STUDIES ON THE NUTRITIONAL SIGNIFICANCE OF THE PORTAL BLOOD IN RUMINANTS I. ON THE MEASUREMENT OF BLOOD FLOW OF HEPATIC VEIN AND PORTAL VEIN BY MEANS OF HEPATIC VEIN CATHETER

STUDIES ON THE NUTRITIONAL SIGNIFICANCE OF THE PORTAL BLOOD IN RUMINANTS

I. ON THE MEASUREMENT OF BLOOD FLOW OF HEPATIC VEIN AND PORTAL VEIN BY MEANS OF HEPATIC VEIN CATHETER

By

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It is a characteristic of the ruminant nutrition that the ruminant has complicated ruminal fermentation previous to the absorption of nutrients into the blood circulation.

As regard to the microbial dissimilation and assimilation for nutrients in the rumen, conversion of ingested carbohydrate (mainly cellulose and hemicellulose) to volatile fatty acids, incorporation of dietal non-protein nitrogen into microbial protein and biosynthesis of many kinds of vitamins are well known. Particularly, it is of interest that volatile fatty acids resulting from rumen fermentation furnish the principal part of utilized energy to the metabolism of ruminants (1, 2, 3).

The ultimate purpose of this study is to know the amounts of volatile fatty acids and other fermentation products which come from the digestive tract and enter the liver.

Generally concentration of volatile fatty acids in the rumen content shows the range from 200 to 900 mg per dl. However, volatile fatty acids are hardly produced when absorption through the rumen wall into the circulation is rapid (4, 5). Therefore it is difficult to know the total amounts of volatile fatty acids produced in the rumen by means of analysis of the rumen content. Hitherto, Shaw (6) used the rumen perfusion method, and Stewartz (7) analysed the rumen contents in order to calculate the amounts of volatile fatty acid produced in the rumen of the cow. Conrad (8), who measured the blood flow of the gastrosplenic vein, informed the amounts of volatile fatty acids absorbed from the forestomach of the calves. The authors under-

took the measurement of the total amounts of volatile fatty acids and other nutrients entering from digestive tract and flowing into the liver, by multiplying the blood flow rate of the portal vein by arterio-veneous differences of the nutrients in the portal blood.

I. Measurement of hepatic blood flow using technique of hepatic vein catheter.

A number of informations have been made concerning the method for measurement of blood flow. Thermostromur (9), bubble-flowmeter (10), rotermeter (11) and electromagnetic flowmeter (12) were used as direct methods for measurement. Recently, indirect methods for measuring of hepatic blood flow are such as clearance of urea (13), bromsulphalein (14) and radioactive colloid (15), which were developed with the aid of the hepatic vein catheter.

We measured the hapatic blood flow, using the technique of hepatic vein catheter prior to measurement of portal blood flow in the goats.

Experimental Procedure

a) *Experimental animals*

The goats of Saanen breed were used as the experimental animals. The goat was grazed on the grass land, but the feeding was interrupted for 24 hours previous to the measurement.

b) *A principle of technique of hepatic vein catheter for measuring of hepatic blood flow.*

The principle of this method is based on Fick's theorem and the calculation of the blood flow rate is expounded by following formula, $HBF = S/H-A$, where HBF is the hepatic blood flow, S is the amounts of a marker which is injected into the blood circulation for a definite period, H and A are concentrations of a marker in the hepatic and arterial blood respectively at the same time. In this case, it is absolutely necessary for the nature of marker to be treated or excreted only in the liver. We used bromsulphalein as the marker. Bromsulphalein has been used as a reagent for diagnosis of the hepatic function concerning on the ability of excretion of foreign substances.

c) *Blood sampling*

Sample of blood from the hepatic vein was taken by the aid of a hepatic vein catheter and arterial blood was obtained from the carotid artery by a syringe. These samplings were done at the same time.

