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Portal vein blood flow rate in the young bovine

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PORTAL VEIN BLOOD FLOW RATE IN THE YOUNG BOVINE

by

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ABSTRACT

Thirty-four young calves were fitted with catheters for dye injection and blood collection to measure portal vein blood flow rate. Initially, double-lumen polyvinyl catheters were established via the anterior mesenteric vein. Separate silicone rubber tubes used in later experiments (one established in the anterior mesenteric vein for dye injection, the other established directly through the portal vein wall for blood collection) were easier to maintain patent and properly positioned. Blood flow rate was calculated by computer analysis of indocyanine green dye-dilution curves. For the seven calves for which values were obtained, the body weight in kg; the number of values obtained; and the range, mean and standard error of flow rate in ml/sec for each calf were, respectively: 66, 8, 19.5 - 73.2, 51.4 $\pm$ 6.5; 62, 25, 8.4 - 39.3, 17.2 $\pm$ 1.4; 73, 12, 29.3 - 73.8, 42.6 $\pm$ 3.8; 100, 44, 39.9 - 100.1, 68.5 $\pm$ 6.7; 105, 33, 45.7 - 130.5, 82.6 $\pm$ 4.2; 138, 6, 73.8 - 82.3, 79.0 $\pm$ 1.0; and 100, 66, 36.6 - 198.6, 102.5 $\pm$ 4.5. Flow rate was highly variable among animals and throughout the day and from day to day within animals. The data suggest that flow rate for a given time should be calculated as the average of three to six values obtained within 15 minutes and should not be used to represent the rate at any other time.

INTRODUCTION

There are limitations to any known method for studying nutrient absorption from the gastrointestinal tract of ruminants. Indirect methods have been used to compare the absorptive ability of different animals, but only qualitative comparisons were possible with such methods.

Calculation of accurate values for absorption rate requires measurement of portal vein blood flow rate and nutrient concentration in the arterial and portal blood. Some attempts have been made to measure portal vein blood flow in conscious and anesthetized sheep and in calves. However, determination of flow rate was usually secondary to other phases of the research, and methods for determining it were not fully developed and perfected. Furthermore, no values have been reported concerning the variability in blood flow rate in a series of determinations made in a short time, throughout the day in any animal, from day to day in the same animal, and in similar animals under different physiological conditions.

The purpose of this research was to develop and perfect a method for measuring portal vein blood flow rate and to study variability in the flow rate.

LITERATURE REVIEW

The methods of Sutton et al. (1962, 1963) are similar to those of other workers who have measured absorptive ability of the rumenoreticulum by indirect methods. Rumen contents were replaced by a solution of volatile fatty acids (VFA) and a nonabsorable marker, polyethylene glycol (PEG); absorptive rate was estimated by relating the change in VFA concentration to the change in PEG concentration in the test solution. Loss of VFA from the solution was assumed to be due primarily to absorption. However, the total concentration of nutrients in the rumen is controlled by factors which influence changes in rumen volume (Hyden, 1961) (salivation, rumen outflow, and water transfer across the rumen wall) and absorption rate (concentration, pH, mixing of rumen contents, and blood flow to the rumen). Measuring the simultaneous changes in all of these factors would be extremely difficult if not impossible, and the methods that have been used to control or eliminate the influence of these factors have upset the normal physiological and biochemical activities in the animal. Attempts to measure absorption rates by indirect methods have been complicated further because it is difficult to obtain a representative sample of rumen contents from which to determine nutrient concentration.

To avoid the disadvantages of the indirect methods, several researchers have turned their attention to developing methods for measuring absorption rate directly. It generally is assumed that the nutrient absorption rate from a tissue can be determined by measuring the concentration difference between the arterial blood supplying the tissue and the

venous blood draining the tissue (corrected for hemodilution or hemoconcentration); the product of concentration difference times venous blood flow rate equals the nutrient absorption rate. A necessary prerequisite for making this calculation is that the venous blood flow rate must be constant and known. Since all blood from the gastrointestinal tract enters the portal vein, calculation of accurate values for absorption rate requires measurement of portal vein blood flow rate and nutrient concentration in arterial and portal blood.

The indicator-dilution method commonly is used for measuring blood flow rates (Wood, 1962). Zierler (1958, 1962) has presented the theoretical considerations of this technique. A known quantity of indicator is introduced into the fluid system, and the fluid is monitored downstream to determine the indicator concentration as a function of time. The indicator usually is introduced by rapid injection, but it can be by continuous infusion. In the portal system, the continuous infusion method incurs analysis problems due to recirculation and hemodilution, and, therefore, only the sudden injection method has been considered further. Calculation of blood flow rate by indicator-dilution methods depends upon the assumptions that the blood and indicator are thoroughly mixed and that all of the indicator eventually leaves the system. According to Zierler (1962) the rate of flow can be determined as follows:

> Let m_i units of indicator be injected at time zero into the entrance to the system, and measure the concentration of indicator at exit as a function of time, c(t). The amount of indicator, dm, leaving the system during a small time interval between time t and time t + dt is the concentration of indicator, c(t), multiplied by the volume of fluid leaving the system during this time interval, and this is the flow, Q, in units of

volume/time, multiplied by the time interval, dt; that is, $dm = c(t)Qdt$.

Because all indicator, m_i, must leave the system, m_i equals the sum of the amounts leaving the system during all such time intervals, or,

$$m_i = \int_0^\infty c(t)Qdt = Q\int_0^\infty c(t)dt$$

whence

$$Q = \frac{m_i}{\int_0^\infty c(t)dt}$$

The unknown flow, Q, through the system is determined by measuring the area under the observed indicator concentration-time curve and dividing it into the known quantity of injected indicator.

Gott et al. (1961) have reported that the indicator-dilution curve consists of two parts; the rising slope which is nearly linear and peaks in a very short time and the descending slope which is exponential. Furthermore, the slopes vary with the rate of flow and are likely to be different for each determination. If enough points can be measured, the formula of each slope can be calculated and the area under the curve can be integrated for flow rate determination.

Evans blue dye (T-1824), indigo carmine, methylene blue, indocyanine green dye, isotonic saline, I^{131}-labelled serum albumin, P^{32}-labelled red blood cells, nitrous oxide, Kr^{85} and other substances have been used as indicators for obtaining indicator-dilution curves (Fox, 1962). Of these, indocyanine green dye has several advantages which are not all found in any of the others (Cherrich et al., 1960): it is nontoxic to the animal; it is rapidly cleared by the liver and, therefore, does not accumulate in

the plasma; it binds rapidly to the plasma proteins; and its concentration can be determined from optical density values for either plasma or whole blood without the use of elaborate equipment.

