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A NEW PROCEDURE FOR THE CONTINUOUS MEASUREMENT OF ENERGY EXPENDITURE IN PIGS AND SHEEP

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Summary

A new procedure has been developed in this laboratory for the measurement of energy expenditure from oxygen consumption in pigs and sheep. Oxygen consumption is calculated from the simultaneous and continuous measurement of cardiac output and the difference in blood oxygen content across the lungs (Fick technique). New technology for measuring cardiac output using transit-time ultrasound and of blood oxygen content in vivo using fibre-optic oximetry has meant that it is now possible to measure continuously the energy expenditure due to high ambient temperature, activity or drug administration in metabolic studies with pigs and sheep.

I. INTRODUCTION

Measurements of energy expenditure have been made with a variety of techniques, based on the knowledge that the oxidation of food leads to energy release as heat, while oxygen is consumed and carbon dioxide and methane produced. The techniques either directly measure heat output using specially designed chambers or indirectly when heat production is calculated or inferred from the quantitative measurement of chemical by-products of metabolism, generally gaseous exchange (see McLean and Tobin 1987).

The connection between energy expenditure and gaseous exchange has been known for over two hundred years (see Kleiber, 1961: Blaxter, 1962). More recently Brouwer (1965) published a formula for predicting heat production from gaseous exchange based on the assumption that heat is produced only by the oxidation of carbohydrates, fats and proteins to carbon dioxide, water, urea and in the case of ruminants, methane. This has been modified by Lawrence and Pearson (1989) such that:

$$H = 16.16\,C + 5.09\,P - 6.5\,U - 2.0\,M \qquad [1]$$

where H = heat production (kJ)
C = oxygen consumed (l)
P = carbon dioxide produced (l)
U = urinary nitrogen (g)
M = methane production (l)

The ratio of CO_2 produced : O_2 consumed is known as the respiratory quotient (RQ) and may vary from 0.7 when an animal is oxidising only fat to 1.3 when producing fat at a maximised rate from carbohydrate.

According to Lawrence and Pearson (1989) the relative importance of O_2 consumption in the Brouwer equation is 77% compared to CO_2 production at 24% with urinary N at 0.5% and methane at 0.7% of the total. If an RQ of 0.9 is assumed, heat production can be calculated from oxygen consumption alone with an uncertainty of 2.4% as follows.

$$H\ (kJ) = O_2 \text{ consumption (l)} \times 20.5 \qquad [2]$$

In this paper we describe a procedure for measuring oxygen consumption and thereby heat production of the whole body based on this simplified equation [2] which requires the direct measurement of cardiac output (l/min), and the difference in oxygen content between arterial and mixed venous blood as first described by Adolf Fick in 1870 (see Zierler 1976).

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Oxygen consumption is calculated in pigs and sheep by applying the ultrasonic blood flow and oximetrix techniques to the Fick equation as follows :

$$QO_2 = CO \times (SaO_2 - SvO_2) \times Hb \times 1.34 / 10$$

where QO_2 = oxygen consumption (ml/min)
CO = cardiac output (l/min)
SaO_2 = arterial oxygen saturation (%)
SvO_2 = mixed venous oxygen saturation (%)
and Hb = blood haemoglobin content (g%).

The artery used in these studies was the saphenous artery. The oxygen saturation in arterial blood is relatively constant (Giles and Gooden, 1991) and was measured manually at intervals throughout the experimental period. The similar voltage output for both the blood flow and the oximetrix meters facilitates the use of a micropower data logger (Tain Electronics, Melbourne) for automatically averaging and recording cardiac output and SvO_2 at selected time intervals (eg one or five minutes)

This technique is obviously not new. What is unique is the combination of new technology which allows the continuous and simultaneous measurement of cardiac output and oxygen content of mixed venous blood.

II. MEASUREMENT OF CARDIAC OUTPUT

Cardiac output in pigs and sheep is measured as the volume of blood flow per unit time through the pulmonary artery. This is measured using an ultrasonic blood flow device developed by Cornell University (Transonic Systems Inc., Ithaca, New York).

(a) Ultrasonic Blood Flowmeter

This flowmeter utilizes an ultrasonic, transit-time principle whereby volume flow is sensed independent of vessel size. Acoustic contact is required between ultrasonic sensor and blood vessel. A flow probe consist of an epoxy probe body containing the ultrasonic sensors and stainless steel brackets (see Figure 1).