A polyethylen tube (5 mm in diameter) was used as a hepatic vein catheter. The catheter was entered from the right jugular vein into the blood circulation and its tip was placed in the hepatic vein located at the left leaf of the liver via the right ventricle of heart. To prevent blood

coagulation, the heparin in saline solution was dropped into the circulation from end of the catheter.

d) *Injection of marker*

Bromsulphalein (BSP) solution (100 mg/dl) was injected constantly for an hour into the circulation from the medial planter vein by a speed-controlled injector. Concentration of BSP in the blood serum was determind colorimetrically (570 mμ) after the addition of N/10 NaOH solution.

Result

The data of estimation on No. 1 goat is shown in Fig. 1.

In Fig. 1, the curve of concentrations of BSP in the arterial blood runs parallel with that of the blood of the hepatic vein during 20 to 30 minutes of the experiment. This suggests that, in this period, the amount of the injected BSP was equal to the amount of the BSP excreted into the goal duct by hepatic function. Hence, by dividing the amount of BSP injected during this period by the difference between the concentration of BSP in the blood of the hepatic vein and that of arterial blood, the blood flow of the liver was calculated. In the same way, the results of measurment of the hepatic blood flow of seven goats are given in Table 1. As shown in

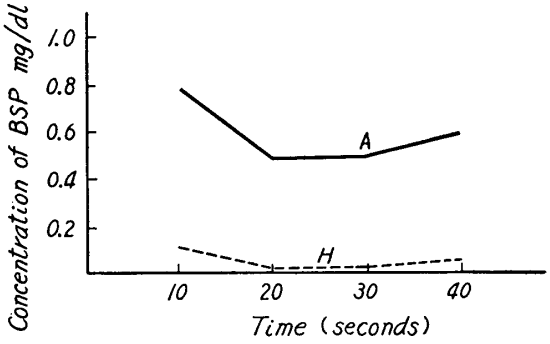

Fig. 1 The concentrations of bromsulphalein in the blood during injection of bromsulphalein by speed-controlled injector.
A : Arterial blood
H : Hepatic venous blood

Table 1. The rates of hepatic blood flow, which was measured by means of BSP clearance using hepatic veins catheter technique, in the goat.

Goat No.	Body Weight (A)	Hepatic Blood Flow (B)	B/A
1	23.0 kg	193 cc/min.	8.4 cc/min./kg
2	34.0	625	18.2
3	21.0	197	9.4
4	16.5	171	10.4
5	20.5	205	10.0
6	33.5	711	21.2
7	20.5	354	17.2
8	24.0	619	25.8

Table 1, the obtained data varied with wide range. It appears that these variations are probably due to the differences of influence resulting from anesthesia and feeding condition in the experimental period. It has been

reported that the hepatic blood flow in the dog (16) was 40 cc per kg per minute and the hepatic blood flow in the man (17, 18) was 860 cc per sqm. per minute. Conrad (19) informed that the blood flow in the gastrosplenic vein of calves was 365 to 872 cc per minute per 100 lb. of body weight. It appears that our data are considerably lower than that of the values measured in the dog, man and calf.

II. Measurement of the rate of portal blood flow

As above mentioned, we measured the amounts of hepatic blood flow by means of the hepatic vein catheter technique. The purpose of this paper is to measure the rate of the portal blood flow.

Ueda (20) informed the procedure in which hepatic blood flow was divided into portal blood flow and hepatic arterial blood flow by the following method.

They injected evans blue from the peripheral vein into the blood circulation and carried out sampling of the blood from the hepatic vein and artery (carotid was used) at intervals of 3 to 5 seconds after evans blue injection. Their procedure is shown schematically in Fig. 2.

