UNIVERSITY OF SOUTHAMPTON

MUSCULAR AND CILIARY MOTILITY OF THE HUMAN FALLOPIAN TUBE AT DIFFERENT TIMES IN THE MENSTRUAL CYCLE

by Frances Norah Rush

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A thesis submitted to the University of Southampton in fulfilment of the requirements for the degree of Master of Philosophy

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE

HUMAN REPRODUCTION AND OBSTETRICS

Master of Philosophy

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The muscular activity was analysed by using isometric measuring techniques with an organ-bath set-up. Tubes examined were from women at different stages in the menstrual cycle, and post-menopausal, pregnant, and post partum. The spontaneous motility, and the response of the tubes to adrenergic and cholinergic drugs was measured, with isthmic and ampullary regions being compared. Puerperal oviducts were also treated with exogenous hormones and the response to drugs again measured.

The isthmic regions had a greater response to adrenergic drugs than the ampullary, but there was little difference in results throughout the cycle. However the pregnant and menopausal tubes showed a smaller response to drugs. The cholinergic drugs had little effect on motility. Progesterone caused relaxation which was more pronounced in the isthmic regions. Oestrogen increased activity in both regions of tube.

Ciliary activity in tubes from menstruating and post partum women were analysed using high speed cine photography. Differences in beat were found throughout the menstrual cycle, with ampulla and isthmus showing a greater activity after ovulation than before, but no difference in the fimbriae.

The cellular composition of the epithelium was examined histologically. No evidence was obtained to suggest a transformation of ciliated to secretory cells throughout the cycle. The number of ciliated cells was greatest in the fimbriae, and decreased towards the uterus, although still comprising more than 50 % of cells in menstruating women. At mid-cycle the epithelium had a flat appearance, but in the secretory phase the secretory cells arched over the ciliated.

In conclusion, movement of the ovum through the human Fallopian tube is probably brought about mainly by muscular contractions, but cilia probably play a larger part than was otherwise thought.

Introduction

The Fallopian tube is of utmost importance in the reproductive system of the female. It forms a connection between ovary and uterus, and is the site of fertilization and early development of the ovum. The passage of the ovum through the tube takes three to four days in the human, and this journey is crucial for the successful further development of the egg. The ovum must arrive at the uterus at the correct stage for implantation or it will degenerate.

The tubes have been well studied in the past in an attempt to elucidate the mechanisms involved in the ovum transport, but no conclusions have yet been reached. There have also been many contradictions and controversies about the results obtained. There are two main forces involved in transport, muscle contractions,,and ciliary action, and it is the relative contributions of these that have caused the arguments.

This present work has sought to investigate aspects of both muscular and ciliary activity, and although it cannot be hoped to provide a definite answer to the problem, the work may help towards answering some of the questions.

A. ANATOMY OF THE FALLOPIAN TUBE

1. Gross anatomy

The fallopian tubes are paired organs lying in the pelvis of the female. As can be seen in figure 1., they form a channel between the peritoneal cavity and the exterior. The tubes are enclosed within the leaves of the broad ligament, and the entire structure os known as the "mesosalpinx".

The fallopian tube is generally thought of as having four regions:

1. The intramural or interstitial portion
This is found in the wall of the uterus. It has an average length of 1.5 - 2.5 cms.
2. The isthmus is the region between the tubal-uterine junction and the ampulla. It is about 2-3 cms. long.
3. The ampulla is 5-8 cms. long.
4. The infundibulum is the trumpet shaped distal end of the tube. It is 1-6 cms. long

In the human oviduct there is only a gradual macro-anatomical difference between the ampulla and isthmic regions, whereas it is more marked in some other species.

a.) Muscular anatomy

This can be divided into two parts, the tunica serosa and the tunica muscularis. The former is the outermost part of the oviduct, and is composed of mesothelium continuous with the peritoneum, and connective tissue. It is well vascularized, and there are smooth muscle fibres present both subperitoneally and around the vessels. The tunica muscularis is inside the serosa, and is basically three layers, an inner and outer longitudinal, and an intermediary circular. The muscle layer in the interstitial and isthmic regions is always thicker than the ampullary portion. The circular muscle is most extensive in the interstitial portion, and the longitudinal muscle also increases in amount towards the uterus.

Interstitial region

The wall of this region of the tube is thick, and is composed

Diagram to show Pelvic Organs in Human Female

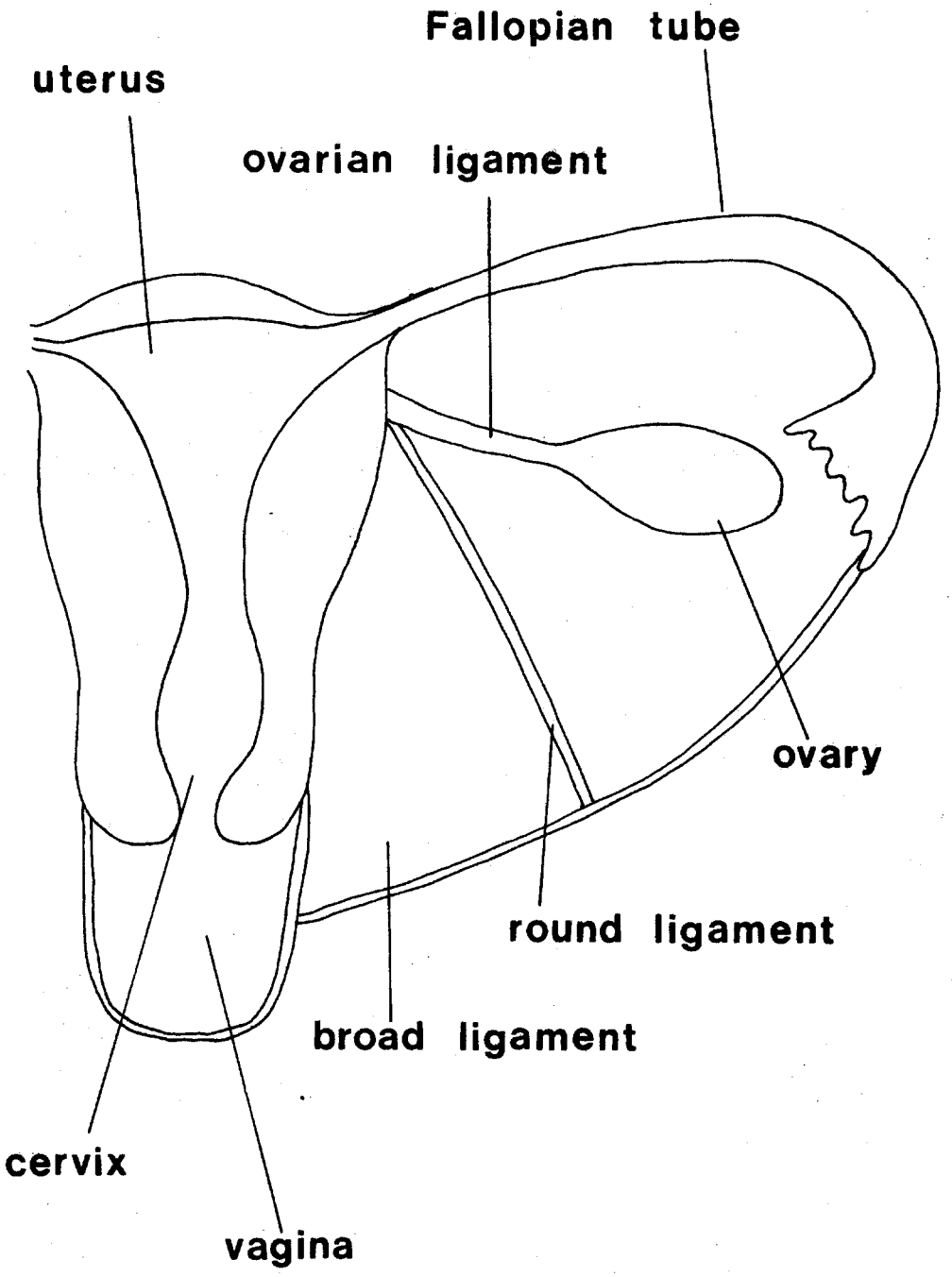


Fig. 1

of three layers, the outer subperitoneal muscle, vasomuscular layer, and autochthonous muscle. The latter musculature consists of four bundles around the tubal lumen, and the complete arrangement enables a constrictor effect to be put on the intramural portion. This may help in the transport of eggs into the uterus, and also the sperm in the opposite direction.

The length of the intramural portion is from 1.5 - 3.5 cms. It often has a convoluted route through the uterine wall. The diameter of the lumen is a matter of some dispute. Sweeney (1962) thought it was 2 - 4 mm., but narrowed to 100 μ when it reached the vascular layer of the uterus. Rocker (1964) thought the lumen might be reduced to 100 μ along the length, under some circumstances. It is probably slightly larger than the lumen of the isthmic region.

Isthmic region

The isthmus contains the heaviest musculature of the extra-uterine tube. There is a well defined inner longitudinal layer, and the circular layer increases in thickness towards the interstitial portion. The average luminal diameter is 400 μ, with a range of 100 μ to 1mm.(Woodruff and Pauerstein 1969), although a lumen of up to 2 mm. has been noted (Rubin 1947). The mucosa is thrown up into three to six primary folds, with four being present most often.

Ampullary region

The muscle in the ampulla is much less well developed than the isthmus. There is no well developed inner longitudinal layer, but instead the muscle bundles are rather sparse and more widely separated by loose, highly vascular connective tissue. The muscle bundles also continue into the lamina propria of the mucosal folds. The circular bundles are also not well defined, but are dispersed among the longitudinal muscle. There is a very complex pattern of mucosal folds, almost filling the lumen. The lumen varies from 1 to 2 mm. at the junction with the isthmus, to up to 2 cms. near the infundibulum.

Schematic drawing of the Human Oviduct

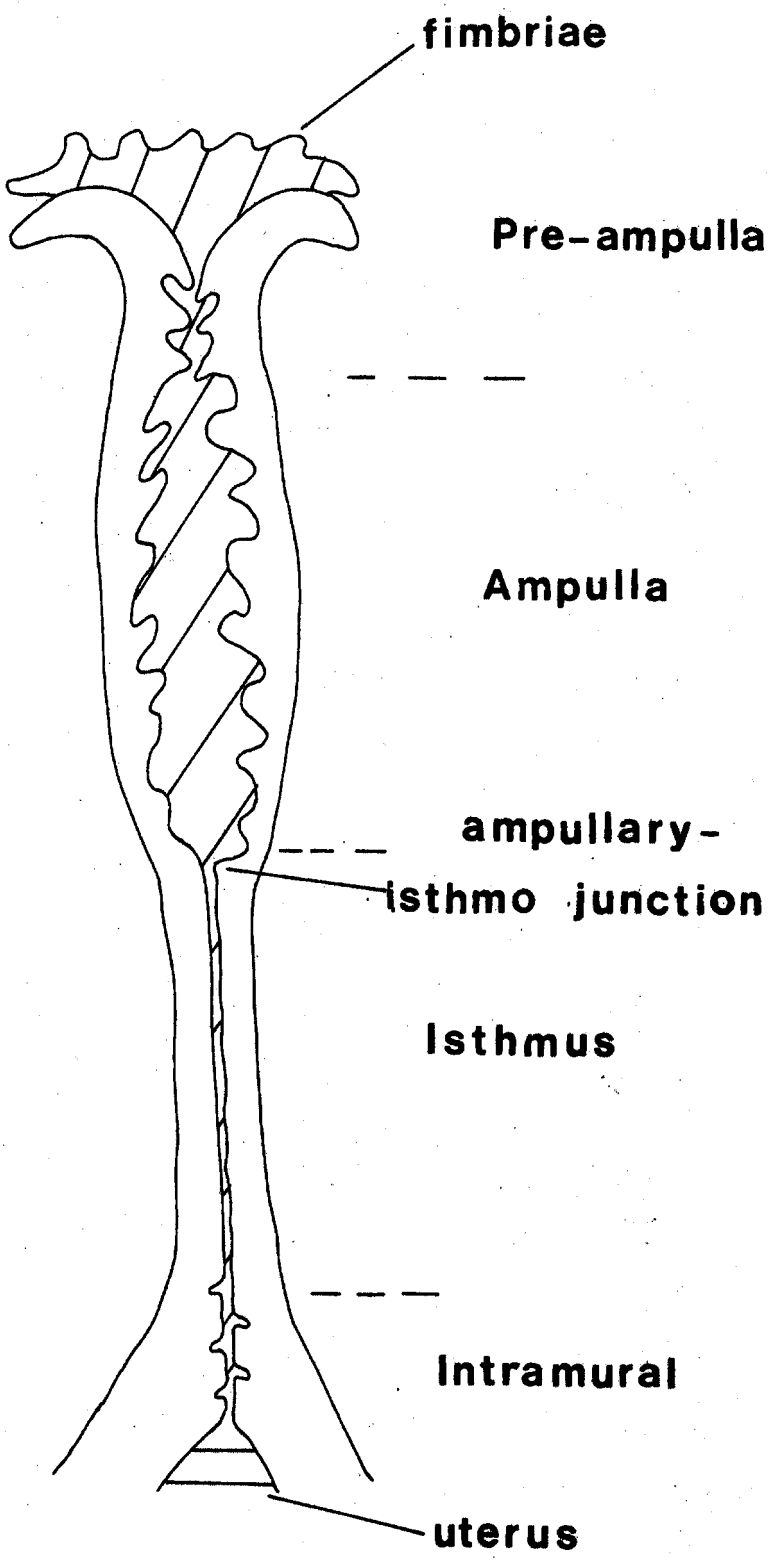


Fig.2

T.S. Isthmus showing well defined inner longitudinal muscle layer and five primary mucosal folds x36

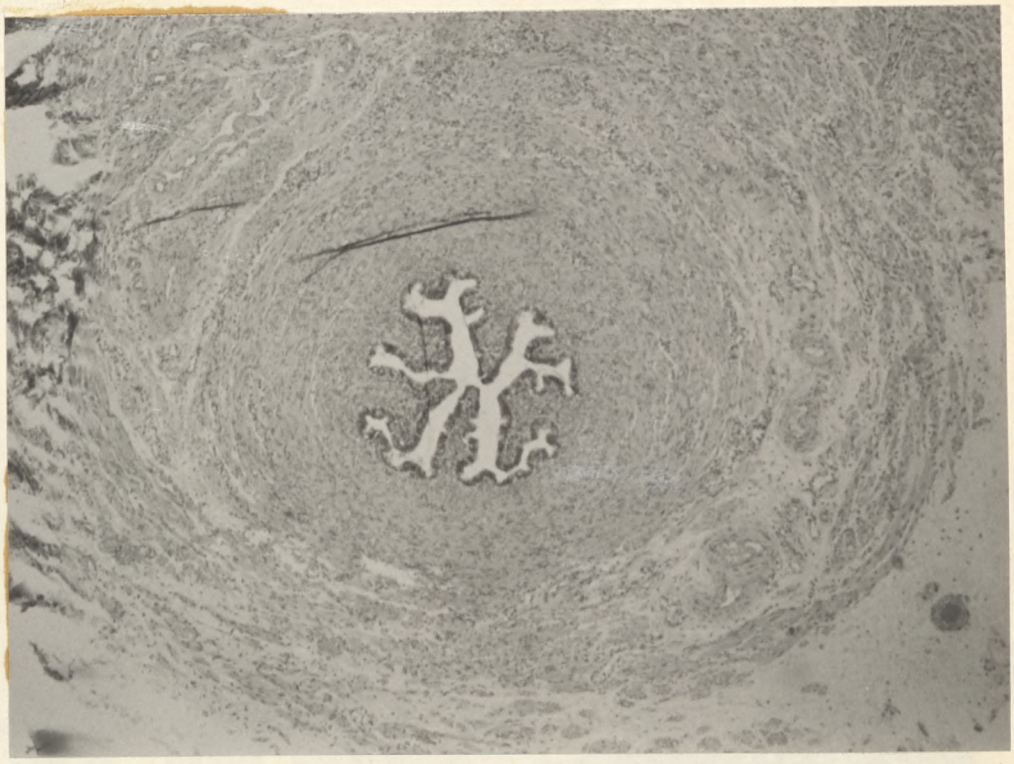


Plate I

T.S. Ampulla showing the complex mucosal folds. The muscular layer is thin, circular and longitudinal layers being difficult to distinguish x36

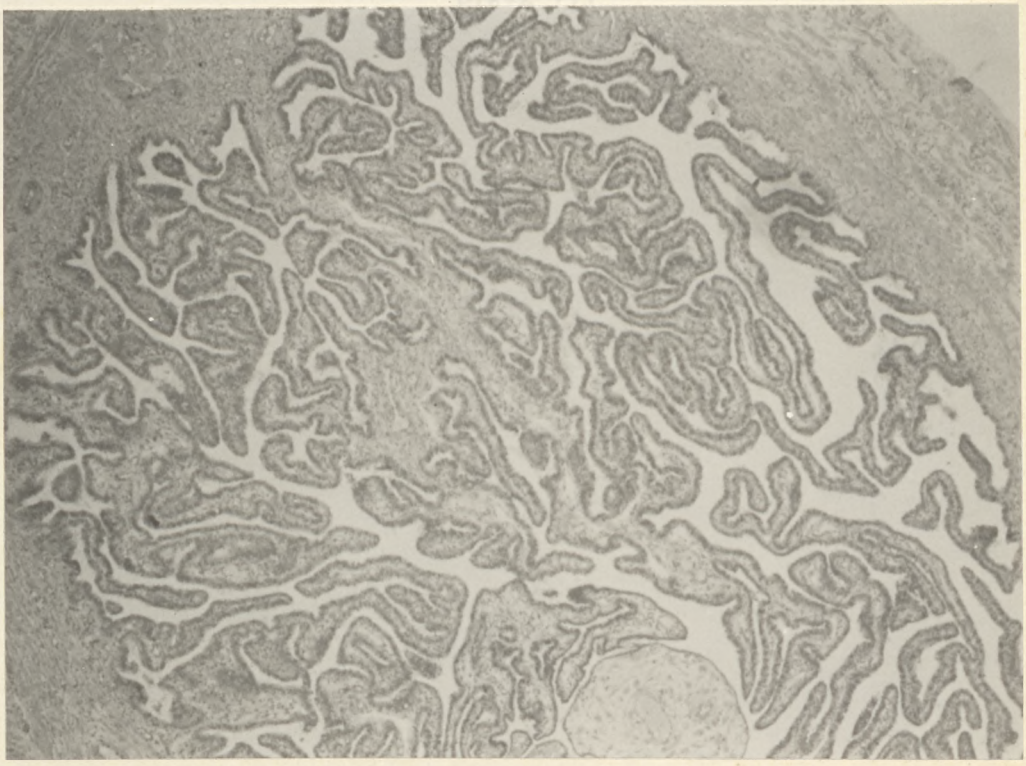
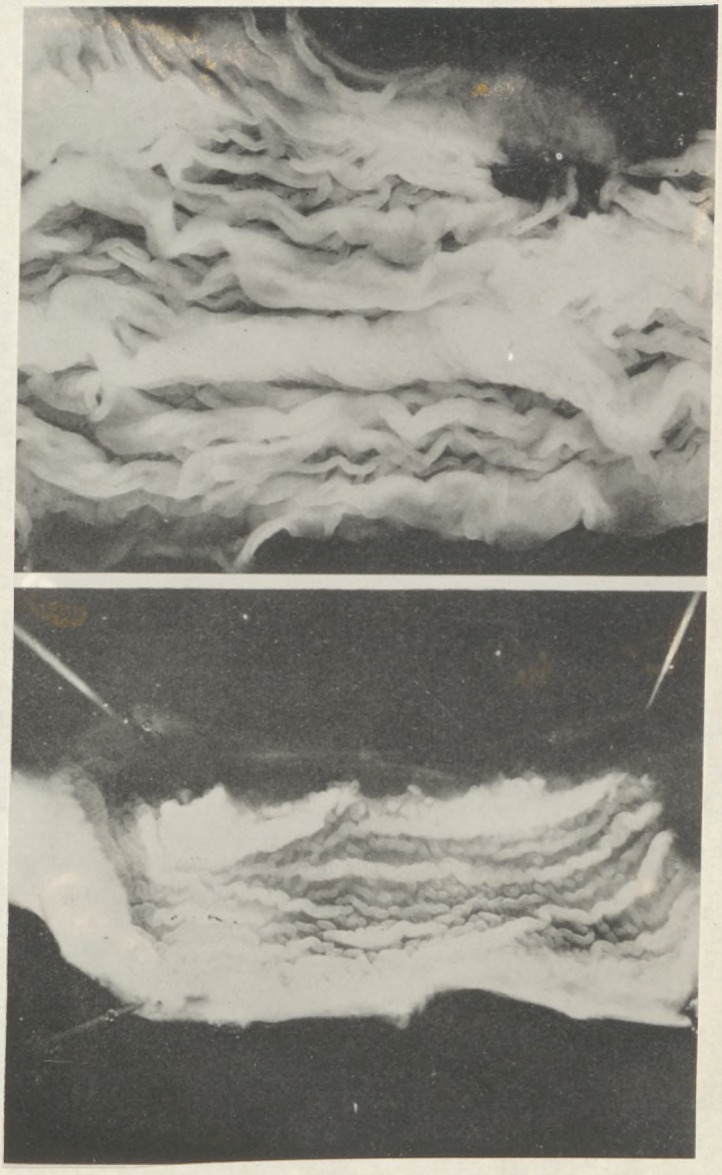


Plate II

Section through fimbriated end of oviduct. This area is composed of mucosal folds, with muscle fibres continuing into the lamina propria of the folds x36

Plate III

L.S. Fallopian tube showing the difference in the complexity of the mucosal folds between the two regions.
Top: ampulla x10 Bottom: isthmus x6



Infundibulum

This is the trumpet shaped end of the oviduct, which terminates in the fimbriae. These are fringes of tissue abundantly covered with cilia, and which are in contact with the ovary at the time of ovulation.

The musculature of the infundibulum is similar to the ampulla, being thin and easily distended. Stange (1952) thought the autochthonous muscle of the tube grouped to form a sphincter at the abdominal opening of the tube.

b.) Muscular sphincters in the fallopian tube

Uterotubal

In some animals, but notably those that are polytocous, there is a barrier between oviducts and uterus (Lee 1928). This can be either in the form of a sphincter muscle, or folds and polyp-like projections, that prevent the passage of fluid from the uterus to the tubes. In humans no anatomic sphincter has been proved to exist (Rocker 1964, Schneider 1942, Lisa et al 1954). However some investigators have found evidence for a barrier dependent on the reproductive state of the female. Hysterosalpingograms carried out throughout the menstrual cycle showed that higher pressures were needed to pass fluid into the tube from the uterus during pre- and post- menstrual phases than at ovulation, although no evidence of a muscular sphincter was found (Hunter et al 1956).

Isthmus

The musculature of the isthmus is better developed than in the surrounding regions of tube, which would enable it to function as a sphincter. As will be seen, the vascular supply and innervation of the isthmus are also different, and the isthmus could act as an adrenergic sphincter.

Infundibulum

As previously mentioned, the autocthonous muscle could be grouped to form a spincter. Stange found that this sphincter

functioned differently throughout the cycle. Woodruff and Pauerstein (1969) demonstrated a functional sphincter. When fluid was pumped slowly into the isthmus via a catheter, the ampulla was markedly distended without much leakage from the abdominal ostium.

c.) Blood supply

The blood supply to the oviducts is derived from the uterine and ovarian arteries, although there is still controversy about the relative contributions of each (Goss 1966, Anson 1966). It is likely that the ampulla has branches from the ovarian artery, whereas the isthmic region receives branches from the uterine. The venous system probably follows the arterial supply. There are interconnected capillary systems in the mucoaa, muscularis and subserosa (Gatsalov 1963). The serosal capillary network in the isthmus is dense, whereas it is sparse in the ampulla.

d.) Lymphatics

The drainage of the isthmic and ampullary regions of the tube are different. The isthmus and utero-tubal junction have a much more elaborate system than the ampulla. Gatsalov (1963) found a more delicate system in the isthmus, and a more variable system in the ampulla.

The lymphatic system appears to alter and develop throughout the reproductive life. During these years the muscularis is drained by a network of capillaries in both circular and longitudinal muscle. These are not developed before puberty, and begin to close after the menopause.

e.) Neuroanatomy

1. Gross Innervation

The tube receives innervation from the autonomic nervous system. This is composed of two systems, the sympathetic and parasympathetic nervous systems, both of which have a dual supply to the tube. The sympathetic system has fibres from the ovarian

plexus serving the ampulla, and from the hypogastric plexus serving the isthmus. The parasympathetic system has vagal fibres from the ovarian plexus running to the ampulla, while the isthmus and interstitial regions receive sacral fibres derived from S_2, S_3, and S_4, and which form the pelvic nerve.

2. Fine Innervation of the Tube

This is different in the proximal and distal regions of the tube. Brundin (1964,1965) investigated the neuroanatomy of rabbit oviducts using a formaldehyde-induced catecholamine fluorescence technique (Falck et al 1962). He found that the isthmic region had a very rich supply of adrenergic nerve terminals in the circular muscle layer, whereas the supply to the ampulla was quite sparse, and found generally around the blood vessels. In the ampulla the supply to the musculature was constant despite the increasing thickness of the muscle wall towards the isthmus. There was a sudden increase in adrenergic nerves at the ampullary-isthmic junction. Owman et al (1966) also working on the rabbit oviduct found the rich supply of nerves was concentrated in a ten millimetre distance form the isthmic-ampullary junction, and did not extend along the isthmic region. In the human this region of dense distribution is not so obvious although the supply to the isthmus is still greater than the ampulla (Woodruff and Pauerstein 1969). Owman et al (1967) carried out assays for adrenaline in the isthmus, ampulla and intramural portion of the human fallopian tube, and found a higher level in the isthmic region.

As there is a parasympathetic nerve supply to the oviduct, it would be expected that a cholinergic innervation could be demonstrated. Jacobwitz and Koelle (1965) examined the cat oviduct using a histochemical technique for acetylcholinesterase, which should reflect the amount of acetylcholine present, and found that the only true cholinergic fibres were in the lamina propria. Owman et al (1966) found no cholinergic innervation to the musculature of the rabbit ampulla, and little to the isthmus.

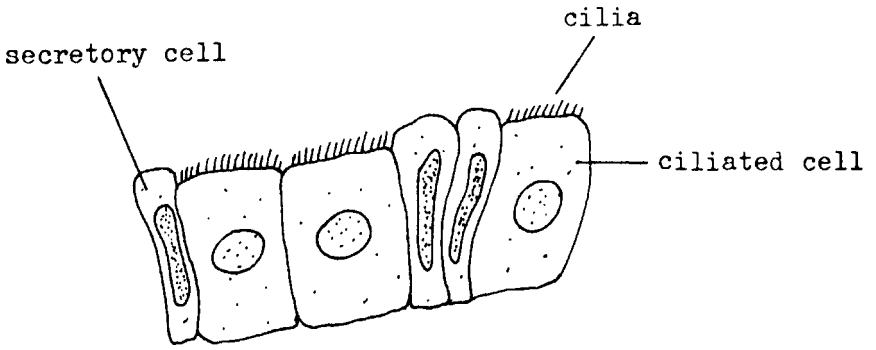
2. Histology of the epithelium

a.) Types of cells

The epithelium of the oviduct is composed of two main types of cell, the secretory and ciliated, although two further kinds have been described by some authors. These are an intercalary or "peg" cell, and "reserve" or "indifferent" cell.

The ciliated cell is relatively square with a finely granular cytoplasm. The nucleus is round or oval in shape, large, and usually found in the centre of the cell. The cilia are attached to a row of basal granules beneath the cell membrane.

Secretory cells have a more coarsely granular cytoplasm, and a large darker, more elongated nucleus. These cells are generally long and narrow, with the long axis of the nucleus parallel to the long axis of the cell.



The "peg" cell has also been called an intercalary cell or "Stiftchenzellen". It is rodlike with a flat compressed nucleus and very little cytoplasm, and is found compressed between adjoining cells. These cells have been thought to be either independent elements or emptied secretory cells, but most authors now consider them to be the latter.

The "reserve" or "indifferent" cells are found at the base of the epithelium, above the basement membrane. They have a

large round nucleus, with the chromatin dumped near the nuclear membrane. There have been many arguments about the role of these cells, but it is possible they may be transformed into other cell types for oviductal growth (Pauerstein and Woodruff 1967). These authors, using fluorescent microscope techniques found that the nuclei of the indifferent cells contained more than the diploid content of D.N.A.. They also used tritiated thymidine, and showed it was taken up by these cells alone, and the labelled cells gradually move through the epithelium. They concluded that these cells possibly play a large role in the replication of the epithelium.

b.) Cyclic variations in the epithelium of the oviduct

There has been a lot of discrepancy in the literature about cyclical variation in the cells of the oviduct epithelium.

Variations in the relative numbers of cells

Earlier authors thought the secretory and ciliated cells alter in numbers and height during the menstrual cycle, with ciliated cells becoming transformed into secretory during the last stage of the cycle.(Moreaux and Schaffer 1913).

Some of the authors that have found changes in the number of ciliated cells throughout the cycle have described processes by which ciliated cells have been transformed into secretory. Balboni (1954) thought that during the late luteal phase of the cycle the distal third of the cell becomes constricted from the rest of the cell and is cast off into the lumen. Two other mechanisms have been described. The whole cells could be passed from the epithelium into the lumen, or cilia could fall out of the cells (Flerko 1954).

There are also disagreements about the fate of the deciliated cells. Schultka (1963) thought they fill up with granules and become active secretory cells. These eventually become "peg" cells which are finally lost from the oviduct. At the beginning of the next cycle new ciliated cells are formed from a cell called a "clear cell" or "Ersatzzelle".These are large, with very clear cytoplasm, and are only found in the oviduct during the first post-menstrual week. Schultka thought the only function of these cells was to produce ciliated cells.

Flerko however thought these cells could become either ciliary

or secretory cells. He thought deciliated cells at the end of the cycle are either lost from the oviduct, or become "Ersatzellen" at the beginning of the next cycle. The depleted secretory cells become "peg cells" which are eventually lost.

More recent studies, and especially electron microscopy studies have failed to show cyclic changes in number (Clyman 1966).

The position in other species may be slightly different. Gupta (1970), working on rabbits found that although the numbers of ciliated and secretory cells remained constant throughout the cycle, the actual numbers of cilia altered. The numbers in the ampulla increased fourteen hours after copulation, corresponding with the time ova would pass through. Similarly at the ampulla-isthmic junction the numbers were at a maximum at twentyfour hours post coitum. The numbers of cilia in the isthmus were reduced at seventy hours post coitum, at the time ova would be in this region. These results were obtained from a light microscope study. In a later report by the same authors using an electron microscope (Shipstone el al 1974), they found that ciliation in the isthmus was in fact increased at seventy hours post coitum.

Brenner (1967a) workes on the oviduct of Rhesus monkey. He could find no evidence either for a transformation of ciliated to secretory cells in the Rhesus monkey, or for the concept of a universal replacement cell. But he found that the ciliated cells of the fimbria and ampulla do show marked cyclic differences. The ciliated cells of the fimbriae appeared to shed and regenerate the cilia during each cycle, but the ampullary ciliated cells fluctuated only in height, and the cilia were not shed.

Variations in the size of cells

Snyder (1924) and Novak and Everett (1928) were some of the first investigators to notice cyclical variations in the size of the epithelial cells in the human oviduct. They found that the height of the epithelium was greatest during the proliferative phase, when it was over 30u. In the luteal phase it was 20u or less, and in pregnancy it was not above 17u. They thought that the variation in height was more pronounced in the ciliated cells. At midcycle both secretory and ciliated cells reached their peak heights, and so the surface was smooth. After ovulation the ciliated cells become broader and lower, and the secretory

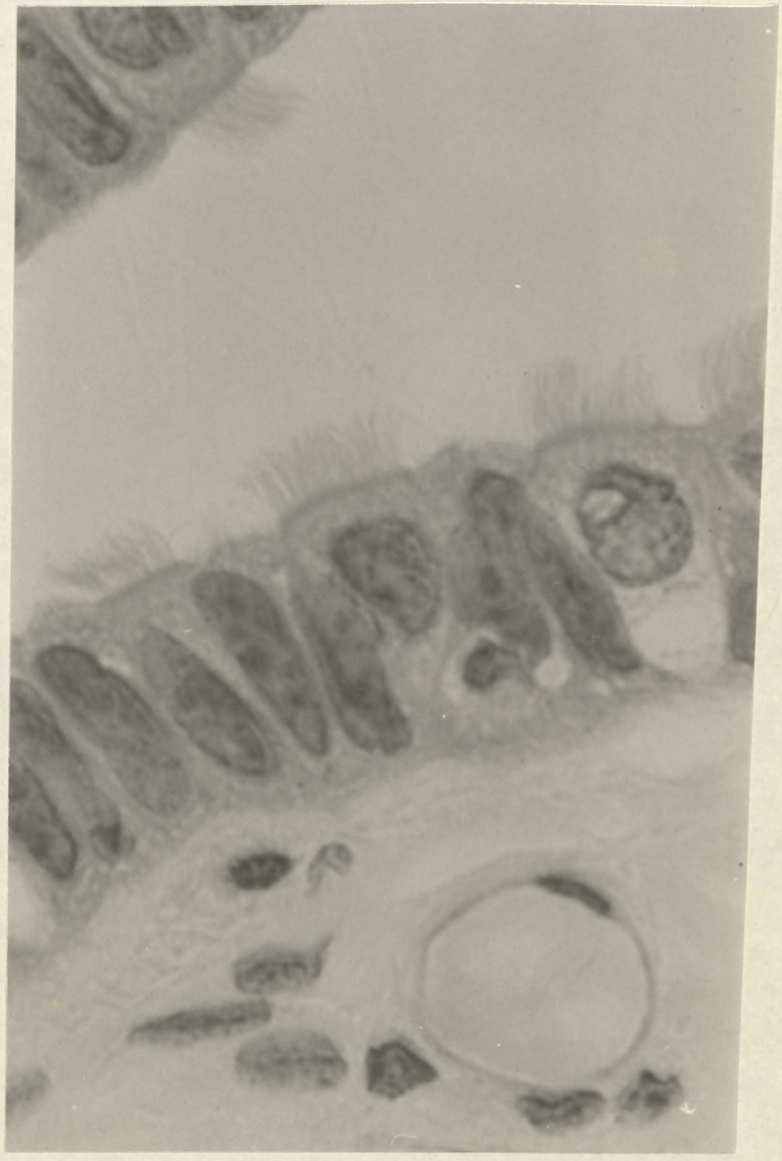
cells protrude above them forming a dome, and giving a rough appearance to the epithelium.Later in the interval phase this dome often breaks off into the tubal lumen leaving the nucleus of the secretory cell with little cytoplasm. During menstruation the epithelium is low and of a smoother appearance. Snyder found that during pregnancy the secretory cells protruded above the ciliated, however Novak and Everett found the epithelium at this time to be at its lowest, and in the later stages almost flattened. The latter authors found that early post menopause there was little atrophic change, and the epithelium resembled the interval phase. After the age of sixty they found areas of flattened epithelium, but there were still some cilia present.

