DIRECTIONAL FLOW OF FALLOPIAN TUBE SECRETIONS IN THE ROMNEY EWE

by

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CONTENTS OF THESIS

SUMMARY

The anatomy of the Fallopian tube and the function of its components, especially muscle and cilia, were discussed in reference to their participation in sperm and ovum transport. The possibility of instantaneous sperm transport in the female reproductive tract and the extent to which the inherent motility of spermatozoa is required were noted as topics of long standing controversy. In contrast, evidence in the literature on the rate of ovum transport was reasonably consistent. While considerable evidence was available to indicate the relative contribution of muscular and ciliary activity only limited data were found on the role of tubal secretions. That the direction of fluid flow was controlled, and possibly regulated by ovarian hormones, was shown by few authors.

The continuous collection of fluid flowing from both ends of the Fallopian tube of the ewe was therefore attempted by placing a cannula in the ovarian end of the ampulla and a further cannula passing through the wall of the corresponding uterine horn and opposed to the utero-tubal junction. By experimentation the technique of cannulation and collection of fluid was improved and a standard procedure adopted. Daily recordings of fluid were made with 40 entire ewes over a period ranging from 3 - 106 days after surgery during the breeding season. Observations were made for oestrous behaviour.

There was an increase in total output of tubal fluid, which commenced on the last day of the oestrous cycle and reached a maximum about day 2 after which a gradual decline occurred. A greater part of the fluid secreted flowed through the ampulla end. Flow through the utero-tubal junction into the uterus remained low for most of the cycle but markedly increased in all cycles 3.9 ± 0.1 days after the onset of oestrus. Thus maximum flow through the isthmus appeared to

coincide with the time during which ova enter the uterus.

To determine the hormonal control of fluid flow a series of experiments on ovariectomised ewes were undertaken. The principle ovarian hormones, oestrogen and progesterone, were given in single, serial, or sequential doses. A 'positive oestrous response' (Robinson & Moore, 1956b) to oestrogen therapy was indicated by the subsequent cornification of vaginal epithelial cells.

A decline in fluid secretion and fluid flow following ovariectomy was counteracted by the administration of oestradiol benezoate. Both ampullar and isthmic flow markedly increased (with single injection of 30, 90 or 500 μg ODB) until it was commensurate with, or exceeded, that which occurred during oestrus in the entire ewe. But, the sequence of maximum ampullar flow and that of isthmic flow was reversed by oestrogen therapy, the difference being further accentuated by each increase in dose level. Furthermore, serial therapy (30 μg ODB daily for 3 days) so extended the duration of isthmic flow that it offset a comparable increase in ampullar flow.

In an attempt to achieve the sequence of fluid flow as it occurred in the oestrous animal the normal hormonal balance was simulated by sequential progesterone-oestrogen therapy. A preparatory treatment with progesterone (10 mg daily for 8 days) depressed fluid secretion and in consequence the flow of fluid out both ends of the Fallopian tube. The response of fluid secretion and isthmic flow to subsequent oestrogen therapy was also reduced. This was reflected by a decrease in response of maximum fluid secretion and isthmic flow and also a reduction in the duration of response. The progesterone therapy, given on the third day after oestrogen, further decreased the duration of response. Thus an increase in isthmic flow, expected to occur with the succeeding progesterone therapy, did not eventuate.

The sequence of fluid flow was essentially the same as that occurring after oestrogen alone.

While the oestrogen-induced isthmic flow was not completely suppressed by preparatory treatment with progesterone it was reduced. Thus it had been surmised that progesterone preceding oestrogen therapy may be necessary to suppress isthmic flow during the oestrogen dominant pre-ovulatory period of the entire ewe.

Supporting evidence on this point was obtained from data on the direction of fluid flow during silent heat. The sequence of maximum ampullar flow and that of isthmic flow in the 'first oestrous' (silent heat) was similar to that which occurred in the oestrogen treated ovariectomised ewe. In contrast, the sequence of fluid flow in the subsequent oestrous cycle was reversed, being similar to that occurring in the oestrous ewe.

It was concluded that the direction of fluid flow was under hormonal control; fluid secretion and isthmic flow being promoted by oestrogen therapy but depressed by progesterone. In addition, progesterone emanating from the waning corpus luteum of a preceding ovulation was considered as playing a decisive role in suppressing isthmic flow during the pre-ovulatory period of the oestrous ewe. However, the hormonal balance necessary to enable the surge in isthmic flow on the third day after ovulation was not determined.

The significance of these results, and their limitations, were then discussed in terms of spermatozoan and ovum transport. It was concluded that tubal secretions have an intergrative function in the transport mechanism. The fluid was considered as a medium through which the effect of ciliary and muscular activity could be transmitted. The movement of ova, spermatozoa and fluid was considered synonymous.

CHAPTER ONE

THE FALLOPIAN TUBE AND ITS FUNCTION IN THE TRANSPORT OF SPERMATOZOA AND OVA

CONTENTS OF CHAPTER

A. INTRODUCTION

Each normally implanted embryo is preceded by the successful transfer of spermatozoa and ova to the ampulla of the Fallopian tube, fertilisation, and subsequent delivery of fertilised ova to the uterus after a suitable period of time. Any malfunction in the mechanisms controlling these processes can seriously interfere with the ability of an individual, or species, to reproduce. It is therefore necessary to gain a closer understanding of the physiological mechanisms controlling these events.

The review presented in this chapter outlines present knowledge on the mechanics, and time relationships, of normal entry and passage of gametes through the Fallopian tube.

B. ANATOMY OF THE FALLOPIAN TUBE

1. Morphology

The Fallopian tubes are bilaterally placed, each being suspended by a mesenteric peritoneal fold, the mesosalpinx. The following regions of each Fallopian tube are easily recognised (Piliero, Jacobs & Wischnitzer, 1965):

1. The funnel shaped infundibulum containing a fimbriated opening, the abdominal ostium, projecting towards and in contiguity with the ovary.
2. An intermediate dilated portion, the ampulla.
3. The isthmus, a constricted and convoluted segment, providing continuity between its uterine orifice, the utero-tubal junction, and the ampulla.

The vascular system of the Fallopian tube has not been clearly defined. Arterial blood originates from anastomoses of the uterine and utero-ovarian arteries in the cow (Hansel & Asdell, 1951), ewe

(Sisson & Grossman, 1953), and appears to be similar in the sow (Oxenreider, McGlure & Day, 1965). The venous system follows a pattern similar to the arterial while lymph drainage occurs via the lumbar and inguinal nodes (Sisson & Grossman, 1953).

Sympathetic innervation links with the ovarian and hypogastric plexus. Recent evidence reported by Brundin (1964a) suggests ampulla innervation is cholinergic, while fluorescent staining techniques (Brundin & Wirsen, 1964b) indicated adrenergic nerve terminals in the isthmus of the rabbit.

2. Histology

The Fallopian tube is organised into 3 layers, a thin peripheral serosa, a muscularis, and the internal mucosa.

The muscularis consists of two poorly defined muscle layers, a circular and a longitudinal layer. The layer of circular muscle is slightly spiralling (Greenwald, 1961), and partly interlocked with longitudinal muscle (Bjork, 1959). The presence of longitudinal muscle in the ampulla of the doe (Black & Asdell, 1958) is doubted by some authors. Greenwald (1961) claimed it appeared a few centimetres before the uterus as two distinct bands which gradually widened to form a complete layer around the uterus. No contemporary reports are available for the distribution of the muscle layers in the Fallopian tube of the ewe.

The internal mucosa is lined by a single columnar epithelium. Sectioning of the ampulla reveals the mucosal lining thrown into a series of longitudinal folds which decrease in height and complexity as one moves into the isthmic region.

Three cell types: "Ciliated", "non-ciliated secretory" and "rod" cells, are present in the tubal epithelium of the guinea pig, rabbit, cat, dog, sheep, monkey and human Fallopian tubes (Frommel, 1886; Nicolas, 1890). The presence of similar and other cells in further

species was confirmed by a series of authors around the turn of the century (cited Hadek, 1955). Hadek confirmed previous suspicions that the rod cells were degenerate secretory cells, but did not support an early contention that secretory cells were transformed ciliated cells. This objection was later favourably supported by colchicine studies in the rabbit (Fredericsson, 1959a) and electron microscopy in the rabbit (Borell, Nilsson, Wersall & Westman, 1956) and human (Clyman, 1966). Secretory cells were found to be most abundant in the infundibulum and ampulla but comparatively few present in the isthmus of the sheep (Hadek, 1955; Restall, 1966c).

The epithelial folds of the rabbit, sheep and human ampulla are covered with an almost continuous layer of cilia, especially nearer the fimbria. They appear less numerous in the mid isthmus but are again predominant on the folds of the utero-tubal junction (Hadek, 1955; Bjork, 1959; Greenwald, 1961a; Harper, 1961b; Clyman, 1966). The earlier evidence of Espinasse (1935) suggesting cilia were absent from the isthmus of the rat and mouse was refuted by the more refined techniques of Alden (1942), Deane (1952) and Borell, Gustavson, Nilsson & Westman (1959).

The main components of the Fallopian tube which are directly implicated with the transport of the gametes have been outlined. The part played by each will be discussed subsequently.

C. TRANSPORT OF SPERMATOZOA IN THE FEMALE GENITAL TRACT

The relative contributions of sperm motility, and uterine and tubal contractions, towards sperm transport through the reproductive tract is still a controversial topic. Differences arose from the discrepancy between the almost instantaneous sperm transport in the female genital tract and the much slower progress of sperm *in vitro*. Reports indicate the mean speed of travel *in vitro* is less than

8 mm/min. (Phillips & Andrews, 1937; Tampion & Gibbons, 1962), although within species this may be increased if an opposing fluid current is created (Yamane & Ito, 1932). Even so, the rate of progress obtained does not account for the rapid transport which has been found to occur in many species. The occurrence of rapid transport, and the ascent of inert particles, has led many authors to propose a more passive role for spermatozoa. Contributions to the opposing views have been reviewed by Reynolds (1939), Dauzier (1958), Bishop (1961) and Hancock, (1962).

1. EVIDENCE FOR PASSIVE TRANSPORT

(a) Time of Transit

Hartman & Ball (1930) and Warren (1938) were among the first to demonstrate the presence of sperm in the Fallopian tube of the rat within 2 minutes of copulation. Subsequent estimates in other animals have placed the time at 15 minutes in the mouse (Lewis & Wright, 1935), 20 min. in the bitch (Evans, 1933), while estimates in humans range up to 3 hr (Chang & Pincus, 1951). Results with cattle and sheep vary considerably. With the cow, Van Demark & Moeller (1950, 1951) claim a few minutes but in contrast Brewster, May & Cole (1940) and Dauzier (1958) consider several hours more appropriate. In the ewe rapid ascent may (Phillips & Andrews, 1937; Schott & Phillips, 1941; Starke, 1949) or may not occur (Green & Winters, 1935; Kelly, 1937; Warbritton, McKenzie, Berliner & Andrews, 1937; Lopyrin & Loginova, 1939). The rabbit, on the other hand, appears to be an anomally as rapid transport has not been reported to occur (Braden, 1953; Turnbull, 1966).

In his review Dauzier (1958) considered that inadequate precautions had been taken against possible sources of sperm contamination. For this reason he was skeptical of results showing rapid transport in the ewe and cow. This criticism can not be disregarded but the

position was perhaps clarified by Mattner & Braden (1963a) when they found the concentration of sperm in the ampulla of the ewe at 8 to 30 minutes was considerably reduced if the animals were mated when subjected to stress.

(b) Passage of Inert Particles

Additional evidence for a passive role of spermatozoa comes from the rapid transport to the ampulla of immotile sperm in the rat (Howe & Black, 1963), ewe (Mattner, 1963c), and cow (Van Demark & Moeller, 1951). Also the passage of radiopaque fluids (rabbit - Krehbiel & Carstens, 1939; Akester & Inkster, 1961; cow - Rowson, 1955), indian ink particles (rabbit - Parker, 1931), ^{131}I labelled microspheres (rabbit - Glover & Wood, 1964), and the ascent of carbon particles (ewe - Mattner, 1963a). In the sow and mare components of seminal plasma, ergothionine, fructose and citric acid, are often found in the uterine horns following mating (Mann, Polge & Rowson, 1955). Using a similar approach Gunn & Gould (1958) detected a zinc labelled component of prostatic fluid present in the Fallopian tube of the rat 1.5 hr after mating.

These observations have not passed unopposed. Noyes, Adams & Walton (1958), Bjork (1959) and Edgar & Asdell (1960) were unable to confirm the entry of radiopaque material into the uterus of the rabbit while Leonard & Pearlman (1939) failed to find non-motile sperm passing through the utero-tubal junction of the rat. However, each of these techniques involve mating, or inseminating, under conditions of possible stress and so preconditioning of the animals is probably a prerequisite for the occurrence of rapid transport.

2. MECHANISMS FOR SPERM TRANSPORT

If the inherent motility of the sperm is not obligatory for transport to the ampulla then it is necessary to seek other means to account for the displacement of sperm in the female genital tract.

Ciliary activity and muscular contractions of the uterus and Fallopian tube have been implicated (Westman, 1926; Parker, 1931).

(a) Muscular Contractions

Using direct observations Westman (1926) concluded that the muscular activity was peristaltic in nature. Subsequent work tended to refute this and considered the contractions to be propagated by a segmentation process (Maeda, 1933, Bjork, 1959) although intermittent peristaltic contractions may occur (Harper, 1961b). However, such peristaltic contractions that do occur are directed from the ampulla to the cervix (Reynold, 1939) and so barely able to induce sperm transport in the ovarian direction.

Segmental contractions encourage dispersal of uterine and ampulla contents as seen following the deposition of indian ink (Parker, 1931) or radiopaque materials (Bjork, 1959). The increase in uterine and tubal activity coinciding with oestrus presumably increases the turbulence of fluid within the genital tract thereby enabling more rapid dispersion of spermatozoa (Parker 1931).

(b) Barriers to Sperm Progress

Three possible barriers to sperm progress exist; the cervix, the utero-tubal junction, and to a lesser extent the narrow confines of the isthmus. In those animals in which sperm are deposited in the vagina during coitus, as occurs in the rabbit, ewe and human (Warbritton et al., 1937; Chang, 1951b; Braden, 1953; Hartman, 1957; Noyes, Adams & Walton, 1958), the mechanisms promoting the passage of sperm across the cervix are not definitely known. One theory, recently discussed by Hartman (1957), involves a transient negative pressure in the uterus which 'sucks' the sperm through the cervix. Millar (1952) in fact recorded a transient negative pressure of 0.7 lb per square inch in the uterus of the mare during coitus but the significance of this find is somewhat clouded if ejaculation normally occurs

directly into the uterus in this species (Braden & Austin, 1953b). The mass uptake of radiopaque fluid from the vagina into the uterus observed by Krehbiel & Carstens (1939) was considered due to vaginal contractions, but the conditions in which the rabbits were stimulated may have been abnormal (Akester & Inkster, 1961).

That the inherent motility of spermatozoa may be necessary for their transport across the cervix (Noyes et al., 1958) has been emphasised by Glover & Wood (1964) when they found only .002% to .04% of the copolymer microspheres deposited in the vagina of the rabbit eventually reached the uterus. This appears considerably less than the percentage of spermatozoa which reach the uterus (Braden, 1953), although a possible difference between strains of rabbits and degree of vulva stimulation cannot be discounted.

The resistance of the utero-tubal junction to sperm transport appears to vary between species. The speed of sperm transport through this region of the genital tract is generally rapid and in only the rat and rabbit (cited Braden, 1953) and possibly the sow (Rigby, 1966) is it considered an obstacle to sperm migration. The passage of radiopaque materials through the junction indicates a role for uterine contractions in the movement of fluids into the isthmus. Alden (1942b) noted that it was uterine fluid which partly filled the isthmus in the rat, while recently (Hawk, 1965) failed to find any sperm in flushings of the Fallopian tube in the ewe following the insertion of a plastic spiral in the corresponding uterine horn.

(c) Anti-peristaltic Contractions

Wintenberger - Torres (1961) found plastic spheres, comparable in size to natural ova, moved in an adovarian direction if placed at the base of the excised isthmus of the oestrus ewe. Whether the in vitro conditions and the nature of the spheres allow their argument for the existence of antiperistaltic contractions to be valid or extrapolated to the transport of spermatozoa is questionable. It

is worthy of note that Lim & Chao (1927), among other earlier authors, considered anti-peristaltic contractions of the isthmus as a possible mechanism for inducing sperm movement towards the ampulla.

(d) Compartmental Theory

Following his investigations in the rabbit, Parker (1931), proposed a 'compartmental' theory to account for sperm movement in the Fallopian tube. He contended that the epithelial folds of the internal mucosa form temporary compartments within which ciliary activity create aduterine and adovarian currents of fluid. The segmental contractions of the tubal musculature ensure a continuous dissolving and reforming of these compartments. Under such conditions fluid streams in one compartment can become part of, or continuous with, fluid streams in adjacent compartments.

Thus the dynamic state of compartmental fluid circulation would act as a media for the gradual dispersion of spermatozoa towards the infundibulum. The validity of this contention remains to be tested.

D. THE TRANSPORT OF OVA THROUGH THE FALLOPIAN TUBE

The pattern of normal ova transport through the rabbit Fallopian tube has been described by Tourneux (1889), Parker (1930), Chang (1951c), Braden (1953), Greenwald (1959a, 1961a), Zimmerman (1959), Harper, Bennett, Boursnell & Rowson (1960), and Harper (1961a,b). It has also been described in the guinea pig (Squier, 1932), the sow (Corner, 1921; Anderson, 1927; Pomeroy, 1955; Perry & Rowlands, 1962; Oxenreider & Day, 1965b), the ewe (Kelly, 1937; Wintenberger, 1953) and the cow (Hamilton & Laing, 1946).

In their reviews on this topic, Anderson (1927), Chang & Pincus (1951a) and Blandau (1961) concluded that the time taken for ova to reach the uterus was remarkably constant between species. Apart from the sow (Oxenreider & Day, 1965b) dog and ferret (cited Hammond & Walton,

1934) the time taken, 3-4 days, appears to be independent of the size of the animal concerned or the length of the Fallopian tube.

1. RATE OF NORMAL TRANSPORT

Since few ova have been flushed from the ovarian half of the ampulla in the rabbit (Black & Asdell, 1959, Greenwald, 1961a) the ewe (Kelly, 1937; Wintenberger, 1953) and sow (Anderson, 1927) these authors concluded that ova transport through the ampulla of these species was rapid. Wintenberger (1953) noted ova had already passed through the upper half of the ampulla by 2.5 hr in the ewe. In the rabbit, where larger numbers of ova released allow more precise estimates, Zimmerman (1959), put the time of ampulla passage at 30-45 minutes. Recently, Harper (1961a), reported that rabbit ova in cummulus, when placed in the ovarian end of the ampulla, were transported to the ampulla-isthmus junction in 5 minutes (range 3.5 - 6 minutes).

It is therefore evident that ova spend the greater part of the 3 days following ovulation traversing the isthmus. The infrequent location of ova in the uterine half of the isthmus (Andersen, 1927; Kelly, 1937; Greenwald, 1959a) indicates ova may also be rapidly transported through this section of the Fallopian tube. However, considerable dissention exists between the reports on the site at which ova are delayed in the isthmus. One faction (Wintenberger, 1953, 1961; Greenwald, 1961a;) consider ova to be retained at the ampulla-isthmic junction for a prolonged period while others (Black & Asdell, 1958, 1959; Noyes, 1959; Edgar & Asdell, 1960) propose that the site of retention is immediately prior to the utero-tubal junction.

2. MECHANISMS FOR OVA TRANSPORT

It will be apparent that two distinct phases of ova transport occur. These two phases: entry of ova into the Fallopian tube, and transport through the ampulla and isthmus, have different underlying mechanisms inducing egg movement.

(a) Entry of ova into the Fallopian tube

The fimbria is known to partially surround the ovary for a brief period during ovulation in the guinea pig, rabbit, sheep, monkey and human (cited Westman, 1952; Doyle, 1956). In these species close approximation of the fimbria and ovary is ensured by an increase in motility and turgidity of the fimbria, and the rhythmic contractions of the musculature of the peritoneal fold and ovarian ligaments (Westman, 1926, 1952; Reynolds, 1939).

The process of ovulation has been directly observed and described in detail in the rabbit (Walton & Hammond, 1928; Hill, Allen & Kramer, 1935; Harper, 1963) and in the ewe (McKenzie & Terrill, 1935, 1937). Rupture of the follicle was explosive in nature and subsequent expulsion of follicular contents took only 30-60 seconds (Hill et al., 1935). However ovulation may not be explosive in all cases (McKenzie & Terrill, 1937) nor the ovum expelled until some minutes after rupture of the follicle (Harper, 1963). The mechanism whereby ova are transferred from the ovarian surface to the ampulla remains to be elucidated. One of the earlier theories involve the rhythmic relaxation and contraction of the ampulla creating a negative pressure and thereby drawing ova into the tube (Westman, 1926). Investigating this possibility Clewe & Mastroianni (1958) and Hafez (1961) placed a ligature around the ampulla immediately below the fimbria of the rabbit. No differences were apparent when a comparison was made of the number of ova reaching the fimbrial portion of the ligated and non-ligated tubes, thus discounting suction as a major factor in the 'pick-up' of ova.

The existence of numerous cilia (Borell et al., 1956) and that rabbit ova in cummulus move in a smooth continuous motion in this region (Harper, 1961b; Wintenberger-Torres, 1961) lends support to the more recent concept (Alden, 1942a,b; Blandau, 1958) which implicates ciliary activity as playing a major part.

(b) Transport through the Fallopian tube

Early discussions on the mechanism of ova transport through the Fallopian tube (Lode, 1893; Sobotta, 1914; Mikuliez - Radecki, 1925; Westman, 1926; and Kuo & Lim, 1928) considered muscular contractions and ciliary activity to be the main factors involved. The relative significance of these two factors remained undecided in subsequent reviews (Parker, 1931; Alden, 1942; Pincus & Chang, 1951; Blandau, 1961). More recently evidence has implicated a physiological ampulla-isthmic sphincter (Greenwald, 1961a), a restriction of the lower isthmus (Black & Davis, 1962) and the utero-tubal junction (Black & Asdell, 1959) may also be involved.

(i) muscular contractions:

Aduterine peristaltic contractions were observed in the human (Rubin & Bendick, 1926) and rabbit (Westman, 1926). In the following year Dryoff (1927) noted clear 'spindel' formations following the injection of a contrast medium into the Fallopian tubes of women. During the succeeding years, after considerable confusion as to the direction and type of contractions, it became evident that the direction of fluid movement depended on where the contrast medium was placed. Thus Nahmacher (1929) reported peristaltic contractions towards the ovaries in the human when contrast medium was placed in the uterus, but in the opposite direction if the medium was placed in the abdominal opening of the ampulla. Conveyance of a contrast medium has been used to confirm the occurrence of tubal contractions in both directions in many experiments since that time (Bjork 1959).

