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Drug Absorption and Metabolism Studies by Use of Portal Vein Infusion in the Rat. II.[1] Influence of Dose and Infusion Rate on the Bioavailability of Propranolol

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Propranolol concentrations in the blood was determined after rapid femoral vein and 50 min portal vein infusion of several doses to rats. No propranolol appeared in the systemic circulation after intraportal infusion of doses less than approximately 2 mg/kg. The mean bioavailability of propranolol was 42% in a dose of 5 mg/kg and increased progressively with increasing dose. However, the area under the blood concentration-time curve after portal vein infusion of propranolol was found to vary with infusion rate. The hepatic elimination of the drug during the first pass through the liver after portal vein infusion was highly dose-dependent in lower doses, and highly rate-dependent in higher doses. Therefore, the bioavailability of the drug was dose and rate-dependent. However, in higher doses, it remained almost constant at a constant infusion rate.

The previous work[1] has shown that the area under the blood concentration-time curve (AUC) after portal vein infusion of propranolol is appreciably less in a dose of 2.5 mg/kg as compared with that observed upon infusion of the equal dose into the femoral vein of the rat, while the AUC's after intravenous and portal vein infusion were not significantly different in a dose of 12.5 mg/kg. Administration of a drug directly into the portal vein is equivalent to oral administration of a drug which is absorbed completely in the absence of drug metabolism in the intestinal wall, since the entire dose reaches the systemic circulation. Therefore, even though propranolol after oral administration is completely absorbed through the gastrointestinal tract, the AUC would be considerably less than the AUC after intravenous administration of an equivalent dose, and accordingly the bioavailability of propranolol may be assessed to be considerably low, if its measurement is based on the comparison with an intravenous injection as a standard.

Shand, *et al.*[3] have shown that the AUC of propranolol is less after oral administration than after intravenous administration to human subjects. Only trace amounts of the drug were detected in the systemic circulation in doses less than 30 mg in human subjects, while the relationship between dose and the AUC was linear over a dose range of 40 to 160 mg.[4] The reduction in the AUC was explained on the basis of a first-pass effect which is attributed to the fact that all of the drug infused into the portal vein is exposed to the liver before reaching the vascular site being sampled.[3,4] It has recently been shown that also in the rat, little drug appeared in the systemic circulation after propranolol administration into the portal vein of doses less than 0.8 mg/kg and that the blood concentration at 30 sec after intraportal administration increased linearly with dose.[5] These findings demonstrate that despite com-

1) Part I: T. Suzuki, Y. Saitoh, S. Isozaki, and R. Ishida, *Chem. Pharm. Bull.* (Tokyo), **20**, 2731 (1972); *idem*, *J. Pharm. Sci.*, **62**, 345 (1973).
2) Location: *a*) *Yayoi-cho, Chiba*; *b*) *Hongo, Bunkyo-ku, Tokyo*; *c*) Present address: *Kyorin Chemical Laboratory, Ukima, Kita-ku, Tokyo.*
3) D.G. Shand, E.M. Nuckolls, and J.A. Oates, *Clin. Pharmacol. Therap.*, **11**, 112 (1970).
4) D.G. Shand and R.E. Rangno, *Pharmacology*, **7**, 159 (1972).
5) D.G. Shand, R.E. Rangno, and G.H. Evans, *Pharmacology*, **8**, 344 (1972).

plete absorption, the fraction of dose, which appears in the systemic circulation, is greatly influenced by dose and that the bioavailability of propranolol is constant above a threshold dose (T), if an apparent dose ($D-T$) was used instead of an actual dose (D) of propranolol.