In order to obtain dye-dilution curves, catheters must be established and maintained patent in the portal system. Various techniques for venous catheterization have been described (Bensadoun and Reid, 1962; Conner and Fries, 1960; Denton et al., 1953; Jackson et al., 1960; Lewis et al., 1957; Moodie et al., 1963; Parker et al., 1963; Schambye, 1955a, 1955b; Shoemaker et al., 1959; and Waldern et al., 1963), but none of the catheters remained functional for any appreciable length of time. Shortly after insertion, the catheters usually became occluded by a fibrin clot and/or connective tissue sheath which rapidly formed at the tip; usually this formation would allow injection of materials but prevent withdrawal of blood.

Moodie et al. (1963) used nylon catheters; Denton et al. (1953), Parker et al. (1963) and Shoemaker et al. (1959) used polyvinyl catheters. All others used polyethylene catheters. Moodie et al. (1963) used a tube-within-a-tube arrangement whereby the inner tube could be removed for cleaning and a solid nylon rod could be used to clean the outer tube. Waldern et al. (1963) left a piano wire in the catheter and used it for cleaning the catheter. Recent reports (Braley, 1966) indicate that medical grade silicone rubber is the material of choice for chronic implantation in the soft tissues; there is less foreign body reaction to this than to other materials, and the body does not react metabolically with it as with some materials, e.g., nylon. However, there is no known

report of research where silicone rubber was used for portal catheterization.

In all of the above studies, the catheters were flushed periodically with saline or heparinized saline, and some were then filled with a strong solution of heparinized saline. Details about maintenance of the catheters were not always reported. Where details were reported, the catheters were flushed two to four times daily, and, if they were filled at all, 400 to 1000 units of heparin per ml was used for filling (Bensadoun and Reid, 1962; Conner and Fries, 1960; Jackson et al., 1960; Lewis et al., 1957; Moodie et al., 1963; Shoemaker et al., 1959; and Waldern et al., 1963).

Schambye (1955a, 1955b) estimated the portal vein blood flow in anesthetized and conscious sheep. P^{32}-labelled erythrocytes and Evans blue dye were used as indicators; they were introduced into a branch of the mesenteric vein by either rapid injection or continuous infusion through a polyethylene catheter. Portal blood was sampled through either a needle or London cannula established in the portal main trunk. Values for flow rate in the conscious sheep ranged from 31.1 to 43.9 ml/(min X kg body weight) and averaged 37.0 as compared to 29.0 ml/(min X kg body weight) in the anesthetized sheep. In vitro and in vivo studies showed that mixing of indicator was thorough and that samples taken from the portal trunk truly represented blood in the stream. Instantaneously injected dyes were rapidly cleared from the area.

Fegler and Hill (1958) used thermodilution to estimate the portal vein blood flow of anesthetized sheep. A catheter was established in the mesenteric vein, and a thermocouple was inserted via a mesenteric branch

or directly through the portal vein wall. Cold saline was injected into the mesenteric vein, and temperature change downstream was monitored with the thermocouple. An average rate was not reported, but flow rate was 30.8% of cardiac output. It was estimated that flow rate in the conscious sheep would be 45.8 ml/(min X kg body weight).

Bensadoun and Reid (1962) used the method of Fegler and Hill (1958) to study the portal vein blood flow of sheep. Feeding caused a great increase in flow rate, but fasting and anesthesia caused depression. The thermodilution method was used because, with saline as the indicator, several consecutive determinations could be made without accumulation of indicator in the blood.

Roe et al. (1966) estimated portal vein blood flow in conscious sheep by using a continuous-infusion, indicator-dilution method. Catheters were established by the method of Conner and Fries (1960), and p-aminohippuric acid was used as the indicator. A mean flow rate of 46 ml/(min X kg body weight) was calculated for 25 sheep.

The only published values for portal vein blood flow in unanesthetized calves are those of Fries and Conner (1961). One polyethylene catheter was established in the mesenteric vein for dye injection; another, with the tip in the portal vein for blood sampling, was inserted via the mesenteric vein (Conner and Fries, 1960). Evans blue dye was injected, and blood was sampled from the portal vein at about 2 second intervals. Average flow rate was 37.8 ml/(min X kg body weight) and values ranged from 9.2 to 65.7 ml/(min X kg body weight). Flow rate was greatly depressed for 1 to 2 days after surgery, and excluding these values gave

an average of 46.0 ml/(min X kg body weight). Deliberately exciting the animal decreased flow rate from 61.3 to 14.4 ml/(min X kg body weight).

Unfortunately, details about the techniques used in the above studies were vague. Except where thermodilution was used, the indicator-dilution curves were constructed from concentration values of blood samples obtained at intervals of about 2 seconds, and the samples were not taken continuously. Therefore, the accuracy of the calculations is questionable, especially if the peak dye concentration occurred less than 2 seconds after the first dye appeared. There was great variation among values for flow rate, but details about such variation were not published. Most values were obtained from a single determination rather than from an average of several, and values for daily variation and the significance of such variation were not reported.

Portal vein blood flow rate must be measured in order to obtain accurate values for absorption rate from the gastrointestinal tract of ruminants. Several researchers attempted to measure this rate by indicator-dilution methods, but it was difficult to establish and maintain catheters for dye injection and blood collection. Blood flow rate was highly variable and averaged about 45 ml/(min X kg body weight). The great variability suggests that it may be necessary to obtain several values from which to calculate an average for a certain time, and values obtained at one time probably do not represent other times in the same or different animals.

PROCEDURE

Calves

Young calves from the Iowa State University dairy herd were used to develop catheterization procedures and indicator-dilution methods for calculating portal vein blood flow rate. The calves, housed in individual pens, were fed diets of either whole milk only or milk, hay and grain (Sutton et al., 1963). Each calf was placed in an adjustable metabolism stall when flow rate was measured. Age, weight, sex, breed and dietary effects were not studied.

Catheters

To obtain indicator-dilution curves, the calves were fitted with one of two types of catheter (Table 1). Initially, the catheters were modified versions (Figure 1:A) of the double-lumen catheters described by Kountz et al. (1964). Two pieces of polyvinyl tubing[a] (0.150 cm I. D. X 0.232 cm O. D.) were fused for 32 cm with the tips 10 cm apart. Both tips were cut at 45° to facilitate insertion, and holes were cut through the sides near each tip by means of a sharpened 10 gauge needle shaft. The injection tip was plugged to obtain a retrograde spray injection which enhanced the mixing of dye with blood; the collection tip was open-ended. In an attempt to prevent clot and sheath formation, some catheters were