Variations in the ultrastructure of the epithelium

Hashimoto et al (1964) found no change in the ciliated cells during the cycle in humans, but found changes in the secretory cells. Clyman (1966) found definite daily changes in the secretory cells in human. At the beginning of the cycle the cells had diminished mitochondria, and a small Golgi apparatus with a limited endoplasmic reticulum. During the cycle the mitochondria increased in number, and the endoplasmic reticulum increased in size. The Golgi apparatus became more tightly packed. Towards mid cycle the cytoplasm increased in volume, swelling the cell surface, and secretory granules appeared beneath the cell membrane. The mitochondria decreased in number, and the Golgi apparatus expanded. From day sixteen to eighteen some of the cells ruptured, extruding their contents into the tubal luman. Hashimoto et al found there were less secretory granules in the ampulla, and distinct secretory changes were found only in the isthmus.

Bajpai et al (1974), working on rabbits found variations in both secretory and ciliated cells. They also found differences between secretory cells of the ampullary and isthmic regions. The ampulla cells had a number of microvilli spread along the luminal surface at oestrous, whereas the isthmic cells are devoid of such structures. From 14 to 70 hours post coitum the microvilli of the ampullary cells become reduced in number, whereas from 24 hours post coitum those of the the isthmus become prominent. These changes would appear to coincide with the arrival of the ova in the different parts of the tube. The secretory granules are more electron dense in the ampulla than the isthmus.

Proliferative phase - the epithelium is tall and even, with ciliated and secretory cells approximately the same height. Ciliated cells are broader than the secretory, with a round nucleus and pale cytoplasm. Secretory cells are long and thin, with an elongated nucleus. x560



Midcycle - both secretory and ciliated cells have reached their peak heights, giving a smooth surface to the epithelium. x144

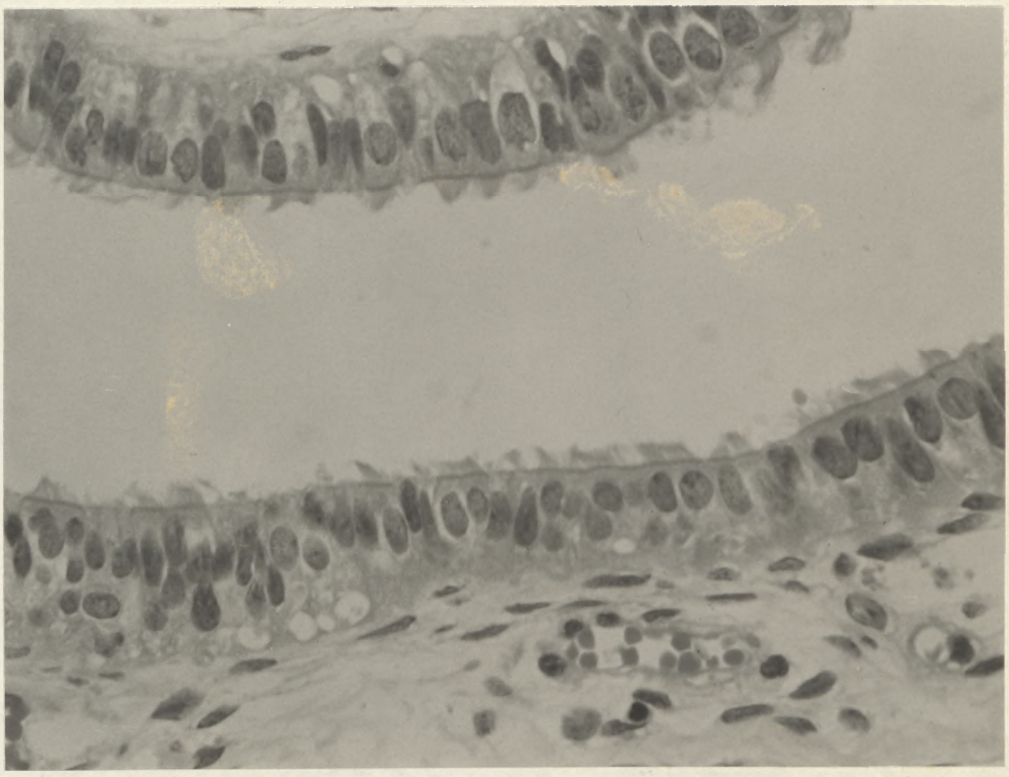


Plate VI

Secretory phase - the secretory cells are much higher than the ciliated, and protrude into the tubal lumen

x144

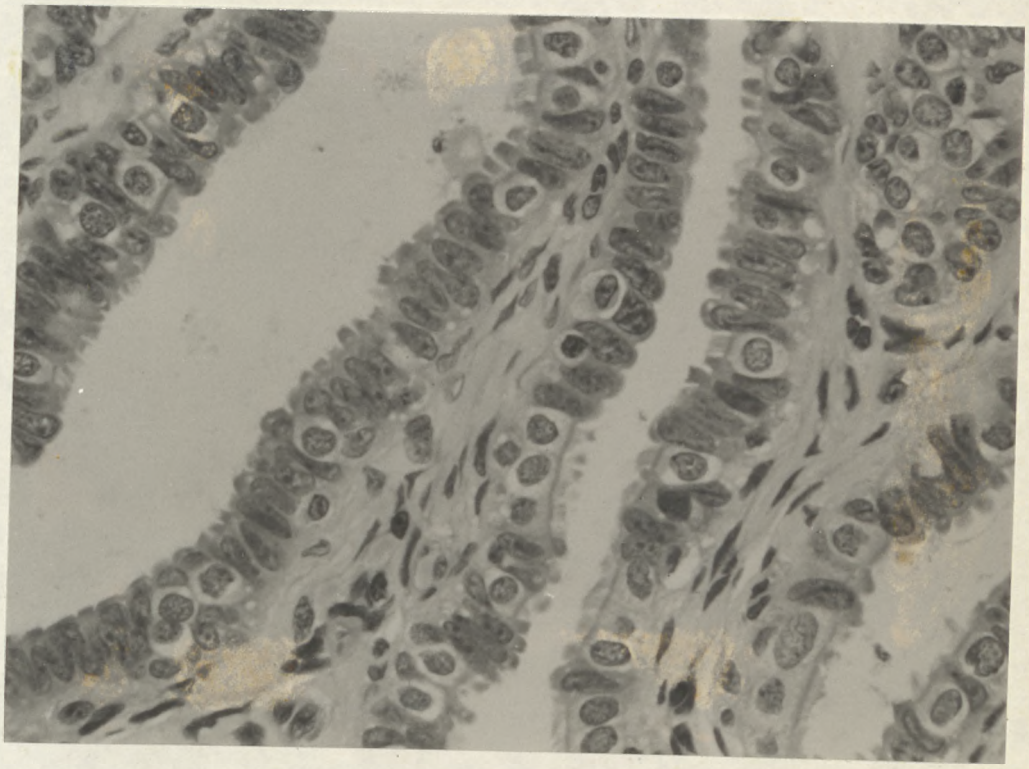


Plate VII

x560

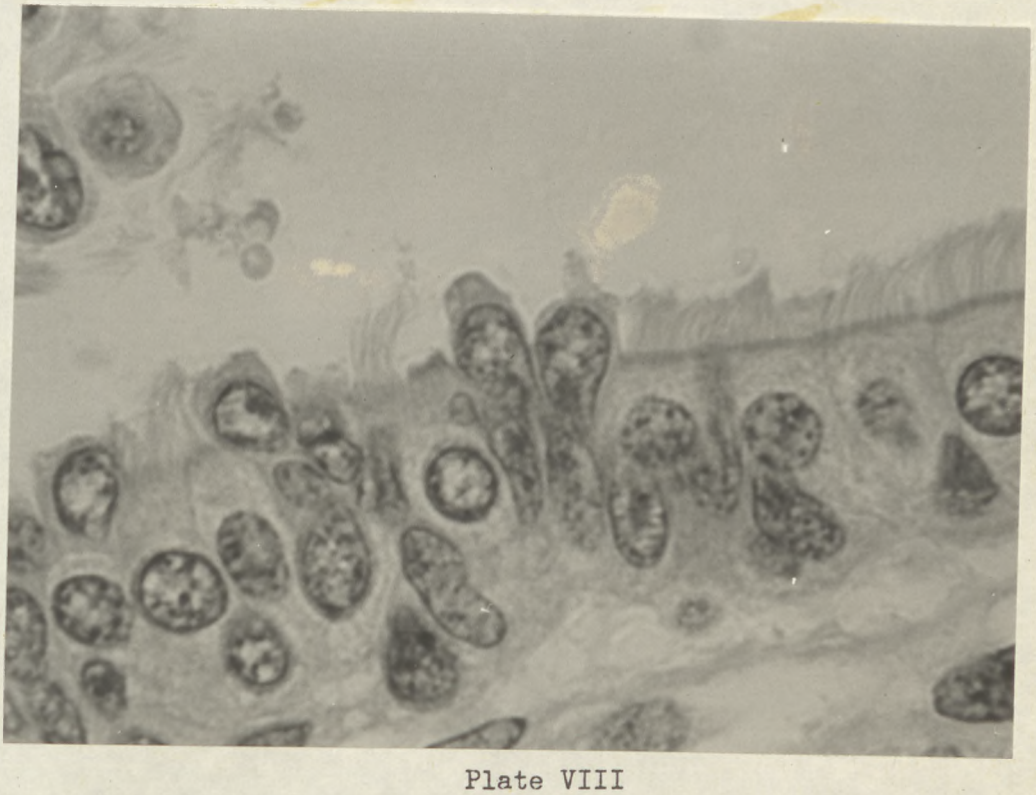


Plate VIII

Late secretory phase - the cupola formed on the secretory cells has broken into the tubal lumen x560

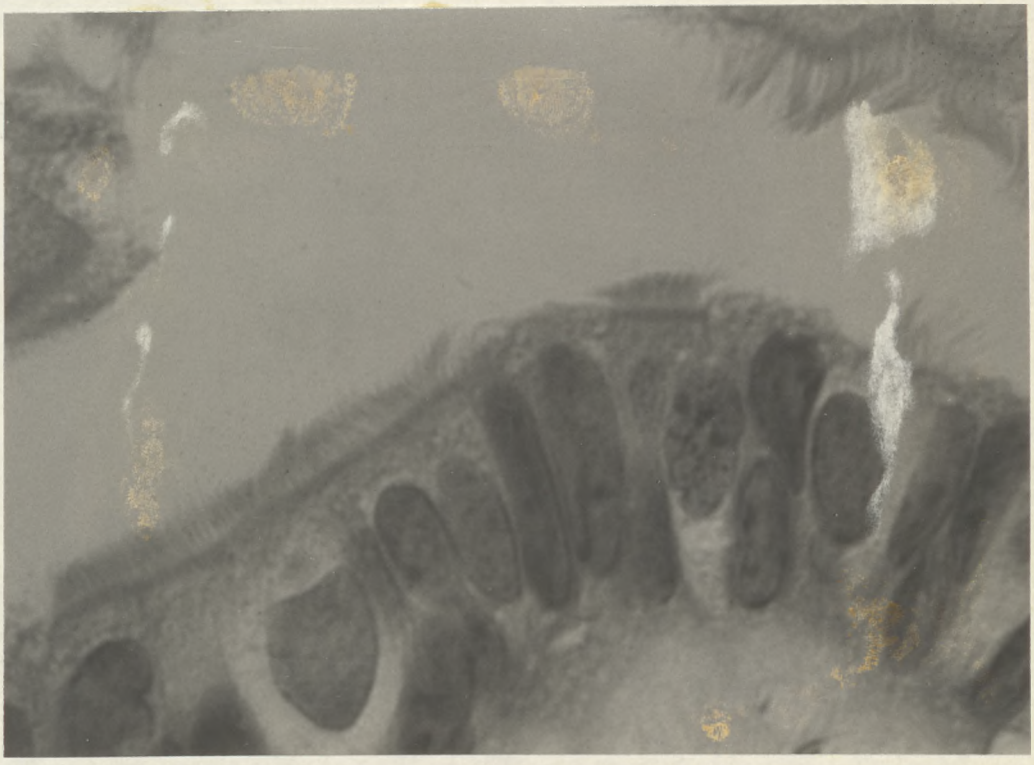


Plate IX

Ciliated cells x800

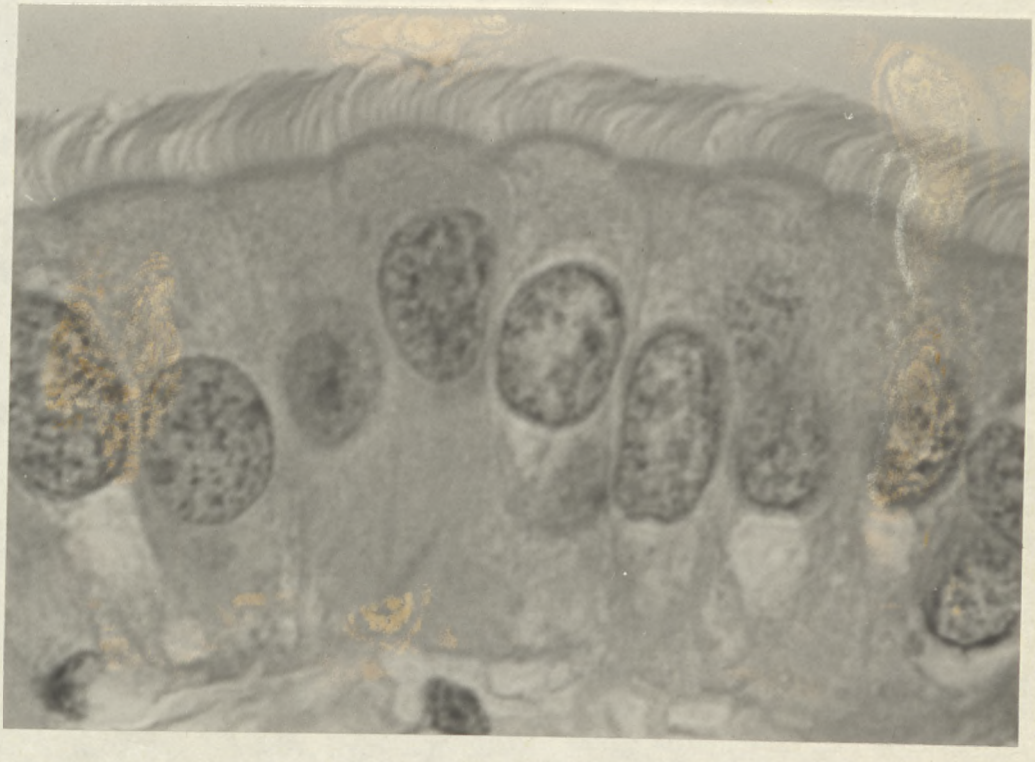


Plate X

Their position in the cell alters throughout the cycle, with a progression from the cell cytoplasm to the tubal lumen.

Shipstone et al (1974) found that the ciliation of the ampulla in the rabbit oviduct increased at 24 to 70 hours post coitum, whereas an increase in the isthmus occurred only at 70 hours. The number of ciliated cells remained constant, with only a difference in the density of cilia. The mitochondria showed similar changes. Their positions altered from supra-nuclear at oestrous, to becoming aggregated in the sub-apical region of the cell after ovulation. The ampullary mitochondria were increased in numbers, with well developed cristae at 24 to 70 hours post coitum, whereas those in the isthmus showed similar changes at 70 hours post coitum.

c.) Histochemistry of the tubal epithelium

Differences in the histochemistry of ciliated and secretory cells further supports the view that they are separate entities. Glycogen has been found in ciliated cells, but the majority of investigators have not demonstrated it in secretory cells (Joel 1939, Fredricsson 1959). Fawcett and Wislocki (1951) did find it in secrerory cells. Joel thought the glycogen appeared in the proliferative phase, and reached a peak at day 22. However Fredricsson found that the level decreased after ovulation, and then returned to the follicular phase levels around day 20. He also found glycogen on the ampullary cells during pregnancy. He thought that the glycogen acted as an energy source for ciliary activity, and found in the rabbit the decrease in levels after ovulation corresponded to the increase in ciliary activity noted by Borell at al (1957).

A PAS positive diastase resistant material has been found in the secretory cells of human, cattle, sheep, rat and mouse oviducts (Fredricsson 1957, Deane 1952, Hadek 1955). This secretion, probably a neutral mucopolysaccharide, is more readily found in the follicular and early luteal phase. Fredricsson noted that it was richest in the isthmus, where the greatest number of secretory cells are found, and least evident in the infundibulum. The exception was during pregnancy when the reverse applied. After menopause it was reduced in all areas of the tube. This material is probably concerned with the nutrition of the ovum through the tube.

Lipids have been found in both ciliated and secretory cells in humans (Fredricsson 1959, Fawcett 1951). They are found either as fine granules, or as vacuoles in the cytoplasm, and are most often present in the epithelium of the infundibulum, with the concentration reaching a peak in the mid-luteal phase.

Alkaline phosphatase was found on the secretory cell surface of humans but not in ciliary cells, by Fredricsson (1959). He found a decrease after ovulation, and also low levels during pregnancy, the puerperium, and post menopausal.

Non-specific esterase has been found in the cytoplasm of both secretory and ciliated cells (Fredricsson 1959), although a more widespread distribution was noted in the secretory cells. An increased activity was found after ovulation.

Amylase has been found in the epithelium (McGeachin et al 1958, Green 1957). This presumably degrades glycogen to provide nourishment for the developing ovum.

d.) Effect of artificially induced hormonal variations on cilia

Very little experimental work has been carried out on the effects on the cilia of experimental procedures such as hypophysectomy and ovariectomy with and without subsequent replacement therapy, and the hormonal treatment of intact animals.

Allen (1928) found that oviducts of untreated immature monkeys have very few ciliated cells, whereas immature oestrogen treated monkeys have a completely ciliated epithelium. He found also that in adult ovariectomized monkeys, oestrogen treatment produced patches of ciliated cells in the oviducts.

Brenner (1967) working with Rhesus monkeys found an almost complete loss of cilia from the oviduct after ovariectomy or hypophyectomy, but similarly to Allen, he found they were restored by oestrogen treatment.

Flerkó (1954) found a similar picture in rabbits. Three to four months after castration the oviducts had a complete loss of cilia. However after oestrogen treatment of 800 units over two weeks, the ciliated epithelium was completely restored.

Very little work has been carried out on the human. Andrews (1951) found that in postpasturient women the oviductal epithelium was low, with an average height of 16 μ. This becomes

lower during the postpartum period, with an average height of ten microns. However women given stilbestrol alone, in a dose of 5 mg/day for five to nine days after delivery have an intense proliferation of the ciliated epithelium, with an average height of twenty to twentyfive microns, compared with ten microns in untreated cases. Women receiving stilbestrol in combination with progesterone showed no change from the untreated cases. Similarly treatment with stilbestrol five to ten days before delivery produced no proliferation of the epithelium, and progesterone alone had no effect. It would appear here that the progesterone inhibits the effect of oestrogen on the growth of the cilia.

B. Egg transport through the fallopian tube

The passage of ova through the fallopian tube from ovary to uterus is still poorly understood. The mechanisms are complex and involve an interaction of many components.However there are thought to be three main forces responsible for the transport. These are:

1. Contraction of the musculature
2. Ciliary activity
3. Oviductal fluid

The ova have to pass through the different areas of the tube which as has been seen have a different musculature, vascular, and nerve supply. Thus transport through each part is not necessarily brought about by the some mechanism.

Transport from the surface of the ovary into the oviductal ostium

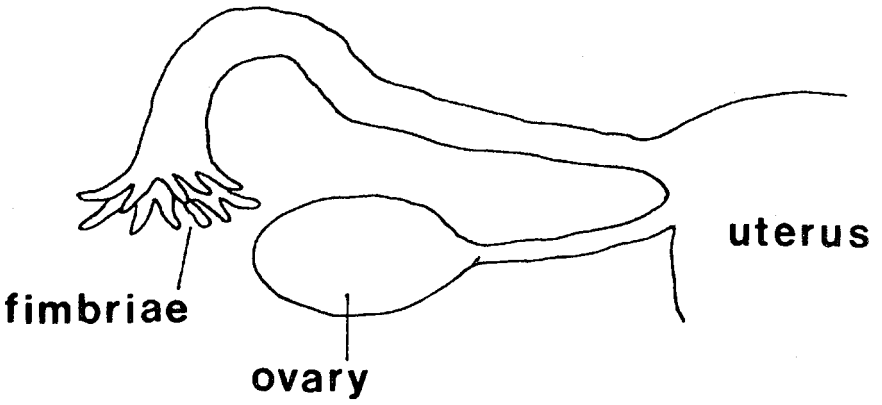
This is poorly understood due both to the short period of time it takes, and also to the technical difficulties involved in making direct observations. The mechanism also differs according to the anatomical variations of the infundibulum in different animal species.

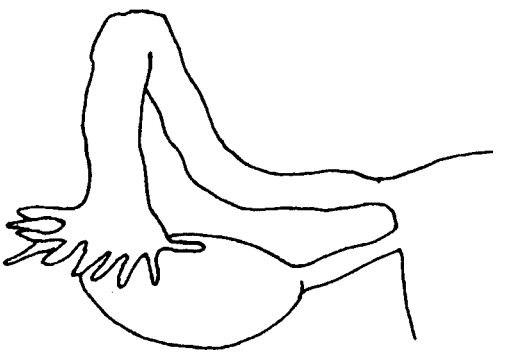
In the Mustelidae and Muridae, the ovaries are enclosed in a membraneous sac. The infundibulum projects only partly into this sac, and the fimbriated tip only partially touches the surface of the ovary (Alden 1942).

In rabbits, sheep, guinea pigs and primates the bursa is open. The contact between the fimbriae and the ovary varies with the stage of the cycle. At ovulation the fimbriae become engorged with blood and actively embrace the ovary. The musculature of the meso-ovarian, meso-tubarium, and tubo-ovarian ligaments also play an active part.Contraction of these ligaments cause the fimbria to slide over the surface of the ovary, and this together with the movements of the fimbriae enable very close contact to be maintained between ovary and tube.

Observa tions have mainly been carried out on the rabbit, and so from them the mechanism in humans must be hypothesised. The followihg description and experimental work is from observations in the rabbit, and it is possible that it is similar in the human due to the similarity in structure in th e two species.

Diagram to show movement of fimbriae over ovary at midcycle





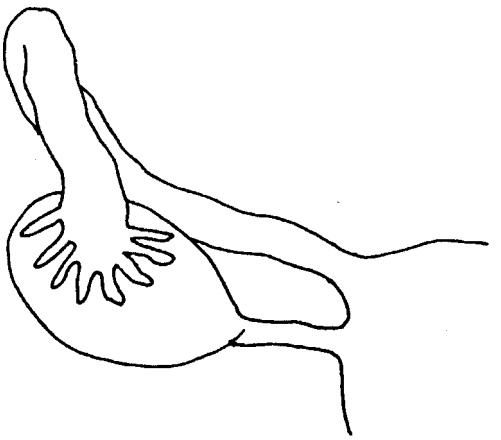


Fig.3

It has been suggested that the ova are transported into the tube by negative pressure being created in the fimbrial region by the vigorous peristaltic activity of the fimbriae and ampulla (Austin 1963). Thus when particles such as dyes are placed near the fimbriae, they are drawn into the tube. There is no experimental evidence that a suction is created, and it has been found that ovum uptake in rabbits and rats was still effective in spite of ligatures close to the infundibulum (Clewe and Mastroianni 1958).

It is probable that transport from the ovary to the oviduct is by means of the cilia. In the rabbit the eggs adhere to the follicles of the ovary and are picked up by the sweeping action of the fimbriae. Ova are carried into the ampulla by the ciliary currents caused by the cilia beating in the direction of the oviduct ostium (Blandau 1967).

If freshly ovulated rabbit eggs are denuded of cumulus and matrix, and are put onto the fimbriae, they are not transported but are rotated on the spot. It seems probable that the cilia hold the cumulus and matrix on the surface of the fimbriae (Blandau 1969).

The action of the cilia is strong enough to carry the eggs into the ampulla. There must be intimate contact between the eggs and cilia, and here the muscular contractions and the membranes attached to ovary and oviducts play a large part.

Transport through the ampulla

Fertilization takes place proximal to the isthmo-ampullary junction. The rate of transport through the ampulla to the site of fertilization is a matter for dispute. All the times given relate to the rabbit, as the rates for humans is unknown due to the ethical problems involved in experimentation.

Greenwald (1961) obtained a time of two hours. He induced ovulation in rabbits by mating them, removed the oviducts between twelve and seventy hours later, cut them into segments and examine d them for ova.

The time is probably much shorter than this. Harper (1961) removed fresh cumuli from rabbits, stained them, and reintroduced them into the oviducts. He then made a cine film of their movements, and found they reached the isthmo-ampulla junction

in from four to twelve minutes.

Similar times were obtained by Blandau (1969) using similar methods. The cine film showed the passage through the ampulla to be discontinuous, with considerable variations in the pattern of muscular contractions. These contractions were segmental, and were probably initiated by the presence of the cumulus. By observation the cilia seemed only directly responsible for the passage through the first few millimetres of tube, with muscle activity responsible for the rest. However as the complex folds of the ampulla are lined with numerous cilia it is possible the cilia may play some role, albeit an indirect one, in the transport of the eggs through the ampulla.

Isthmo-ampulla junction

Eggs are retained at this junction for two to three days following ovulation, although as has been mentioned earlier no anatomical sphincter has been demonstrated.

Transport through the isthmus

The ova remain in the isthmus for twentyfour to forty-eight hours, moving gradually towards the uterus. Little information on the egg transport has been obtained due to the thickness of the muscular walls which make it difficult to observe objects in the lumen. By the time the ova reach the isthmus they are free of the cumulus and corona radiata, thus making them even more difficult to see.

The isthmus has a complex pattern of movement, with almost continuous peristaltic and antiperistaltic motions. The epithelium has fewer ciliated cells, and is thrown up into fewer folds, and so there is less likelihood of the cilia playing much part in transport.

General theories relating to egg transport

There are three forces thought to be involved in ovum transport. These are: muscular contractions, ciliary action, and the oviductal fluid.

Muscular

This is probably the most important force in the movement of ova through the tube. As has been seen, the different sections vary in their musculature, and so there should be a difference in its contribution to transport. It would be expected that muscular contractions would play a greater part in the isthmus.

Cilia

The whole length of tube contains ciliated cells, but once again these vary in their distribution, and it would be thought, in the relative contribution to movement of ova.

Both muscular and ciliary activity will be expanded further in the relevant sections.

Oviductal fluid

This fluid is produced by the secretory cells. The effect on transport has been studied by Koester (1969), but the subject has been ignored by the majority of authors. Koester used radioactive sulphur given intravenously in rabbits. This is excreted by secretory cells, and so the amount of secretion can be determined using autoradiography. He found the main secretion was always in the mucosa of the isthmus. Secretion was maximal on the first postovulatory day, and was much decreased by the third.

Thus most secretion is in the narrowest part of the tube during the period of closure of the utero-tubal junction. Koester postulates that definite flow patterns are found inside the tube, with the direction of flow from isthmus to ampulla, and with the secretions of the ampulla having a lower velocity than those of the isthmus. In the ampulla the propelling force of the cilia is stronger than the velocity of the fluid, and so ova are propelled towards the uterus. However at the isthmo-ampulla junction the tube bore is smaller, the fluid velocity rises, the number of ciliated cells is decreased, and so the balance between cilia and flow of secretion is altered, which causes the egg to

remain static at the junction.

After three days the oestrogens are replaced by progesterone and secretion decreases. The ciliary stroke increases, and the ova can enter the isthmus.

Morphological changes in the epithelium also help in this process. Under the influence of oestrogen the secretory cells arch over the ciliary cells. Koester postulates that in the ampulla this has no effect due to the small number of secretory cells. However in the isthmus the effect is mechanical due to the large number of secretory cells. These occlude the tips of the cilia preventing contact with the ova. Under the influence of progesterone the ciliated cells project over the secretory, and can now exert their forward driving force.

This hypothesis supports the view held by Greenwald (1963) that it is a blockade at the ampullary-isthmic junction that regulates egg transport, and not muscular activity.

MUSCULAR MOTILITY

MUSCLE MOTILITY

Introduction

a.) The effect of hormones on egg transport in the tube

Activity of the oviduct at different stages of the cycle

It has long been realised that there are cyclic variations in the movement of fallopian tubes. Corner (1923) found that the tubal muscle of pig had small rapid contractions pre- and post-ovulation. Seckinger and Snyder (1923) found rapid contractions in the human tube at mid-cycle, and less frequent contractions at other times of the cycle. Repeated observations of vigorous contractions of the tube at the time of ovulation have since been reported in many species (Davids 1948 - human, Seckinger and Corner 1923 - monkey, Black and Asdell 1958 and 1959 - rabbit).

Various activity patterns have been described, mainly by the earlier workers and again with a lot of contradiction. A wave-like contraction was seen by Hirschberg (1924). Miculicz-Radecki (1925) found no peristalis in the human tube, whereas Kok (1926) described well defined peristalsis in the pig. He could find no movements adjacent to the fimbriated end, but in the proximal third of the tube found peristaltic waves gradually increasing in strength with contractions lasting up to one and a half minutes. He could find no movement for three days after ovulation.

Generally long distance peristaltic waves were seldom seen, but instead contractions were localized and only travelled short distances. The contractions were usually abovarian. Black and Asdell (1958) noted segmental contraction in the rabbit, but Rubin and Bendick (1926) reported writhing of the entire human oviduct.

Strong tubal activity for six to fourteen hours after ovulation was seen by Westman (1926), working with rabbit tubes. This was followed by a period of relaxation while the ovum was in the tube. Similarly Burdick et al (1942), also using rabbits, found that the entire tubal musculature was more relaxed when the ovum was in the ampulla. They also noted peristaltic and

anti-peristaltic movements of the tube which may have been missed by earlier observers. More recently Blandau (1966) described segmental contractions producing a "forward-backward motion".

From these observations it would appear that the endogenous hormones play a part in initiating tubal movement.

Hormones present during the menstrual cycle in the human

In order to evaluate experiments involving exogenous hormones and to elucidate the effects of endogenous hormones, it is necessary to know the amounts of hormones present during the menstrual cycle. These are summarized in table 1.

Before menstrual bleeding begins there is an increase in luteinising hormone (LH) and follicle stimulating hormone (FSH), and a new set of follicles start to develop. Oestrogen and progesterone are at a low level. In the middle third of the follicular phase, the amount of oestradiol begins to increase slowly. At about day ten there is a more rapid increase, and a peak is reached about day fourteen, a day before a LH peak. LH also has a slow rise corresponding to that of oestradiol, and at the same time FSH levels slowly fall. Both LH and FSH have a rapid increase about day thirteen, and reach a peak at day fifteen. Ovulation follows sixteen to twentyfour hours later. After the peak of oestradiol, this hormone undergoes a rapid fall, to reach its lowest level about fortyeight hours after the LH peak. It then begins to rise again to give a maximum about day nineteen, in the middle of the luteal phase, although these levels are about half that of the first peak.

After ovulation the levels of LH and FSH both drop rapidly at first, and then more gradually, to reach a low a few days before menstruation.

Progesterone has a low concentration in the blood until a day before the LH peak. Levels then begin to rise, and a peak is reached about day nineteen, corresponding to the second oestadiol peak. After three days it undergoes a rapid decline, becoming barely detectable at day one of menstruation.