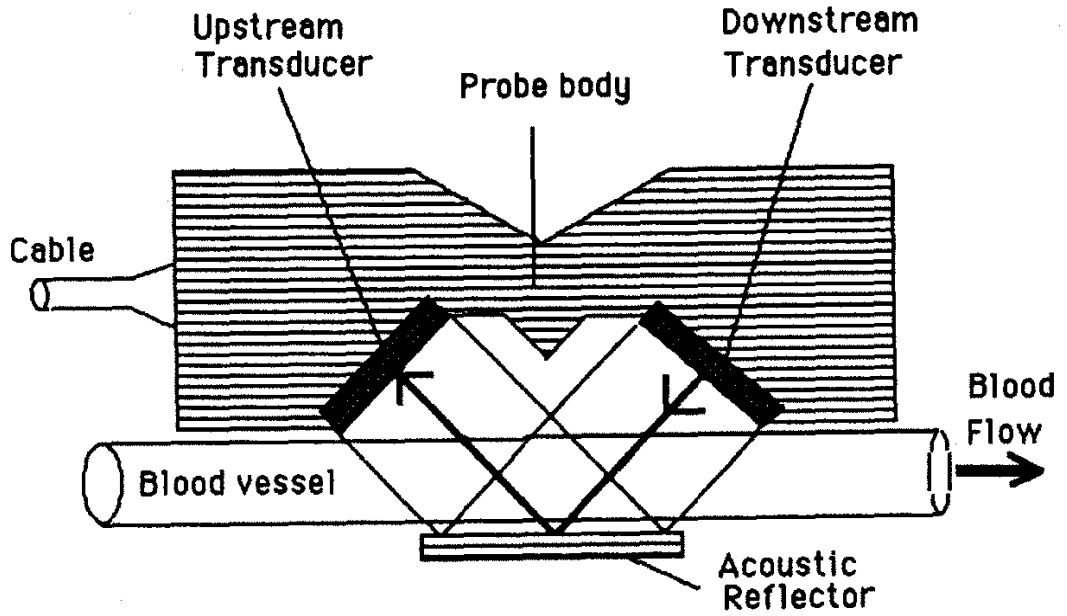

Figure 1. Diagram of ultrasonic blood flow probe

The brackets screw into place onto the body probe body, leaving a rectangular acoustic window. The probe is designed to sense the volume flow of liquid passing through this window, irrespective of where the flow occurs within the window. During surgical implantation the blood vessel is positioned within the acoustic window and the probe firmly sutured onto surrounding tissues to prevent it tugging on the vessel. Measurements can begin once the cavities between the

probe and vessel are filled with fibrous tissue, a process that takes three to five days. The probe body and reflector align two ultrasonic transducers with the vessel under study. Electronic circuitry within the flowmeter operates this probe through the following cycles:

(b) Upstream transit-time measurement cycle

An electrical excitation forces the downstream transducer to emit a burst of ultrasonic vibrations. This burst passes through and around the vessel, bounces off the "acoustic reflector" (a section of the stainless steel bracket enclosing the vessel), passes through the vessel again and is received at the upstream transducer. The upstream transducer converts the received acoustic vibrations into electrical signals. The flowmeter analyses this received signal to produce an accurate measure of the time it took for the burst of ultrasound to pass from one transducer to the other (the "transit-time").

(c) Downstream transit-time measurement cycle

The transmit-receive sequence of the upstream cycle is repeated, but now the transmitting and receiving functions of the transducers are inter-changed. Thus the flow is now traversed by the ultrasonic burst in the downstream direction. Again, the flowmeter derives from this transmit-receive sequence an accurate measure of the transit-time. Just as the flying time of an aeroplane is affected by the winds it encounters, so is the transit-time in the probe affected by motion of its conducting medium, in this case, the flow of blood. In the upstream cycle, a part of the sound burst travels against the flow on part of its course, which increases the total transit time by a certain amount. In the downstream cycle the burst travels along the same section of flow, but this time the transit time is decreased by the same amount. The ultrasonic flow probe is designed in such a way that this shift in transit time is proportional to the volume flow of liquid through the vessel. Flowmeter circuitry then subtracts the downstream transit time from the upstream transit time, resulting in a difference signal proportional to volume flow. This difference is subsequently scaled to correspond with the predetermined calibration factor of the probe, and displayed as the absolute volume rate of flow through the probe in l/min in the case of cardiac output.