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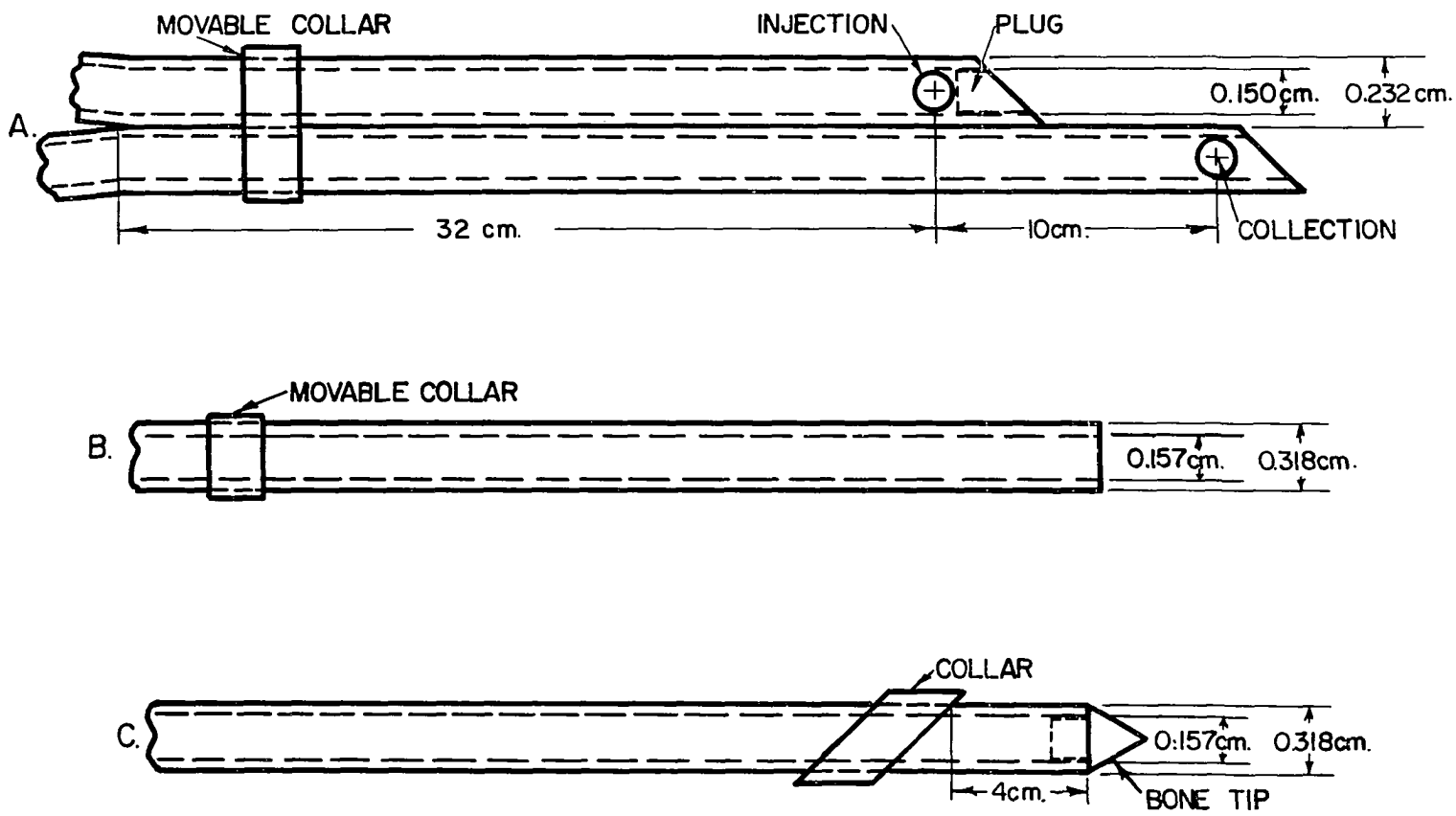
[a]Surco Flexible Transparent Tubing - Formulation S-1 Clear, Surprenant Mfg. Co., Clinton, Mass.

Figure 1. Catheter design used for injecting dye and collecting blood in portal vein blood flow experiments: A, double-lumen, polyvinyl catheter; B, silicone rubber injection catheter; C, silicone rubber collection catheter


A.
MOVABLE COLLAR
INJECTION
PLUG
0.150cm.
0.232 cm.
32 cm.
10cm.
COLLECTION
B.
MOVABLE COLLAR
0.157cm.
0.318cm.
C.
COLLAR
0.157cm.
0.318cm.
4cm.
BONE TIP

coated with a graphite[a], benzyl alkyl ammonium chloride and heparin[b] (GBH) solution (Gott et al., 1964). A snug, movable collar was fitted over the catheter for fastening it in the vein.

Later, separate silicone rubber tubes[c] (0.157 cm I. D. X 0.318 cm O. D.) were used as catheters; one was inserted directly through the wall of the portal main trunk for blood collection, and the other was inserted via a branch of the anterior mesenteric vein for dye injection. The collection tube (Figure 1:C) was fitted with a small silicone rubber collar about 4 cm from the tip. The collar was cut at a 45° angle so that, when the catheter was fastened in place, the tip was not butted directly against the opposite wall of the vein. To facilitate insertion, a bone tip with a sharp point was fitted to the end of the collection tube (Figure 1:C). The injection tube (Figure 1:B) was fitted with a movable collar for fastening it in the vein.

Surgery

Prior to surgical establishment of the catheters, each animal was fasted 36 to 72 hours to allow emptying of the gastrointestinal tract.

Anesthesia was induced by administering 3% Surital[d] sodium via the

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[a]DJ - 600, Acheson Colloids Co., Port Huron, Mich.

[b]Courtesy of Dr. H. M. Bryant, Upjohn Co., Kalamazoo, Mich. and Dr. R. F. Elliott, American Cyanamid Co., Princeton, New Jersey.

[c]Medical Grade Silastic, Dow Corning Corporation, Medical Products Division, Midland, Mich.

[d]Parke Davis & Co., Detroit, Mich.

jugular vein. Immediately, the calf was placed on the surgical table in lateral recumbency on the left side, and the trachea was intubated. The endotracheal tube was attached to a closed-circuit anesthesia machine, and anesthesia was maintained with Fluothane[a].

The double-lumen, polyvinyl catheters were established via the anterior mesenteric vein with the collection tip in the portal vein (Conner and Fries, 1960). About 30 to 40 cm of tubing resided in the vein. The catheter was exteriorized by the procedure of Dougherty et al., 1965.

In order to establish the silicone rubber catheters, an incision about 40 cm long was made over the 12th rib, the periosteum was stripped from the rib, and the rib was removed along with its costal cartilage. Entrance to the peritoneal cavity was through the rib resection. Care was exercised during this phase of the operation to prevent penetration of the thorax at the dorsal aspect of the incision. The portal vein, which enters the liver at the portal fissure, was located ventral to the posterior vena cava and medial to the gall bladder. Location of the portal vein was facilitated by tracing the fissure between the caudate and dorsal lobes of the liver medially and ventrally to its base. This fissure was readily seen upon retraction of the incision. The dorso-lateral surface of the portal vein close to the liver was dissected free of surrounding tissue for about 5 cm. With the bone tip in place, the catheter was forced cranially through the wall of the portal vein. The elasticity of the wall prevented loss of blood through the wound. The catheter was

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[a]Fluothane, a halothane anesthetic, Ayerst Laboratories, New York, New York.

secured by suturing the rubber disk to the vein wall and suturing the surrounding connective tissue together around the disk. The catheter was then flushed with saline to dislodge the bone tip, and continuous infusion of anticoagulant was begun as described below. The injection catheter was established in the same manner as the double-lumen catheter (Conner and Fries, 1960); the objective was to establish the tips about 10 to 15 cm apart. Both catheters were exteriorized directly through the incision (Figure 2); about 30 cm of each was left free in the peritoneal cavity.