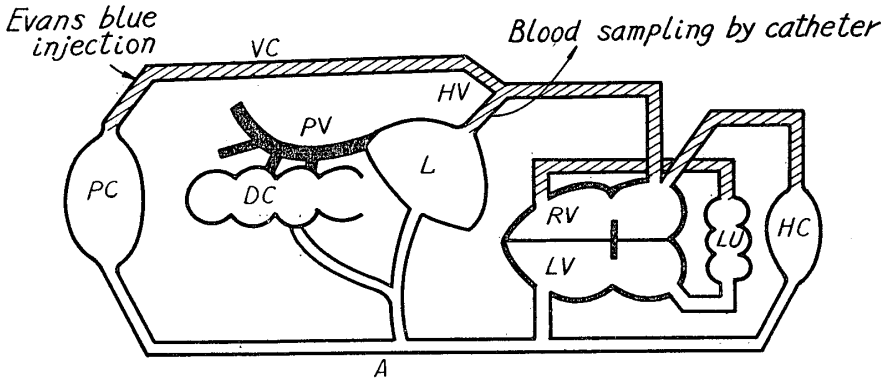

Fig. 2 Schematic illustration of Ueda's method for separating hepatic blood flow into protal and hepatic arterial blood flow.

HC : Head Circulation	PV : Portal Vein
LU : Lung	DC : Digestive Cavity
RV : Right Ventricle	VC : Vena Cava
LV : Left Ventricle	PC : Peripheral Circulation
HV : Hepatic Vein	A : Aorta
L : Liver	

From the results of determinations of evans blue in the blood serum on each samplings, the curves of dye concentration as shown in Fig. 3.

They described that the first (HA) of the two peaks found in the curve of evans blue concentration in the blood taken from the hepatic vein was due to evans blue of arterial blood and the second (PV) of the two peaks were due to evans blue of portal blood, hence the comparison of the area (A′) of the peak of evans blue in the arterial blood with the area (H′) of the first peak

in the curve of evans blue of blood from hepatic vein, may give the rate of dilution of the concentration of evans blue in blood of hepatic artery by portal blood in which evans blue is not contained at this time.

Hence, following equation was given,

$$\frac{CA \times ta}{CH \times th} = \frac{A + P}{A}$$

$$\frac{CA \times ta}{CH \times th} - 1 = \frac{P}{A}$$

CA : Mean concentration of evans blue in arterial blood.

CH : Mean concentration of first peak of evans blue in hepatic venous blood.

ta : half of time which evans blue passed through an arterial blood.

th : half of time which evans blue passed through the hepatic venous blood flow.

A : rate of hepatic arterial blood flow.

P : rate of portal blood flow.

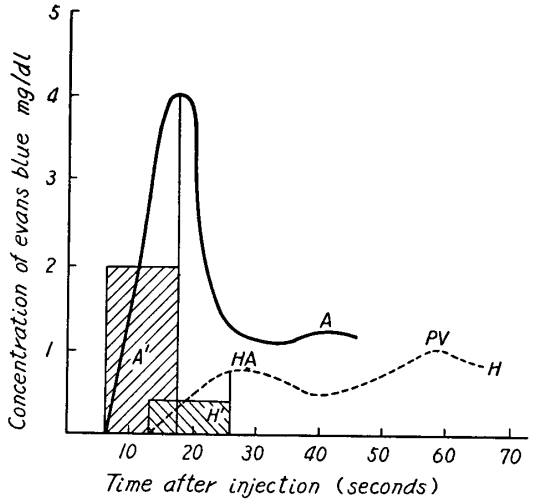

Fig. 3 The concentrations of evans blue in the blood of hepatic vein (H) and peripheral arterial blood (A).

By means of Ueda's procedure, we measured the rate of portal blood flow in the goat.

Experimental Procedure

The goat was anesthetized with pentobarbithal. To get the blood samples from the hepatie vein, the catheter was inserted into the hepatic vein located at the left leaf of the liver as above mentioned. Another polyethylen tube, for the injection of dye, was inserted into the medial planter vein. Evans blue was injected quickly into the blood circulation from the tube, blood samplings were carried out from hepatic vein and carotid artery with intervals of each 3 seconds.

Blood serums were diluted by physiological saline and concentrations of evans blue in the serum were determined colorimetrically (610 mμ).