(c) Surgery

The thoracic surgery required to place the ultrasonic blood flow probe around the pulmonary artery is similar in pigs and sheep but the degree of difficulty is much higher in the porcine due to difficulties with endotracheal intubation because of laryngospasm, drug interaction during anaesthesia and the inaccessible position of blood vessels. A diagram showing the placement of the blood flow probe in the pulmonary artery is shown in Figure 2.

Following surgery, which includes catheterisation of a saphenous artery, the blood flow probe is connected to a bench top meter which provides a direct read-out of cardiac output after approximately three days. This time period is required to allow tissue build up around the probe resulting in the exclusion of air and hence acoustic coupling between the pulmonary artery and the probe. The animals return to normal feed intakes within three to seven days following surgery.

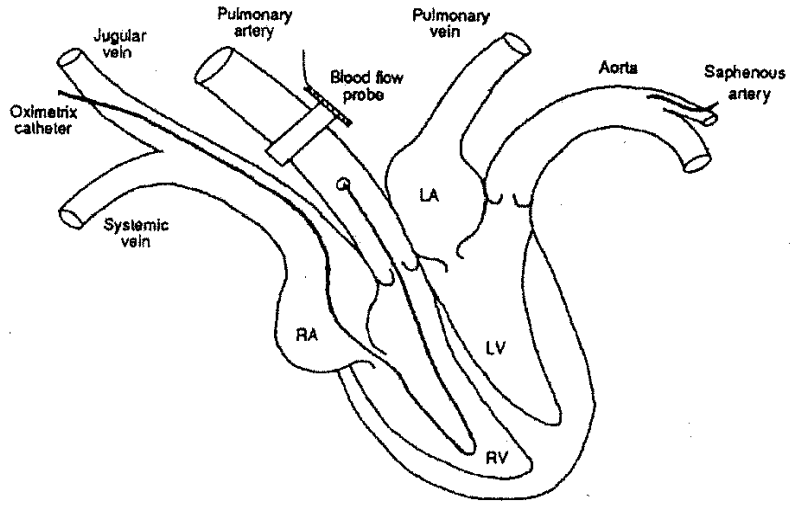

Figure 2. Diagram of the heart showing surgical placement of the ultrasonic blood flow probe around the pulmonary artery.

This technique of measuring cardiac output has been carried out in 20 pigs (60-90kg liveweight) and 10 sheep (35-45kg) over periods of two to six months, respectively.

We have compared the transit time ultrasonic blood flow method with dye-dilution using the jugular vein (Lush et al. 1989) and with the stop watch / measuring cylinder technique using the portal vein (Neutze et al. 1989). In both cases no significant difference in blood flow between the techniques was observed. It would appear that the ultrasonic blood flow method accurately monitors blood flow in a range of vessels including the portal vein (Lush et al. 1990) and the pudic artery supplying the mammary gland (Maas et al. 1991).

III. MEASUREMENT OF BLOOD OXYGEN CONTENT

Placement of catheters in the pulmonary artery (mixed venous blood) and any other artery in the body (arterial blood) will allow the measurement of oxygen uptake by the lungs. This is achieved by manually sampling blood at regular intervals and measuring oxygen saturation photometrically (Hemoximeter; Radiometer, Copenhagen). A number of laboratories have used this technique over the past 15 years. The continuous in vivo measurement of blood oxygen saturation in human medicine was described over 25 years ago (Gamble et al. 1965) using fibre optic technology. A light beam of three specific wave lengths is transmitted by a fibre optic bundle to the tip of a specially designed catheter. The reflected light is then transmitted back to a monitor by a second fibre optic bundle. The difference in light intensity between the afferent and efferent fibre optic bundles is related to blood colour and hence oxygen saturation which is displayed continuously on a monitor (Oximetrix, Abbott Laboratories, North Chicago). Using this technology in combination with an ultrasonic blood flow meter, we have developed a system which can be used to continuously measure oxygen consumption. Manual blood sampling from the pulmonary artery is required from the oximetrix catheter once or twice a day to calibrate the oxygen saturation using a hemoximeter as the reference. At the same time arterial blood samples are required to determine arterial oxygen saturation and haemoglobin concentration.