Graphs to show serum concentrations of gonadotropins and sex steroids during normal 28-day human menstrual cycle

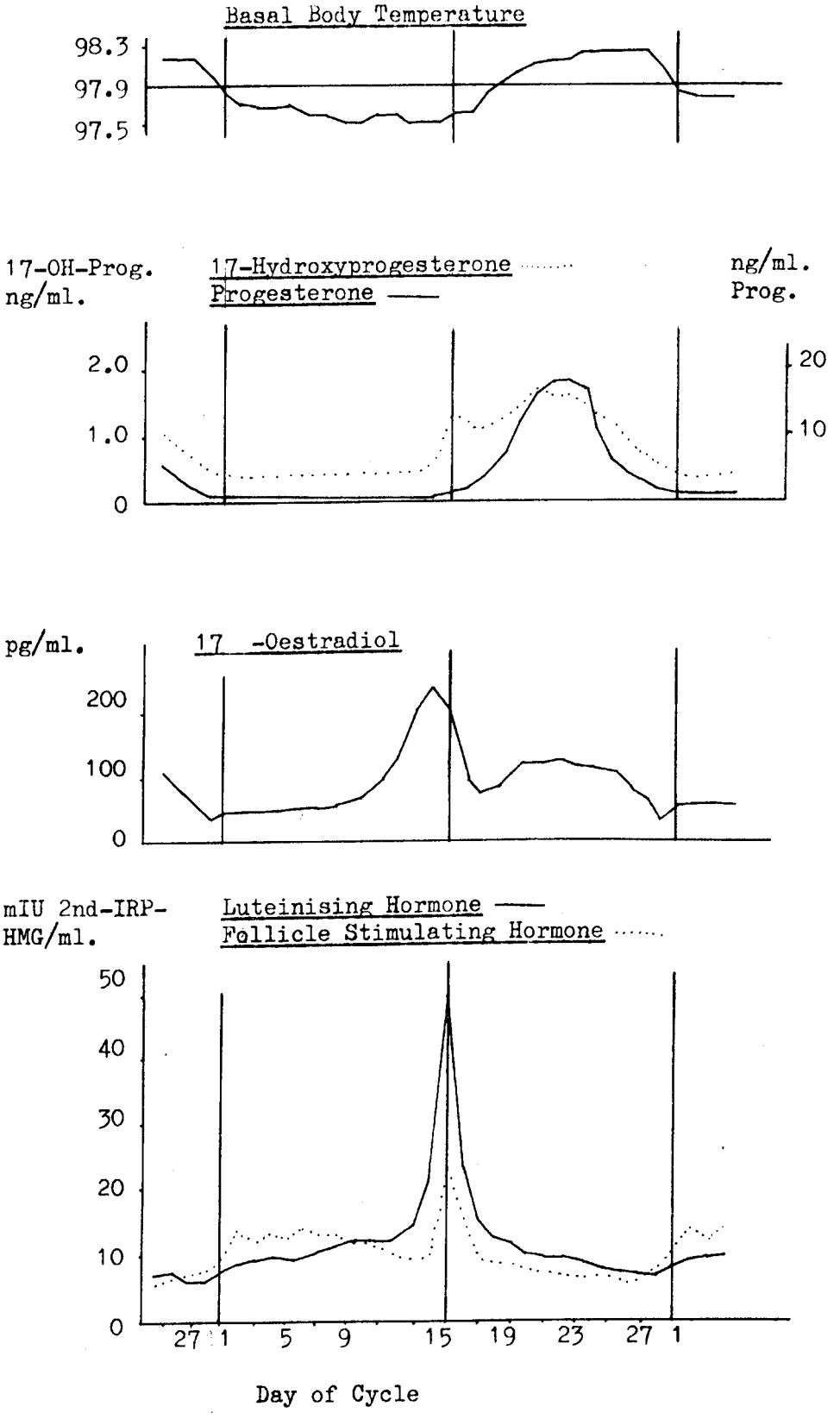


Table 1 - Midgley et al (1973)

Levels of Sex Steroids found in Peripheral Blood in Women

Progesterone

	ng/ml
Proliferative	1.0 - 5.3
Secretory	6.0 - 20.0
Ovariectomized women	0.39 - 1.0
Pregnancy 11 - 42 weeks	20.0 - 300.0
Postpartum day 1	3.5 - 6.5
2 - 3	1.0 - 2.0

Oestrogens

	Oestrone pg/ml	Oestradiol pg/ml	Oestriol pg/ml
Proliferative			
End of flow	230	100	
"normal" follicular	200	130	250
Secretory			
Midcycle	580	260	
Ovulation peak	700	280	370
Pregnancy			
12 - 13 weeks	2680	910	1060
37 weeks - term			20,000

(Gold 1968, Fuchs and Klopper 1971)

17-hydroxyprogesterone has a higher blood level than progesterone. Levels are constant until it undergoes a first increase, corresponding to the increase in LH and FSH. At ovulation the level drops slightly, but then undergoes a second increase and decline at the same time as oestradiol and progesterone.

Effect of exogenous hormones on egg transport

Many experiments have been carried out in an attempt to elucidate the effect of exogenous hormones on egg transport. The experiments have, by necessity been performed mainly on small laboratory animals,chiefly rabbits which are ideal as they are reflex ovulators. Generally the experiments follow a similar plan, with the transport of ova and/or ovum models being examined. Control rabbits are killed at different times after mating or ovulation induced by the injection of human chorionic gonadotropin. The oviducts are removed, and searched for the ova, and so a normal rate of transport can be arrived at. Test animals are subjected to various procedures, for example the pre-ovulation injection of hormones, inducing ovulation, and determing the rate of transport through the tubes.

Unfortunately the results have been contradictory, and a few examples of the results obtained show the conflicting ideas.

1. Injected oestrogen causes a phenomenum termed "tube locking". Egg transport is prevented in intact rabbits and mice (Burdick and Pincus 1935).
2. Large doses of oestrogen accelerate egg transport in mice (Greenwald 1967).
3. Small doses of oestrogen injected at the time of mating cause acceleration of egg transport in rats (Wu et al 1971).
4. Large doses of oestrogen injected at the time of mating cause retention of eggs for up to six days (Greenwald 1961).
5. Progesterone injected into spayed rabbits restored egg transport to that of an animal at oestrous (Wu et al 1971).
6. Progesterone injected into spayed rabbits caused a delay in egg transport (Bjork 1959).

7. Progesterone injected into rabbits three days before induction of ovulation by gonadotropins accelerates transport (Chang 1966).
8. Progesterone injected into rabbits before ovulation has no effect on transport (Langley et al 1968, Black and Asdell 1959).
9. Increased progesterone retards egg transport through the ampulla due to the depressant effect on muscle activity in rabbits (Harper 1966).
10. Egg transport is not normal if ovaries are removed one to twelve hours after finding the vaginal plug in mice (Whitney and Burdick 1939).
11. Oviduct muscle remains relatively quiet under the influence of oestrogen continuosly present in small (physiological) quantities (Boling and Blandau 1968).(Working on rabbits).
12. Oviduct muscle which has remained relatively quiet under the influence of oestrogen begins to contract more vigorously when oestrogen is withdrawn (Boling and Blandau 1968).
13. Oviduct muscle that has remained relatively quiescent under the influence of oestrogen begins to contract vigorously a few hours after the injection of progesterone (Boling and Blandau 1968)

This controversy has arisen mainly because unphysiological dosed of hormones have been used in some of the experiments. These cause unphysiological results. Thus oestrogen can cause both tube-locking in the ampulla, and accelerated transport to the uterus depending on the species of animal used, the time of treatment, and the dose and potency of the hormone. It is usually assumed that oestrogen is directly and primarily responsible for increased muscle activity, however in the normal pre-ovulatory animal, when oestrogen is at a physiological level the oviducts are quiescent (Boling and Blandau 1968). Muscle contraction may be initiated at the time of oestrogen withdrawal, and in fact reactions attributed to oestrogen may be associated with their withdrawal, rather than as a result of their direct action.

Normal transport probably depends on a balance between oestrogen and progesterone. Blandau (1966) considered that oestrogen was the primer, and progesterone the mover of the tubal musculature.

Experiment 1. Spontaneous motility of the Fallopian tube at different stages in the menstrual cycle

Materials:

Fallopian tubes were obtained from women undergoing a salpingectomy either at the same time as hysterectomy, or for sterilisation purposes in the puerperal period. Only tubes with a normal macroscopic appearance were used. In ovulating women, the tubes were classified according to the phase of the cycle by taking into account the woman's menstrual pattern and the endometrial histology.

The numbers of tubes examined were as follows:
17 proliferative, 13 secretory, 17 puerperal, 6 pregnant, 6 post-menopausal

Method:

After removal the tubes were put immediately into Krebs solution (see appendix) at a temperature of 37°C. The tube was tested as soon as possible after removal. A length of tube approximately two cms. was taken from either the isthmic or ampullary end of the tube. It was dissected free of the peritoneal covering and underlying connective tissue, and a loop of cotton tied around each end. This preparation was transferred to a ten ml. organ bath filled with Krebs solution, and kept at a temperature of 37°C by means of a thermostatically controlled water bath. The organ bath was continuously aerated by bubbling through a mixture of 95% oxygen and 5% carbon dioxide. A resting tension of five grams was placed on the preparation, and a period of about thirty minutes allowed for the spontaneous motility to become established as a constant wave form. The Krebs solution was replaced at ten minute intervals. The spontaneous motility was recorded isometrically with a Leeds and Northrup recorder which charted the amplified responses.

Detail of organ bath set-up

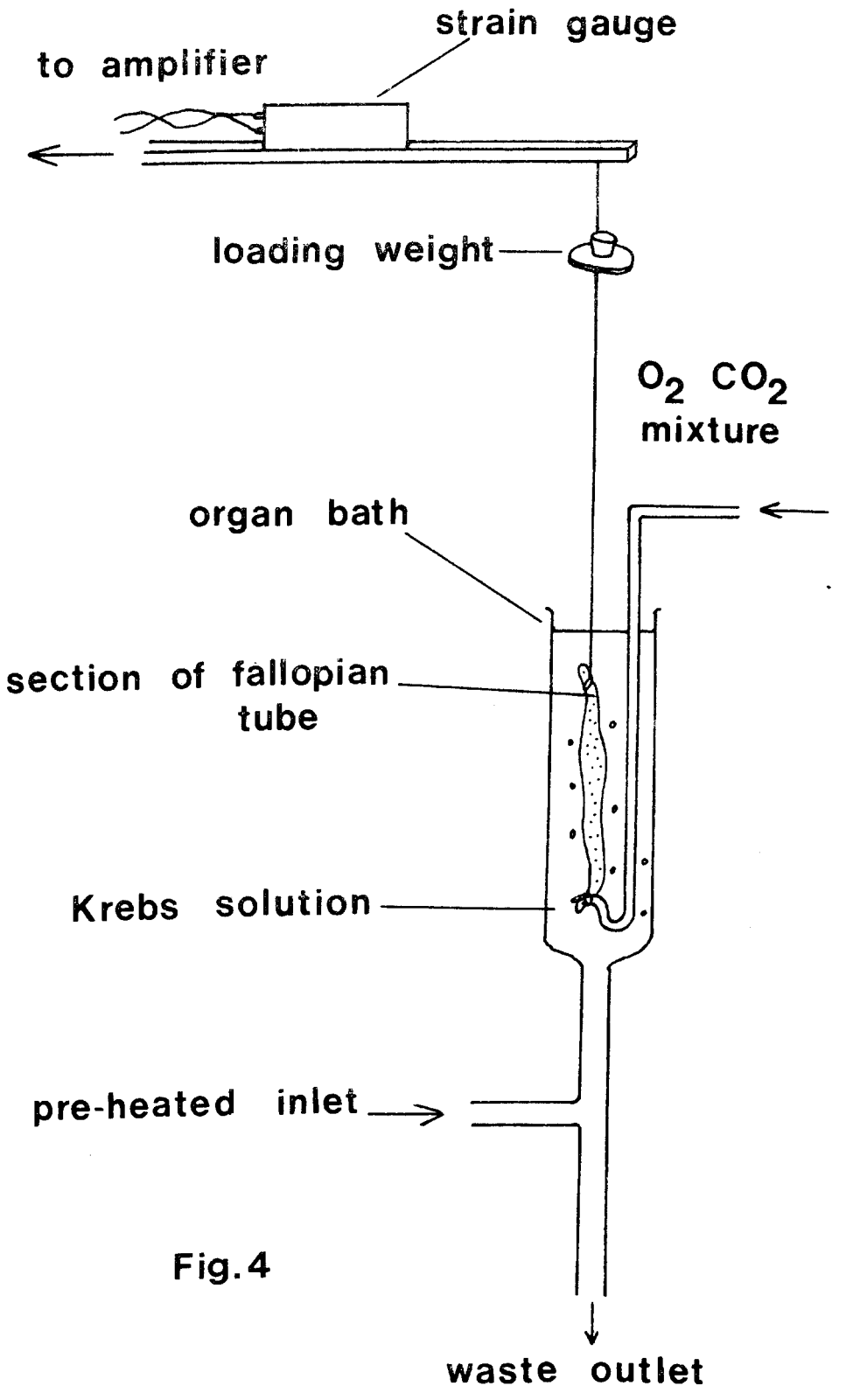


Fig.4

Parameters used in the measurement of the response

Spontaneous motility

Three parameters were used in the measurement of the spontaneous motility.

1. Frequency

The number of contractions during a standard time of three minutes. This standard time corresponded to a distance of one centimetre on the recorder tracing.

2. Average intensity

The average height of all contractions over one millimetre during the standard time.

3. Activity units

The product of average intensity and frequency.

Experiments 2 and 3

Five parameters were used. In addition to the three described above, two extra were used.

4. Tonus

The measurement of the variation of the base line.

5. Amplitude maximum

The measurement of the variation of a line drawn through the highest point of all contractions.

Both tonus and average intensity have two results, the first and second measurement. On addition of a drug there is often an immediate response followed by a modified response.

All parameters were measured from the recorder tracing.

Expression of results

Spontaneous motility

The results for each parameter as shown in the appendix are direct measurements from the recorder tracing.

Experiment 2.

For every parameter, for example frequency, the results obtained for the spontaneous motility were subtracted from the results obtained after the addition of the drug. Thus if the drug caused an increase in frequency the end result was positive, if a decrease resulted, the result was negative. For the tonus, the spontaneous motility was taken as zero.

Experiment 3.

The final result was obtained from four readings. Thus the response of the tube to a drug (reading a.) was found as in experiment 2. The response to a drug under continuous hormone stimulation (b.) was found in a similar way, by subtracting the result for each parameter from the spontaneous motility. The figures for a. were subtracted from the figures for b. to give the end result. This end result was either positive, showing that the hormone enhanced the response that was obtained by using the drug alone, negative, showing that the response was diminished, or zero, indicating that there was no change in the response.

The method of obtaining the results can be expressed as follows:

Experiment 2.

Result = figure obtained after addition of drug - figure obtained for spontaneous motility

Experiment 3.

Result = (figure obtained after addition of drug + hormone - spontaneous motility + hormone (b.)) - (figure obtained after addition of drug - figure obtained for spontaneous motility (a.))

Histograms to show spontaneous motility of human fallopian tubes

Fig.5

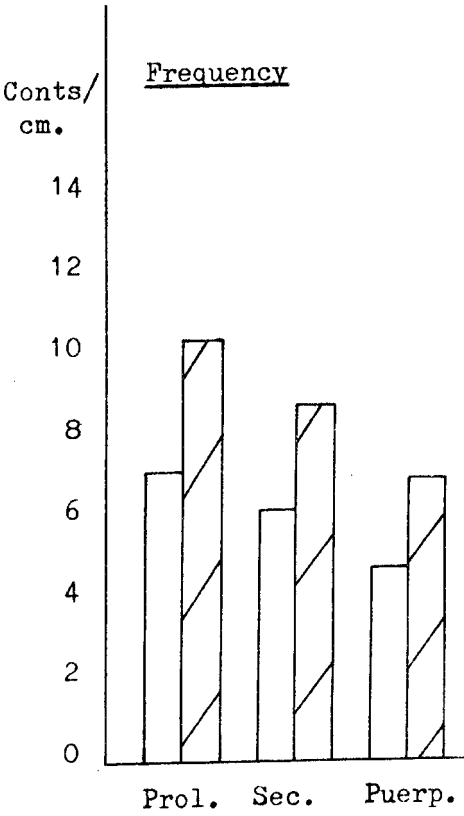
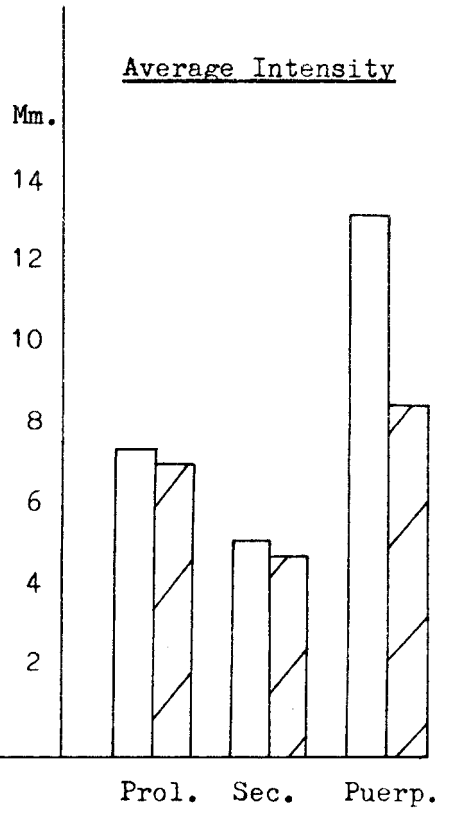


Fig.6

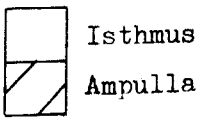


Significant results:

Proliferative, Secretory, and Puerperal - ampulla > isthmus

Ampulla - Proliferative > Puerperal

Isthmus - Proliferative > Puerperal



Spontaneous Motility

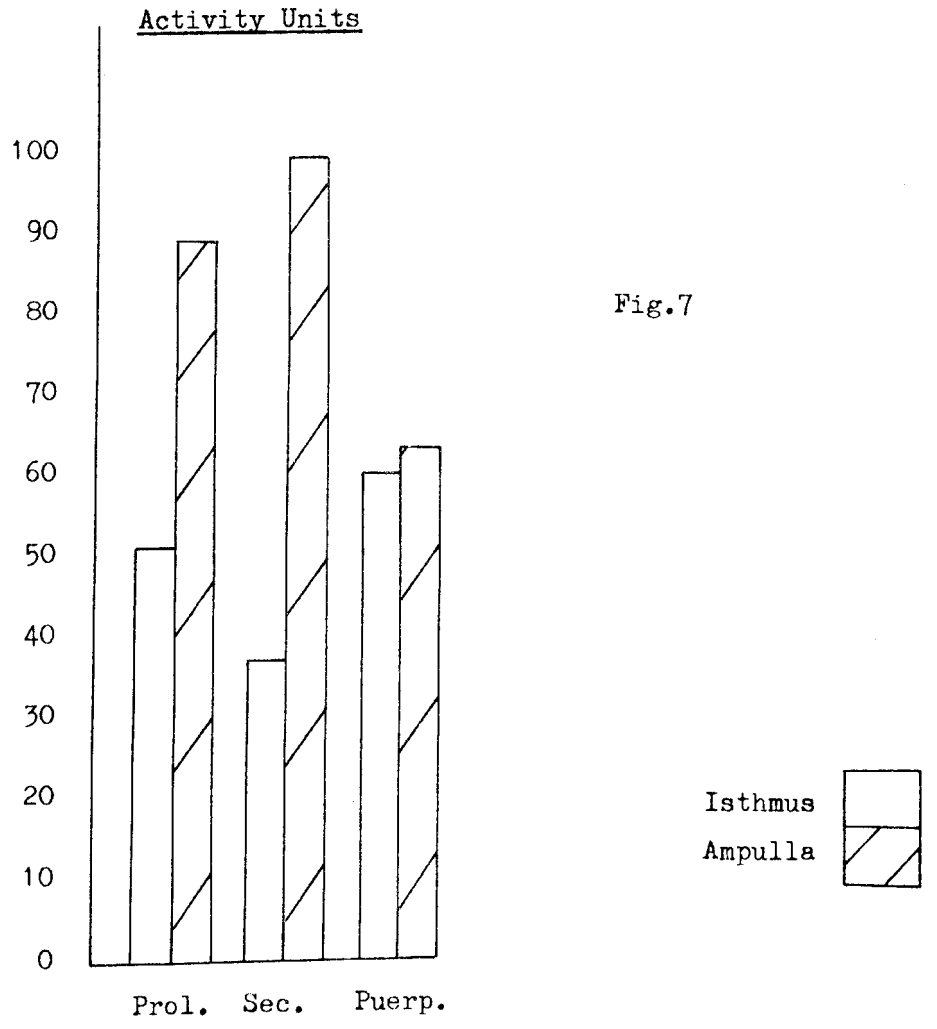


Fig.7

Significant results:

Proliferative, Secretory - ampulla isthmus

Ampulla - Secretory Puerperal

Isthmus - Puerperal Secretory

Spontaneous motility of the human Fallopian tube - Results

In all tubes the ampullary region contracted at a greater frequency than the isthmic site, differences being statistically significant in all cases. The frequency of the proliferative tubes was greater than the other two environments, with the puerperal tubes showing the least frequency. The difference was significant between proliferative and puerperal tubes in both regions.

The average intensity of contractions showed less difference between regions in any of the environments, although the intensity of the isthmus of the proliferative and puerperal tubes was greater than the ampulla. However the intensity of the ampulla from the secretory phase was significantly greater than the isthmus. There was no difference in the intensity of tubes from the different environments.

There was a difference between the activity units in the two regions. In all cases the results for the ampullary region were greater than the isthmic, this being significant in the proliferative and secretory tubes. The activity of the isthmic region of the tube was greatest during the puerperium, and lowest during the secretory phase. The difference between these two phases was significant. However the secretory tubes showed the greatest activity of the ampullary region, with the puerperium the least. Once again the difference between secretory and puerperal tubes was significant.

Discussion

The results suggest that there are definite differences in oviducal muscular activity between the ampullary and isthmic regions of the tube, and between tubes under different hormonal conditions. The frequency of the ampullary region was significantly greater than the isthmic region in all tubes, and the activity units of the ampulla from menstruating women were also higher than the isthmus. The secretory phase was the only group that showed a lower average intensity in the isthmus.

When considering the effect of the hormonal environment on the spontaneous motility, although there are small differences in the results between groups, they only become significant between menstruating and puerperal tubes, with the latter results lower than the former. It appears that throughout the cycle there are continuous small contractions of the tube, but they do not increase in the secretory stage, after ovulation. Black and Asdell (1958), working on the rabbit tube found that contractions were uniform until the fourth day post-ovulation, when they became less regular, and the intervals between them increased. Earlier investigators Breipohl (1938), and Wislocki and Guttmacher (1924), working with human and sow respectively, both found an increase in contractions in the proliferative phase, and a peak at ovulation, with a slow decrease during the luteal phase.

Method used for the statistical analysis of muscle motility results

Spontaneous Motility

For each parameter the results of every hormonal environment i.e. puerperal, proliferative, and secretory, and every site i.e. isthmus and ampulla, were compared, using an analysis of variance. This gives a figure for the probability of there being a significant difference between:

1. Sites for that parameter
2. Hormonal environments
3. An interaction between sites and hormonal environments

In the case of a difference being found, for example between sites for the parameter of frequency, the sites for each hormonal environment are compared with each other by the Student's t test. Thus the frequency of the ampullary puerperal tubes is compared with the frequency of the isthmic puerperal tubes etc.

Results of statistical analysis

Spontaneous motility

Frequency

Analysis of variance

	s.s.	d.f.	m.s.	v.r.	test
Inter-action sites & hormonal environments	391.29	5	78.26	13.40	P 0.0001
Between sites	220.60	1	220.60	37.77	P 0.0001
Between hormonal environments	143.34	1	143.34	24.54	P 0.0001
Residual	514.03	88	5.84		
Total	905.32	93			

Average Intensity

	s.s.	d.f.	m.s.	v.r.	test
Int. sites & hormonal environments	45393.6	5	9078.7	5.18	P 0.001
Between sites	29420.9	1	29420.9	16.80	P 0.001
Between hormonal environs.	852.6	1	852.6	0.49	P N.S.
Residual	154167.4	88	1751.9		
Total	199561.0	93			

Activity Units

	s.s	d.f.	m.s.	v.r.	test
Int. sites & hormonal environs.	663.3	5	132.7	2.92	P 0.05
Between sites	0.1	1	0.1	0.00	N.S.
Between hormonal environs.	189.2	1	189.2	4.16	P 0.05
Residual	4001.5	88	45.5		
Total	4664.8				

Differences in spontaneous motility

Particular differences between sites and hormonal environments have been tested for by the Student's t test.

Differences between sites

In each case the isthmic region has been compared with the ampulla.

	t value	probability
Frequency		
Proliferative	3.2655	0.005
Secretory	3.7837	0.005
Puerperal	4.1845	0.005
Average Intensity		
Proliferative	1.999	N.S.
Secretory	3.300	0.005
Puerperal		
Activity Units		
Proliferative	2.1992	0.05
Secretory	5.0532	0.005
Puerperal	0.0444	N.S.

Spontaneous Motility - Differences between hormonal environments

In each case two hormonal environments have been compared with each other to determine any significant difference between populations.

	t value	probability
Frequency		
Ampulla - Proliferative/Secretory	1.3855	N.S.
Proliferative/Puerperal	4.1140	0.005
Isthmus - Proliferative/Puerperal	3.0967	0.005
Proliferative/Secretory	1.1087	N.S.
Activity Units		
Ampulla - Proliferative/Secretory	0.5769	N.S.
Proliferative/Puerperal	1.6329	N.S.
Secretory/Puerperal	2.4658	0.025
Isthmus - Proliferative/Secretory	1.0782	N.S.
Proliferative/Puerperal	0.6121	N.S.
Secretory/Puerperal	2.5036	0.025

Experiment 2. Effects of drugs on the motility of the Fallopian tube at different stages in the menstrual cycle

Materials:

These were the same as those used in experiment 1.

Method:

A muscle preparation was set up in the organ bath as described for the spontaneous motility. After this motility had been recorded for about twenty minutes, the organ bath was emptied and replaced with one of three drugs, at a temperature of 37°C. Changes in motility were continually recorded. After five minutes the drug was removed, and the tissue washed out with fresh Krebs solution. The spontaneous motility was then recorded for about twenty minutes with regular changes of Krebs solution, before a second drug was added. No drug was added until the spontaneous motility was back to normal.

This whole procedure was repeated, adding a total of three drugs, and testing both ampullary and isthmic regions of every tube.

Drugs tested:

Acetylcholine chloride
Adrenaline acid tartrate
Nor-adrenaline acid tartrate

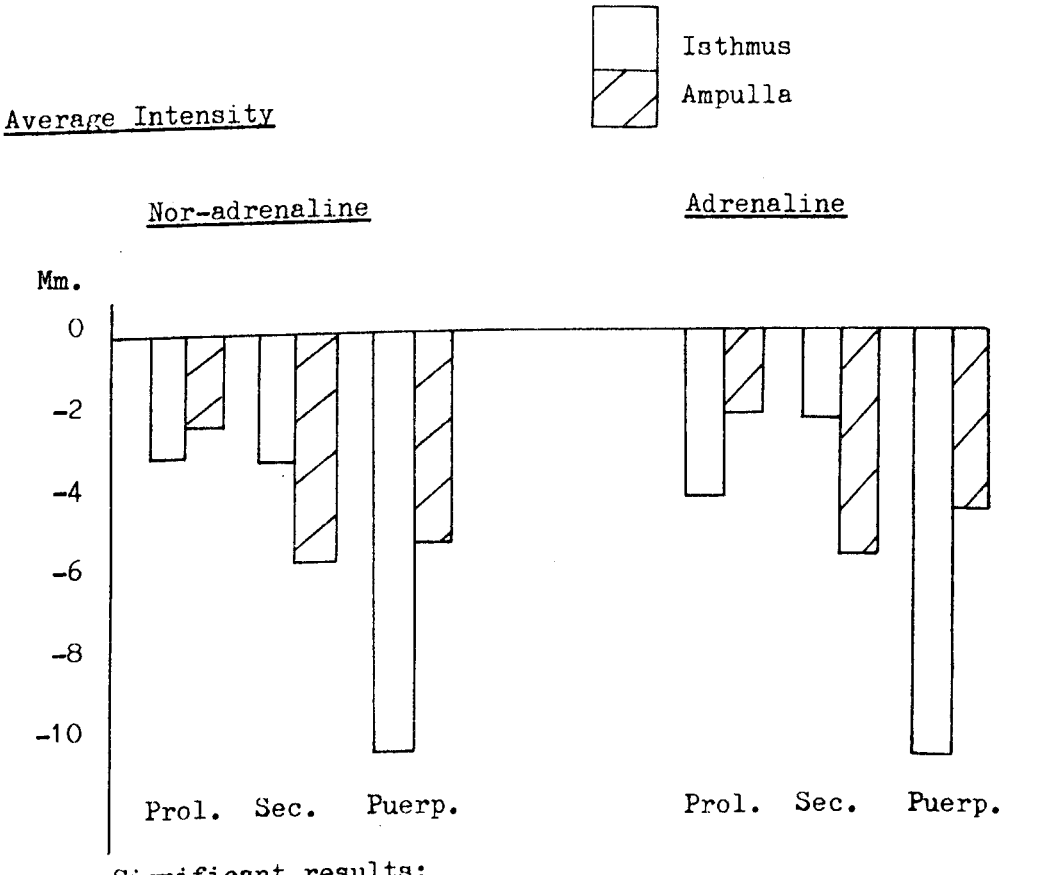
These were all given in a dose of 1.0 µg per ml., and all made up in Krebs solution. This dose was used following experiments of five tubes in each group, in which doses of the drugs from 0.5 µg per ml., to 5.0 µg per ml. were found to produce similar results. The doses are expressed in terms of the base.

To avoid misleading reults due to drugs always being added in the same order, the following sequence was used:

1. First tube in each group:
Nor-adrenaline, acetylcholine, adrenaline
2. Second tube in each group:
Adrenaline, nor-adrenaline, acetylcholine
3. Third tube in each group:
Acetylcholine, adrenaline, nor-adrenaline
4. Fourth tube in each group:
Acetylcholine, nor-adrenaline, adrenaline

Histograms to show the effects of drugs on the human Fallopian tubes at different stages in the menstrual cycle

Due to the large numbers of results, to avoid confusion only those groups of results showing a significant difference have been represented by histograms. Parameters or drugs not shown have not been altered appreciably, or have had little effect.
The results for the pregnant and post-menopausal tubes have not been shown by histograms as they were not statistically analysed due to the small numbers in each group.



Significant results:

Isthmus - Prol. Puerp.

Fig.8

Isthmus - Prol. Puerp.
Sec. Puerp.

Fig.9

Tonus - first measurement

Fig.10 Nor-adrenaline

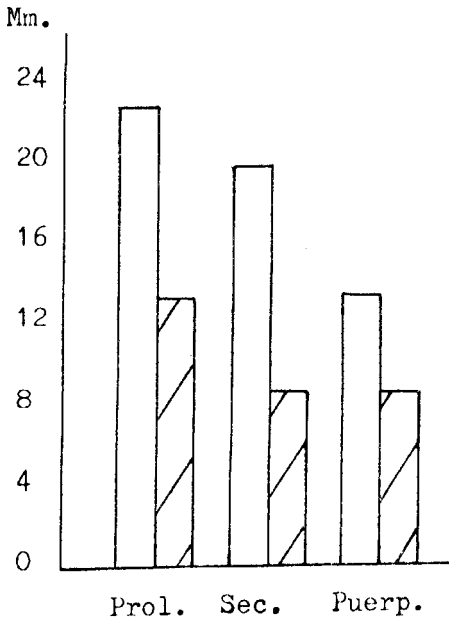
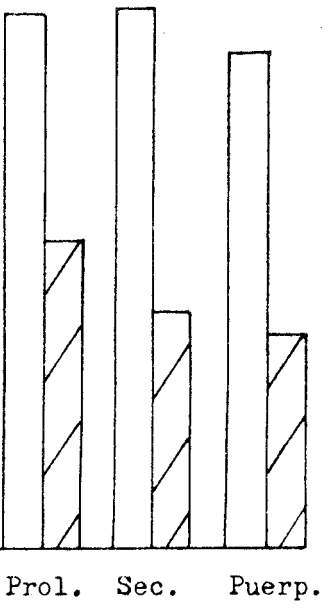


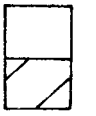
Fig.11 Adrenaline



Significant results:

Secretory - isthmus > ampulla

All isthmic regions > ampulla



Tonus - second measurement

Fig.12 Nor-adrenaline

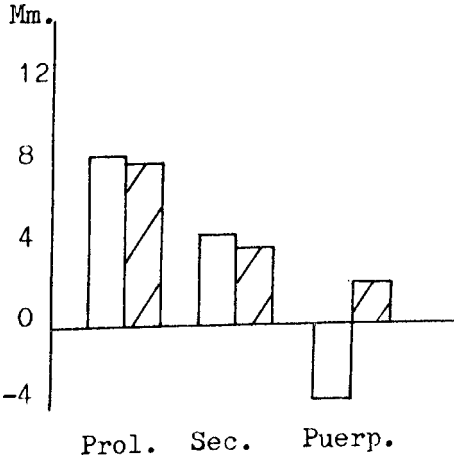
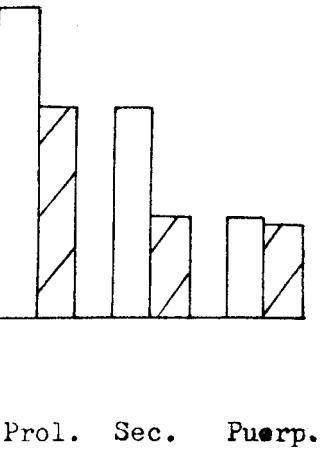


Fig.13 Adrenaline



Significant results:

Isthmus - Prol.> Puerp.

