APPARATUS FOR THE CONTINUOUS COLLECTION OF SHEEP OVIDUCT FLUID

D. L. BLACK, R. T. DUBY AND J. RIESEN

Department of Dairy and Animal Science, University of Massachusetts, Amherst, Massachusetts, U.S.A.

(Received 21st March 1963)

Summary. An apparatus has been constructed for the collection of oviduct fluids from sheep. The use of this equipment makes possible the collection of relatively large amounts of oviduct fluid for biochemical analysis.

INTRODUCTION

Little is known about the biochemical environment afforded the ova and spermatozoa by the oviduct. It has long been established that the oviduct possesses secretory cells which undergo pronounced changes during the reproductive cycle. Bishop (1956) demonstrated that secretion by the oviduct is an active process which is influenced by the hormonal state of the animal. In rabbits, he found the highest rate of secretion as well as the greatest secretory pressure at the time of oestrus. In the past, biochemical study of oviduct fluid has been hindered by the small amount present at any one time. Clewe & Mastroianni (1960) solved this problem by devising a method for continuous collection of rabbit oviduct fluid. With their method it was possible to collect it from unanaesthetized rabbits over a prolonged period of time and to sample the secretion at intervals for biochemical analysis.

It was the purpose of this experiment to attempt collection of oviduct secretions from sheep. During the experiment it was necessary to construct a collection apparatus suitable for this animal. The construction of this apparatus and its method of use are described in this paper.

MATERIALS AND METHODS

Initially, attempts were made to collect sheep oviduct fluid by the method used by Clewe & Mastroianni (1960) on rabbits. Basically, their apparatus consists of a long small-diameter glass tube folded upon itself several times, embedded in plastic, and attached to the animal. Considerable difficulty was encountered in reading the contents in this apparatus because the column of fluid within the collecting tubing invariably contained bubbles. Since the animals in our study were allowed the freedom of individual pens, their activity played an important part in breaking the column, so that the amount of secretions could not be read; a broken column also made it difficult to recover the oviduct fluid completely.
Because of these difficulties, a collection apparatus was constructed which more closely met our needs. Basically, it consisted of four parts: an inlet tube through which oviduct secretions could enter the collection chamber, a collection chamber, a vent to keep the pressure within the system at atmospheric level and an outlet tube through which the collected fluids could be aspirated from the collection chamber (Text-fig. 1). The entire tubular structure was embedded in plastic.

More specifically, the collection chamber consisted of a shell vial (outer diameter 12 mm, inner diameter 10 mm, length 4.5 cm) from which the bottom had been removed. Both ends of the collection chamber were closed with rubber stoppers. Through one hole in the top stopper a short piece of glass tubing (outer diameter 3 mm, inner diameter 1.5 mm, length 12 mm) entered the collection chamber. A piece of plastic tubing approximately 12 mm long, with one end sealed, was fitted over the end of the glass tubing. After embedding in plastic, a small hole was drilled into the plastic block to penetrate the plastic tubing, thus forming a vent. The inlet tube consisted of a second piece of glass tubing of similar size which was introduced through the top stopper of the collection chamber. This tube was L-shaped with both arms of the L about 12 mm in length. A piece of gum rubber tubing 5 cm in length (outer diameter 7 mm, inner diameter 3 mm) was fitted over the end of the inlet tube.

At the bottom of the collection chamber, a glass tube of the size used for the inlet was introduced through the rubber stopper. After leaving the collection chamber, this tube was bent so that it emerged from the plastic block at approximately the same level as the intake tube. As with the intake, the gum rubber tubing over the glass tubing was the only portion which emerged from the plastic block.

The entire tubular structure was set in plastic by methods commonly employed for embedding biological materials. Turtox Embedding Plastic* was

* General Biological Supply House, 8200 South Hoyne Avenue, Chicago 20, Illinois, U.S.A.
found to be satisfactory for this purpose. There is, however, no reason why any of the plastics designed for embedding biological materials should not be suitable. After casting the plastic block, two holes were drilled through each corner and used in suturing the apparatus to the skin. The final dimensions of the collection apparatus were approximately $7 \times 7 \times 1.5$ cm and the entire structure weighed 80 to 100 g.