Results

a) *A Result of the goat (No. 1)*

The curve in Fig. 4 showed a result of the experiment in goat No. 1.

In arterial blood, evans blue appeared at 9 seconds after its injection and

the maximum concentration occured at 20 seconds. Subsequentely, the concentration of evans blue decreased sharply.

On the other hand, in the blood of the hepatic vein, evans blue appeared at 27 seconds after its injection, increased gradualy and then the curve leveled off at 47 seconds. A little later its concentration somewhat increased again.

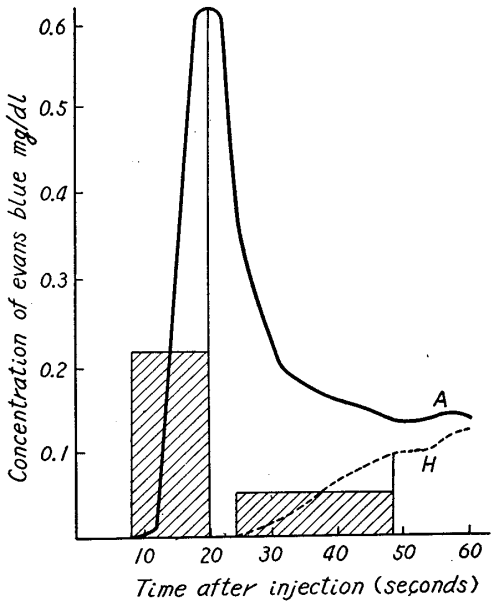

Fig. 4 The concentrations of evans blue in the blood of hepatic vein (H) and artery (A) after injection of evans blue in the goat No. 1.

As Ueda said, it appears that the curve of evans blue in the blood of the hepatic vein contains two peaks, but it is very difficult to draw a line between the first and the second peak of the curve.

Hereupon, when the hepatic blood flow was divided into portal blood flow (P) and blood flow of the hepatic artery (A) from the result shown in Fig. 4, the ratio of P to A was 1.16. As mentioned above, hepatic blood flow of the portal vein and hepatic artery were 106.1 cc and 91.4 cc per minute respectively.

b) *Result of experiment in the goat (No. 2)*

Fig. 5 contains the result of experiment using goat No. 2

In arterial blood, evans blue appeared at 4 seconds after its injection and increased quickly until the maximum at 12 seconds, but the level decreased sharply during the next 4 seconds.

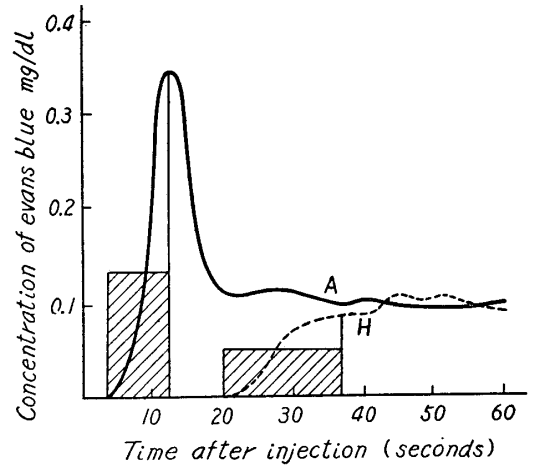

Fig. 5 The concentrations of evans blue in the blood of hepatic vein (H) and artery (A) after injection of evans blue in the goat No. 2.

In blood circulation of the hepatic vein, evans blue appeared at 20 seconds after the injection, with subsequent increase, and the level arrived to a balance at 36 seconds. At 40 seconds, evans blue level apparently increased again.

According to the obtained data, the ratio of blood flow of the portal vein to that of hepatic artery was calculated as 0.4.

