was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a crystalline residue, whose TLC is shown in Fig. 1a. It was repeatedly recrystallized from MeOH to afford XIa (7 mg). mp 257—264° (decomp.), UV $\lambda_{max}^{max}m\mu(\log\varepsilon)$: 217 (4.18), IR ν_{max} cm^{-1}: 3410 (OH), 1796 (sh), 1771 (sh), 1731, 1626 (butenolide). *Anal.* Calcd. for $C_{23}H_{33}O_4Cl$: C, 67.55; H, 8.13. Found: C, 67.33; H, 8.05.

Reduction of 3β-Acetoxy-14-chloro-15-oxo-5β,14β-card-20(22)-enolide (IXb) with $NaBH_4$——To a solution of IXb (300 mg) in MeOH (150 ml) was added $NaBH_4$ (300 mg) at 0° for 10 min, and the reaction mixture was allowed to stand at 0° for 1.5 hr. After addition of AcOH (1.5 ml) and H_2O (50 ml) it was concentrated *in vacuo* and the product was extracted with $CHCl_3$. The organic layer was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a crystalline residue, whose TLC is shown in Fig. 1b. Repeated fractional crystallizations of the residue from aectone–ether afforded Xb (63 mg), which was identical with an authentic specimen in the usual criteria (TLC, mixed melting point, IR), and XIb (180 mg). mp 160—166° (decomp.), $[\alpha]_D^{25}$ −14.9° (c=1.35, $CHCl_3$), UV $\lambda_{max}^{max}m\mu(\log\varepsilon)$: 217 (4.20), IR ν_{max} cm^{-1}: 3480 (OH), 1803 (sh), 1778 (sh), 1741, 1730 (sh), 1628 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{35}O_5Cl$: C, 66.57; H, 7.82. Found: C, 66.86; H, 8.09.

A solution of XIb (5 mg) and CrO_3 (1.5 mg) in AcOH (0.3 ml) was allowed to stand at 20° for 18 hr. To the reaction mixture was added MeOH (0.5 ml) and then H_2O (1 ml). The product was extracted with $CHCl_3$, and the organic layer was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* gave a crystalline residue which was recrystallized from MeOH to afford IXb (2 mg), mp 200—203° (decomp.), identical with an authentic sample in the usual criteria (TLC, mixed melting point, IR).

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Drug Absorption and Metabolism Studies by Use of Portal Vein Infusion in the Rat. I. Pyloric Vein Cannulation and Its Application to Study of First-Pass Effect on Bioavailability of Propranolol

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The rate and extent of drug absorption into the systemic circulation have been estimated by pharmacokinetic analysis of plasma concentration-time data or urinary excretion data.[2,3] The percent of absorption can be assessed by comparison of the relative areas under the plasma concentration-time curves after oral and intravenous administration. This method is based on the presumptions that the distribution and elimination of a drug may be expressed in terms of first-order kinetics within the dose ranges studied and that the parameters of these processes remain constant after administering the same quantity of drug by different routes. Thus, the resultant areas are independent of the route of administration and proportional to the dose even when given by different routes. However, it has recently been shown that the areas under the blood level-time curves for aspirin[4] and lidocaine[5] after infusion into a peripheral vein were considerably great as compared with results observed after infusion of an equal dose

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2) J.G. Wagner and E. Nelson, *J. Pharm. Sci.*, **52**, 610 (1963).
3) J.C.K. Loo and S. Riegelman, *J. Pharm. Sci.*, **57**, 918 (1968).
4) P.A. Harris and S. Riegelman, *J. Pharm. Sci*, **58**, 71 (1969).
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into the hepatic portal vein of the dog. Dollery, *et al.*[6] have shown that substantial differences in drug action for two drugs, propranolol and isoproterenol, arise in man and animals after oral and intravenous administration, since these drugs are differently metabolized when given by different routes. In addition, Shand, *et al.*[7] have reported that in the same human subjects, the estimated percentage of oral dose of propranolol reaching the systemic circulation was calculated to be 16 to 60 from the ratio of the areas under the plasma concentration curves after oral and intravenous administration, and that this probably resulted from appreciable metabolism of propranolol during its first passage through the liver.