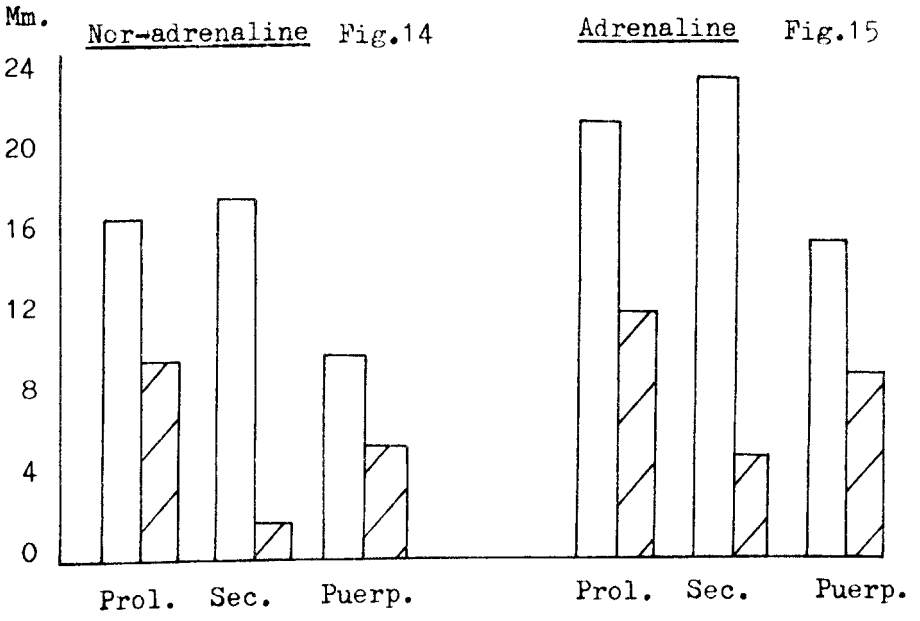
Ampulla - Prol.> Puerp.
Prol.> Sec.

Secretory - isthmus> ampulla

Isthmus - Prol.> Puerp.

Ampulla - Prol.> Puerp.
Prol.> Sec.

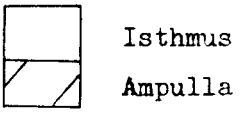
Amplitude Maximum - first measurement



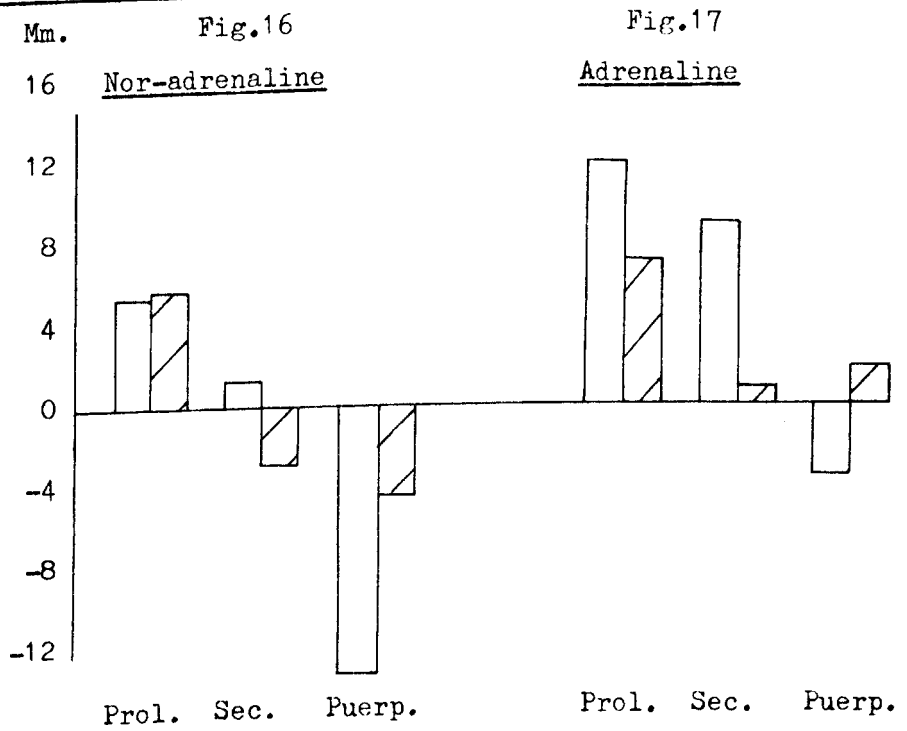
Significant results:

Secretory - isthmus > ampulla

Secretory - isthmus > ampulla



Amplitude Maximum - second measurement



Significant results:

Puerp. - ampulla > isthmus
Isthmus - Prol. > Puerp.
Sec. > Puerp.

Sec. - isthmus > ampulla
Isthmus - Prol. > Puerp.
Sec. > Puerp.
Ampulla - Prol. > Sec.

Results

Nor-adrenaline

The frequency of contractions was statistically unaffected in any preparation although an increase was produced in every tube except the proliferative isthmic region. There was no statistical difference between either hormonal environments or regions of tube.

The average intensity was decreased in all tubes. There was no statistical difference between sites, but the puerperal isthmic tubes had a lower amplitude than the proliferative isthmic.

The activity units also showed a fall in all tubes, but there was no difference between either sites or hormonal environments.

The tonus was increased significantly in all tubes from menstruating women, there being a large initial increase followed by a drop to a lesser but still significant increase in all sections. The puerperal tube produced a different pattern, with an initial increase in tonus in both ampullary and isthmic regions, but then followed by a decreased tone in the isthmic part. The first measurement was not significantly different between environments, but the isthmic region of the secretory tubes showed a greater increase than the ampulla. Other site differences were non-significant. The second measurement also had significant differences between isthmus and ampulla of the secretory tubes, and also between the isthmic region of proliferative and puerperal tubes, and the ampulla of proliferative/puerperal, and proliferative/secretory tubes, the proliferative having a greater tonus in all cases.

Adrenaline

The frequency was increased in all tubes except the isthmus of the secretory, but there were no site or hormonal differences.

Average intensity was decreased in all tubes. There were no site differences, but the intensity of the puerperal tubes was significantly lower than the proliferative and secretory.

The activity units were significantly decreased in all tubes, but there was no difference between sites or hormonal environments.

The first measurement for tonus showed an appreciable increase in all tubes, decreasing in all to a smaller but still significant increase. There were significant differences between sites in all three environments, the isthmus having a greater increase. As with nor-adrenaline, the proliferative isthmic region had a greater increase than the isthmic puerperal, and similarly with the ampullary region of the proliferative/puerperal, and proliferative/secretory tubes.

The first reading for amplitude maximum showed an increase, but the second, while showing an increase for the proliferative phase tubes, and the isthmic part of the secretory tubes, showed no significant change from baseline in the remainder. The first measurement showed a difference between proliferative and secretory isthmic and ampullary regions, and the second measurement showed a difference between the sites in the secretory tubes. The isthmic region always had a greater reading than the ampullary. There was also a difference between proliferative/puerperal and secretory/puerperal in the isthmic region of the second measurement, the puerperal tubes showing a decrease. The proliferative ampullary tubes had a greater increase than the secretory ampullary.

Acetylcholine

The frequency was not altered significantly. The average intensity was slightly decreased in all tubes except the isthmic puerperal, although this was only significant in the isthmic secretory.

The activity units were decreased in all tubes except the isthmus of the proliferative and puerperal.

The tonus was increased for all types of tube, in both measurements.

The amplitude maximum was increased in all tubes, but the increase was only significant in the first reading, the second measurement reverting to readings which were not significantly different from base line recordings.

There was no significant difference between sites or hormonal environments for any of the parameters.

Results of the addition of drugs to pregnant and menopausal tubes

These two groups were not compared statistically with the other three groups due to the small number of samples in the former.

Both pregnant and menopausal tubes showed less response to drugs than the other groups, although the pattern of response was similar. The frequency of contractions in menopausal tubes was decreased in all except the isthmic region, on addition of adrenaline. The pregnant tubes had a decreased response in the isthmic region, but an increase in the ampulla.

The average intensity was decreased in both regions of both groups, but the decrease was less than that found in menstruating or puerperal women. Similarly the activity units were decreased, but again less than in the other three groups.

The first and second measurements in the two groups were increases in both ampulla and isthmus, with all drugs, but the increase was less than in the other three groups. The first measurement of amplitude maximum was similarly increased, but less than the other groups. In menopausal groups the isthmus appeared to have a greater response than the ampulla, whereas whereas both regions of the pregnant tubes showed a similar response. All drugs in both regions produced an increased second amplitude maximum measurement with menopausal tubes, but the isthmic region of the pregnant tubes showed a decrease on addition of nor-adrenaline and adrenaline, and an increase in the ampullary region.

In the majority of parameters the isthmic and ampullary regions showed no difference in their responses to drugs.

Results of analysis of variance

In orded to save space, the figures for the sum of squares, mean of squares, degrees of freedom, and variance ratio have been omitted.

Addition of drugs to oviducts from different hormonal environments

	Between sites-hormonal environs.	Between sites	Between hormonal environments
Frequency			
Nor-adrenaline			
Probability	N.S.	N.S.	N.S.
Adrenaline			
P	N.S.	0.05	N.S.
Acetylcholine			
P	N.S.	N.S.	0.05
Average Intensity			
Nor-adrenaline			
P	N.S.	N.S.	0.05
Adrenaline			
P	N.S.	N.S.	0.05
Acetylcholine			
P	N.S.	N.S.	N.S.

	Between sites-hormonal environs.	Between sites	Between hormonal environments
Activity Units			
Nor-adrenaline			
P	N.S.	N.S.	N.S.
Adrenaline			
P	N.S.	N.S.	N.S.
Acetylcholine			
P	N.S.	N.S.	N.S.
Tonus - first measurement			
Nor-adrenaline			
P	N.S.	0.01	N.S.
Adrenaline			
P	N.S.	0.01	N.S.
Acetylcholine			
P	N.S.	N.S.	N.S.
Tonus - second measurement			
Nor-adrenaline			
P	0.05	N.S.	0.025
Adrenaline			
P	0.05	0.05	0.025
Acetylcholine			
P	N.S.	N.S.	N.S.

	Between sites-hormonal environs.	Between sites	Between hormonal environments
Amplitude Maximum - first measurement			
Nor-adrenaline			
P	N.S.	0.01	N.S.
Adrenaline			
P	0.05	0.01	N.S.
Acetylcholine			
P	N.S.	N.S.	N.S.
Amplitude Maximum - second measurement			
Nor-adrenaline			
P	0.05	0.05	0.005
Adrenaline			
P	N.S.	0.05	0.01
Acetylcholine			
P	N.S.	N.S.	N.S.

Addition of drugs to oviducts from different hormonal environments

From the results of the analysis of variance it can be seen that there are some differences between sites and hormonal environments for some of the parameters and drugs. The Student's t test has been used to test for these particular differences.

Results

Differences between sites

In each case the isthmic region has been compared with the ampulla.

	t value	probability
Frequency		
Adrenaline		
Proliferative	1.4961	N.S.
Secretory	1.0257	N.S.
Puerperal	1.0234	N.S.
Tonus - first measurement		
Nor-adrenaline		
Proliferative	1.4570	N.S.
Secretory	5.1032	0.005
Puerperal	1.2488	N.S.
Adrenaline		
Proliferative	2.2637	0.05
Secretory	3.8651	0.005
Puerperal	2.6678	0.025
Tonus - second measurement		
Adrenaline		
Proliferative	1.3169	N.S.

	t value	probability
Secretory	2.1464	0.05
Puerperal	0.6357	N.S.
Amplitude Maximum - first meas.		
Nor-adrenaline		
Proliferative	1.6647	N.S.
Secretory	3.8847	0.005
Puerperal	1.2798	N.S.
Adrenaline		
Proliferative	2.1472	0.05
Secretory	3.8219	0.005
Puerperal	1.6371	N.S.
Amplitude Maximum - second meas.		
Nor-adrenaline		
Proliferative	1.2653	N.S.
Secretory	1.5534	N.S.
Puerperal	2.6351	0.025
Adrenaline		
Proliferative	1.0456	N.S.
Secretory	2.6440	0.025
Puerperal	1.4713	N.S.

Differences between hormonal environments

In each case two hormonal environments have been compared with each other to determine whether the two populations are statistically different.

	t value	probability
Frequency		
Acetylcholine		
Ampulla - Proliferative/Puerperal	1.3844	N.S.
Average Intensity		
Nor-adrenaline		
Isthmus - Proliferative/Puerperal	2.3822	0.01
- Secretory/Puerperal	1.9966	N.S.
Adrenaline		
Isthmus - Proliferative/Puerperal	1.9019	0.05
- Secretory/Puerperal	2.2010	0.05
Tonus - second measurement		
Nor-adrenaline		
Isthmus - Proliferative/Puerperal	2.7379	0.01
Ampulla - Proliferative/Puerperal	2.3411	0.05
- Proliferative/Secretory	2.2800	0.05
Adrenaline		
Isthmus- Proliferative/Puerperal	2.4006	0.025
Ampulla - Proliferative/Puerperal	2.8133	0.01
- Proliferative/Secretory	2.7273	0.01
Amplitude Maximum - second meas.		
Nor-adrenaline		
Isthmus - Proliferative/Puerperal	4.0712	0.005
- Secretory/Puerperal	3.4825	0.005

	t value	probability
Ampulla - Secretory/Puerperal	1.2431	N.S.
<u>Adrenaline</u>		
Isthmus - Proliferative/Puerperal	2.8974	0.01
- Secretory/Puerperal	2.9503	0.01
Ampulla - Proliferative/Secretory	2.5006	0.025

Discussion

This experiment has attempted to examine the effect of nor-adrenaline, adrenaline, and acetylcholine on the human tubal motility, and to see how the response varies during the menstrual cycle.

Adrenergic nerve terminals have been observed in the human fallopian tube by fluorescent microscopy (Brundin and Wirsen 1964), and adrenergic receptors have demonstrated by both perfusion techniques (Rosenblum and Stein 1966), and measurement of the tubal motility (Sandberg et al 1960, Nakanishi et al 1967). The receptors can be of two types, those causing a relaxation of the tissue (beta receptors), and those causing a contraction (alpha receptors). Nor-adrenaline is largely an alpha adrenergic receptor excitant, although the effect on oviduct muscle has been a matter of debate. Adrenaline is an excitant of both alpha and beta receptors.

Although the effect on tubal motility of the three drugs has been looked at before, there are conflicting results, and the majority of previous reports have dealt only with the tonus or frequency of the contractions. In many cases the results have not been adequately analysed. This present study seeked to look at more parameters, and to subject the results to more intensive analysis.

It was found firstly that there is a great deal of variation between the response of individual tubes, and in many cases the results are not easy to interpret, as different parameters sometimes point to a different response, making it difficult to obtain an overall assessment of the drug action.

Adrenaline caused an increase in frequency in all tubes except the isthmic secretory, but the average intensity was decreased, leading to a decrease in activity units. There was no significant difference in action between sites, although in the majority of cases the response of the isthmus was more pronounced than the ampulla. The tonus was increased in all tubes, with a greater increase in the isthmus, and no difference between the menstruating tubes, but a difference

between these and the puerperal tubes. The amplitude maximum had an initial increase in the isthmus, although the second measurement had varying responses in the different tubes.

Kok (1927) found that when maturing follicles were present in the rabbit ovary, adrenaline caused a relaxation of the ampulla and a rise in tonus of the isthmus, whereas after ovulation the isthmic region contracted, and the ampulla relaxed but had a secondary increase in tonus. The present study on human tubes supports the first part of his observations, however the secretory tubes in human were not statistically different from the proliferative, making the responses to adrenaline the same throughout the menstrual cycle. Differences occur however between the menstrating tubes and the puerperal. The latter are less responsive, although increases in tonus still occur.

The differences in response between regions does point to a difference in motility. The response by the isthmus was more pronounced than the ampulla. Anatomical studies show that the isthmic and ampullary regions of the tube have a different density of adrenergic nerve innervation. The supply to the ampulla is rather sparse, whereas there is a dramatic increase at the ampullary-isthmic junction and the isthmic region (Brundin 1965, Owman et al 1967). Thus it would be thought that the response to adrenergic drugs in the two regions would be slightly different.

There are also differences in opinion regarding the action of nor-adrenaline on the tube. Brundin (1964) found the frequency and intensity of the rabbit tubal contractions were increased in the isthmus but not in the ampulla. Sandberg et al (1960) found the tonus in the human tube was increased regardless of cycle, and with no difference between region. The present study showed that nor-adrenaline had a similar effect to adrenaline. The frequency was increased in all tubes except the isthmus of the proliferative, but the average intensity in all tubes was decreased in contrast to the results of Brundin. The tonus was increased in all tubes from menstruating women, and the isthmus of the secretory tubes had a greater increase in tonus than the ampulla.

The isthmus of the secretory tubes similarly had a greater increase in amplitude maximum than the ampulla, there also being a difference between the ampullary tubes of menstruating women, with the proliferative result being higher than the secretory. This was also found in the second tonus measurement, between the same tubes.

To relate these differences in response to the movement of the ovum, in the proliferative phase of the cycle, before ovulation there is more movement in the ampulla of the tube than in the secretory phase. It would be thought the reverse would be true, with greatest movement in the ampulla taking place shortly after ovulation. To check this it would be necessary either to test tubes from women at ovulation, which would be very difficult, or else to try to simulate conditions by the addition of exogenous hormones.

However a decrease in movement in the ampulla of the secretory stage could point to a greater involvement by the cilia in the movement of the ovum in this area than was otherwise thought.

The isthmic region of the secretory tubes show a greater increase in both tonus and amplitude maximun than the ampulla. This would be expected, due to the arrival of the ovum in the isthmus during the secretory phase.

Acetylcholine had the least effect on the motility of the Fallopian tubes. This would be expected as little cholinergic innervation has been found in the tube (Nakanishi et al 1967). Various authors have found a response with acetylcholine, although these have not all been analysed statistically. Murakami (1932) and McKinney (1932) both found it stimulated the oviduct in human and fowls respectively. Sandberg et al (1960) found the tonus in humans was increased throughout the cycle in all regions of tube, but he found a difference in amplitude, with the secretory tubes showing a decrease and the proliferative an increase. Brundin (1964) found the drug caused an increase in motility in rabbits, but this effect was more pronounced in the ampulla. Davids and Bender (1950), using an insufflation technique found a relaxation throughout the rabbit oviduct upon addition of acetylcholine.

In the present study it was found that the drug had little significant effect on motility, but in general a stimulatory effect was produced. The results agreed with those produced by Sandberg et al in principle. The intensity of the isthmic secretory tubes was decreased more than from other phases and regions, although a decrease was found in all but the isthmic puerperal tubes. No difference could be found between regions.

Discussion of the addition of drugs to pregnant and menopausal tubes

Both groups had a smaller response to drugs than menstruating or puerperal women. There was also less difference in the reactions of isthmic and ampullary tubes. Both pregnant and menopausal women have different hormonal levels to those found in either menstruating or puerperal women. During pregnancy there are very high levels of progesterone, while after the menopause there are only very low levels of hormones present.

Miller and Marshall (1965) found that the addition of progesterone to oestrogen-primed rabbits caused an alteration of the uterine response to hypogastric nerve stimulation from excitation to inhibition. It may be possible that progesterone is responsible for the decreased response of the pregnant tubes. Nakanishi and Wood (1968) found that the isthmic region of the tubes of pregnant women showed no difference in response to nerve stimulation or nor-adrenaline than the ampulla. This would indicate that during pregnancy the isthmic region could not act as a sphincter.

Similarly the lessened response of menopausal tubes, and the fact that there was no discernible difference between ampulla and isthmus also indicates that the isthmus no longer acts as a sphincter. In the absence of ovulation or menstruation this would not matter. Nakanishi and Wood also found a decrease in response with menopausal women. They thought this could be due either to an increase of beta-receptors in the tube, a decrease of alpha-receptors, or the withdrawal of endocrine influences on the tube.

Experiment 3. Effects of exogenous hormones and drugs on the motility of the Fallopian tube

Materials:

Fallopian tubes from women undergoing a salpingectomy in the puerperal period were used.

Method:

One of a pair of tubes was taken, and a muscle preparation set up as before, with the spontaneous motility allowed to equilibrate for twenty minutes. The tube was then tested with nor-adrenaline, adrenaline, and acetylcholine using a similar technique to experiment 2.

The second tube was then used. The experiment was repeated, but instead of using plain Krebs solution, and drugs made up in Krebs solution, a hormone solution was used instead. This hormone was made up in Krebs solution. Similarly the drugs were made up in Krebs solution + the hormone. The tube was thus bathed from the onset of the experiment in a hormone solution. The drugs plus hormone were added in the same order as that used in the first part of the experiment.

Both ampullary and isthmic regions of the tube were tested. Due to the time factor, each pair of tubes could only be tested with one hormone.

Hormones used:

Progesterone - at a dose of 15 ng/ml.
Oestrogen - oestradiol monobenzoate 12ng/100 mls. and 100ng/100 mls.

Frequency

Fig.18

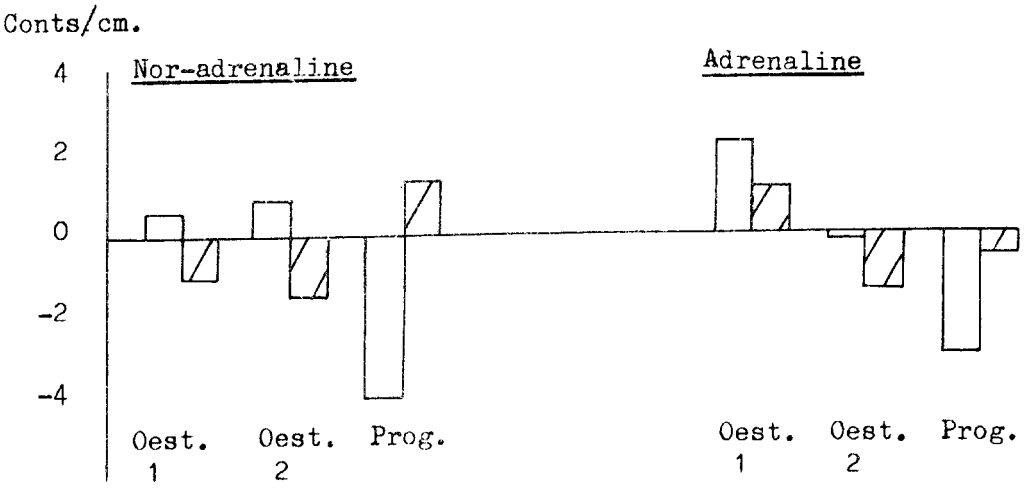
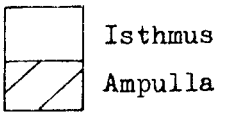


Fig.19

Significant results:

Progesterone - isthmus

Progesterone - isthmus



Average Intensity

Fig.20

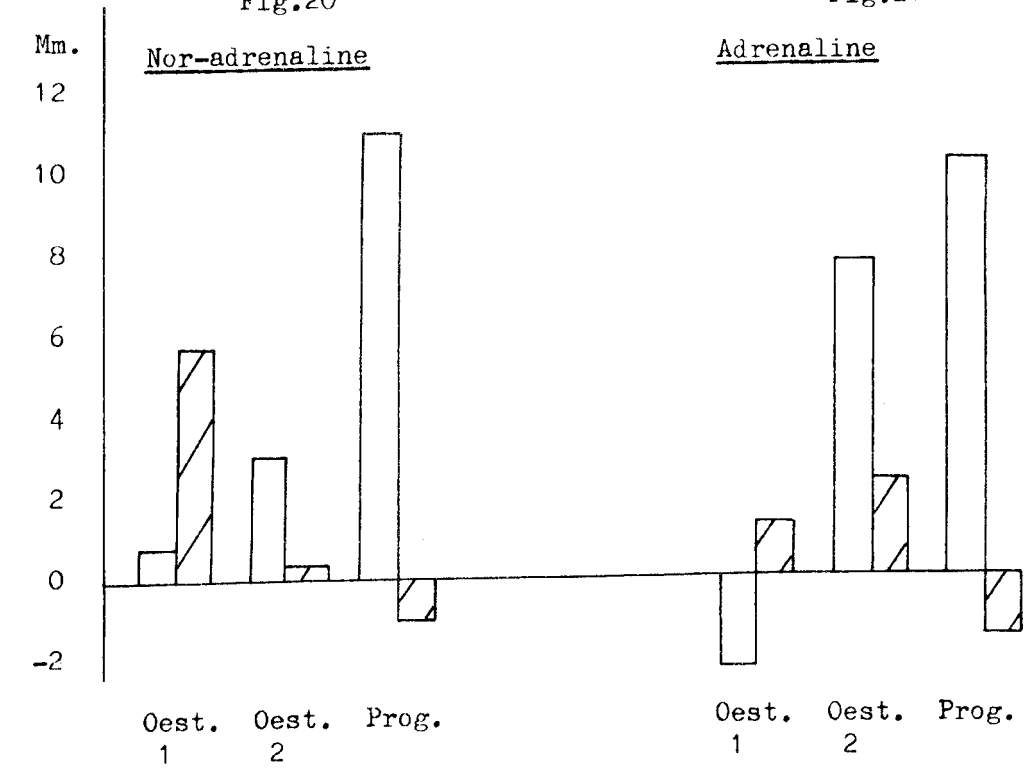


Fig.21

Significant results:

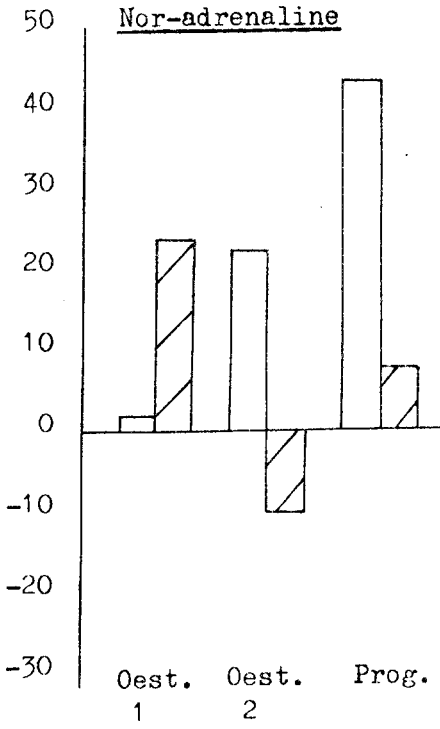
Oestrogen 1, Progesterone - isthmus

Oestrogen 2 - ampulla

All three - isthmus and ampulla

Activity Units

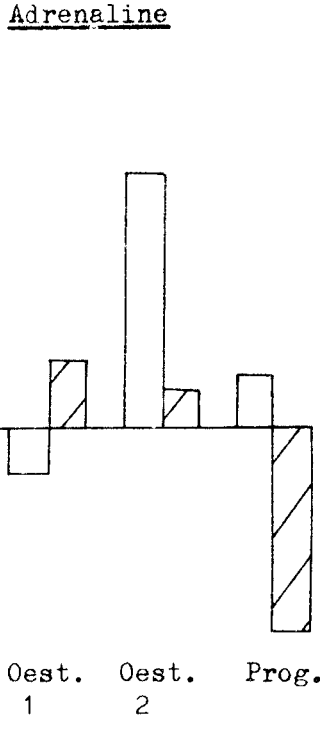
Fig.22



Significant results:

Oestrogen 1 - ampulla

Fig.23



Oestrogen 2 - isthmus

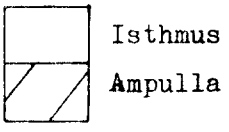
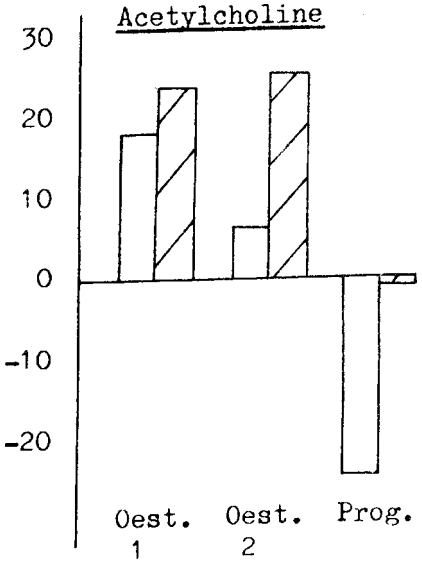


Fig.24



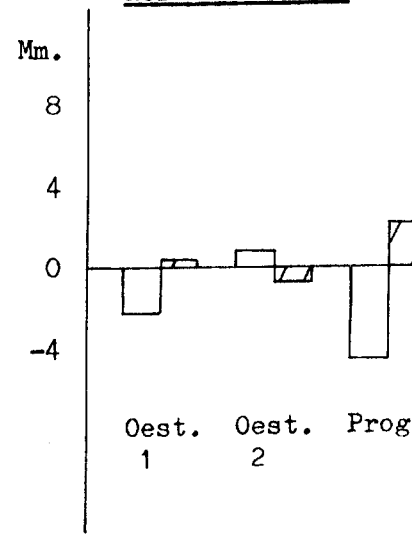
Significant results:

Oestrogen 1 , Progesterone - isthmus

Tonus - second measurement

Fig.25

Nor-adrenaline

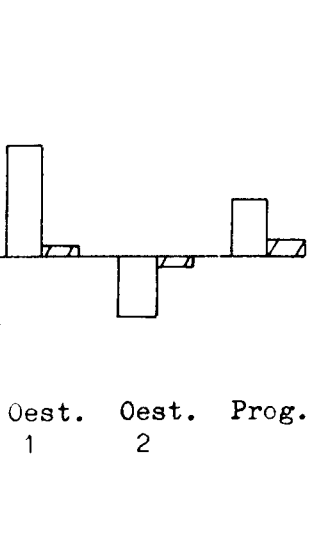


Significant results:

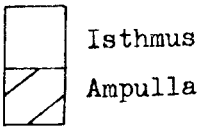
Oestrogen 2 - isthmus

Fig.26

Acetylcholine



Oestrogen 1, Progesterone-isthmus



Results:

All the hormones added caused some modification of the tubal response to drugs, this effect being more noticeable in the isthmic region.

Nor-adrenaline

Progesterone caused a significant decrease in the frequency of the contractions in the isthmic region of the tube, and a non-significant increase in the ampullary region. Neither of the concentrations of oestrogen had a significant effect on frequency, but they both caused an increase in the isthmus and decrease in the ampulla.

Average intensity was increased in all cases except the ampullary region under the influence of progesterone. Significant differences were found in the isthmic region with progesterone and oestrogen 1, and in the ampulla with oestrogen 1.

Activity units were similarly increased in all cases except one, only the decrease occurred in the ampulla with oestrogen 2. Statistically different results were found only in the ampulla under the influence of oestrogen 1.

The results for the first tonus measurement were all non-significantly different from the response without exogenous hormones. Progesterone aand oestrogen 2 tended to give a slight decrease in tonus, while oestrogen 1 gave an increase. The second measurement gave a different result. Decreases were found with the isthmic region of oestrogen 1 and progesterone, and the ampulla of oestrogen 2, while the other groups gave slight increases. Significant results were obtained with the isthmic region of the oestrogen 1 tube only.

There were no significant differences in either the first or second measurements of the amplitude maximum. The first was decreased in all cases except the ampulla with progesterone, and the second measurement increased in all cases except the progesterone ampulla.

Adrenaline

Reductions in frequency were obtained with oestrogen 2 and progesterone, with both regions, oestrogen 1 causing an increase. Significant differences were obtained in the isthmic region

with progesterone.

Average intensity was increased in both regions with oestrogen 2, in the isthmus with progesterone, and in the ampulla with oestrogen 1, other tubes being decreased. Significant results were obtained in all cases, with all hormones.

Activity units were increased in all tubes except two, the isthmus with oestrogen 1, and ampulla with progesterone, but only the isthmic oestrogen 2 increase was significant.

Tonus followed a similar pattern to that obtained with noradrenaline, with a couple of minor variations. There were no significant results.

Amplitude maximum also had a similar pattern to the noradrenaline result, with no significant results.

Acetylcholine

There was little significant difference on the results upon the addition of exogenous hormones, and little significant difference between the hormones.

Progesterone caused a decrease in frequency in both regions while the other drugs caused an increase. The isthmic regions of the tubes with added progesterone, and the larger dose of oestrogen both showed a decrease in frequency, all other areas showing an increase.

The average intensity in the ampullary region was increased with all three hormones, and that in the isthmus was increased with oestrogen 1, and decreased with the other two hormones.

Activity units were decreased with progesterone, but increased with the other hormones. The first tonus measurement showed a similar pattern to the activity units, while the second measurement was reduced with oestrogen 2, and increased with the other two preparations.

The amplitude maximum was increased in the first measurement, with all the hormones, while the second measurement was increased in all cases except the isthmus of the progesterone and oestrogen 2 tubes.

The only significantly different results with acetlycholine were the activity units, the isthmic regions on addition of progesterone and oestrogen 1, and both concentrations of oestrogen, in the isthmic region with the second measurement of the amplitude maximum.

Effect of exogenous hormones on the response of the oviduct to drugs

	Nor-adrenaline		Adrenaline		Acetylcholine	
	Isthmus	Ampulla	Isthmus	Ampulla	Isthmus	Ampulla
Frequency						
F	3.62	0.73	3.42	0.49	2.41	0.40
Average Intensity						
F	6.52	3.97	4.11	13.75	0.33	0.32
Activity Units						
F	1.04	1.95	1.99	0.54	6.23	1.68
Tonus - first measurement						
F	1.35	2.95	2.16	1.30	3.74	0.86
Tonus - second measurement						
F	3.08	1.59	0.54	0.10	5.49	0.40
Amplitude Maximum - first measurement						
F	1.23	0.86	0.21	0.99	1.35	0.69
Amplitude Maximum - second measurement						
F	1.08	1.04	0.66	0.30	0.36	0.81

The five per cent critical value of F corresponding to a numerator of 39 and a denominator of 42 is 1.68 (from tables of the F distribution). Thus any value of F above 1.68 can be regarded as showing that some difference exists between the groups giving that figure, and these should be further tested.