Regarding to the ratio of blood flow of the portal to hepatic artery, many investigations using the dog and the man showed the value of 3 to 7. Our results on the goat differ from those data, probably owing to the accuracy of the procedure of measurement employed rather than those factors related with the different animals used. In the curve of evans blue of the blood of the hepatic vein shown in Figs. 2 and 3, it is very difficult to distinguish the first peak from the second, these peaks were expounded as an arterial and portal peak respectively by Ueda. Hence, comparing the area of evans blue which appeared in the arterial blood with that of the first peak which appeared in blood of the hepatic vein, it is difficult to set up a clear datum line of the area. It is suggested that this is due to the fact that the evans blue injected from the peripheral vein into circulation is diluted with the blood from the general circulation in the heart, therefore, only a few portions of injected evans blue may arrive at the portal system.

Hence, if the evans blue was injected from the posterior aorta nearby the coeliac artery, an appearence of a clear peak of evans blue in the blood of hepatic vein would be expected.

Experimental procedure

The goat anesthetized with pentobarbithal (25 mg/kg).

To obtain the blood sample from the hepatic vein, a catheter was placed from the jugular vein to the hepatic vein, according to the procedure above mentioned. And to inject evans blue into the circulation, another catheter

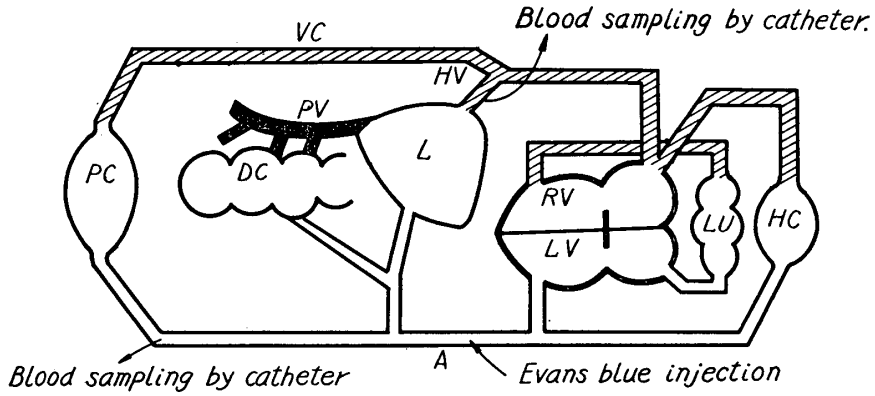

Fig. 6 Schematic illustration of modification of Ueda's procedure to separate the hepatic blood flow into portal and hepatic arterial blood flow.

HC : Head Circulation	PV : Portal Vein
LU : Lung	DC : Digestive Cavity
RV : Right Ventricle	VC : Vena Cava
LV : Left Ventricle	PC : Peripheral Circulation
HV : Hepatic Vein	A : Aorta
L : Liver	

was inserted from the right carotid artery into the blood circulation and was

put quickly into the posterior aorta near by the coeliac artery. Evans blue solution was injected speedily into the blood circulation from this catheter. (Fig. 6.) After injection of evans blue, blood samplings from the hepatic vein and posterior aorta were carried out at intervals of each 3 seconds. Evans blue in the blood serum was determined colorimetrically.

Results

The result of measuring by modification of Ueda's method is shown in Fig. 7.

In the arterial blood, the evans blue that appeared at the first 3 seconds after its injection reached the maximum at the next 3 seconds and then its falling occured sharply. On the other hand, in the blood of the hepatic vein, the evans blue appeared at 6 seconds after its injection, the maximum occuring at 15 seconds. Subsequently, the level fell until 30 seconds, and it was maintained in balance during a period of 30 to 42 seconds.