In most instances, administration of a drug directly into the portal vein may be equivalent to oral administration, if one assumes complete drug absorption and an absence of drug metabolism in the intestinal wall. However, after intravenous administration of a drug, only about 30% of the drug-containing blood traverses the liver in the first circulatory pass,[4] although all the drug molecules absorbed after oral administration usually must traverse through the liver before reaching the vascular site being sampled for analysis. The reduction in area after portal vein infusion may be attributed to a significant degree of metabolism during the first passage of a drug through the liver before the systemic circulation. Bioavailability has been defined, therefore, as a term indicating the relative amount of an administered drug reaching the systemic circulation, which refers primarily to the venous blood (excepting the hepatic portal blood during the absorptive phase) and arterial blood.[8] The purpose of this report is to present a method of pyloric vein cannulation in the rat, which is useful for drug absorption and metabolism studies, and to investigate the influence of the first-pass effect on the bioavailability of propranolol after portal vein infusion.

Result and Discussion

Pyloric Vein Cannulation

A male Wistar rat weighing 200 to 240 g was anesthetized lightly with ether at suitable intervals, and the abdomen was opened through a midline incision. The pyloric vein was exposed by pushing aside the duodenum of the intestine, and a minimal amount of connective tissue was removed from the pyloric vein to facilitate ligation. The pyloric vein was then ligated approximately 0.7 cm distal to the junction of the hepatic portal vein and pyloric vein. A cannula (polyethylene tubing, i.d. 0.04 cm, o.d. 0.06 cm, length 7 cm), filled with heparin solution (200 units/ml) and closed with an arterial clamp at the other end, was inserted into the pyloric vein, approximately 0.2 cm proximal to the ligature, after careful venous puncture using a needle point. The cannula was gently guided upward into the portal vein so that its tip projected several millimeters into the vein (Fig. 1). The cannula was secured by ligation with nylon suture, and a syringe was attached to the end of the cannula. It was then confirmed that the portal blood could be detected in the cannula with a slight suction. The abdominal incision was closed, and the syringe was replaced by another syringe containing infused solution. At the end of the infusion experiment, it is desirable to inject methylene blue solution at an appropriate concentration into the cannula and to observed the liver stained with the dye in order to check for lack of leakage during infusion.

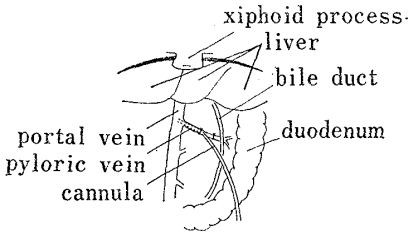

Fig. 1. Pyloric Vein Cannulation

Mesenteries were retracted to show the cannulation.

6) C.T. Dollery and D.S. Davies, *Ann. N.Y. Acad. Sci.*, **179**, 108 (1971).
7) D.G. Shand, E.M. Nuckolls, and J.A. Oates, *Clin. Pharmacol. Therap.*, **11**, 112 (1970).
8) "Guidelines for Biopharmaceutical Studies in Man," ed. by APhA Academy Sciences, Washington, D.C., 1972, p. 17.

First-Pass Effect on the Bioavailability of Propranolol

Figures 2 and 3 show the mean blood concentrations of propranolol after rapid infusion into the femoral vein and after constant infusion during 50 min into the portal vein at doses of 2.5 and 12.5 mg/kg propranolol. Each point represents the mean and standard error of the estimation from three rats after intravenous infusion or five rats after portal vein infusion. The blood concentration-time curve of propranolol after intravenous infusion was described in all rats by a biexponential equation of the form $C=Ae^{-\alpha t}+Be^{-\beta t}$. Table I lists the estimated parameters A, B, α, β, and the total areas under the curves calculated from these parameters. The area under the blood concentration-time curve was directly proportional to the dose administered, since the ratio of the mean area at a dose of 2.5 mg/kg to that

