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STUDIES ON THE DIRECT VISUALIZATION OF THE BOVINE OVARIES THROUGH A RETAINED CANNULA IN THE PARALUMBAR FOSSA

SUMMARY: Three types of cannulae (ebonite, stainless steel, and polyethylene) varying in design and construction were fitted into the flanks of six cows. The first two of these cannulae communicated directly with the peritoneal cavity. The third type was incorporated in the fascia on top of the peritoneum; with this type the peritoneum had to be incised for each observation and then afterwards sutured.

The ovaries were observed through a modified human bronchoscope. The cows' tolerance of the cannulae varied, the maximum time a cannula was left in a cow's flank being nine weeks.

In all cases successful ovarian sightings were made and in one cow the development of an ovarian follicle was followed to maturation. Successful photographic technique was established. It was concluded that in the bovine the retained cannula is indispensable for continuous endoscopic ovarian observations and photography.

INTRODUCTION

Since its introduction into medical diagnostic techniques, the direct visualization of abdominal and pelvic organs through the use of a culdoscope in human patients has progressed considerably (Decker, 1952). Early studies in veterinary medicine by McEntee (1949) in the observation of bovine ovaries by means of a culdoscope met with only limited success. Megale, Fincher, & McEntee (1956), however, reported the successful visualization of bovine ovaries through a retained cannula. Betteridge & Raeside (1962) adapted this technique for use in the sow and showed that tolerance

of the cannula was satisfactory. The main obstacle in both studies was tissue reaction and the formation of fibrin, but it was claimed that this could be controlled by the use of proteolytic enzymes.

The technique of rectal palpation of genitalia has yielded invaluable information on cyclic morphological changes in the ovary of the bovine. However, the direct visualization of the ovaries with a bronchoscope through a retained cannula and the recording of these observations by photography open new horizons in the study of normal ovarian function and the reaction of the ovaries to treatments or pathological changes.

MATERIALS AND METHODS

The materials consisted of three cannulae of varying design, a bronchoscope with built-in light source, and photographic equipment comprised of a modified perspex tube and camera. The cannulae, bronchoscope, and all surgical instruments were sterilized before use by autoclaving or chemical means.

Experimental animals

Six Jersey cows six to eight years and two crossbred ewes four to six years were used for the experimental project. All animals were stall fed throughout the project and all were in good health.

Cannulae

Three different cannulae (Fig. 1) with the characteristics listed in Table 1 were used.

TABLE 1

	Cannulae		
	Ebonite	Stainless Steel	Polyethylene
Construction	Turned from solid block	Cut and welded	Turned, cut, and glued
Dimensions: Length of shaft	5.4 cm	5.6 cm	5.7 cm
Internal diameter of shaft	2.5 cm	9.0 cm	9.0 cm
Shaft thickness	0.3 cm	1.0 cm	0.6 cm
External diameter of flanges	6.3 cm	14.0 cm	14.0 cm

The bronchoscope

Observations were made through a bronchoscope 47.7 cm in length with an internal diameter of 1.3 cm and an external diameter of 1.5 cm. It was modified so that the light source could be passed down a recess in the side of the principal tube. Power was supplied by 2 x 2.5 volt batteries. This instrument could be dismantled easily for sterilization (Fig. 2).

Photographic equipment

(*a*) The perspex tube. The tube (Fig. 3) was made from clear perspex, its dimensions being:

Length	$63\frac{1}{2}$ cm	2 ft 1 in
Internal diameter	3.3 cm	$1\frac{3}{4}$ in

To produce an area of opacity at one end the tube was roughened with fine abrasive paper to a length of 4 cm. Light when applied to the opposite end of the tube caused the area of opacity to glow and form a pool of light at the end of the tube. This effect provided an excellent source of light for location of the ovaries. The light transmission property was used in subsequent photography through the tube.

(*b*) The camera. The camera used to photograph the ovaries was a Nikkon camera equipped with a medical Nikkon lens and ring flash (i.e., the flash is built into the camera encircling the lens). The focal length was 200 mm with 1/6 and 1/4 close-up attachment. Exposures were made at a set distance of 2 ft 1 in, i.e. the length of the perspex tube. The apparatus was F 16. The film used was Ilford FP 3, 35 mm type, developed at 68°F for 10 minutes.

Surgical technique

In all animals the site selected to insert the cannula was the left paralumbar fossa; this area was shaved and sterilized prior to surgery. Anaesthesia of the site was obtained using a combination of paravertebral T13 to L4 and local infiltration with 2 per cent Xylocaine (Astra).

Cows 1, 2, and 3

The first incision was made 10 cm anterior to the proposed cannula site. This was sufficiently large to admit the operator's hand and the cannula shaft into the peritoneal cavity. The second incision was made approximately 10 cm anterior and ventral to the tuber coxa. This was sufficiently large to allow the end of the shaft to be forced through so that a tight fit of the peritoneum, muscle, and skin would be obtained around the shaft. The operator passed the cannula through the first incision into the peritoneal cavity and then manoeuvred the shaft of the cannula partly through the smaller incision. The external flange and protective cap were then screwed into position and the first incision closed, No. 3 chromic gut being used for peritoneum and muscle and heavy Vetafil (Bengen & Co.) mattress sutures for the skin.