After either method of exteriorization, two Polyotic Oblets[a] were placed in the peritoneal cavity and the incision was closed. The exteriorized catheters were fitted to 15 gauge needle hubs, and the needle hubs were attached to BD, MS09-T1, one-way valves (the handles were removed from the valve stopcocks to prevent accidental opening). The valve assembly was then fastened to the skin with stay sutures (Figure 3), and the wounds were dusted with Polyotic Powder[a]. In order to prevent general infection, 5 ml of Antibiotic Combination I[b] was injected intramuscularly on the day of surgery and on each of the two succeeding days.

During either method of catheterization, the tubes were continually infused with a solution of 500 ml of 5% dextrose in saline containing 10,000 units of Varizyme[c] and 10,000 units of heparin; about 150 to 250 ml of this solution was infused during surgery.

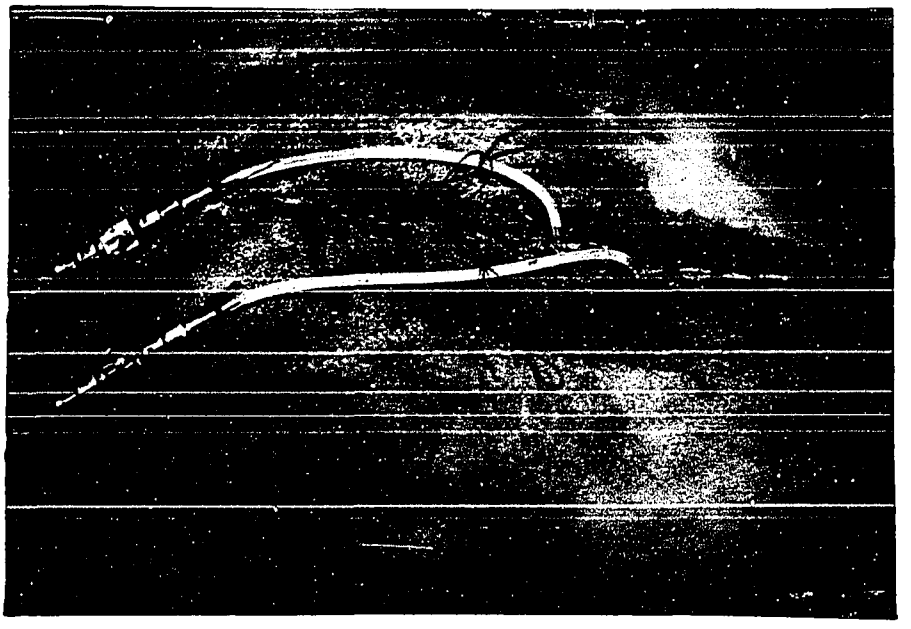
[a]A tetracycline hydrochloride, American Cyanamid Co., Princeton, N.J.

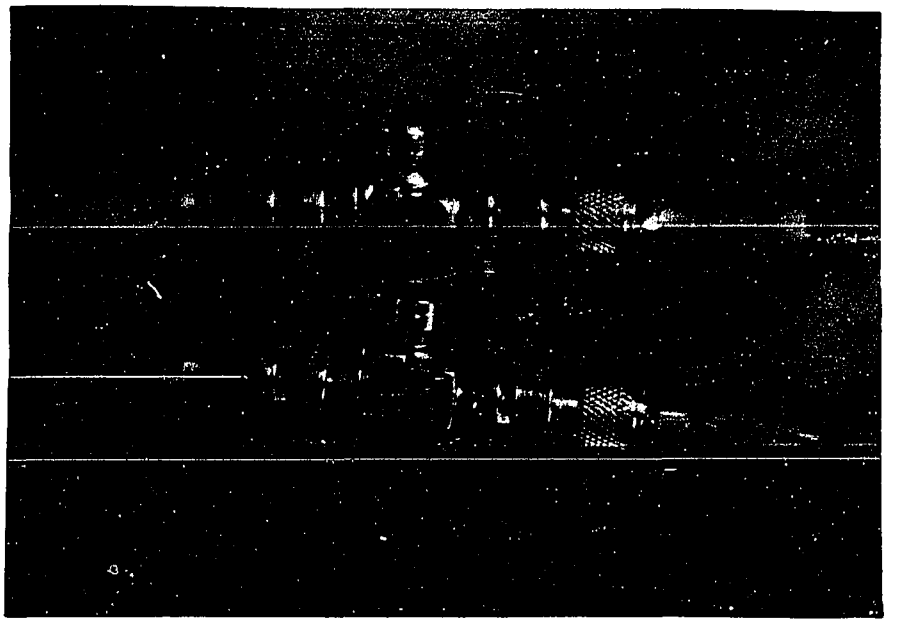
[b]400,000 units penicillin in dihydrostreptomycin, 0.5 mg/2 cc, Corvell division of Eli Lilly & Co., Indianapolis, Ind.

[c]Courtesy of Dr. R. F. Elliott, American Cyanamid Co., Princeton, N.J. Varizyme contains streptokinase, streptodornase and human plasminogen; the level indicated here and in other parts of the text is that of the streptokinase activity.

Figure 2. Exteriorized, silicone rubber catheters

Figure 3. Valve assembly *in situ* for attaching exteriorized catheters to skin





Strict asepsis was maintained during surgery.

Catheter Maintenance

Anticoagulants were used postoperatively to increase the functional lifetime of the catheters. Twenty ml of saline was flushed through the catheter, blood was withdrawn if the tube was open, 40 ml of saline was used to flush the catheter and the tube was filled with a solution of saline containing 500 units of heparin and 5,000 units of Varizyme per ml. For two days after surgery, the catheters were flushed at 8-hour intervals. Subsequently, the catheters were flushed at 12-hour intervals for five days and then at 24-hour intervals. No Varizyme was infused after the second day.

Dye Injection

Indocyanine green dye[a] was used as the indicator for blood flow rate determinations. The dye was diluted to 5 mg per ml, and 1 ml was injected for each determination. Dye concentration in the blood was calculated from plasma optical density which was determined at 800 mμ in a Bausch & Lomb Spectronic 20 colorimeter equipped with a flow-thru cuvette.

