

Fasting and the Volume of Drug Distribution in the Rats

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An attempt was made to elucidate the mechanisms of the drug absorption from intestine in the fasted rats.

During fasting, weight of the body decreased gradually, whereas arterial hematocrit increased in proportion to the duration of food deprivation. It was observed that not only volume of blood plasma but also interstitial fluid volume decreased. These results could be interpreted as suggesting a decrease of distribution volume. In spite of the less absorption of drug from intestine, blood concentration of the fasted rats was higher than that of the controls. Volume of distribution was lost approximately 40% of the initial values during fasting for 60 hours.

From these results, it was concluded that the diminished volume of distribution induced by fasting is one of the main causes which may bring about the decrease of drug absorption from intestine in the fasted rats. Other possible mechanisms were also discussed.

Absorption of substances, whether nutrients or drugs, from the intestine is influenced by numerous factors which are thought to be divided largely into two categories, namely 1) factors related to physico-chemical properties of drugs and 2) factors concerning the physiological conditions of the experimental animals. Since Brodie and his co-workers²⁾ presented the pH-partition hypothesis, attentions have been concentrated on the elucidation of the relation between the physico-chemical properties and the transport of drugs through epithelial layer of the gastrointestinal tract of the animals and many lines of evidences have been accumulated. On the other hand, relatively little attention has been paid to the effect of the latter factors on drug absorption.

Deprivation of food from experimental animals exerts numerous influences on the physiological conditions of the subjects. These alterations do not always bring proper conditions to the absorption studies. A recent study in this laboratory has shown that the intestinal absorption from rats of a variety of drugs was decreased upon fasting. The purpose of this investigation to be described here was to elucidate some of the mechanisms of the reduction of the intestinal absorption of drugs in the fasted rats, with particular attention to the alteration in the volume of body fluid of rats.

Experimental

Materials—Sulfanilamide and reagents were obtained from the commercially available sources of analytical grade (Nakarai Chem. Co. Ltd., Kyoto, Japan) and used without further purification.

Animals—Male albino rats of Wistar strain weighing 110 to 175 g were used in all the absorption experiments.

In case of fasting, rats were deprived of food but free access to tap water at all times during the deprivation. Details are described in the previous paper.³⁾

Analytical Methods—Sulfanilamide was estimated colorimetrically as described previously.³⁾

1) Location: a) Shogoin, Sakyo-ku, Kyoto; b) Yoshida, Sakyo-ku, Kyoto.

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, B.B. Brodie, and L.S. Schanker, *ibid.*, **125**, 275 (1959).

3) T. Komuro, S. Kitazawa, and H. Sezaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 400 (1974).

Absorption Experiments—The animals were anaesthetized with pentobarbital. 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 experiments. Intestinal absorption was conducted by the *in situ* recirculation methods. Forty milliliters of the test solution containing sulfanilamide (1 mM) was perfused for one hour at a rate of about 5 ml/min. Tonicity of the solution was adjusted to 0.9% by adding sodium chloride. Details are described previously.³⁾

Wet and Dry Weight of Intestine—After the experiments, the entire small intestine was removed by cutting both ends, those are the proximal end of the duodenum and the distal end of the ileum, and manually stripping the mesentery from the intestine. The isolated intestine was hung up for a few minutes to remove any fluid remained inside of the lumen and then surface was gently blotted with a filter paper. In case of the dry weight, the small intestine was dried in the oven at 110° for 5 hours and further over silica gel to reach a constant weight.

Estimation of Hematocrit—Polyethylene tubing of about 0.5 mm diameter was cannulated into the femoral artery of animal and about 50 μ l of arterial blood was depleted and contained in the glass capillary, where blood coagulation was prevented by the intravenous injection of aliquots of heparin sodium solution before experiments. The other end of the capillary was sealed with clay. By the centrifugation of blood for 5 min at 12000 rpm using a centrifuge (HEMATO KH-120, Kubota Manufacturing Industry Co. Ltd., Osaka, Japan), plasma and erythrocyte were separated each other, where the percentage of the packed erythrocyte volume was called "Hematocrit". The hematocrit (Ht) is calculated from the equation: $Ht = (V_e/V_b) \times 100 \cdots 1$, where V_b =volume of whole blood, V_e =volume of erythrocyte and, in addition, volume of blood plasma (V_p) = $V_b - V_e$.