In priori to Westman's peristaltic contractions Parker (1931) and Maeda (1933) introduced the term 'segmentation' to describe the contractions which they observed in the Fallopian tube of the rabbit. Segmentation is presumably synonymous with Alden's (1942a) pendular contractions which caused violent back and forward movements of tubal

contents in the ampulla of the rat. Similar 'pendulum' or 'segmental' movements in the Fallopian tube of the mouse (Whitney & Burdick, 1936; Burdick, Whitney & Emerson, 1942) the rabbit (Black & Asdell, 1958; Bjork, 1959; Harper, 1961b; Inkster, 1964) the pig (Seckinger, 1923) and sheep (Wintenberger-Torres, 1961) have been reported.

In contrast, Wintenberger-Torres (1961) have reported antiperistaltic activity in the *in vitro* isthmus of the ewe. These singularly directional contractions were considered to aid sperm transport (refer page 7) and also prevent ova from entering the isthmus until the third day following ovulation. Supporting evidence under *in vivo* conditions may not be easily obtained.

(ii) *ciliary activity*:

Some authors, particularly Grosser (1918), maintained that tubal cilia were the only means of transporting ova through the Fallopian tube. Parker (1931), Anderes (1941), Borell, Nilsson & Westman (1957) and others, have discussed the relative importance of cilia in ova movement. Employing high speed cinematography Borell *et al.*, (1957) found cilia beat toward the uterus at a rate of 1500 beats/min. during oestrus in the rabbit, then increased to 1600 at 24 hr, and 1800 beats/min. at 48 hr after copulation. Since maximum ciliary activity coincided with the time during which ova enter the isthmus the authors concluded that ciliary activity was significantly involved in the movement of ova through the isthmus. Previously Parker (1931) pointed out that cilia would only be effective in moving the ovum towards the uterus if the epithelial folds within the tube maintained a steady pressure thus ensuring reasonable ciliary contact. Even so, cilia have been observed to rotate a cluster of ova in the ampulla of the mouse (Burdick *et al.*, 1942). Reversal and reanastomoses of a small section of the tube in the rabbit prevented pregnancy but reanastomoses without reversal did not (Kuo & Lim, 1928). Kuo & Lim extrapolated these

results to indicate that reversal of ciliary beats disrupted the transport of ova, although disruption of sperm transport, or muscle activity, cannot be ignored as possibilities. It is perhaps significant that ovum movement is most rapid in the ovarian and uterine quarters of the Fallopian tube where cilia concentrations are greatest.

(iii) restrictive action of the isthmus:

The exact location and cause of the physiological sphincter in the isthmus remains uncertain. While anatomic sphincters at the utero-tubal junction have been demonstrated in many species (Anderson, 1927) none have been demonstrated at the ampullar-isthmic junction (Greenwald, 1961a).

Muscular activity of the Fallopian tube increases as the time of ovulation approaches but quickly declines following ovulation (Westman, 1926). Intraluminal pressure changes in the isthmus and ampulla of the rabbit were recorded by Greenwald (1963b). Contractions of the ampulla at oestrus, and the two succeeding days, were rapid but relatively weak and highly irregular in amplitude. The isthmic contractions occurred at the same rate as those of the ampulla but were more uniform and of greater amplitude. Over the next three days however, isthmic contractions became progressively less frequent with powerful prolonged contractions being interspersed with others of lower amplitude. This decrease in the frequency of isthmic contractions coincided with the isthmic passage of ova. Greenwald concluded that the initial intense muscle activity in the isthmus presumably created a restrictive action on ovum entry into this region. Under in vitro conditions Brunden (1964a) induced marked increases in intraluminal pressure in the ampulla and isthmus using doses of acetylcholine and noradrenaline respectively. The failure of increases in ampulla pressure reaching the uterine strain guage transducer, and the converse, indicated a functional isthmic occlusion in the rabbit. Indeed, this

was the case even when the two records were taken from points 1.5 cm apart and separated only by the ampulla-isthmic junction (Brunden, 1964c).

The accumulation of tubal secretions in the oestrous rabbit (Black & Asdell, 1959) sheep (Edgar & Asdell, 1960a) and cow (Black & Davis, 1962) following ligation at the ovarian end introduced further supporting evidence of an occlusive mechanism. While Black & Asdell (1959) failed to find histological evidence of their proposed oestrogen-induced oedema at the utero-tubal junction, limited evidence was later obtained with the ewe (Edgar & Asdell, 1960a). In contrast, removal of the utero-tubal junction and part of the isthmus, or the application of nicotine to these regions did not reduce the fluid distension in the cow (Black & Davis, 1962). Whether the fluid is eventually released into the uterus by a diminution of the oedematous condition or a relaxation of the isthmic musculature was not resolved.

In support of these findings, Stavorski & Hartman (1958) while carrying out an extensive investigation using utero-tubal insufflation, found the isthmus to be more important than the utero-tubal, or isthmic-ampulla junction, in resisting the passage of gas.

It is thus possible that the constriction of the isthmus is only a function of its small diameter and convoluted nature although a full explanation is still required for the cyclic nature of the phenomenon.

E. PURPOSE OF THE INVESTIGATION

It is apparent that the Fallopian tubes are highly specialised organs with distinct anatomic regions intergrated in function so as to move ova and spermatozoa in contrasting directions during similar periods in time. The part played in these advents by the muscular and ciliary activity of the tube has received considerable attention in the past. In contrast, knowledge on the movement of fluid secreted into

the lumen of the female genital tract is extremely limited. Since Woskressenski in 1891 postulated the presence of an active secretion in the Fallopian tube of the rabbit emphasis has been on investigating the role of genital fluids as media through which early metabolic and developmental changes of ova and spermatozoa may be prompted (Mastroianni, 1962). Thus the quantity of fluid secreted and changes in some of its components during the oestrous cycle have been investigated only in a limited number of species.

The direction of flow of tubal secretions, and their possible role as a medium in which ova and spermatozoa are conveyed, do not appear to have been measured quantitatively. This investigation was therefore primarily concerned with devising, and testing, a method which would allow continuous collection and quantitative assessment of the direction of fluid flow from the Fallopian tube in the ewe. Two parameters, flow through the ovarian end of the ampulla and through the utero-tubal junction into the uterus, were to be measured in the same animal on successive days of the normal oestrous cycle. Summation of these two parameters would provide information on the quantity of fluid secreted by the Fallopian tube.

CHAPTER TWO

DIRECTIONAL FLOW OF FALLOPIAN TUBE SECRETIONS IN THE ROMNEY EWE

CONTENTS OF CHAPTER

A. INTRODUCTION

METHODS OF INVESTIGATING NORMAL TUBAL TRANSPORT

1. Progress of Spermatozoa and Ova

Techniques for defining the rate of progress of sperm and ova in the genital tract are few in number. One commonly used method has been to determine the distribution of sperm, and location of ova, at known time intervals following mating. The genital tract of the slaughtered animal is removed and the Fallopian tube sectioned into an even number of equal segments. The distribution of sperm in each segment, (Blandau & Money, 1944; Blandau & Odor, 1949; Braden, 1953; Mattner, 1963b) or location of ova, (Assheton, 1895; Anderson, 1927; Chang, 1951c; Greenwald, 1959a, 1960, 1961a; Harper, Bennett, Boursnell & Rowson, 1960) indicates their rate of progress.

The simplicity of the method is desirable but certain limitations are apparent. The location of ova is imprecise although where a lower recovery is acceptable then it can, in part, be overcome by increasing the number of segments. Use of polytocous species to provide a distribution pattern through one or more segments can also give a better measure of mean progress. This problem of precisely locating ova *in situ* was partly overcome by the application of autoradiography for tracing radioactive resin spheres (Harper *et al.*, 1960) and by Lang (1965) who employed a variation of the transillumination technique to locate glass beads within the extirpated Fallopian tube. When such artificial ova move in an experimentally reproducible manner (Harper *et al.*, 1960) the advantages are readily apparent (cf Bennett & Rowson, 1961).

Further inaccuracies arise from being unable to determine the precise time of ovulation following mating. In the ewe this is

particularly relevant as the time of ovulation is extremely variable (McKenzie & Terrill, 1937; Asdell, 1946). The problem has been partly overcome by transferring ova to the Fallopian tube and observing their progress by transillumination (Harper, 1961a). Ova, foreign to the recipient (Lode, 1894; Wintenberger-Torres, 1961; Bennett & Rowson, 1961), and artificial ova (Anderes, 1941; Wintenberger-Torres, 1961), have also been used.

2. The Movement of Fallopian Tube Secretions

With the introduction of Lipiodol as a contrast medium in hysterosalpingography during the 1920's a series of investigations were carried out on the movement of such fluids using X-ray photography or cineradiography. Experimentally the technique was deployed to record muscular activity of the tubes, but in combination with clinical testing of tubal patency (Decker & Decker, 1954; Buxton & Mastroianni, 1957) some information on fluid movement can be gleened. The bi-directional movement of fluid has been noted during serial radiography (Krehbiel & Carstens, 1939; Rowson, 1955; Bjork, 1959) and cine-radiography (Akester & Inkster, 1961; Inkster, 1964).

More recent studies have associated the secretion of fluid within the Fallopian tube with the mechanism of ovum movement through the isthmus and entry into the uterus. Thus in the rabbit (Black & Asdell, 1959), sheep (Edgar & Asdell, 1960), and the cow (Black & Davis, 1962) the experiments involved ligation of the ovarian end of the tube. In each case fluid accumulated in the lumen for 3 days after the onset of oestrus but then rapidly disappeared. Black & Asdell (1958) suggested that ova are carried into the uterus by 'a fluid surge' but Black & Davis (1962) later reported that with the cow the fluid surge preceded the expected time of ovum transport into the uterus by 24 hours.

In view of these reports an attempt was made to quantitatively

assess gross fluid movement by using a modification of the cannulation technique described by Clewe & Mastroianni (1960). This technique (Bellve, 1967) was adopted since it allowed repeated observations to be made on the same animal and avoided the disadvantages of methods involving post mortem examination of fluid in the genital tract. A preliminary report has been presented for publication (Bellve & McDonald, 1967).

B. METHODS AND MATERIALS

1. Animals

Forty 4- and 6-year-old Romney ewes were used during April - July 1966. The ewes were kept in individual pens arranged so that a vasectomised ram was in contact with each ewe but prevented from mating by the wire netting partition (Fig. 1). Observations for oestrous behaviour and flow of vaginal mucus were made at frequent intervals during daylight hours. The onset of oestrus was recorded when the ewe's behaviour and the activity of the ram suggested mating would occur. Where oestrous behaviour was doubtful (5 cycles) oestrus was presumed to have occurred when characteristic changes in the flow of mucus were detected (Radford & Watson, 1955). The ram was periodically replaced in an attempt to ensure active interest in the ewes.

2. Operative Procedure

Each animal was tranquillized with chlorpromazine and anaethetised with nembutal. The genital tract was exposed by a mid ventral incision anterior to the mammary glands and the size and number of corpora lutea and follicles noted.

The abdominal wall was punctured with a stainless steel trochar at a point 15 cm lateral to the dorsal mid line and 15 cm anterior to the

stifle joint. Two cannulae, filled with isotonic saline containing heparin and penicillin, were passed through the trochar and into the peritoneal cavity. The trochar was then withdrawn and the flank wound closed with a purse string suture leaving about 6 cm of each cannula protruding.

The flanged end of one cannula was ligated into the fimbrial end of the ampulla by a single atraumatic No. 6 braided silk ligature, while the other cannula was passed through the uterine wall approximately 4 cm below the utero-tubal junction and ligated into a position opposed to this junction, (Fig. 3). During insertion of the ligatures care was taken to avoid undue interference with blood vessels. Penicillin was placed in the peritoneal cavity and the body wall closed.

A collection device was placed 5 cm below the point where the cannulae emerged on the flank and sutured to the skin at each corner, (Fig. 2). The cannulae, previously marked for identification, were connected to the appropriate collection chamber. Fluid accumulating in the collection chambers was removed daily at 8.30 a.m.

3. Collection of Tubal Secretions

Two collection devices both similar to that described by Black, Druby & Reisen (1963) were used.

Type 1: The first (Fig. 5) consisted of two glass collection chambers (4 mm int. diam., 7 mm ext. diam.) each sealed at both ends by small rubber stoppers. Two metal tubes (part of a 14 guage hypodermic needle) passed through the upper stopper and one through the lower stopper. A 4 cm length of vinyl tubing (int. diam. 1.2 mm) was connected to each metal tube. Both collection chambers were embedded in a block of plastic (9 x 4 x 1 cm) by a method commonly used for embedding biological materials. Each of the vinyl tubes emerge from the plastic block;

one at the top of each chamber formed an air vent, the other the inlet for tubal fluids; the third tube emerging from the bottom of each chamber allowed withdrawal of the fluid by adjusting the regulating screw. Calibration marks on the collection chambers were used for the measurement of fluid volume prior to its withdrawal.

Type 2: The second collection device (Fig. 6) consisted of two polythene chambers (7 mm int. diam., 9 mm ext. diam.) 10 cm in length attached to a block of plywood (10 x 6 x 0.6 cm). To the base of each collection chamber was fitted a thimble shaped rubber cone and to the top a polyethylene adaptor. A fine hole at the top of each collection chamber allowed maintenance of atmospheric pressure. Fluid accumulating in each collection chamber was removed and measured by using a 1 ml hypodermic syringe inserted through the rubber cone.

4. Operations

Three groups of animals were operated on as follows;

Group 1: Twenty ewes were cannulated with 43 cm lengths of transparent vinyl tubing (1.2 mm int. diam., 1.6 ext. diam.). Six of these animals had 'type 1' collection device, the remainder 'type 2'.

Group 2: Ten ewes were cannulated with 43 cm lengths of translucent polyethylene tubing (1.5 mm int. diam., 2.5 ext. diam.). Type 2 collection device was used.

Group 3: Ten ewes were cannulated as with group 1 but using cannulae of 1.8 mm int. diam. and 2.5 ext. diam. connected to a 'type 2' collection device.

5. Post-Mortem and Histology

Ten ewes in which the cannulae functioned successfully were slaughtered at the completion of their recording periods and the

Fallopian tubes fixed in Bouins fluid. Tissues from the mid ampulla and mid isthmus of the cannulated and non-cannulated Fallopian tubes of each animal were embedded in paraffin wax; sections were cut at 8 - 10 μ and stained with haematoxylin and eosin. Measurements of epithelial cell height (5 fields in each of 2 sections) were made on each sample of tissue.

PLATES

Figures 1 to 6

Fig. 1 Ten experimental ewes in close association with a centrally placed vasectomised ram.

Fig. 2 A ewe with collection device *in situ*

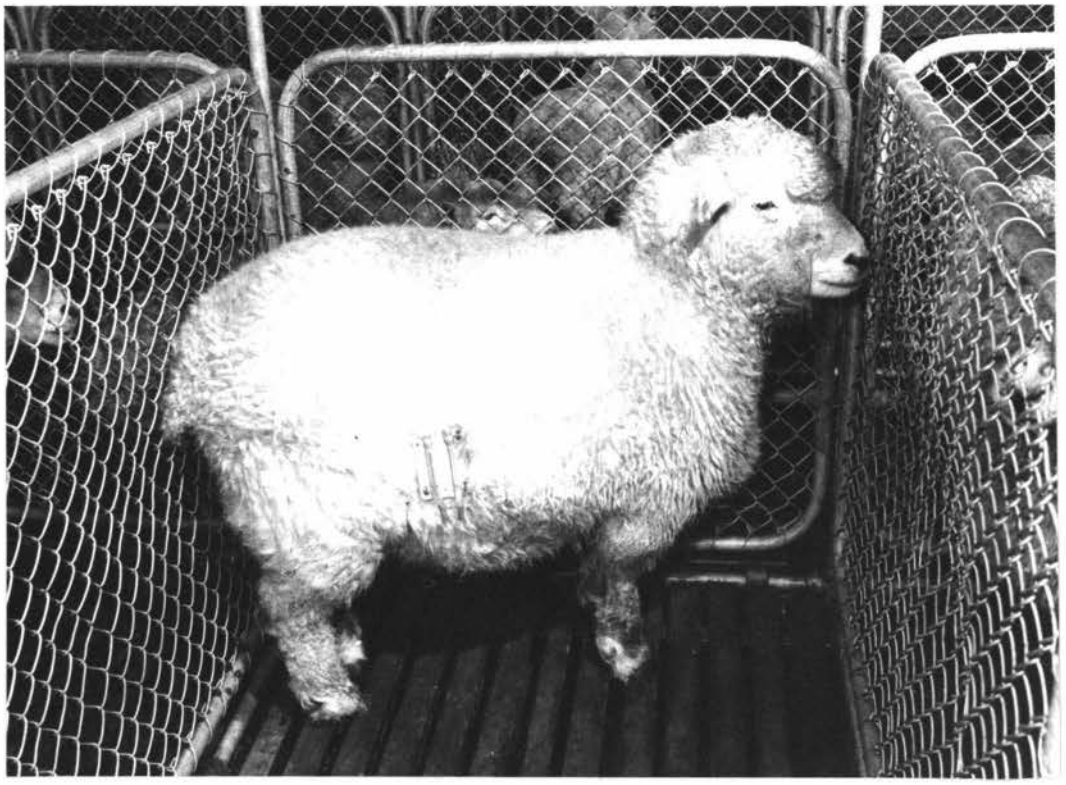


Fig. 3 - Excised genital tract of a ewe. The right Fallopian tube, dissected, with the ampullar and isthmic cannulae ligated into position.

Fig. 4 - Excised genital tract of a ewe. The right uterine horn and Fallopian tube being surrounded by an antibody tissue reaction to the cannulae. The non-cannulated side of the tract remained unaffected.

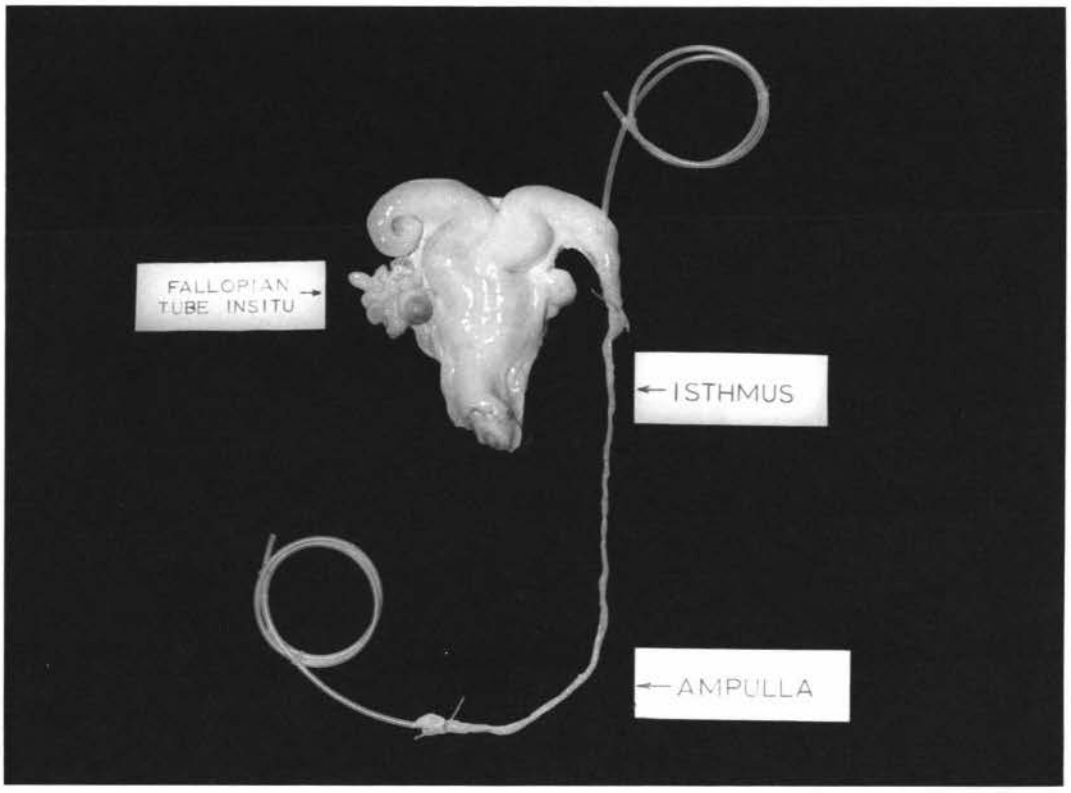
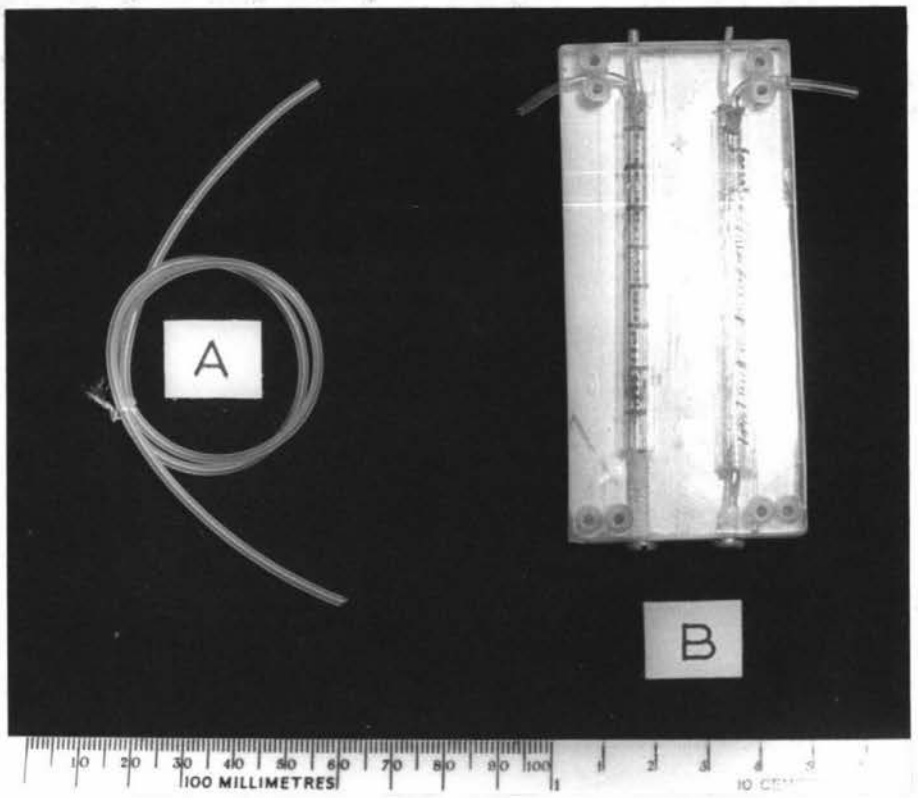
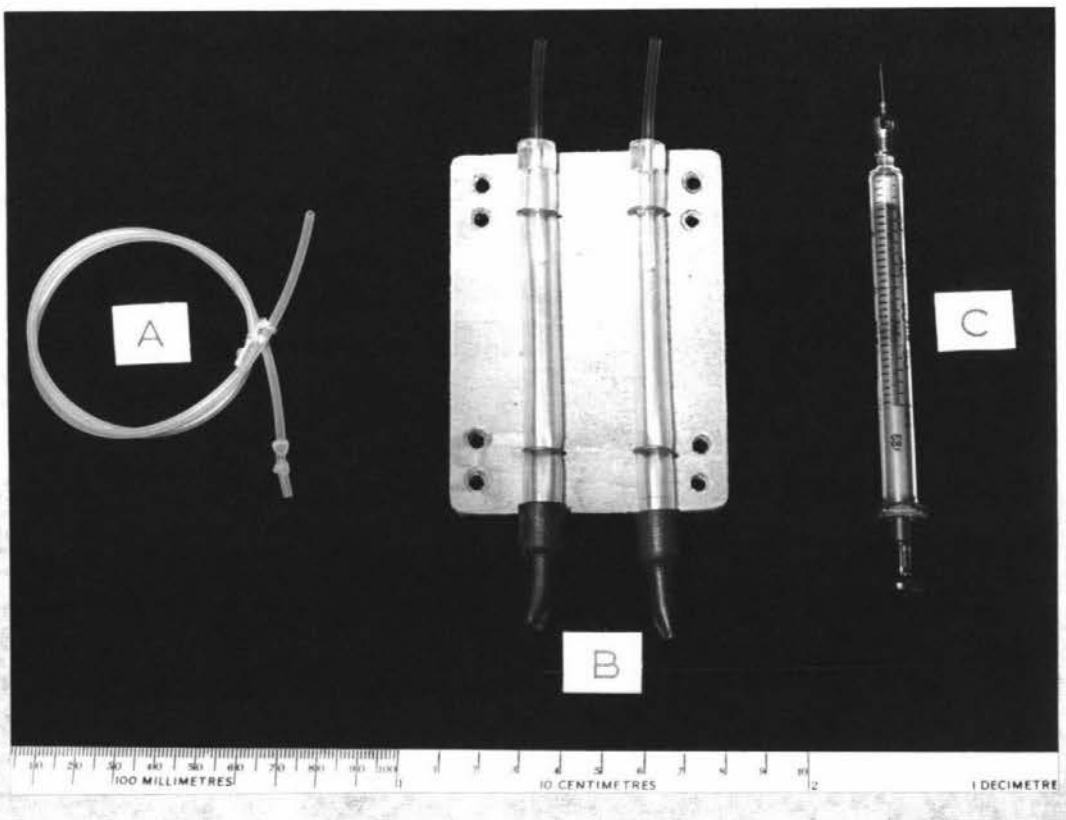

