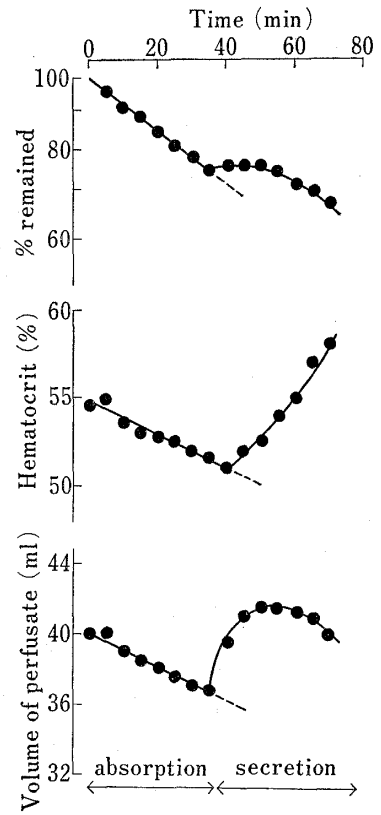

Fig. 5. Effect of Perfusate's Tonicity on Intestinal Absorption of Sulfanilamide, Femoral Arterial Hematocrit and Perfusate Volume in the 60-Hour-fasted Rat

A single addition of 1.5 ml of 15% saline solution into the perfusate was made 35 minutes after the beginning of the perfusion experiment.

dicates that both of the inflow and the outflow of the fluid were less than that of the control. This evidence clearly demonstrated that the drug did not affect apparently the transmucosal bidirectional fluid movement which was caused by the osmotic difference even in such a condition of fasting.

The second possibility was the fasting-induced inhibitory function of the epithelial layers to fluid transpass. To clarify the possibility, the perfusion experiments were undertaken with isotonic solution and the results are illustrated partly in Fig. 4 and Fig. 5. The fluid inflow was observed both in the fasted and the control rats and the Ht value decreased with decreasing the volume of perfusate, and the drug remained in the perfusate decreased. A parallel relationship was observed between these three variables. However, suggestive phenomena were found when the isotonic perfusion was switched to hypertonic solution, where a single addition of 1.5 ml of 15% saline solution into the perfusate was made 35 minutes after the beginning of the perfusion experiment. As illustrated in the rest of Fig. 4 and Fig. 5, the volume of perfusate increased in both the fasted and the control animals and reflecting these reductions in the transmucosal fluid movement, the rates of the drug absorption were observed diminished correspondingly. In the time-course-observations of Ht, however, different behaviours were elucidated between the fasted and the control. In the former case the Ht value increased consistently during the hypertonic perfusion, but in the latter case the Ht value decreased with a scarce jump at the time of the switching, while the fluid moved toward the intestinal tract. These exactly opposite phenomena seemed to be easily explained with differences in water content observed in both the fasted and the control.

The control animals had enough water in fluid compartments of body and the fluid would be transfer into the circulation to compensate the reduction of fluid moved from the circulation system toward the intestinal lumen by the hypertonic perfusion. In the fasted animals, such a fluid transfer for the compensation

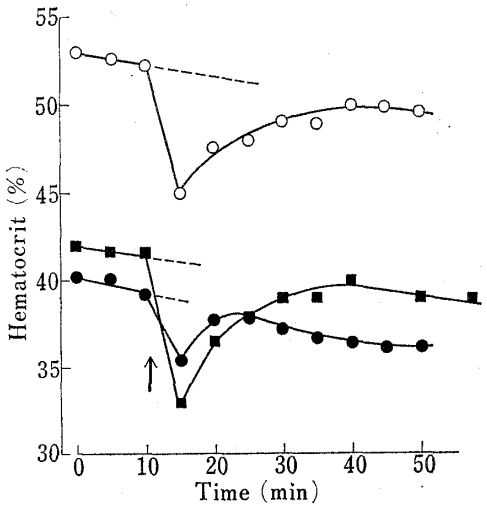

Fig. 6. Effect of Saline Injection on Hematocrit in the 60-Hour-fasted and Control Rats

Arrow indicates the injection of 0.9% saline solution. Intestinal absorption experiment was not conducted in these cases.