Student's t test

This has been used to test for particular differences where the results from the F distribution show that some variation exists.

In all cases for each hormone and each parameter, the reaction to a drug before adding that hormone is compared with that obtained after.

Progesterone = 15 ng/ml

Oestrogen 1 = 12 ng/100 mls oestrogen 2 = 100 ng/100 mls

	Progesterone		Oestrogen 1		Oestrogen 2	
	t value	prob.	t value	prob.	t value	prob.
Frequency						
Isthmus						
Nor-ad.	4.9962	0.005	0.3656	N.S.	1.9514	N.S.
Adren.	4.9591	0.005	1.6811	N.S.	1.3717	N.S.
Acetyl.	1.4139	N.S.	0.9999	N.S.	0.5008	N.S.
Average Intensity						
Isthmus						
Nor-ad.	3.1284	0.01	4.7909	0.005	1.3280	N.S.
Adren.	4.6411	0.005	2.5800	0.025	4.4341	0.005
Ampulla						
Nor-ad.	1.6680	N.S.	2.7116	0.025	0.0571	N.S.
Adren.	2.7877	0.025	1.9999	0.05	4.7883	0.005
Activity Units						
Isthmus						
Adren.	0.3873	N.S.	0.5117	N.S.	2.6404	0.025
Acetyl.	2.0988	0.05	2.1435	0.05	0.4033	N.S.
Ampulla						
Nor-ad.	0.2373	N.S.	2.5109	0.025	0.3112	N.S.

	Progesterone		Oestrogen 1		Oestrogen 2	
	t value	prob.	t value	prob.	t value	prob.
Tonus 1st. meas.						
Isthmus						
Adren.	0.7584	N.S.	1.5905	N.S.	0.8081	N.S.
Acetyl.	0.1737	N.S.	1.1163	N.S.	0.5593	N.S.
Ampulla						
Nor-ad.	0.0379	N.S.	0.4640	N.S.	0.8796	N.S.
Tonus 2nd. meas.						
Isthmus						
Nor-ad.	1.6640	N.S.	0.9628	N.S.	2.7605	0.025
Acetyl.	0.8842	N.S.	2.1054	0.05	2.0914	0.05

Discussion:

The adrenergic receptors are influenced by the sex steroids, but there has been a great deal of dispute as to the effect of oestrogen and progesterone on tubal motility and ovum transport. The effect varies with the dosage, time of administration, and method of administration, and it is differences in these three points that have given rise to the conflict.

In the present experiment the response to the adrenergic drugs was modified by the exogenous hormone present, and also the effect did differ between regions, although these differences were not always significant. Under the influence of progesterone the isthmic region had fewer, larger contractions on addition of both nor-adrenaline and adrenaline, whereas the ampulla had more, smaller contractions with nor-adrenaline, and fewer, smaller ones with adrenaline.

Taking the seven parameters into account, the results obtained with adrenergic drugs and exogenous hormones on the fallopian tube can be tabulated as follows:

		Nor-adrenaline	Adrenaline
Isthmus	- Progesterone	Relaxation	Relaxation
	Oestrogen 1	Contraction	"
	Oestrogen 2	"	"
Ampulla	- Progesterone	"	"
	Oestrogen 1	"	Contraction
	Oestrogen 2	Relaxation	"

Progesterone tended to produce relaxation of the tube, whereas oestrogen had a more variable effect. The smaller dose caused contraction with nor-adrenaline in both ampulla and isthmus, but with adrenaline only the ampullary region contracted. The larger dose produced both relaxation and contraction. However, when only the results significantly different from those obtained before adding a hormone are considered, there is a lot of difference between ampulla and isthmus. The isthmic tubes under the influence of progesterone had fewer, larger contractions, with decreased activity units, but the ampullary tubes only had a smaller average intensity, other results showing no

variation. The tubes under the increase of both concentrations of oestrogen showed an increase in motility, this being more pronounced in the isthmus.

The larger dose of oestrogen corresponds to that present at midcycle, and the smaller is equivalent to the venous blood level in the proliferative phase. The smaller dose brought about a greater tubal contraction than did the larger. Seitchik et al (1968) thought the adrenergic receptors helped the tube to function as a sphincter. Thus under alpha-receptor dominance the isthmus would contract, thus delaying ovum transport at the ampullary-isthmic junction. Beta-receptor dominance would allow the sphincter to relax, and allow the ovum to pass down the tube. The difference in response found in the two regions does add support to this theory.

However results from experiments carried out on rabbits, counting the progression of ova in the tube after injections of hormones (Pauerstein et al 1974) indicates that oestrogen increases muscular activity, but decreases the rate of transport of ova through the tube. The ova had a prolonged pause at the ampullary-isthmic junction when compared to the controls. Progesterone appeared to exert its influence at the ampullary-isthmic junction and proximal isthmus. Ova were never found in the ten percent of the oviduct closer to the uterus when the tube was under progesterone control.

Langley et al (1968) used a combination of hormones and autonomic drugs, again looking at the location of ova in the oviduct of rabbits. They found that oestrogen increased muscular activity, but decreased the rate of movement of ova through the tube. They also found that in rabbits primed with progesterone and adrenaline, ova were located near the ovarian end of the tube. They postulated that the beta-receptors were sensitized by progesterone.

It appears from the present experiments that a combination of oestrogen with autonomic drugs brings about greater tubal motility, whereas progesterone with the same drugs relaxes the tube. The increased contractility of the tube under the influence of oestrogen could, as previously indicated, enable the isthmus

to exert its "sphincter effect" to delay transport, whereas progesterone would have the reverse effect.

Acetylcholine had very little significant effect on the motility of the tubes under either oestrogen or progesterone influence, although an increase in motility was found more often than a decrease. Nakanishi and Wood (1967) found acetylcholine produced a contractile effect, as the response was blocked by pretreatment with atropine. They found no pharmacological evidence of a cholinergic nerve supply. Cholinergic agents had no effect on the contractions.

CILIARY MOTILITY

CILIARY MOTILITY

Introduction

a.) Structure of cilia

The cilia found in the mammalian oviduct are basically similar in structure to those found throughout the animal kingdom. Each one is a thin motile projection from the surface of the cell, about 5 μm in length, and 0.2 μm. in diameter in the human. Each has a characteristic arrangement of microtubules forming the axoneme. This consists of nine circumferentially arranged double fibrils (subfibres A and B) surrounding a pair of single fibrils. All are enclosed in a membrane that is continuous with the cell surface. Each microtubule is composed of a number of linear protofilaments which run unbranched the length of the tubule. It is thought that the central tubules each have thirteen filaments, subfibre A thirteen, and subfibre B ten filaments (Warner and Satir 1973). Subfibre A is a complete tubule, while B shares three of the filaments of A.

Each doublet is skewed and lies in a plane about ten degrees to the tangent of the axoneme radius as extended through the centre of subfibre A.

A and B subfibres of adjacent doublets are separated by a distance of approximately 175-200 Angstrons, and in this space attached to each subfibre A is a pair of dynein arms. Also attached to A is a radial link which joins the doublets to a sheath around the two central microtubules. Each link is about 360 Angstrons long, and ends in an opaque head near the central sheath. It is thought that a fine linkage, an inter-doublet link, connects the terminal portion of the inner dynein arm to the adjacent B subfibre.

Each cilium is firmly rooted into the cytoplasm of the cell by a basal body. Each is a cylinder with a wall formed by nine triplet microtubules, and surrounded by electron dense material. The doublet subfibres of the axoneme are direct continuations of the A and B subfibres of the basal body triplet microtubules. The triplets are at an angle with a tangent to the circumference. This varies from 30-45 degrees at the base of the basal body, to 10-20 degrees at the top.

Diagram of LS Cilium

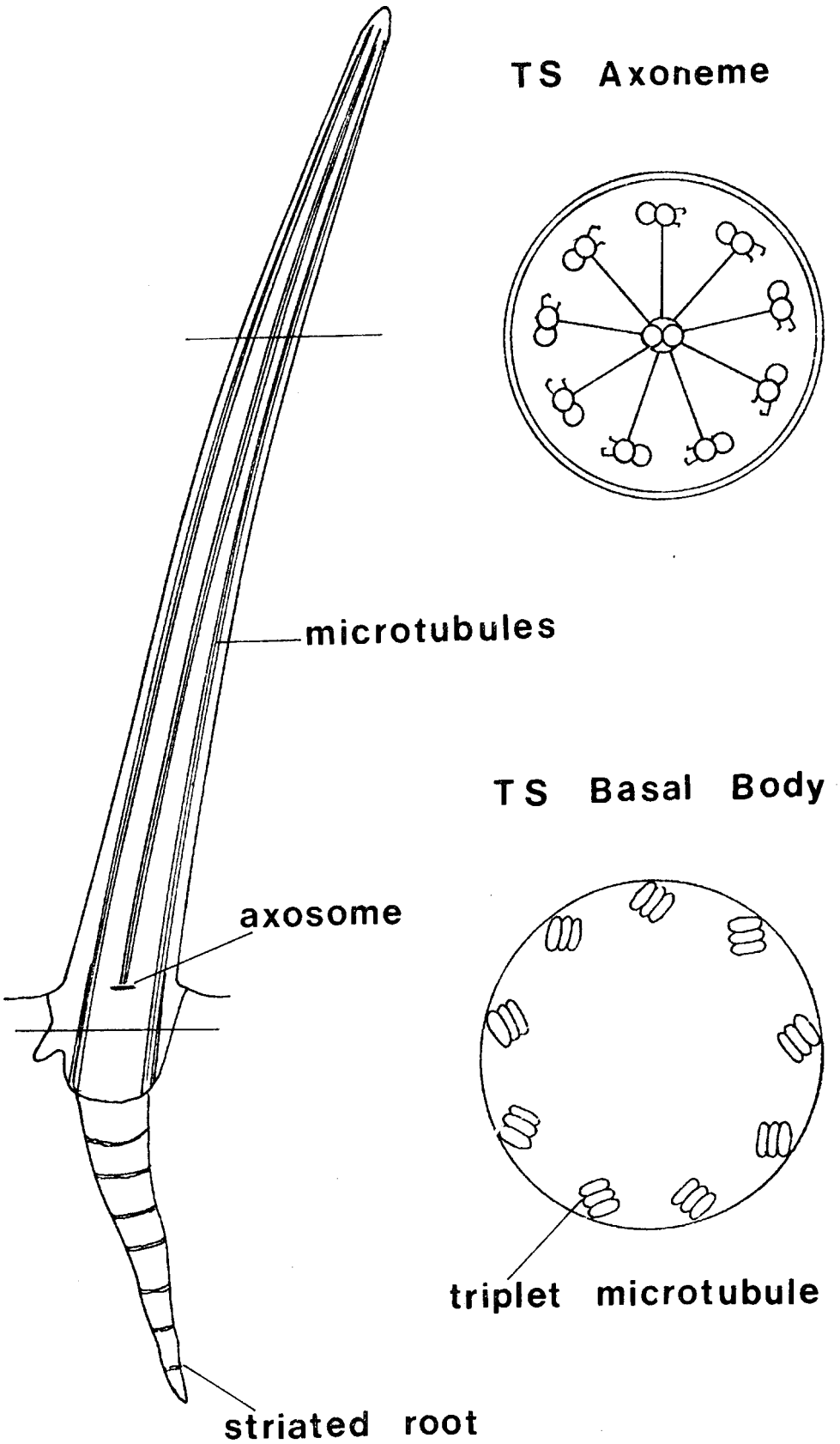


Fig. 27

Diagram of portion of typical 9 + 2 axoneme

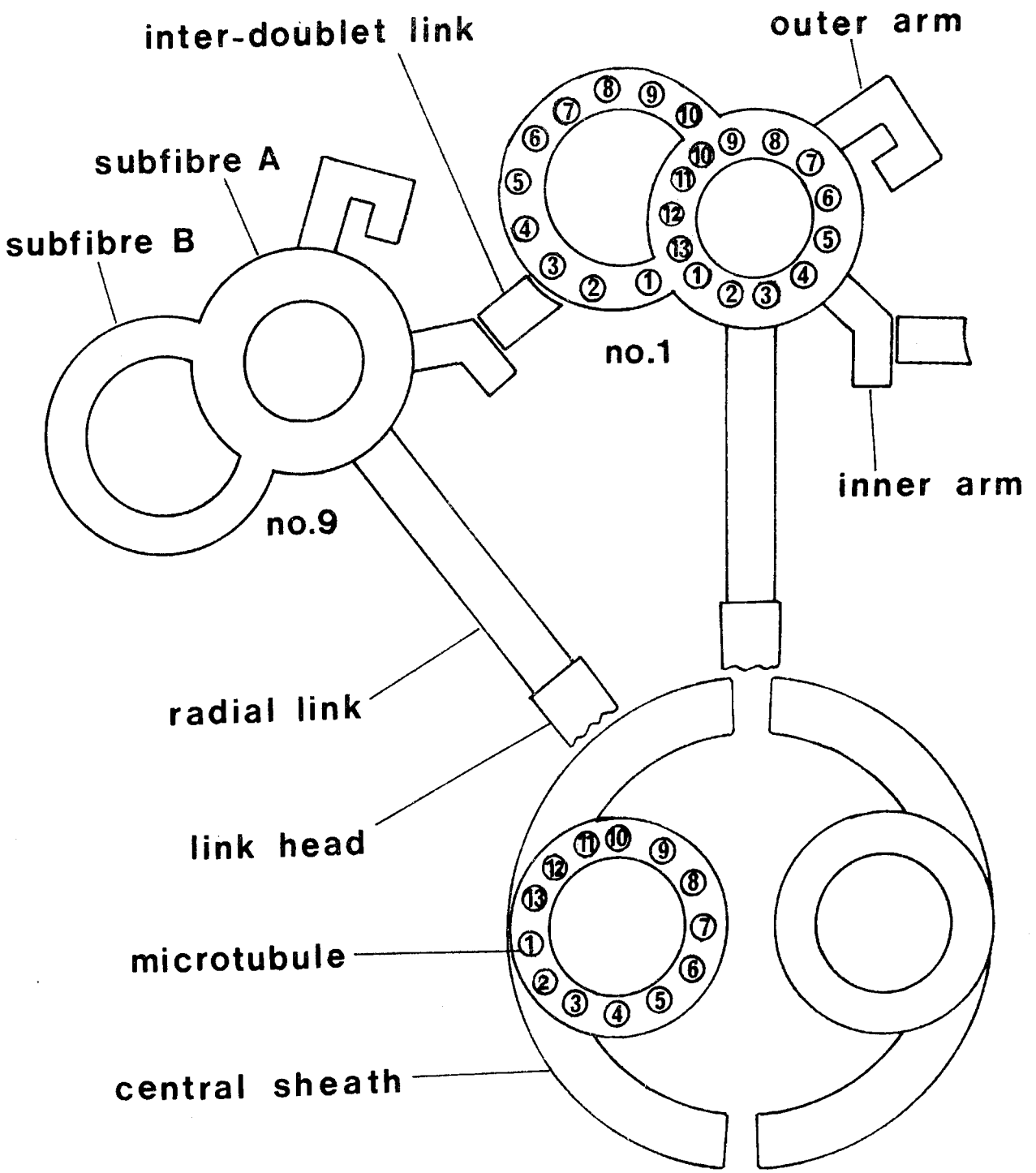


Fig. 28

Electron dense material sometimes forms nine radiating spokes attached to a hooked process of the A subfibre, but these spokes do not always persist.

b.) Biochemistry of the axoneme

The biochemistry of cilia has been studied so far only in a few species, comprising some Protozoa and a few invertebrates. To examine the biochemistry the cilia must first be isolated from the tissue, generally by treatment with 10% ethanol cotaining ethylene diamine tetraacetic acid (EDTA), followed by calcium chloride and high speed centrifugation (Watson and Hopkins 1962, Gibbons 1965). The impermeable ciliary membrane must be broken into, and Gibbons (1963) found it was removed by teatment with digitonin, leaving the 9 + 2 structure and the arms.

The arms, central pair of fibres, secondary filaments and matrix material were solubilized, and ultra-centrifugation showed 4-6 S, 14 S, and 30 S components. Centrifugation in a sucrose gradient showed that only the 14 S and 30 S components possess ATPase activity (Gibbons 1965). These proteins were termed dynein due to their relationship to mechano-chemical function. They provide the enzymatic energy for axoneme function.

The arms contain 30 S dynein. Either form of dynein is very specific for the terminal phosphate of ATP. Other nucleoside triphosphates are hydrolysed at about 10% of the ATPase rate.

Both 14 S and 30 S dynein are activated by the same divalent cations although the response differs. Thus calcium activates both forms equally well, as does manganese. Magnesium activates the 14 S type three times more than the 30 S. Iron, cobalt and nickel similarly activate both, but again 30 S is less active. Strontium, zinc, cadmium and beryllium are ineffective with both.

14 S dynein has an optimum pH of 9, whereas 30 S has two optima, pH 6,and pH 8.5. 14 S is inhibited by increasing ionic strength, whereas 30 S is activated.

The 14 S particle is a globule 85-90 by 140 Angstrons with a molecular weight of 540,000 - 600,000. 30 S is probably a

polymer of 14 S units (Gibbons and Rowe 1965).

The microtubules are composed of a protein called tubulin . this has a monomer weight of 55,000 daltons, and under physiological conditions probably exists as a 6 S particle of two monomers.The amino acid composition grossly resembles muscle actin, although there are some important differences.There are differences in the gross morphology of the polymerized form, the presence of different nucleotides in dissimilar ratios, different molecular weights, and a drastic dissimilarity in nearlt 90% of the primary structure.

Even though tubulins from the central pair and the outer fibres of cilia from different species have nearly identical properties, there are differences in morphology and differences in solubilities, indicating chemical alterations.

The outer fibres are not identical in structure or properties to the central pair. The latter are more easily solubilised. Dynein arms are attached only to the A subfibre of the outer doublets, and at specific sites. A is a complete microtubule with B an outgrowth of A. However the central pair exist as singlet tubules and do not bear auxiliary structures.

There are three other classes of protein which are of importance in the functioning of cilia.

The membrane of cilia is impervious. The structure of the protein is not known yet although different workers have obtained components with a high molecular weight. Witman et al (1972) had a figure of over 170,000 daltons from Chlamydomonas flagella, and Stephens and Linch (1969) found two components of 115,000 and 60,000 daltons in gill cilia of molluscs.

The matrix of the axoneme is composed of many minor proteins of unknown significance, but it also contains adenylate kinase (Brokaw 1961). It is this protein which catalyses the formation of ATP: 2 ADP = AMP + ATP.
It sediments at 10 S with a molecular weight of about 35,000 daltons. Muscle myokinase is smaller with a molecular weight of about 21,000 daltons, and it is more stable.

The third type of protein is that responsible for the symmetry of the axoneme, and which provides mechanical constraints. These architectural proteins form the radial links which connect the outer fibres to the central pair, the inter-doublet links which connect the outer fibres, and the central sheath surrounding the central pair. The biochemistry of the protein

is little understood yet, although it appears to have a molecular weight of 150,000 - 165,000 daltons. It has been given the name nexin, from the Latin word nexus, meaning a tie binding together members of a group (Stephens 1971).

Ciliary Motility - Objectives of Study

Although the majority if investigators have favoured a muscular answer to the problem of transport through the Fallopian tube, there are many factors that point to a ciliary involvement as well. Shipstone et al (1974) found that mitochondria became aggregated in the sub-apical part of ciliated cells in the isthmus as well as the ampullary regions, after ovulation in rabbits. They also found that the number of cilia in the isthmus increased as the ovum was about to enter that region of the tube. They took these facts as an indication that cilia also play a part in the movement of the ova through the isthmus, a role that has hitherto been dismissed.

Similarly, the amount of glycogen present in the ciliated cells in the oviducts of rabbits and humans decreased after ovulation (Fredricsson 1959). The author thought this glycogen probably supplied the energy needed for ciliary activity.

A further structural detail was noted by Nilsson and Reinius (1969). The cilia of the human oviduct have a cross-striated rootlet, with a distance between the striations of about 450 Angstrons. They thought this could indicate a contractile protein, and wondered if it would endow the cilia with some functional specificity such as beating frequency.

Borell et al (1957) were one of the few investigators to attempt a numerical analysis of ciliary motility. They induced ovulation in rabbits, and measured the ciliary activity by filming at different intervals after copulation. They found that the rate of ciliary beat gradually increased by about twenty percent on the second and third day after copulation. This corresponds to the time of ovum movement through the tube. They found no difference between the regions of the tube. In pregnant rabbits, the degree of activity had decreased to the figure obtained from non-copulated animals during oestrous. They concluded that ciliary activity plays a major role in transportation of the ova through the Fallopian tubes in rabbits.

No study has yet been carried out to determine whether the same applies to humans, and if the ciliary beat alters throughout the menstrual cycle. Thus the present study was instigated, to try to find the answer to this problem, and to further elucidate the role of cilia in the Fallopian tube.

CILIARY MOTILITY

Practical considerations

To examine ciliary motility many technical difficulties must first be overcome. The cilia are microscopical in size, poorly refractile, and are moving too quickly to be registered by the human eye.

Two different ways of dealing with the problem have been evolved. Methods have been developed that only enable movement to be observed, whereas the second group of methods actually enable the motility to be measured.

1. Observation methods

One of the first investigators to study ciliary movement was Engelmann in 1868. He timed the rate of movement of a small sphere that was suspended by a silk thread on the ciliated surface of a frog's pharynx. From this he extrapolated the movement of the cilia. The majority of investigators since then have used modifications of this method. A more complicated device was used by Inchley in 1921. He used a "cilioscribe". The mucous membrane of the frog's pharynx was pulled horizontally against a glass rod, the distance it was pulled being an indication of the ciliary activity.

Observation techniques are still in use. They are generally only for in vitro use, and the ciliated membrane usually examined is the trachea of the rabbit of the oesophagus of the frog. The organ to be examined is generally placed in a moistened temperature regulated chamber, opened longitudinally, and particles placed on the ciliated membrane. The distance travelled by the particles in a certain time can be measured, and from this the transport velocity found out. The particles used vary with the investigators, but recently poppy seeds have been used (Gokhale et al 1968). Gaddum-Rosse and Blandau (1973) investigated ciliary activity in mammalian oviducts using lycopodium spores, which are about 25 μ in size, 15 μ and 25 μ microspheres, spores of the slime mould Fuligo septica (8 - 10 μ), fixed rabbit erythrocytes, and rabbit eggs. The

latter were either freshly ovulated and thus in cumulus masses, or else two days old, which are devoid of cumulus and surrounded instead by mucoid material.

However all these observation techniques can only measure the transport velocity of the particles, and this need not be directly related to ciliary activity. There are many other factors that can affect transport of particles. Changes in the viscosity, pH and quantity of the secretion covering the ciliated membrane can all affect transport in spite of normal ciliary motility. Similarly muscle fibres in the mucosa can alter the rate of transport. Thus these techniques can only give a rough guide to the ciliary activity, and more accurate methods must be used to give a clearer picture.

2. Registration Methods

Registration techniques are preferable for the study of ciliary activity. In these the actual number of beats of the cilia can be counted. There are three basic methods:
a.) Measurement by filming the cilia and counting the number of beats on the developed projected film.
b.) Measurement by means of stroboscopy.
c.) Measurement using a photoelectric cell.

a.) Photography

This is a relatively good method of determining ciliary activity, although there are many technical difficulties involved in producing a film of adequate quality for analysis. There are six basic requirements when photographing cilia that together will give undistorted images:
1. Optical devices have to transform phase differences at the protoplasmic boundaries of cilia into differences in light amplitude strong enough to be detected by photographic emulsions.
2. Numerical apertures of objectives and condensers should be such as to resolve single cilia.
3. The duration of exposure has to be very short in order to obtain sharp images of the moving cilia.
4. The intensity of illumination should not change the normal functioning of cells, but must be sufficient for photographic exposure.
5. Appropiate sensitivity of the photographic emulsion should

be combined with sufficient resolving properties.
6. Any mechanical impairment of the free motion of cilia has to be excluded.

Once these criteria are met, exact observations can be made. One disadvantage with this method is that the cilia are generally filmed for only a brief period of time, and so temporary variations are not easily registered.

b.) Stroboscopy

To measure the frequency of movement of an object using a stroboscope, the light frequency must be adjusted until the object remaines stationary. However in the analysis of cilia this is extremely difficult. For the technique to be effective solitary cilia must be examined. When working with mammals this is impossible to achieve. Dalhamn (1956) attempted to use the technique to analyse the beat of rat cilia, but found that it was impossible to adjust the stroboscope adequately due to the high beat frequency of the cilia. It is however feasible to use a stroboscope to analyse the cilia beat of lower animals such as mussels (Mytilus edulis) where every cilium can be distinguished, and the frequency is low (Gray 1930).

c.) Photoelectric cell

This method measures ciliary activity in the following way. A preparation of cilia is made using a glass slide so the cilia can be viewed in profile, and an area about 0.5 µm is examined through an aperture. The light reflected from the moving cilia is projected onto a photoelectric cell, from where the impulses can be measured by an oscilloscope. Fluctuations in the light intensity caused by the moving cilia are detected. The photocurrent can be amplified and recorded.

This method has the advantage that the movement of one particular group of cilia can be followed over a period of time. However only one cell can be examined at one time due to the size of the aperture. This makes it difficult and time consuming to gain an overall view of ciliary movement in a tissue. At the present time this method can be used only for in vitro experiments, as the animal's movements during in vivo

work tend to produce artefacts.

Previous investigators have used a variety of animals to study ciliary motility. Mussels and oysters were at first used abundantly. These animals have long, fairly slow moving cilia in the gill regions, and so are easier to study. Valuable information about the ciliary beat was gained from these species. However ciliary movement is often used as an indicator in toxic inhalation experiments, and it is doubtful whether results obtained from these species can be directly related to vertebrates.

Work has also been carried out using higher animals, with the cilia of respiratory epithelium in frog and rabbit being most extensively studied. In the majority of these experiments however the transport velocity of secretions has been used as an indicator of ciliary activity, and direct observations have not been used. Very few studies have been made of the cilia in the female reproductive tract.

Photography of Ciliary Activity

The development of the technique

In view of the technical difficulties involved, the method for the photography of oviductal cilia had to be worked out on a more or less trial and error basis, using various combinations of the available equipment, until the best possible results were obtained.

1.) The first camera tried was a 16 mm. Bolex cine camera. This was used with a stroboscope light source that theoretically allowed a camera speed of up to 128 frames per second to be used. Unfortunately the results were most unsatisfactory as it proved very difficult to synchronise the camera shutter and stroboscope. Different shutter angles were tried but with no success.

2a.) The second camera was a Vinten high speed 35mm. cine camera. This was used with a Watson monocular microscope equipped with a x40 objective and a x10 eyepiece. The light source was a quartz iodine projector lamp of 10 amps, 24 volts. To achieve the greatest amount of illumination the light had to be focussed on the mirror of the microscope, and reflected up into the condenser. To do this, an 85 mm. Nikon lens with an f1.8 aperture was placed between the light source and the microscope. All possible care was taken to prevent loss of light, by covering the apparatus with black cloth.
A camera shutter angle of 60 degrees was used, and a speed of 250 frames per second. The film gate of the camera was ten inches from the eyepiece of the microscope.

The exposed film was developed in D76 developer (see appendix). The film was divided in two in the darkroom before developing, one half being developed for twenty minutes, and the second for forty minutes, to see if the contrast could be improved.

The resultant film was quite promising. Cilia could be seen, but they were not in focus. The exposure was adequate.

2b.) The same equipment as in 2a. was used with the exception that the projector lamp was replaced by an endoscope light source used without the fibre optic connection. This gives a more intense light, and is cooler, thus fewer filters would be needed to achieve the desired temperature of 37°C. This means that more light is available for the camera.

The film was again quite good, and cilia could easily be seen, but once again they were not completely in focus.

2c.) A Vickers M41 Photoplan microscope was used instead of the Watson. This microscope has built in optics for photography, and so filming does not have to be carried out through the eyepiece. It also has a better condenser, which helps to give better resolution. A x45 objective was used. In an attempt to obtain better focusing, the shutter angle on the camera was altered to 20°. The film gate was again ten inches from the microscope. The endoscope light source and 85 mm. lens were used as before.

This time good results were obtained. The cilia were distinct and in focus, and the pattern of movement was easily discernible.

2d.) To find out if more contrast could be obtained a shutter angle of 5° was used. All other equipment was as in 2c.. This angle was found to be too small as it did not allow enough light into the camera.

2e.) A shutter angle of 20° was again used. The distance between film gate and microscope was altered to twelve inches. This combination also did not allow enough light for filming.

2f.) A shutter angle of 20° was used, and a distance of eight inches between camera and eyepiece.

Unfortunately at this point the camera developed a technical hitch and broke down irretrievably!

Although good results could have been obtained with this Vinten camera, there were three disadvantages. Firstly as it was a 35 mm. camera and would have been run at 250 frames per second, the film costs would have been high. Secondly, projection facilities for 35 mm. cine film are more difficult to find and use than for 16mm. film. Thirdly, focusing is extremely difficult

as the camera has no separate eyepiece for viewing.

3a.) The third camera used was a 16 mm. Beaulieu cine camera. The endoscope light source and Vickers microscope were used as before. Shutter speeds of 64 and 32 frames per second were tried out.A x45 objective was used on the microscope. The film used was Kodak 4 X Negative, 400 ASA.

Good results were obtained although the magnification was not sufficient.

3b.) The set-up was as in 3a., with a x45 objective, but a tube length of 240 mm. was used. Plus X Reversal film, 40 ASA was substituted for 4 X Negative. The camera speed was 64 frames per second.

Fairly good results were obtained, although the focusing and resolution could have been improved.

3c.) A tube length of 212 mm. with the x45 objective was tried, and the 4 X Negative film.

From then on different combinations of film, objectives, and tube lengths, plus various methods of focusing, and different developing times were experimented with in an attempt to improve contrast and resolution of the cilia. The following method was chosen as it gave good consistent results.

Equipment for the cinematography of oviductal cilia

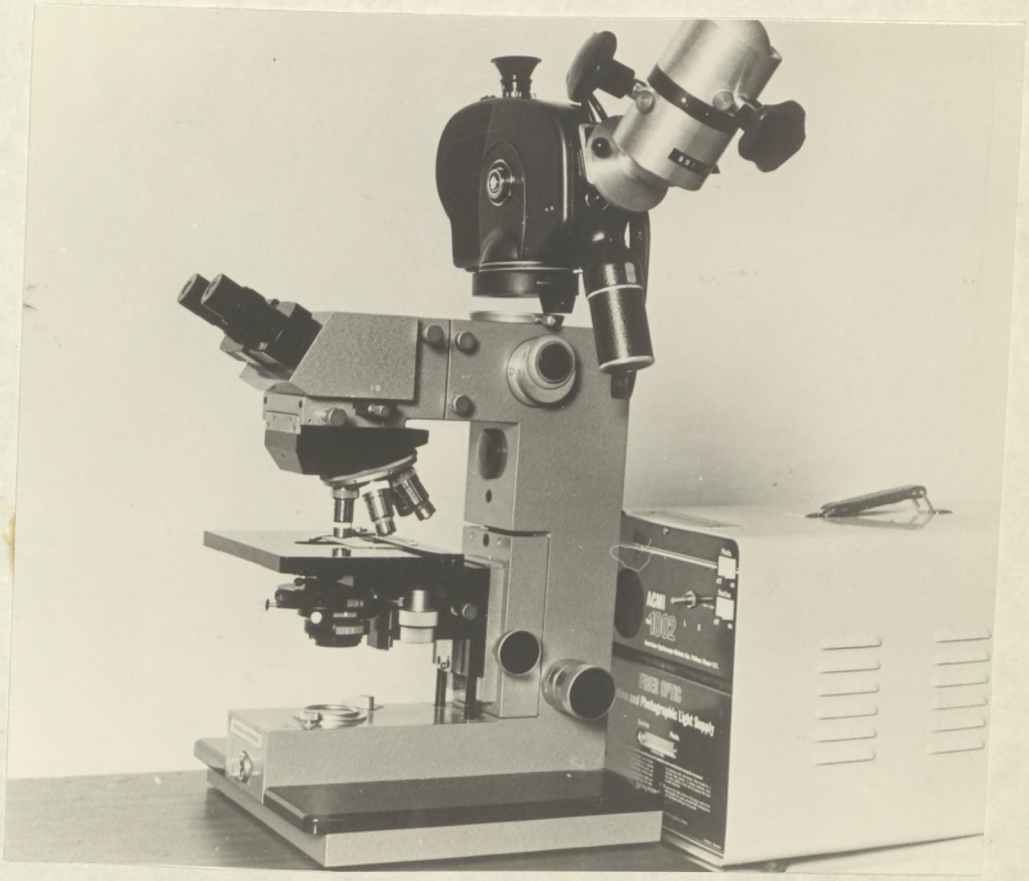
Camera

Beaulieu 16 mm. cine camera with a speed of 64 frames per second. This camera has a ground glass screen for easier focusing. The film was Kodak Plus X Reversal 7276, 40 ASA, 17 Din

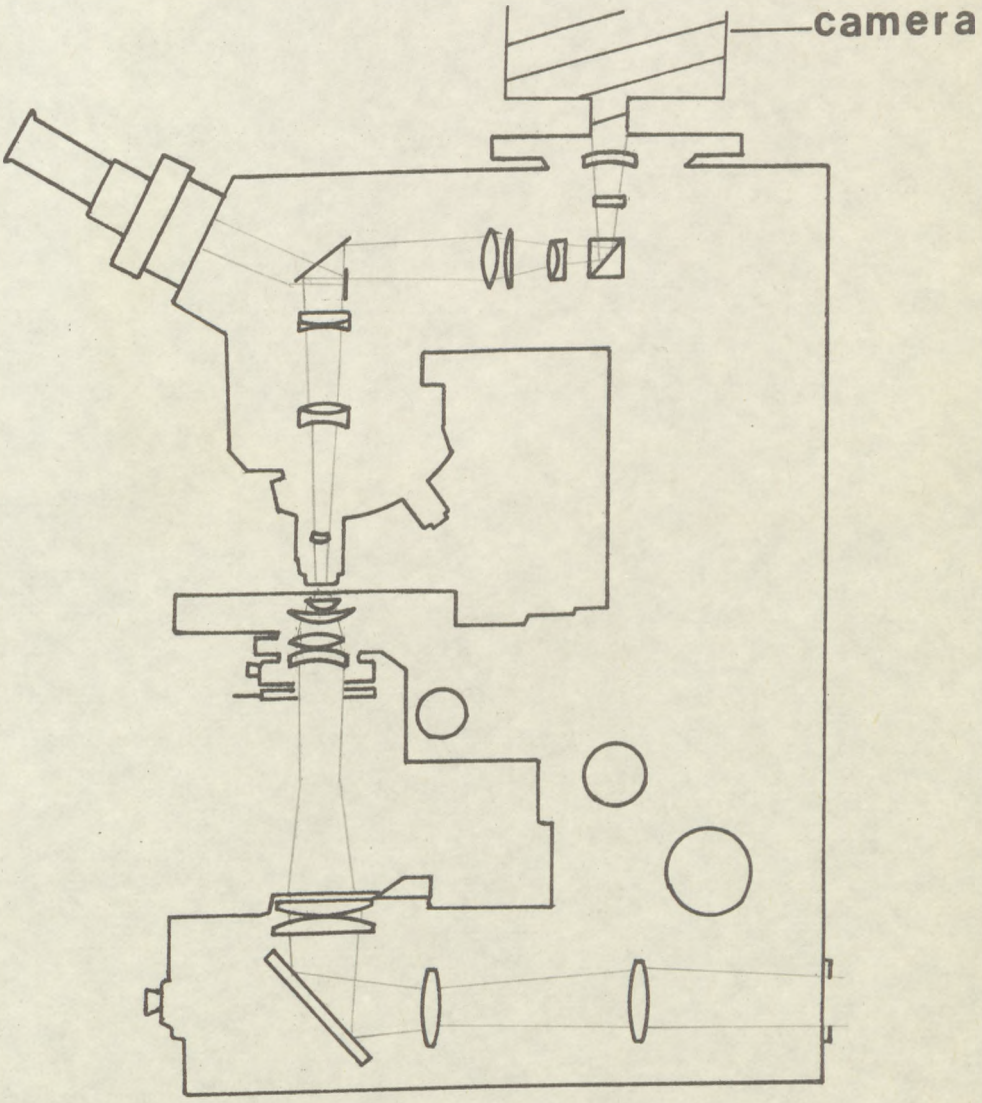
Light source

Acmi fibre optic unit used without the fibre optic connection. The lamp used was a high intensity xenon arc lamp of 300 watts, with an integral reflector.
A glass filter was used between the light source and the microscope so that the temperature of the microscope stage was 37°C. The temperature was monitored by using a thermocouple

Photographic set-up used for filming cilia, showing camera, microscope, and light source



Schematic diagram of microscope showing optical arrangement for transmitted light



—— light path

Fig. 29

connected to a data processing unit giving a continuous digital temperature readout.

