Clearance by the hepatic portal circulation of the products of digestion and metabolism in the ruminant gut

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The Fick principle states that the uptake or release of any substance by an organ is equal to the product of blood flow through that organ and the changes in concentration of the substance in question as it passes through.

This simple principle has been exploited by physiologists in order either to estimate blood flow through an organ from the rate of clearance of a marker, or to measure the uptake and release of naturally occurring substances when blood flow can be measured by other means.

In ruminant nutrition it is, of course, most important to be able to measure with precision the way in which the products of digestion are removed from the gut, and carried through the hepatic portal circulation for metabolism in the liver or other tissues. It is also important to be able to measure quantitatively the nature and extent of metabolism of different compounds in the large cell mass that constitutes the wall of the digestive tract itself.

The rate at which some substance S leaves the lumen of the gut can be measured using the Fick principle. The concentration of S is measured in samples taken from the gut at appropriate points and the rate of flow of digesta is measured by total collection or the use of a marker. This approach is discussed elsewhere in this symposium (MacRae, 1974). At best the method can estimate very precisely what is leaving the gut. In order to find out what happens thereafter it is necessary to measure the rate at which substance S appears in the portal circulation. The difference between the amount leaving the gut and that entering the portal circulation is, of course, that which is metabolized in the gut wall. If one also can measure the rate at which S leaves the liver then the metabolism of S in the liver can be assessed as well.

Fig. 1 illustrates very schematically the circulation to the gut and liver in the sheep and indicates the sites at which it is possible to measure flow and concentrations in order to measure the movement of compounds through the gut and liver. The arterial blood enters the mesenteric circulation and the liver usually, although not invariably, via two main trunks, the coeliac and the anterior mesenteric arteries. A representative sample of arterial blood can be obtained from either artery or from the aorta but total arterial blood flow to the gut cannot be measured at any one point.

The venous blood draining the gut collects via two main trunks, the gastrosplenic and anterior mesenteric veins, which join very close to the liver into the single short
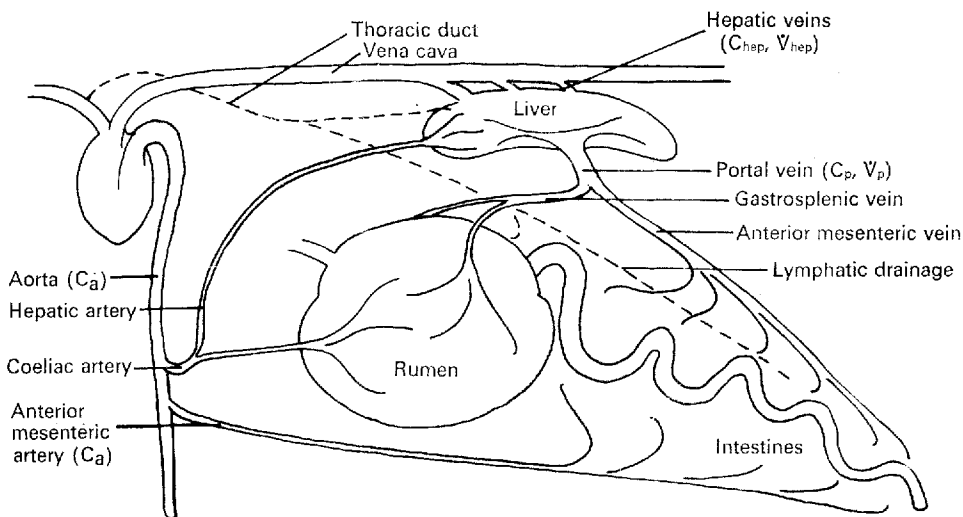
Fig. 1. Schematic diagram of the mesenteric and hepatic circulation in the ruminant. Sampling points for: arterial concentration, C_a; hepatic concentration, C_{hep}; portal concentration, C_p; hepatic blood flow, $\dot{V}_{hep}$; portal blood flow, $\dot{V}_p$.

portal vein which almost immediately breaks up into vessels supplying different parts of the liver. Total blood flow through the visceral circulation and the concentration of S in the venous effluent can therefore be measured at the porta of the liver. How representative this sample may be depends on the extent of mixing of blood in the portal vein. Garner & Singleton (1953) and Heath (1968) have suggested that in acute preparations mixing is incomplete, so that blood from the gastrosplenic vein preferentially enters the left side of the liver, although the anterior mesenteric vein is distributed more evenly. In our experience radio-opaque materials injected into a ruminal or mesenteric vein appear to diffuse throughout the portal vein. Temperature traces in the portal vein during continuous thermal dilution (q.v.) usually, but not invariably, suggest good mixing.