Estimation of Blood Plasma and Erythrocyte Volume—Hematocrit change caused by one rapid, intravenous injection of plasma obtained from non-fasted rats (donors) was conveniently used to estimate an approximate volume of blood plasma and erythrocyte of the control and 60-hour-fasted rats. Injection of the plasma of donor rats brings about an increase of total blood plasma of the injected rat which is immediately followed by the fall in the hematocrit in proportion to the magnitude of blood volume. After one intravenous injection of plasma, changed hematocrit (Ht') is given as follows: $Ht' = \{V_e/(V_b + V_p)\} \times 100 \cdots 2$, where V_p =volume of the injected plasma. Accordingly, the volume (ml) of whole blood, blood plasma and erythrocyte was calculated using equations of 1 and 2 as follows:

$$V_b = \frac{V_p \cdot Ht'}{Ht - Ht'}, \quad V_e = \frac{V_p \cdot Ht'}{Ht - Ht'} \times \frac{Ht}{100}, \quad \text{and} \quad V_p = \frac{V_p \cdot Ht'}{Ht - Ht'} \times \left(1 - \frac{Ht}{100}\right).$$

Results and Discussion

Effect of Fasting on the Weight of Whole Body and Organs

When rats were deprived of food but free access to tap water prior to the experiment, it was observed on the second day of the deprivation that external appearances of animals became gradually lean with a coarse coat of fur. Body weight of the animals (average 170 g) decreased gradually and about 57 g of the initial weight was lost after fasting for four days as presented in Table I. This indicates that the body weight decreased at a mean rate of 14 g per day.

TABLE I. Effect of Fasting on Body Weight and Weight of Organs in the Rats

	Control	4-Day-fasted	% difference
Whole body, ^{a)} g	170.4 \pm 11.3	113.5 \pm 6.7	-33.4
Small intestine, ^{b)} g	7.81 \pm 0.78	3.02 \pm 0.27	-61.3
Liver, g	7.30 \pm 1.17	3.38 \pm 0.41	-53.6
Spleen, mg	514.2 \pm 53.3	258.7 \pm 120.5	-49.7
Heart, mg	695.0 \pm 18.7	491.8 \pm 31.4	-29.2
Kidneys, mg	693.3 \pm 86.3	552.9 \pm 32.5	-20.2
Adrenal glands, mg	34.8 \pm 6.2	38.7 \pm 8.8	+11.2

a) Whole body weight for 1-, 2- and 3-day-fasted rats are 147.7 \pm 5.7, 135.7 \pm 5.2 and 126.0 \pm 6.9 g, respectively.

b) Small intestine is the part from the proximal end of the duodenum to the distal end of the ileum and expressed as wet weight. The figures are the mean \pm S.D. The positive and negative values for the differences indicate that the values for the fasted animals are respectively greater or less than the values for the control animals.

Influence of fasting was also observed in the internal organs. The small intestine lost 61% of its initial weight during four days, liver and spleen lost about 50% during the same period of fasting, heart lost about 30% and in case of kidneys, about 20% of the initial weight was lost. On the contrary, adrenal glands did not lose or even gained weight as presented in Table I. From these results, it is apparent that percentage lost in the weight of these internal organs are not always the same in spite of the same period of fasting. This suggests that the effect of fasting on the internal organs is not uniform and that the small intestine is one of the most susceptible organs to fasting.

Ju and Nasset⁴⁾ and Desai⁵⁾ demonstrated a disproportionately greater and more rapid loss of weight from the liver and small intestine than the body as a whole during total fasting, whereas Addis, *et al.*⁶⁾ reported a little loss from heart. Our results obtained are in good agreement with those observations. Therefore, conditions of the studies presented here seem to be appropriate. As for adrenal gland, this organ responds rapidly to such a stress as fasting, and weight increase found in this study is thought to be reasonable, although recent studies have led to results not always consistent with each other.⁷⁾

It is well known that the internal organs keep a certain amount of fluid and functions of these organs are considered to be dependent on the maintenance of an appropriate fluid environment.⁸⁾ Therefore, effect of fasting on the tissue fluid was examined. Figure 1 illustrates the relation between wet and dry weight of the whole intestine obtained from the control and the fasted rats. The linearity of the plots indicates that the relative fluid content of the intestine is kept constant with or without food deprivation. In other words, 1.25 g of dried intestinal tissue of the control rats had contained 6.56 g of fluid before drying and 2.82 g of fluid had been kept in 0.55 g of dried intestine of 72-hour-fasted rats and thus relative fluid content of these intestines becomes approximately 84%.