FALLOPIAN TUBE INSITU
ISTHMUS
AMPULLA

Fig. 5 - 'Type 1' collection device with glass collecting chambers embedded in plastic (B) and the transparent vinyl cannulae (A).

Fig. 6 - Type 2 collection device with polythene chambers mounted on a plywood block (B). Translucent polyethylene cannula (A) and 1 ml hypodermic syringe (C) also shown.


A
B
100 MILLIMETRES


A
B
C
100 MILLIMETRES
10 CENTIMETRES
1 DECIMETRE

C. RESULTS

1. Collection Device

Of the two collection devices 'type 2' proved to be the more practical. The complexity of the 'type 1' model led to the following disadvantages; (a) construction was time consuming, (b) the calibration marks were difficult to read, (c) both inlet and outlet tubes frequently blocked with cellular debris, (d) adequate cleaning was difficult and (e) in some the outlet-regulating-control leaked.

2. Period of Collection

As judged by the presence of a continuous column of fluid in the cannulae and accumulation of fluid in the collection chambers, both cannulations were successful in all ewes, at least initially. With the 40 ewes the average period following surgery during which fluid was obtained from both cannulae was 35.6 days (range 3 - 106 days). However the degree of success achieved in recording normal oestrous cycles from each animal differed between the three groups.

Group 1:

Cannulation was successful in the 20 ewes for an average period of 23.5 days (range 3 - 106 days). There were 8 ewes which did not contribute fluid through both cannulae during a complete oestrous cycle for a variety of causes i.e. failure to maintain a flow of fluid for the full experimental period (5 ewes), salpingitis (2 ewes), and damage to cannula (1 ewe). Cannulation eventually failed in the remaining 12 animals. Post-mortem examination usually revealed cannulae blocked by cellular detritus although in five animals one or both cannulations had also come adrift prior to slaughter.

Group 2:

Initially, fluid secretion from these animals appeared normal but between the range of 9 and 19 days fluid secretion

increased and was maintained at an extremely high level. Large quantities of cellular detritus and a yellow colouration of the fluid became evident. Microscopic examination of the fluid indicated an excessive concentration of leucocytes while post-mortem examination revealed large tissue growths surrounding the cannulae and part of the genital tract (Fig. 4). Records on daily fluid secretion from these animals up to the time of slaughter (range 36 - 46 days) were considered abnormal and subsequently disregarded.

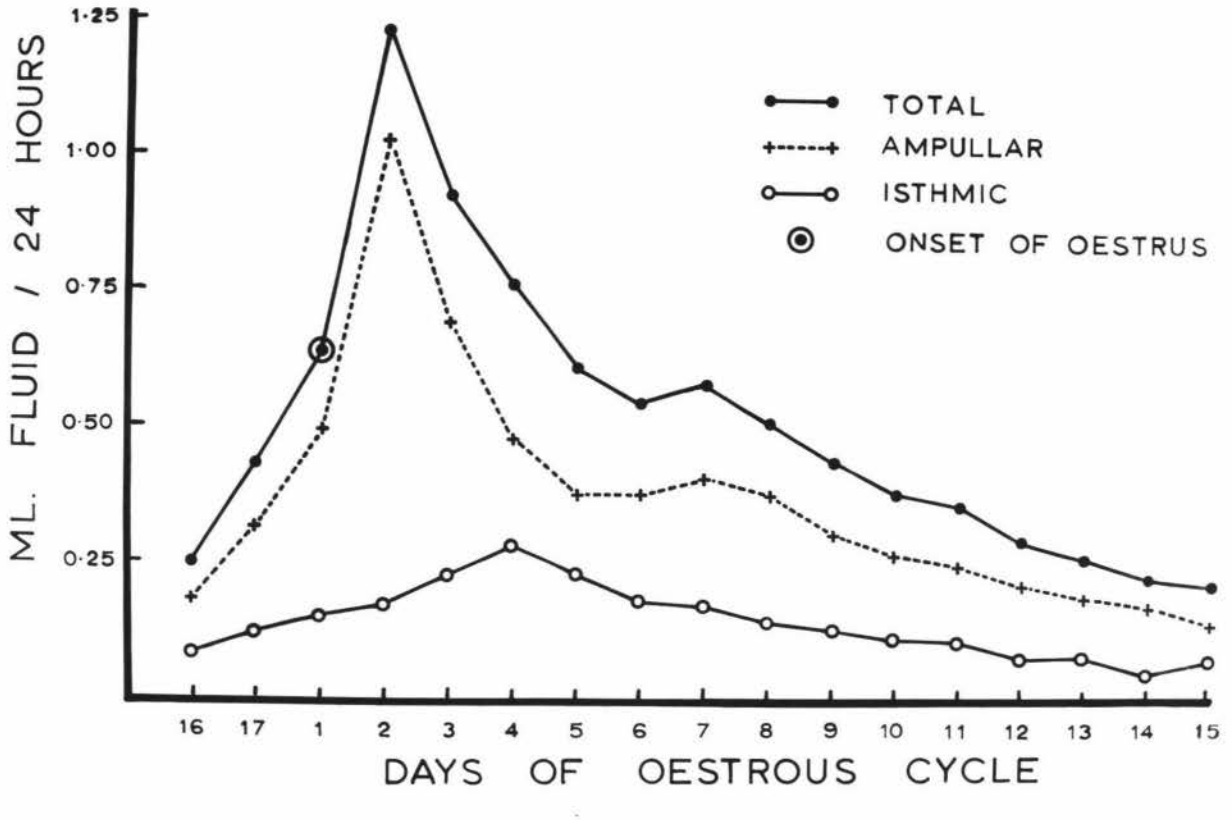
Group 3:

The average period of successful cannulation with this group of 10 animals was 53.8 days (range 25 - 94 days). Of these, three failed to contribute records for a complete oestrous cycle. Two of these ewes exhibited oestrus 3 - 4 days after surgery, but cannulation at the isthmic end failed prior to a full recording of the subsequent oestrous cycle. The third ewe died on day 20; post-mortem examination did not reveal the cause of death.

The remaining 19 successful animals from groups 1 and 2 provided data on fluid secreted daily during a total of 26 oestrous cycles. Data has not been included in the analysis where oestrus occurred within 7 days of operation, since it seemed that secretion of fluid at this critical period of the study would still be seriously influenced by surgery.

3. Fluid Secreted

The mean daily fluid secreted is given in Table 1 and graphically shown in Text-fig. 1. Fluid secretion increased on the last day of the oestrous cycle, reaching a maximum of 1.18 ml/24 hr (range 0.77 - 1.61 ml) on day 2, that is about 24 hr after the onset of behavioural oestrus. Thereafter, the amount of fluid produced showed a sharp decline until about day 6.



Text-fig. 1. Total output of tubal fluid and fluid flow through the ovarian (ampullar) and uterine (isthmic) ends of the Fallopian tube in the ewe.

In all oestrous cycles recorded, except for two (different sheep) there was a transient rise in the fluid secreted within the range of 6 - 10 days (mean day 7). On succeeding days fluid secretion again gradually declined reaching a low level of 0.20 ml/24 hr (range 0.03 - 0.46 ml) on day 15.

Following the analyses of variance daily means of fluid secreted were ranked in ascending order and differences tested (Table 2) using Duncan's Multiple Range test (Duncan, 1955). For simplicity only the differences between the means of successive days of the cycle are considered. On this basis differences between adjacent days from 16 to 5 inclusive were significant ($P < 0.01$).

TABLE 1

MEAN ± S.E. OF FLUID SECRETED, AMPULLAR FLOW, AND ISTHMIC FLOW (ml/24 hr)

Fluid Source \ Days	16	17	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ampullar flow	.17 ±.03	.31 ±.03	.49 ±.05	1.01 ±.05	.69 ±.05	.48 ±.05	.37 ±.04	.37 ±.04	.41 ±.05	.37 ±.04	.30 ±.03	.27 ±.04	.24 ±.04	.21 ±.03	.18 ±.03	.16 ±.02	.13 ±.02
Isthmic flow	.08 ±.02	.12 ±.03	.14 ±.03	.16 ±.03	.23 ±.04	.28 ±.03	.23 ±.03	.17 ±.02	.17 ±.02	.14 ±.02	.13 ±.02	.11 ±.02	.10 ±.02	.07 ±.04	.08 ±.01	.05 ±.01	.07 ±.01
Fluid secreted	.25 ±.04	.43 ±.04	.64 ±.05	1.18 ±.05	.92 ±.06	.76 ±.07	.06 ±.06	.55 ±.05	.57 ±.06	.50 ±.05	.43 ±.04	.38 ±.04	.35 ±.05	.29 ±.04	.25 ±.03	.21 ±.02	.20 ±.02

TABLE 2

TEST* OF SIGNIFICANCE BETWEEN MEANS OF FLUID SECRETION, AMPULLAR FLOW, AND ISTHMIC FLOW

Fluid Source	DAYS OF CYCLE WITH MEANS RANK IN ASCENDING ORDER
Ampullar flow	15 14 16 13 12 11 10 9 17 8 6 5 7 4 1 3 2
Isthmic flow	14 15 12 13 16 11 10 17 9 8 1 2 7 6 3 5 4
Fluid secreted	15 14 16 13 12 11 10 17 9 8 6 7 5 1 4 3 2

* Duncans' Multiple range test (1955)

The means of days not underscored by a common line are significantly different at the 5% level (----) or 1% level (_____).

The analyses of variance of fluid secreted daily and also total volume of fluid secreted over each individual oestrous cycle are presented in Table 3. The main source of variation in daily fluid secretion accrued from differences between days of the cycle ($P < 0.01$). Data from three ewes with 4, 3 and 3 cycles respectively, indicated that considerable variation occurred between cycles within sheep ($P < 0.01$). Whether variation also occurs between sheep was not proven ($P > 0.05$) by the present results.

4. Direction of Flow

The mean flow per 24 hr through the abdominal tubal ostium and into the abdominal cavity (designated ampullar flow) and through the utero-tubal junction into the uterus (isthmic flow) is given in Table 1 and graphically shown in Text-fig. 1.

Ampullar flow closely paralleled fluid secretion and for each day of the oestrous cycle the greater part of the fluid secreted entered the peritoneal cavity. Maximum ampullar flow of 1.01 ml/24 hr (range 0.55 - 1.44 ml) occurred about day 2 of the cycle, while a minimal mean flow of 0.13 ml/24 hr (range 0.01 - 0.35 ml) occurred on day 15 of the cycle. Duncan's multiple range test indicated that the adjacent days 16, 17, 1, 2 and 3 differed significantly ($P < 0.01$) in their mean flow. Analyses of variance (Table 3) again indicated 'day' of the cycle was the major source of variation ($P < 0.01$) while a minor source of variance was due to differences between cycles within sheep ($P < 0.01$).

Changes in the rate of flow through the utero-tubal junction also occurred in a cyclic manner. Peak isthmic flow of 0.28 ml/24 hr (range 0.17 - 0.84 ml) occurred 3.9 ± 0.1 days (range day 3 - 6) following the onset of oestrus; but for the greater part of the cycle, flow of fluid into the uterus remained low. The increase in isthmic flow taking place in most animals was transient in nature seldom exceeding 3 days

in duration. In 16 of the cycles fluid from the isthmic cannula during these three days was red in colour contrasting markedly with the clear appearance of this fluid over the remainder of the cycle, and fluid of the ampullar flow. The mean isthmic flow on days 2, 3 and 4 of the cycle were the only adjacent days significantly different ($P < 0.05$). However, peak isthmic flow on day 4 was significantly greater ($P < 0.01$) than the flow on either day 2 or day 6. Day of the cycle was the major source of variation in isthmic flow as was the case with ampullar flow. But variation from cycle to cycle within sheep was a relatively more important source of variation for isthmic flow than for ampullar flow.

TABLE 3

SUMMARY OF ANALYSES OF VARIANCE FOR FLUID SECRETED AND FLUID FLOW (ml/24 hr)

(Data 19 ewes, 20 x 17-day cycles; 6 x 16-day cycles)

Source	d.f.	Fluid secreted M.S.	Ampullar flow M.S.	Isthmic flow M.S.
Between days	16	1.840**	1.256**	0.109**
Between sheep	18	0.248	0.265	0.034
Between cycles, within sheep	7	0.141**	0.204**	0.049**
Error	394	0.046	0.025	0.011

** $P < 0.01$

5. Histology

Examination of the sections taken from the mid ampullar and mid isthmus did not reveal any morphological differences between the cannulated and non-cannulated Fallopian tube of the same animal. Mean epithelial cell height of cannulated versus non-cannulated Fallopian tubes did not differ significantly for the mid ampulla ($F < 1$) or the mid isthmus ($F < 1$).

D. DISCUSSION

It would appear that when a lumen size less than 1.5 mm internal diameter is used for either the cannulae or in the collection device they become prone to blockage from cellular debris present in normal tubal secretions. For this reason cannulae used in Group 1 and the 'Type 1' collection device were discarded. The larger internal diameter of the other cannulating material and 'Type 2' collection device appeared to overcome this problem. Unfortunately the cannulating material used in Group 2 animals caused an inflammatory foreign-body reaction. Similar reactions to polyethylene have been found to occur in both animals and man (Grindley, 1948a; Grindley & Mann, 1948b), but the tissues are not usually irritated by the pure material (Yeager & Cowley, 1948). An impurity in the tubing was therefore a possible cause of the inflammatory response. The vinyl tubing used in Group 3 animals proved satisfactory and so used in suceeding experiments.

The present investigation confirms previous findings of a well defined cyclic pattern of fluid secretion in the Fallopian tube of the ewe. The levels recorded at oestrus are less than reported by Black, Druby & Reisen (1963) and Perkins, Goode, Wilder & Henson (1965), but greater than reported by Restall (1966b). The minimum dioestrous levels appear to be lower and occur later in the cycle, than reported by these authors.

Maximum fluid flow through the ovarian end of the Fallopian tube occurred about 24 hr after the onset of oestrous behaviour. Although the time at which ovulation occurs in the ewe is somewhat variable (Asdell, 1946) it is probable that this peak flow of fluid in the ovarian direction occurs near the time of ovulation. In contrast to the flow of fluid through the ovarian end, maximum flow through the utero-tubal junction and into the uterus occurred about 3.9 days (94 hr) after the

onset of oestrus. Since ova enter the uterus between 77 and 96 hr after the onset of oestrus in the ewe (Clarke, 1934) it is probable that passage of the ovum into the uterus coincides with this period of maximum flow. The gradual increase in fluid flow over days 1, 2 and 3 is probably indicative of a gradual decline in the occlusive mechanism in the isthmus, thus permitting passage of fluid through the utero-tubal junction into the uterus. It is also noteworthy that the occlusion is not complete even at the period of maximum follicular activity and presumably high levels of endogenous oestrogen.

The origin of the red colouration in the maximum isthmic flow remains unknown but significantly none of this colouration ever appeared in the ampulla flow. This presumably indicates that movement in the ovarian direction from the distal isthmus was not taking place. In which case uterine contractions may play a role in the movement of fluid towards the ovary.

The factors causing the wide variation between cycles of individual animals in volume of fluid secreted, ampullar flow, and isthmic flow, over each oestrous cycle remains to be examined. The possibility exists that much of this is due to a variability in the number of follicles and corpora lutea functioning during any given oestrous cycle and in consequence the level of oestrogen and progesterone circulating (e.g. Edgar & Ronaldson, 1958).

The experimental technique described provides evidence on the pattern of fluid secretion and movement in the Fallopian tube but does so within certain limitations. Apart from the presence of the cannula, which apparently causes no morphological changes in the epithelium, ligation at each end of the tube prevents the possible entry of fluid from other sources. The influx of uterine and peritoneal fluid is known to occur while follicular fluid may also contribute during the process of ovulation. The extent to which these fluids contribute

towards tubal secretion and its influence on the transport of sperm and ova remains to be clarified.

CHAPTER THREE

THE EFFECT OF OESTROGEN THERAPY ON THE DIRECTIONAL FLOW OF TUBAL SECRETIONS IN THE OVARIECTOMISED EWE.

CONTENTS OF CHAPTER

A. INTRODUCTION

The transport of gametes within the Fallopian tube is dependent upon the balance of ovarian hormones since ovariectomy hinders progress of both ova (Corner, 1928; Adams, 1958; Noyes, Adams & Walton, 1959b; Harper, 1964) and spermatozoa (Noyes, 1959a; Noyes, Adams & Walton, 1959c; Mattner & Braden, 1963a). However, the mechanisms by which the ovarian hormones, oestrogen and progesterone, regulate tubal transport is a subject of continuing speculation. The role of oestrogen appears particularly complex and since many of the earlier experiments were primarily concerned with the effects of oestrogen on implantation, tubal transport being a side issue, they do little to clarify the problem.

Much of the experimental evidence is based on the rabbit (Courier & Raynaud, 1934; Burdick & Pincus, 1935; Pincus & Kirsch, 1936; Csapo, 1955; Greenwald, 1957; 1959b, 1961b; Black & Asdell, 1958; Noyes, Adams & Walton, 1959b; Harper, 1961b, 1964) but the rat (Dreisbach, 1959), mouse (Burdick, Whitney & Pincus, 1937a), guinea pig (Kelly, 1931; Deansley, 1961, 1963), cat (Courier & Gross, 1935;) and sheep (Edgar & Asdell, 1960a) have also been investigated. Much of this work was done before crystalline hormones became readily available and it is now evident that, with few exceptions, investigators used quantities of hormone which exceeded physiological levels. In addition, a considerable variation in the dosage, time of injection in relation to oestrus, and whether the recipient animals were entire or ovariectomised, has probably led to the controversy which exists on the retarding or accelerating effect of oestrogen therapy.

1. Oestrogen Therapy in the Entire Animal

Corner (1928) conducted one of the earliest experiments. He noted that post coital ablation of the luteal tissue in the rabbit, leaving

follicular tissue functional, caused the disappearance of ova from the tubes and uterine horns prior to day 5. That similar destruction of corpora lutea in the rabbit (Adams, 1965a) caused accelerated ovum transport if all corpora lutea were destroyed provided confirmation of this phenomenon.

In contrast, the injection of the oestrogenic compound 'Theelin' prevented the descent of fertilised ova through the Fallopian tube in both mice and rabbits (Burdick & Pincus, 1935). This 'tube locking' characteristic was shared by several other commercial oestrogenic extracts when given at dose levels ranging from 5 to 25 R.U. At the lower dose level of 5 R.U., Amniotin, Progynon and Theelin failed to have any effect. However, where 100 to 500 R.U. of Progynon B were injected into mice (Burdick & Whitney, 1937b; 1938) and 5000 R.U. in rabbits (Whitney & Burdick, 1938) accelerated tubal transport of ova occurred.

More recently, Greenwald (1957), reported tubal retention of ova in only a few instances where 5 µg daily of oestradiol benzoate (ODB) was given to rabbits for three days following mating. Similarly, two injections of 5 µg ODB 48 hr apart, and similar doses of oestrone and stilboestrol definitely accelerated ovum transport. In two subsequent investigations Greenwald (1961a,b) found that up to 25 µg of oestradiol cyclopentylpropionate (ECP) accelerated tubal transport in the doe but 250 µg induced tube locking. It appears evident, however, that the latter response need not occur in all species. In the hamster (Greenwald, 1961b) guinea pig (Deansley, 1961, 1963) and rat (Banik & Pincus, 1964) the passage of ova was accelerated at all dose levels of oestrogen ranging up to 250 µg. Furthermore, Banik & Pincus did not recover any ova from the utero-tubal junction area, the region supposedly concerned with the retention of ova (Black & Asdell, 1959; Edgar & Asdell, 1960a).