Microscope

Vickers M41 Photoplan microscope with a x100 oil immersion objective.The microscope was set up to use transmitted light. This ensures that the resultant film has sharp images of the cilia. Previous workers Dalhamn (1956), who did some of the earliest work on the photography of tracheal cilia, and Borell et al (1957), who filmed rabbit oviductal cilia, both used incident light. By this means the cilia can only be visualized as a series of light reflections from the mucosa, and so cannot be clearly seen.

Materials

Fallopian tubes from women undergoing either hysterectomy or post partum sterilisation were used. Only tubes that were normal in appearance were used.

The numbers of tubes examined in each group were as follows: 8 puerperal, 8 proliferative, 8 secretory.

Method

On removal in the operating theatre the fallopian tubes were put in tissue culture medium (see appendix) at 37°C. Filming was carried out as soon as possible, and in all cases within half an hour of removal. In the laboratory each tube was opened longitudinally with scissors, and a piece of epithelium measuring approximately 2 $mm.^2$, with underlying tissue was removed. In most cases this latter operation was carried out using a dissecting microscope. The piece of epithelium was transferred to a glass slide, and a drop of tissue culture medium placed on it. A glass cover slip was gently lowered onto it, and the whole preparation put onto the microscope stage.

Filming was carried out immediately. A suitable area of epithelium was found using a x10 objective. The x100 oil

immersion objective was put into place and focused carefully, looking through the eyepieces of the microscope, and using the fine adjuster. The beam-splitter was then pushed in thus enabling the light to be directed to the camera and not the eyepieces. The camera was held in a tripod over the microscope (see photograph). The focusing was checked on the ground glass screen of the camera.

Ten feet of film at a shutter speed of 64 frames per second was shot. Epithelium from the fimbrial, ampullary, and isthmic regions was filmed, and each region had three different parts of the epithelium examined. From each preparation two different fields were filmed. Thus each tube had eighteen pieces of film taken.

Care was taken at all stages not to let the temperature of 37°C. alter. While filming was being carried out, the rest of the tube was kept in tissue culture medium in a water bath at 37°C.

To avoid possible discrepancies due to the same area of tube always being filmed first, filming was carried out using the following sequence:-

Fallopian tube 1.
Fimbria, ampulla, isthmus
Fallopian tube 2.
Isthmus, fimbria, ampulla
Fallopian tube 3.
Ampulla, isthmus, fimbria

Stages in the estimation of ciliary motility

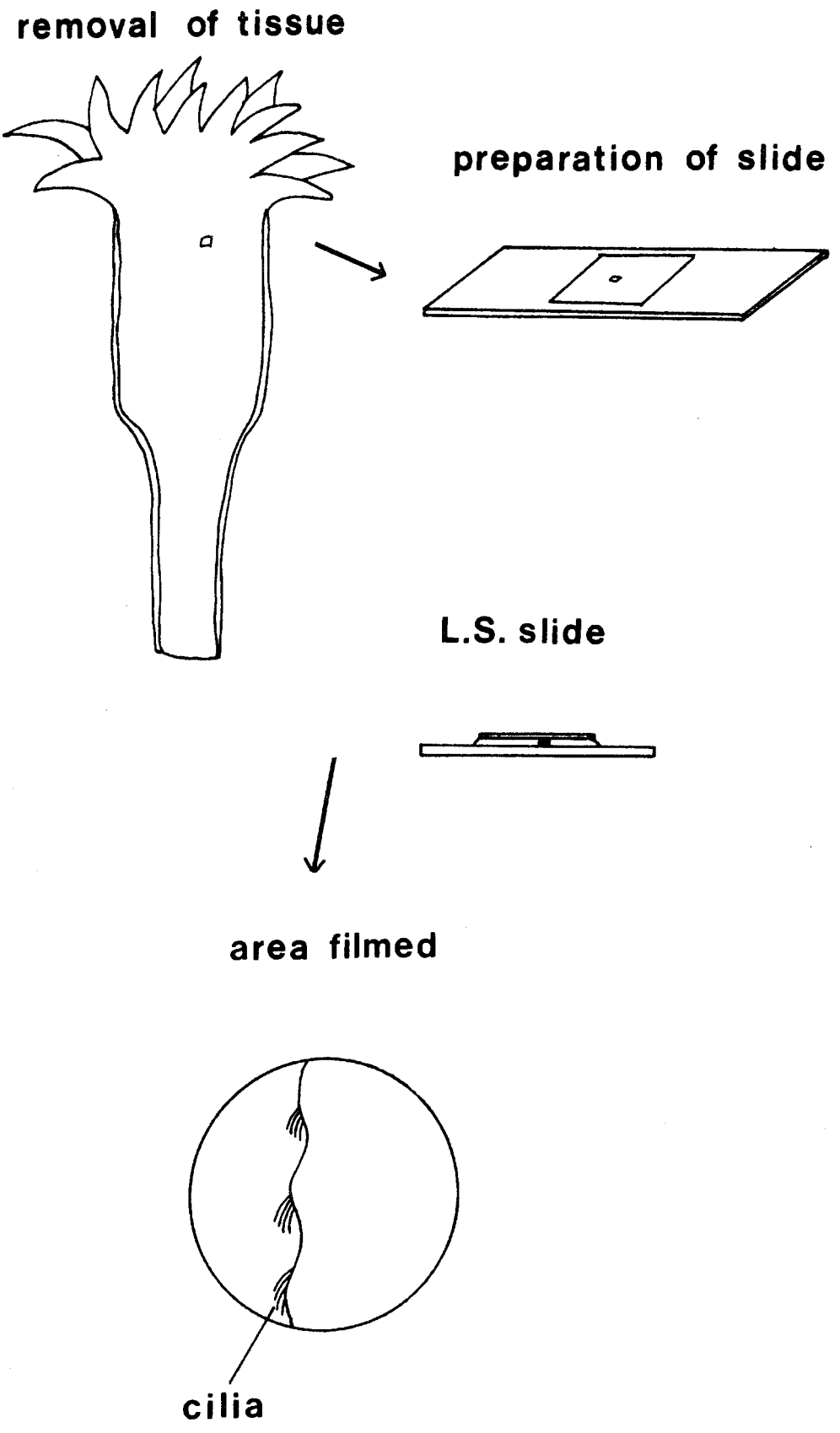


Fig. 30

Analysis of the filming

The film was projected onto a screen by a Bell and Howell 16 mm. cine projector, and using a speed of 15 frames per second. At this speed the cilia appeared to be moving moderately slowly and could be easily counted. The number of beats in each piece of film were counted, the time taken being measured with a stop-watch, so that the number of beats over a period of time was obtained. Each beat comprised one complete movement ie a forward and a return stroke.

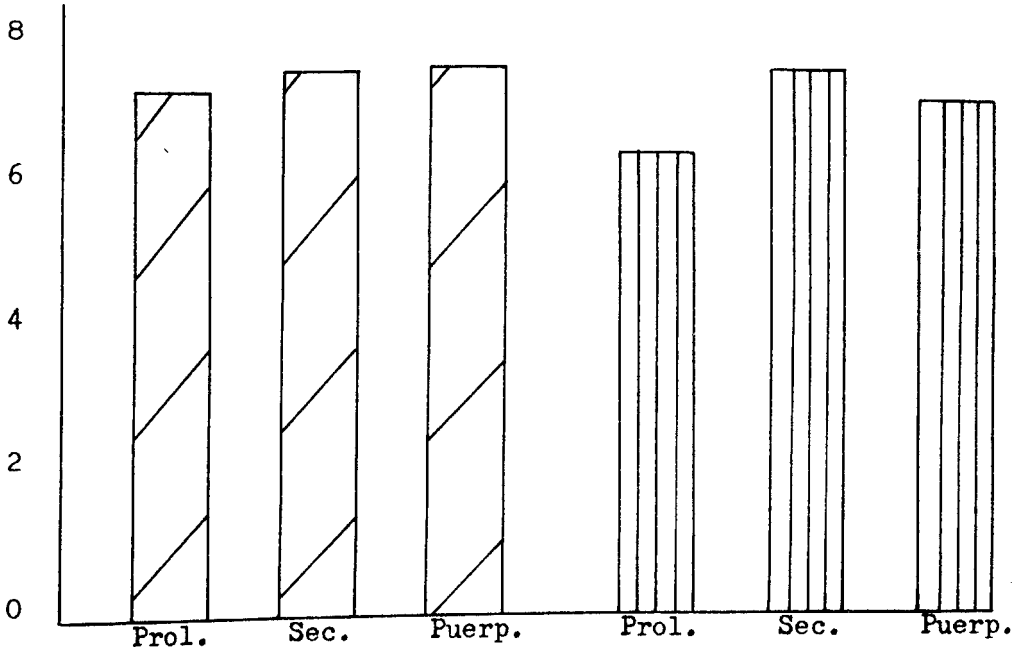
The cilia in general were evenly spaced over the epithelium in small clumps, and so each part of film contained many groups of cilia. To get as representative figure as possible, all the groups that could be easily seen were counted. Each group was counted five times. Thus for each small section of film, representing one area of epithelium, up to 50 counts could be made.

From the results of the counts over a known period of time, the ciliary beats per second can be worked out.

$$\text{beats/second} = \frac{\text{camera shutter speed x no. beats counted}}{\text{projector speed x time in counting}}$$

Histograms to show ciliary motility - differences between types

Fig.31



Significant differences:

None

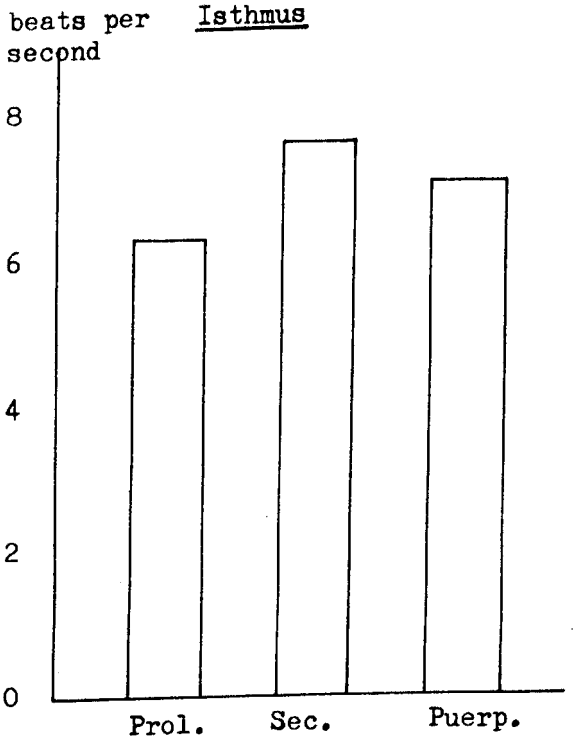
Fig.32

Secretory > Proliferative

Puerperal > Proliferative

Secretory > Puerperal

Fig.33



Significant results:

Secretory > Proliferative

Puerperal > Proliferative

Secretory > Puerperal

Prol. = proliferative

Sec. = secretory

Puerp.= puerperal

Ciliary motility - differences between regions

Fig.34

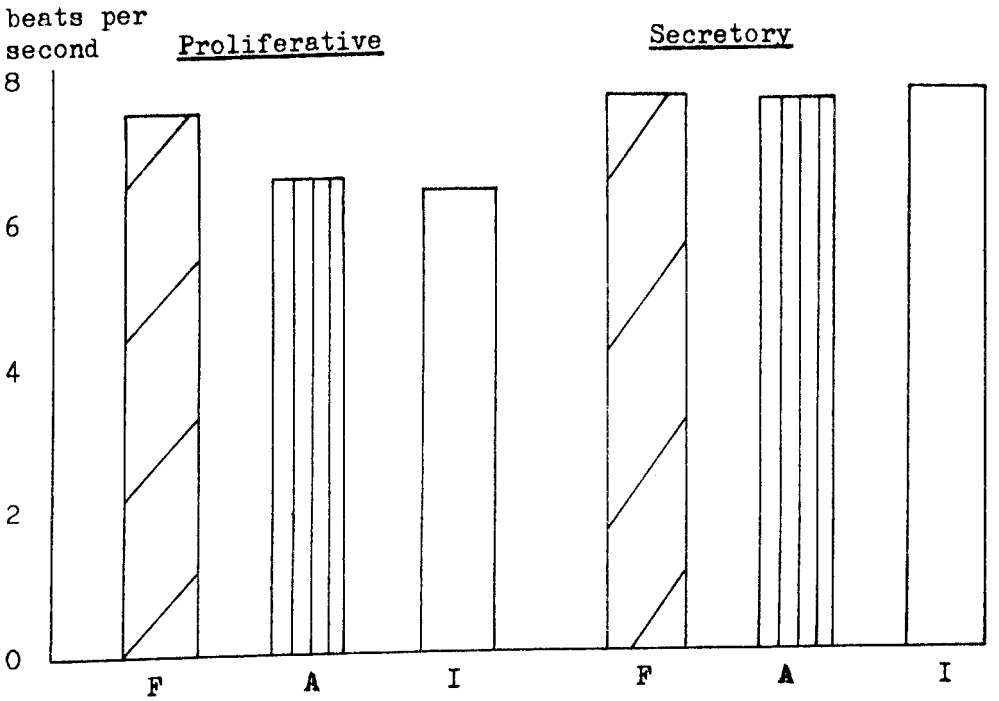


Fig.35

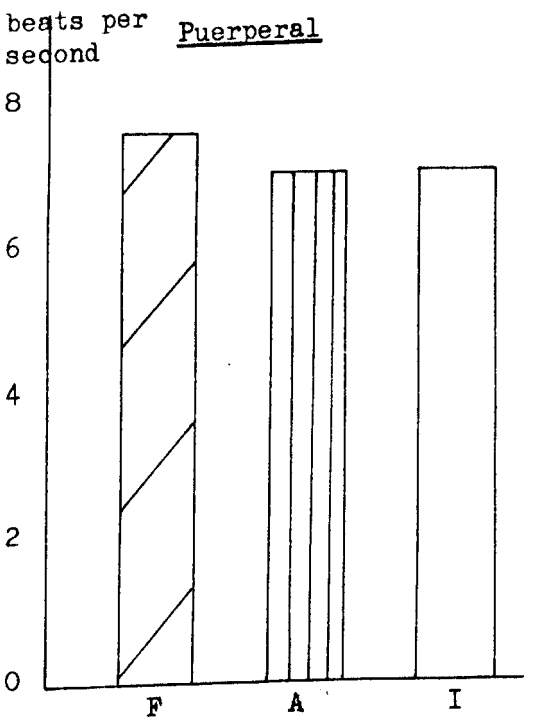
Significant differences:

Fimbria > ampulla

Fimbria > isthmus

None

Fig.36



F = Fimbria

A = Ampulla

I = Isthmus

Significant differences:

Fimbria > ampulla

Fimbria > isthmus

Ciliary Activity

Results

The statistical analysis showed:

1. There was a significant difference between types (puerperal, secretory, proliferative).
2. There was a significant difference between regions (fimbria, ampulla, isthmus).
3. There was a type/region interaction.
4. There was no person and region interaction within types.

The number of beats per second was highest in the secretory phase of the cycle, and lowest in the proliferative. In the proliferative and puerperal phases the cilia beat more quickly in the fimbrial region, with the rate in the ampulla and isthmus being equal. The isthmic cilia from the secretory tubes beat faster than the cilia in the fimbrial and ampullary regions, there being no difference between these latter two. Apart from the secretory cilia, in which the differences in regions were not significant, the differences outlined above were significant at the 0.005 level.

There was no difference between the beat of the fimbrial cilia in the three types, but there were significant differences in both the ampullary and isthmic cilia. The cilia in the ampullary region of the secretory tubes beat faster than those in both the puerperal and proliferative tubes, and similarly the puerperal ampullary cilia beat faster than the proliferative. The isthmic cilia followed the same pattern as the ampullary.

The results for the interactions showed that for people in any one type, the difference from region to region is the same. The differences between the regions vary with the type.

Ciliary Activity

Results of statistical analysis

a = 3 (proliferative, secretory, puerperal)

b = 8 (number of people in each group)

c = 3 (fimbria, ampulla, isthmus)

n = 3 (number of measurements in each region)

$\lesssim\lesssim\lesssim\lesssim Y^2_{ijkn} = 10{,}201.4770$

$G = 1471.73$

$\lesssim\lesssim\lesssim\lesssim Y^2_{ijkn} - \frac{G^2}{abcn} = 173.750$

$\lesssim_i \lesssim_j T^2_{ij..} = 91{,}058.131$

$\lesssim_k T^2_{..k.} = 722{,}904.16$

$\lesssim_i \lesssim_j \lesssim_k T^2_{ijk.} = 30{,}550.933$

$\mathcal{C} = \frac{G^2}{abcn} = 10{,}027.727$

$S_B = 89.843$	$\frac{S_B}{ab-1} = 3.906$	persons and regions ignoring types
$S_C = 12.608$	$\frac{S_C}{c-1} = 6.304$	
$S_{BC} = 53.466$	$\frac{S_{BC}}{(c-1)(ab-1)} = 1.162$	
$S_E = 17.833$	$\frac{S_E}{abc(n-1)} = 0.124$	

$\lesssim_i T^2_{i...} = 724014.29$

$\lesssim_i \lesssim_k T^2_{i.k.} = 241874.24$

$S_A = 28.027$ $\quad \frac{S_A}{a-1} = 14.014$

$S_{B(A)} = 61.816$ $\quad \frac{S_{B(A)}}{a(b-1)} = 2.944$

$S_{AC} = 50.366$ $\quad \frac{S_{AC}}{(a-1)(c-1)} = 12.592$

$S_{BC(A)} = 3.100$ $\qquad \frac{S_{BC(A)}}{a(b-1)(c-1)} = 0.074$

Analysis of variance

	d.f.	s.s.	m.s.	test
S_A (types)	2	28.027	14.014	$F(2,144) = 113$
$S_{B(A)}$ (persons in types)	21	61.816	2.944	$F(21,144) = 23.7$
S_C (regions)	2	12.608	12.608	$F(2,144) = 102$
S_{AC} (interaction types& regions)	4	50.366	12.592	$F(4,144) = 102$
$S_{BC(A)}$ (person & region int. within types)	42	3.100	0.074	$F(42,144) = 0.597$
Error	144	17.833	0.124	
Total	215			

In all cases except the person and region interaction within types, the analysis of variance showed that a significant difference was present in the ciliary activity. A Student's t test was carried out to determine where these differences lay.

Results

Differences between types		
Fimbria	t value	probability
Proliferative/Secretory	1.1263	N.S.
Proliferative/Puerperal	1.8213	N.S.
Secretory/Puerperal	0.8642	N.S.
Ampulla		
Proliferative/Secretory	11.2246	0.005
Proliferative/Puerperal	4.4365	0.005
Secretory/Puerperal	6.7396	0.005
Isthmus		
Proliferative/Secretory	12.8243	0.005
Proliferative/Puerperal	6.5172	0.005
Secretory/Puerperal	5.9871	0.005
Differences between regions		
Proliferative		
Fimbria/ampulla	10.0083	0.005
Ampulla/isthmus	0.0426	N.S.
Fimbria/isthmus	10.2112	0.005
Secretory		
Fimbria/ampulla	0.0627	N.S.
Ampulla/isthmus	0.1243	N.S.
Fimbria/isthmus	0.1347	N.S.
Puerperal		
Fimbria/ampulla	5.3214	0.005
Ampulla/isthmus	0.0213	N.S.
Fimbria/isthmus	5.2093	0.005

Discussion:

From the results obtained there are differences between the rate of ciliary beat at different times in the menstrual cycle, and also within different regions. Prior to ovulation, in the proliferative phase, the cilia beat slower than after ovulation, and this difference was significant in both the ampullary and the isthmic regions although not in the fimbriae. The reasons for this can only be postulated. It is thought that the cilia in the fimbrial region play a large part in the movement of the ovum from the ovary into the tube. Similarly the ampullary cilia help in the movement through the first few millimetres of ampulla. It is generally thought that after this stage movement through the remainder of the oviduct is mediated primarily by muscle motility. However it now seems possible that the cilia play a larger role than was otherwise thought.

The increase in ciliary beat as the ovum passes through the ampullary and isthmic regions could be for two reasons. It could aid in the propulsion of the ovum, or it could help in the movement of secretions. Koester (1969) found that the main secretion was in the isthmus, but this was at its peak on the first post-ovulatory day, and much decreased by the third day. He thought a decline in secretion rate preceded an increase in ciliary beat, which led to the passage of ova into the isthmus.

The rate of ciliary beat in the puerperal tubes was also significantly higher than the proliferative tubes. During the puerperium there are still increased amounts of hormone present which may have a stimulatory effect on the cilia.

Borell et al (1957) found that the ciliary beat of rabbit oviducts increased twenty percent after ovulation. They concluded that ciliary activity plays a major role in the transportation of ova through the Fallopian tubes in rabbits. They found an average ciliary beat of twenty beats per second. In this present study a rate of approximately seven beats per second was obtained.

The rate in the human increased sixteen percent after ovulation in the ampulla, and eighteen percent in the isthmic region.

At the time of passage of the ova through the tubes it appears that mutual touching of ovum and cilia could increase ciliary activity, although this does not necessarily mean that the cilia play a greater role in transport.

Cellular Composition of the Human Oviduct Epithelium - Objectives of Study

In spite of numerous investigations that have been carried out on the tubal epithelium, two things are still not clear:
1. The actual ratio of secretory to ciliated cells has not been quantified throughout the tube.
2. The relative numbers of ciliated cells throughout the cycle have not been assessed to determine if there is a variation.

Although the ratio of secretory to ciliated cells throughout the tube cannot specifically point to a ciliary involvement in ovum movement, it would be a helpful guide in trying to assess the role of both cilia and muscle in transport.

A figure for the numbers of cells throughout the cycle would help to put an end to the old controversy regarding transformation of ciliated to secretory cells throughout the cycle.

Materials:

Human Fallopian tubes were obtained from women undergoing hysterectomy, or post-partum salpingectomy. Only tubes with a normal appearance were used. The tubes from menstruating women were classified according to the phase of the cycle by taking into account the woman's menstrual pattern, and the endometrial histology.

The numbers of tubes in each group were as follows:
6 proliferative, 6 secretory, 6 puerperal, 6 post-menopausal. Each tube was complete, with fimbriae, ampulla, and isthmus.

Method:

On removal in the operating theatre, the tubes were put into tissue culture medium. In the laboratory they were transferred to 10% formal saline and left overnight. Three cubes of tissue approximately 2mm. x 2mm. x 2mm. were then cut from each of the three regions of tube, fimbriae, ampulla, and isthmus, and each cube embedded and processed following the schedule for plastic sections as shown in Appendix

The resultant slides were examined using a Vickers Photoplan M41 microscope with a x100 oil immersion objective. The cells of the epithelium were classified and counted according to type, ciliated or secretory. For each slide at least 500 cells were counted, and the percentage of each type of cell found. Epithelium from the fimbriae, ampulla, and isthmic regions was examined. For every section of the ampullary region two counts were made. The cells of the inside epithelium were counted separately from the cells of the outside edge, as it appeared that the latter had a greater proportion of ciliated cells.

Results:

The one-way analysis of variance showed that there was no significant difference between the percentage of ciliated and secretory cells in the fimbriae, inside or outside of the ampulla, in the four hormonal environments, proliferative, secretory, puerperal, and post-menopausal.

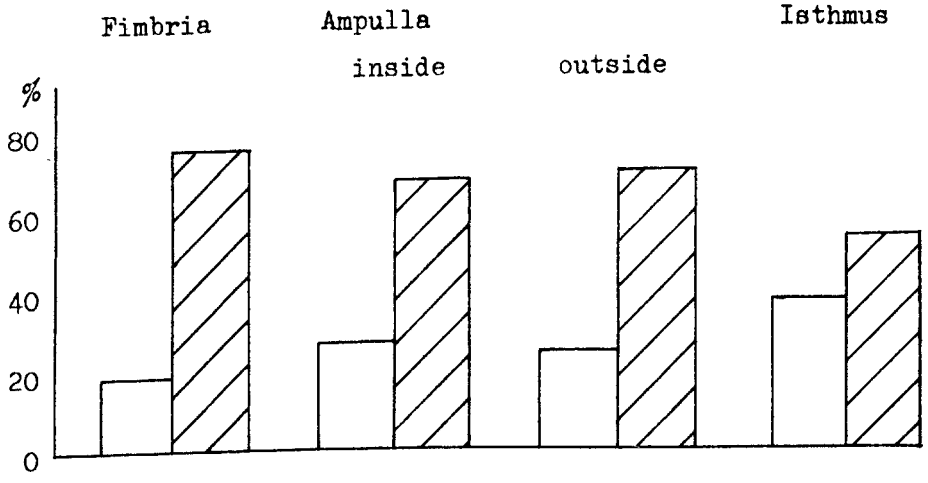
However there was a difference in the isthmic region. The Student's t test showed that the percentage of ciliated cells in the proliferative and secretory phases was higher than the puerperal. There was also a greater number of ciliated cells in the menopausal state when compared to the puerperal. There was no difference in the percentage of the cells in the proliferative, secretory, and menopausal tubes.

There was of course, a great deal of difference in the cell composition of each of the regions. The percentage of ciliated cells increased towards the fimbrial region. The epithelium of the outside of the ampulla had a greater number of ciliated cells when compared with the inside. In the fimbrial region there was still a high number of ciliated cells. In the proliferative, secretory, and post-menopausal tubes there were over 50% ciliated cells. It was only in the puerperal tubes that the number dropped, to 41%.

Histograms to show the percentage of secretory and ciliated cells in the oviduct epithelium in women

Fig.37

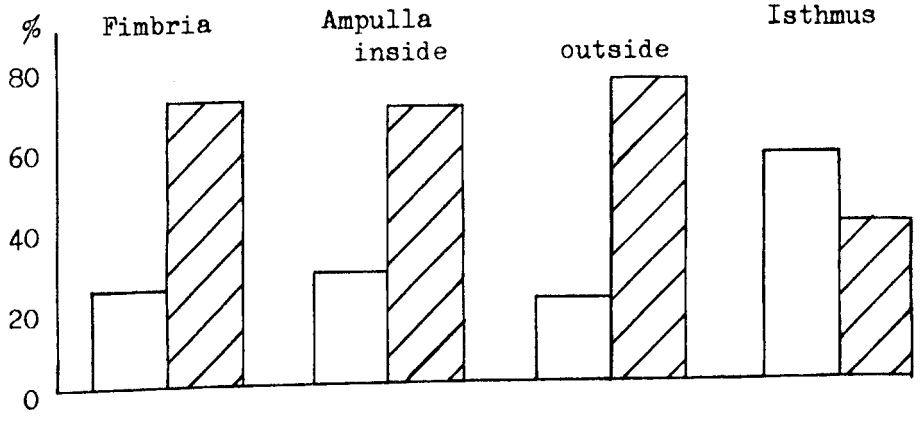
Proliferative



There were no significant differences between the results obtained in the proliferative, secretory, or post-menopausal tubes, and so only a histogram for the proliferative region has been shown

Fig.38

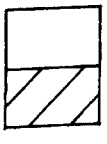
Puerperal



Significant differences:

Number of secretory cells in isthmus greater than number in other phases of cycle

Number of ciliated cells in isthmus less than in other phases of cycle



Secretory cells

Ciliated cells

Statistical analysis of the numbers of cells in the epithelium

A one way analysis of variance was used firstly, to determine whether there was any significant difference between the percentage of secretory and ciliated cells in each region at different stages of the menstrual cycle. On finding a difference, a student's t test was carried out on the respective numbers. Both analyses were as used to investigate the effect of exogenous hormones on muscle motility, and further details can be found in this section.

Results

One way analysis of variance

Fimbria	s.s.	d.f.	m.s.
Between groups	157.12	3	52.375
Within groups	1807.5	20	90.375
Total	1964.62	23	
F = 0.579			
Ampulla - outside			
Between groups	19.12	3	6.37
Within groups	1594.50	20	79.72
Total	1613.62	23	
F = 0.079			
Ampulla - inside			
Between groups	127.46	3	42.49
Within groups	1571.50	20	78.57
Total	1698.96	23	
F = 0.541			
Isthmus			
Between groups	1148.46	3	382.82
Within groups	1267.46	20	63.37
Total	2415.92	23	
F = 6.04			

The fimbria and both inside and outside ampulla show no significant differences between the percentage of secretory and ciliated cells throughout the cycle.
The F value for the cells in the isthmic regions is greater than that corresponding to the one percent critical value, and so the students's t test has been applied.

Students t test

Proliferative / puerperal

t value = 6.1187
Degrees of freedom = 5
Probability = 0.005

Puerperal / post-menopausal

t value = 4.2258
Degrees of freedom = 5
Probability = 0.01

Secretory / proliferative

t value = 1.1623
Degrees of freedom = 5
Probability = not significant

Secretory / post-menopausal

t value = 0.8631
Degrees of freedom = 5
Probability = not significant

Discussion:

From the results obtained it seems unlikely that during the menstrual there is any transformation of ciliated into secretory cells or vice versa. For any given region of tube there was no difference in the percentage of ciliated and secretory cells in the proliferative, secretory, or post-menopausal states. However, the percentage of ciliated cells in the isthmic region of menstruating women was significantly higher than the same region of puerperal tubes. Andrews (1951) found that in the epithelium of the oviduct in women, the average height at the time of delivery was low, at 16 μ. This became lower in the post-partum period, dropping to an average height of 10 μ. Also the ciliated cells were decreased in numbers. Women receiving stilboestral from the day of delivery had an intense proliferation of the ciliated epithelium, with cells reaching 20 - 25 μ in height. Women given progesterone and stilboestral together showed no proliferation , the progesterone inhibited the effect of the oestrogen. Progesterone alone had no effect on the epithelium. Treatment with stilboestral 5 - 10 days before delivery also had no effect. It appears that growth of the ciliated epithelium in the human is stimulated by exogenous oestrogen, and inhibited by progesterone.

As was expected, there was a greater number of ciliated cells in the fimbriae, and the numbers decreased towards the uterus, although the isthmic region in menstruating women still had more than 50% ciliated cells. The outside edge of the epithelium in the ampulla had a greater proportion of ciliated cells than the inside, which would presumably help in the transport of secretions.

Muscular and Ciliary Activity of the Human Fallopian Tube

Discussion

This work aimed to try to clarify the role of muscle and cilia in the movement of the ovum through the Fallopian tube in humans.