Although McManus, *et al.*⁹⁾ reported that weight decrease in the small intestine induced by a short period of fasting for 15 to 16 hours was not due to changes in water content, there

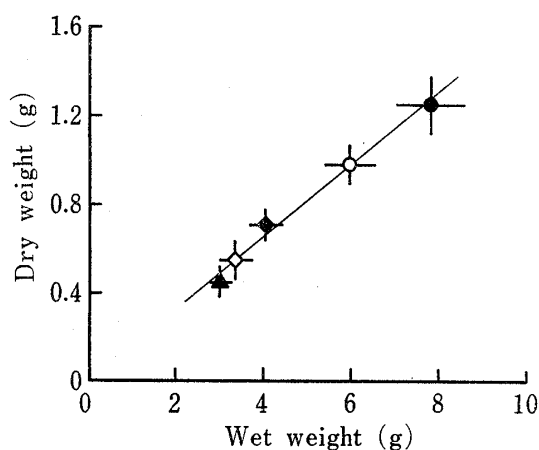


Fig. 1. Relation between Wet and Dry Weight of Rat Small Intestine

●: control, ◇: fasted for 3 days,
○: fasted for 1 day, ▲: fasted for 4 days,
◆: fasted for 2 days,

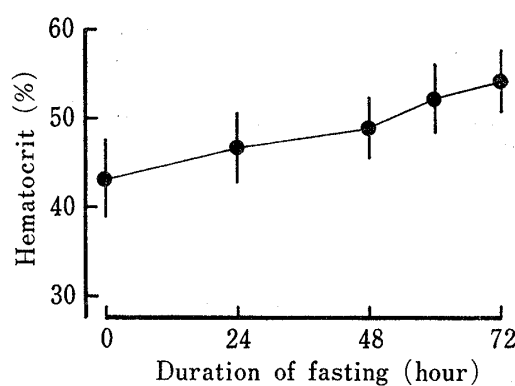


Fig. 2. Time Course of Changes in the Hematocrit of Rats during Fasting

- 4) J.S. Ju and E.S. Nasset, *J. Nutr.*, **68**, 633 (1959).
- 5) I.D. Desai, *Can. J. Biochem.*, **49**, 170 (1971).
- 6) J. Addis, L.J. Poo, and W. Lew, *J. Biol. Chem.*, **115**, 111 (1936).
- 7) M. Steiner, H.R. Bourges, L.S. Freedman, and S.J. Gray, *Am. J. Physiol.*, **215**, 75 (1968); C. Bouill  and Assenmacher, *Endocrinol.*, **87**, 1390 (1970).
- 8) A.C. Guyton, "Function of the Human Body," 3rd ed., W.B. Saunders Company, Philadelphia, 1969, p. 44.
- 9) J.P. McManus and K.J. Isselbacher, *Gastroenterology*, **59**, 214 (1970).

appears little report supporting that relative water content of the small intestine is kept constant for such a long period of fasting as represented here. The present finding is of most interesting in view of an effect of fasting on body fluid since weight decrease of the organs was due not only to the decrease of tissue itself but also to the decrease of tissue fluid and further, absolute amount of the loss of tissue fluid was by far larger than that of the tissue itself. Such a reduction of tissue and fluid during fasting is thought to be taken place not only in the intestine but also in all the other internal organs of the body with different degrees.

As the total body fluid is known to be equal to 50 to 60% of the body weight, it is conceivable that such decrease in the fluid content will influence the distribution of drug in the fasted rats.

Effect of Fasting on the Volume of Extracellular Fluid

Since blood is one of the most important components of the body fluids, effect of fasting on the volume of blood was examined using hematocrit as a valuable index distinguishing the normal conditions from the abnormal ones of fluid distribution in the body. Arterial hematocrit of non-fasted rats was 43% on average, whereas that of the 72-hour-fasted animals was about 54% and increased in proportion to the duration of fasting as illustrated in Fig. 2. Since erythrocytes in the circulating blood have a long lifespan of approximately 120 days,¹⁰⁾ the increase of hematocrit suggests the fall of plasma fraction of blood. To clarify this point, volume of blood plasma and erythrocytes of both control and 60-hour-fasted rats were calculated following the methods described elsewhere. The results indicate that the volume of erythrocytes of the fasted rats was not so significantly different from those of the controls but the volume of blood plasma decreased considerably from the average control value of 7.3 ml to 5.1 ml in the fasted animals. These values coincide with the increase of hematocrit of fasted rats mentioned earlier.