The oximetrix catheter (pulmonary artery, 7.5F, or 2.5mm OD) is inserted into the pulmonary artery via the jugular vein in both pigs and sheep (see Figure 2.) using the Seldinger technique (Seldinger, 1953) as described in pigs by Schwartz and Smallgood, 1977) with the aid of a catheter introducer (8.5F with a 13 cm sheath, W.A. Cook Aust, Brisbane). Once clear of the introducer, the latex balloon at the tip of the catheter is inflated with air to allow easy movement of the catheter through the heart chambers and into the pulmonary artery. We discovered that a pressure transducer was not needed to verify correct placement in the pulmonary artery since the balloon inflated with air caused interference when passed through the acoustic window of the blood flow probe.

Experience has shown that the oximetrix catheters remain patent for up to two weeks in pigs and sheep. If the fibre-optic bundles become damaged or the catheter tip becomes covered with a blood clot, it has proved possible to withdraw the catheter and replace it with a new one. This is more difficult in the pig but up to six catheters have been placed in a sheep in the same jugular vein over a period of six months. The oximetrix catheter has a number of other useful features built in, such that deep body temperature can be measured from a thermocouple built into the tip of the catheter and cardiac output can be measured by thermal dilution. In the latter case the accuracy appears less than that using the ultrasonic probe and it lacks the ability to measure flow continuously. However it may be useful where surgery is not feasible or desirable.

IV. MEASUREMENT OF OXYGEN CONSUMPTION AND HEAT PRODUCTION

(a) Pigs

These techniques have been used to measure cardiac output and SvO_2 in pigs and to compare the results with those obtained using a head box (Gile et al. 1989). The results are shown in the following table where the oxygen consumption of 3 female pigs (average liveweight, 60kg)

was measured over the same two hour period with a head box and with the new procedure based on the Fick principle where mixed venous oxygen saturation was measured manually using a hemoximeter from blood sampled at intervals of 30 minutes.

Pig number		1	2	3		
Technique					Mean	SEM
Head Box	O_2 consumed (ml/min)	428	378	321	378	30.9
Fick	Cardiac output (l/min)	8.8	7.2	6.3	7.4	0.73
	AvO_2 (ml/100ml blood)	5.27	5.35	5.26	5.29	0.03
	Blood Hb (g%)	9.0	7.9	8.2	8.4	0.33
	O_2 consumed (ml/min)	464	383	333	393	38.4

It can be seen that mean values for oxygen consumption yielded by the head box (378 ml/min) and the Fick procedure (393 ml/min) over the same two hour period were not significantly different. Limitations of the head box, apart from the need to use trained animals, include the difficulty of matching conditions within the box to those of the environment at temperatures above thermoneutral. The new Fick procedure described above has been shown to be ideally suited to studies with growing pigs (Giles et al. 1990) maintained at raised ambient temperatures for periods up to 12 days.

(b) Sheep

Preliminary results during exercise and following drug administration have been obtained from three merino wethers (average liveweight, 35kg) prepared with ultrasonic probes and oximetrix catheters (Gooden et al. 1991).

(c) Exercise

Figure 3 shows cardiac output, SvO_2 and QO_2 of the whole body for one of these sheep. Each value is the mean ± sem for three replicates.