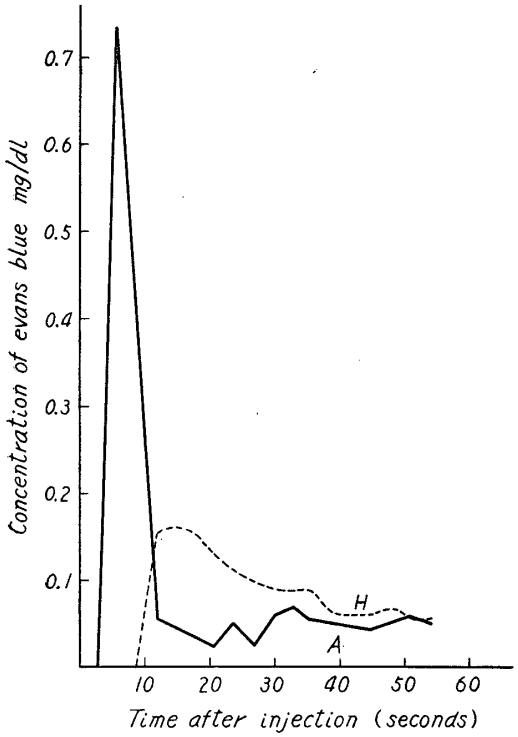

Fig. 7 The concentrations of evans blue in the blood of hepatic vein (H) and artery (A) after injection of evans blue into posterior vena cava in the goat.

It appears that the curve of the evans blue in the blood of the hepatic vein contains two peaks. In spite of our modification, however, the obtained result resembled that in the case when the evans blue was injected into the peripheral vein. That is to say, it is difficult to draw a clear line of demarcation between the peak and the peak.

Discussion

The hepatic blood flow was measured by means of the hepatic vein catheter technique using BSP clearnace in the goat.

The results obtained varied from 8.4 to 25.8 cc per min. per kg. It appears that the variation is due to the degree of accumulation of food in the digestive tract or to the degree of anesthetization during the measurement. No information has been reported on the rate of hepatic blood flow in the goat. The hepatic blood flow that has been measured for the dog and the man exceeds considerably our data. This difference is not clearly explained.

The purpose of this paper was to measure the rate of the portal blood flow. The procedure of evans blue clearance described by Ueda was employed. The ratio of the blood flow of the portal to the hepatic artery was 1.16 and 0.4. These values were considerably less than the other measure for the dog and man. (21, 22)

However, disregarding the data stated above, we do not hold that the rate of the portal blood flow in the goat was peculiarly less than that of the portal blood flow in other animals. We prefer to think that this difference was due to the obscurity of the border of two peaks of the evans blue which appeared in the blood of the hepatic vein. For this reason, an accurate comparison between the area of the peak in the arterial blood and that of the first peak in the blood of hepatic vein was difficult.

Thereupon, we modified Ueda's procedure and the evans blue was injected from the posterior aorta, not from the peripheral vein, into the blood circulation. It is because of that the injected evans blue was not diluted by the blood of other circulation systems and not divided into various pathways of circulation, and so considerable amounts of evans blue may reach to the digestive tract and liver. However, the data obtained resembled the data resulting from the measurement by means of Ueda's method. Mellinkoff (23), who used radioiodinated serum albumin as a marker, determined the cardio-portal circulation time by external scintillation counting. His hepatic scintillogram was similar to the curve of the evans blue in the blood of the hepatic vein which we measured for the goat. And so the first peak was indistinguishable from the second peak. These data indicate that it is very difficult to separate the hepatic blood flow into the rate of portal blood flow and arterial blood flow by means of Ueda's procedure.

Summary

According to the characteristic of the nutrition of the ruminant brought to light, it has became an important problem to know the amount of fermentation products resulting from the microbial activity in the rumen.

We attempted to measure the amount of volatile fatty acids and other nutrients entering from the digestive tract into the liver, by multiplyng the rate of the portal blood flow by arterio-venous differences of the contents of the nutrients between the portal and carotid arterial blood. In this paper, the methods for measuring the rate of the portal blood flow in the goat were examined. At first, the hepatic blood flow was measured by means of Bromsulphalein clearance method using hepatic vein catheter technique and the blood flow rate of 8.4 to 25.8 cc per kg was obtained.