It has been generally recognized that despite the fact that animals had free access to water, fasting produces an increase in hematocrit and a decrease of the volume of circulating

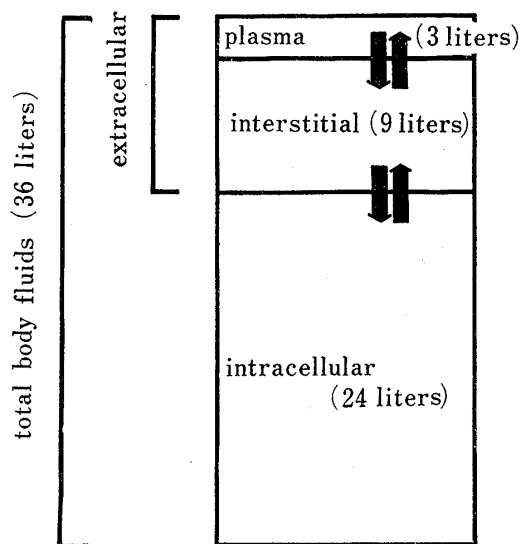


Fig. 3. Diagrammatic Representation of the Body Fluids

The total water in the average 60 kilogram man is about 36 liters, or 60 per cent of his total body weight. Of this, about 25 per cent is in the interstitial spaces. In case of rats weighing about 150 to 200 g, interstitial fluid is known to be about 23 per cent of the body weight. (J. Katz, *et al.*, *Clin. Sci.*, **39**, 705 (1970)).

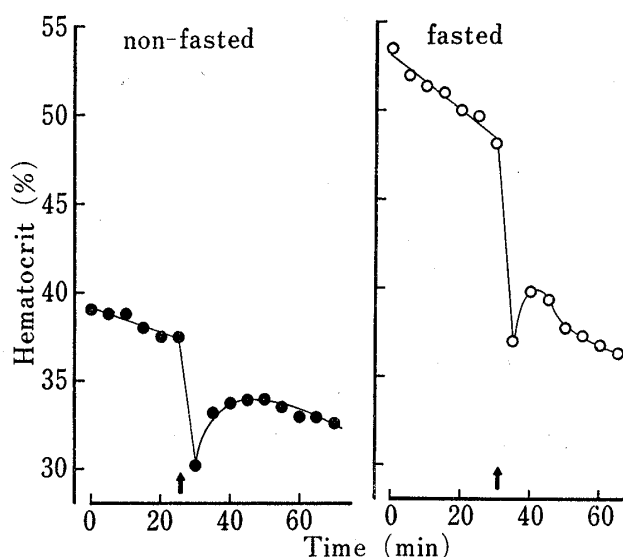


Fig. 4. Time Course of Changes in the Hematocrit before and after Injection of 0.9% NaCl Solution in the Renal Ligated Rats

Arrow indicates the intravenous administration of 5 ml of 0.9% NaCl solution. In both experiments, intestinal recirculation was conducted with 0.9% NaCl solution at a rate of 5 ml/min.

10) B.A. Brown, "Hematology, Principles and Procedures," Lea and Febiger, Philadelphia, 1973, p. 27.

blood of experimental animals.¹¹⁾ Magharabi and Haines¹²⁾ demonstrated that dehydration-induced changes in the fluid compartment are not always the same in different animals and suggested that in the laboratory rats water is lost proportionately from all fluid compartments. Figure 3 represents diagrammatically various fluid compartments of the body. From this diagram and above discussions, it is suggested that effect of fasting on interstitial fluid where drugs are rapidly distributed, is too large to be neglected in the rats. To confirm this point, the role of interstitial spaces in controlling the volume of blood was examined using renal ligated rats. Under condition of perfusing intestine with 0.9% sodium chloride solution, the other solution was administered intravenously to rat and hematocrit change was periodically observed. As shown in Fig. 4, in the control rats, elimination of the excess amount of fluid outward from blood was not affected so largely as the influence of renal ligation appears. In other words, when the volume of blood becomes too much, fluid leaks into the extravascular spaces, namely, interstitial spaces. Under the experimental conditions, fluid-retaining capacity of the spaces seems to be so large that hematocrit can be rapidly returned to an intended level in spite of cessation of renal function. On the contrary, in fasted rats, administration of fluid into blood produced the decrease in hematocrit much abruptly and increased the volume of fluid component of blood. It is conceivable, therefore, that the interstitial spaces could absorb not so much amount of fluid from blood in the fasted rats. The mechanisms by which such extreme expansion of blood volume lasts so long time remained obscure. However, there are some evidences that plasma albumin plays a major role in the regulation of the size of the interstitial spaces¹³⁾ as well as the retention of fluid in the body, and that prolonged fasting decreases the amount of the plasma albumin significantly.¹⁴⁾ Accordingly, fasting might retard the shift of water from blood to interstitial spaces.