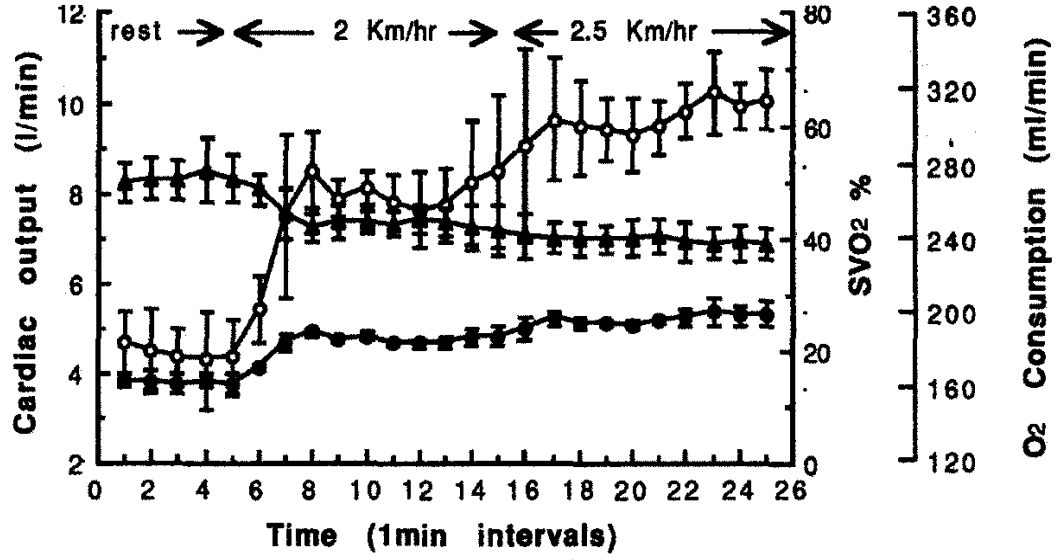

Figure 3. Cardiac output •—•, SvO_2 s—s and QO_2 o—o of sheep 2 during exercise on a treadmill at 2.0 and 2.5 km/hr.

Blood flow rapidly increased from a resting, standing value of 3.8 l/min to 4.8 l/min during a walk of two km/hr and 5.2 l/min at 2.5 km/hr. This represents an increase of 26% and 37% compared to the resting value. During these periods QO_2 increased from 180 ml/min at rest to 270 and 315 ml/min at two and 2.5 km/hr respectively representing an increase of 47% and 69% compared to the resting period.

Figure 4 also shows the effect of exercise on sheep 2 but in this example the treadmill has been inclined at a 9° slope.
As expected the cardiac output increased from 3.8 l/min while standing to six l/min at two km/h to 7.9 l/min during exercise at three km/h up an incline. The comparable decreases in SvO_2 were 64% to 50% to 47% respectively. As a consequence of doing work sheep 2 increased its heat production from 2.6 kJ/min at rest to 5.3 at 2km/h to 7.9 kJ/min at 3 km/h. Heat production was calculated by multiplying oxygen consumption by 20.5 as described previously (equation [2]).

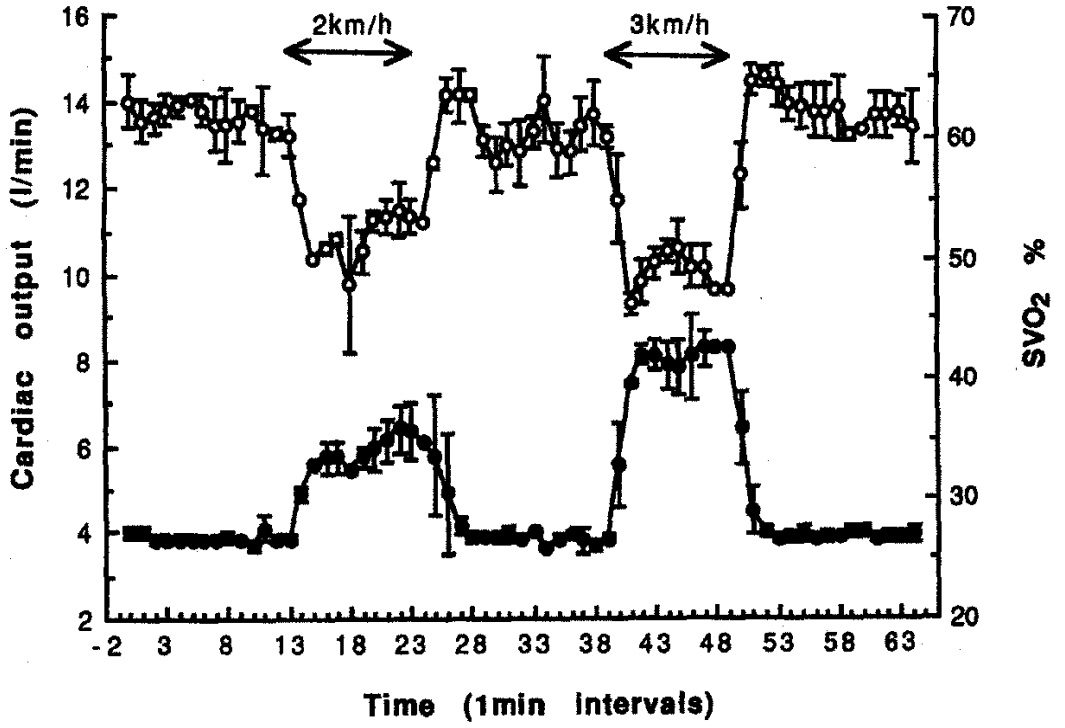

Figure 4 Cardiac output •—• and SvO_2 o—o saturation of sheep 2 while at rest and during exercise up an incline of 9° at 2 and 3 km/h.