Special, 1 ml injection pipettes (Robinson et al., 1955) and a variable speed, variable-time injection pump[b] were used to achieve rapid dye injection (Figure 4). The pump was set to inject the dye and flush 10

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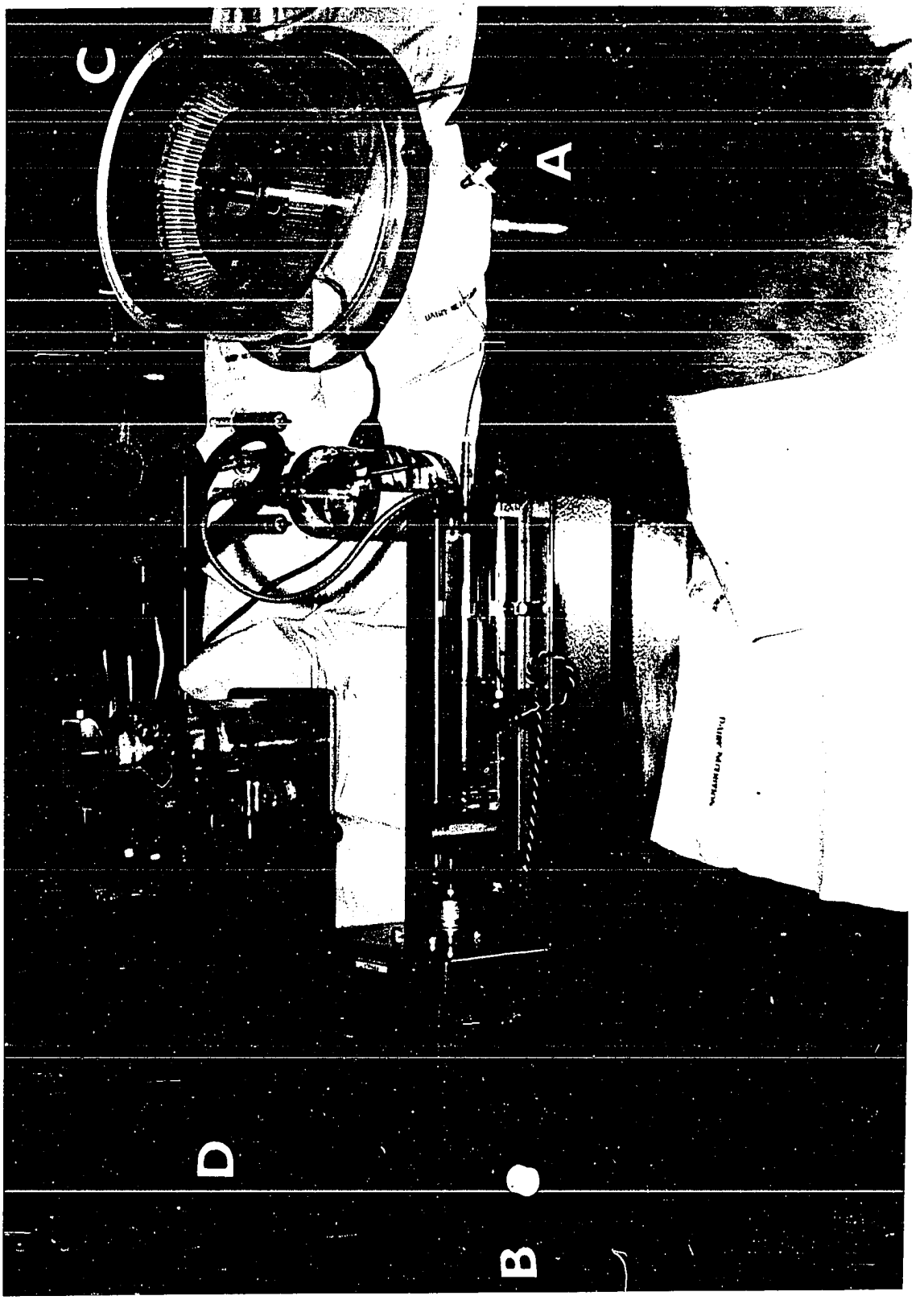
[a]Cardiogreen, Hynson, Wescott, and Dunning, Baltimore, Md.

[b]Specially made for high-speed dye injection by Harvard Apparatus Co., Inc., Dover, Mass.

Figure 4. Equipment for dye injection and blood collection used for measuring portal vein blood flow rate: A, injection pipette; B, injection pump; C, sample collector; D, vacuum pump attached to collector


C
A
D
B

ml of saline into the vein in 1.5 seconds; thus, most of the dye was injected in less than 0.2 seconds.

Blood Collection

In the early phases of the research, a Brewer pipetting machine was used to collect 2 ml samples of blood at 2 second intervals. This method did not give enough observations on the dye-dilution curve for accurate analysis, and, therefore, the collection process was refined further.

A sample collector was made after the design of Rothe and Sapirstein (1954) with certain modifications (Figure 4). A plexiglass disk was made with a well in the center and 60 holes spaced around the perimeter. The disk was attached to a 1 or 2 rpm motor so that a continuous flow of blood into the holes of the rotating disk would give samples at 1.0 or 0.5 second intervals, respectively. Rothe's apparatus was made for collecting arterial blood under pressure; collecting venous blood necessitated enclosing the disk under a vacuum in order to get samples of adequate size. A sliding, 10 gauge needle hub in the container wall was attached by a tube to the collection catheter. By moving the rod, the blood could be directed into either the center well or the holes around the perimeter. When determining blood flow rates, the blood was directed into the center well until the injection pump was activated; then, the rod was moved over the perimeter, twenty samples were collected, and the blood flow was redirected into the center well. Since only twenty samples were needed for a flow rate determination, one disk was used for three successive determinations. For maximal use of the collecting disk, preliminary studies were made to

determine how rapidly the dye appeared in the blood samples after injection. Two to four samples of blood without dye were collected each time flow rate was determined in order to have a blank with which to set the colorimeter.

To prevent coagulation, a cotton swab soaked lightly with a solution containing 500 units of heparin per ml was dipped into each hole, and the disk was allowed to dry before samples were collected. Immediately after each determination the collection catheter was flushed with saline containing 10 units of heparin per ml, and at the end of an experiment both catheters were filled with a solution containing 500 units of heparin per ml.

Calculation of Flow Rate

Two methods were used to calculate flow rate from the values for dye concentration. A computer program, developed by the Numerical Analysis section of the Statistical Laboratory at Iowa State University, was used to analyze the values and calculate the formula for each slope of the indicator-dilution curve (Atkinson, 1966). A graphical plot of the data was used to determine which points fitted the rising slope and which fitted the descending slope. On the assumption that the rising slope is linear and the descending slope is exponential (Gott et al., 1961), the last two points of the rising slope were fitted to the formula $Y(1) = A + BT$. $Y(1)$ = concentration on the linear slope at time T; B = slope of the straight line; A = $Y(1)$ at $T = 0$. The succeeding points were fitted to the formula $Y(2) = CexpD(T-TP)$. $Y(2)$ = concentration on the descending line at time T; C and D are constants determined for each curve; TP = time

of peak concentration determined by extrapolating the two curves to their point of intersection. The formula for each slope, the area under each slope, the total area under the curve, the flow rate, and the degree of fit of the data points to the exponential formula were determined.

The method of Lewis (1953) was used to calculate flow rate values against which to compare the computer values.