Cow 4

Because of the larger dimensions of the stainless steel cannula a different insertion technique was employed. A single vertical incision which was large enough to admit the shaft of the cannula into the peritoneal cavity was made directly over the proposed site. It was necessary to remove an elliptical piece from the skin on either side of the cannula to ensure a neat fit. The cannula was positioned in the dorsal part of the incision, the peritoneum and muscle being in direct contact with the shaft and internal flange. A continuous suture of No. 3 chromic gut was used to close the peritoneum and muscle at the ventral end of the incision. The skin over this was closed with three mattress sutures of heavy Vetafil.

At this stage the outer flange and cap could be screwed into position. Because of the rigidity of the stainless steel a sterile gauze packing impregnated with sulphanilamide powder was placed between the skin and outer flange. This helped to minimize pressure necrosis in the surrounding tissue but daily dressings of the packing were required (Fig. 4).

Cows 5 and 6

A modified surgical technique was used in an attempt to reduce peritoneal reaction. A vertical skin incision 8 in long was made in the mid paralumbar fossa. The muscle layers were separated by blunt dissection down to the peritoneum. The fascia on top of the peritoneum (fascia transversalis) was carefully dissected on both sides of the incision to allow the insertion of the inner flange of the cannula, which now rested on the peritoneum. The peritoneum was not penetrated. The skin ventral to the cannula was closed up to the cannula by three mattress sutures of heavy Vetafil.

An antibiotic mixture containing 5 x 10^6 I.U. of benethamine, procaine, and sodium penicillin and 5 g of streptomycin was administered intramuscularly.

Sulphanilamide powder was dusted around and behind the outer flange of the cannula.

Cannulation of sheep

An ebonite cannula was fitted into the left paralumbar fossa of each ewe, endodural anaesthesia being used in this case. The surgical technique was identical to that carried out in cows 1, 2, and 3.

Method of observation

The procedure in cows 1, 2, and 3 was to insert the sterilized bronchoscope through the cannula and locate the ovaries. This technique, however, proved difficult and had to be aided by rectal palpation. In cow 4 the arm of the operator, after meticulous sterilization, was inserted through the cannula and the ovary located. The bronchoscope was then inserted along the arm (Fig. 5) and by gentle manipulation examination of the entire surface of the ovary was possible. Both ovaries were easily examined by this method.

Procedure in cows 5 and 6 involved incision of the peritoneum and then the introduction of the endoscope. The peritoneum had then to be sutured with 2/0 surgical gut after each observation.

In cows 5 and 6 successful attempts were made to take photographic records of the ovaries *in situ*. The perspex tube was placed against the ovary holding it in position. The light transmission properties of the tube enabled light from the ring flash of the camera to be transmitted to the ovary in quantities sufficient for adequate exposure. The blurring of the exposures caused by mist forming on the cold surface of the tube was overcome by preheating the tube in hot antiseptic solution. A series of satisfactory exposures resulted.

The ebonite cannula in cows 1, 2, and 3

The ebonite cannula was maintained in these cows for six, seven, and nine weeks respectively and during this period attempts were made to observe the ovaries at weekly intervals.

Cow 1

The first observation was made one week after surgery. On this occasion a clear view of part of the surface of the left ovary was obtained. On subsequent observations the left ovary was located only once and the right ovary was not located at any stage. To aid the location of the ovaries rectal palpation and manipulation of the ovaries was carried out while observations were being attempted through the bronchoscope. Towards the end of the six-week period a clear serous peritoneal fluid accumulated in the abdominal cavity. Whenever the cap was removed a white film of fibrin-like material was observed around the cap and the inside of the shaft but it was not until the fourth week that this material partially covered the opening. For further observations this material had to be broken down. The skin area around the cannula did not heal completely and repeated cleansing and applications of sulphanilamide powder were employed. A slight elevation of body temperature was recorded at this stage.

After the six-week period the cannula was removed and samples of the accumulated peritoneal fluid were submitted for bacteriological examination; this showed a profuse growth of α-haemolytic *Streptococci* spp. and a moderate growth of coliforms. A fibrous ring was well established around the site when the cannula was removed. During this period the cow lost condition.

Cows 2 and 3

In cows 2 and 3 topical and systemic proteolytic enzymes were used (Betteridge & Raeside, 1962) in an attempt to reduce the fibrin formation. This was found to make very little difference to tissue reaction in the latter stages, although it did appear to depress the fibrin formation in the first few weeks. In the case of cows 2

and 3 location of the ovaries by rectal palpation became easier but even then damage to the ovaries and surrounding tissue resulted. The cannulae were retained in these animals for seven and nine weeks respectively.

One cow was observed on heat and a follicle was recognized on the left ovary. This was detectable by rectal palpation. Endoscopic examination on the following day showed that the follicle had ruptured; it was assumed that ovulation had occurred. The developing corpus luteum was followed each day for five days but the fibrin formation obliterated further observations.

The stainless steel cannula in cow 4

The stainless steel cannula was maintained in cow 4 for three weeks. No observations were made until seven days after surgery. Observations were subsequently made on the ninth and eleventh days. On the fourteenth day daily injections of 40 mg of progesterone in peanut oil were given and daily observations commenced. Diagrammatic recordings of both ovaries were made for six days (Fig. 6). The left ovary contained one corpus luteum and two follicles and during the period of observation these follicles regressed to approximately 50 per cent of their original size. The corpus luteum appeared lighter in colour and slightly bigger on the sixth day.