injection volume of saline
○: fasted rat: 3 ml (2.2 ml/100 g body weight)
●: control rat: 3 ml (1.8 ml/100 g body weight)
■: control rat: 6 ml (3.6 ml/100 g body weight)

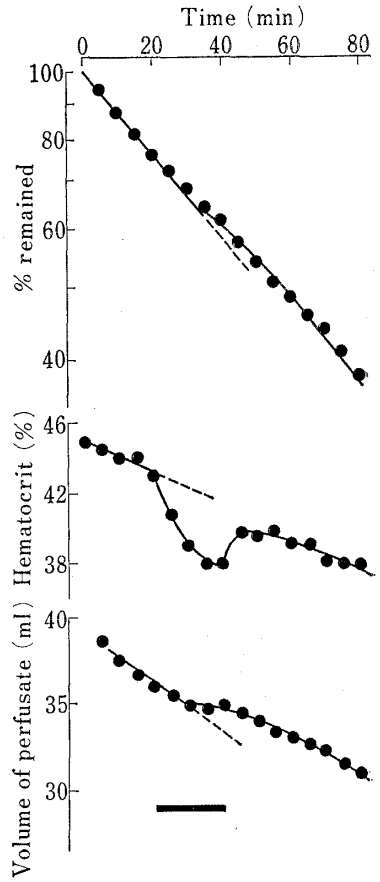

Fig. 7. Effect of Intravenous Saline Infusion on Intestinal Absorption of Sulfanilamide, Femoral Arterial Hematocrit and Perfusate Volume in the Control Rat

Bar indicates a period of the infusion.

might not occur because of a diminished water content due to the fasting. These explanations were reasonable and appeared not to be irrelevant. However, considering the results that the increase in Ht in the fasted was so fast, it could be possible that homeostatic fluid transfer from fluid compartments into the circulation might be scarcely brought about during all the period of the perfusion experiments.

The other possibilities should be taken into account. There should be a barrier from fluid compartments to the circulation and a function of the barrier should be diminished in the fasting state, and as the result of this low function, the fluid transfer into the circulation might be considerably inhibited. To elucidate these relations, the following experiments were undertaken.

Intravenous Infusion of Saline in the Rats

First, responses to a single intravenous administration of isotonic saline solution in the fasted and the control rats were investigated without conducting any intestinal perfusion, as shown in Fig. 6. Disappearance profiles of the injection solution from the circulatory blood are represented as a periodical changes in arterial Ht. Fluid infusion brought about a fall in Ht in the both states of rats. In the control rat, the reduction in the Ht value per 3 ml of the injection was about 3.7%, but the fasted animal displayed about two fold reduction of about 7.3%. After a temporal reduction of the Ht value, it increased progressively and finally to a predicted line in the both cases, however, there was a very interesting difference between the two cases in a point of a recovery of Ht required for reaching a predicted level which was expected as a broken line. In the non-fasted state, the recovery time was about 15 minutes, whereas in the state of fasting, it took about 30 minutes.

When the volume injected was calculated per 100 g of body weight, the control rat received less

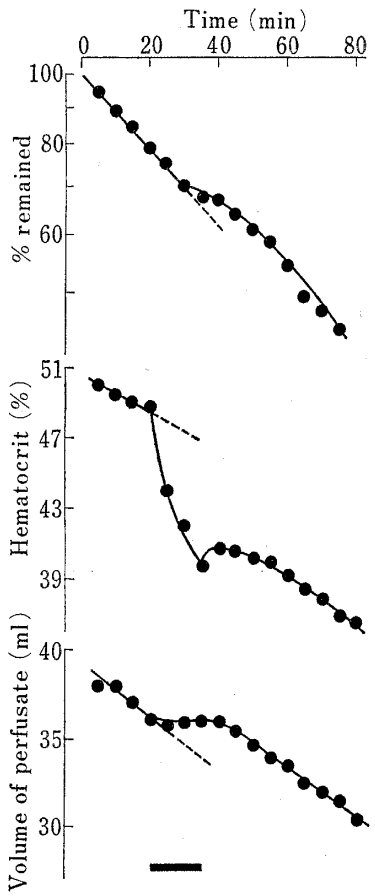

Fig. 8. Effect of Intravenous Saline Infusion on Intestinal Absorption of Sulfanilamide, Femoral Arterial Hematocrit and Perfusate Volume in the 60-Hour-fasted Rat

Bar indicates a period of the infusion.