RESULTS AND DISCUSSION

Thirty-four calves were used to develop indicator-dilution methods for calculating portal vein blood flow rate. Catheters for injecting dye and sampling blood were established surgically; postoperative recovery was rapid and uneventful, and no infection or other undesirable effects occurred as a direct result of the surgical procedure.

Many catheters had a short functional lifetime, even though they were flushed periodically with anticoagulants (catheters were considered functional only if blood could be withdrawn) (Table 1). The silicone rubber catheters were patent longer than the polyvinyl catheters. All of the polyvinyl catheters were covered to some extent with a fibrous tissue sheath. The sheath appeared to originate either at the point of insertion, near the catheter tip, or in both places, apparently as a result of injury to the intima. The groove where the polyvinyl tubes were fused usually was well-covered by the sheath, perhaps due to its uneven surface. The holes through the side of the collection and injection tips were especially amenable to thrombus and sheath formation. The GBH coating used on some of the polyvinyl tubes was not satisfactory because it flaked off during and after establishment of the catheters. Gott et al. (1964) have indicated that GBH coatings were less effective on flexible plastics than on rigid plastics. There was no noticeable sheath formation on the silicone rubber catheters. In all calves, there were thrombi close to the catheter tips at the time of sacrifice, regardless of the type of catheter used. Neither the presence nor the size of thrombi appeared to be related to any treatment (diet, anticoagulant, length of time catheters had been

Table 1. Maintenance of portal vein catheters

Polyvinyl catheters			Silicone rubber catheters		
	Functional[a] (days)			Functional[a] (days)	
Calf[b]	Injection tip	Collection tip	Calf[b]	Injection tip	Collection tip
5500	1	1	5487	10[c]	35
6030-7	45	17	7095-2	10[c]	18
7085-1	22[d]	17	5846	12[d]	12[d]
7021-5	20	21[d]	5850	52	70[d]
7070-3	17[d]	1			
5680	5	56[d]			
5675	40	43	Mean	21	32
6100-1	18[d]	18[d]			
7065-4	1	14			
6087	2	17			
7103	13	2			
5757	5[d]	0			
5751	0	14			
7074-5	13[d]	11			
6105	4	9[d]			
7091-2	8	9[d]			
7106-1	18	18			
6097-2	13	14			
7059	1	4			
7067-6	2	1			
6110-1	14	14			
6050-6	6[d]	6[d]			
6052-5	12[d]	12			
7049-5	14	14			
Mean	12	14			

[a]Catheters were considered functional only if blood could be withdrawn.

[b]Calves are identified in this and subsequent tables by herd number.

[c]Calf bit tube to render it nonfunctional.

[d]Calf was sacrificed while catheter was still functional.

established, or catheter design). In some cases, a catheter was nonfunctional for 2 or 3 days but then became functional. Apparently there was a temporary occlusion which was cleared by flushing or movement of the catheter. The reason the silicone rubber catheters remained patent longer is not clear. It may have been due to the difference in chemical composition of the tubing, size of the tubing, design of the catheter, method of establishing the catheter or any combination of these.

With one calf in which the double-lumen catheter was used, the blood flow rate dropped from about 45 ml/sec during the first three days of the experiment to about 15 ml/sec during the next two days. The calf was sacrificed the next day, and it was found that the collection tip was no longer in the portal main trunk. All other calves in which the double-lumen catheters were used were sacrificed after the last determination of blood flow rate, and the collection tip was found to be in the portal main trunk. When it became apparent that the collection tip of the double-lumen catheter (or any catheter established via the anterior mesenteric vein) might move out of the portal main trunk, direct catheterization of the portal vein and separate catheterization of the anterior mesenteric vein was adopted. Having the collection tip secured in the portal main trunk gave greater assurance that the calculated values represented the blood flow rate in that vein. The characteristics of the dye-dilution curves and other factors discussed later indicate that the double-lumen catheter could be used for calculating blood flow rates in other systems where the location of the collection tip can always be determined. Gott et al. (1961) indicated that, with any method of catheterization, the tips should

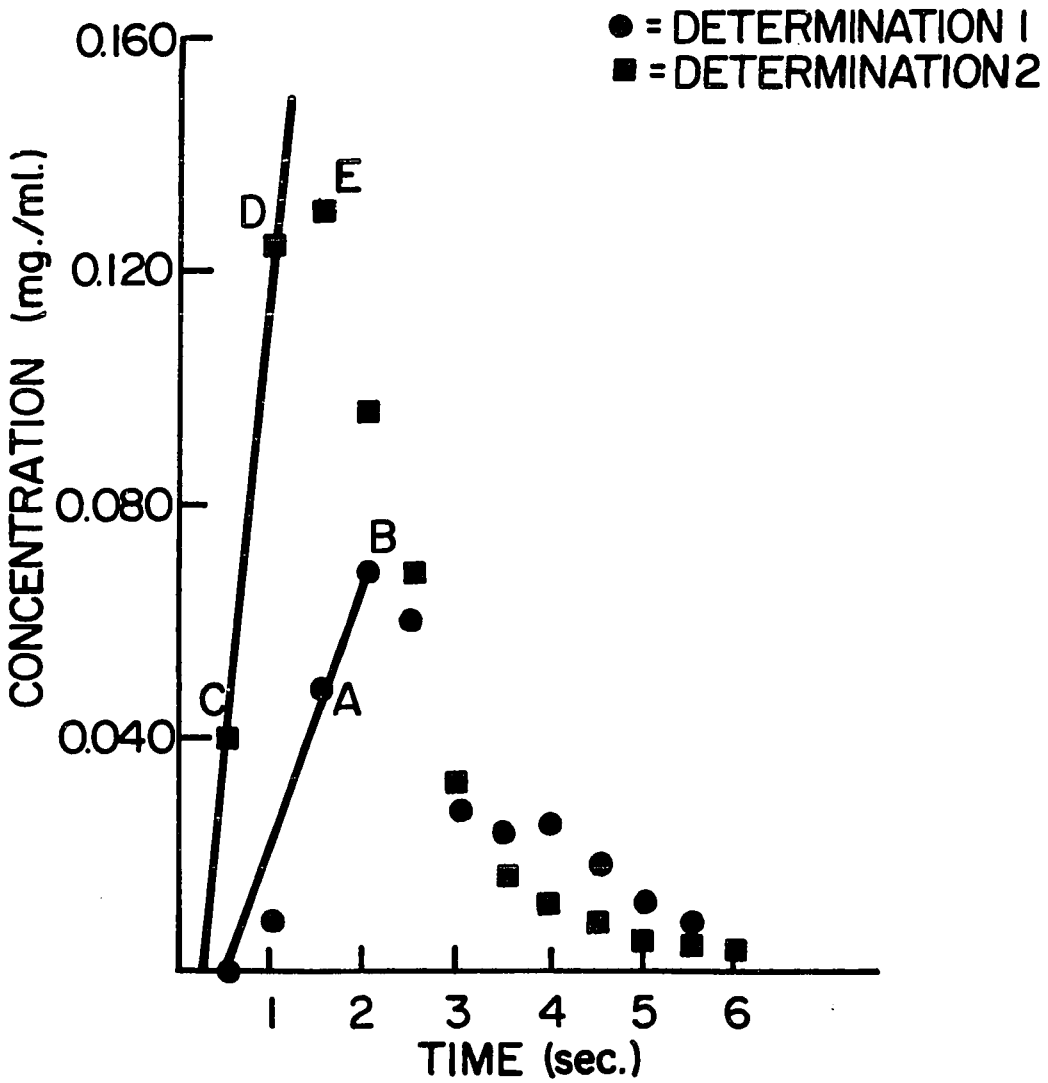
be no farther apart than is necessary to assure thorough mixing of the dye and blood, or the dye-dilution curve will have characteristics different from those described above.