On the right ovary a large follicle had formed on one pole and at the opposite pole a smaller follicle with what appeared to be a haemorrhagic area surrounding it was observed. In this case the larger follicle remained unaltered and the smaller follicle slowly increased in size with the haemorrhagic area around it apparently regressing in three days. At this point the fibrin development prevented further observations. Examination of the cannula with the cap removed twenty-four hours after surgery showed a hyperaemic area of about 12 cm in diameter on the rumen wall in apposition to the cannula. Another seven days after surgery the area was covered by fibrinous deposits as well as adhesions from the wall of the rumen to the cannula. During the period of daily observations it was apparent that the peritoneal fluid and fibrinous deposits were increasing. Further adhesions from the rumen wall to the surrounding cannulated area had developed and fibrinous deposition occurred across the pelvic inlet; this made the location and observation of the ovaries difficult (Fig. 7).

Cows 5 and 6

In cows 5 and 6 the continued entrance and suturing of the peritoneum resulted in the formation of a thick fibrous and highly vascularized tissue.

The flexible nature of the cannula proved to be successful and tissue tolerance was better than with the previous two cannulae. Both cows remained in excellent condition throughout the test period which extended to thirty-three days.

No daily observations were made with the bronchoscope. However, a series of successful ovarian exposures was taken over a period of four days.

The cannulated ewes

The cannulation of the ewes was carried out to estimate tissue reaction when the cannula was left undisturbed for a period of two weeks. The cap was removed at the end of this period and it was observed that the tissue reaction had covered the internal opening. The animals were sacrificed for post mortem examination and it was found that the tissue reaction had completely isolated the cannula from the peritoneal cavities.

Photographic records

A series of photographs (Figs. 8-10) demonstrates the type of material that can be obtained in specific ovarian studies. The degree of clarity is demonstrated by clearly defined patches of froth on the ovaries (Fig. 8).

DISCUSSION

The retained cannula is a useful adjunct in the study of ovarian activity in the bovine. The ebonite cannula although larger than that used by Megale *et al.* (1956) proved to be too small to allow regular and complete ovarian observations. Difficulty was experienced in locating the ovaries; visual obstruction by the mesovarian ligament and other internal organs was encountered, although in the case of cows 2 and 3 the technique of locating the ovaries improved considerably. Reaction of the animals to the ebonite cannula was slight and no adhesions to internal organs resulted. The accumulation of peritoneal fluid could be attributed to the low grade peritonitis present for the greater duration of the observations, this being substantiated by the fact that a profuse growth of α-haemolytic *Streptococci* spp. was recovered from the peritoneal fluid. This infection was accompanied by slight elevation of body temperature. It was found that asepsis was extremely difficult to maintain when observations were being made daily, as air-borne bacteria have free access to the peritoneal cavity, particularly when the cap is removed for long periods because of the difficulty in locating the ovaries. This would probably be a significant factor contributing to the presence of the peritonitis. Ideally, observations should be made in a dust-free, heated room, thus minimizing the sources of irritation to the peritoneum. Also observations should be carried out as quickly as possible by one experienced worker. Ideal conditions for observation were not available during these periods as the observations were made in an open cow bail. On removal of the cannula it was found that a fibrous ring had formed under the cannula reducing healing between the peritoneum and muscle. This is a desirable factor in closure of the tissues around the cannula.

As a result of the difficulty experienced in locating the ovaries when the ebonite cannula was used, the stainless steel cannula was designed to allow the admission of the operator's hand. The ovaries could now be completely and accurately studied by carefully rotating them in front of the bronchoscope. The utmost care was exercised during these manipulations to ensure that as little trauma as possible was sustained. Small haemorrhagic spots appeared on the ovaries after a number of examinations and may have been due to handling or the heat of the bronchoscope bulb. It is not known whether this procedure could have an effect on ovarian activity and long-term examination would be necessary to elucidate this point.

The principal disadvantage of this technique was the large amount of fibrinous exudate which was produced by interference with the internal organs. It was found that the greatest reaction had occurred wherever the arm and bronchoscope had been in contact with the internal organs. The use of costly proteolytic enzymes in later studies did very little to reduce the formation of fibrin after eighteen days.

In cows 5 and 6, fitted with the flexible polyethylene cannula which did not penetrate the peritoneum, less local tissue reaction was observed. No systemic reaction occurred in these cows and they remained in good condition.

However, after the peritoneum has been incised and sutured several times it becomes thick and vascular and difficult to handle. This therefore limits the number of observations and entries that can be made. However, for short-term studies it appears to be ideal. This technique of quick laparotomy has been used by Lamond (1963) as a means of diagnosing early pregnancy in ewes. A modification of this laparotomy technique could probably be applied to bovines for short-term ovarian observations. To increase the period of ovarian study observations could be started as soon as possible after the cannula has been established. Alternatively, the cannula could be left in position until sufficient tissue reaction has taken place to completely isolate it, as was found in the ewe. The fibrinous layer covering the opening could then be broken down to allow passage of the arm, care being taken to ensure that the internal edges of the cannula are not exposed. This technique might then reduce trauma caused by the cannula.

The early photographic studies are satisfactory, but they have to be improved for

specific ovarian studies. The area of opacity on the tube could possibly be reduced to decrease the amount of light above the ovary and obtain a clearer picture. A tube of greater diameter could be used to expose a larger area of the surface of the ovary.

ACKNOWLEDGMENTS

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Appreciation is also expressed to R. Cameron, G. Davis, J. Danalis, and I. Maguire for their valuable contributions and to I.C.I. who kindly donated the polyethylene cannula.