The previous work[1] has suggested that the bioavailability of propranolol given intraportally in doses of 2.5 and 12.5 mg/kg is dependent upon dose and possibly also upon the rate of infusion, since both the intraportal doses were given over an interval of 50 min. The purpose of this investigation is to show quantitatively the effect of the route of administration upon the bioavailability of propranolol, using the portal vein infusion technique in the rat.[1]

Experimental

Materials——Propranolol and all other chemicals used in this study were the same as those reported previously.[1]

Animal Experiments——Male Wistar rats weighing 200 to 250 g were used. The rats were anesthetized lightly with ether at suitable intervals as needed during surgery. In a constant-infusion experiment into the hepatic portal vein in doses of 1.0 to 12.5 mg/kg, an aqueous propranolol solution (1.14 ml) was administered at a constant rate *via* a pyloric vein catheter using an infusion pump (Natsume Model KN-1H).[1] A rapid intraportal infusion in a dose of 2.5 mg/kg was given over 30 sec. A rapid intravenous infusion of propranolol (0.5 ml) in doses of 1.0 to 12.5 mg/kg was given into the right femoral vein over 30 sec. Blood samples (0.1—0.3 ml) were taken at various times from the aorta through a catheter inserted into the left femoral artery. An equal volume of blood to the drawn blood sample was transfused each time through a catheter inserted into the left femoral vein in order to avoid circulatory disturbances resulted from blood loss. The blood for transfusion was prepared by adding 0.4 ml of 10% sodium citrate to 4.6 ml of blood taken from another rat of the same strain.

Analytical Methods——Blood concentrations of propranolol were determined spectrophotofluorometrically by a minor modification of the method of Shand, *et al.*[3] This analytical method was described in the previous paper.[1]

Calculation of Bioavailability——The bioavailability of propranolol was calculated from comparison of the AUC's after portal vein and intravenous administration of an equal dose. The blood concentration-time curve of propranolol after rapid intravenous infusion was described in all rats by a biexponential equation of the form, $C=Ae^{-\alpha t}+Be^{-\beta t}$. Here, the coefficients A and B represent the intercepts on the ordinate obtained by an extrapolation to zero time of the two linear segments, whose slopes may be defined as $-\alpha/2.3$ and $-\beta/2.3$ ($\alpha>\beta$), when the concentrations of drug (C) are plotted as a function of time (t) on a semilogarithmic paper. The AUC after rapid intravenous infusion was calculated by $A/\alpha+B/\beta$. The AUC's other than rapid intravenous infusion were calculated using the trapezoidal rule. The area for the tail end was calculated by C_t/β. C_t is the blood concentration at the last time point (t), and β is an estimate of the slope of the line which is the best fit to the terminal natural log-linear data after the end of infusion.

Result

Figure 1 shows the mean AUC's of propranolol after intravenous infusion over 30 sec into the femoral vein and after constant infusion over 50 min into the hepatic portal vein of rats in a dose range of 1 to 12.5 mg/kg of propranolol. The AUC of propranolol given intravenously was found to be directly proportional to dose. On the other hand, the relationship between dose and the AUC was not linear after infusion into the portal vein, and no propranolol was found in the systemic circulation below a dose of approximately 2 mg/kg. In addition, the mean AUC obtained after portal vein infusion was less than the mean AUC of propranolol given intravenously, and the former approached the latter with increasing dose. The previous paper[1] has shown that the mean AUC's in doses of 2.5 and 12.5 mg/kg after portal vein infusion were 7.8 and 91% of the mean AUC's in the equal doses after intravenous infusion, respectively. The AUC after portal vein infusion was significantly different in a dose of 2.5 mg/kg, but not significantly different in a dose of 12.5 mg/kg from the AUC's after the corresponding intravenous infusion.[1] The observed data indicate that the differences between the AUC's after intravenous and portal vein infusion are significant in doses of 5.0 and 7.5 mg/kg, but not significant in a dose of 10 mg/kg (Fig. 1). The insert curve in Fig. 1 shows the bioavailability of propranolol plotted against dose, which is assessed from com-