Indocyanine green dye was satisfactory for measuring portal blood flow rate. The dye was rapidly cleared by the liver, and there was no recirculation or buildup of dye in the blood. The blank colorimeter reading was the same for the last determination as for the first, even after 21 determinations in one day or six in 15 minutes. Therefore, blood flow rate could be measured frequently, and several consecutive values could be obtained to increase the accuracy of the mean value calculated for any time period. In the present experiment the indocyanine green dye, which is bound to the plasma proteins rapidly and has an absorption maximum at 800 mμ, was read in the plasma. It can be read, however, in whole blood because the ratio of reduced-/oxy-hemoglobin does not influence the background optical density (Cherrich et al., 1960). Consequently, in a densitometer designed for continuous flow of whole blood through the optical system, optical density could be read continuously and directly in the blood being collected (Hanson and Tabakin, 1964).

With the calibrated dye injection pipettes and the injection pump, rapid injection of a known quantity of dye was achieved. The dye-dilution curves (Figure 5) had characteristics similar to those of the theoretically ideal curve (Gott et al., 1961), and the variance of consecutive determinations made in a short time was low. Therefore, the mixing of dye with blood was thorough, and injection was uniform among trials.

With the Brewer pipetting machine, peak dye concentration often

Figure 5. Dye-dilution curves obtained when measuring portal vein blood flow rate: B and E are peak concentration values for the respective curves; lines C-D and A-B represent the linear slopes of the respective curves


● = DETERMINATION 1
■ = DETERMINATION 2
0.160
0.120
0.080
0.040
CONCENTRATION (mg./ml.)
A
B
C
D
E
1
2
3
4
5
6
TIME (sec.)

occurred in the first sample which contained dye, and the dye was virtually cleared from the system after five or six samples had been collected. Also, there was no blood collected while the sample was being ejected from the syringe, so the values obtained were not from continuous sampling. The values thus obtained were not adequate for calculating flow rate. As a result, the continuously rotating disk was used to obtain concentration values at shorter time intervals. Even at half-second intervals, the dye peak still occurred in one of the first three samples which contained dye (Figure 5), and few samples were collected before dye disappeared from the system.

All values for dye concentration were plotted (Figure 5), and a straight line was drawn to fit the points on the rising slope as nearly as possible. The last two points on the line were analyzed by computer to determine the formula for the line. If the experimental value for a given time did not fit on the straight line, a value that did fit on the line for that time was used. In some cases, the peak dye concentration was not greatly different from the value immediately preceding it (points D and E Figure 5). On the assumption that the peak value really belonged on the descending slope, then, the value preceding it was used as the second point on the linear slope. If the peak concentration occurred in the first dye sample, the formula for the linear curve was computed by using zero concentration and the peak value. Subsequent points were fitted to the exponential formula.

The assumptions of the computer program appear to be valid for several reasons. The assumption of linearity in the rising slope is

supported by the fact that the peak dye concentration always occurs in the second or third sample in which dye occurs. The fact that only 6 of 174 curves analyzed had an exponential R-square of less than 0.97 (the lowest value was 0.88) supports the assumption of an exponential descending slope. Furthermore, there was close agreement between the flow rate values calculated for the same curves by two different methods (Table 2).

Table 2. Comparison of portal vein blood flow rates calculated from the same data by different methods

Determination	Method	
	Lewis (1953)	Computer analysis
	(blood flow rate, ml/sec)	
1	79.1	72.1
2	61.8	64.1
3	84.2	88.8
4	75.0	77.9
5	57.0	56.0
6	72.2	80.1
7	69.8	64.9
8	57.0	55.4
9	89.7	96.8
10	66.7	66.4
Mean	71.3	72.3
$S_{\bar{x}}$	3.5	4.3

Continuous monitoring of dye concentration would improve the accuracy of analyzing the rising slope of the concentration-time curve because there would be more points from which to construct the curve. On the other hand, it probably would not improve the accuracy of analyzing the

exponential slope because the degree of exponential fit already is high. The greatest advantage to continuous monitoring of dye concentration would be that many more flow rate determinations could be made in a short period of time. Analysis of dye concentration (a tedious process under the conditions of this experiment) would be facilitated greatly, and less blood would have to be withdrawn from the calf for the analysis.

Most of the values obtained for portal vein blood flow rate (Tables 3, 7) were similar to those from which Fries and Conner (1961) calculated an average of 46.0 ml/(min X kg body weight). The variation among the values obtained in this study was large.

Table 3. Average portal vein blood flow rates, range and standard error

Calf	7091-2[a]	7106-1[a]	6097-2[a]	6050-6[a]	6052-5[a]	7095-2[b]	5850[b]
Weight (kg)	66	62	73	100	105	138	100
Number of values	8	25	12	44	33	6	66
Range (ml/sec)							
Low	19.5	8.4	29.3	39.9	45.7	73.8	36.6
High	73.2	39.3	73.8	100.1	130.5	82.3	198.6
Mean (ml/sec)	51.4	17.2	42.6	68.5	82.6	79.0	102.5
$S_{\bar{x}}$	6.5	1.4	3.8	6.7	4.2	1.0	4.5

[a]Catheterized with double-lumen catheters.

[b]Catheterized with separate catheters.

Previous researchers have not made several flow rate determinations from which to calculate an average value for a given time. In this study, it was possible to obtain several consecutive flow rate values at intervals of about 2 minutes. The large variation among values obtained within a short time (Table 4) suggests that the average of several consecutive values should be used as the more accurate value for flow rate

Table 4. Variation in consecutive portal vein blood flow rates determined in a 15 minute period; values listed in order in which they were obtained

	Calf				
Order	5850[a]	7095-2[a]	6052-5[b]	6052-5[b]	5850[a]
	(blood flow, ml/sec)				
1	71.3	79.5	49.7	62.1	157.5
2	80.4	79.8	89.4	87.0	143.9
3	50.1	73.8	73.3	62.5	82.5
4	129.6	77.8	61.8	71.3	88.2
5	83.5	82.3	45.7	68.6	126.1
6	109.7	80.6	50.4	62.3	167.3
Mean	87.4	79.0	61.7	68.9	127.6
$S_{\bar{x}}$	11.5	1.0	6.9	3.9	14.5

[a]Catheterized with separate catheters.