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FIG. 1 (a).—The assembled ebonite cannula

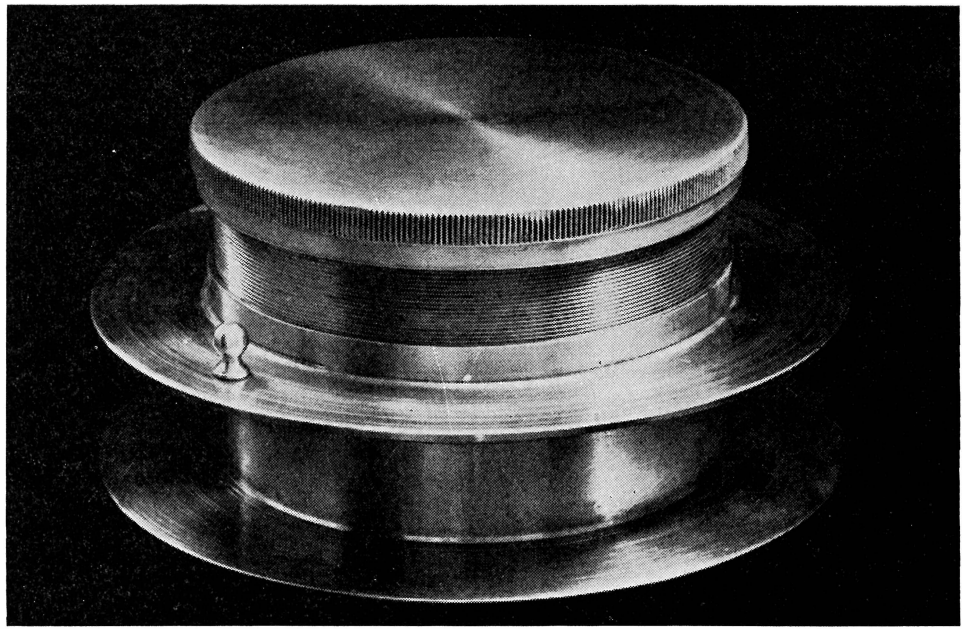


FIG. 1 (b).—The assembled stainless steel cannula

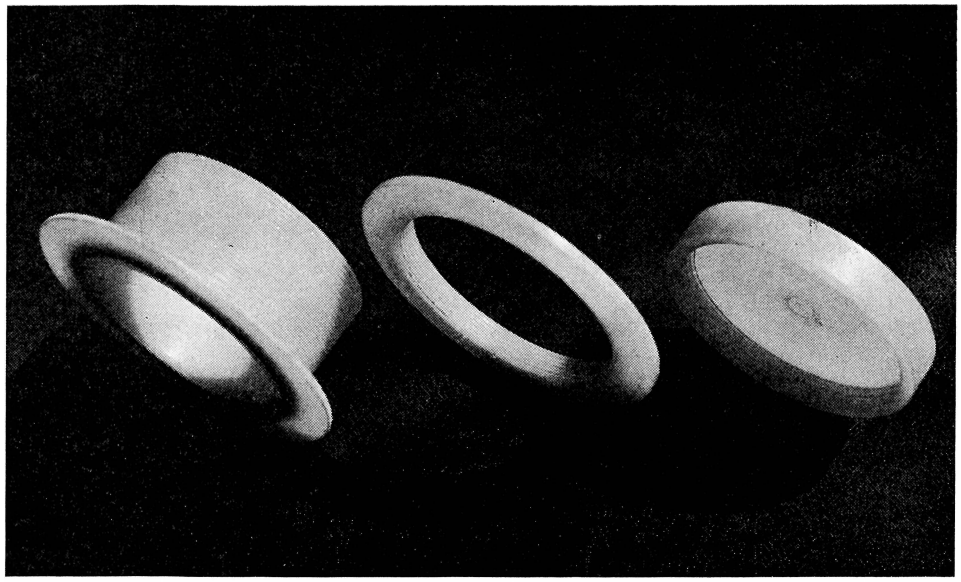