While ovum movement in the uterine direction appears to be accelerated, the ascent of spermatozoa in the Fallopian tube is also enhanced by the subcutaneous injection of 10 µg ODB in the doe (Chang & Bedford, 1961).

2. Oestrogen Therapy in the Ovariectomised Animal

Results from oestrogen therapy to the ovariectomised animal closely parallel those obtained from the entire animal. With rabbits, ovariectomised 35 days prior to treatment, ODB at doses between 0.1 and 4.0 µg had variable effects on tubal transport of ova after transfer (Noyes et al., 1959b). Low doses enhanced ova transport yet the 2.0 to 4.0 µg probably caused ovum retention in many instances. These results are not conclusive however, as a large proportion of the transferred ova were not recovered and the site of retention nor its duration beyond 78 hr assessed. These criticisms are more relevant when considering the results obtained by Harper (1964). He found that similar dose levels accelerated ova transport and only with a few rabbits were a small number of the artificial ova still in the tubes at 56 hr after transfer. These few were located at the ampullar-isthmic junction.

Oestrogen therapy in the ovariectomised animal also enhances the passage of spermatozoa. This has been observed in the rabbit (Noyes, Adams & Walton, 1959c) although the higher dose levels which caused the retention of ova, also prevented the ascent of spermatozoa. This latter effect does not appear to occur in the ewe (Mattner & Braden, 1963a).

After consideration of these results, and that the administration of oestrogen has been reported to delay the passage of tubal fluid into the uterus from the ligated Fallopian tube (Black & Asdell, 1958; Edgar & Asdell 1960a; Black & Davis, 1962) it was considered pertinent to investigate the hormonal factors controlling the direction of fluid flow in the ovariectomised oestrogen primed ewe.

B. MATERIALS AND METHODS

1. Animals

Eleven mature Romney ewes were used during the 1966/67 anoestrous season. The ewes were kept in individual pens. Vaginal smears were taken daily during treatment periods and stained using the technique originally described by Shorr (1940). Smears were examined and classified by the technique of Robinson & Moore (1956b).

2. Operative Procedure

The procedure used has been described in the previous chapter. In the course of each operation the ewes were ovariectomised and the ovaries examined. All animals were operated on within a period of two days.

Cannulating material, and the collection apparatus, was that used for Group 3 animals outlined in Chapter 2. During the periods of treatment fluid collection was made at 12 hr intervals.

3. Treatments

Following the operation each ewe received no treatment for an interval of 15 to 16 days. At the end of this period three successive experiments were carried out. A 7 day period was allowed between the conclusion and the onset of succeeding experiments.

The experiments were carried out as follows:

Experiment 1. Two groups of 4 ewes were given 30 µg and 90 µg, respectively, of oestradiol benzoate (ODB).

Experiment 11. Four ewes were treated with 500 µg of ODB.

Experiment 111. Ten ewes were given 90 µg of ODB; as a single injection to 4 animals and 30 µg daily for 3 days in the remaining 6 animals.

During the course of experiments 1 and 11, two ovariectomised ewes were left untreated. Subsequent to experiment 1 ewes were allocated

to treatment groups in the succeeding experiment after consideration of previous treatment.

The oestradiol benzoate (Organon) was dissolved in warm peanut oil at a concentration of 200 µg/ml. Of this preparation sufficient was diluted to 30 µg/ml for each experiment. Injections were given intramuscularly at 9.00 a.m. on the day of treatment.

In those ewes receiving oestrogen, the duration of secretory response and fluid flow were examined. 'Duration of response' was regarded as the number of days from the time of oestrogen administration to the return to pretreatment levels. The time of maximum secretion, and maximum fluid flow, was also calculated from the time of hormone injection.

C. RESULTS

1. Collection of Fluid

Cannulation and fluid collection was successful for the duration of the experiments (68 days) in all but one ewe. In this animal the isthmic cannulation failed on day 11 and the ewe was subsequently discarded.

2. Vaginal Response

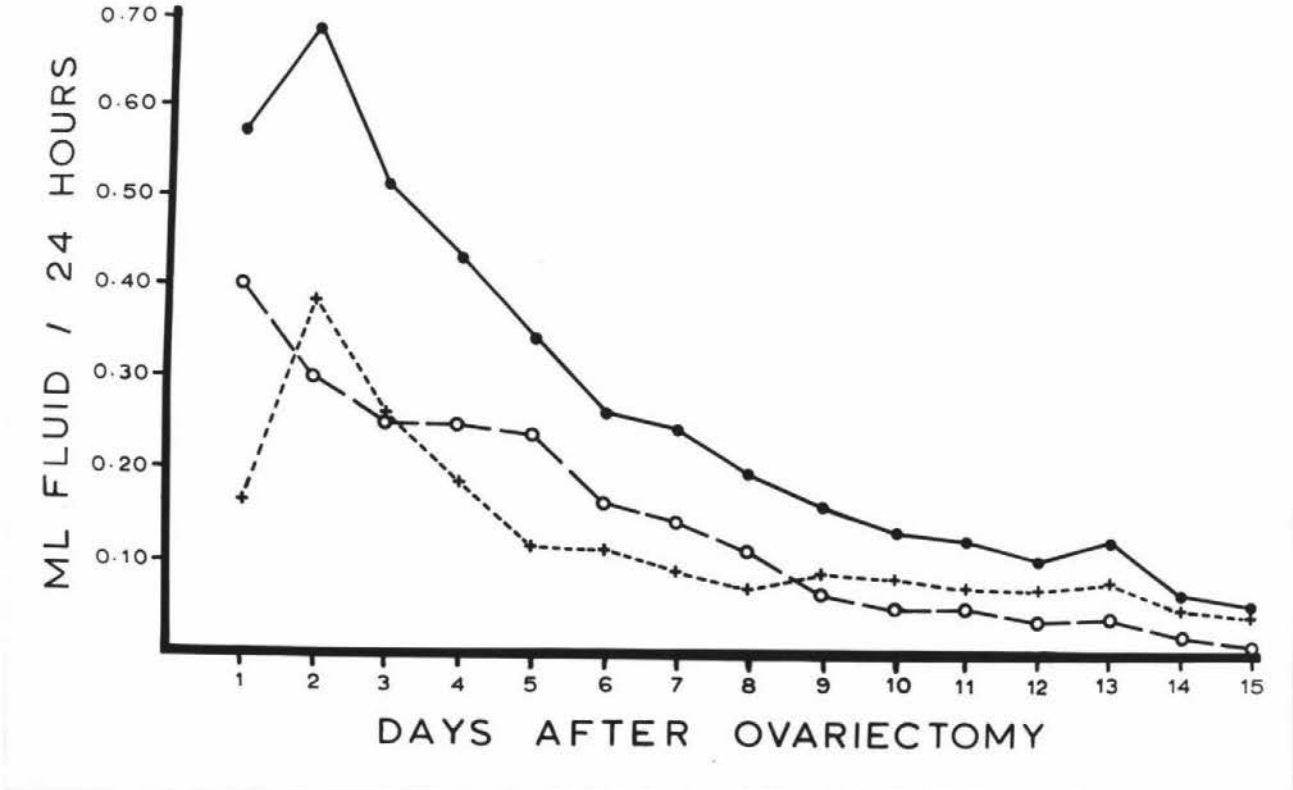
Following the injection of ODB each ewe secreted copious quantities of vaginal mucus and the epithelial cells became cornified 3 to 5 days post injection. Two ewes, on the 30 µg dose level, failed to give a 'Grade I' response, one being classified 'Grade 2' and the other 'Grade 3'. Both these ewes secreted lower quantities of tubal fluid than the average within the 30 µg treatment group. Cornification was not observed to occur with the untreated ovariectomised animals.

3. Effect of Ovariectomy

Mean fluid secretion, ampullar and isthmic flow are given in Table 4 and graphically presented in Text-fig. 2. The data were subjected

to analyses of variance (Table 6). A significant variation between animals ($P < 0.01$), most noticeable during the immediate post-operative period, caused an apparent elevation in fluid secretion and ampullar flow on day 2. Isthmic flow did not appear to be markedly influenced during this period but between animal variation ($P < 0.01$) was still an important factor during the first 8 days.

Fluid secretion and its two components, isthmic and ampullar flow, declined significantly ($P < 0.01$) after ovariectomy. This was particularly apparent between days 2 and 6 inclusive. Subsequent to day 7 the decline became more moderate reaching a static level about day 11. Subsequent to day 11 isthmic flow of each individual animal became progressively more spasmodic until, on day 15, it was recorded in only one animal.



Text-fig. 2. Mean fluid secretion (●——●), ampullar flow (+ ----- +) and isthmic flow (O—— ——O), following ovariectomy on day 0.

TABLE 4

DAILY MEANS (+ S.E.) OF FLUID SECRETION, AMPULLAR FLOW AND ISTHMIC FLOW (ml/24 hr) IN THE OVARIECTOMISED EWE

Fluid Source \ Days after Ovariectomy	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ampullar flow	.17 ±.07	.40 ±.03	.27 ±.06	.19 ±.04	.12 ±.03	.11 ±.03	.09 ±.04	.07 ±.02	.07 ±.02	.08 ±.02	.07 ±.02	.07 ±.01	.08 ±.01	.05 ±.01	.05 ±.01
Isthmic flow	.42 ±.16	.31 ±.11	.26 ±.09	.26 ±.08	.25 ±.08	.16 ±.04	.15 ±.03	.12 ±.02	.07 ±.01	.05 ±.02	.05 ±.01	.04 ±.02	.04 ±.01	.02 ±.01	.01 ±.01
Fluid secreted	.59 ±.16	.71 ±.16	.53 ±.12	.45 ±.10	.36 ±.09	.27 ±.05	.25 ±.03	.20 ±.02	.15 ±.02	.13 ±.02	.12 ±.03	.11 ±.02	.12 ±.02	.07 ± .02	.06 ±.01

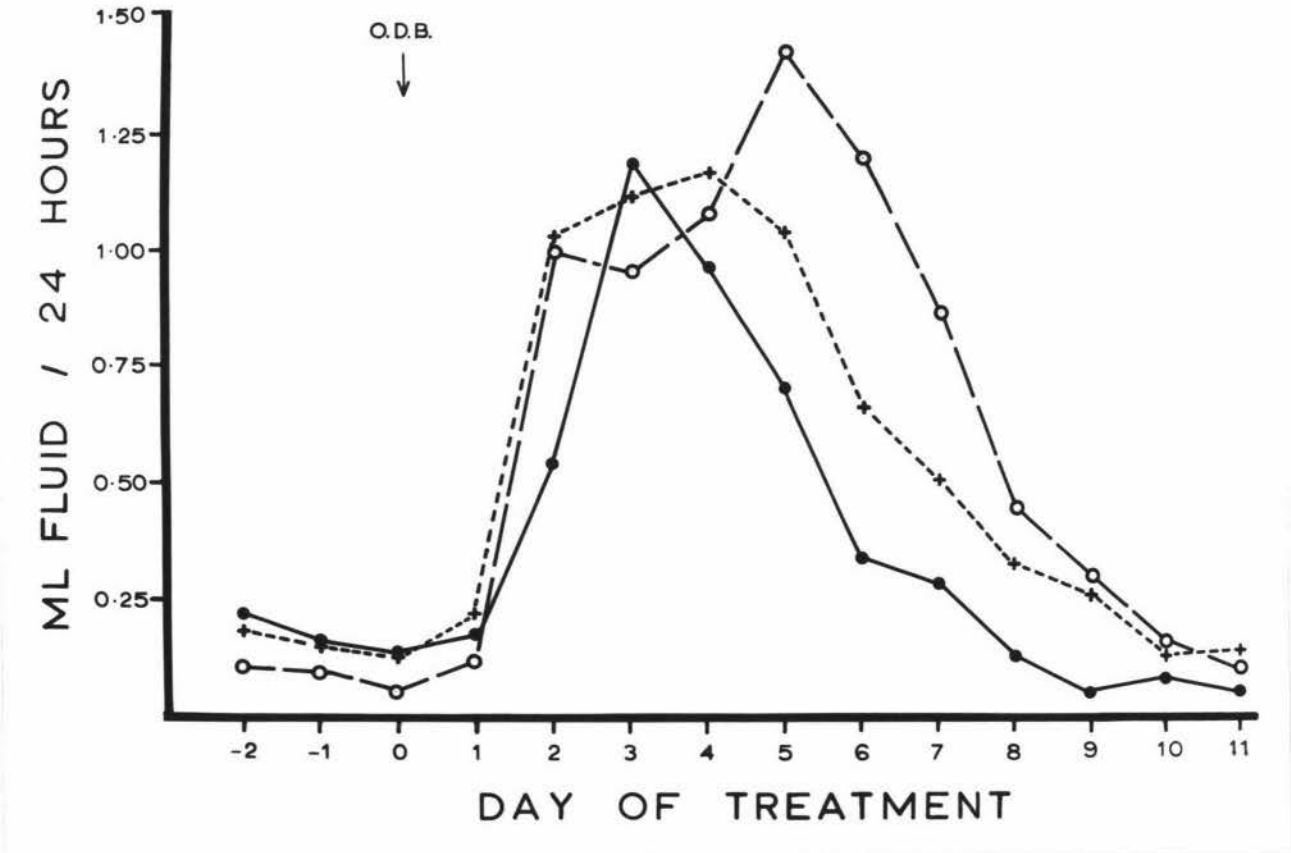
4. OESTROGEN THERAPY IN THE OVARIECTOMISED EWE

SECTION A

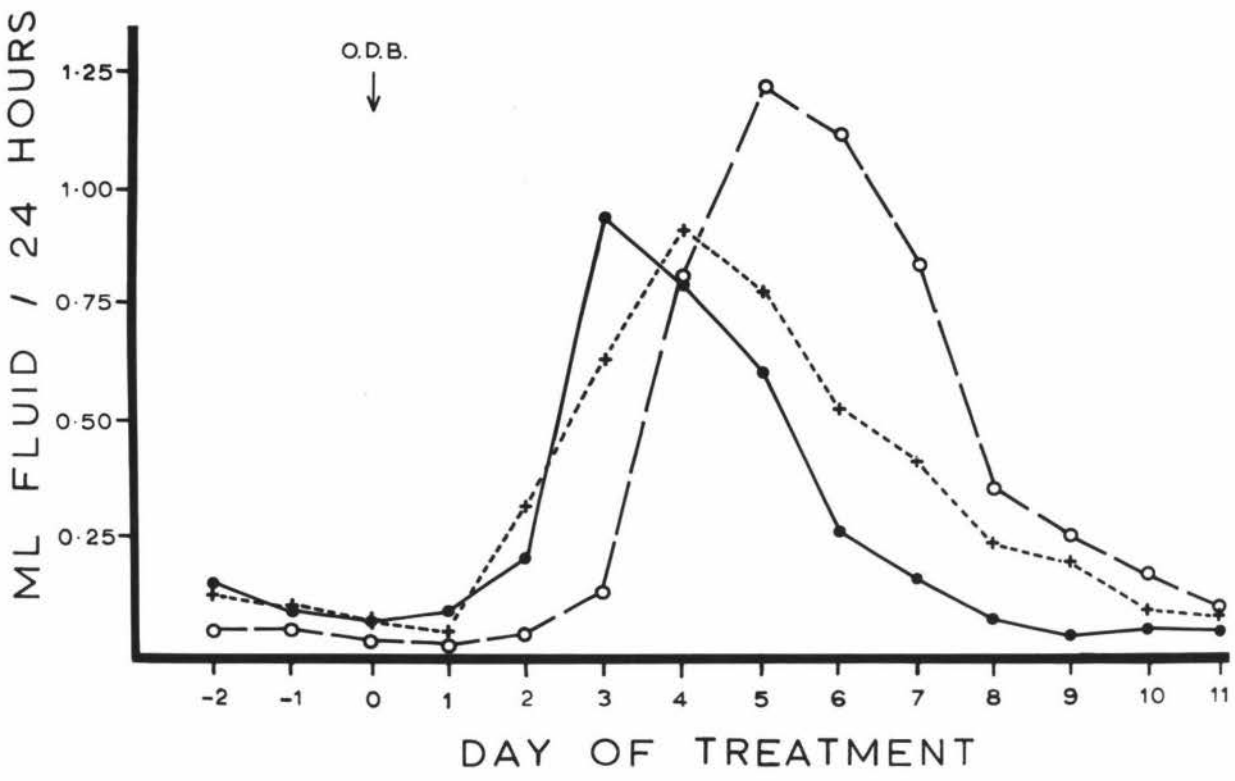
EFFECT OF DOSE LEVEL

(a) Fluid Secreted

Injection of oestradiol benzoate increased fluid secretion on 30, 90 and 500 µg dose levels. Means (± S.E.) for each dose level are given in Table 7 and graphically presented in Text-fig. 3. Fluid secretion increased from approximately 0.16 ml/24 hr in the ovariectomised pretreatment period to a maximum of 1.19, 1.18 and 1.44 ml/24 hr following the injection of 30, 90 and 500 µg ODB, respectively. Maximum fluid secretion and length of response following



Text-fig. 3. Mean fluid secretion following a single intramuscular injection of oestradiol benzoate at 30 µg (●——●), 90 µg (+ ----- +), and 500 µg (O—— ——O) dose levels.



Text-fig. 4. Mean ampullar flow following a single intramuscular injection of oestradiol benezoate at 30 µg (●———●), 90 µg (+ ----- +), and 500 µg (O—— ——O) dose level.

treatment occurred at progressively later times as the dose level increased (Table 5). In both instances, however, the difference between the 90 µg and 500 µg was not substantial.

The analyses of variance (Table 6) showed a highly significant treatment x day interaction. Major differences between treatments being apparent on days 2 to 9 inclusive during which two distinct types of interaction occurred. On days 2 to 4 inclusive, a part of the treatment x day interaction was due to a change in the order of treatment means. In contrast, on days 5 to 9, the interaction is represented by differences between treatment means not occurring at other stages of the treatment period.

TABLE 5

DURATION OF RESPONSE AND TIME OF MAXIMUM FLUID SECRETION, AND FLUID FLOW OF THE OVARIECTOMISED OESTROGEN TREATED EWE

FLUID	DOSE LEVEL (μg)	DAY OF MAXIMUM FLOW (Mean ± S.E.)	DURATION OF RESPONSE (Days ± S.E.)
Fluid secretion	30	3.21 ± 0.32	7.00 ± 0.61
	90	3.90 ± 0.64	9.75 ± 0.76
	500	5.01 ± 0.81	10.45 ± 0.83
Ampullar flow	30	3.40 ± 0.54	6.96 ± 0.55
	90	4.02 ± 0.68	9.03 ± 0.88
	500	5.33 ± 0.69	10.42 ± 0.76
Isthmic flow	30	2.12 ± 0.22	5.33 ± 0.42
	90	2.26 ± 0.22	5.92 ± 0.54
	500	2.33 ± 0.35	7.25 ± 0.72

TABLE 6

SUMMARY OF ANALYSES OF VARIANCE FOR FLUID SECRETED, AND FLUID FLOW, IN THE OVARIECTOMISED AND OVARIECTOMISED OESTROGEN TREATED EWE (ml/24 hr)

SOURCE	d.f.	FLUID SECRETION M.S.	AMPULLAR FLOW M.S.	ISTHMIC FLOW M.S.
OVARIECTOMISED				
Days	14	7.71**	7.59**	4.84**
Sheep	7	5.52**	5.77**	3.21**
Error	98	.042	.010	.026
TREATMENTS				
Between treatments	2	0.618	0.191	0.123
Between days	13	1.664	0.796*	0.336**
Tr. x days	26	1.795**	0.334**	0.108*
Error	126	0.161	0.060	0.063

* $P < 0.05$ ** $P < 0.01$

TABLE 7

DAILY MEANS (± S.E.) OF FLUID SECRETED PRIOR TO, AND AFTER, OESTROGEN INJECTIONS (ml/24 hr)

Oestrogen \ Days	-2	-1	0*	1	2	3	4	5	6	7	8	9	10	11
30 µg	.22 ±.11	.15 ±.09	.14 ±.07	.18 ±.04	.55 ±.11	1.19 ±.31	.98 ±.20	.72 ±.21	.37 ±.08	.30 ±.05	.13 ±.03	.07 ±.03	.10 ±.03	.07 ±.02
90 µg	.17 ±.05	.15 ±.07	.12 ±.06	.23 ±.10	1.04 ±.62	1.12 ±.38	1.20 ±.13	1.05 ±.31	.69 ±.13	.52 ±.06	.34 ±.06	.28 ±.07	.14 ±.05	.16 ±.06
500 µg	.10 ±.20	.09 ±.10	.06 ±.02	.03 ±.01	.12 ±.03	1.01 ±.31	.96 ±.15	1.08 ±.19	1.44 ±.32	1.22 ±.23	.90 ±.22	.47 ±.14	.32 ±.10	.17 ±.03

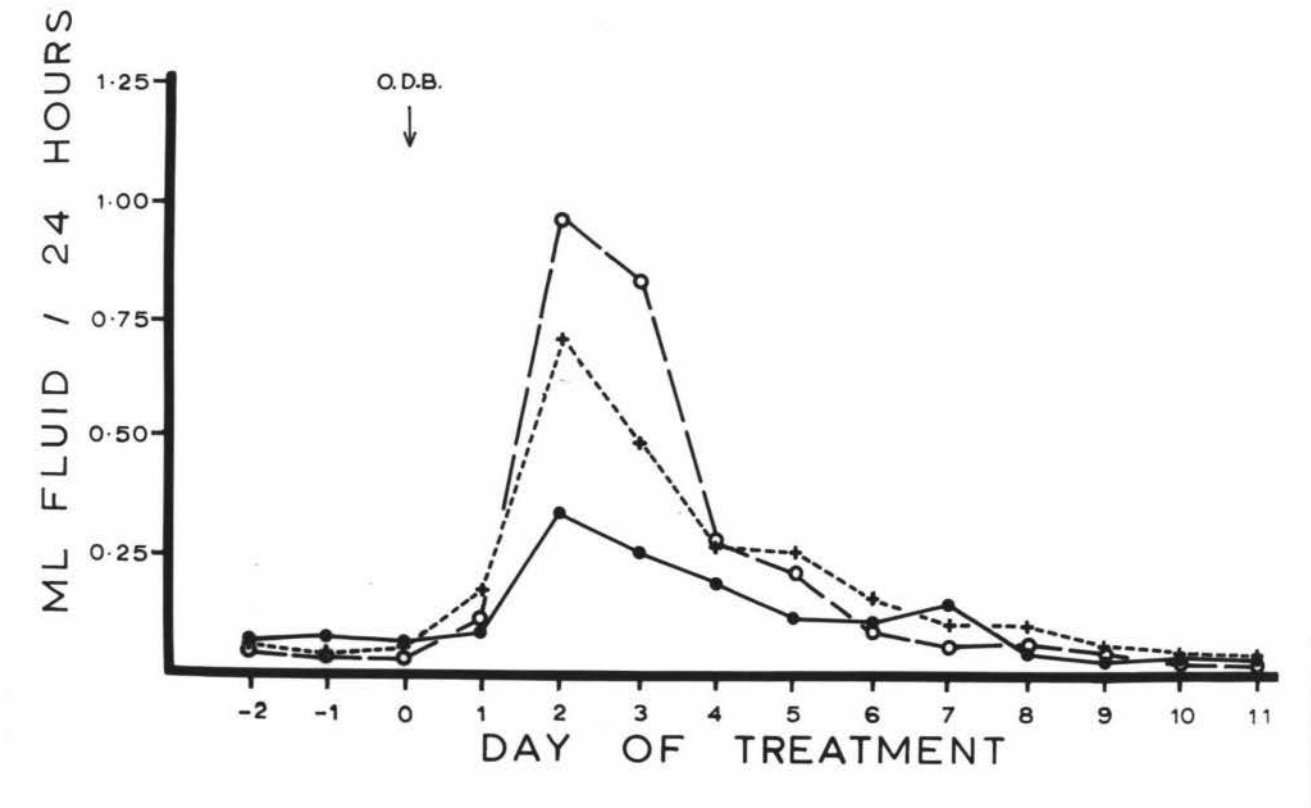
* Day 0 being day of oestrogen injection.