Effect of Fasting on the Volume of Distribution

Because of the decreased volume of blood as well as interstitial fluid in fasted rats, it was expected that the volume of distribution¹⁵⁾ of drugs might be decreased by fasting, where volume of distribution is represented as the volume of body fluids which hold the substance in solution at the same concentration as the plasma. With an aim of illustrating to what extent the volume of distribution decreased in the 60-hour-fasted rats, sulfanilamide which was used as a marker drug was injected intravenously with the dose of 30 mg/kg and blood concentration changes were periodically determined and plotted as depicted in Fig. 5. The volume of distribution in the steady state (V_{ss} , ml) of the control and fasted rats was calculated from the equation: $V = D/C$ where D = dose, C = blood concentration of drug at time 0, the ordinate intercept. In case of the controls, body weight was about 170 g and the ordinate intercept was 32.0 $\mu\text{g/ml}$. On the other hand, in the fasted rats, body weight and the ordinate intercept were about 130 g and 40.6 $\mu\text{g/ml}$, respectively. From these data, volume of distribution is calculated to be about 159 ml for controls and about 96 ml for fasted animals. These results indicate that the 60-hour-fasted rats lost about 40% of the initial volume of distribution. This was further substantiated from the investigation of blood concentration after drug absorption from intestine.

Figure 6 shows the time course of intestinal sulfanilamide absorption and the corresponding blood concentration profiles in the control and fasted rats. It appears from Fig. 6 that the absorption rate of sulfanilamide was significantly reduced in the fasted rat compared to the

- 11) J.A. Archambeau, P. Stryckmanns, and H. Brenneis, *Radiat. Res.*, **36**, 396 (1968); H. Maagøe, *Metabolism*, **17**, 133 (1968); H.L. Taylor, A. Henschel, O. Mickelsen, and A. Keys, *J. Appl. Physiol.*, **6**, 613 (1954); S.E. Dicker, *Biochem. J.*, **44**, 274 (1949).
- 12) F. Mogharabi and H. Haines, *Am. J. Physiol.*, **224**, 1218 (1973).
- 13) M.A. Rothschild, M. Oratz, and S.S. Schreiber, *Am. J. Digest. Dis.*, **14**, 711 (1969).
- 14) M.A. Rothschild, M. Oratz, and S.S. Schreiber, *Gastroenterology*, **64**, 324 (1973).
- 15) J.G. Wagner, *Ann. Rev. Pharmacol.*, **8**, 67 (1968).

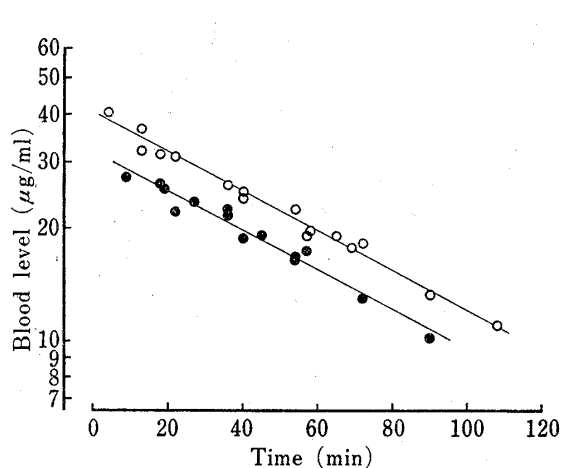


Fig. 5. Blood Level after Intravenous Administration of Sulfanilamide

●: controls, ○: 60-hour-fasted rats

non-fasted control. In spite of the decrease of absorption, blood level of the drug as well as area under the curve in the fasted rat was significantly larger than that of the control. In the bioavailability study of drug, it is generally assumed that the higher blood level of a drug or area under the curve will reflect the better absorption of the drug.¹⁶⁾ However, under condition of fasting, it is not always so. Such extremely high blood level of a drug in fasted animals may be disadvantageous to absorption from intestinal tract, since absorption rate of passively transported drug such as sulfanilamide is proportional to the concentration difference between blood and intestinal perfusate.