FIG. 1 (c).—The cannula disassembled

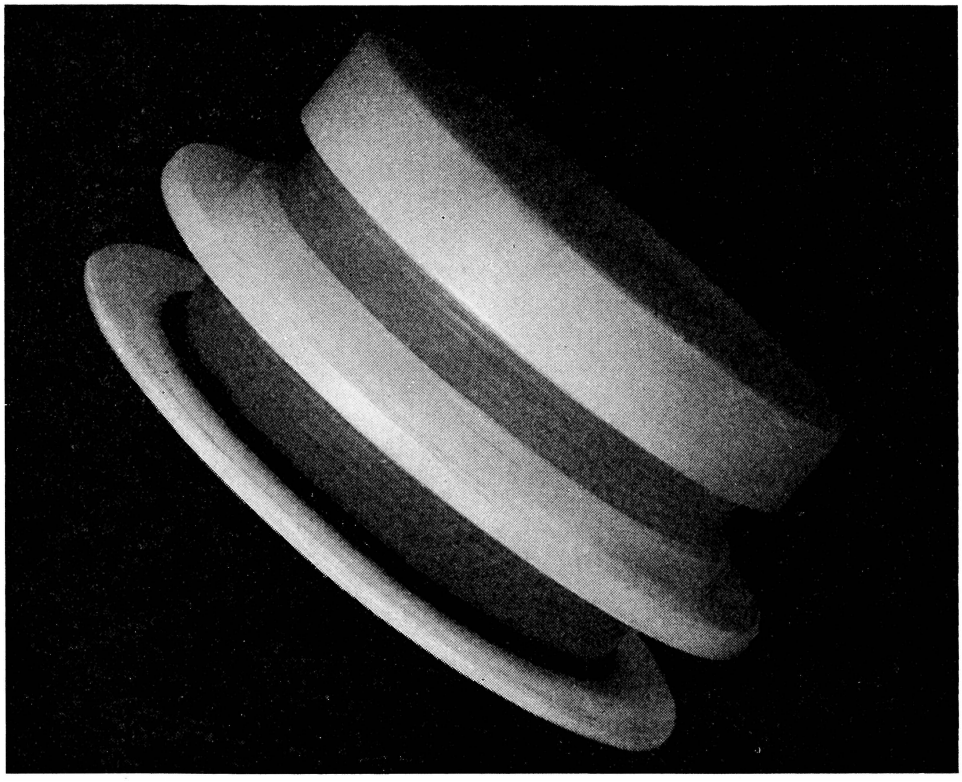


FIG. 1 (d).—The cannula assembled

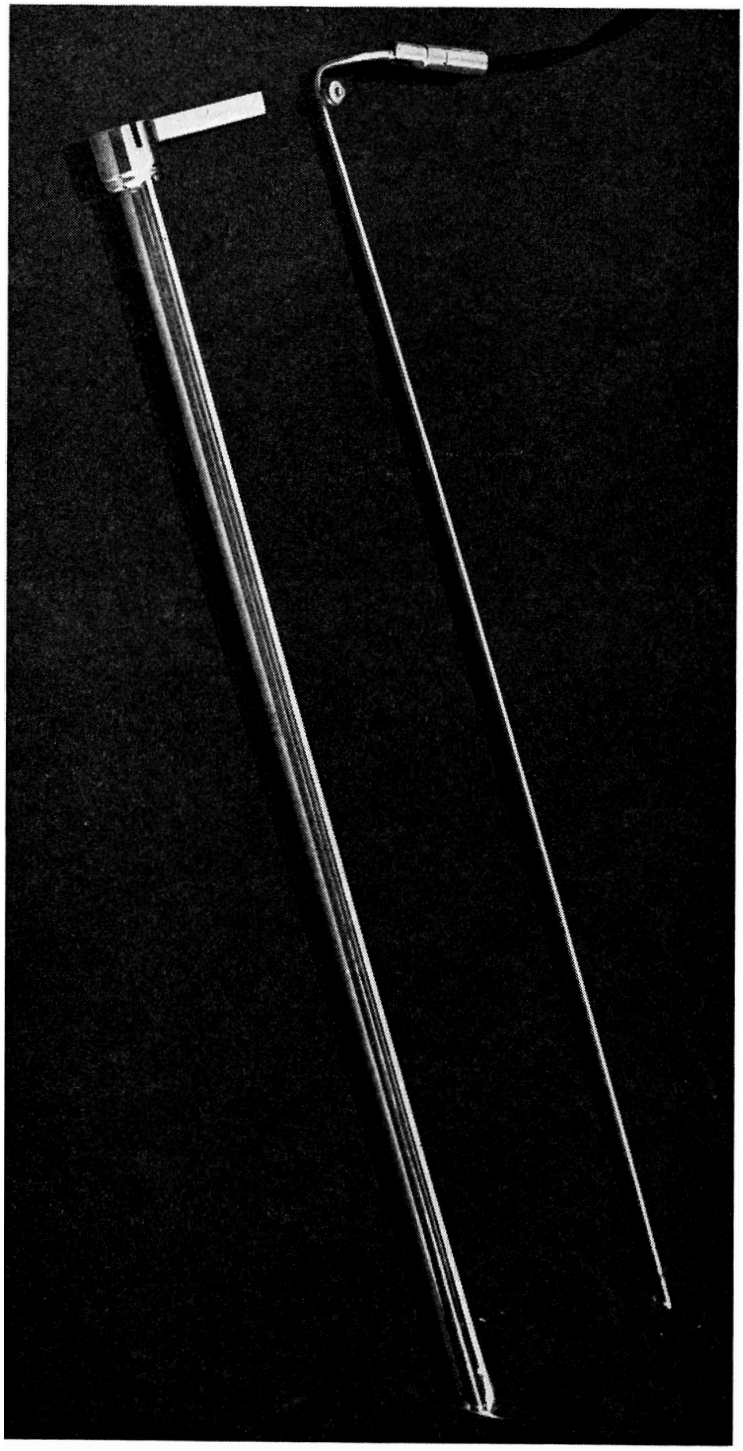


FIG. 2.—The bronchoscope used for observation in its component parts