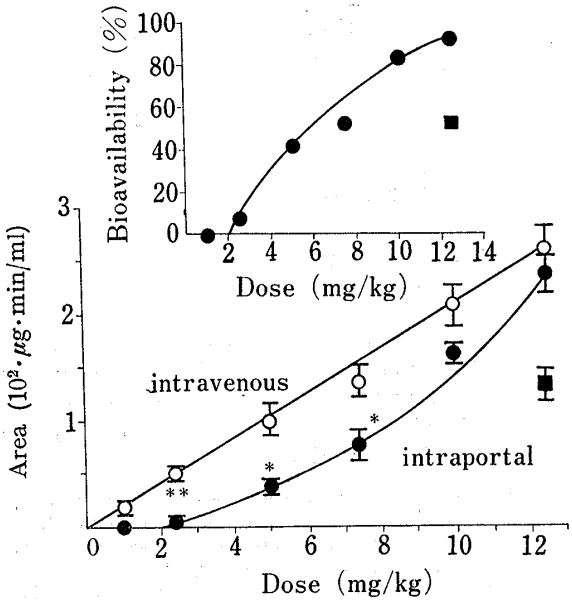


Fig. 1. Mean Areas under the Blood Concentration-Time Curves after Intravenous and Intraportal Infusion of Various Doses of Propranolol in Rats
Insert: Relationship between Administered Dose and Bioavailability

Intravenous doses were given over 30 sec into the femoral vein, and intraportal doses were given at constant rates over 50 min into the hepatic portal vein. The data in doses of 2.5 and 12.5 mg/kg are taken from the previous paper.[1] Vetrical bars represent standard errors of the estimation from three rats after intravenous infusion or from five rats after portal vein infusion. Statistically significant at $p<0.01$ (**)[6] and $p<0.05$ (*) when compared with the area after intravenous administration. A solid square (■) represents the area or the bioavailability of propranolol in a dose of 12.5 mg/kg at the same infusion rate as that of 5 mg/kg.

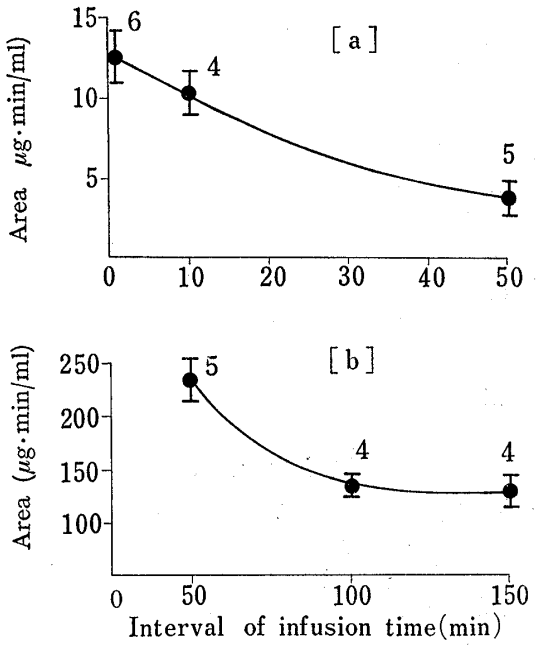


Fig. 2. Relationship between Interval of Intraportal-Infusion Time and Area under the Blood Concentration-Time Curve

Mean ± SE with number of rats [a] in a dose of 2.5 mg/kg and [b] in a dose of 12.5 mg/kg.

parison with the AUC in an equal dose on the regression line of the AUC of intravenous infusion on dose shown in Fig. 1.

The effect of infusion rate on the AUC was examined by way of infusion of propranolol in an equal dose into the portal vein at three different rates. Figures 2a and 2b show the AUC's in doses of 2.5 and 12.5 mg/kg plotted against infusion rate. The AUC of propranolol after portal vein infusion was found to decrease with decreasing infusion rate. An analysis of variance was performed for the mean AUC at each dose, and there were significant differences ($p<0.005$) within the AUC's for the infusion rates. On the other hand, the mean AUC's after 50 min intravenous infusion were 108 and 93% of those after 30 sec intravenous infusion in doses of 2.5 and 12.5 mg/kg, respectively. The differences between the mean AUC's in the equal doses for infusion intervals of 30 sec and 50 min could not be regarded as significant, regardless of wide variation in infusion rate.

The mean blood concentration-time curves of propranolol in a dose of 2.5 mg/kg after rapid portal vein and intravenous infusion are shown in Fig. 3. The time course for the concentration after rapid portal vein infusion typically passes through a maximum value,

6) The mean AUC's in doses of 2.5, 5.0, and 7.5 mg/kg after intravenous and portal vein infusion were compared according to the Aspin-Welch method, since the variances of the AUC's after these infusion were significantly different. Consequently, significant difference ($p<0.001$) in dose of 2.5 mg/kg in the previous paper[1] was found to be corrected to significant difference ($p<0.01$).