(b) Direction of Fluid Flow

(i) Ampullar flow

The mean increase in ampullar flow (Table 8) for each dose level of ODB is graphically presented in Text-fig. 4. Fluid flow increased from approximately 0.05 ml/24 hr during the pretreatment period to 0.94, 0.91, and 1.22 ml/24 hr on 30, 90 and 500 µg dose levels, respectively. Similar to the treatment effect on fluid secretion, time of maximum ampullar flow, and duration of response, became progressively later as the dose level increased (Table 5).

Analyses of variance (Table 6) indicated a significant variation between treatments resided within the treatment x day interaction



Text-fig. 5. Mean isthmic flow following a single intramuscular injection of oestradiol benezoate at 30 µg (●———●), 90 µg (+ ----- +), and 500 µg (O—— ——O) dose levels.

($P < 0.01$) and is mainly evident as a change in the order of treatment means within days.

(ii) Isthmic flow

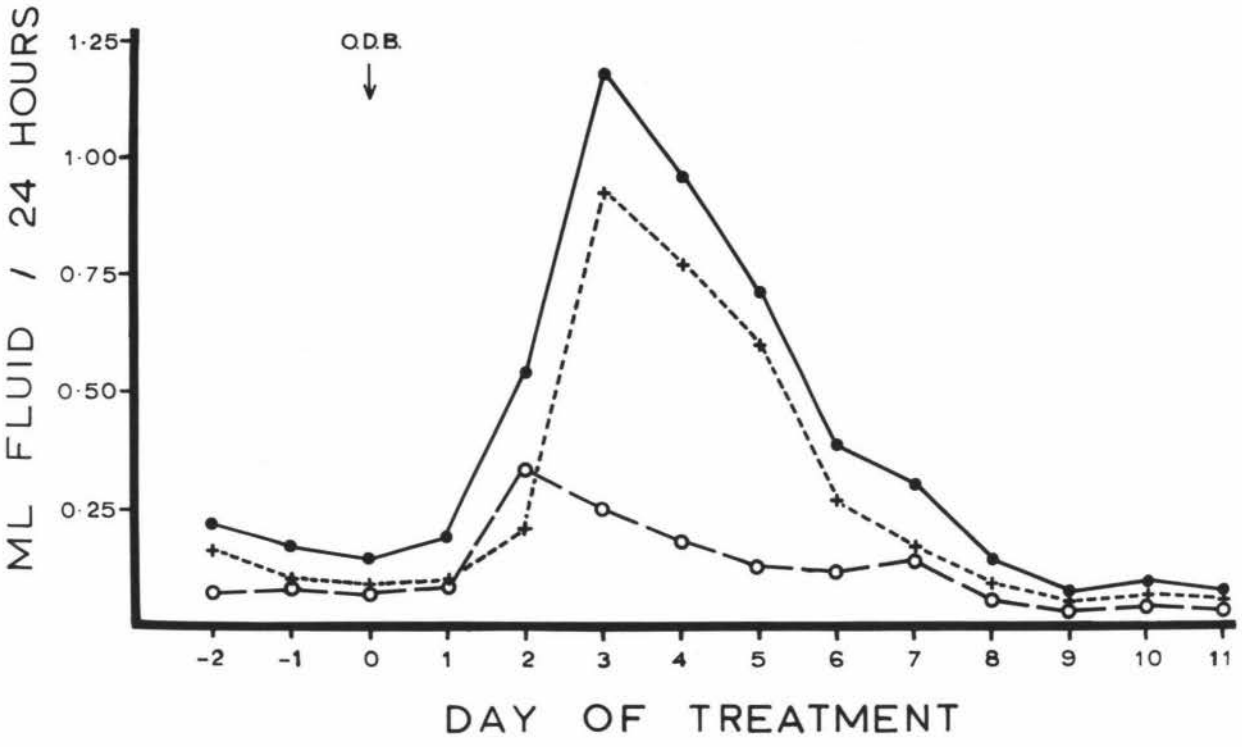
Isthmic fluid flow was also temporarily increased by treatment with ODB. From a level of 0.03 ml/24 hr in the period prior to treatment isthmic flow increased to a maximum of 0.34, 0.71, 0.97 ml/24 hr on 30, 90, and 500 µg respectively (Table 9, Text-fig. 5). In contrast to fluid secretion and ampullar flow, peak isthmic flow occurred earlier and was not markedly retarded by increased dose level. While the response declined fairly abruptly a differential effect of dose level was still measurable between 5 and 7 days after the injection (Table 5).

Differences between days, due to treatment (Table 6), were significant ($P < 0.01$). In addition, only a small proportion of total variation resides within the treatment x day interaction ($P < 0.05$) and this being due to differences between treatments occurring on some days (1 to 4 inclusive) and not on others. A change in the order of treatment means within days, a predominant component of the day x treatment interaction in fluid secretion and ampullar flow, does not occur in the data on isthmic flow.

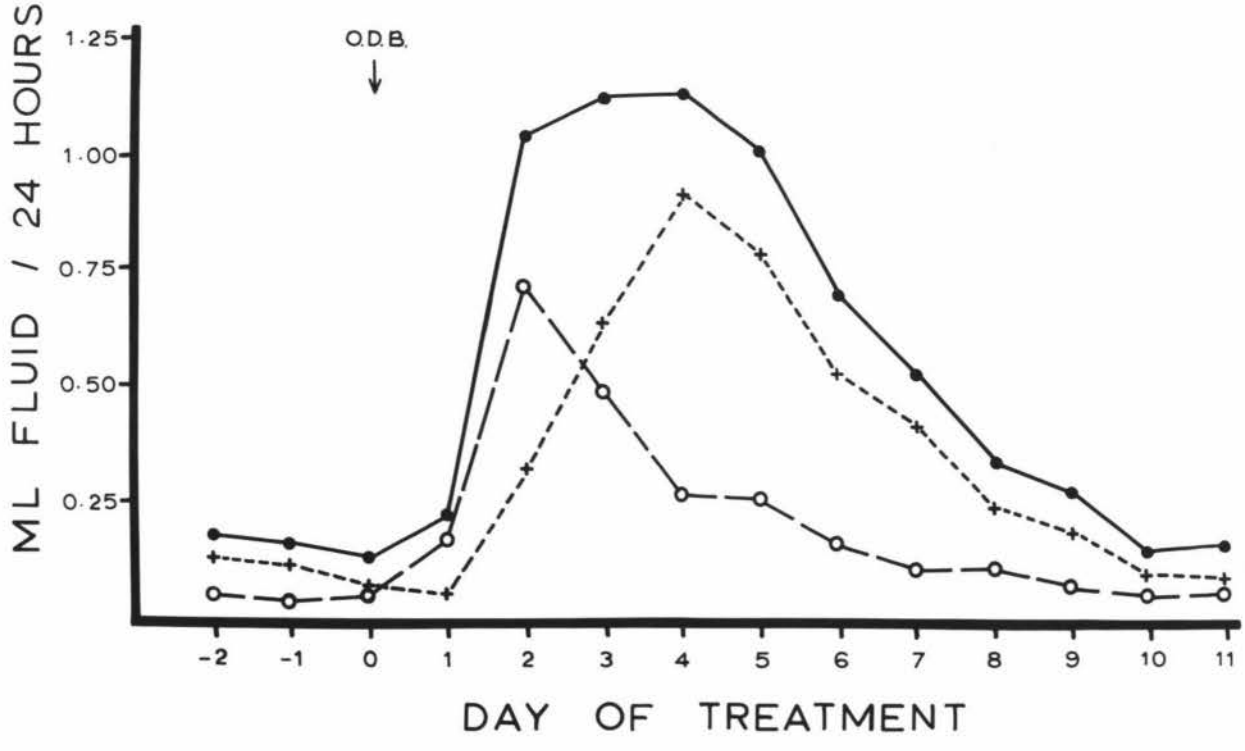
(iii) Time of peak isthmic and ampullar flow

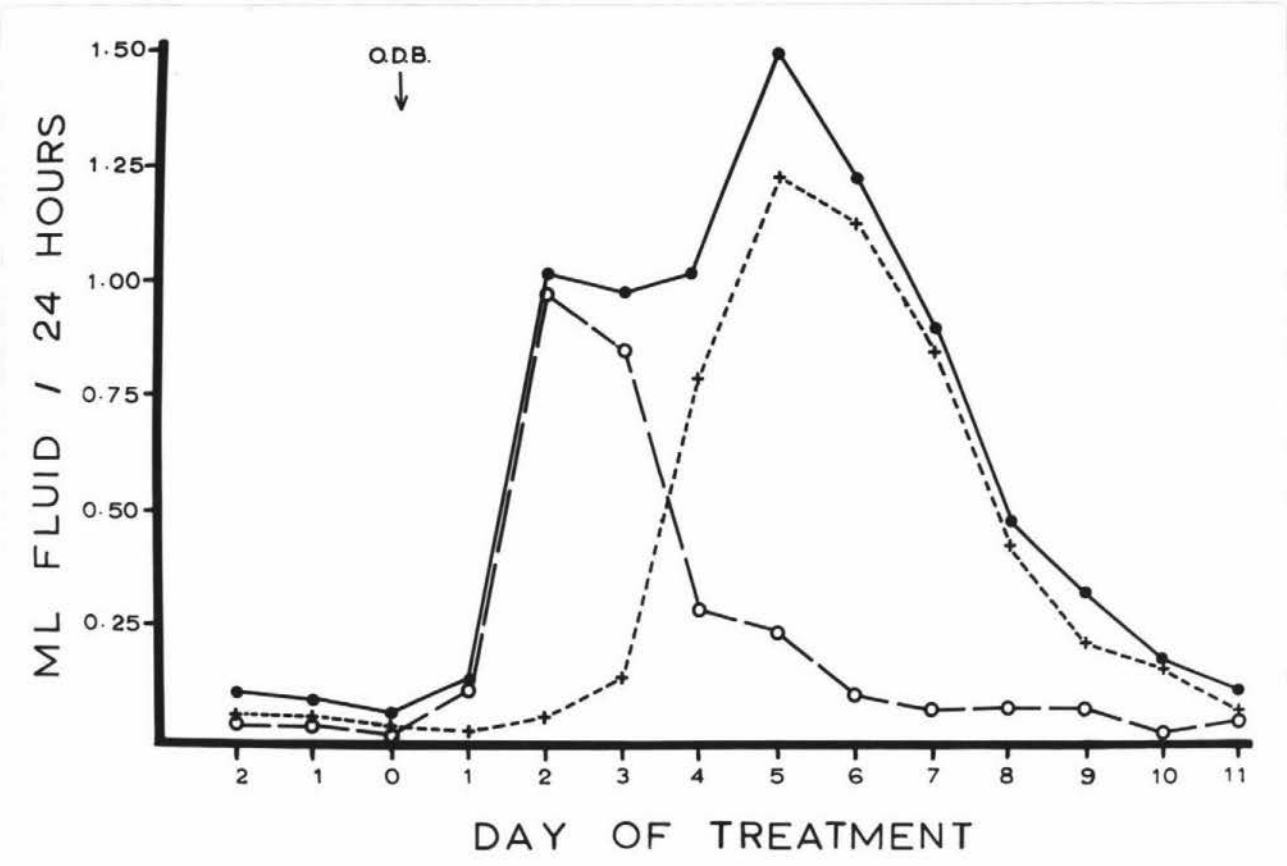
A comparison of isthmic and ampullar flow for each ODB dose level is presented in Text-fig. 6, 7, and 8, respectively.

At all dose levels maximum isthmic flow preceded that of ampullar flow. In addition, the time interval between these two peaks became greater as the dose level increased. These were 1.28 ± 0.45, 1.76 ± 0.48 and 3.00 ± 0.65 days for the 30 µg, 90 µg and 500 µg ODB, respectively (derived from Table 5).



Text-fig. 6 and 7. Mean fluid secretion (●——●), ampullar flow (+ ----- +), and isthmic flow (O—— ——O), following the injection of 30 µg ODB (above) and 90 µg ODB (below).





Text-fig. 8. Mean fluid secretion (●———●), ampullar flow (+ ----- +), and isthmic flow (O—— ——O) following the injection of 500 µg ODB.

TABLE 8

DAILY MEANS (± S.E.) OF AMPULLAR FLOW PRIOR TO, AND AFTER, OESTROGEN INJECTIONS (ml/24hr).

Oestrogen \ Days	-2	-1	0*	1	2	3	4	5	6	7	8	9	10	11
30 µg	.15 ±.04	.09 ±.03	.08 ±.04	.09 ±.04	.21 ±.07	.94 ±.30	.79 ±.22	.60 ±.19	.27 ±.06	.16 ±.05	.08 ±.02	.04 ±.02	.06 ±.02	.05 ±.02
90 µg	.12 ±.03	.12 ±.04	.07 ±.02	.05 ±.01	.32 ±.17	.63 ±.22	.91 ±.09	.78 ±.22	.53 ±.11	.42 ±.04	.24 ±.05	.19 ±.06	.09 ±.04	.08 ±.03
500 µg	.05 ±.02	.05 ±.02	.03 ±.01	.05 ±.01	.02 ±.01	.04 ±.03	.13 ±.04	.80 ±.27	1.22 ±.21	1.12 ±.26	.83 ±.23	.40 ±.15	.26 ±.12	.16 ±.03

* Day 0 being day of oestrogen injection.

SECTION B

EFFECT OF SERIAL THERAPY

Data on fluid secretion, ampullar and isthmic flow, resulting from a single injection of 90 µg ODB in experiments 1 and 111 were subjected to analyses of variance (Table 12). No significant differences were evident between the two groups. The data therefore pooled for comparison with the serial oestrogen treatment (3 x 30 µg ODB).

(a) Fluid Secretion

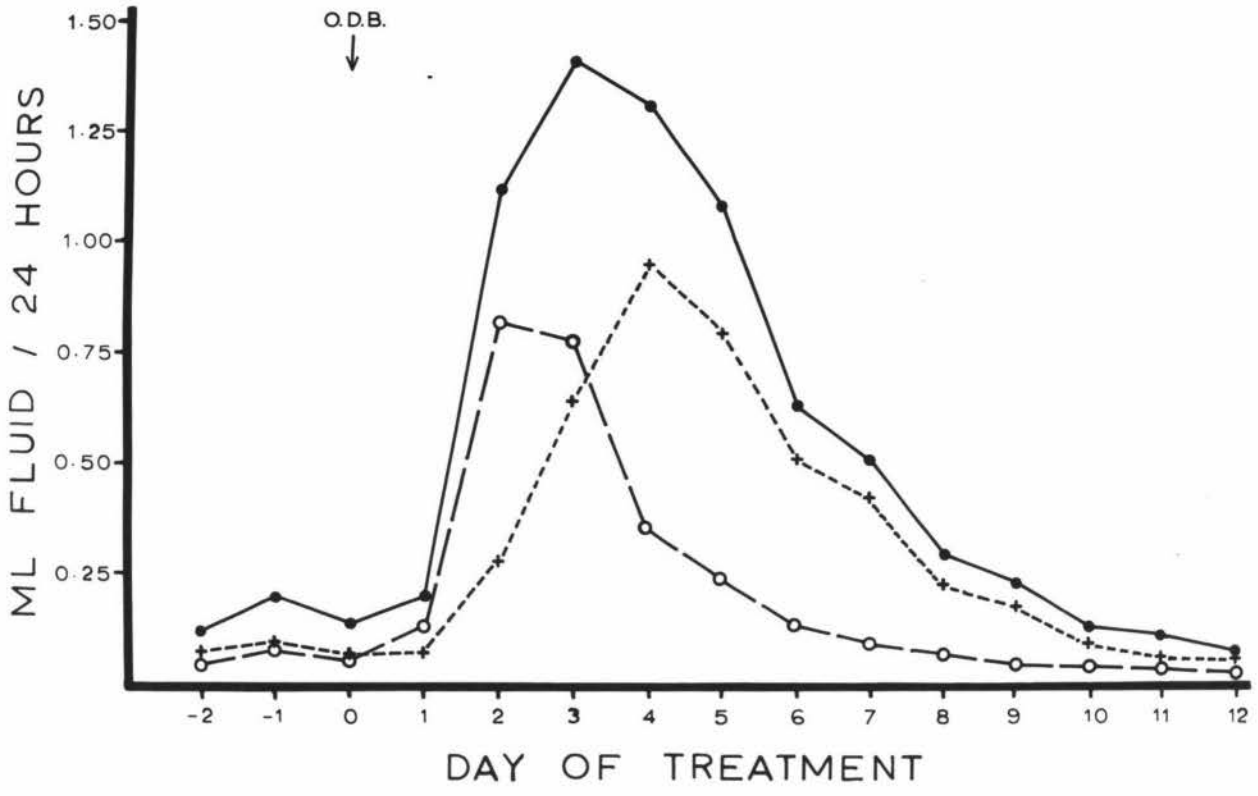
That injection of 30 µg ODB on each of three consecutive days substantially increased fluid secretion beyond that obtained from a single injection of 90 µg ODB (Table 10) is evident when comparing Text-figures 9 and 10. Fluid secretion was increased to 1.41 and 2.27 ml/24 hr with 90 µg and 3 x 30 µg doses respectively. While a difference was not evident between the treatments in the time taken to reach maximum secretion rate (Table 11), the 3 x 30 µg group maintained this high level for about 4 days. The duration of response was also extended by the serial therapy, 12.05 $\pm$ 1.42 days compared with 9.5 $\pm$ 0.63 days on the single injection.

Analysis of variance (Table 12) indicated a significant treatment x day interaction ($P < 0.01$). However, testing main effects against the interaction also showed significant variation between days ($P < 0.01$), and treatments ($P < 0.01$), in addition to the interaction effect.

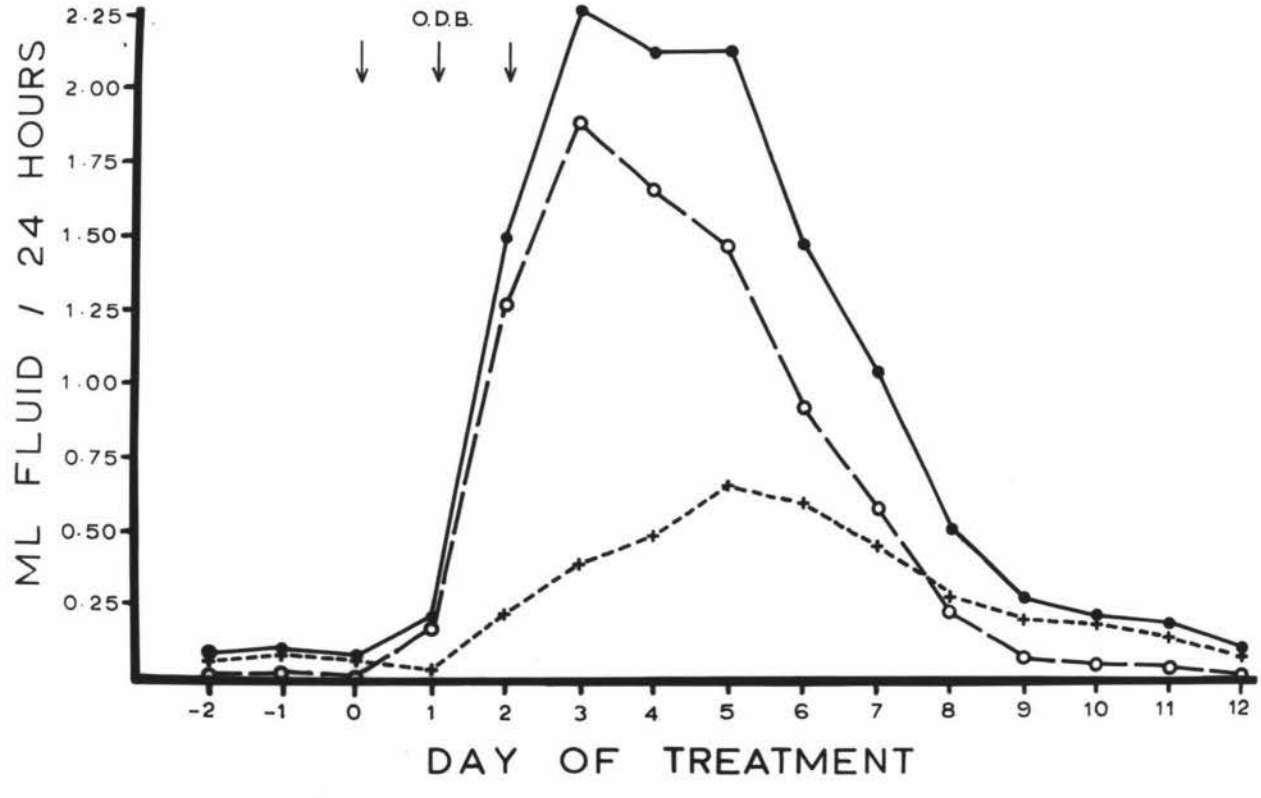
(b) Direction of Fluid Flow

(i) Ampullar flow

This source of fluid was increased by both treatments, the between days mean square being highly significant (Table 12). While a difference in total flow over the duration of each treatment was



Text-fig. 9 and 10. Mean fluid secretion (●——●), ampullar flow (+ ----- +), and isthmic flow (O—— ——O), following a single injection of 90 µg ODB (above) and 30 µg ODB on each of 3 consecutive days (below).