After the tubular structure was fixed in plastic, the collection chamber was calibrated by introducing distilled water in 0.1 ml increments into the collection chamber via the outlet tube. After each 0.1 ml addition, the plastic block was scored at the bottom of the meniscus with a sharp instrument. These scores were later filled in with India ink and the entire surface of the block given several coats of a spray plastic. Before use, the inside of the collection apparatus was coated with silicone.

Tygon plastic tubing (outer diameter 2 mm, inner diameter 1 mm) was used to connect the oviduct to the collection apparatus. At mid-ventral laparotomy under general anaesthesia, the slightly flanged end of a length of this tubing was inserted into the ovarian end of the oviduct and held in place with two ligatures of No. 0 plain catgut suture. The free end of the plastic tubing was then exteriorized through a puncture wound of the abdominal wall in the flank area, and the skin tightly closed around the tube with a purse-string suture. The free end of the plastic tube was flanged with heat and inserted into the rubber tubing forming the inlet; a linen ligature held the plastic tube firmly in place. Before closing the abdomen, a small amount of air was forced into the collection apparatus while the catheterized oviduct was observed. Distension of the oviduct indicated patency of the system and insured against occlusion of the plastic tubing as the result of excessively tightened ligatures. After the tube from the oviduct to the collection device was found to be open, the abdomen was closed in the usual manner.

The collection apparatus was attached to the skin of the animal with No. 4 linen suture through the holes drilled in the plastic. An attempt was always made to secure the apparatus to the animal below or level with the normal position of the oviducts to minimize any pressure gradient. Cotton plugs were inserted into the vent and outlet tube to prevent the entry of foreign material.

Oviduct fluid from the collection chamber was aspirated by introducing the hub of a 1 ml tuberculin syringe into the outlet tube and applying negative pressure. Long-term collection required periodic cleaning of the apparatus. This was accomplished by occluding the rubber intake tube at its junction, with the plastic tubing catheter and by means of a hypodermic syringe and needle inserted in the rubber tubing, flushing through the device a saline solution followed by 70 % alcohol, ethyl ether and finally a stream of air to complete drying.

RESULTS AND DISCUSSION

The apparatus has been successful, and oviduct secretions have been collected continuously through five complete oestrous cycles in three ewes. Secretory rates varied from 0.01 ml/hr during di-oestrus to 0.085 ml/hr on the day of oestrus.
The rate of oviduct secretion for one ewe over a 35-day period is presented in Text-fig. 2. The increased secretory rate observed at the time of oestrus is in agreement with data presented for rabbits (Bishop, 1956; Clewe & Mastroianni, 1960), which show that the greatest rates of secretion occur under the influence of oestrogen.

Text-fig. 2. Graphed data obtained from one ewe over a period of 35 days. E = Oestrus; OP = Operation.

No discomfort to the animals was observed during the periods of observation. In some of the earlier preparations, trouble was encountered in keeping the collection apparatus on the animal. It was found that the linen suture securing the device to the skin of the animals was being cut by the sharp edges of the plastic block. Removal of the sharp edges alleviated the difficulty.

The collection chamber was sufficiently large to prevent separation of the column of fluid. No difficulty was encountered in reading the amount of fluid within the chamber or in aspirating the full amount. While the calibrations of the chamber of this apparatus were of greater increments than used by Clewe & Mastroianni (1960), the larger capacity of the chamber permitted greater volumes of oviduct fluid to be obtained for biochemical analysis and allowed collection at less frequent intervals.

ACKNOWLEDGMENTS

The authors wish to express their appreciation for the assistance given by Dr A. Kumar and Mr J. Whiton McDaniel in carrying out this study.

This investigation was supported by the North-eastern Cooperative Regional Project NE-41 'Endocrine Factors Affecting Reproduction and Lactation' and by funds from the Animal Husbandry Research Division, A.R.S., Beltsville, Maryland, U.S.A.

REFERENCES