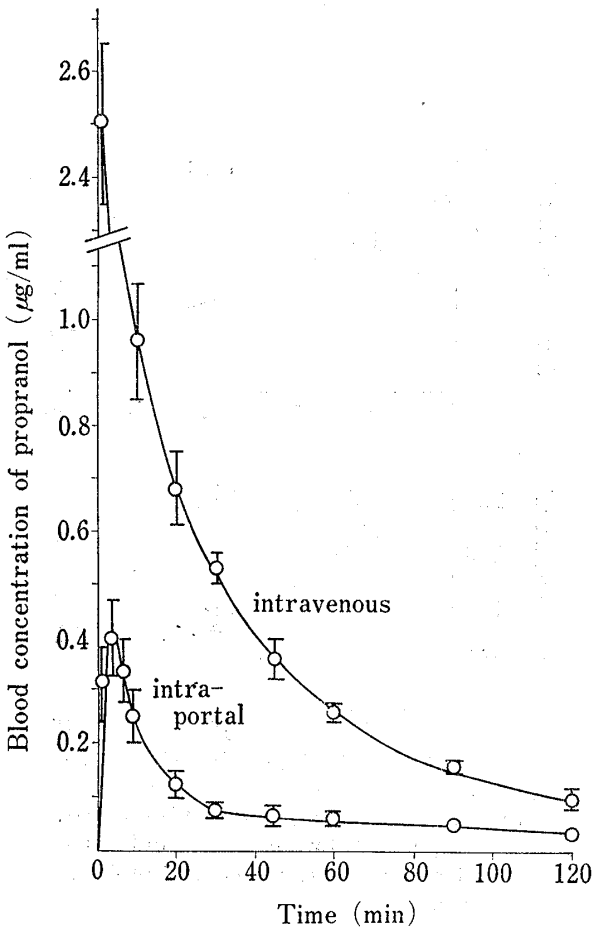
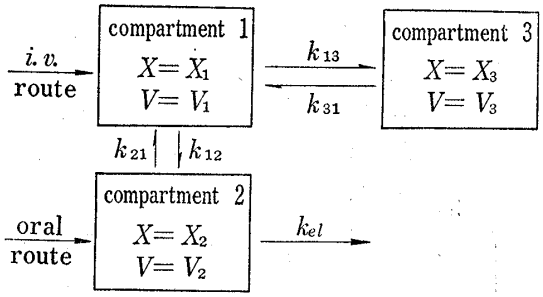


Fig. 3. Mean Blood Concentration-Time Curves of Propranolol after Intraportal and Intravenous Infusion over 30 sec in a Dose of 2.5 mg/kg (mean ± SE, $n=6$)

Mean blood concentrations ± SE at 4 and 7 min after rapid intravenous infusion of propranolol are 1.17 ± 0.12 and 0.92 ± 0.13 μg/ml, respectively, which are omitted from this figure. A mean blood concentration of 2.94 μg/ml at zero time was estimated by fitting to a biexponential equation on the basis of the graphical analysis described in the Experimental.



Chatr 1. Three-Compartment Open Model Proposed to explain the Influence of Route of Administration on the Area under the Blood Concentration-Time Curve by Gibaldi and Feldman[7]

Compartment 1 represents the vascular system being sampled and certain tissues not including the liver. Compartment 2 represents the hepatoportal system. Compartment 3 represents a group of tissues which are less rapidly accessible to the drug than the tissues involved in Compartment 1. Elimination is assumed to occur in the hepatoportal system (Compartment 2). X is the amount of drug in a given compartment, and V is the volume of the compartment. The rate constants in processes designated by arrows are assumed to be first-order.

while that after rapid intravenous infusion decreases monotonously. These facts indicate that the blood concentration of propranolol is clearly dependent on the route of administration, and the hepatoportal system is adequately described as a compartment which is kinetically distinct from the vascular site being sampled. The three-compartment open model shown in Chart 1 has been proposed to explain the influence of route of administration on the AUC, in which the hepatoportal system was treated as a compartment distinct from the central blood pool.[7] If the blood concentration-time curve after rapid portal vein infusion of propranolol is analyzed on the basis of this compartment model, it may be given by the following triexponential equation:[7]

$$C = \frac{\text{dose}}{V_1} \times [C_1' e^{-\pi t} + C_2' e^{-\alpha t} + C_3' e^{-\beta t}] \qquad (1)$$