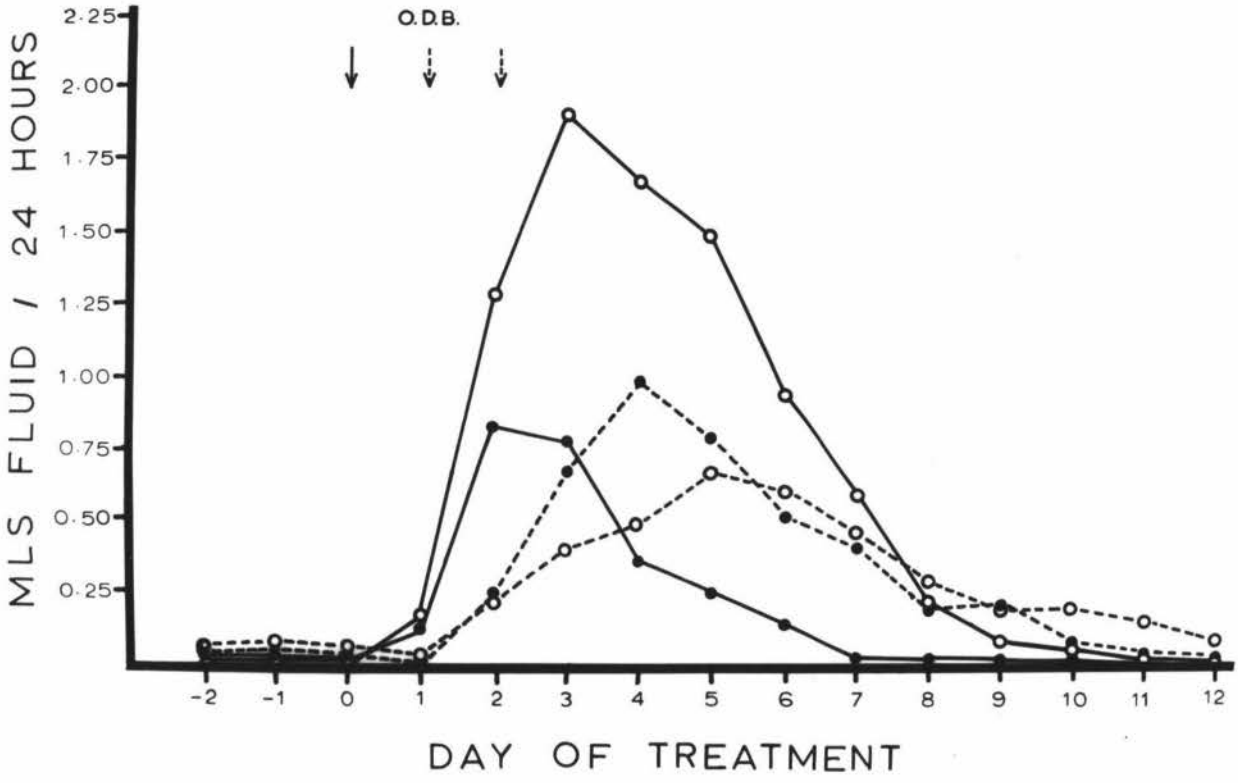
not significant, a difference in the time of peak flow, 4.01 and 5.45 days for the 90 µg and 3 x 30 µg dose levels respectively, is partly evident from the treatment x day interaction ($P < 0.01$) with major differences being confined to days 3 and 4.

(ii) Isthmic flow

Both treatments increased isthmic flow (between days, $P < 0.05$) but in contrast to the single injection of 90 µg ODB, serial injection caused a substantial increase, being 0.81 and 1.88 ml/24 hr respectively (Text-fig. 11). Peak isthmic flow and the duration of response was also prolonged by the serial treatment. This is again evident, by analysis, as a difference between treatments ($P < 0.05$) beyond the treatment x day interaction ($P < 0.01$).

(iii) Time of peak isthmic and ampullar flow

Single, or serial, injection of 90 µg ODB caused peak isthmic flow to precede peak ampulla flow by 1.85 ± 0.38 and 2.43 ± 0.46 days, respectively. Despite the greater fluid secretion following serial therapy, ampullar flow was only a minor portion with the greater quantity coming through the isthmic cannulae. The single injection of 90 µg ODB, while inducing fluid secretion to a lesser extent, had a greater ampullar flow than that resulting from sequential therapy.



Text-fig. 11. Comparison of ampullar flow (broken lines) and isthmic flow (continuous lines) after the administration of 90 µg ODB as a single injection on day 0, (——●——) or serial injection on days 0, 1 and 2 (——○——).

TABLE 10

DAILY MEANS (± S.E.) OF FLUID SECRETION, AND FLUID FLOW, FOR 90 µg ODB (ml/24 hr).

SINGLE INJECTION*

DAYS / FLUID SOURCE	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12
Ampullar flow	.068 ±.02	.10 ±.03	.07 ±.04	.07 ±.03	.29 ±.08	.64 ±.10	.95 ±.06	.79 ±.10	.51 ±.06	.41 ±.03	.23 ±.03	.18 ±.03	.09 ±.02	.06 ±.03	.06 ±.02
Isthmic flow	.05 ±.02	.09 ±.03	.07 ±.03	.12 ±.05	.81 ±.26	.77 ±.21	.35 ±.10	.24 ±.06	.13 ±.05	.09 ±.03	.07 ±.03	.05 ±.02	.04 ±.02	.04 ±.02	.03 ±.03
Fluid secretion	.11 ±.03	.19 ±.05	.13 ±.05	.19 ±.05	1.11 ±.33	1.41 ±.26	1.30 ±.10	1.03 ±.16	.63 ±.07	.51 ±.04	.30 ±.04	.23 ±.04	.13 ±.03	.11 ±.04	.08 ±.03

SERIAL INJECTION+

Ampullar flow	.08 ±.03	.09 ±.02	.07 ±.02	.04 ±.01	.22 ±.05	.39 ±.08	.50 ±.09	.66 ±.08	.60 ±.16	.47 ±.06	.29 ±.05	.21 ±.04	.19 ±.03	.15 ±.03	.09 ±.02
Isthmic flow	.01 ±.004	.01 ±.005	.01 ±.005	.17 ±.06	1.28 ±.15	1.88 ±.52	1.66 ±.17	1.47 ±.32	.89 ±.22	.59 ±.17	.24 ±.07	.08 ±.03	.04 ±.02	.04 ±.02	.01 ±.006
Fluid secretion	.08 ±.03	.10 ±.02	.08 ±.02	.21 ±.06	1.50 ±.19	2.28 ±.20	2.13 ±.22	2.13 ±.15	1.48 ±.19	1.06 ±.18	.52 ±.08	.28 ±.05	.22 ±.03	.18 ±.03	.10 ±.02

* 90 µg ODB as a single injection on day 0

\+ 90 µg ODB as 30 µg injections on days 0, 1 and 2.

TABLE 11

DURATION OF RESPONSE AND TIME OF MAXIMUM FLUID SECRETION, AND FLUID FLOW, FOLLOWING REPEATED OESTROGEN TREATMENT

SOURCE	DOSE LEVEL (µg)	DAY OF MAXIMUM (Mean ± S.E.)	DURATION OF RESPONSE (Days ± S.E.)
Fluid secretion	90	3.72 ± 0.45	9.50 ± 0.63
	3 x 30	3.58 ± 0.92	12.05 ± 1.42
Ampullar flow	90	4.01 ± 0.38	9.38 ± 0.72
	3 x 30	5.45 ± 0.72	11.59 ± 1.20
Isthmic flow	90	2.16 ± 0.19	5.80 ± 0.35
	3 x 30	3.02 ± 0.26	8.66 ± 0.75

TABLE 12

SUMMARY OF ANALYSES OF VARIANCE FOR FLUID SECRETED, AND FLUID FLOW, IN THE OVARIECTOMISED EWE FOLLOWING SINGLE, AND SEQUENTIAL, OESTROGEN THERAPY.

SOURCE	d.f.	FLUID SECRETED M.S.	AMPULLA FLOW M.S.	ISTHMIC FLOW M.S.
90 µg (Expt. 1) v. 90 µg (Expt. 11)				
Between groups	1	0.004	0.001	0.010
Between days	13	1.846**	0.710**	0.552**
Group x days	13	0.074	0.003	0.065
Error	84	0.140	0.026	0.080
90 µg (pooled) v. 3 x 30 µg				
Between treatments	1	5.558**	0.052	6.6457*
Between days	14	5.619**	0.874**	2.512*
Treatment x days	14	0.573	0.074	0.947
Error	180	0.109	0.022	0.072

* $P < 0.05$ ** $P < 0.01$

DISCUSSION

Fluid secretion

The volume of fluid secreted into the lumen of the Fallopian tube declined markedly following ovariectomy thus confirming previous reports with the rabbit (Bishop 1956; Mastroianni, Beer, Shah & Clewe, 1961a) and the ewe (Restall, 1966b). The initial rapid decline through to day 6 tended to parallel the decline which occurred with the rabbit following ovariectomy (Mastroianni et al., 1961b), but is partly obscured by possible post surgery effects on days, 1, 2 and 3.

Subsequent to day 14, mean fluid secretion varied between .05 and .09 ml/24 hr which, on preliminary evidence, appears to be one quarter to one third of that secreted by the intact anoestrous ewe. Since ovariectomy did not eliminate fluid secretion indicates that some portion of the secretion is not under ovarian control. This fraction could be a transudate as suggested by Bishop (1956) since few secretory cells are present in the Fallopian tube of the ovariectomised rabbit (Borell et al., 1956) and ewe (Restall, 1966b).

The low quantity of fluid secreted in the ovariectomised ewe could be brought to a level commensurate with that of normal oestrus by the injection of 30 µg of oestradiol benzoate. This stimulation of fluid secretion by oestrogen therapy in the ovariectomised animal has been reported previously (Courier, 1925; Bishop, 1956; Mastroianni et al., 1961b and Restall, 1966b). However, with the Australian Merino, 30 µg of ODB failed to give a response equal to that of the intact oestrus ewe (Restall, 1966b) but between animal variation in response would be sufficient to account for the difference.

Higher levels of oestrogen did not cause any further substantial increase in fluid secretion. Thus 90 µg and 500 µg ODB induced only a slight increase over that obtained from 30 µg. In contrast, serial

injection of 90 µg ODB caused a considerable increase over that obtained from a single injection of the same quantity. This effect is presumably due to the prolonged stimulation of the basal cell population, and subsequent differentiation, leading to a greater population of secretory cells (Bertalanffy & Lau, 1963; Bullough, 1965). It is equally possible that the prolonged response obtained with 90 µg and 500 µg ODB, compared with 30 µg, may have been due to the slower absorption of oestrogen as the volume of hormone and vehicle increased with the higher dosages.

Direction of fluid flow

Considerable variation between animals in the direction of fluid flow during the period immediately after the operation obscured any definite changes incurred by ovariectomy. However, the variation is itself sufficient to demonstrate that the direction of flow need not be normal following surgery and several days may be required before normal flow is resumed. The eventual effect of ovariectomy on fluid flow is also obscured by the rapid concurrent decline in fluid secretion. It is therefore difficult to decide whether the significant decline in isthmic flow is due to a failure of the mechanism which decides the direction of flow or the low quantity of fluid present at this time.

The volume of fluid in the ampullar flow, following a single injection of 30 or 90 µg ODB, approximated that obtained with the intact animal while the response to 500 µg ODB appeared to be somewhat excessive. Isthmic flow was also substantially increased by the higher levels of oestrogen, this being particularly so with serial therapy. It is clear from the results that the oestrogen treatments undertaken did not lead to the sequence of fluid flow as it occurred in the normal intact oestrous ewe (Text-fig. 1). The sequence of maximum flow from each source being completely reversed by oestrogen therapy and further accentuated by each increase in dose level. Furthermore, serial oestrogen

therapy grossly accentuated this difference in that the duration of isthmic flow was so extended as to offset any comparable increase in ampullar flow.

CHAPTER FOUR

THE EFFECT OF OESTROGEN-PROGESTERONE SEQUENTIAL THERAPY ON THE DIRECTIONAL FLOW OF TUBAL SECRETIONS IN THE OVARIECTOMISED EWE

CONTENTS OF CHAPTER

A. INTRODUCTION

The role of progesterone, and its interactions with oestrogens, in regulating the tubal transport of ova and spermatozoa has been the subject of few investigations. Furthermore, interpretation of early observations is problematical being in part confused by experiments where endogenous ovarian hormones are supplemented by exogenous hormones. It is inevitable that the administration of progesterone to the entire animal must supplement endogenous progesterone secreted by corpora lutea and synergise or antagonise the action of follicular oestrogen. The inference being that such treatment must be regarded as a joint action between the hormones and not an effect due to one alone. This point is exemplified by the postulates of Wislocki & Snyder (1933), Robinson (1951b), and Willet (1953). That accelerated ovum transport occurred following induced or super ovulation was common to each observation. But whether this was caused by an increased progesterone output from a larger number of corpora lutea or excessive oestrogen due to greater follicular activity cannot be determined. It is apparent then that the hormonal situation underlying the imposed treatment needs consideration.

1. Progesterone therapy

In the progesterone dominated pseudo-pregnant rabbit transport of both spermatozoa and ova is hindered. This is particularly so during days 5 to 12 after the onset of pseudo-pregnancy (Austin, 1949). In contrast, the injection of 2 mg progesterone (Chang & Bedford, 1961) and 10 mg or 50 mg (Black & Asdell, 1959) on each of three days preceding ovulation, or a single injection of 25 mg 5 hr *post coitum* (Greenwald, 1961a), enhanced the rate of ovum transport in the intact rabbit.

Observations with the ovariectomised animal show that progesterone

retards transport (Adams, 1958) being comparable to that occurring in the untreated ovariectomised animal (Harper, 1964). It is interesting that these results parallel those obtained with the pseudo-pregnant rabbit since oestrogen is virtually absent in both circumstances.

2. Oestrogen-progesterone therapy

Enucleation of all corpora lutea in the rabbit caused a proportion of ova to enter the uterus at an accelerated rate (Adams, 1965a, 1965b). But, leaving two corpora lutea intact was sufficient to counteract this accelerating effect of follicular oestrogen so that the limited progesterone released enforced a 'normal' rate of transport. These observations confirmed the similar but more variable results reported by Corner (1928). Similarly, the presence of newly formed corpora lutea ensured normal ovum transport in the pseudo-pregnant doe (Austin, 1949).

Austin considered an optimal balance between the two hormones was necessary for normal transport. This balance would appear to be a dynamic one since several studies have stressed the role of decreasing oestrogen and increasing levels of progesterone to provide normal transport (Wimpfheimer & Feresten, 1939; Anderes, 1941; Alden, 1942). Hartman (1939) also emphasised that progesterone diminishes oviducal contractions following ovulation so that ova are swept rapdily into the uterus at the appropriate time.

Interactions between oestrogen and progesterone have been studied using constant and simultaneous doses of each hormone (Greenwald, 1961a; Harper, 1964) and variable doses in sequential therapy (Harper, 1965a). Greenwald injected 10 µg ODB with either 1 mg or 100 mg of a progestational compound (Delalutin) into intact rabbits on 4 days subsequent to coitus. Both treatments led to 'tube locking' as occurred with a similar treatment where ODB was given alone (refer page 33).

With the ovariectomised rabbit, Harper (1964), observed the tubal

passage of artificial ova following the injection of fixed doses of oestrogen and progesterone. As these constant dosages did not induce normal transport he subsequently compounded the treatments to simulate the physiological hormonal situation after ovulation (Harper 1965a). Each rabbit received a preparatory treatment of 2.0 µg ODB/day for 3 days then 2.0 µg ODB and 0.5 mg progesterone/ rabbit on the 4 th day as the spheres were inserted. Transport of the spheres within the first 24 hr was comparable to that in the normal intact animal. But when the preparatory treatment was followed by varied doses of progesterone, subsequent passage was not normal. Of the spheres which reached the isthmic ampullar junction only a small proportion eventually entered the uterus.

As reported in the previous chapter treatment with oestrogen failed to promote the normal flow of tubal fluid. It was therefore considered necessary to investigate the effects of sequential therapy with progesterone and oestrogen. Optimistically it was thought that such treatment might lead to a sequence of fluid flow similar to that occurring in the intact oestrous ewe.

B. METHODS AND MATERIALS

1. Animals

Ten 5 and 6 year old Romney ewes were used during January, 1967. Each animal was ovariectomised and tubal fluid collected by procedures indicated in the previous chapter. Vaginal smears were taken daily and stained (Shorr, 1940) for the presence of cornified epithelial cells.

Fluid was collected daily at 8.30 a.m. and 8.30 p.m.

2. Treatments

A suitable period was allowed between ovariectomy and the start of treatment. Two ovariectomised animals were left untreated and

acted as controls. The remaining 8 animals were given intramuscular injections of 10 mg progesterone daily for 8 days as a preparatory treatment. On the day following the last injection (designated as day 0) these animals were divided into two equal groups, those in one group receiving 30 µg ODB, and the other group 90 µg ODB, as a single intramuscular injection. No further treatment was given until day 4. At this time two animals were randomly selected from each of the two groups and again treated with progesterone. This consisted of 5 mg progesterone on day 4 and 10 mg on each of 8 succeeding days.

Hormone injections were given following the morning collection of fluid. The ODB was prepared and administered as outlined in the previous chapter. Progesterone (British Drug Houses Ltd) was dissolved in peanut oil at a concentration of 2 mg/ml.

3. Statistical analysis

To enable separation of treatment effects the data were considered in three sections, each being independently subjected to analyses of variance. Data on fluid secreted, and the two parameters of fluid flow, were collated for the 8 days of preparatory treatment; the analyses of variance thereby testing responses due to daily constant doses of progesterone. Similarly, analyses of variance of data from days 1, 2, 3 and 4 detected differences which occurred between the two ODB dose levels after treatment with progesterone. Data subsequent to day 4 tested possible effects of progesterone when following oestrogen therapy. In each case main effects were tested against the components of significant first-order interactions.

C. RESULTS

The cannulation and collection of tubal fluid was successful in all animals for the duration of the experiment (20 days).

The occurrence of leucocytes as a major cellular component of the

vaginal smear, and the absence of cornified epithelial cells, indicated treatment with progesterone was successful. Similarly, oestrogenic domination following oestrogen therapy was confirmed by the presence of cornified epithelial cells; each animal was classified as having a 'positive oestrous response' (Robinson & Moore, 1956b).

For convenience each phase of treatment, preparatory progesterone, and sequential therapy with oestrogen and progesterone, will be discussed separately. However, means (± S.E.) of fluid secreted and fluid flow for the full course of treatment are given in Table 13 while a summary of the analyses of variance for each phase is presented in Table 14.

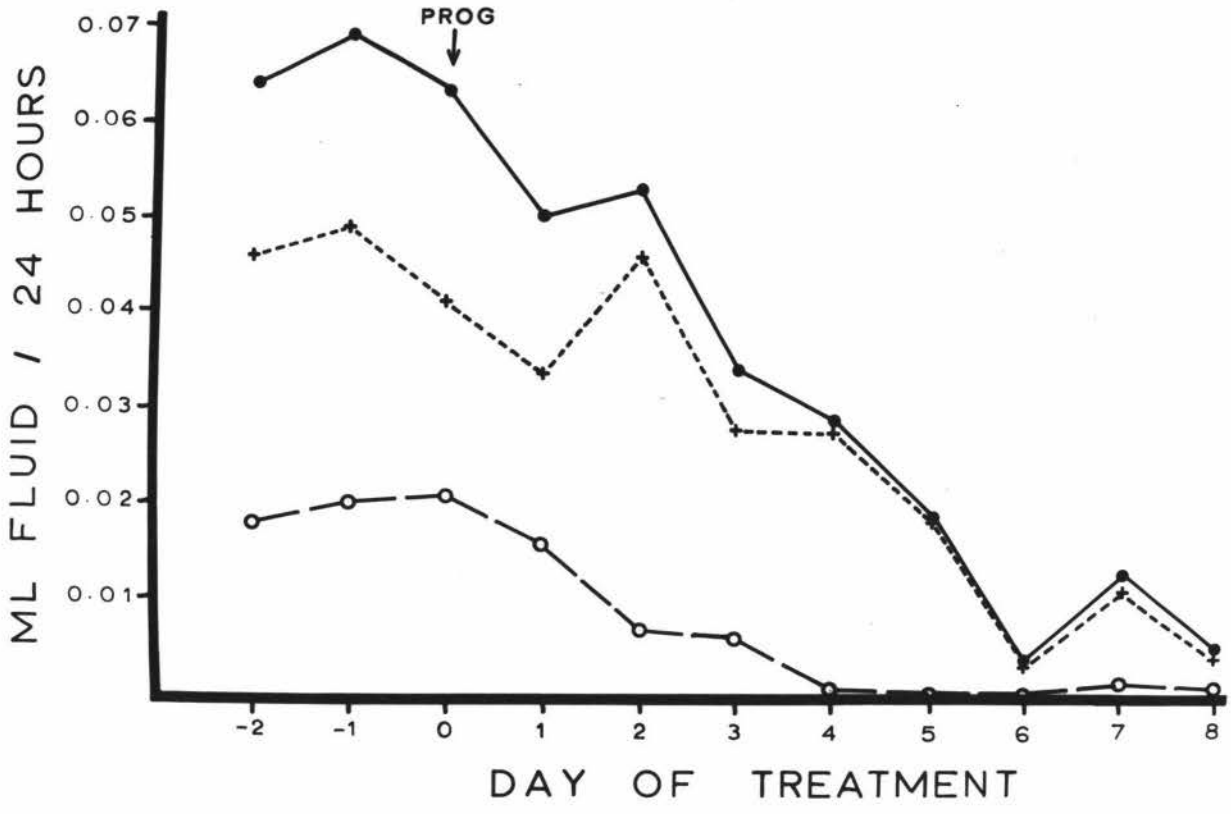
1. PREPARATORY PROGESTERONE

Mean fluid secretion, and fluid flow, are presented graphically in Text-fig. 12. Analyses of variance of the data showed that a significant decline ($P < 0.01$) in fluid secretion had occurred during the treatment period. This decline was evident even though considerable variation existed between animals ($P < 0.01$). The within animal variation, and the extremely low volume of both ampullar and isthmic flow, prevented any statistical confirmation of their downward trend.