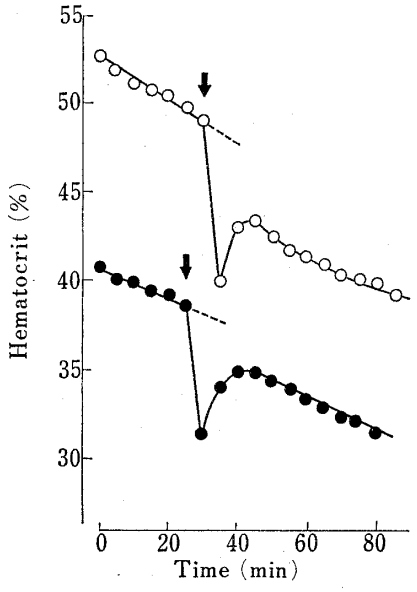

Fig. 9. Effect of Intravenous Saline Injection on the Femoral Arterial Hematocrit in Rats with Ligatures of Renal Arteries and Veins of Birateral Kidneys

●: control rat, ○: 60-hour-fasted rat
Arrows indicate the infusion of 0.9% saline solution of 2.8 ml/100 g body weight.

volume than the fasted one. Another Ht profile shown in Fig. 6 is a case of 6 ml-injection, or 3.6 ml/100 g of body weight, of isotonic saline to the control rat, where the injection volume expressed by 'per 100 g of body weight' is about 1.6 times that of the fasted one, 2.2 ml/100 g of body weight. Interestingly, the recovery time was about 30 minutes. These suggested a retardation of fluid disappearance from the circulation in the fasted rat as compared with the control animal.

Next, under a condition of intestinal perfusion experiment with isotonic drug solution, a certain amount of saline was infused in the femoral vein at a rate of 0.25 ml/min (total volume injected: 2.8 ml/100 g of body weight). Changes in a volume of perfusate, Ht and drug absorption obtained from the control and the fasted rats are illustrated in Fig. 7 and Fig. 8, respectively. As is evident from Fig. 7 and Fig. 8, the volume of perfusate was increased and retardations in the absorption rate of the drug were observed in both of the rats. These evidences suggested that the infused saline solution moved transcapillary in the direction from the circulation to lumen. Of course, Ht decreased consistently during the infusion in both cases. However, differences were observed in the time course changes after the infusion has ceased. Hematocrit in the control recovered immediately to a predicted value within ten minutes, on the other hand, in the fasted the recovery was not noticed within the period of the experiment. These differences in a capability of the recovery suggested that there should be some difficulties in the water transfer from the circulation into fluid compartments in the fasted animal. Some comments might occur that the circulation volume expansion induced by the saline infusion in the fasted rat was due to a lack in functions of urinary excretion of water in the circulation which was caused by the fasting. Our attentions were focused to pursuit of the Ht changes under a condition of blocking the renal functions, which was carried out by ligations of renal vessels in the bilateral kidneys. As demonstrated in Fig. 9, it was evident that Ht value in the fasted was not observed to recover to the predicted value, while in the control the Ht recovered within 20 minutes. All of these evidences demonstrated, more or less, that the reduction of water flow from the circulation to fluid compartments in peripheral tissues was brought about by the fasting.

Discussion

Several reports in literatures cited that fasting might produce numerous physiological and biochemical changes which affect functions of animal. Gastrointestinal drug absorption is not an exception. Levine, *et al.* reported in 1965[8] that 3-day-fasting produced a decrease in intestinal absorption of passively transported substances in rats and suggested that the reduced absorption would be due to non-specific factors such as reduction in the surface area of the intestinal tract and in the mesenteric blood flow. Diamond, *et al.* also demonstrated in 1970[9] that prolonged starvation might inhibit the absorption by virtue of an induced diminution in the rate of intestinal blood flow.

