S. Afr. J. Anim. Sci. 4, 93–95 (1974)

AUTOMATIC COLLECTION OF BOVINE BLOOD SAMPLES

D.H. Hale

Receipt of MS 29.10.73.

Department of Agriculture, University of Rhodesia, Box MP. 167, Mount Pleasant, Salisbury

OPSOMMING: OUTOMATIESE VERSAMELING VAN BEESBLOEDMONSTERS

'n Tegniek vir die outomatiese versameling van nekaarbloed van koeie wat vasgemaak is, word beskryf. In hierdie stelsel word bloed aanhoudend deur 'n cannula met 'n dubbel opening gepomp terwyl 'n antikoagulant deur die tweede opening toegevoeg word. Verdunde bloed word versamel in 'n fraksieversamelaar wat aan 'n tydskakelaar gekoppel is.

SUMMARY

A technique is described which allows automatic collection of jugular venous blood from tethered cows. In this system, blood is pumped continuously from an intravenous cannula which has a double lumen while an anticoagulant is pumped through the second opening. Diluted blood is collected in a fraction collector which is fitted with a time-switch.

Although hormone levels have been measured in body-fluids other than blood, end organ response depends upon blood levels of the relevant hormone. Consequently, blood levels of a particular hormone reflect most accurately the respective endocrine status of the animal. Further, rates of secretion of hormones change rapidly and the halflife of hormones in blood is often very short. This indicates that very frequent measurement of hormonal blood levels is not only desirable but indeed essential to assess accurately amounts of hormone secreted and the period of hormone release. These remarks are particularly pertinent to hormones associated with reproduction– follicle stimulating hormone, luteinizing hormone, prolactin and the gonadal steroids.

In recent years, development of radioimmunoassays has rendered feasible not only the measurement of hormone levels in large numbers of blood samples, but has also obviated the necessity to withdraw large volumes of blood for each determination. On the other hand, in the absence of several skilled technicians, collection of large numbers of samples of blood at very frequent intervals constitutes a major problem. Furthermore, frequent handling of experimental animals is highly likely to influence the levels of hormones which are being measured.

Techniques have been developed to allow collection of frequent blood samples by manual withdrawal of blood from indwelling cannulae (Anderson & Elsley, 1969; McGilliard, 1972). Continuous withdrawal of blood by use of an arterovenous shunt is combined with extracorporal dialysis in acute and chronic renal failure (Merrill, 1965). Blood has been taken continuously from the portal vein of anaesthetized dogs (Jungblut, Lohman, Schober & Turba, 1955). However, a simple technique was required which would allow collection of small samples of blood at frequent intervals over several days from unanaesthetized cows without the assistance of a team of technicians. This paper describes a technique which allows the automatic collection of blood samples from tethered cows.

Procedure

In principle, the system involves continuous collection of blood from the jugular vein of a tethered cow.

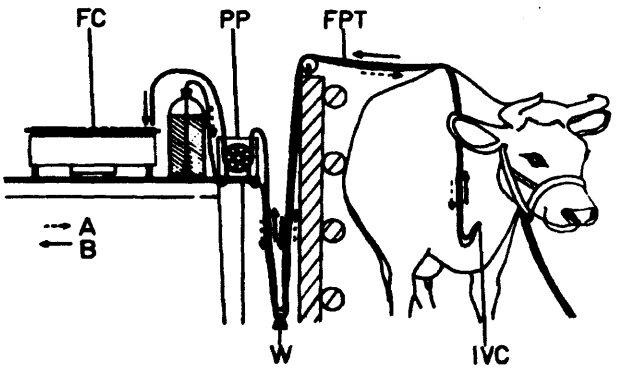

Fig. 1. *Automatic collection of bovine blood samples: Blood (B) is pumped from and anticoagulant (A) is pumped to an intravenous cannula (IVC) through fine polythene tubing, (FPT) by a peristaltic pump (PP). A fraction collector (FC) allows collection of blood at fixed time intervals. A system of a weight (W) and pulleys allows movement of the cow*

Blood is pumped from an intravenous cannula to a fraction collector which regulates frequency of sampling. Simultaneously, anticoagulant is pumped to the intravenous cannula to maintain patency of the system.

Secretory products of the anterior pituitary are transported to the appropriate target organs mainly via the jugular vein. Further, the jugular is a large, easily accessible vein which is relatively close to the point at which the animal is tethered. Consequently, movement of the cannula is reasonable restricted, thus facilitating its connection to a metering pump and fraction collector by fine, flexible polythene tubing. A system of a weight and pulleys maintains tension on the polythene tubing and allows for movement of the cow.

The apparatus comprises three main units:-

(1) A fraction collector
(2) A metering pump
(3) An intravenous cannula.

Fraction collector

The fraction collector is fitted with a time switch to allow collection of blood samples at fixed time intervals. Tubes in the fraction collector are enclosed in a tank which is cooled either by ice bags or by a refrigeration unit. Temperature of the cooling tank is +4°C.

Metering pump

A pump with two channels is used to regulate withdrawal of blood from the animal and at the same time to pump anti-coagulant to the cannula. In the present investigations, a modified LKB Perspex peristaltic pump was used. Flow rate of blood and anticoagulant is regulated by the internal diameter of the silicone rubber peristaltic tubing and by different reduction gearboxes in the pump.