2. SEQUENTIAL THERAPY

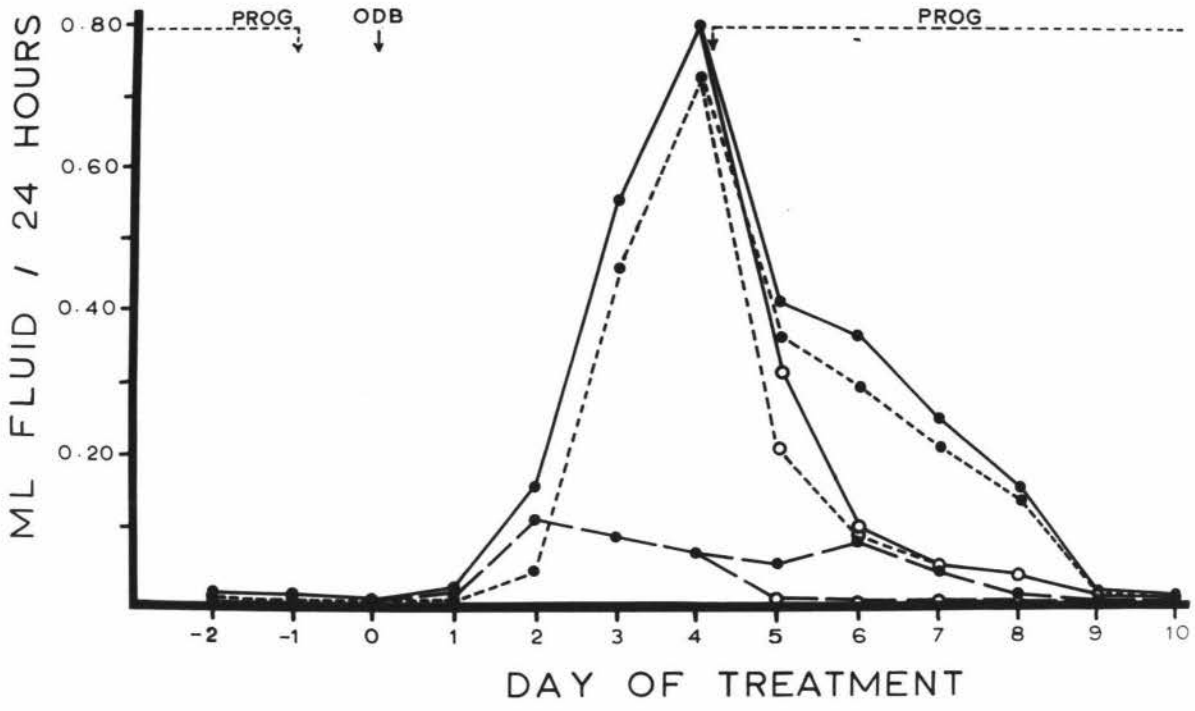
(a) Fluid secretion

Following the course of preparatory progesterone, treatment with ODB at 30 µg (Text-fig. 13) and 90 µg (Text-fig. 14) increased the level of fluid secretion to 0.80 and 0.72 ml/24 hr respectively. While the increase on both treatment was significant ($P < 0.01$) no difference was apparent in the total quantity of fluid secreted over days 1, 2, 3 and 4. Subsequent to day 4 a decline in fluid secretion occurred (days, $P < 0.01$) comparable on both dose levels of ODB and independent of the concurrent treatment with progesterone. However, the decline in



Text-fig. 12. Fluid secreted (●——●), ampullar flow (+ ----- +), and isthmic flow (O—— ——O) during preparatory treatment with daily doses of progesterone (10 mg) commencing on day 0.

fluid secretion was further enhanced by the daily injection of progesterone ($P < 0.01$). This effect of progesterone is again indicated by a reduction in the 'duration of response' to ODB (Table 15) in recipient animals.



Text fig. 13 and 14. Effect of 30 µg ODB (above) and 90 µg ODB (below) on fluid secretion (●——●), ampullar flow (●----●) and isthmic flow (●— —●), when preceded by progesterone therapy. The effect of daily injections of progesterone (——O——) and no progesterone (——●——) commencing on day 4 of treatment, is also shown.

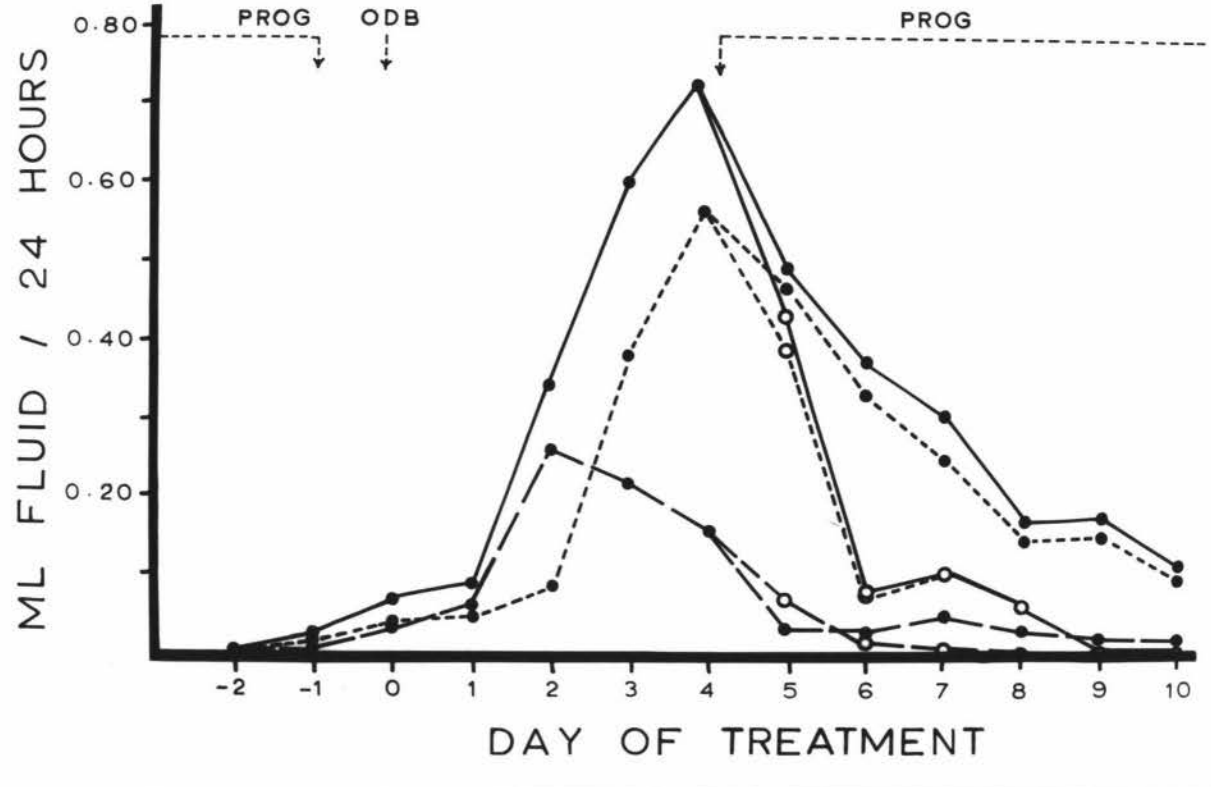


TABLE 13

MEAN ± S.E. OF FLUID SECRETED AND FLUID FLOW DURING SEQUENTIAL HORMONE THERAPY (ml/24 hr).

Treatment	Preparatory Progesterone (10mg daily)								Oestrogen		No Treatment			Progesterone (0mg or 10mg daily)						
Days / Fluid	1	2	3	4	5	6*	7*	8*	Dose µg	1	2	3	4	Dose mg	5	6	7	8	9	10
Ampullar flow	.04 ± .004	.04 ± .003	.05 ± .003	.03 ± .02	.03 ± .01	.02 ± .01	.01 ± .001	.01 ± .001	30	.01 ± .002	.04 ± .01	.46 ± .11	.74 ± .26	0	.37 ± .09	.30 ± .10	.22 ± .08	.15 ± .05	.05 ± .02	.01 ± .005
														10	.22 ± .07	.10 ± .03	.05 ± .01	.05 ± .02	.01 ± .005	- -
									90	.04 ± .01	.09 ± .02	.38 ± .14	.56 ± .19	0	.46 ± .15	.34 ± .14	.25 ± .12	.15 ± .07	.16 ± .05	.09 ± .03
														10	.39 ± .11	.08 ± .03	.10 ± .02	.06 ± .01	- -	- -
Isthmic flow	.02 ± .01	.02 ± .01	.01 ± .005	.006 ± .003	- -	- -	- -	- -	30	.02 ± .001	.12 ± .03	.10 ± .03	.07 ± .02	0	.05 ± .02	.07 ± .02	.04 ± .02	.01 ± .005	- -	- -
														10	.01 ± .002	- -	- -	- -	- -	- -
									90	.05 ± .01	.26 ± .05	.22 ± .05	.15 ± .04	0	.03 ± .02	.03 ± .01	.06 ± .02	.03 ± .01	.02 ± .005	.05 ± .03
														10	.07 ± .02	.01 ± .005	- -	- -	- -	- -
Fluid Secreted	.06 ± .01	.06 ± .02	.05 ± .02	.03 ± .01	.03 ± .02	.02 ± .01	.01 ± .001	.01 ± .001	30	.03 ± .001	.16 ± .02	.56 ± .13	.80 ± .31	0	.42 ± .10	.37 ± .11	.26 ± .08	.16 ± .05	.05 ± .01	.01 -
														10	.32 ± .09	.10 ± .03	.05 ± .02	.05 ± .02	.01 ± .005	- -
									90	.09 ± .02	.34 ± .08	.60 ± .15	.72 ± .12	0	.49 ± .13	.36 ± .11	.30 ± .10	.17 ± .08	.18 ± .10	.14 ± .06
														10	.44 ± .11	.09 ± .03	.10 ± .04	.06 ± .02	- -	- -

* Data for days -2, -1 and 0 of figures 4.2 and 4.3 being in total the same as days 6, 7 and 8, respectively, presented above.

TABLE 14

SUMMARY OF ANALYSES OF VARIANCE FOR FLUID SECRETION AND FLUID FLOW DURING SEQUENTIAL HORMONE THERAPY (ml/24 hr)

Source	d.f.	Fluid secreted M.S.	Ampullar flow M.S.	Isthmic flow M.S.
Preparatory progesterone				
Days	9	.0043**	.0020	.0006
Sheep	7	.0047**	.0001	.0008
Error	63	.0013	.0013	.0004
Oestrogen				
Days	3	.6413**	.6651**	.0329**
Treatments	1	.0043	.0351	.0435**
Days x treatments	3	.0431	.0307	.0009
Error	24	.0144	.0153	.0041
Progesterone				
Days	5	.1570**	.1357**	.0011
Oestrogen	1	.0253	.0165	.0011
Progesterone	1	.2352**	.1599**	.0066**
Days x oest.	5	.0029	.0016	.0007
Days x prog.	5	.0095	.0086	.0007
Prog. x oest.	1	.0032	.0460	.0004
Prog. x oest. x days	5	.0060	.0009	.0002
Error	24	.0119	.0121	.0002

*P< 0.05 **P< 0.01

(b) Direction of Flow

(i) Ampullar flow

The injection of 30 µg and 90 µg ODB increased ampullar flow to a maximum of 0.74 ml and 0.56 ml/24 hr on days 4.15 and 4.38, respectively. The increase in ampullar flow was significant ($P < 0.01$) for both dose levels of ODB but a difference between them was not evident.

Ampullar flow, being the major outlet for tubal fluid, also declined after day 4 ($P < 0.01$) the rate of decline being further increased by the concurrent administration of progesterone ($P < 0.01$). During this period a significant progesterone x oestrogen interaction ($P < 0.05$) indicated an effect due to dose level of the previous ODB treatment which is not accounted for by the variance incurred by the progesterone and no progesterone treatment. That this component of variance accrues from differences occurring on day 9 and 10 between the ODB dosages is evident from a comparison of Figures 13 and 14. Thus 90 µg ODB, compared with 30 µg, considerably prolonged the 'duration' of ampullar flow in the absence of progesterone (Table 15).

(ii) Isthmic flow

Oestrogen therapy substantially increased ($P < 0.01$) isthmic flow to 0.12 ml and 0.25 ml/24 hr on the 30 µg and 90 µg dose levels, respectively. However, a real difference between the two treatments ($P < 0.01$) is apparent in that a greater volume of fluid passed through the isthmic cannula following treatment with 90 µg ODB.

The decline in isthmic flow after day 2 continued throughout the last phase of progesterone therapy causing a further decline ($P < 0.01$) in isthmic flow.

(iii) Time of peak isthmic and ampullar flow

In all animals treated isthmic flow preceded ampullar flow by 1.09 ± 0.75 and 2.09 ± 0.84 days on 30 µg and 90 µg ODB, respectively

(derived from Table 15). However, on the 30 μg ODB treatment isthmic flow was only a minor outlet for the fluid secreted. An increase in mean isthmic flow on day 6, caused by one animal in the 30 μg ODB - no progesterone group, was not significant.

TABLE 15

DURATION OF RESPONSE AND TIME OF MAXIMUM FLUID SECRETION, AND FLUID FLOW, FOR SEQUENTIAL OESTROGEN-PROGESTERONE THERAPY. PRECEDED BY PREPARATORY PROGESTERONE.

TREATMENT	DAY OF MAXIMUM (Mean ± S.E.)			DURATION OF RESPONSE (Days ± S.E.)		
	Fluid secretion	Ampullar flow	Isthmic flow	Fluid secretion	Ampullar flow	Isthmic flow
30 μg ODB	3.88±0.26	4.15±0.22	2.25±0.18	7.25±0.88	7.00±0.74	6.5 ±0.43
30 μg ODB + prog.	-	-	-	6.72±0.62	6.75±0.60	3.75±0.28
90 μg ODB	4.38±0.28	4.46±0.25	2.38±0.21	9.5 ±0.95	9.25±0.96	7.75±0.49
90 μg ODB + prog.	-	-	-	7.5 ±0.52	7.25±0.61	6.75±0.35

DISCUSSION

Fluid secretion in the Fallopian tube of the rabbit has been shown to decline with the onset of pregnancy (Bishop, 1956; Mastroianni & Wallach, 1961b). While progesterone, when given to the oestrogen primed ovariectomised rabbit (Mastroianni et al., 1961a) and ewe (Restall, 1966b), also decreased the secretion of tubal fluid. That this antagonistic action occurs when progesterone is given before or after oestrogen therapy is confirmed by the present results. However, in contrast to the findings of these authors, the administration of progesterone also depressed fluid secretion in the ovariectomised animal. It is interesting to note that a similar trend may have occurred with the rabbit (Mastroianni et al., 1961a). Since the decline was not evident in the untreated ovariectomised controls it is presumed that a part of the fluid secreted by the ovariectomised animal may be stimulated by oestrogenic compounds from extra-ovarian sources (Bulbrook & Greenwood, 1957a, 1957b).

It is evident that oestrogen therapy preceded by preparatory treatment with progesterone did not give the sequence of fluid flow which was found to occur in the intact oestrous animal (Text-fig. 1). However, in the comparison with the comparable doses of oestrogen alone (Text-fig. 6 and 7), it is equally apparent that progesterone decreased peak and total isthmic flow thereby allowing a greater proportion of the fluid secreted to pass through the ampulla orifice. If, therefore, progesterone therapy had been extended so as to merge with the injection of oestrogen a greater suppression of isthmic flow may have resulted.

An elevation of isthmic flow, expected to accrue as a result of the concluding progesterone therapy, did not eventuate. In fact, contrary to expectation, isthmic flow declined more rapidly in those

animals which received progesterone subsequent to day 3.

Since fluid secretion increased on day 16 in the intact oestrous animal (Text-fig. 1) and that progesterone levels in ovarian vien blood of the ewe are still substantial at this time (Edgar & Ronaldson, 1958) indicates that a dynamic ratio of oestrogen to progesterone occurs in the intact animal. It would appear then that the dose levels used, the sequence, and hence the changing proportion of the two hormones, did not approximate those which occurred in the intact oestrous ewe. Thus the results obtained were similar to those recorded with the injection of oestrogen alone.

CHAPTER FIVE

THE DIRECTIONAL FLOW OF TUBAL SECRETIONS IN THE ENTIRE EWE DURING SILENT OESTRUS.

CONTENTS OF CHAPTER

A. INTRODUCTION

It was found that peak isthmic flow preceded that of ampullar flow in the ovariectomised ewe following oestrogen therapy while the converse occurred in the intact oestrous animal. The mechanism which prevents a similar premature surge in isthmic flow in the intact ewe during the pre-ovulatory period, when oestrogen is in dominance, remains obscure. Sequential therapy in the ovariectomised animal indicated that progesterone, when proceding oestrogen therapy, considerably depressed isthmic flow. Thus it was surmised that in the intact oestrous animal progesterone from the corpus luteum of the previous ovulation may play a decisive role in this regulatory mechanism.

Ovulation without oestrus, silent heat, is a common occurrence with the ewe at the beginning and end of the breeding season (Grant, 1933; Millar 1935; McKenzie & Terrill, 1937). The failure to exhibit oestrous behaviour in the latter case, may be due to insufficient oestrogen (McKenzie et al., 1937), and at the beginning of the season due to the absence of a corpus luteum (Hammond & Parkes, 1942; Hammond, 1945). Although spurious ovulations can occur during anoestrum (Roux, 1936), it is evident that in most instances the first ovulation of the breeding season is not preceded by a corpus luteum.

An attempt was therefore made to obtain evidence on the direction of fluid flow from the Fallopian tube during ovulation in the intact ewe but in the absence of progesterone from a waning corpus luteum.

B. METHODS AND MATERIALS

Six 5 year old Romney ewes were used during March and April, 1967. Cannulation and collection of tubal fluid was carried out by the standard procedure. In the course of each operation the ovaries

were examined to ensure that no corpora lutea were present. During the subsequent period of fluid collection vaginal smears were taken daily and stained (Shorr, 1940) for the presence of cornified epithelial cells. Each smear was classified by the method of Robinson & Moore (1956b).

As behavioural oestrous is not exhibited during silent heat the occurrence of ovulation was initially determined by a sharp elevation in fluid secretion followed by partial or complete cornification of the vaginal epithelial cells. Fluid collection was thus continued until the approach of the third ovulatory period. At this time each animal was slaughtered and the ovaries recovered for examination.

Collation of data on fluid secretion and fluid flow presented an acute problem. Considerable variation between animals in the time at which maximum cornification of vaginal epithelial cells occurred (Robinson & Moore, 1956b) deleted this as a possibility. The time at which fluid secretion began to increase in the pre-ovulatory period of the oestrous ewe, in relation to the onset of oestrous behaviour, was found to be less variable (re - chapter 2). It was on this basis then that the data of each individual oestrous cycle of silent heat (designated "first oestrous") and subsequent heat (designated "second oestrous") were collated. Numerical allocation of days for silent heat are, therefore, only arbitrary and bear no known relation to the time of ovulation.

C. RESULTS

Cannulation and the collection of tubal fluid was successful with 5 ewes for the full experimental period (53 days). In the remaining animal fluid ceased flowing through the isthmic cannula 9 days after surgery.

At the time of operation each ewe was considered in anoestrus

since no corpora lutea or graafian follicles were evident. On concluding the experiment examination of the ovaries revealed two distinct generations of corpora lutea; from a recent and preceding ovulation. Age of the corpora lutea, as judged by morphological appearance, was comparable with that derived from data on the time at which fluid secretion increased in the pre-ovulatory period of each oestrous cycle.

The daily means (± S.E.) for fluid secreted and fluid flow during the 'first' and 'second' oestrous cycles are given in Table 16 and presented graphically in Text-figures 15 and 16, respectively.

1. Fluid Secretion

As expected the major source of variation ($P < 0.01$) was due to differences in fluid secretion which occurred between certain days in both the first and second oestrous cycle. While the level of maximum fluid secretion did not differ between the two cycles it is evident that the total volume secreted during each was greater in the first oestrous cycle ($P < 0.05$). This is evident as a later and more prolonged fluid secretion with the first oestrous. Despite this difference the interaction term was not significant.