The previous report by the authors[10] presented the evidences that although apparent reduction was observed in the intestinal sulfanilamide absorption, blood concentration of the drug was apparently higher in the fasted animals than that of the controls. The findings suggested that the volume of fluid, at least the fluid in which the drug might distribute, would be reduced in the fasted animals, or that some defects might occur in the mechanism of a fluid transfer, the term of which indicates homeostatic movement of fluid between fluid compartments in the body. Based on these estimations, our attentions had concentrated to elucidate the mechanism by which the intestinal drug absorption had been reduced.

8) R.J. Levine, H. Newey, and D.H. Smyth, *J. Physiol.*, **177**, 58 (1965).
9) L. Diamond, J.T. Doluisio, and W.G. Crouthamel, *Europ. J. Pharmacol.*, **11**, 109 (1970).
10) T. Komuro, S. Kitazawa, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 909 (1975).

Before starting our experiments, informations concerning physiological states of the fasted animals such as body weight, water intake, Ht and urinary sodium excretion should be provided to determine an appropriate period of the fasting. As illustrated in Fig. 1, our results showed a significant fall in a daily water intake during food deprivation. Since Adolph reported in 1947[11] that the rat reduced water intake when food was restricted, a number of studies[12] had demonstrated the correlation between food and water intake and those findings were in fair agreement with our results obtained. Moreover, all of these results and previously reported results[10] indicated consistently that the volume of fluid in the fasted rats decreased with increasing the period of the fasting. Judging from the data obtained in water intake and urinary sodium excretion, which were thought to present a balance of intake and output of water in the animal, volume of fluid in the fasted rats seemed to become a steady state after the fasting reached to 60 hours. The animals still responded normally to stimulations so far as the period of fasting, but became somewhat insensible after 72-hour-fasting. There observed a severe reduction in body weight and a markedly slow behaviour in motion. Blood vessels in most parts of body were observed to become dark reddish progressively after such a long period of fasting in rats. Considering these observations, 60 hours would be appropriate to set the period of fasting.

In the present work, sulfanilamide absorption and transmucosal fluid movement were demonstrated to be reduced in the fasted rats as compared to the control, as shown in Fig. 1 and also in Fig. 2. There may be some doubts that difference itself of body weights between the both rats might affect the drug and fluid absorption from intestine. However, careful examinations reported previously[10] revealed that the percentage absorption and also fluid movement in the fasted rats were even more reduced than the expected value from the body weight loss. On the other hand, in clinical medicine, a dosage might be often made with attention to a patient's body weight at a beginning of clinical therapeutics and without any attention to a change of his body weight during the course of therapeutics. Under these background, the present experiment was made to get further insight into the inhibition of absorption brought about by fasting, with attention to body fluid transfer.

There should be many valuable methods in experiments to study the change in water balance in an animal body which was brought about by unphysiological conditions or diseases. Pursueing the Ht change might be one of the methods, in fact, Ht measurement has been made in investigations of changes of fluid transfer in body by other investigators.[13]

The method employed in the present study was consisted of intensive monitoring of both the ratio in the transmucosal fluid movement and the Ht value of the subjected animals during the course of the intestinal perfusion experiments. As observed in Fig. 4 to Fig. 8, the changes in all of these indices occurred without any retardations with predicted directions and extents. Therefore, applying the method in both of the fasted and the control animals, the rate and extent of the transmucosal fluid movement, and homeostatic fluid transfer into and out of blood were able to be observed simultaneously.

Although the present study was initiated with the purpose to investigate how the drug absorption would be modified in the fasted animal, many results obtained on the course of the investigation revealed remarkable differences concerning the transmucosal fluid movement and the water transfer in the subjected animals as well, which was of interest physiologically. In spite of the body being in the most parts constituted of water, or fluid, so much attentions have not been paied to the role of the fluid in intestinal absorption and in general the body fluid has been recognized to be merely a medium of drug transfer from blood to active sites in the body

11) E.F. Adolph, *Am. J. Physiol.*, **151**, 110 (1947).
12) S. Lepkovsky, R. Lyman, D. Fleming, M. Nagumo, and M.M. Dimic, *Am. J. Physiol.*, **188**, 327 (1957); L.J. Cizec and M.R. Nocenti, *ibid.*, **208**, 615 (1965); M.I. Gregersen, *ibid.*, **102**, 344 (1932).
13) P.C. Weiser and F. Grande, *Am. J. Physiol.*, **226**, 1028 (1974); J. Lister, M.S. Win, and R.P. Altman, *Surg.*, **60**, (1966); I.F. McNeill, J.P. Williams, and F.D. Moore, *J. Surg. Res.*, **3**, 322 (1963).