Where, the symbols of coefficients and exponents are the same as those of Eq. 6 in reference 7. The mean blood concentrations of propranolol after rapid portal vein infusion of 2.5 mg/kg propranolol were plotted as a function of time on a semilogarithmic paper. Using back-extraporation procedures,[8] the mean blood concentration-time curve was resolved into three exponential components. The values of π, α, and β were evaluated to be 0.431, 0.166, and 0.033 min^{-1}, respectively. The corresponding coefficients of the three exponential terms were evaluated to be −0.903, 0.537, and 0.366 μg/ml. The rate constants based on this linear three-compartment open model were calculated from the values of exponents and coefficients

7) M. Gibaldi and S. Feldman, *J. Pharm. Sci.*, **58**, 1477 (1969).
8) B. Alexanderson, *Europ. J. Clin. Pharmacol.*, **4**, 82 (1972).

of the fitted triexponential equation.[9] The estimated rate constants, k_{12}, k_{21}, k_{13}, k_{31}, and k_{el}, which are defined in Chart 1, were 0.189, 0.098, 0.119, 0.102, and 0.122 min^{-1}, respectively.

Discussion

The quantitative assessment of bioavailability can be determined by comparing the total area under the blood, plasma or serum concentration-time curve (AUC) after giving a drug orally with the AUC following intravenous administration of an equal dose, if the proportionality between dose and the AUC is valid, regardless of the route of administration.[10] If the AUC ratio is less than unity, the drug is incompletely available. The reduction in AUC after hepatic portal vein infusion can be explained on the basis of the first-pass effect in the liver. This results from the fact that all of the infused drug into the portal vein must pass through the liver, where it can be eliminated before reaching the systemic circulation, while less than 30% of the drug pass through the liver in the first circulatory pass after usual intravenous administration.[11] Thus, a fraction of an intraportal dose is removed by the liver before entering the systemic circulation.

By using compartment-model or clearance-concept analysis, Eq. 2 was derived, which allows an estimate of bioavailability of a completely absorbed drug through the gastrointestinal tract.[12,13] The fraction (θ) of the absorbed dose appearing in the systemic circulation after first-pass elimination can be expressed as:

$$\theta = 1 - \frac{(\text{dose})_{i.v.}}{V_B(\text{AUC})_{i.v.}} \qquad (2)$$

where V_B is blood flow rate entering into the liver. Equation 2 indicates that the relationship between dose and the AUC after portal vein infusion is linear and extrapolated through the origin, if liver metabolism is the major route of elimination. These pharmacokinetic analyses of the first-pass effect were based on the assumption that the hepatic extraction ratio of the drug is unaltered by dose and the same during the first passage as that for the drug which has reached the systemic circulation.

The available evidences suggest that the reduction in the AUC of propranolol after oral administration results from appreciable metabolism during its first passage through the liver.[3-5] Gibaldi, *et al.*[12] have analyzed the first-pass effect of propranolol by substituting 10 mg intravenous dose data for $(\text{dose})_{i.v.}/(\text{AUC})_{i.v.}$ in Eq. 2. The mean bioavailability after oral administration of propranolol was calculated as 37%, which is in excellent agreement with a mean experimental value of 32% for 80 mg oral dose data, if only 70% of the oral propranolol is assumed to be actually absorbable to the hepatoportal system at a liver blood flow of 1.7 liter/min. Equation 2 was derived also from the equation, $(\text{AUC})_{p.o.}/(\text{AUC})_{i.v.}=k_{21}/(k_{21}+k_{el})$, based on the linear three-compartment open model, in which the hepatoportal system is defined as a compartment distinct from the compartment containing the blood in order to explain the influence of route of administration on the AUC.[12] A value of the relative AUC for rapid intraportal and intravenous infusion is estimated to be 0.45 by substituting the corresponding values observed in the Result for k_{21} and k_{el} into the above original equation. However, as shown in Fig. 1, the mean relative AUC is not a constant but varies from 0 to 0.90

9) The rate constants were obtained by solving the simultaneous six equations, Eqs. 7a—9a and Eqs. 17a—19a, in the Appendix of Reference 7. The volume of distribution of Compartment 1 (V_1) was approximated by 213 ml calculated from the estimated mean blood concentration, 2.94 μg/ml, immediately after rapid intravenous infusion of 2.5 mg/kg.