The spontaneous muscular motility indicated that there were continuous small contractions throughout the menstrual cycle, with the ampullary region in menstruating women showing a greater activity than the isthmus. The tubes from menstruating women showed a greater activity than those from the puerperium.

Upon addition of adrenergic drugs, the response of the tubes in ampullary and isthmic regions did differ, with the isthmus having a greater response. This would be expected due to the distribution of the adrenergic nerves in the areas, with the isthmus having a greater innervation (Brundin 1965). However this differential response has not always been found (Sandberg et al 1960). Cholinergic drugs had little effect in motility, and this agrees with studies of cholinergic innervation to the tube, with little evidence to show a well defined distribution.(Nakanishi et al 1967).

There was not a great deal of difference in the results between tubes from different stages of the menstrual cycle, but this would only be likely to show up if tubes from ovulating women were examined. Unfortunately it would be difficult to obtain such tubes. There was however a difference between pregnant/ menopausal tubes and those from menstruating women. The former showed a smaller response to drugs. It appears that very low or high levels of hormones both cause a relaxation of the tube.

The effect of the addition of exogenous hormones on the response to drugs was varied. Progesterone tended to produce relaxation of the tube, a small amount of oestrogen was stimulatory, and a large dose of oestrogen produced both relaxation and contraction. Again there was a difference in activity

between the two regions. Progesterone tended to produce relaxation, with the isthmic region showing fewer, larger contractions, and decreased activity units, and the ampulla having less relaxation. Oestrogen tended to increase activity in both regions of the tube.

These results support the views of Seitchik et al (1968) who thought adrenergic receptors helped the tube to function as a sphincter, with tubes under alpha-receptor dominance showing contraction, and causing a delay at the ampullary-isthmic junction. Under beta-receptor dominance the tube would relax and allow the ovum to pass down the tube.

Thus muscle activity in the human Fallopian tube as determined by in vitro methods does differ throughout the cycle, between regions, and under the influence of exogenous hormones.

The results obtained for ciliary activity are most interesting as they show a difference in the rate of beat at different times in the menstrual cycle, and also within different regions. The cilia of the ampullary and isthmic regions show a greater activity after ovulation than before. While this cannot be taken as conclusive evidence that the cilia help in the transportation of ova through the tube, it seems likely that they play a greater part than was otherwise thought.

The number of ciliated cells in the isthmic region of the tube was greater than the number of secretory in all groups except the puerperal. Many authors state that the isthmic region contains few ciliated cells, without providing numerical evidence. The figure obtained is larger than was otherwise thought, and together with the increase in ciliary motility after ovulation, does indicate that the movement of the ovum through the isthmus cannot be considered to be wholly brought about by muscular activity.

The examination of the sections of the Fallopian tube showed clearly the difference in the size of the cells throughout the menstrual. In the proliferative phase ciliated and secretory cells are of equal height, thus giving the epithelium a smooth appearance. The ciliated cells reach their maximum

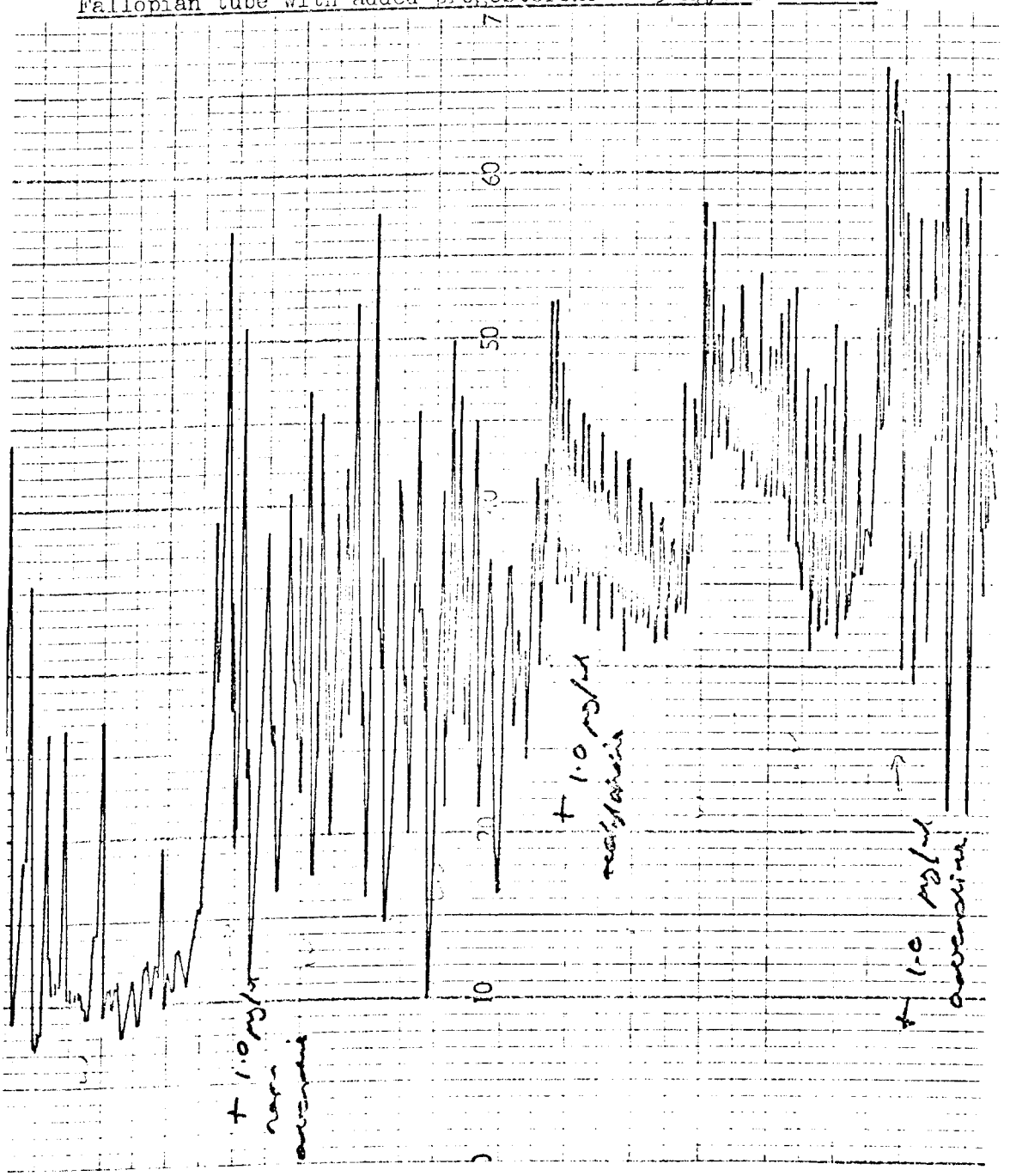
height at ovulation, at the time of the passage of the ovum through the tube. The cilia then protrude into the lumen, above the secretory cells. After ovulation, in the latter part of the menstrual cycle, the ciliated cells become broader and lower, while the secretory cells protrude above them forming a dome above the border of the ciliated cells. This would have the effect of nullifying any effect the cilia might have in transportation.

In conclusion, the results indicate that the cilia play a more than passive role throughout the menstrual cycle in women, although movement of the ovum is probably brought about mainly by muscular contractions of the tube.

APPENDIX

Examples of the recorder tracings from the muscle motility studies

Fallopian tube with added progesterone - 15 ng/ml. Isthmus

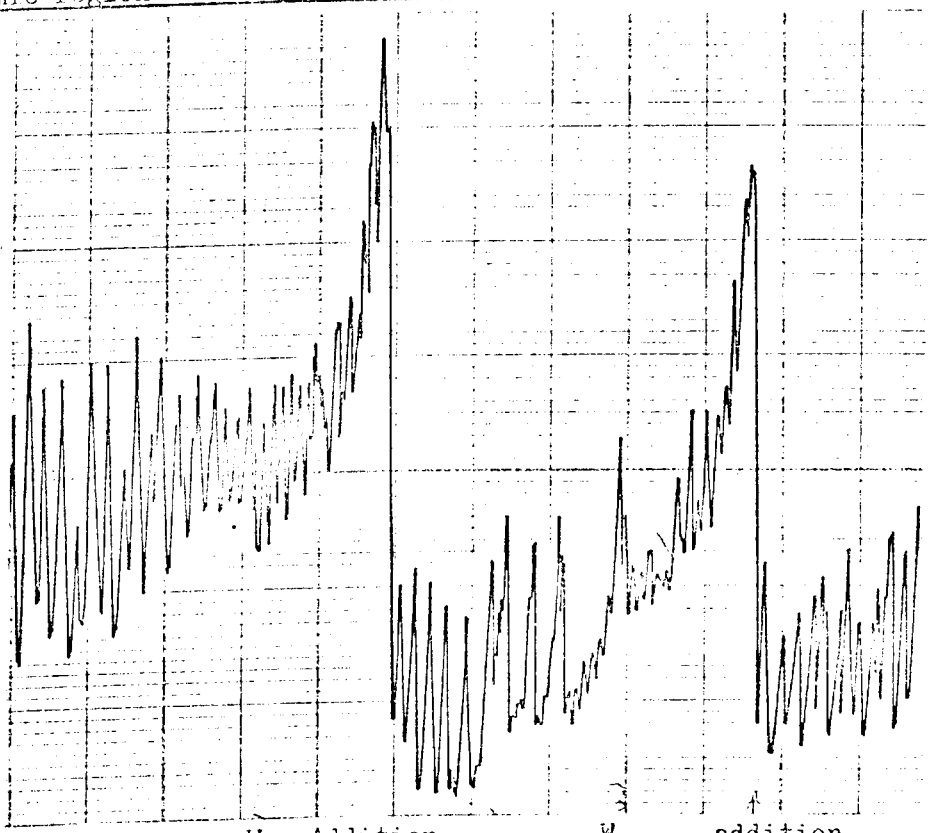


W Addition of nor-adrenaline W W addition of acetylcholine W W addition of adrenaline

W= wash with Kreb's solution + added hormones if necessary

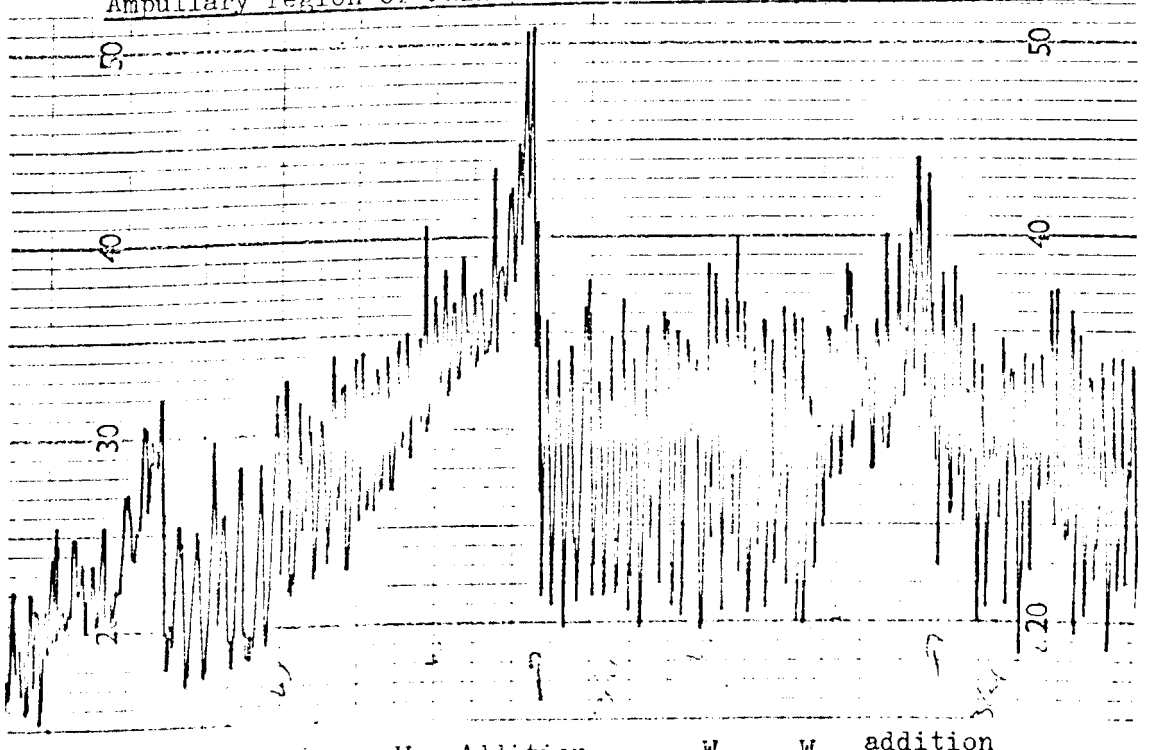
The tracing should be read from right to left

Isthmic region of Puerperal Fallopian tube



W = wash with Kreb's solution + added hormones if necessary

Ampullary region of Fallopian tube + oestrogen - 100 ng/100ml.



One - way analysis of variance as used for muscle motility results

Method

Given independent random samples of sizes n_1, n_2,..., n_k, from k populations and X_{ij} is the jth observation of ith population with

$$n_1 + n_2 + \ldots + n_k = n.$$

Suppose that X_{ij} are independently $N(\mu_i, \sigma^2)$, $i = 1,\ldots, k.$

and $X_{ij} = \mu_i + e_{ij}$

$(i = 1,\ldots k;\quad j = 1,\ldots n_i)$

e_{ij} are independently $N(0, \sigma^2)$

Then the F-test for the null-hypothesis,

H_0; $\mu_1 = \mu_2 = \ldots = \mu_k$

can be written as:

$$F = \frac{SSB/k-1}{SSE/n-k} = \frac{A}{B} = \frac{n-k}{k-1} \quad \frac{\sum_{i=1}^{k} \frac{1}{n_i}\left(\sum_{j=1}^{n_i} X_i\right)^2 - \frac{1}{n}\left(\sum_{i=1}^{k}\sum_{j=1}^{n_i} x_{ij}\right)^2}{\sum_{i=1}^{k}\sum_{j=1}^{n_i} x_{ij}^2 - \sum_{i=1}^{k} \frac{1}{n_i}\left(\sum_{j=1}^{n_i} x_{ij}\right)^2}$$

with k-1 and n-k degrees of freedom

Results of Muscular Motility of the Human Fallopian Tube

Table A.1a. Spontaneous Motility

Isthmus

Proliferative			Secretory			Puerperal		
Freq.	A.Int.	A.Unit	Freq.	A.Int.	A.Unit	Freq.	A.Int.	A.Unit
7	7.8	39	8	6.0	48	6	18.9	94
7	12.7	89	7	12.3	86	5	14.2	66
9	7.6	69	5	7.9	39	4	4.9	20
8	3.4	27	7	3.5	24	5	13.8	69
16	2.3	37	9	8.3	74	7	8.6	60
6	4.8	29	3	3.0	9	7	6.8	47
7	2.3	16	13	4.2	55	7	9.6	67
7	8.9	48	7	3.1	22	4	19.7	69
6	38.3	229	7	4.5	32	6	18.4	110
8	2.8	23	5	5.9	29	5	5.2	26
9	7.1	64	6	4.4	26	5	6.4	32
3	4.8	14	5	2.3	12	3	36.9	111
7	5.9	41	4	3.1	12	6	1.7	11
3	8.1	24	5	12.0	60	2	28.9	58
8	5.2	42				4	24.2	97
11	6.5	71				5	17.9	89
						6	6.9	42
Mean 7.6	8.0	53.9	6.5	5.7	37.7	5.1	14.3	62.8

Table A.1b. Spontaneous Motility

Ampulla

Proliferative			Secretory			Puerperal		
Freq.	A.Int.	A.Unit	Freq.	A.Int.	A.Unit	Freq.	A.Int.	A.Unit
5	13.0	65	13	8.3	107	5	9.1	41
14	5.4	75	8	21.9	175	9	13.9	137
8	7.9	63	7	14.7	103	8	6.1	49
11	5.5	60	9	4.8	43	7	8.8	62
16	6.6	105	8	11.7	94	7	6.7	47
13	4.4	58	7	14.4	101	5	5.5	27
12	8.9	107	12	5.2	62	9	10.7	97
10	7.0	70	9	13.9	126	7	9.2	65
10	11.1	111	11	11.0	121	7	20.0	140
9	30.0	270	10	4.6	46	7	4.9	34
18	4.5	82	11	17.1	188	6	18.7	112
14	7.8	110	10	8.4	84	7	9.9	69
10	3.7	37	12	9.4	112	11	5.7	63
10	15.5	155				7	7.5	52
11	5.2	57				11	10.1	111
7	9.6	67				5	1.9	9
12	9.2	110				9	4.7	42
Mean 11.2	9.1	94.2	9.8	11.2	104.8	7.5	9.3	68.1

TABLE A. 1 PROLIFERATIVE

FREQUENCY CONTRACTIONS/CM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-7	-5	-4	0	1	4
2	6	0	0	6	9	0
3	-7	3	1	2	10	-2
4	0	0	0	2	3	-2
5	-9	-4	-1	-4	0	6
6	2	4	0	0	9	4
7	-3	5	4	-2	0	-1
8	3	7	0	5	3	3
9	-5	-4	-3	2	6	-2
10	2	5	-1	2	5	-1
11	1	3	3	4	1	0
12	3	1	1	0	-3	-4
13	-1	3	-2	3	0	-1
14	-1	-2	0	-1	0	-3
15	2	0	-2	2	3	-1
MEAN	-0.9	1.1	-0.3	1.3	3.1	0.0

TABLE A. 2 PROLIFERATIVE

AVERAGE INTENSITY MM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-8.0	-9.0	-5.0	-7.0	-4.6	-4.2
2	-2.6	0.0	0.0	-4.2	-0.6	0.0
3	0.7	-7.5	-0.5	-2.0	-0.9	-0.6
4	0.0	0.0	0.0	-0.2	-2.8	1.1
5	0.0	-1.6	-0.9	1.7	0.6	-4.6
6	0.0	0.9	-1.6	-1.8	-1.0	-0.9
7	-1.6	0.1	0.2	0.2	-1.0	0.5
8	-1.7	0.5	-0.3	-2.1	-0.5	0.0
9	-1.1	-0.4	-0.4	3.2	-1.1	-3.8
10	-0.4	-7.1	-1.7	1.1	-1.9	0.3
11	-24.5	-31.4	-7.4	-17.6	-10.7	-2.3
12	-0.7	-2.6	0.1	-3.2	-1.1	-0.7
13	-1.2	-3.0	3.5	-2.6	-1.9	0.3
14	-3.5	-0.5	-1.3	0.9	-1.9	-0.6
15	-2.9	-3.8	-0.3	-0.7	-2.6	1.6
MEAN	-3.1	-4.4	-1.0	-2.3	-2.1	-0.9

TABLE A. 3 PROLIFERATIVE ACTIVITY UNITS

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-50	-40	-15	-15	-22	19
2	-10	0	0	-40	20	0
3	-92	-16	7	-11	45	-23
4	0	0	0	4	-19	-2
5	-70	-41	9	5	2	-3
6	7	27	-5	-32	26	-1
7	-35	-8	-6	-5	-12	1
8	5	24	-2	2	19	27
9	-13	-12	-8	52	22	-52
10	7	-53	-17	30	-44	-6
11	-135	-157	51	-31	-103	-23
12	-2	-24	3	-74	-25	-16
13	-16	-9	9	-7	-29	0
14	-4	-5	-3	-5	-75	-25
15	-22	-34	-12	3	-24	12
MEAN	-28.6	-23.2	0.7	-3.3	-14.6	-6.3

TABLE A. 4 PROLIFERATIVE

TONUS MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	18	32	17	5	7	12
2	-4	10	5	18	15	12
3	8	10	9	12	15	8
4	5	12	6	7	14	4
5	25	25	2	17	21	0
6	17	26	8	17	25	16
7	16	30	14	6	8	4
8	20	28	4	10	11	5
9	36	42	4	10	16	0
10	54	63	14	31	42	16
11	47	55	28	36	31	20
12	53	63	13	4	5	2
13	31	40	12	25	25	16
14	10	13	6	15	15	11
15	10	12	6	0	16	7
16	-5	2	0	5	-9	11
MEAN	21.3	28.9	9.2	13.6	16.2	9.5

TABLE A. 5 PROLIFERATIVE

TONUS MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-4	15	17	5	2	12
2	18	15	3	15	20	9
3	-4	1	5	8	12	5
4	-2	2	9	6	10	8
5	0	6	6	0	6	4
6	-4	5	2	9	15	4
7	7	17	12	12	21	15
8	8	13	4	4	5	-2
9	16	30	2	5	6	-5
10	25	45	9	20	26	3
11	23	26	21	13	8	-4
12	31	47	5	4	4	2
13	0	10	6	10	10	11
14	2	2	6	0	4	5
15	13	14	14	13	16	13
MEAN	8.7	16.5	8.1	8.3	11.0	5.3

TABLE A. 6 PROLIFERATIVE

AMPLITUDE MAXIMUM MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	0	0	0	0	-3	7
2	25	20	3	18	23	9
3	-5	11	0	7	11	14
4	8	10	9	11	12	8
5	3	12	5	10	12	-3
6	28	26	0	16	20	5
7	17	26	9	18	25	17
8	14	19	15	11	13	12
9	20	29	3	5	7	2
10	36	42	5	11	8	-9
11	53	52	11	27	25	14
12	22	20	16	10	14	10
13	50	60	12	5	3	4
14	35	35	3	20	21	21
15	10	11	6	9	13	13
16	5	10	7	0	4	8
17	-9	0	0	0	9	13
MEAN	18.4	22.5	6.1	10.5	12.8	8.5

TABLE A. 7 PROLIFERATIVE

AMPLITUDE MAXIMUM MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-9	-9	-5	0	-3	7
2	17	15	3	10	18	9
3	-5	0	0	0	11	3
4	-2	2	9	5	8	8
5	-2	5	5	2	5	-3
6	-2	9	0	9	12	2
7	8	17	12	14	21	13
8	10	14	3	2	5	-2
9	16	28	0	10	4	-9
10	30	36	8	21	14	0
11	-9	0	-4	0	2	-4
12	29	45	4	2	3	0
13	3	7	3	6	10	13
14	-4	-2	7	0	-4	0
15	13	19	3	15	13	13
MEAN	6.2	12.4	3.2	6.4	7.9	3.3

TABLE A. 8 SECRETORY FREQUENCY CONTRACTIONS/CM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	5	3	0	2	-1	1
2	-2	-2	2	-1	-1	-3
3	-2	-3	-3	2	-2	1
4	3	1	1	1	-2	2
5	0	-4	-2	0	3	3
6	2	-2	1	-1	1	0
7	1	-1	0	1	0	2
8	-1	-2	1	1	3	4
9	5	4	-1	-1	4	-2
MEAN	1.2	-0.7	-0.1	0.4	0.6	0.9

TABLE A. 9 SECRETORY

AVERAGE INTENSITY MM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-6.9	1.6	-0.7	-2.1	0.5	0.7
2	-0.5	-1.7	-2.0	-3.6	-18.1	6.6
3	-5.5	-8.7	-5.7	-4.6	-6.6	-6.9
4	-4.2	-1.1	-0.5	-7.6	-10.8	-2.9
5	-3.0	-2.0	-2.7	-0.9	-2.9	-1.1
6	-0.3	-1.5	-2.1	-3.3	-1.5	-1.5
7	-2.5	-1.2	-2.3	-6.4	-5.2	7.4
8	-6.2	-4.1	0.8	-1.1	-1.1	-2.2
9	-0.2	-3.2	-5.1	-11.7	-8.7	-5.8
MEAN	-3.3	-2.4	-2.3	-4.6	-6.9	-0.6

TABLE A.10 SECRETORY ACTIVITY UNITS

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-3	41	-9	0	3	15
2	-9	-15	-3	-105	-81	-27
3	-56	-66	-70	-27	-28	-55
4	-11	-2	0	-43	-74	-1
5	-39	-23	-38	-10	-19	-3
6	2	-12	-19	-40	-2	-12
7	-17	-13	-9	-35	-72	110
8	-39	-14	6	-8	0	-15
9	8	-13	-42	-134	-46	-98
MEAN	-18.2	-13.0	-20.4	-44.7	-35.4	-9.6

TABLE A.11 SECRETORY TONUS mm. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	22	20	8	8	9	4
2	32	35	6	0	5	11
3	10	15	6	8	4	-4
4	27	37	13	7	5	12
5	19	23	12	16	17	12
6	29	42	15	15	23	13
7	26	37	15	20	25	24
8	21	15	15	8	13	9
9	16	23	2	6	11	12
10	20	17	18	8	15	19
11	21	50	8	4	6	9
MEAN	22.1	23.5	10.7	9.1	12.1	11.0

TABLE A.12 SECRETORY TONUS MH. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	10	14	8	2	5	4
2	0	11	6	-0	3	4
3	0	3	6	7	11	6
4	2	-3	0	6	7	4
5	-2	5	6	3	2	-4
6	11	23	9	5	2	6
7	8	13	7	12	11	8
8	14	32	11	7	0	4
9	3	5	15	0	10	15
10	2	6	7	4	6	5
11	3	3	-10	-2	4	5
12	2	11	13	4	2	5
13	-3	22	8	2	1	8
MEAN	4.3	11.2	6.6	3.8	4.9	5.4

TABLE A.13 SECRETORY

AMPLITUDE MAXIMUM MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	12	24	0	12	7	6
2	29	25	0	-25	-20	15
3	7	8	-2	-4	-6	-9
4	25	35	12	0	-8	8
5	15	18	9	14	15	10
6	30	40	14	13	12	7
7	33	36	17	8	26	25
8	12	7	18	3	12	11
9	16	27	-14	0	3	3
10	10	14	11	6	6	5
11	21	48	8	-3	4	10
MEAN	19.1	25.6	6.6	2.4	4.6	8.3

TABLE A.14 SECRETORY

AMPLITUDE MAXIMUM MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	2	24	0	0	3	6
2	3	3	0	-12	-20	-3
3	0	-1	6	-6	7	7
4	2	24	0	5	8	0
5	-6	-3	-9	-4	-6	-9
6	8	24	9	-5	-8	0
7	8	11	4	11	12	2
8	14	31	11	5	7	7
9	0	6	11	-13	6	23
10	-4	2	5	0	6	11
11	5	3	-14	-15	-6	-2
12	-9	0	2	3	-1	-3
13	-3	24	8	-6	-3	6
MEAN	1.5	11.4	2.5	-2.8	0.4	3.5

TABLE A.15 PUERPERAL

FREQUENCY CONTRACTIONS/CM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	0	4	0	6	5	-2
2	0	3	4	-3	1	2
3	3	2	2	2	0	10
4	2	2	0	0	1	2
5	3	0	0	3	0	0
6	2	3	1	-2	1	1
7	-7	0	0	5	-1	2
8	3	2	0	1	3	1
9	2	1	1	-3	2	1
10	2	3	1	3	1	1
MEAN	1.0	2.0	0.9	1.2	1.3	1.8

TABLE A.16 PUERPERAL

AVERAGE INTENSITY MM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-5.3	-26.8	0.0	-4.4	-8.3	-4.0
2	-2.7	-16.1	-1.6	-11.8	-8.0	-4.7
3	-0.5	-2.4	1.0	-0.2	-0.5	3.0
4	-5.7	-5.7	-0.4	-3.9	-5.0	-9.0
5	0.5	-3.0	1.3	-4.7	-2.3	0.3
6	-11.6	-10.6	15.4	-4.0	-2.1	-0.2
7	-7.2	-8.9	-1.3	-5.4	-5.0	-4.0
8	-18.1	-6.9	0.2	-8.4	-6.9	1.5
9	-18.6	-17.3	-4.2	-21.6	-14.5	-7.7
10	-2.8	-1.9	0.2	-1.6	-1.3	-1.9
11	-6.8	-3.0	-1.6	-4.7	-17.8	-9.3
12	-39.5	-45.0	17.2	-13.0	0.3	0.0
13	-1.2	3.1	-0.4	0.0	-5.9	0.0
14	-19.3	-13.5	0.0	-5.3	-5.9	2.6
15	-32.2	-19.6	-2.7	-2.6	1.1	3.0
16	-16.1	-13.2	-2.8	3.2	3.5	4.5
17	-1.5	-0.4	0.0	-0.9	-1.1	-1.1
MEAN	-11.1	-11.2	0.8	-5.4	-4.7	-1.6

TABLE A.17 PUERPERAL

ACTIVITY UNITS

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-32	-64	0	-10	-12	-30
2	-19	-45	20	-165	-51	-33
3	-9	-4	4	-32	-5	35
4	-32	-32	-19	-28	-31	-50
5	-21	-18	8	-46	-23	6
6	-55	-50	-4	-73	-33	-21
7	-35	27	8	-55	-42	20
8	-130	-104	-25	-162	-108	-32
9	-5	-3	1	-11	2	-10
10	-32	-13	0	-31	-81	-54
11	-70	-121	104	-65	11	6
12	-6	27	10	-11	-93	0
13	-70	-27	0	-24	-9	18
14	-91	-78	11	-19	14	-59
15	-141	-32	3	45	48	52
16	-3	3	-20	24	24	-11
MEAN	-46.9	-33.4	6.3	-41.4	-24.3	-10.2

TABLE A.18 PUERPERAL

TONUS MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	0	40	40	6	5	7
2	10	26	14	4	8	7
3	16	30	18	18	18	15
4	28	40	18	13	10	8
5	20	30	3	12	16	15
6	0	5	0	0	6	4
7	2	7	3	9	19	20
8	47	45	11	5	15	6
9	4	11	8	7	7	5
10	-2	0	0	19	17	11
11	45	70	17	16	19	20
12	25	37	5	8	8	8
13	0	0	8	6	6	0
14	15	25	48	6	4	8
MEAN	15.0	26.1	13.8	9.2	11.3	9.6

TABLE A.19 PUERPERAL

TONUS MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-8	5	0	5	8	3
2	-32	0	30	-3	0	0
3	6	11	2	4	1	5
4	-8	-8	0	0	0	0
5	-12	-15	-14	4	8	7
6	-8	7	16	3	4	8
7	-7	10	9	7	8	8
8	11	21	12	5	5	5
9	-9	0	0	-7	-3	4
10	7	15	0	6	11	9
11	0	0	3	5	7	10
12	12	10	11	4	7	2
MEAN	-4.0	4.7	5.7	2.7	4.7	5.1

TABLE A.20 PUERPERAL

AMPLITUDE MAXIMUM MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	20	15	0	8	8	5
2	0	30	38	6	7	10
3	26	22	15	4	6	8
4	15	20	-5	5	8	3
5	8	25	3	0	8	7
6	18	23	19	10	16	10
7	11	30	20	10	5	10
8	19	31	7	9	16	11
9	-37	-24	-7	-25	-13	-5
10	10	7	4	5	13	7
11	21	0	25	2	30	26
12	3	13	7	7	2	5
13	-21	-17	0	7	16	12
14	36	50	16	19	20	20
15	15	26	-11	15	12	12
16	5	0	8	7	12	0
17	6	18	10	2	3	4
18	20	28	22	8	16	12
19	16	12	58	6	4	8
20	25	35	7	6	12	3
MEAN	10.8	17.2	11.8	5.5	10.1	8.4

TABLE A.21 PUERPERAL

AMPLITUDE MAXIMUM MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-40	-25	28	-15	-10	-5
2	10	12	4	-4	0	8
3	-10	-10	-5	-5	-4	-10
4	-8	-12	-14	0	8	7
5	-18	-14	24	-2	4	8
6	-6	3	10	-4	10	-4
7	-8	17	12	-8	0	10
8	-37	-24	-7	-25	-13	-5
9	6	15	0	5	11	2
10	-9	-3	4	0	-6	2
11	-30	-26	10	-6	15	3
12	1	6	4	0	-6	3
13	-21	-17	0	-6	-4	6
14	-35	15	-5	8	14	-5
15	-25	3	-11	13	12	7
16	0	0	-4	7	12	0
MEAN	-14.5	-3.7	3.1	-2.6	2.7	1.7

TABLE A.22 MENOPAUSAL

FREQUENCY CONTRACTIONS/CM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	0	5	0	-9	-8	0
2	-6	0	0	0	0	0
3	-5	-4	-6	0	0	-4
4	-1	2	0	-7	-3	-1
MEAN	-3.0	0.8	-1.5	-4.0	-2.7	-1.2

TABLE A.23 MENOPAUSAL

AVERAGE INTENSITY MM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-1.7	-2.8	0.0	-6.9	-8.7	-2.0
2	0.0	0.0	0.5	0.0	0.0	0.6
3	0.5	-0.4	-0.4	-0.7	0.1	-1.3
4	-0.2	-0.1	-1.5	0.7	0.6	0.9
MEAN	-0.3	-3.1	-0.3	-1.7	-2.0	-0.5

TABLE A.24 MENOPAUSAL

ACTIVITY UNITS

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-12	8	0	-20	-22	-14
2	-12	0	2	0	0	4
3	-5	-8	-10	-12	3	-32
4	-9	-5	-11	-15	-5	8
MEAN	-9.5	-1.2	-4.7	-11.7	-6.0	-8.5

TABLE A.25 MENOPAUSAL

TONUS MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	8	11	2	4	5	8
2	15	23	6	10	12	12
3	14	22	7	8	8	6
4	11	36	8	7	17	6
MEAN	12.0	23.0	5.7	7.2	10.5	8.0

TABLE A.26 MENOPAUSAL

TONUS MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-9	2	2	-3	0	8
2	5	11	6	10	12	12
3	14	22	14	4	8	6
4	11	25	0	7	17	6
MEAN	5.2	15.0	5.5	4.5	9.2	8.0

TABLE A.27 MENOPAUSAL

AMPLITUDE MAXIMUM MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	7	8	2	4	5	6
2	18	20	7	10	12	12
3	15	21	7	7	7	4
4	12	30	14	7	15	3
MEAN	13.0	19.7	7.5	7.0	9.7	6.2