TABLE 16

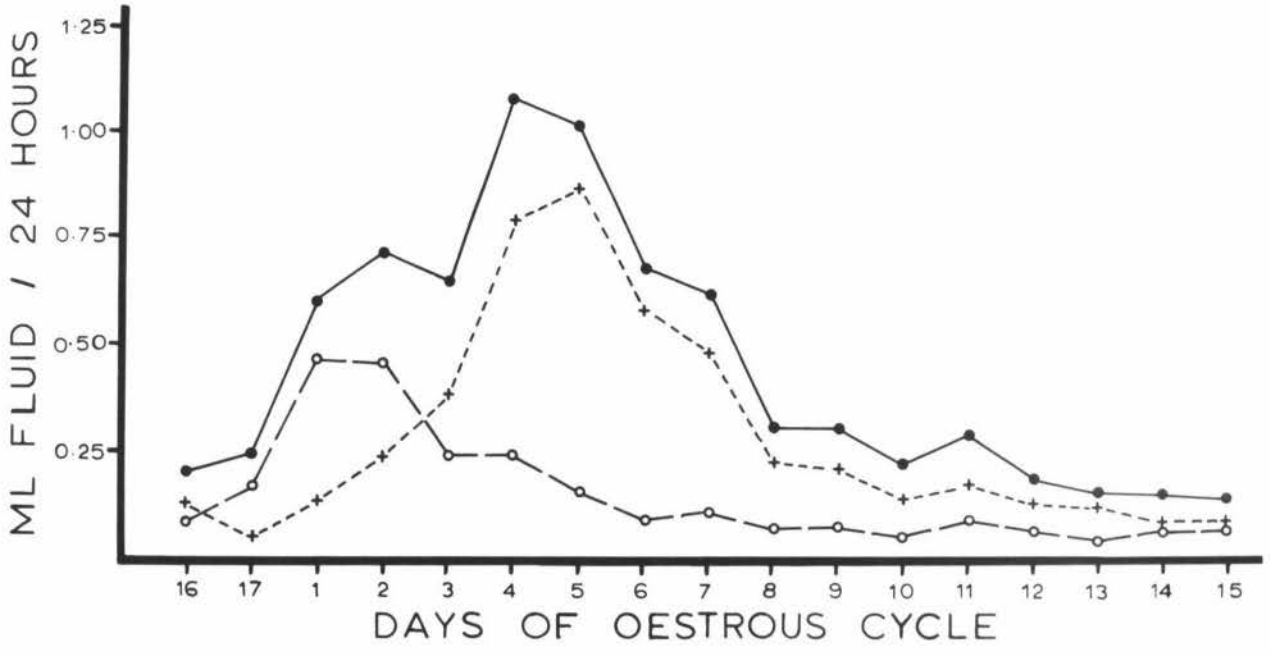
DAILY MEANS (± S.E.) OF FLUID SECRETED, AND FLUID FLOW (ml/24 hr)

'FIRST OESTROUS'

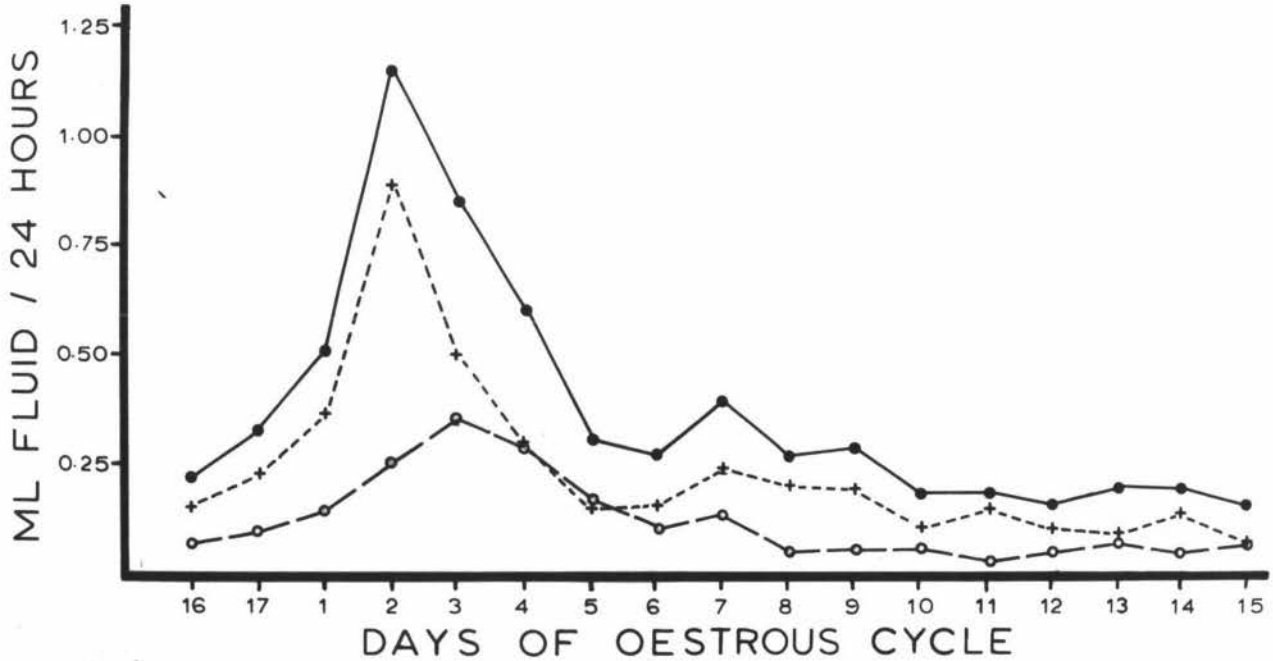
Days / Fluid Source	16	17	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ampullar Flow	.09 ±.04	.07 ±.03	.13 ±.04	.24 ±.06	.38 ±.15	.82 ±.17	.86 ±.20	.58 ±.09	.49 ±.08	.23 ±.06	.22 ±.05	.15 ±.04	.19 ±.03	.13 ±.02	.12 ±.02	.10 ±.01	.09 ±.01
Isthmic flow	.14 ±.04	.18 ±.08	.47 ±.16	.46 ±.15	.25 ±.06	.26 ±.08	.15 ±.09	.10 ±.04	.12 ±.04	.08 ±.04	.09 ±.03	.07 ±.04	.10 ±.04	.07 ±.01	.05 ±.01	.05 ±.01	.05 ±.01
Fluid secretion	.22 ±.06	.25 ±.11	.61 ±.20	.71 ±.18	.63 ±.10	1.08 ±.21	1.01 ±.24	.67 ±.25	.61 ±.09	.31 ±.08	.30 ±.09	.22 ±.06	.29 ±.08	.19 ±.03	.16 ±.03	.16 ±.03	.14 ±.03

'SECOND OESTROUS'

Ampullar flow	.15 ±.06	.22 ±.08	.37 ±.07	.89 ±.06	.50 ±.11	.29 ±.07	.15 ±.05	.16 ±.08	.25 ±.13	.21 ±.09	.22 ±.06	.12 ±.05	.15 ±.05	.11 ±.03	.10 ±.03	.15 ±.03	.09 ±.02
Isthmic flow	.07 ±.01	.10 ±.04	.14 ±.03	.25 ±.05	.35 ±.07	.29 ±.08	.16 ±.03	.11 ±.03	.14 ±.04	.06 ±.02	.07 ±.02	.06 ±.02	.03 ±.01	.06 ±.02	.08 ±.02	.05 ±.02	.06 ±.02
Fluid secretion	.22 ±.05	.32 ±.07	.51 ±.05	1.14 ±.09	.85 ±.12	.59 ±.08	.31 ±.03	.27 ±.03	.39 ±.04	.27 ±.04	.29 ±.04	.18 ±.04	.18 ±.04	.17 ±.03	.18 ±.03	.20 ±.03	.15 ±.03



Text-fig. 15 and 16. Mean fluid secretion (●——●), ampullar flow (+ ----- +) and isthmic flow (O—— ——O) during the 'first oestrous' (above) and 'second oestrous' (below).



2. Fluid Flow

The analysis of variance (Table 17) confirmed an oestrous cycle x day interaction in ampullar flow ($P < 0.01$). The interaction is readily apparent as a difference in the order of day means either side of day 4. While an interaction term is not evident in the data on isthmic flow a difference between days of both oestrous cycles was significant ($P < 0.01$).

Moreover the pattern of fluid flow differed markedly between the two oestrous cycles. In the 1st oestrous cycle peak isthmic flow preceded that of ampullar flow by 1.75 days ($\pm$ 0.95). By contrast, the converse occurred in the 2nd oestrous cycle where peak isthmic flow succeeded that of ampullar flow by 1.08 $\pm$ 0.5 days.

TABLE 17

SUMMARY OF ANALYSES OF VARIANCE FOR FLUID SECRETED AND FLUID FLOW (ml/24 hr)

Source	d.f.	Fluid secreted M.S.	Ampullar flow M.S.	Isthmic flow M.S.
Between days	16	0.728**	0.277	0.106**
1st V's 2nd Oestrous	1	0.258*	0.074	0.059
Oestrous x days	16	0.090	0.233**	0.025
Error	136	0.054	0.029	0.015

* $P < 0.05$ ** $P < 0.01$

DISCUSSION

It was ascertained that the 'first oestrous' of these experimental ewes was not preceded by a functional corpus luteum. Hence it is assumed that any differences which occurred in the 'first oestrous', when compared with subsequent oestrous cycles, is due to the absence of progesterone exuding from a waning corpus luteum. However, it is probable that other differences in the hormonal axis do exist. Because of this possibility, and the nebulous manner in which the present data was collated, the results must be considered with caution. Thus absolute values of fluid secretion and fluid flow are of little consequence. Equally so, it is only a surmise that the greater amount of fluid secreted in 'first oestrous' is due to a lower level of progesterone antagonism (Mastroianni et al., 1961a; Restall, 1966b; re - Chapter 4) andnot a greater oestrogen output. Similarly, the longer duration of response could be caused by either, a more prolonged release of oestrogen with ovulation occurring later than would be expected, or, less progesterone in circulation at that time.

Significantly however, the sequence of fluid flow is markedly different between the two cycles. In the 'first oestrous', where peak isthmic flow preceded that of ampullar flow, the sequence is the same as that recorded in the oestrogen primed ovariectomised animal (Text-figures 6 and 7) indicating that the results from oestrogen therapy are not entirely divorced from reality. In contrast the sequence of fluid flow in the 'second oestrous' is similar to that which occurred in the normal oestrous animal (Text-fig. 1).

It is possible then that progesterone emanating from the waning corpus luteum of a preceding ovulation is sufficient to prevent an increase in isthmic flow in the intact animal during the period of oestrogen dominance prior to ovulation. While these results indicate

possible humoral factors which may be responsible for the suppression of isthmic flow during this period, it did not clarify the hormonal balance necessary to ensure the subsequent passage of fluid through the isthmus.

CHAPTER SIX

GENERAL DISCUSSION

CONTENTS OF CHAPTER

A. MECHANISMS DETERMINING THE DIRECTION OF FLUID FLOW

Few theories have been postulated to explain the mechanisms which regulate the direction of flow of Fallopian tube secretions. Evidence supporting the existence of an oestrogen induced oedema (Black and Asdell, 1958, 1959) and flexure of the utero-tubal junction (Edgar & Asdell, 1960a), or a functioning occlusion of the isthmus (Black & Davies, 1962) are mainly derived from experiments which involved ligation of the fimbrial end of the Fallopian tube. The fluid distension which thereby occurred during the preovulatory period and its subsequent diminuition about day 4 of the oestrous cycle is, presumably, a function of an isthmic restriction and/or the volume of fluid secreted. The latter possibility, that it was solely due to the increased fluid secretion being in excess of isthmic flow during the preovulatory period, has been deleted by the present results. It is evident then that at some region of the isthmus a mechanism exists which prevents an increase in isthmic flow concurrent with the increase in fluid secretion.

In addition, it has been reported that the administration of oestrogen after mating, with the rabbit (Black & Asdell, 1958, 1959), ewe (Edgar & Asdell, 1960a) and cow (Black & Davis, 1962), prolonged the duration of the tubal distension indicating that the mechanism was under hormonal control. The results from oestrogen therapy on ovariectomised ewes showed, contrary to expectation, that peak isthmic flow preceded that of ampullar flow following the injection of 30, 90 and 500 µg ODB. Since 30 µg ODB has been found to induce a normal behavioural oestrus, and associated vaginal cycle, in the ovariectomised ewe (Robinson, 1955; Robinson, Moore & Binet 1956a; Robinson & Moore, 1956b; Moore & Robinson, 1957) then 90 µg and 500 µg can be considered

in excess of that required for a physiological response. But despite the dose levels used, isthmic flow increased significantly with each increase in the dosage of oestrogen. Thus any isthmic occlusion which may have been present, was not able to prevent fluid flow into the uterus.

It may be argued that the oestrogen induced restriction of the isthmus and/or utero-tubal junction may have caused the decline in isthmic flow subsequent to day 3. However, it was found that serial therapy further prolonged the duration of isthmic flow indicating that the oedematous condition (Black & Asdell, 1958, 1959; Edgar & Asdell, 1960a) was not a major factor causing this decline of isthmic flow in the ovariectomised oestrogen primed ewe.

The decline in muscular activity of the Fallopian tube following ovariectomy, and the return of this activity after injection of oestrogenic compounds, has been demonstrated by many investigators (Westman, 1926; Reynolds, 1932; Wimpfheimer & Ferresten, 1939; Bernstein & Feresten 1940; Ichijo, 1960; Greenwald, 1963b). With the rabbit, this effect of oestrogen is not evident until 10 to 15 hr after the injection and maximum muscular activity seldom attained within the first 24 hr (Reynolds, 1932; Greenwald, 1963b). The duration of response appears to be only 2 to 4 days for short acting oestrogens (Reynolds, 1932) although the contractions may persist for longer periods if ECP is used (Greenwald, 1963b). Small doses of ODB are as effective as large doses in most instances (Wimpfheimer & Feresten, 1939) and no differences in frequency or amplitude of the muscular contractions were evident between 25 µg and 250 µg of ECP (Greenwald, 1963b). Extrapolation of such isolated information to the ewe must be given due reservation but it would appear possible that in the oestrogen primed ovariectomised ewe isthmic flow coincided with the onset of increased muscular activity. The decline in isthmic flow about day 3 would be,

if this was the case, due to the return of weak and irregular contractions. Since fluid secretion was still high and isthmic flow diminished at this time the ampullar orifice would, in consequence, then become the main fluid outlet. The slow conveyance of contrast medium in the ovariectomised rabbit, and its rapid movement which coincided with the onset of muscular contractions following oestrogen therapy (Bjork, 1959), supports the contention.

As progesterone priming, prior to oestrogen therapy, appears necessary to increase the height of isthmic epithelial cells in the ovariectomised ewe (Restall, 1966b) the possibility of an isthmic occlusion occurring and acting in the entire ewe can not be disregarded. The investigation of this possibility showed that a course of preparatory progesterone, when preceding the injection of 30 or 90 µg ODB, depressed isthmic flow. That complete suppression of isthmic flow occurred during the preovulatory period in the entire oestrous animal (Text-fig. 1 and 16) but not in the same period of 'first oestrous' (Text-fig. 15) confirmed the role of progesterone. It is possible then that progesterone may facilitate the function of oestrogen in causing an oedematous condition. Significantly, the fluid distension incurred by ligation has been investigated solely with entire animals which are primed by an endogenous source of progesterone.

To oppose this concept it is equally plausible that the partial suppression of isthmic flow may have been due to the antagonistic action of progesterone on tubal motility (Wimpfheimer & Feresten, 1939; Ichijo, 1960; Greenwald, 1963b). Since muscular activity of the Fallopian tube in the intact animal is considerable during the preovulatory period (Westman, 1926; Greenwald, 1963b) it is difficult to concede that this is the case. Rather, progesterone may modify the type of contraction, so that they become more tonic in nature (Ichijo, 1960; Greenwald, 1963b), and by this means prevent the passage

of fluid through the isthmus. That the application of nicotine to the isthmus (Black & Davis, 1962) partially reduced fluid distension supports the proposition. Equally, such an occlusion would occur if the muscular contractions of the isthmus were antiperistaltic at this time (Winterberger - Torres, 1961).

On day 4 in the oestrous cycle of the entire ewe this occlusion ceased to exist and an increasing proportion of the tubal fluid passed through the utero-tubal junction. Considering the difference in lumen size and pressures between the ampulla and isthmus (Brundin, 1964a, c) some propelling mechanism would appear essential to direct fluid down the isthmus. It is worthy of note that Burdick et al., (1942) observed peristaltic activity in the uterus and tubo-uterine region of the rat was greatly increased on the third day. Such activity was not confirmed by Greenwald (1963b) but a change in the type of muscular contractions of the isthmus was recorded at this time. Whether such contractions do exist, and in conjunction with a diminuition of the isthmic oedema, promote fluid movement through the isthmus has yet to be resolved.

B. THE ROLE OF TUBAL SECRETIONS IN THE TRANSPORT OF SPERMATOZOA AND OVA.

1. Sperm Transport

It has been postulated that the cervix, in vaginally inseminated animals, and the utero-tubal junction act as barriers which limit the number of spermatozoa reaching the Fallopian tubes. Muscular contractions of the genital tract appear capable of maintaining a gradient in spermatozoa concentration, directed either caudally or cranially. While uterine contractions may also promote dispersal of spermatozoa within the uterus only limited evidence is available to indicate their participation in projecting spermatozoa through the utero-tubal junction.

That such passive transport of spermatozoa does occur is evident from many reports (Krehbiel & Carstens, 1939; Van Demark & Moeller, 1951; Rowson, 1955; Akester & Inkster, 1961; Howe & Black, 1963; Mattner & Braden, 1963a; Mattner, 1963c) although their inherent motility may be an added advantage (Dauzier, 1958; Mattner, 1963c). It is plausible then to surmise that secretions of uterine origin pass through the utero-tubal junction, a surmise which is amply supported by the passage of radiopaque fluids through this region (Krehbiel & Carstens, 1939; Rowson, 1955; Akester & Inkster, 1961) and by observation (Alden, 1942b). Since in the absence of effective uterine contractions spermatozoa fail to reach the Fallopian tubes (Hawke, 1965) it is apparent that uterine contractions may be involved.

In this investigation the technique used to determine isthmic flow prevented any influence from opposing pressures of the uterus. That such aduterine movement of fluid does occur is exemplified by the progress of indian ink (Parker, 1930) and radiopaque fluids (Bjork, 1959). The fact that fluid movement can, therefore, occur in both directions is intriguing. A concept of unidirectional fluid flow cannot be substantiated.

Segmental contractions of the Fallopian tube (Maeda, 1933) would ensure the 'pendulous' like movement of tubal fluid as observed by Bjork (1959) and in consequence dispersion of tubal contents. A similar state of turbulence would exist in the lumen of the uterus even though the waves of contraction are less frequent and more singularly directed (Reynolds, 1939). As uterine and tubal contractions are not necessarily synchronous (Reynolds, 1939) an alternate interchange of uterine and tubal fluid across the utero-tubal junction is feasible and would guarantee transport of spermatozoa across this barrier. To ensure this interchange an alternating pressure gradient must exist. Furthermore it is necessary that the respective pressure

waves, in addition to transiently exceeding each other, must also overcome the resistance of the utero-tubal junction (Anderson, 1928).

The slower progress of spermatozoa during anoestrum and dioestrum (Green & Winters, 1935; Thibault, 1952) and the lower level of isthmic flow during these periods in the ewe could be compatible with the present results. Similarly, at high doses of oestrogen sperm transport in the rabbit is hindered (Noyes, 1959b) possibly due to an excessive isthmic flow. However, until the movement of fluid and pressure differentials across the utero-tubal junction are assessed, and the influence of the ovarian hormones determined, full evaluation of isthmic flow in sperm transport must wait future developments.

The relative contribution of the isthmus and ampulla to tubal secretions is unknown. That the number of secretory cells in the isthmus is less than in the ampulla (Hadek, 1955; Restall, 1966b) only suggests that any fluid contribution of the isthmus would be proportionately reduced. In consequence the extent of fluid interchange between these two regions cannot be established. The lower concentration of spermatozoa found in the ampulla (Braden, 1953) indicates the interchange is not complete but as tubal fluid has 'free' access to the peritoneal cavity ampullar flow would continually deplete the number of spermatozoa in this region.

The loss of spermatozoa to the peritoneal cavity, as evident from the reports of Edgar & Asdell (1960b) and Mattner (1963c), is offset by a continual progression from the cervical pool (Quinlan, Marē & Roux, 1933; Stark, 1949; Dauzier, 1958). The lifespan of spermatozoa is limited (Green & Winters, 1935; Dauzier, 1958), and while many of those reaching the Fallopian tube are subsequently ingested by phagocytes (Hammond & Asdell, 1926; Austin, 1957), degenerate spermatozoa surviving this process would be removed from the site of fertilisation by the adovarian flow of fluid through the ampulla.

2. Ova Transport

Peak ampullar flow in the entire oestrous animal would appear to coincide with the time of ovulation (Asdell, 1946). This movement of fluid would therefore be in the opposite direction to that of the ovum in its passage through the fimbria and down into the ampulla. On this basis it appears that fluid movement plays little part in the mechanism of ovum pickup and subsequent transport. It should be emphasised, however, that the measurements of fluid collected were made at 24 hourly intervals, thus, a transient change in the direction of flow, at or after the time of ovulation, would not be detected. Any such reversal if it did occur is probably not important in ovum pickup since ova may enter the fimbrial portion of the Fallopian tube in the rabbit previously ligated at the abdominal tubal ostium (Clewe & Mastroianni, 1958). Thus it seems likely that cilia (Borell, Nilsson & Westman, 1957) and/or muscular contractions (Greenwald, 1963b) are capable of projecting the ovum forward against the opposing current. It is also feasible that the cumulus matrix, known to surround the preovulatory ovum, may facilitate the action of cilia in moving the ovum and preventing its loss into the abdominal cavity. That glass beads are rejected if placed on the fimbria of the ewe (D.R. Lang pers. comm.) and a cumulus coating facilitated the movement of resin spheres down the ampulla of the ewe (Bennett & Rowson, 1961) lend support to this concept.

In contrast to ampullar flow, the period of maximum flow through the utero-tubal junction is in the same direction, and coincides with, the passage of ova in the oestrous ewe (Clarke, 1934). Despite the gradual rise and decline in muscular contractions 24 - 36 hr after an injection of oestrogen only a few observations have been reported on the movement of ova at different times after such treatment. In some instances acceleration of ovum movement occurred within 30 hr after

the injection of ODB into mice (Burdick & Whitney, 1938): and in the rat, about 48 hr after injection of the relatively insoluble ECP (Greenwald, 1961b) and between 20 and 24 hr after treatment with oestrone (Banik & Pincus, 1964). It would appear probable then that the passage of ova into the uterus coincides with the period of peak isthmic flow in the oestrogen primed animal.

A study of ova transport in the rabbit where a progesterone oestrogen sequential therapy was employed (Harper, 1965a) indicated that transport through the isthmus was hindered, not enhanced, by the concluding progesterone treatment. This evidence is again comparable with the reduction of isthmic flow which was recorded with those ewes receiving a similar sequential therapy. Equally consistent is the negative effect of progesterone and ovariectomy on both ova transport (Adams, 1958, 1965a; Harper, 1964, 1965a) and isthmic flow. If then the time of peak isthmic flow indicates when ova pass through the isthmus one could expect to find rapid ova transport occurring during 'silent' oestrous. This could well be the case since Dutt (1954) and Hart & Laffey (1959) have indicated that part of the embryonic loss which occurred early in the ewe's breeding season may have been due to a faster rate of egg transport.

Reports on the effect of oestrogen on ova transport appear to be at variance but it is apparent that oestrogen before mating has different effects from that starting afterward (Pincus & Kirsch, 1936; Chang & Bedford, 1961). In addition, if treatment is daily after mating (Greenwald, 1957) or is a single dose of long acting oestrogen (Greenwald, 1961a; 1963a) ova are retained in the Fallopian tube, but if a single dose of a short acting oestrogen is given (Whitney & Burdick, 1938; Greenwald, 1957, Banik & Pincus, 1964) accelerated transport occurs.

That isthmic flow was similar with both single or multiple injections of ODB appears incongruous with such reports. However, it is also apparent, that much of the controversy has arisen where entire animals have been used. Oestrogen promotes tubal motility (Greenwald, 1963b) but whether oestrogen can cause the tubal occlusion in the absence of progesterone has not been demonstrated. Thus in its absence, or where the level was insufficient, tube locking of ova (Harper, 1964, 1965a) and a restriction on isthmic flow may not occur.

Both oil (Black & Asdell, 1958; Ichijo, 1960) and ova (Burdick, Whitney & Emerson, 1942; Harper, 1965b) have been observed to move in a pendular motion indicating that the movement of tubal fluid and ova may be synonymous. It is interesting to note that Squier (1932) and Burdick et al., (1942), using transmitted light, observed that ova within the Fallopian tube "shifted to and fro with perfect ease" and that each behaved "like a free body in water". Thus fluid could in fact transmit the effect of muscular contractions to the ovum, with each being rapidly displaced toward the uterus when oestrogen is dominant and more slowly when under the influence of progesterone alone.

CHAPTER SEVEN

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