10) "Guidelines for Biopharmaceutical Studies in Man," ed. by APhA Academy Sciences, Washington, D.C., 1972, p. 17.

11) P.A. Harris and S. Riegelman, *J. Pharm. Sci.*, **58**, 71 (1969).

12) M. Gibaldi, R.N. Boyes, and S. Feldman, *J. Pharm. Sci.*, **60**, 1338 (1971).

13) M. Rowland, *J. Pharm. Sci.*, **61**, 70 (1972).

with dose. Shand, *et al.*[4] have claimed that the assumption of the constant hepatic clearance for dose and route of administration can not be applicable to the disposition of propranolol, because the relationship between dose and the AUC after oral administration was shown to increase linearly with dose above a threshold dose. It can be said from these facts that the bioavailability of propranolol is dose-dependent and increases with increasing dose. The use of Eq. 2 will lead to a higher estimate of the bioavailability of propranolol in lower doses.

The essentially complete hepatic extraction of propranolol in doses less than approximately 2 mg/kg and the significant reduction in the AUC in several doses exceeding this threshold dose agree with the observations, which Shand, *et al.*[5] obtained by measuring propranolol concentrations 30 sec after rapid intraportal administration to rats. The propranolol concentration 30 sec after intraportal infusion increased linearly with dose over a dose range of 1.25 to 5 mg/kg and the slope of the straight line was less than that after intravenous administration.[5] Similar data have been shown after oral administration of propranolol to human subjects.[4] However, as shown in Fig. 1, the AUC after administration into the portal vein of doses exceeding 5 mg/kg approaches progressively the AUC after intravenous administration of an equal dose. These data suggest that the first-pass effect becomes difficult to observe with increasing intraportal dose, since the difference in the AUC between intravenous and intraportal administration is attributed to the hepatic extraction of propranolol on an initial pass through the liver during the process of introduction to the systemic circulation. Here, attention should be called to the variation in intraportal-infusion rate. The rate of infusion into the portal vein under the experimental conditions shown in Fig. 1 increased with incresing dose, since all the intraportal doses were given over an interval of 50 min. It is shown in Fig. 2, as would be expected, that the first-pass effect is dependent on rate of infusion, and becomes greater progressively with decreasing infusion rate. The bioavailability of propranolol in Fig. 1 and that calculated from the data in Fig. 2 in various doses are replotted against infusion rate in Fig. 4. It can be presumed from this figure that the AUC in higher doses is largely dependent on infusion rate. This finding suggests that the bioavailability of propranolol in higher doses could be predominantly expressed as a function of infusion rate. Actually, the AUC after intraportal infusion of 12.5 mg/kg propranolol can be estimated to be decreased by about 45% from Fig. 2a, when the intraportal dose was given at the same infusion rate as that of a dose of 5 mg/kg. The bioavailability of propranolol in a dose of 12.5 mg/kg approached that in a dose of 5 mg/kg as shown with a solid square in Fig. 1. This observation can reasonably be explained by considering that the AUC after portal vein infusion of propranolol at a constant rate is proportional to the apparent dose, which is obtained by substracting the threshold dose from an actual dose.

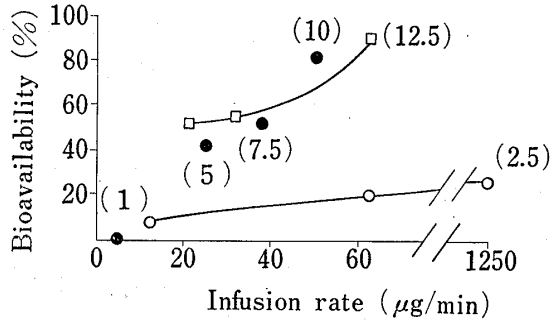


Fig. 4. Relationship between Intraportal Infusion Rate and Bioavailability of Propranolol

The biovailability of propraonlol in Fig. 1 and that calculated from the data in Fig. 2 in various doses are replotted against infusion rate. Each plot is labelled as to the number (mg/kg) of a dose in parentheses, and —○— and —□— are in doses of 2.5 and 12.5 mg/kg, respectively.