Not all the products of absorption and metabolism in the gut wall are cleared into the portal vein. A small proportion is drained by the lymphatic system into the thoracic duct. Flow rate in the thoracic duct of the sheep is about 2–3 ml/min (Yoffey & Courtice, 1956) compared with a blood flow in the portal vein of 1·5–4·5 l/min (q.v.). Thus the contribution of lymph vessels to the drainage of most constitutents from the gut wall is very small indeed, although certain substances like long-chain fatty acids are transported predominantly, if not exclusively, in this way (Mayerson, 1963).

In the sheep, blood leaves the liver through several hepatic veins. There is no common pathway in which one can measure flow and concentrations. The peculiar problems of measuring flow and sampling concentrations in the hepatic and portal circulations are considered below.

Measurement of blood flow by dilution methods

The principle of all the dilution methods is to inject a known amount of marker into the blood and measure at an appropriate point downstream the extent to which it is diluted by the blood. The marker may be a chemical, a radio-isotope or the addition of 'coolth' registered by temperature sensors. The most popular chemical markers probably are indocyanine green (Wangness & McGilliard, 1972) or *p*-amino-hippuric acid (PAH) (Katz & Bergman, 1969). The classic approach to the measurement of, for example, cardiac output, using indocyanine green, has been to inject rapidly a known volume and then sample blood at precisely measured intervals over the next 30–40 s before recirculation of the injectate begins. Unfortunately blood cannot always be drawn that readily through the long catheters one implants into the portal circulation, and the chances of getting a precise integration of the magnitude and time course of the passage of the dye are slim. Sampling time is less critical in the PAH technique, since PAH is rapidly cleared by the kidneys. It may therefore be infused continuously into the mesenteric circulation. Samples taken from the aorta, portal and hepatic veins then provide a good measurement of average rather than instantaneous portal and hepatic venous flow in the sheep (Katz & Bergman, 1969), although in the pig there is apparently poor mixing of PAH in portal venous blood (Anderson, 1974).

Thermal dilution techniques depend on the injection of physiological saline, usually at room temperature, and measurement of the cooling it produces in the blood downstream. In the single-injection technique (Fegler, 1957; Bensadoun & Reid, 1962) a known volume of saline injected rapidly produces, if all goes well, an exponential fall and recovery in blood temperature which must be integrated. In the continuous thermal dilution technique described by Linzell (1966) and adapted by us (Webster & White, 1973) for measurement of portal venous flow in the sheep, cool saline is infused at a constant rate of about 1 ml/s for 30 s. This produces, ideally, a fall in the temperature of the blood downstream within about 10–15 s to a new steady state, which is established before the onset of recirculation of any coolth. This technique has been described elsewhere (Webster & White, 1972, 1973) and will not be repeated here. The reasons why we consider it the most attractive of the dilution methods are briefly as follows.

(1) The dilution of the marker is measured instantaneously in situ. No sampling of blood is required and the result can be calculated within seconds of completing the infusion.

(2) When blood temperature during infusion falls to a new plateau, one can be reasonably sure that good mixing has occurred. If a satisfactory plateau does not occur, the measurement is discarded. This is not possible with sampling techniques.

(3) Frequently repeated infusions of about 30 ml NaCl are usually well tolerated by sheep, although sometimes they react as if the injectate contained pyrogen. Measurements are seldom repeated at intervals of less than 10 min, but on occasions over sixty measurements have been made in the space of 12 h.

(4) Sodium chloride and thermocouples are cheap.

Measurement of blood flow by dilution of radio-isotopes has up to now been neither better nor worse than using chemical markers, since samples of blood have had to be withdrawn for counting. ^{85}Kr is a particularly useful isotope since about 85% of continuously infused Kr is cleared by the lungs and thus the extent of recirculation is small. There has recently come onto the market a series of silicone radioactivity detectors which are small enough to be implanted chronically into the portal and hepatic veins of a sheep. If the counting efficiency of these crystals is satisfactory it should be possible to obtain a continuous record of blood flow in these vessels by continuously recording activity during infusion into the mesenteric circulation of a solution of saline containing ^{85}Kr.