and/or merely a site where drugs should exist or distribute after the administration. Contrary to such a general notion, fluid partition in the body, where body fluid is taken into account kinetically, is assumed to become important in considering the intestinal absorption of drugs, since the rate and extent of fluid transfer in the body which encountered unphysiological and diseased conditions are more or less participated in the absorption, as mentioned before. In the present work, one of the main objects was the difference in both the transmucosal fluid movement and the fluid transfer across the capillary wall between the control and the fasted animals. As had been presented in Fig. 4 and Fig. 5, a process of fluid transfer from fluid compartments to the circulation was blocked by the fasting and the results depicted in Fig. 7, Fig. 8, and Fig. 9 indicated suggestively an inhibition of fluid transfer through the same process while the direction was reverse. This is stated in another way as follows: in the non-fasted animal, when circulatory blood volume falls too much incident to transmucosal fluid outflow, transcapillary refilling of the circulation is occurred rapidly and resulting in repartition of the body fluid between the interstitial fluid and the blood plasma, while the expansion of the circulation produced by the transmucosal fluid absorption brings about both the fluid shifts across the peripheral capillaries into the interstitial spaces and renal fluid excretion, following a recovery of predicted volume of the circulation. And on the other hand, in the state of fasting, such a homeostatic response observed above is thought to be considerably delayed or its capability seems to be much lowered by the fasting and this results in a fall of drug absorption from intestine.

Till now, the mechanism of transcapillary refilling has been studied comprehensively and the main factors concluded in the process appear to be explained reasonably by Starling's hypothesis.[14] It is apparent that both albumin and electrolytes in blood plasma play an important role in the partition of water in body. As reported by Dicker in 1949,[15] plasma concentration of protein, chloride and sodium were still in normal ranges in rats in the state of fasting, however the dehydration observed in the fasted rats caused a significant decrease in absolute amount of plasma in the circulation. Concerning the plasma, as circulatory plasma albumin is in general consumed as an energy source without any supply of nutritions, the decreased volume of plasma induced by fasting is apparent to be dependent on the duration of the fasting, which reflected in the changes of Ht as indicated in our results. As for the role of plasma, Rothscild, *et al.* reported in 1969[16] that albumin plays a major role in the regulation of the size of the extravascular spaces, or interstitial spaces because of its colloid osmotic functions.

Considering these evidences inclusively, there appears to bring about a fall in capillary pressure in the fasted rats and furthermore a fall in the tissue oncotic pressure as well as an increase in the tissue pressure is also speculated. Alterations in these factors consequent on fasting would therefore appear to affect restrictively the fluid repartition in the body. This is thought to be one of the important reasons why intestinal hypotonic perfusion solution could not be absorbed in the fasted rats so largely as in the non-fasted animals and this will be also the reason of the fasting-induced depression of sulfanilamide absorption from intestine in the fasted rats.

Above discussions should be summarized as follows. The organism in the non-fasted state is recognized to be able to respond any disorder of body fluid rapidly and greatly to maintain homeostatically the required constancy of the internal environment, whereas in the fasted rats capability of the organism to maintain an appropriate fluid environment seems to be in general lowered, presumably owing to a fall in the absolute amount of available body fluids both in the circulation and the interstitial spaces.

14) E.E. Starling, *J. Physiol.*, **19**, 312 (1896); F.H. Scott, *Am. J. Physiol.*, **44**, 298 (1917).
15) S.E. Dicker, *Biochem. J.*, **44**, 274 (1949).
16) M.A. Rothscild, M. Oratz, and S.S. Schreiber, *Am. J. Digest. Diseas.*, **14**, 711 (1969).

Although the data reported here do confirm that fasting impairs the repartition of water between fluid compartments in the body, thus secondary followed by the depression of drug absorption from intestine, the *in vivo* actions of fasting on the physiological functions of the organisms are rather complex in nature. Further studies will be required to investigate in detail the connection between physiological characteristics of fasting and intestinal absorption of drugs.