Use of 0,8 mm I.D. tubing to pump anticoagulant and 1,8 mm I.D. tubing to pump blood results in a final dilution of 1,6 ml of diluted blood to 1 ml of actual blood. Use of a 1:200 reduction gearbox results in collection of 8,5 ml/h of blood diluted with anticoagulant or 5,3 ml/h of actual blood. Dilution of blood is checked by haematocrit of undiluted and diluted blood. Obviously flow rates and dilutions vary with experimental requirements and availability of equipment.

Intravenous Cannula

Essentially, the cannula comprises two flexible tubes, one lying inside the other. Anticoagulant is pumped between the outer and inner tubes to the tip of the cannula. Blood is pumped along the inner tube away from the tip of the cannula towards the fraction collector.

Flow rate in the inner tube is greater than the flow rate in the outer tube. Thus, not only is the anticoagulant from the outer tube drawn into the inner tube, but also blood from the jugular vein is drawn into the inner tube to make up the difference in flow rates.

This basic assembly is inserted through an outer nylon cannula (Portex FG8) for three reasons. Firstly, the outer nylon cannula provides enough rigidity to overcome the tendency of tubes to kink due to movements of the neck and skin. Secondly, the outer nylon cannula can be introduced easily into the jugular vein using an 8 gauge MacGregor needle. This operation can be carried out conveniently in a suitable crush with neck bale. The cannula is sutured in position, plugged with a rubber cap and filled with anticoagulant by injection through the rubber cap. The animal can then be moved to the stall where actual sampling is to take place. Finally, the outer nylon cannula facilitates removal and reinsertion of the main cannula if it is necessary to check patency of the inner tubing. The two tubes of the basic cannula project ±2 cm into the vein beyond the end of the outer nylon cannula.

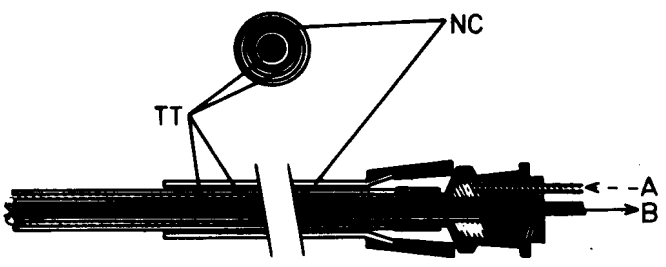

Fig. 2. *Intravenous cannula for automatic collection of bovine blood samples: Anticoagulant (A) is pumped between the outer and inner Teflon tubes (TT) and withdrawn, together with blood (B) from the vein inside the inner tube. The Teflon tubes are inserted through an outer nylon cannula (NC).*

Practical considerations

1. *Design of Cannula*

Design of cannula must ensure that anticoagulant reaches the very tip of the cannula. The tip of the inner tube must lie between 1,0 and 0,5 mm inside the tip of the outer tube. The internal diameter of the inner tube must be sufficiently small (in the present studies 0,4 mm) to ensure adequate mixing of blood and anticoagulant at the prevalent flow rate.

2. *Material for construction of cannula*

Nylon, polyvinyl chloride and polythene tubing were all used for construction of cannulae. Irrespective of material used, and despite attempts to coat tubing with silicone (Repelcote, Hopkin & Williams, London) either blood coagulated and blocked the cannula or a local phlebitis developed which prevented blood from entering the cannula. Nylon was particularly unsuccessful in this respect. This problem was eventually overcome by use of polytetrafluoroethylene tubing (PTFE, Teflon, Baird & Tatlock, London).

3. *Insertion of Cannula*

During the insertion of the teflon cannula, anticoagulant is pumped through both inner and outer teflon tubes to ensure displacement of any small clots which may have formed in the outer nylon cannula. Flow in the inner teflon tube is subsequently reversed to collect blood.

4. *Anticoagulant*

Initially, heparin was used as the anticoagulant because of its low toxicity in the event of the anticoagulant passing into the blood stream. However, even at a concentration of 250 i.u./ml of anticoagulant or ±150 i.u./ml diluted blood, heparin did not completely inhibit formation of thrombi which eventually blocked the collection apparatus. Subsequently, sodium citrate (3,8%) has proved satisfactory as an anticoagulant.

5. *Asepsis*

Ideally, blood should be withdrawn under complete asepsis. Practically, this is almost impossible. Nevertheless it is of great importance to minimise introduction of the micro-organisms which cause local phlebitis. In the present study, provided reasonable asepsis was maintained, local use of antibiotics has not proved as advantageous or as necessary as has been reported elsewhere (McGilliard, 1972). However, intramuscular injection of 1,000,000 units of penicillin (Ultracillin, C.A.P.S., Salisbury) upon insertion of the outer nylon cannula has been useful in minimising the effects of any infection.

Using this technique, blood samples have been collected from ranch cows for up to 3 days at intervals of 15 minutes and up to 5 days at intervals of 1 hour

Acknowledgements

I am very grateful to the Rhodesian Cattle Producers Association for financial support, to the Department of Anatomy, University of Rhodesia, for the equipment and to H.I. Hale (Pvt) Ltd., for animals used in this study.

References

ANDERSON, D.M. & ELSLEY, F.W.H., 1969. A note on the use of indwelling catheters in conscious, adult pigs. *J. Agric. Sci. Camb. 72,* 475.

JUNGBLUT, P.W., LOHMAN, B., SCHÖBER, R. & TURBA, F., 1955. A method for continuous taking of blood from the portal vein in dogs. *Experentia, 11,* 241.

McGILLIARD, A.D., 1972. Permanent implantation of arterial and venous catheters and lymphatic shunts. *J. Dairy Sci. 55,* 1191.

MERRILL, J.P., 1965. *The treatment of renal failure.* London: William Heineman.