Clearance techniques

This approach uses the Fick principle to estimate blood flow through an organ from the rate of clearance by that organ of a marker substance. There is no substance to my knowledge that is exclusively cleared by the tissues of the gut wall. Blood flow through the liver can, however, be estimated from the clearance of bromo-sulphthalein (BSP) (Bradley, Ingelfinger, Bradley & Curry, 1945). Although BSP is not cleared exclusively by the liver, Katz & Bergman (1969) obtained good agreement between measurements of hepatic venous outflow using BSP clearance and PAH dilution. The advantage of BSP is that it can be injected into a peripheral vein and is sure to be thoroughly mixed before sampling.

Physical flowmeters

Blood velocity or mass flow can be measured using electromagnetic (Wetterer, 1963) or ultrasonic, Döppler shift (Vatner, Franklin & Van Citters, 1970) flowmeters. The overwhelming advantage of these techniques for most studies of blood flow per se is that the transducers are placed around rather than inside the blood vessel, and thereby the problems of clotting and other tissue reactions within the vessel are avoided. Tissue reactions around the vessel are more likely to interfere with the signal from electromagnetic than from Döppler transducers. On the whole I think these approaches are more suited to the measurement of flow in arteries than veins, although both techniques have been used to measure portal blood flow (Durotoye & Grayson, 1971; Hume, 1971). However, when one is measuring uptake or clearance of a substance, one must have a catheter inside the vessels to sample blood, so the advantage of an external flow transducer largely disappears.

Surgical approaches

There are numerous reports on different approaches to the catheterization of blood vessels in the hepatic portal circulation (Moodie, Walker & Hutton, 1963; Waldern, Frost, Harsch & Blosser, 1963; Katz & Bergman, 1969; McGilliard & Thorp, 1971; Mason & White, 1971). The surgical approach is essentially straightforward and these papers primarily describe more or less successful approaches to the difficult problem of maintaining patency in the sampling catheters after recovery

from surgery. Most authors favour polyvinyl catheters, although Moodie *et al.* (1963) claim good success with nylon. Our success rate with polyvinyl catheters has been variable. Generally we get little or no phlebitis around the catheters, less apparently than Moodie *et al.* (1963) but in a frustrating number of instances the catheter will permit injection but not withdrawal. Clearly either a flap of fibrin or the wall of the vessel is acting as an effective one-way valve.

Dr White and I insert catheters (internal diameter 1·0 or 1·5 mm) into the arcades of the mesenteric arteries and veins and position them with assistance from Mr Wenham using X-radiography with image intensification. The portion of each catheter outside the vessel is allowed to curve around the ventral and caudal region of the abdomen and emerges through a stab incision on the back (Mason & White, 1971). The advantage of this approach is that the surgical interference is minimal and the post-operative recovery of the sheep is nearly always excellent. The disadvantage is that the catheters are long (600–800 mm) and it is not possible to insert and remove a guide wire or smaller catheter into the cannulated vessels, since they cannot be pushed through the bends in the vinyl catheters. When the portal vein is cannulated directly using a short catheter (<400 mm) having a wider bore (2·4 mm) it is possible to insert another catheter in an attempt to withdraw blood when all else fails. However, this is not always successful either.

Sampling of blood leaving the liver in the sheep is complicated by the multiplicity of hepatic veins which are unlikely all to carry blood of identical composition. Of the techniques described for the cannulation of hepatic veins in sheep (Harrison, 1969; Katz & Bergman, 1969) and cattle (Simmonds & Baird, 1973) that of Katz & Bergman would seem to be the least acrobatic since the catheter is inserted through a hepatic branch on the reasonably accessible visceral surface of the ventral lobe of the liver. Moodie *et al.* (1963) were able to obtain a genuinely representative sample of hepatic venous blood in conscious sheep by the use of a snare to obstruct the posterior vena cava between the entry of the renal and hepatic veins. This is rather a severe approach. An alternative approach which Dr White and I have tried is to measure blood flow and composition at sites in the posterior vena cava just before and after entry of the hepatic veins and estimate the contribution of the hepatic circulation by difference. First indications are, however, that mixing is not very good.