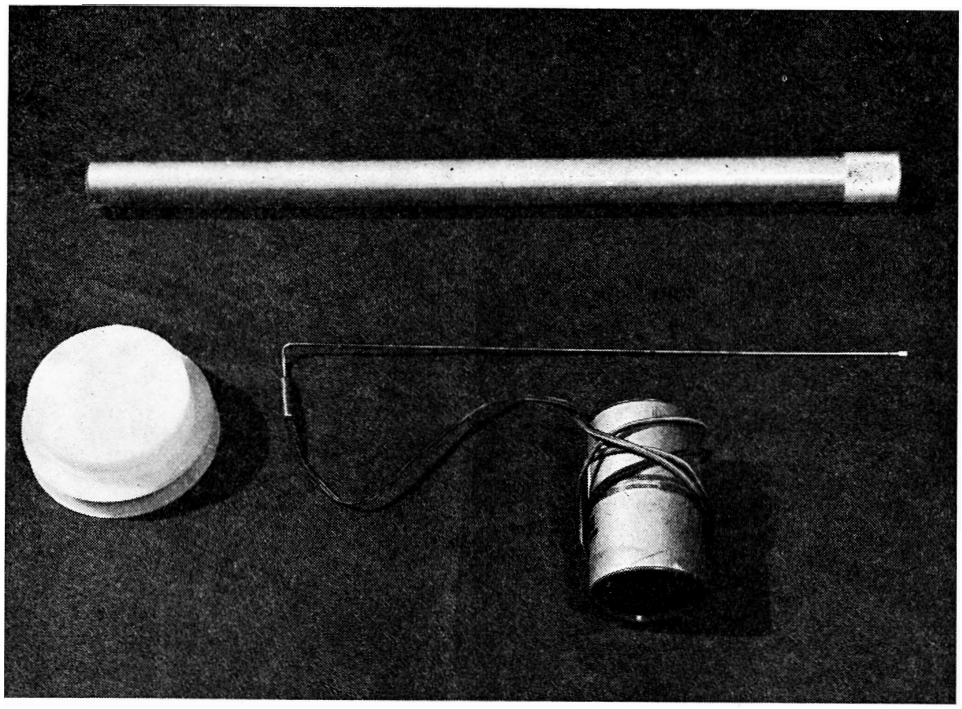


FIG. 3.—The perspex tube, the light source, the cannula

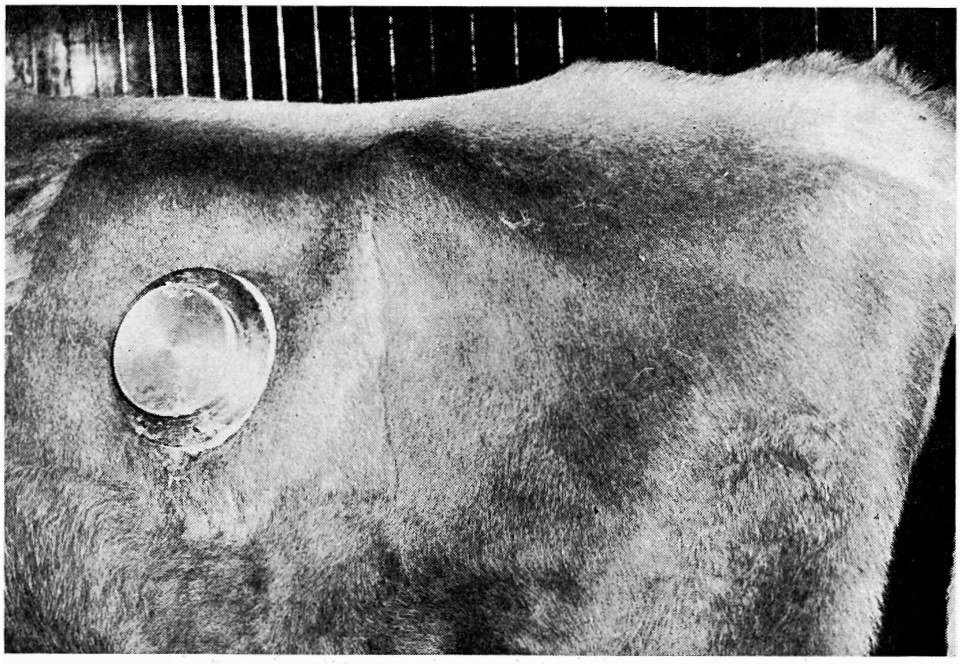


FIG. 4.—Cow 2: close view of stainless steel cannula; note gauze packing between outer flange and skin.