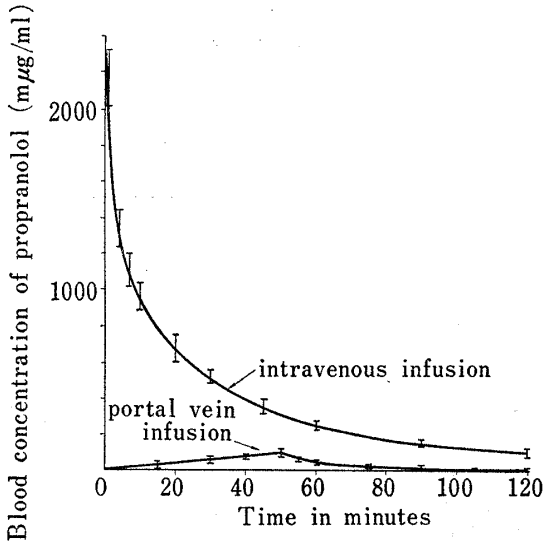

Fig. 2. Mean Blood Concentration-Time Curves of Propranolol in Rats after Intravenous and Portal Vein Infusion of 2.5 mg/kg Propranolol

Intravenous doses were given within 30 sec into the femoral vein, and intraportal doses were given at a constant rate during 50 min into the hepatic portal vein. Vertical bars represent standard errors of the estimation from three rats after intravenous infusion or from five rats after portal vein infusion.

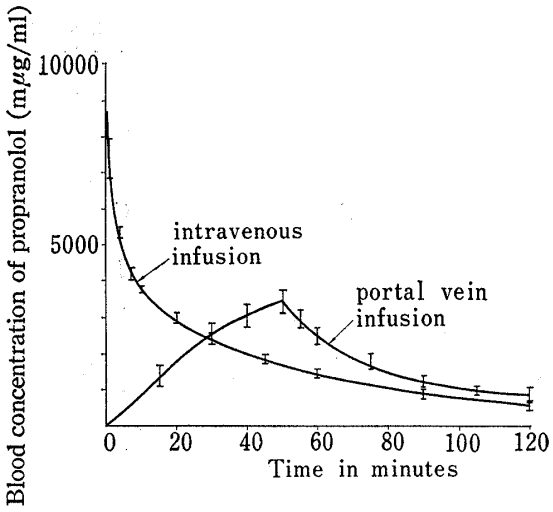

Fig. 3. Mean Blood Concentration-Time Curves of Propranolol in Rats after Intravenous and Portal Vein Infusion of 12.5 mg/kg Propranolol

Intravenous doses were given within 30 sec into the femoral vein, and intraportal doses were given at a constant rate during 50 min into the hepatic portal vein. Vertical bars represent standard errors of the estimation from three rats after intravenous infusion or from five rats after portal vein infusion.

Table I. Pharmacokinetic Parameters of the Two Exponential Components and Areas under the Blood Concentration-Time Curves after Rapid Intravenous Infusion of Propranolol

Dose (mg/kg)	Rat	A (mμg/ml)	B (mμg/ml)	α (min^{-1})	β (min^{-1})	Area[a] (mμg·min/ml)	Relative area
2.5	A	1280	863	0.296	0.0203	46837	
	B	1954	1240	0.427	0.0266	51193	
	C	1175	1047	0.207	0.0192	60208	
	Mean	1470	1050	0.310	0.0220	52746	1.0
12.5	D	5623	4677	0.378	0.0214	233427	
	E	4027	3548	0.249	0.0126	297760	
	F	3162	4677	0.205	0.0199	250450	
	Mean	4271	4301	0.277	0.0180	260546	4.9[b]

a) Calculated as $(A/\alpha+B/\beta)$.
b) Compared with the area for a dose of 2.5 mg/kg.

at a dose of 12.5 mg/kg was 4.9. On the other hand, the mean areas for the portal vein infusion curves were found to be 7.8% (2.5 mg/kg) and 90.9% (12.5 mg/kg) of those for the corresponding intravenous infusion curves (Table II). The area at the lower dose de-

Table II. Areas under Blood Concentration-Time Curves after Constant Intraportal Infusion of Propranolol during 50 min