The continuous thermal dilution technique in practice

It is not the purpose of this paper to present detailed results of current experiments, merely to illustrate ways in which the technique is being used. Most studies so far have concentrated on measurement of total heat production and aerobic metabolism in the digestive tract of sheep eating different quantities of diets differing in physical and chemical form. In these experiments total heat production is estimated from the product of flow and the temperature rise in the blood as it traverses the visceral circulation. The validity of this method was checked by inserting an immersion heater in the rumen of sheep with fistulas. The recovery of the externally

applied heat increment was 98% ($\pm 4 \cdot 8\%$) (Webster & White, 1973). Aerobic metabolic rate in the gut is estimated from the difference in oxygen content between arterial and portal venous blood. In sheep starved for over 2 d portal blood flow was on average about 1·7 l/min, total heat production estimated by temperature difference 53·5 kJ/h, and aerobic heat production estimated from oxygen consumption was 54·8 kJ/h (Webster, Osuji, White & Ingram, 1974). In these circumstances, anaerobic heat production by rumen fermentation would be very small. These results indicate therefore that there is no serious systematic error in the approach. When sheep were offered increasing amounts of food the increase in heat production in the portal-drained viscera was associated with a parallel increase in portal blood flow, since the arteriovenous differences in temperature and oxygen content increased only slightly. Examples of the effect of increasing food intake on blood flow in the portal vein and heat production in the gut wall are shown in Fig. 2.
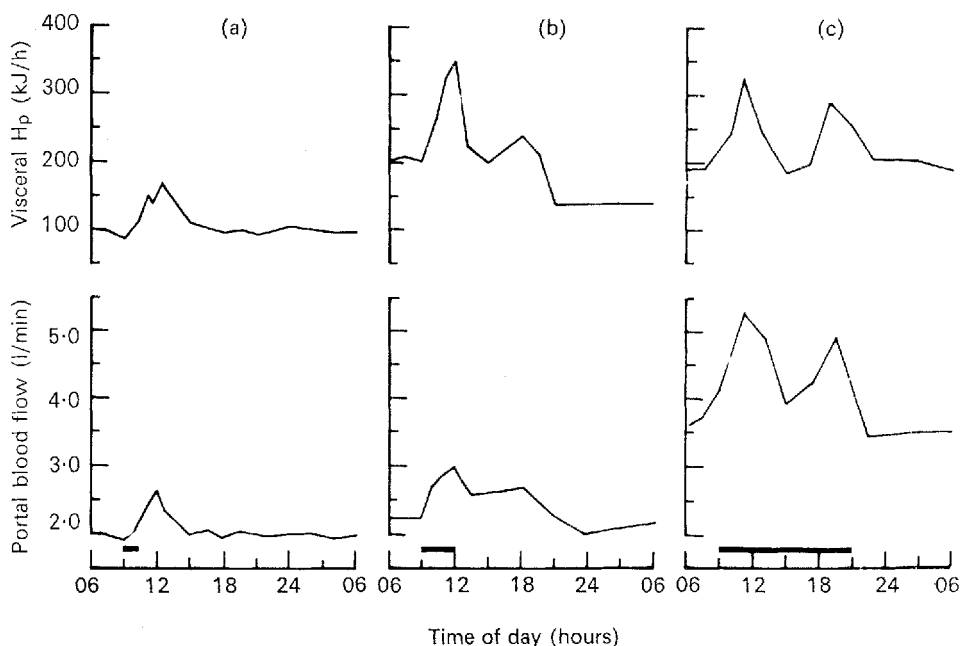

Fig. 2. Portal blood flow and visceral heat production (H_p) in a sheep eating (a) 900, (b) 1500 and (c) 2250 g dried lucerne pellets daily, offered in a single meal at 09.00 hours. The horizontal bars at the bottom of each graph indicate the length of time it took the sheep to consume its entire ration.

Other current studies using this technique include that reported by Dr Weekes at this meeting (Weekes & Webster, 1974) which estimates the metabolism of volatile fatty acids in the gut wall from the difference between that leaving the gut and that entering the portal vein. Studies of amino acid absorption in the sheep (M. I. Chalmers, unpublished results) and pig (Farrell, Jones, Webster & White, 1974) are also in progress. The precision of these techniques is limited primarily by the precision with which one can measure the very small differences that exist between the concentration of, for example, a single amino acid in arterial and portal venous

blood. If a sheep absorbed 12 mg/h of an individual amino acid into the portal circulation when portal flow was 2 l/min, the arterio-venous difference in concentration of that amino acid would be 0·1 µg/ml. Problems of measurement of differences this small are outside the scope of this review. Preliminary results suggest that it is not impossible.

In conclusion, then, the measurement of clearance by the hepatic portal circulation of the products of digestion and metabolism in the ruminant gut is theoretically sound, surgically straightforward, and acceptably precise. It is, however, bedevilled by the problem of maintaining permanently indwelling catheters. How successful the technique is in practical terms is a question of attitude. In this pursuit it pays to expect nothing. In that way, one can frequently be very pleasantly rewarded.

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