Relation between Volume of Distribution and Intestinal Drug Absorption

It is apparent from the foregoing results and discussions that fasting brought about the decrease of the distribution volume, resulting in the increase of hematocrit. Effect of this phenomenon on drug absorption was studied and the result is illustrated in Fig. 7 which shows the relation between the hematocrit obtained from the control and fasted rats and the sulfanilamide absorption in the corresponding rats. Drug absorption is obviously related to hematocrit, thus volume of distribution.

If the volume of distribution is nearly parallel to body weight, absorption should be proportional to the weight of body. This body weight dependency of drug absorption was examined using non-fasted rats of various body weights and the results are depicted in Fig. 8, which reveals a close relation between body weight and percentage absorption of sulfanilamide from intestine and/or fluid movement, where the fluid movement is expressed as the ratio of final volume of perfusate to the initial one.

In the non-fasted rats, it is suggested from the results that drug absorption, distribution volume and body weight are closely related one another.

This relation was further examined in the fasted animals and the result was shown in Fig. 8. The data obtained from 60-hour-fasted rats fall exactly on the regression line, which

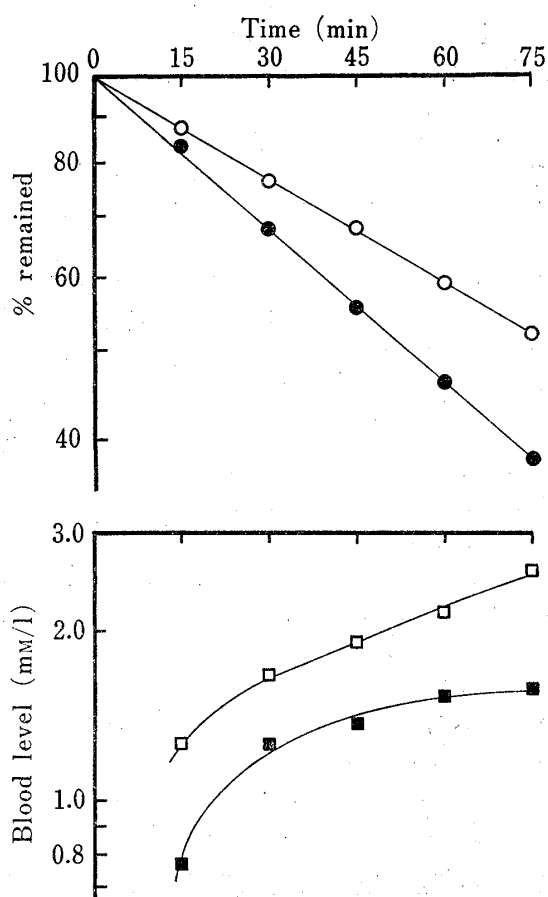


Fig. 6. Relation between Intestinal Absorption and Blood Level of Sulfanilamide in the Control and Fasted Rats

●■: control, ○□: 60-hour-fasted rat

16) M. Rowland, S. Riegelman, and W.L. Epstein, *J. Pharm. Sci.*, **57**, 984 (1968).

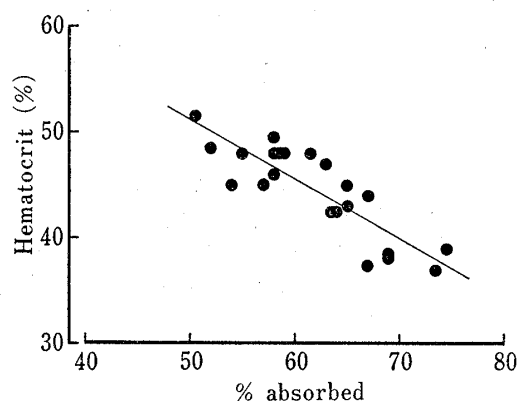


Fig. 7. Relation between Hematocrit and Intestinal Absorption of Sulfanilamide

(correlation coefficient: $r=0.85$)