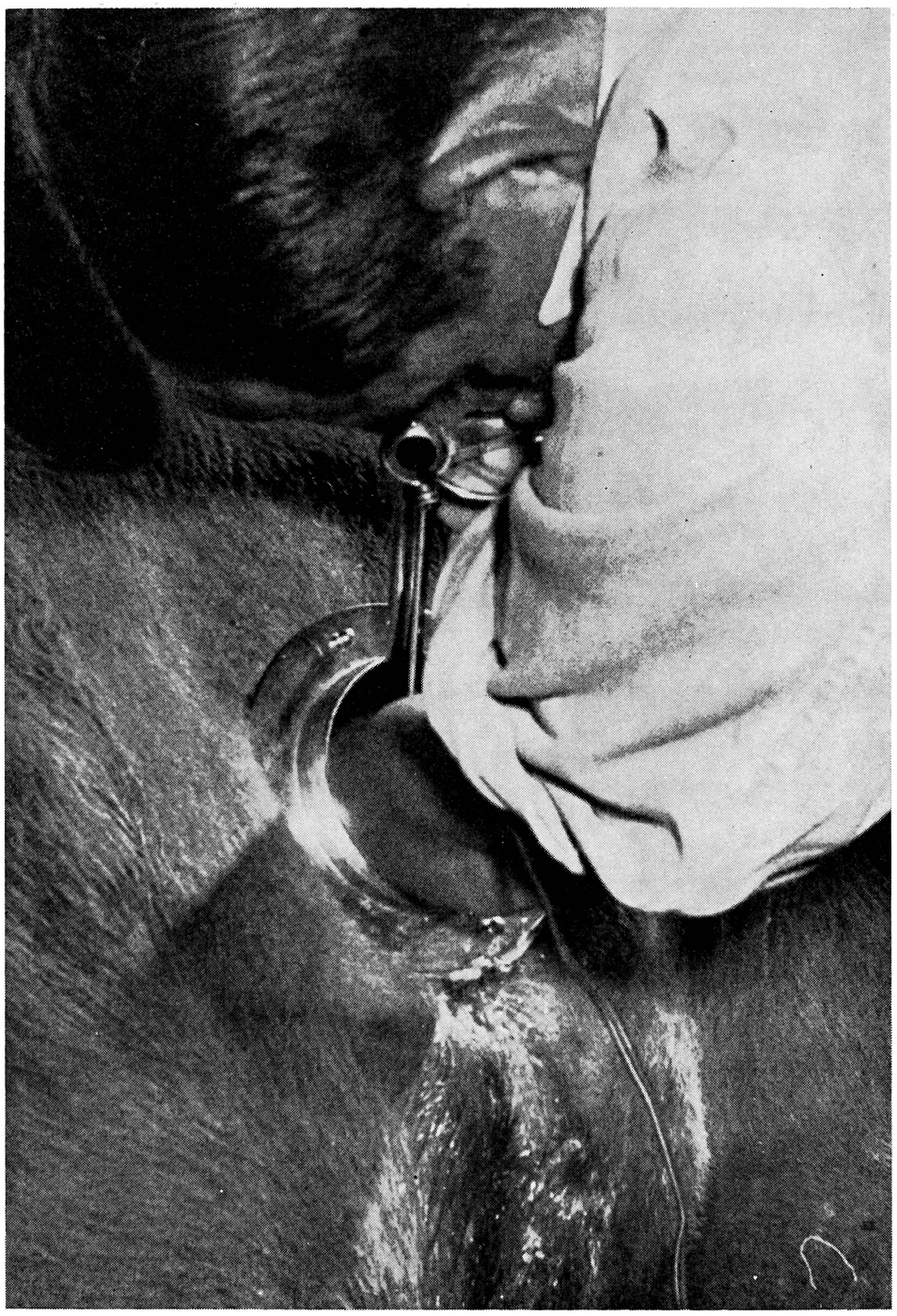
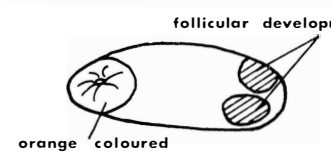
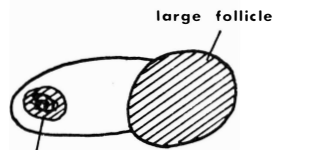
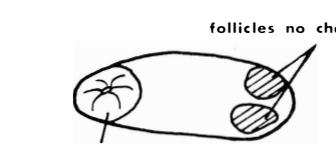
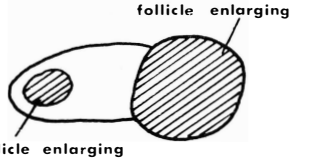
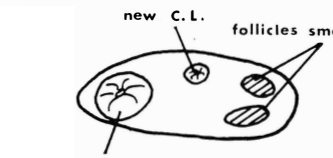
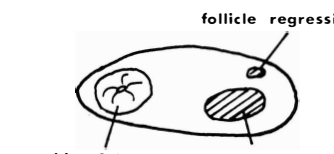
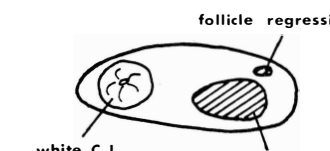
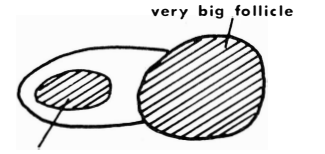


FIG. 5.—Observation of ovaries through the stainless steel cannula. The operator uses left hand to locate the ovary and holds the bronchoscope in the right hand.

1/7/63 Cannula surgically inserted.

15/7/63 Commenced daily progesterone injections.

	Left Ovary	Right Ovary
15/7/63		
16/7/63		
18/7/63	As above	As above
19/7/63		As above
20/7/63 21/7/63	No observations	
22/7/63		As above
23/7/63		

At this stage small haemorrhagic areas noticed over surface of both ovaries. It is thought this was due to trauma. The fibrin deposition at this stage prevented further observation—the cow showed signs of peritonitis.

FIG. 6.—Diagrammatic sketches of left and right ovaries of the bovine as seen through the endoscope after the daily intra-muscular injection of 40 mg progesterone in peanut oil.

FIG. 7.—Stainless steel cannula with cap and outer flange removed twenty days after surgery. Note adhesions and fibrin from rumen wall to cannula and accumulation of fluid. Also note good healing of skin around the shaft.