Next, by means of Ueda's procedure which is based on the dilution of evans blue by portal circulation, the ratio of portal blood flow to hepatic

arterial blood flow was estimated and the value of 1.16 and 0.4 were obtained. However, for the reason of obscurity of the boundary of the two peaks of the evans blue which appeared in the blood of hepatic vein, it is very difficult to estimate accurately the ratio of portal blood flow to blood flow of hepatic artery.

Accordingly, we modified Ueda's procedure and injected evans blue from the posterior aortic artery, not from the peripheral vein, into the blood circulation. However, the obtained results were similar to the results shown in Ueda's procedure.

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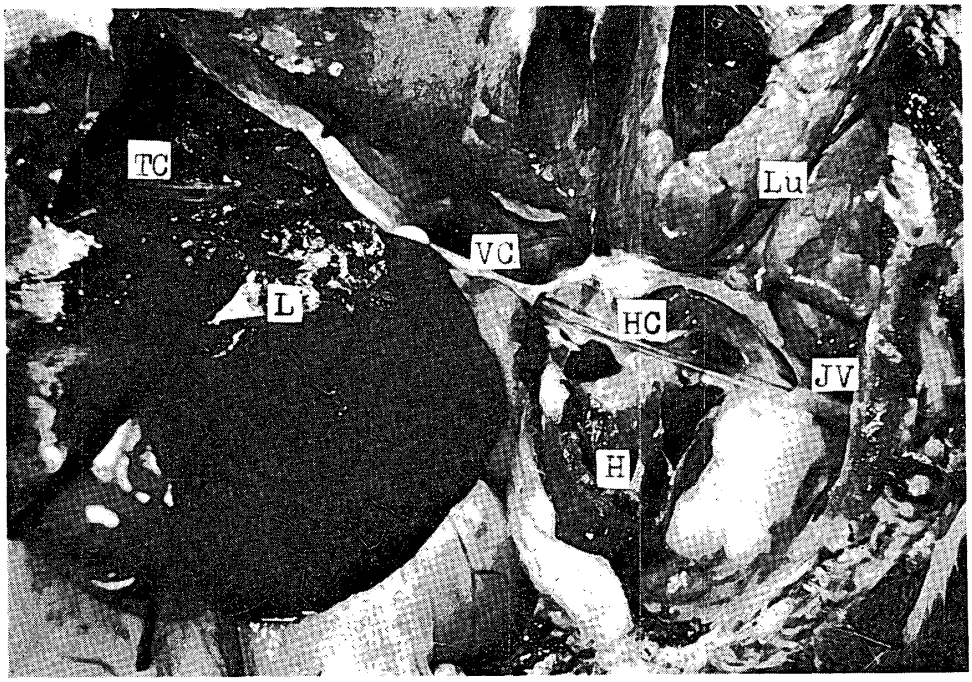

Plate 1. The collection of the hepatic venous blood by hepatic vein catheter in the goat.

L : Liver. VC : Vena Cava.
H : Heart. HC : Hepatic vein Catheter.
Lu : Lung. TC : Tip of the Catheter.
JV : Jugular vein.