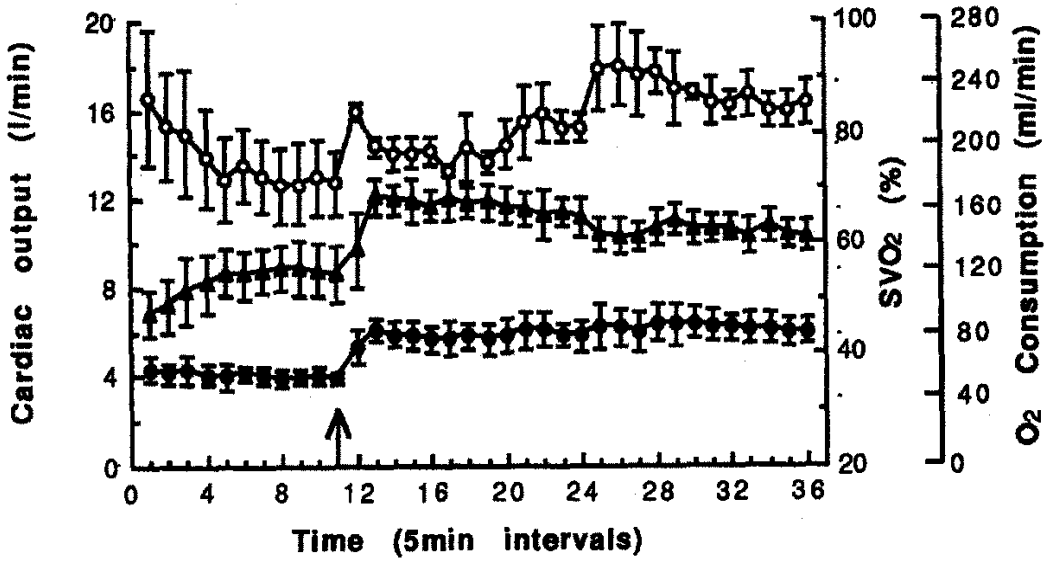

Figure 5. Cardiac output •—•, SvO_2 s—s and QO_2 o—o in 3 sheep following injection with 5 mg/kg liveweight of clenbuterol. Each value is the mean ± sem.

It is hoped in future work to train sheep to run at speeds of 7-9 km/h at 0° incline or 3-4km/h at 9° incline in order to determine the anaerobic threshold, ie that level of activity above which aerobic energy production must be supplemented by anaerobic catabolism (see Pethick et al. 1991).

(d) Drug administration

Changes in the cardiac output and SvO_2 of the 3 sheep (standing) following the injection of clenbuterol (5mg) are shown in Figure 5.

The initial response to the drug was a marked increase in cardiac output followed by an immediate increase in SvO_2. This compensation resulted in only a minor increase in QO_2 in the short-term. As the SvO_2 returned to normal levels cardiac output remained elevated resulting in a gradual increase in QO_2.

VI. CONCLUDING COMMENTS

In conclusion, the examples presented indicate that the Fick technique is able to monitor immediate changes in QO_2. The use of blood flow probes, oximetrix catheters and data loggers to record cardiac output and SvO_2 has allowed measurement to be carried out with minimal labour and minimal disturbance to the pigs and sheep which is very important in metabolic studies requiring steady state conditions in unstressed animals.

The new procedures have two major disadvantages. Surgical intervention is required, and measurements can be made only in restrained animals. We hope to (a) develop other methods of accurately measuring cardiac output using thermodilution without the need for surgery and to (b) find a suitable telemetry system which would allow the use of the Fick technique with unrestrained animals. In this way measurement of the energy cost of grazing could be attempted. An exciting area where this technology has accelerated progress is in the measurement of oxygen consumption by individual tissues such as the portal drained viscera (Lush et al. 1990) and the mammary gland (Maas et al. 1991) of the sheep, rather than the whole body.

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