Dose (mg/kg)	Rat	Area[a] (mμg·min/ml)	Ralative area[b]
2.5	G	2468	
	H	4000	
	I	5983	
	J	5683	
	K	2513	
	mean	4129	0.078
12.5	L	161141	
	M	213612	
	N	268070	
	O	277642	
	P	263720	
	mean	236837	0.909

a) Calculated using the trapezoidal rule. The area for the tail end at a dose of 12.5 mg/kg was calculated by C_t/β. C_t is the blood concentration at time t, and the rate constant β was estimated from the terminal slope of a semilogarithmic plot of the blood concentrations of propranolol after the end of infusion.

b) Compared with the areas for the corresponding intravenous doses. Significantly different at a dose of 2.5 mg/kg ($p<0.001$) and not significantly different at a dose of 12.5 mg/kg ($p<0.4$).

creased significantly as compared with the area observed upon infusion of the equal dose into the peripheral vein, while the areas for the hepatic portal vein infusion and peripheral vein infusion at the higher dose were not significantly different. These findings show that a fraction of the intact drug may reach the systemic circulation at lower intraportal doses. The reduction in area under the blood concentration-time curve after portal vein infusion must be attributed to the fact that a significant degree of metabolism occurred during the first passage of propranolol through the liver.

Hayes and Cooper[9] demonstrated that in the dog and monkey, the metabolic pathway of propranolol was different after oral and intravenous dosings, and that the presence of a pharmacologically active metabolite, 4-hydroxypropranolol, was observed after oral dosing but not intravenous dosing of propranolol. Furthermore, propranolol after intravenous administration was shown to be concentrated in the lung and, to a lesser extent, in the liver, kidney, brain and heart in dogs. Since there is such a higher degree of tissue uptake by the lung, the liver will receive only a part of propranolol after intravenous administration. The rate for introduction of a drug into the liver may influence on its metabolic rate and the quantitative relation between its metabolites. The difference in the metabolic pathway and the first-pass effect of propranolol arising from the different route of administration would appear to result from this mode of characteristic drug distribution.

The portal vein infusion using the pyloric vein cannulation overcomes several disadvantages inherent in the use of direct cannulation into the portal vein, since an intact blood supply to the liver can be maintained during surgery. Administration of a drug into the portal blood has been attempted only in the dog to study differences in areas under blood level-time curves as a function of route of administration.[4,5] The method presented in previous work was based on cannulation into a branch of the splenic vein in the dog.[4] The method of portal

9) A. Hayes and R.G. Cooper, *J. Pharmacol. Exptl. Therap.*, **176**, 302 (1971).

vein infusion in the rat is simple and practical as compared with that in the dog. This procedure in the rat has proven useful in drug absorption and metabolism studies. Details of these studies will be presented in a subsequent paper.

Experimental

Materials——Propranolol[10] was used as its hydrochloride and racemic compound throughout this study and without further purification (mp 162—163°). All other chemicals used in this study were of reagent grade.

Animal Experiments——An intravenous infusion of propranolol (0.5 ml) at a dose of 2.5 or 12.5 mg/kg was given to the right femoral vein within 30 sec. An infusion of propranolol into the portal vein at the same doses was given at a constant rate of 0.0228 ml/min during 50 min by means of an infusion pump (NATSUME Model KN-1H). Blood samples (0.1—0.3 ml) were taken at various times through the cannula inserted into the left femoral artery. An approximately equal volume of blood to sampled blood was transfused each time through the cannula inserted into the left femoral vein, since the blood loss under the conditions described would have possibility of leading to circulatory disturbances. 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.*[7] One milliliter of distilled water was added to 0.1 to 0.3 ml of whole blood. The mixture was then alkalinized with 1 ml of 1N NaOH, and extracted into 12 ml of *n*-heptane containing 1.5% isoamyl alcohol. After centrifuging, 10 ml of the heptane phase was extracted into 1 ml of 0.1N HCl, and the fluorescence of the acid phase was determined on a HITACHI Model 203 spectrophotofluorometer (maximum excitation at 295 mμ and maximum emission at 360 mμ).

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10) Kindly furnished by Sumitomo Chemical Co., LTD.