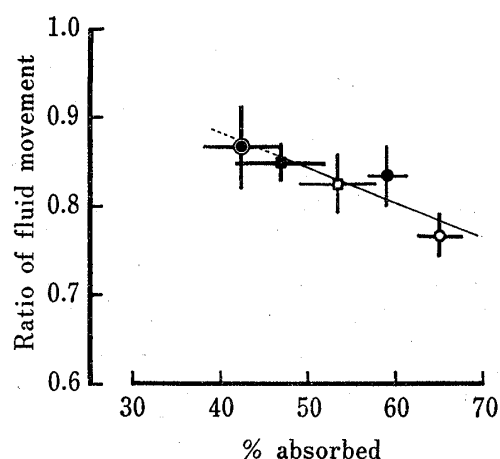


Fig. 8. Relation between Body Weight and Intestinal Absorption of Sulfanilamide

●: 60-hour-fasted rats
controls, mean body weight
■: 117 g □: 132 g ●: 147 g ○: 162 g

indicates that decreased absorption caused by fasting is clearly due to the reduction in the volume of distribution. Accordingly, it is conceivable from these results that the decrease in the volume of distribution is one of the causes which may bring about the reduction of drug absorption from intestine in the fasted rats. However, careful examination of the data reveals that the percentage absorption in the fasted rats was even more reduced than the expected extent from the body weight loss since 60-hour-fasted rats weighed about 130 g, and in the corresponding non-fasted controls sulfanilamide absorption was about 53% as shown in Fig. 8. In order to get further insight into this marked inhibition of absorption brought about by fasting, plots of body weight difference versus % absorption difference are depicted in Fig. 9. In case of the non-fasted rats, there is a close relation between body weight difference and difference of the percentage absorption of drug and regression line is described as a straight line graphically, whereas in the fasted rats, this relation is not represented as a straight line and the regression curve is largely deviated from the controls. It is, therefore, obvious from the results that the other mechanisms may be involved in the intestinal drug absorption of the fasted rats.

Levin, *et al.*¹⁷⁾ reported that 3-day-fasting produced a decrease in intestinal absorption of passively transported substances in rats and assumed that the decrease in intestinal absorption would be due to non-specific factors, such as a decrease in surface area or a reduction in blood flow to the intestine. Recently, Diamond, *et al.*¹⁸⁾ had investigated the possible

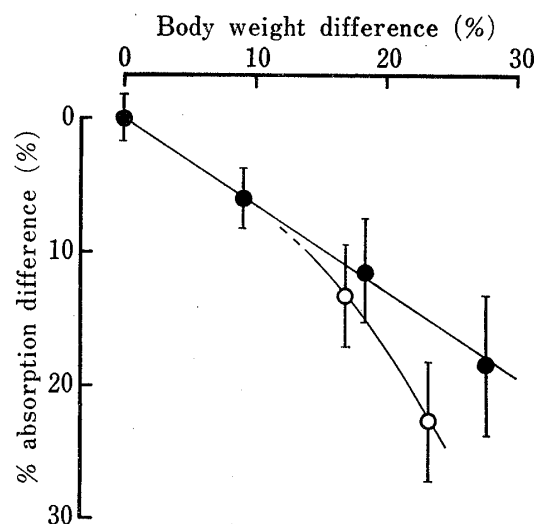


Fig. 9. Relation between Body Weight Difference (%) and Difference in the Intestinal Absorption of Sulfanilamide (%) in the Control and Fasted Rats

In case of fasted animals, abscissa indicates the percentage of body weight loss caused by fasting to the initial weight.

●: control, ○: fasted

17) R.J. Levin, H. Newey, and D.H. Smyth, *J. Physiol.*, **177**, 58 (1965).

18) L. Diamond, J.T. Doluisio, and W.G. Crouthamel, *Europ. J. Pharmacol.*, **11**, 109 (1970).

effects of fasting on mesenteric blood flow using dogs and demonstrated that prolonged inanition might inhibit the intestinal drug absorption process by virtue of an induced diminution in intestinal blood perfusion. In these connections, similar results are obtained by Hayton.¹⁹⁾ He reported the reduced rate of sulfanilamide absorption in irradiated rats and suggested that irradiation could directly alter the permeability of the intestinal mucosa as well as surface area and/or blood flow to the mucosa.

In the present studies, a meaningful relationship between body fluid and intestinal drug absorption has been demonstrated. It appears, however, that numerous physiological and/or biochemical changes that occur during periods of fasting might interact complicatedly upon the intestinal absorption profiles. Further studies will be required to determine the effect of fasting on the drug absorption from intestine.

19) W.L. Hayton, *J. Pharm. Sci.*, **63**, 645 (1974).