The mean clearance of propranolol in rats was calculated to be 2.5 ml/g liver/min from blood concentration data after intravenous administration of 2.5 mg/kg propranolol by Shand, *et al.*[5] A significant degree of extrahepatic elimination in the rat was presumed from the fact that this value is actually greater than an estimate of liver blood flow of 1.2 ml/g liver/min. In our study, the mean clearance of propranolol was calculated to be 12.2 ml/min from the slope of the regression line of the AUC after intravenous infusion of propranolol on dose. A hepatic clearance of approximately 1.5 ml/g liver/min is estimated, if propranolol

is metabolized exclusively in the liver and the rat liver is assumed to weigh 8 g. This estimate suggests that a considerable degree of hepatic elimination occurs, and the hepatic extraction might be almost complete as shown in the dog.[14] This estimate suggests also that according to Eq. 2, the AUC of propranolol after portal vein infusion is estimated to be negligible over the entire dose range, if the hepatic extraction ratio of propranolol during the first passage through the liver after portal vein infusion is the same as that for propranolol which has reached the systemic circulation. Actually, however, the hepatic extraction ratio is shown to decrease progressively, as the liver is exposed to increasing doses (Fig. 1). Consequently, the bioavailability obtained from the relative AUC in this paper does not always indicate the relative amount of propranolol infused that reaches the systemic circulation, and the hepatic extraction ratio of propranolol during the first passage will not be estimated from the relative AUC, since the mean clearance of propranolol after the first passage through the liver becomes different from that after intravenous administration.

Hepatic extraction is considered to consist of hepatic drug binding and the subsequent drug metabolism. Shand, *et al.*[5] have shown that hepatic propranolol concentrations of propranolol are much greater after portal vein administration than after intravenous administration to rats. This agrees with our findings that hepatic propranolol concentrations immediately after 50 min portal vein infusion is much greater than immediately after 50 min intravenous infusion above a dose of 5 mg/kg.[15] It can therefore be presumed that considerable amounts of propranolol eliminated before reaching the systemic circulation after portal vein infusion are present unchanged in the liver. In the dog and monkey, one of the metabolites of propranolol, 4-hydroxypropranolol was observed in the systemic circulation, when propranolol was injected directly into the portal vein, but not when the same dose was administered intravenously.[16] In the rat, our data show that 4-hydroxypropranolol was detected in the systemic circulation after portal vein infusion, but not in the systemic circulation after intravenous infusion.[15] The terminal half-life of propranolol after 50 min intraportal infusion was not significantly dose-dependent over a dose range of 5 to 12.5 mg/kg, and also that after rapid intravenous infusion was not significantly dose-dependent over a dose range of 2.5 to 12.5 mg/kg. However, there was a significant difference between the means (34 and 44 min) of the respective terminal half-lives after intraportal and intravenous infusion. The terminal half-life in a dose of 2.5 mg/kg after intraportal infusion was 22 min, and significantly different from those in higher doses after portal vein infusion. From the facts mentioned above, it therefore would seem that the dose-dependent hepatic elimination of propranolol after portal vein infusion is related to the dependence of a quantity of hepatic uptake of the drug on dose and infusion rate, and also to the alteration of pattern of the subsequent metabolism. Quantitative *in vivo* studies on the liver uptake and metabolism of propranolol during the first passage through the liver are now in progress.

Acknowledgement The authors thank Miss H. Komuro for her excellent technical assistance.

14) D.G. Shand, G.H. Evans, and A.S. Nies, *Life Sci.*, **10**, 1417 (1971).
15) S. Isozaki, Y. Saitoh, and T. Suzuki, unpublished data.
16) A. Hayes and R.G. Cooper, *J. Pharmacol. Exptl. Therap.*, **176**, 302 (1971).