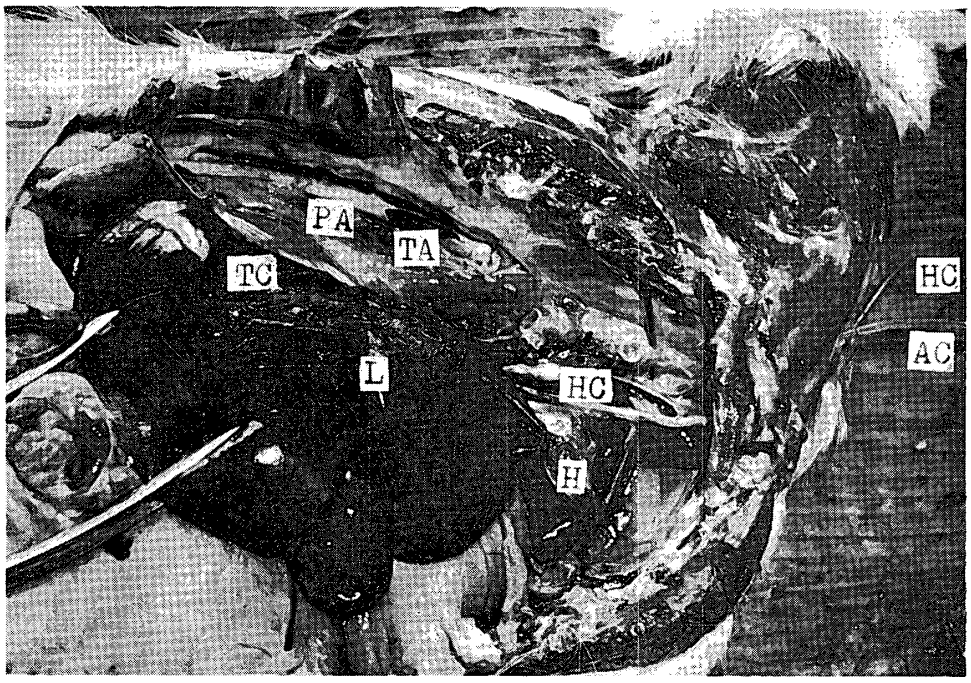

Plate 2. The injection of evans blue solution into aortic blood circulation by aortic catheter and the collection of the hepatic venous blood by hepatic vein catheter.

L : Liver. AC : Aortic Catheter.
H : Heart. HC : Hepatic Vein Cathter.
PA : Posterior Aorta. TC : Tip of Hepatic Vein Catheter.
JV : Jugular Vein. TA : Tip of Aortic Catheter.

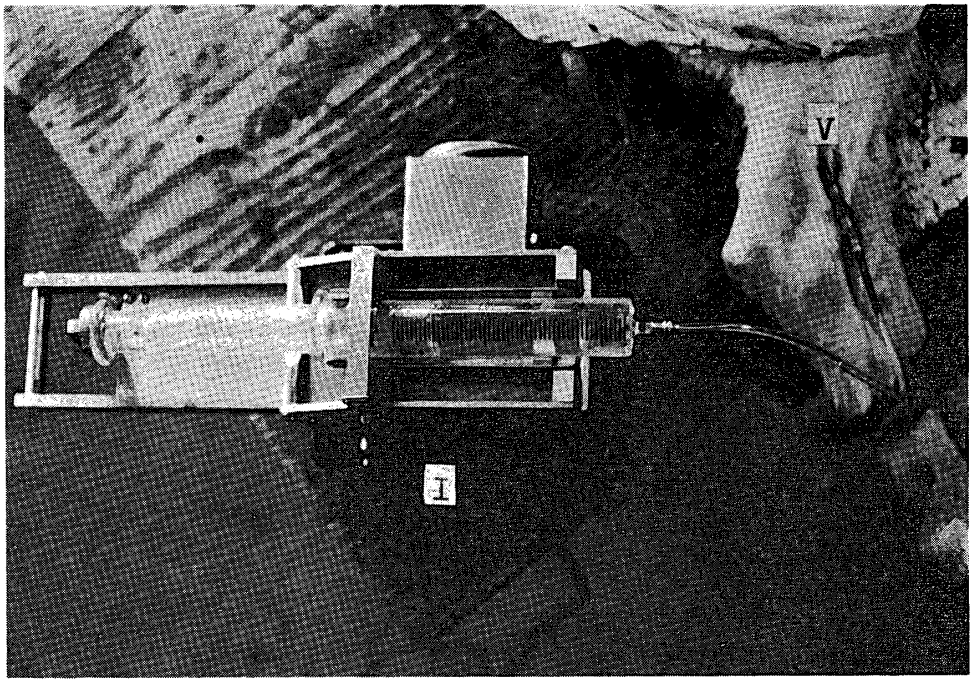

Plate 3. The injection of Bromsulphalein solution from medial planter vein into blood circulation by a speed-controlled injector.
I : Speed-controlled injector. V : Medial Planter Vein.