[b]Catheterized with double-lumen catheters.

at a given time. Some workers have applied flow rates determined for one animal to research with another. In the present study, flow rate varied greatly from day to day (Table 5) and throughout the day (Table 6) in the same animal. These data suggest that blood flow rate should be determined

Table 5. Variation in portal vein blood flow rate on different days as determined at the same time of day in one calf

	Date (1966)			
	3/14	3/15	3/16	3/22
Time of day	1435	1440	1435	1405
Number of values	3	3	3	6
Mean blood flow rate (ml/sec)	13.9	21.4	27.6	12.6
$S_{\bar{x}}$	1.0	2.4	5.8	0.9

Table 6. Variation in portal vein blood flow rate throughout the day in one calf

	Time				
	0830	1005	1128	1300	1435
Number of values	3	3	3	3	3
Mean blood flow rate (ml/sec)	124.5	106.9	91.2	75.1	67.9
$S_{\bar{x}}$	1.5	11.8	9.5	4.9	8.0

each time it is used, and it certainly would be erroneous to apply values from one animal to another. The reasons for the variation among values for portal vein blood flow rate are not clear and will require more definitive study.

Nutrient absorption from the gastrointestinal tract may be determined directly provided the arterio-portal concentration difference of the

nutrient(s) (corrected for hemo- concentration or dilution) and portal vein blood flow rate are known. The nutrient concentration and flow rate of the portal vein blood can be determined with the methods developed in this research. Since blood flow rate is a major parameter in making a direct calculation of nutrient uptake and since it appears to vary considerably within a given animal, it becomes of paramount importance to clearly delineate the nature of this variation and the physiological and biochemical factors which influence portal vein blood flow.

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APPENDIX

Table 7. Blood flow values

Calf	Weight kg	Diet[a]	Date	Time[b]	Determination 1	2	3	4	5	6	Mean	$S_{\bar{x}}$[c]
					(ml/sec)							
7091-2[d]	66	MHG	3/7/66	1015	62.4	59.5	69.6	51.9			60.9	3.7
			3/8/66	1035	19.5	35.4	73.2				42.7	15.8
			3/10/66	1015	39.9							
7106-1[d]	62	M	3/14/66	1435	14.8	12.1	14.8				13.9	1.0
			3/15/66	1440	25.8	20.7	17.6				21.4	2.4
			3/16/66	1435	39.3	20.9	22.7				27.6	5.8
			3/19/66	0650	8.9	14.0					11.5	
				0750	16.9	18.1					17.5	
				0950	15.0	21.3					18.7	
				1250	13.0	9.7					11.4	
				1650	20.3	28.0					24.2	
			3/22/66	1405	13.8	8.4	13.1	14.2	14.1	12.5	12.6	0.9
6097-2[d]	73	MHG	4/7/66	1320	57.4	45.7	38.3				47.1	5.6
			4/8/66	1400	73.8	29.3	34.1				45.7	14.1
			4/9/66	1350	33.3	54.9	33.0				40.4	7.2
			4/11/66	0710	40.1	31.5	40.3				37.3	2.9

[a]Calves received diets of milk only (M) or milk, hay and grain (MHG) (Sutton *et al.*, 1963).

[b]A series of determinations was made at 2 to 5 minute intervals beginning at the time listed.

[c]$S_{\bar{x}}$ was not calculated for less than 3 values.

[d]Catheterized with double-lumen catheters.

Table 7. (Continued)

Calf	Weight kg	Diet[a]	Date	Time[b]	Determination 1	2	3	4	5	6	Mean	$S_{\bar{x}}$[c]
					(ml/sec)							
6050-6[d]	100	M	6/12/66	1120	58.1	75.8	61.0				64.9	5.5
				1405	66.3	70.7	47.0	39.9			55.5	7.4
			6/13/66	1325	59.5	86.4	63.2	55.3			66.1	7.0
				1430	73.0	73.7	79.7	51.8			69.5	6.1
			6/14/66	0650	72.9	64.1	88.8				75.0	7.0
				0805	77.9	56.0	80.1				71.3	7.7
				0900	64.9	55.4	96.8				72.3	12.5
				1100	66.4	63.0					64.7	
				1300	61.3	51.2	78.3				63.6	7.9
			6/15/66	0650	53.7	81.2	64.0				66.3	8.0
				0755	46.0	89.8	75.9				70.5	12.9
				0900	69.7	56.8	71.1				65.8	4.5
				1100	100.1	86.5	67.8				84.8	9.4
				1300	57.7	85.8	69.0				70.8	8.2
6052-5[d]	105	MHG	6/25/66	1315	62.1	87.0	62.5	71.3	68.6	62.3	68.9	3.9
			6/27/66	0915	49.7	89.4	73.3	61.8	45.7	50.4	61.7	6.9
			6/29/66	0850	74.4	71.4	96.9				80.9	8.0
				1000	80.7	90.3	130.5				100.5	15.3
			7/3/66	0830	121.5	125.7	126.4				124.5	1.5
				1005	128.6	87.8	104.4				106.9	11.8
				1128	106.7	73.9	93.0				91.2	9.5
				1300	65.3	80.2	79.9				75.1	4.9
				1435	76.8	52.0	75.1				67.9	8.0

Table 7. (Continued)

Calf	Weight kg	Diet[a]	Date	Time[b]	Determination 1	2	3	4	5	6	Mean	$S_{\bar{x}}$[c]
					(ml/sec)							
7095-2[e]	138	MHG	8/26/66	1100	79.5	79.8	73.8	77.8	82.3	80.6	79.0	1.0
5850[e]	100	MHG	11/24/66	1222	74.6	75.9	123.5	117.5	113.1		100.9	10.6
			11/25/66	0934	177.5	122.4					150.0	
				1130	50.8	52.3	36.6				46.6	5.0
				1330	96.2	70.2	79.7				82.0	7.6
				1528	57.6	76.9	78.3				70.9	6.7
				1728	70.0	65.8	58.2				64.7	3.5
				1929	47.4	92.8	142.3				94.1	27.4
			11/27/66	0934	85.1	85.8					85.5	
				1126	107.3	143.0	123.1				124.5	10.3
				1325	95.6	103.7					99.7	
				1525	73.6	85.1	105.6				88.1	9.4
				1728	85.2	96.5					90.9	
				1925	90.4	144.1					117.3	
			12/1/66	0856	166.5	144.0	92.7	198.6			150.5	22.4
			12/2/66	0839	97.8	96.2	62.6	73.5	146.6	63.0	90.0	13.0
			12/3/66	0854	71.3	80.4	50.1	129.6	83.5	109.7	87.4	11.5
				1057	73.2	112.9	152.7				112.9	22.9
				1252	160.7	110.6	132.9	123.2	160.7		137.6	10.1
				1449	157.5	143.9	82.5	88.2	126.1	167.3	127.6	14.5

[e]Catheterized with separate catheters.