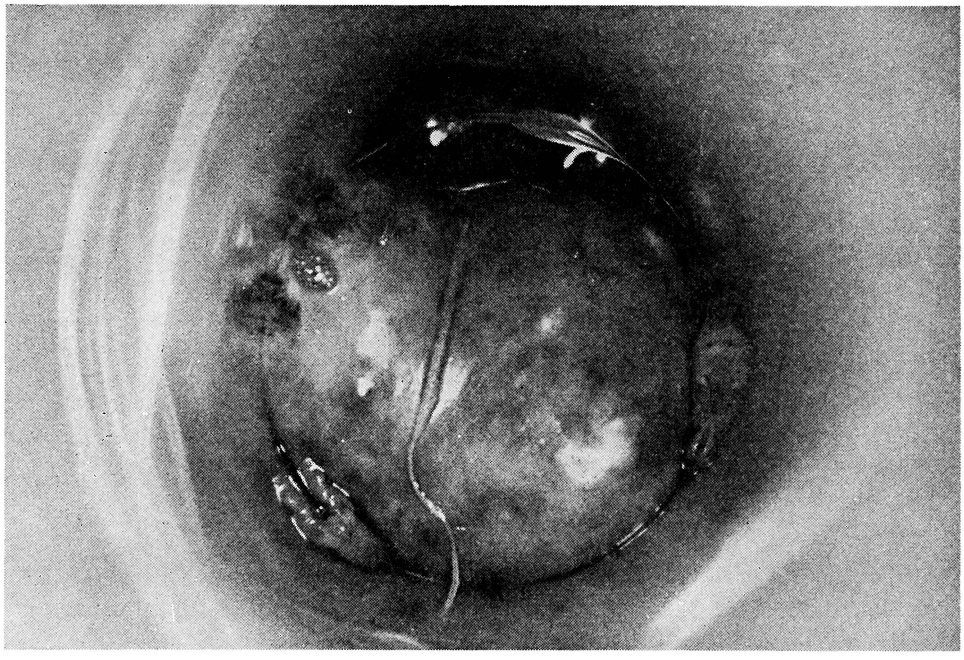


FIG. 8.—Surface of ovary with froth patches and part of fimbria showing. Note fold of tissue across surface of ovary.

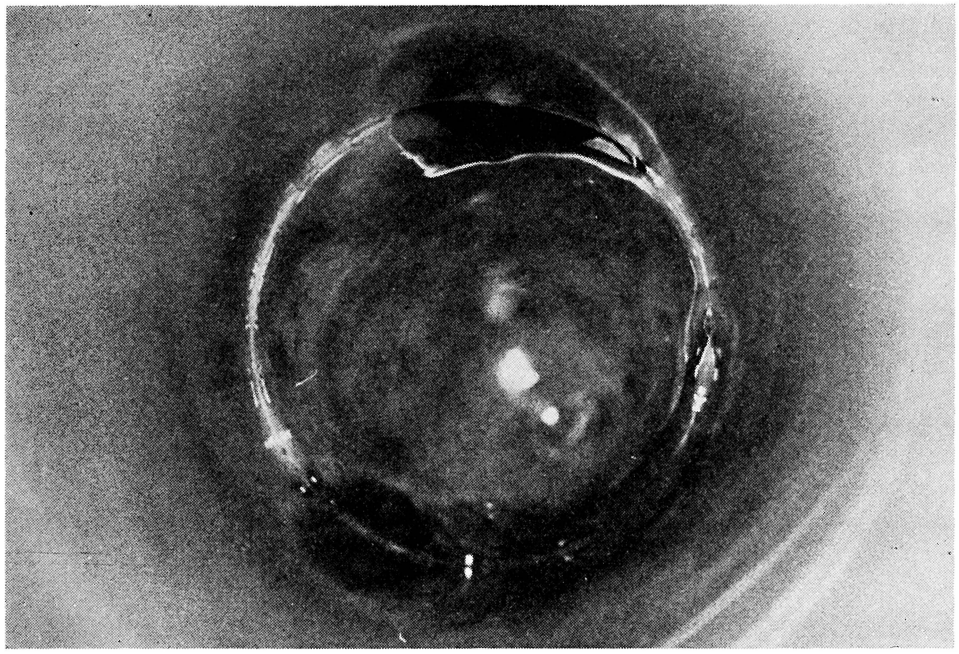


FIG. 9.—Surface of the ovary showing a follicular development.

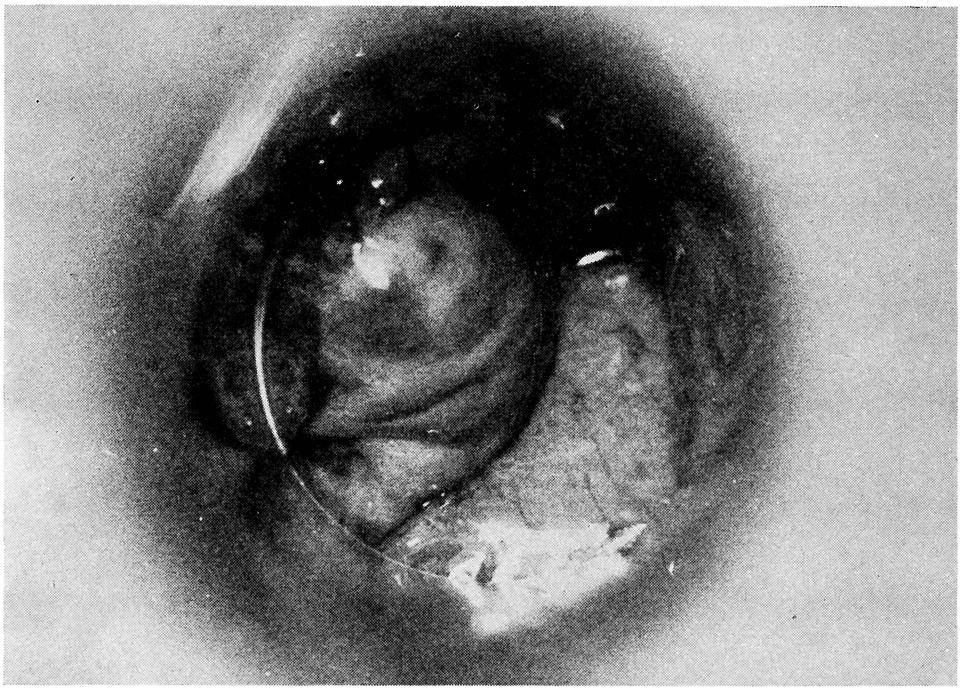


FIG. 10.—Note only small portion of infundibulum on right hand side of photograph. Rest shows surface large and small intestines.

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