TABLE A.28 MENOPAUSAL

AMPLITUDE MAXIMUM MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-10	0	2	-11	-7	6
2	5	11	7	10	12	12
3	15	21	14	3	7	4
4	6	18	-7	7	15	3
MEAN	4.0	12.5	4.0	2.2	6.7	6.2

TABLE A.29 PREGNANT

FREQUENCY CONTRACTIONS/CM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-6	-2	0	0	3	-2
2	2	3	-1	-2	2	1
3	3	2	3	4	1	1
4	-5	-3	2	0	4	1
5	-3	-3	1	5	5	-1
6	-9	-9	-7	3	0	1
MEAN	-3.0	-2.0	-0.3	1.7	2.5	0.2

TABLE A.30 PREGNANT

AVERAGE INTENSITY MU.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	12.7	-1.7	0.0	0.0	-4.1	1.7
2	-2.0	-2.0	-1.1	-4.6	-1.9	-1.7
3	-5.6	-1.4	-2.8	-5.4	-6.8	0.3
4	-2.7	-4.5	-4.0	-0.8	1.5	-0.9
5	-1.0	-1.5	-1.2	0.0	-0.3	-0.4
6	-1.6	-6.4	-4.6	1.9	-2.5	3.9
7	-1.0	-1.5	-1.5	-4.5	0.0	1.0
MEAN	-0.2	-2.7	-2.2	-1.9	-2.0	0.6

TABLE A.31 PREGNANT

ACTIVITY UNITS

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-19	-18	5	-29	-6	-4
2	-12	-12	-10	-41	-8	-6
3	25	-21	-25	-18	-19	-3
4	-9	-24	-18	2	16	-2
5	-22	-19	-4	0	2	-4
6	-13	-43	-7	-41	11	10
7	-12	-22	-20	-38	0	0
MEAN	-8.9	-22.7	-11.3	-23.6	-0.6	-2.0

TABLE A.32 PREGNANT

TONUS MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	0	22	1	0	2	0
2	20	20	22	8	10	12
3	19	23	16	13	17	21
4	4	7	2	4	5	5
5	2	4	3	13	17	16
6	0	0	2	6	5	3
MEAN	7.5	12.7	7.7	7.3	9.3	9.5

TABLE A.33 PREGNANT

TONUS MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	0	0	1	0	2	0
2	-2	8	11	0	5	5
3	8	11	12	9	8	9
4	-3	-2	0	2	3	2
5	-2	0	0	8	8	7
6	0	0	2	3	3	3
MEAN	0.2	2.8	4.3	3.7	4.8	4.3

TABLE A.34 PREGNANT

AMPLITUDE MAXIMUM MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	8	15	0	-7	-3	0
2	15	20	21	5	10	15
3	18	17	11	12	22	18
4	5	8	0	3	5	4
5	-4	-10	3	14	15	14
6	-2	0	0	0	5	3
MEAN	6.7	8.3	5.8	4.5	9.0	9.0

TABLE A.35 PREGNANT

AMPLITUDE MAXIMUM MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	0	-6	0	-7	-3	0
2	-6	2	10	-8	0	5
3	8	8	7	9	11	0
4	-3	-4	0	2	2	3
5	-4	-10	-10	10	4	7
6	-2	-2	0	-4	3	3
MEAN	-1.2	-2.0	1.2	0.3	2.8	3.0

TABLE A.36 PROGESTERONE EFFECT

FREQUENCY CONTRACTIONS/CM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-4	0	-4	1	-6	-5
2	-6	-2	-1	3	-1	2
3	-3	-5	-2	3	-3	-5
4	-2	-4	-7	3	1	0
5	-2	-2	2	1	1	-3
6	-6	-3	5	0	7	2
7	-1	-2	-2	7	5	11
8	-5	-5	-4	-1	-1	2
9	-10	-6	-6	-6	-8	-10
MEAN	-4.3	-3.2	-2.1	1.2	-0.6	-0.4

TABLE A.37 OESTROGEN 1 EFFECT

FREQUENCY CONTRACTIONS/CM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	1	1	-2	-12	1	3
2	-5	8	2	4	4	-1
3	5	7	4	4	1	-3
4	-6	2	-1	2	0	8
5	1	1	0	1	0	-5
6	-3	-6	3	-6	-3	-4
7	6	5	-1	-4	-4	-2
8	0	5	5	-8	-3	3
9	6	-1	-2	9	15	2
MEAN	0.6	2.4	0.9	-1.1	1.2	0.1

TABLE A.38 OESTROGEN 2 EFFECT

FREQUENCY CONTRACTIONS/CM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	3	-2	1	-4	-3	-2
2	-1	-2	1	-6	-5	-10
3	0	-3	4	0	-3	1
4	-3	-1	-4	0	-4	-2
5	-4	0	1	0	0	-3
6	4	0	0	1	-5	3
7	-3	1	2	-2	-3	8
8	8	4	0	-1	-2	3
9	11	-3	-2	3	9	9
10	-3	1	4	-4	-1	-2
11	-1	3	0	-6	0	4
MEAN	1.0	-0.2	0.6	-1.7	-1.5	0.8

TABLE A.39 PROGESTERONE EFFECT

AVERAGE INTENSITY MU.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	13.2	15.4	12.8	-0.2	0.0	-2.1
2	5.6	6.6	0.9	-1.0	0.8	5.3
3	10.3	24.9	2.3	-0.1	-1.8	0.1
4	11.2	10.2	9.8	-0.9	-1.2	6.2
5	21.0	7.5	-21.5	-5.8	-0.1	7.6
6	23.8	10.3	-47.2	1.3	-2.4	4.1
7	-5.2	7.1	-21.8	-2.3	-2.5	-5.7
8	-0.2	-1.4	7.9	-0.7	1.5	0.9
9	33.0	20.0	35.3	-0.3	1.3	-2.1
10	3.0	8.8	-2.0	-1.2	-2.4	1.2
MEAN	11.6	10.9	-2.4	-1.1	-1.6	1.6

TABLE A.40 OESTROGEN 1 EFFECT

AVERAGE INTENSITY MM.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	8.3	22.5	2.5	6.9	-1.8	12.1
2	4.4	-13.8	-2.9	-0.1	-3.2	-0.6
3	4.6	-4.1	6.0	7.4	-0.2	3.9
4	2.0	-0.8	7.4	11.1	5.0	5.4
5	0.8	-0.2	1.4	13.6	-2.1	3.7
6	4.3	-15.7	1.3	2.0	6.4	-1.7
7	3.8	-7.6	4.2	0.9	3.9	2.7
8	-21.2	0.5	11.9	6.9	2.1	1.6
MEAN	0.9	-2.4	4.0	6.1	1.3	3.4

TABLE A.41 OESTROGEN 2 EFFECT

AVERAGE INTENSITY MU.

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	22.0	14.0	13.3	4.8	1.5	3.7
2	1.1	6.0	7.2	8.4	6.9	2.5
3	0.0	3.8	0.0	-0.9	-2.5	-4.7
4	7.9	5.0	-10.4	-3.7	19.8	9.2
5	-3.9	7.8	-18.0	-3.2	6.4	3.2
6	10.2	15.1	5.0	-1.4	7.6	-3.1
7	3.2	20.2	15.4	-3.1	-11.7	-2.8
8	1.7	10.5	0.0	3.2	-1.3	-3.4
9	-9.4	2.6	2.5	-5.2	-3.8	-1.2
10	0.6	3.9	-23.4	2.1	2.6	5.2
11	2.2	0.7	-3.1	3.3	1.3	1.0
MEAN	3.2	8.1	-1.0	0.4	2.4	0.9

TABLE A.42 PROGESTERONE EFFECT

ACTIVITY UNITS

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	127	142	89	3	-13	-32
2	-46	18	-23	0	-15	72
3	-4	11	-14	-11	-46	-6
4	0	-45	-6	23	-16	-62
5	165	40	-54	-40	-73	-12
6	171	-144	-163	11	-3	51
7	52	91	-85	42	-33	10
8	-48	-42	5	-10	14	17
9	-11	-5	2	53	-60	-49
MEAN	45.1	7.3	-27.7	7.9	-27.2	-1.4

TABLE A.43 OESTROGEN 1 EFFECT

ACTIVITY UNITS

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-4	96	-1	70	6	70
2	47	0	7	8	-25	-9
3	-52	-23	-4	74	4	60
4	-4	-22	28	27	30	15
5	-3	-15	19	-3	-25	17
6	-11	-64	5	-11	51	34
7	88	0	74	-8	-4	26
8	-41	-18	24	42	37	-19
MEAN	2.5	-5.7	19.0	24.9	9.2	24.5

TABLE A.44 OESTROGEN 2 EFFECT

ACTIVITY UNITS

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	60	20	79	30	-4	41
2	-9	16	22	-16	-13	75
3	0	34	9	0	-27	-29
4	-12	-27	-34	-34	41	84
5	13	30	-29	-29	-8	43
6	125	57	13	-8	37	25
7	14	123	77	6	21	7
8	33	40	0	29	-14	-17
9	0	-7	-18	-80	7	8
10	12	52	-61	1	8	19
MEAN	23.6	33.8	5.8	-10.1	4.8	25.6

TABLE A.45 PROGESTERONE EFFECT

TONUS MU. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-33	-15	-44	-2	-1	-1
2	7	12	-3	3	-8	-1
3	-40	-57	-13	-6	-6	-1
4	-13	-24	0	2	3	5
5	4	20	23	3	7	-4
6	4	-9	3	-3	5	-3
7	-12	24	0	-1	-2	5
8	0	7	3	2	2	4
9	-38	-43	3	-15	-17	2
MEAN	-13.4	-9.4	-3.1	-1.9	-1.9	0.7

TABLE A.46 OESTROGEN 1 EFFECT

TONUS MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	20	25	10	4	9	11
2	12	-5	0	2	0	2
3	-7	24	20	1	2	2
4	-19	4	-2	3	3	1
5	5	4	-2	3	-4	1
6	-7	-10	3	-1	0	0
7	8	17	-8	-7	-11	-8
8	-3	-5	4	3	1	-2
9	3	7	2	0	-7	4
MEAN	1.3	6.8	3.0	0.9	-0.8	1.2

TABLE A.47 OESTROGEN 2 EFFECT

TONUS MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-31	-15	-24	-14	11	-8
2	-6	-23	-9	7	15	7
3	0	-1	2	0	0	5
4	-25	-30	-4	-9	-6	-8
5	20	32	8	-1	1	-2
6	8	8	16	8	11	15
7	15	10	-7	3	3	8
8	-4	-22	-5	-4	3	1
9	-1	-10	2	-2	0	-3
10	2	22	13	-1	-6	-4
11	-1	-5	-6	-6	6	4
MEAN	-2.1	-3.1	-1.3	-1.7	3.5	1.4

TABLE A.48 PROGESTERONE EFFECT

TONUS MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	0	8	1	-1	-3	0
2	1	-3	-2	8	-3	1
3	-34	-16	42	0	-5	0
4	-1	-11	0	0	0	-3
5	-9	0	-2	7	5	-2
6	2	-3	-2	2	2	1
7	-1	3	-9	2	0	5
8	-4	-5	-2	0	-1	3
9	0	3	-2	1	-8	1
MEAN	-5.1	-2.7	2.7	2.1	-1.4	0.7

TABLE A.49 OESTROGEN 1 EFFECT

TONUS MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	4	-34	0	3	4	11
2	4	-1	2	2	6	2
3	1	7	18	0	4	0
4	-13	0	-2	2	5	3
5	-12	5	8	-1	-4	-2
6	-1	-3	3	-3	3	-2
7	-5	5	-5	-4	-6	-7
8	6	2	12	0	-1	-2
9	-6	-2	12	2	-3	0
MEAN	-2.2	-2.3	5.3	0.1	0.9	0.3

TABLE A.50 OESTROGEN 2 EFFECT

TONUS MU. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	0	-8	-4	-15	-18	-14
2	2	-12	-8	7	10	6
3	0	-9	2	0	-5	0
4	-20	-20	-9	-8	-10	-13
5	0	12	-7	-6	0	0
6	7	12	3	4	7	5
7	3	3	-11	-4	-1	2
8	4	-1	-7	3	2	0
9	2	-2	0	9	7	-1
10	-2	6	0	0	-8	-3
11	-1	3	4	1	4	2
MEAN	0.6	-1.5	-3.4	-0.8	-1.1	-1.5

TABLE A.51 PROGESTERONE EFFECT

AMPLITUDE MAXIMUM MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-20	7	-13	1	-2	-3
2	-32	-9	-2	1	1	4
3	-47	-22	-20	-6	-5	1
4	-7	-17	2	3	3	2
5	8	-2	-5	2	1	-1
6	-11	4	-15	-2	6	4
7	1	6	0	5	-4	1
8	-8	1	6	3	3	4
9	-3	-5	9	5	6	2
MEAN	-13.2	-4.1	-4.2	1.3	1.0	1.6

TABLE A.52 OESTROGEN 1 EFFECT

AMPLITUDE MAXIMUM MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-10	10	4	-5	4	11
2	14	-2	-7	3	2	3
3	3	11	13	1	2	2
4	-22	-37	-2	3	3	1
5	-17	-11	-12	6	-1	2
6	-7	-10	8	-3	-2	3
7	-2	-4	-8	-13	0	-3
8	23	25	7	2	2	-1
9	4	13	13	0	-2	0
MEAN	-1.6	-0.6	1.8	-0.7	0.9	2.0

TABLE A.53 OESTROGEN 2 EFFECT

AMPLITUDE MAXIMUM MM. 1ST MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	-16	6	-8	-11	-19	-5
2	-24	-11	-10	4	10	5
3	0	-4	8	1	-2	5
4	-21	-39	-8	2	-3	-4
5	37	28	3	-7	-3	-6
6	8	19	4	-4	10	12
7	15	14	6	4	-7	7
8	-7	-21	-4	4	-2	2
9	-16	-9	-8	1	-5	1
10	11	9	-4	0	-4	-1
11	1	-13	-7	2	6	-2
MEAN	-1.1	-1.9	-2.5	-0.4	-1.7	1.3

TABLE A.54 PROGESTERONE EFFECT

AMPLITUDE MAXIMUM MN. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	37	27	36	-2	-5	-2
2	6	-11	20	-15	1	1
3	-34	-12	-10	-8	-6	0
4	14	-5	13	-6	-3	-10
5	21	1	-38	0	-7	10
6	50	7	-41	7	1	5
7	-11	-14	-17	7	1	1
8	-10	-11	7	1	0	2
9	54	20	16	0	1	4
MEAN	14.1	0.2	-1.6	-1.8	-1.9	1.2

TABLE A.55 OESTROGEN 1 EFFECT

AMPLITUDE MAXIMUM MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	30	-70	-2	3	-2	11
2	20	21	-5	17	10	16
3	10	-27	11	0	0	-1
4	-5	-19	-2	11	-2	17
5	-10	12	7	8	2	1
6	0	-1	4	-2	1	0
7	-15	-22	-5	-2	0	3
8	20	10	23	1	1	8
9	-11	13	0	-3	-7	-8
MEAN	4.3	-9.2	3.4	3.7	0.3	5.2

TABLE A.56 OESTROGEN 2 EFFECT

AMPLITUDE MAXIMUM MM. 2ND MEASUREMENT

	ISTHMUS			AMPULLA		
NO.	NOR-AD	ADREN	ACETYL	NOR-AD	ADREN	ACETYL
1	27	14	40	-15	-16	-8
2	-5	-5	-2	15	10	0
3	0	-14	3	0	-5	6
4	-1	-50	-11	-7	8	-9
5	-5	20	-21	-8	0	-10
6	15	22	8	3	19	22
7	3	15	6	4	-3	1
8	7	13	-6	11	-3	2
9	-3	1	-1	3	2	2
10	-10	-1	-28	0	-6	1
11	1	-6	0	7	2	3
MEAN	2.6	1.7	-1.1	1.2	0.7	0.9

Sequence of frames from one of the 16mm. cine films of ciliary activity in the human Fallopian tube. Cilia can be seen in profile against the edge of the epithelium, and their position alters during the sequence. Upon projection they appear much clearer, and the movement can be easily followed.
Camera shutter speed 64 frames per second.

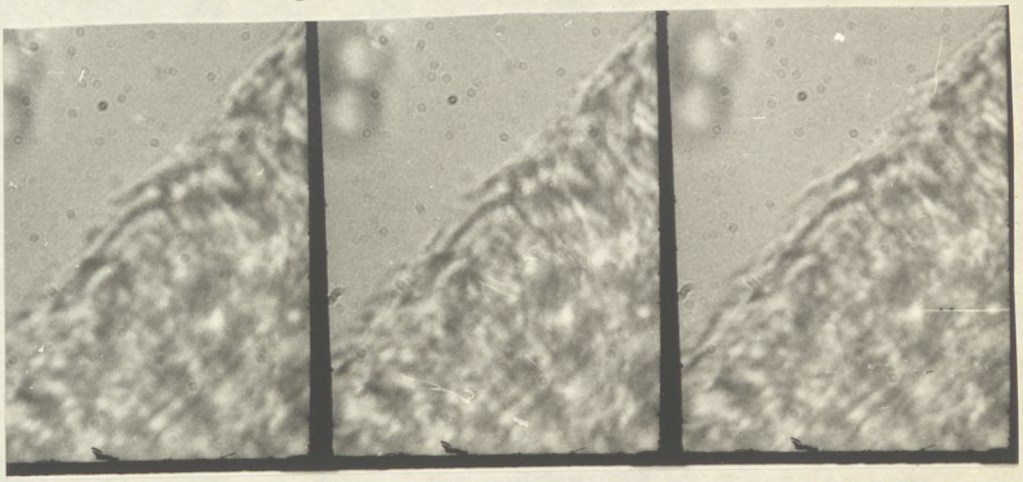
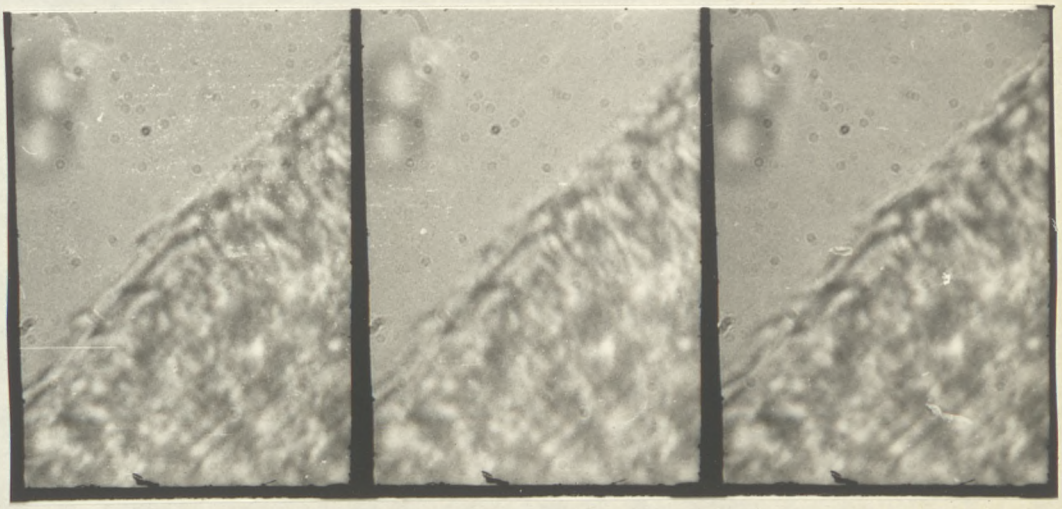
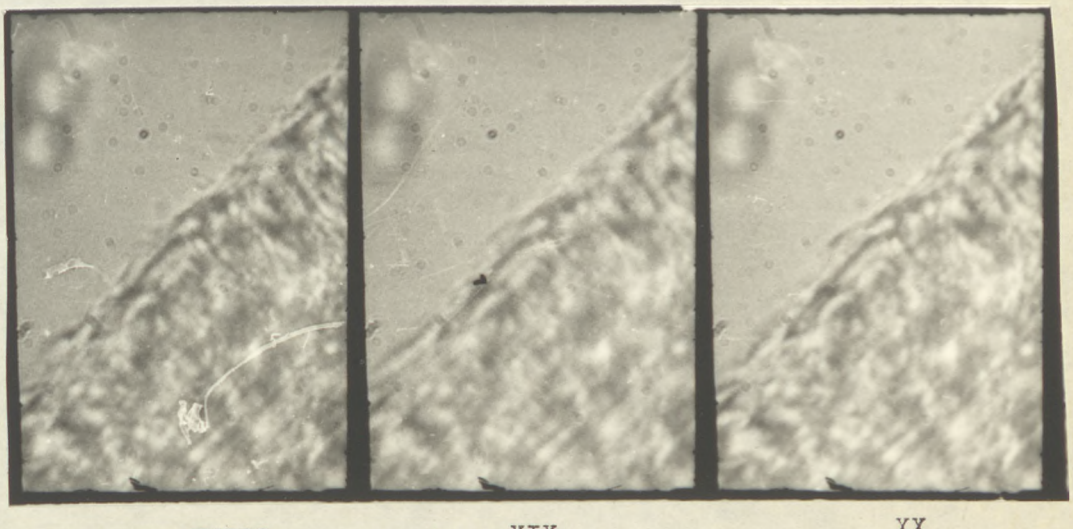


Plate XII XIII XIV



XV XVI XVII



XVIII XIX XX

Results of the ciliary activity of Human Fallopian Tube

PROLIFERATIVE beats per second

No.	Fimbria	Ampulla	Isthmus
1	8.36	6.52	6.49
	9.53	6.93	6.52
	8.08	7.32	6.93
2	6.20	6.72	4.84
	6.64	5.68	5.39
	6.42	6.35	4.91
3	7.40	3.96	6.16
	7.20	4.96	6.24
	7.53	4.12	5.84
4	6.71	5.70	6.67
	6.21	5.62	5.51
	6.32	5.21	5.20
5	7.21	6.75	6.51
	6.93	6.10	6.21
	6.84	6.21	6.03
6	7.51	6.52	5.92
	6.82	5.93	5.71
	7.21	6.14	5.63
7	6.71	5.90	5.51
	6.82	6.71	6.63
	6.53	6.50	6.21
8	6.32	6.53	6.44
	6.88	6.21	5.90
	6.51	5.90	5.79
Mean	7.04	6.02	6.00

Table A.58

SECRETORY beats per second			
No.	Fimbria	Ampulla	Isthmus
1	8.20	7.90	7.77
	8.12	8.33	8.02
	7.98	8.02	8.13
2	6.52	6.00	6.83
	6.20	6.12	6.72
	6.41	6.32	6.31
3	7.64	6.52	6.62
	7.72	6.12	6.02
	7.53	6.43	6.15
4	7.16	6.88	6.69
	6.64	6.72	7.51
	6.99	6.71	7.09
5	5.68	7.92	7.56
	5.96	7.24	7.16
	5.72	6.88	7.08
6	8.01	8.30	8.67
	8.37	7.90	9.48
	8.21	8.10	8.87
7	6.93	6.82	7.01
	7.91	7.85	6.72
	7.54	7.63	6.98
8	6.82	6.72	7.31
	7.13	6.92	7.02
	7.05	7.52	7.45
Mean	7.18	7.16	7.30

Table A.57

PUERPERAL beats per second			
No.	Fimbria	Ampulla	Isthmus
1	7.04	6.40	6.68
	6.86	6.58	6.86
	6.91	6.71	6.71
2	6.12	6.03	7.44
	5.92	5.82	6.92
	6.07	6.58	7.22
3	7.60	4.20	6.48
	7.08	5.12	6.68
	7.23	4.97	6.20
4	7.00	7.20	7.48
	8.40	6.56	6.96
	7.91	6.91	6.04
5	7.80	7.64	6.36
	8.04	7.64	5.40
	7.90	7.52	5.92
6	7.80	7.20	6.76
	7.48	7.28	5.80
	7.51	6.93	6.24
7	6.56	6.64	6.00
	7.08	6.72	6.73
	6.63	6.52	6.52
8	7.16	8.32	7.92
	8.00	7.52	8.40
	7.72	7.92	7.21
Mean	7.24	6.70	6.70

Table A.59

Statistical analysis of ciliary motility

Analysis of variance

Model

$$Y_{ijk} = \mu + a_i + b_j + c_k + (ac)_{ik} + (bc)_{jk} + \varepsilon_{ijkn}$$

μ = overall mean

a_i = type effect — ie proliferative, secretory, puerperal

b_j = person effect

c_k = region effect — ie fimbriae, ampulla, isthmus

$(ac)_{ik}$ = interaction of type and region

$(bc)_{jk}$ = interaction of person and region

ε_{ijkn} = error on each measurement

Method

Calculate:

a.) Sum of squares of all observations $= \sum\sum\sum\sum Y_{ijkn}^2$

b.) Sum of observations $= G$

c.) Total sum of squares $= \sum\sum\sum\sum Y_{ijkn}^2 - \frac{G^2}{abcn}$

d.) $T_{ij..}$ = total for each person (over all regions)

e.) $T_{..k.}$ = total for each region

f.) $T_{ijk.}$ = total for each person on each region

Let $= \frac{G^2}{abcn}$

Analysis for persons and regions ignoring types

	Sum of squares	d.f. degrees of freedom	mean squares
B persons over all regions	$\Sigma_i \Sigma_j T^2_{ij../cn} - \gamma = S_B$	ab-1	$\frac{S_B}{ab-1}$
C regions	$\Sigma_k T^2_{..k./abn} - \gamma = S_C$	c-1	$\frac{S_C}{c-1}$
BC person/ region inter-action	$\Sigma_i \Sigma_j \Sigma_k T^2_{ijk./n} - \gamma - S_B - S_C = S_{BC}$	(c-1)(ab-1)	$\frac{S_{BC}}{(c-1)(ab-1)}$
error	Total $- S_B - S_C - S_{BC} = S_E$	abc(n-1)	$\frac{S_E}{abc(n-1)} = \hat{\sigma}^2$
Total	$\Sigma_i \Sigma_j \Sigma_k \Sigma_n Y^2_{ijkn} - \gamma$	abcn-1	

Also calculate

$S_A = \Sigma_i T^2_{i.../bcn} - \gamma$ = sum of squares due to types

$T_{i...}$ = total for each type

$S_{B(A)} = \Sigma_i \Sigma_j T^2_{ij../cn} - \Sigma_i T^2_{i/bcn}$

= sum of squares due to differences among people of the same type

$S_{AC} = \Sigma_i \Sigma_k T^2_{i.k.}/bn - \mathcal{C}$

$T_{i.k.}$ = total for each region in each type

S_{AC} = sum of squares due to interaction of region and type

$S_{BC(A)} = S_{BC} - S_{AC}$

= sum of squares due to interaction of person and region within one type

Analysis of variance including types

	sum of squares	degrees of freedom	mean squares
types	S_A	a - 1	$S_{A/(a-1)}$
persons within types	$S_{B(A)}$	a(b-1)	"
regions	S_C	c - 1	"
type/region interaction	S_{AC}	(a-1)(c-1)	"
from last table	S_E	abc(n-1)	$\hat{\sigma}^2 = \frac{S_E}{abc(n-1)}$
person/region interaction within types	$S_{BC(A)}$	a(b-1)(c-1)	$S_{A/(a-1)}$
		abcn - 1	

To test

1. Is there any difference between types?

$$F\ (a-1,\ abc(n-1)\ ) = \frac{S_{A/(a-1)}}{2}$$

2. Is there any difference between regions?

$$F\ (c-1,\ abc(n-1)\ ) = \frac{S_{C/(c-1)}}{abc(n-1)}$$

3. Is there any type / region interaction?

$$F\ (\ (a-1)(c-1),\ abc\ (n-1)\ ) = \frac{S_{AC/(a-1)(c-1)}}{\hat{\sigma}^2}$$

4. To test for a particular difference between, for example type 1 and type 2

Student's t test

$$t_{(abc(n-1)\ )} = \frac{T_{1/bcn} - T_{2/bcn}}{\sqrt{\frac{2\hat{\sigma}^2}{bcn}}}$$

T_1 = total for type 1

T_2 = total for type 2

$\frac{T_1}{bcn}$ = average for type 1

$\frac{T_2}{bcn}$ = average for type 2

Percentage of secretory and ciliated cells in the epithelium

Proliferative

No.	Fimbria		Ampulla Inside		Ampulla Outside		Isthmus	
	sec.	cil.	sec.	cil.	sec.	cil.	sec.	cil.
1	14	86	15	85	10	90	30	70
2	9	91	14	86	18	82	47	53
3	21	79	31	69	27	73	52	48
4	32	68	39	61	28	72	32	68
5	31	69	28	72	32	68	37	63
6	15	85	44	56	39	61	48	52
Mean	20	80	29	71	26	74	41	59

Table A.60

Secretory

1	23	77	34	66	38	62	49	51
2	10	90	21	79	16	84	38	62
3	14	86	29	71	25	75	47	53
4	20	80	35	65	35	65	65	35
5	28	72	37	63	33	67	54	46
6	24	76	29	71	23	77	42	58
Mean	18	82	28	72	21	79	42	58

Table A.61

Puerperal

1	21	79	18	82	9	91	54	46
2	13	87	17	83	14	86	65	35
3	22	78	28	72	29	71	57	43
4	38	62	38	62	19	81	55	45
5	15	85	31	69	22	78	61	39
6	49	51	44	56	39	61	64	36
Mean	26	74	29	71	22	78	59	41

Table A.62

Post-Menopausal

No.	Fimbria		Ampulla				Isthmus	
			Inside		Outside			
	sec.	cil.	sec.	cil.	sec.	cil.	sec.	cil.
1	21	79	33	67	23	77	47	53
2	22	78	25	75	19	81	53	47
3	19	81	31	69	25	75	42	58
4	15	85	22	78	32	68	48	52
5	25	75	33	67	20	80	31	69
6	30	70	29	71	26	74	45	55
Mean	22	78	29	71	24	76	44	56

Table A.63

Formulae of solutions used

Krebs solution - used in muscular motility studies

NaCl	-69 g.
KCl 10% solution	-35 mls.
$MgSO_4.7H_2O$ 10% solution	-29 mls.
KH_2PO_4 10% solution	-16 mls.
Glucose	-20 g.
$NaHCO_3$	-21 g.
1 M-$CaCl_2$	-25.2 mls.

Made up to 10 litres with distilled water

Medium 199 with Earle's salts - used in ciliary motility studies

	mg/L
NaCl	6800.0
KCl	400.0
$MgSO_4.7H_2O$	200.0
NaH_2PO_4	140.0
Glucose	1000.0
Phenol red	20.0
$CaCl_2$ (anhyd.)	200.0
$NaHCO_3$	2200.0
L-Arginine HCl	70.0
L-Histidine HCl	20.0
L-Lysine monohydrochloride	70.0
DL-Glutamic acid monohydrate	150.0
DL-Tryptophan	20.0
DL-Phenylaline	50.0
DL-Methionine	30.0
DL-Serine	50.0
DL-Threonine	60.0
DL-Leucine	120.0
DL-Isoleucine	40.0
DL-Valine	50.0
DL-Aspartic acid	60.0
DL-Alpha-Alanine	50.0
L-Proline	40.0
L-Hydroxyproline	10.0
Glycine	50.0
L-Glutamine	100.0

Schedule for processing film

Loading film into the camera, removing film from the camera, and developing the film were all carried out in a dark room.

1. Remove film from camera, and unwind film from spool
2. Put film into deep tank containing Kodak D76 developer for ten minutes, with constant agitation to ensure even development.
3. Remove film and put into a wash of one part acetic acid/one part water, for two minutes.
4. Put film into deep tank containing fix - Ilford Hypam, for ten minutes.
5. Wash film in running tap water for half an hour.
6. Put in Kodak Photoflo for a few seconds. This enables the water to drain off the film evenly.
7. Dry film in drying cabinet and re-wind onto spool.

Preparation of plastic sections

1. Fix tissue in 10% formal saline overnight.

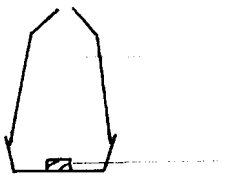
2. Cut small sections of tissue approximately 2mm. x 2mm. x 2mm.

3. Dehydrate: 70% industrial methylated spirits overnight
   90% IMS 3 changes ¼-½ hour each
   100% IMS " " " " "

4. Glycol methacrylate solution A overnight, in fridge.

5. Put tissue into small plastic embedding pots, first having cut the tops off the pots. Put a label in each pot. Fill each pot through the hole in the top with a mixture of 42 parts glycol methacrylate solution A, 1 part solution B. Leave at least one hour at room temperature to harden. Care must be taken during this procedure to ensure that the block of tissue remains at the bottom of the pot during embedding, and does not rise to the surface. If it shows a tendency to rise, it may be possible to push it down with a mounted needle, before the plastic has set too much. Once hardened the block is removed from the embedding pot and stored in a desiccator until needed.

Embedding pot



6. Cut sections 1 to 2 u in thickness using a Sorvall JB4 microtome. The cut sections are floated out on a waterbath, and mounted on a slide by immersing the slide, and bringing it up under the section, so the section adheres to the slide.

Staining schedule for plastic sections

1. 1% Erhlich's haemotoxylin 15 - 20 minutes (in fridge)

2. Wash in running tap water 2 minutes
3. Rinse in acid alcohol
4. Blue in tap water 5 minutes
5. Stain in ½% Phloxine 5 minutes
6. Rinse in tap water
7. Dehydrate: 70% Industrial methylated spirits 5 minutes
   90% IMS 3 changes 5 minutes each
   100% IMS 3 changes 5 minutes each
7. Clear in xylol 2 minutes
8. Mount in neutral mounting medium

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