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A STUDY OF THE FACTORS CONTROLLING EMBRYO TRANSPORT IN THE OVIDUCT OF THE MOUSE

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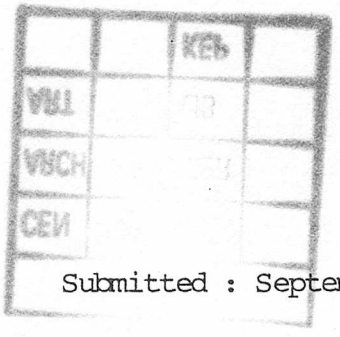

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Abstract

A Study of the Factors Controlling Embryo Transport in the Oviduct of the Mouse

Kenneth L. Grieve

The time course of tubal transport through the oviduct of the mouse has been examined in some detail. It comprises a multi-factorial system, consisting of ciliary activity, fluid elaboration and muscular contractility, alterations in which may adversely affect fertility. This study has examined this process from a variety of viewpoints.

The results indicate that a volume of fluid passes from the oviduct between Days 1 and 2 of pregnancy, the time at which embryos normally pass into the isthmus, but the role of this fluid is uncertain. Administration of oestradiol by injection into one bursa ovarica produces effects which are more pronounced than SC administration and are laterally biased.

An *in vitro* system has been developed in which embryo transport occurs from Day 2 and Day 3 onward for up to 16 hours, but does not occur from Day 1 onward. Isthmic transport *in vitro* can be manipulated pharmacologically, and the presence of alpha-adrenergic receptors is suggested. The musculature is proposed as the most important factor in isthmic transport.

Removal of ovaries and adrenal glands resulted in a retardation of tubal transport which can be reversed by progesterone administration and measurement of serum progesterone levels indicates that progestogenic activity is required for normal tubal transport. Administration of testosterone also caused delay which could be reversed by progesterone administration which confirms that continued progesterone activity throughout the tubal transport phase is required. The role of progesterone is discussed, but further work on tubal progesterone and oestradiol receptor levels and their interactions are required for a definitive result.

To Fiona,

with all my love

A C K N O W L E D G E M E N T S

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Contents

General Introduction

Embryo Transport in the Mouse

The term embryo transport is used to describe the physical passage of newly ovulated ova from the ovary, through the oviduct into the lumen of the uterus. The oviduct has several physiological functions:-

1. Transport of sperm to the site of fertilisation and provision of an environment suitable for sperm survival and capacitation.
2. Transport of ova from the site of release on the ovary to the site of fertilisation and provision of an environment suitable for fertilisation.
3. Transport of fertilised ova from the site of fertilisation to the uterine lumen and provision of an environment suitable for embryo development to the stage of morula.
4. Correct timing for all of the above.

Since the developmental stage of both embryo and endometrium at the time of entry of the fertilized ova into the uterus determines whether or not implantation occurs, normal oviduct function is vital for fertility, and agents which disrupt these processes will also adversely affect fertility. Therefore examination of the physiological factors controlling embryo transport through the oviduct may be of value in the treatment of infertility of tubal origin and in the control of fertility for contraception.

The Anatomy of the Oviduct

Descriptions of the anatomy of the mouse oviduct have been reported by several authors (Agduhr, 1927; Burdick et al., 1942; Dirkson, 1971, 1975; 'Espinasse, 1935; Humphrey, 1968A; Reinius, 1969, 1970;), as for the rat (Alden, 1942A; Deane, 1952). The comparative anatomy of the oviduct of various species has been reviewed by a number of authors (Beck et al., 1974; Gould, 1974; Nilsson and Reinius, 1969; Pauerstein and Eddy, 1979).

The oviducts are paired, convoluted smooth muscle structures, derived embryonically from the Mullerian (paramesonephric) duct, which histologically consist of three distinct layers, an outer Tunica Serosa, a Tunica Muscularis and an inner Tunica Mucosa (Beck et al., 1974). The variation in composition of these three layers along the length of the oviduct allows division of the eight loops of the mouse oviduct into 5 distinct segments, as defined by Reinius (1969).

1. The Preampulla. This consists of the fimbria and first loop of the mouse oviduct. The muscularis contains two to three layers of muscle cells, mainly longitudinally orientated. The mucosa has a scanty lamina propria, and has high folds, which can occlude the lumen. The endothelial layer is mostly made up of ciliated cells. Reinius (1969) reported the ratio of ciliated cells to non-ciliated cells to be 5-10 to 1.

The fimbria of the mouse consist of low folds of tissue and are diminutive (Humphrey, 1968A), as are those of the rat (Alden, 1942A). However, in man, rabbit and others, the fimbriae consist of long, fluted, finger-like projections. The role of these two anatomical types is discussed later.

2. The Ampulla. This consists of the second loop of the mouse oviduct. The muscularis is thin and highly distensible. The epithelium has small, sparse longitudinal folds, and consists of non-ciliated cells with 'frequent' ciliated cells (Reinius, 1969). The diameter of the mouse ampulla changes from 300 to 500 um when ova are present in the lumen. When the loop is not distended, the epithelial folds meet in the lumen.

The muscularis of the human oviduct is also mainly longitudinally orientated (Pauerstein et al., 1979) whilst Greenwald (1961) reported the muscularis of the rabbit to be slightly spiralling circular muscle, with no longitudinal muscle visible. Beck et al., (1974) reported three different arrangements of the muscularis in the ampullae of the forty-one species examined.

3. Ampullary-Isthmic Junction. This is formed by the narrowing of the loop prior to the isthmus. It is debateable whether or not this can be described as a distinct anatomical region or merely the transition between ampulla and isthmus. However, the local thickening of the muscularis of the inner curve, which is mainly circular in orientation, and the long mucosal folds which close the lumen of the isthmus when the ampulla is dilated, suggest the existence of the ampullary-isthmic junction (AIJ) as a discrete anatomical region.

4. Isthmus. Loops three to seven make up the isthmic region of the mouse oviduct. The isthmus has thicker, more muscular walls than the ampulla. The muscularis is reported by Reinius (1969) as being mainly circular in orientation, whilst Burdick et el., (1942) report both circular and longitudinal orientations (by light microscope examination) and Beck et al., (1974) report an inner circular and outer longitudinal layer of smooth muscle. The epithelium has longitudinal folds only in Loop 3, next to the AIJ, the rest having low circular folds. Reinius (1969) reported two kinds of non-ciliated cells present, and ciliated cells located in the epithelial grooves, a fact confirmed by Dirksen (1975). Humphrey (1968A), however, did not find any ciliated cells in this region. Greenwald (1961) reported cilia to be present but uncommon in the isthmus of the rabbit, and Alden (1942A) reported ciliated cells present in the isthmus of the rat, a fact confirmed by the electron microscope study of Odor (1953).

5. Junctura. Reinius (1969) defines the junctura as the last loop of the extramural oviduct and the intramural oviduct, including the colliculus tubarius. Other authors (Pauerstein and Eddy, 1979; Humphrey, 1968A) have tended to include Loop 8 of the oviduct as part of the isthmus, and considered the intramural section of the oviduct as the "utero-tubal junction". However, the arrangement of the mucosa and muscularis of this area suggest a collective term would be more suitable. In the junctura the muscularis is thicker than that of the isthmus and is mainly circularly arranged, although the intramural muscular layers diminish towards the colliculus tubarius. In the mouse, Reinius (1969) reported that the external smooth muscle layer is longitudinal in orientation and is contiguous with the uterine smooth muscle. In the human, the three layers of uterine smooth muscle are reported to continue into the muscularis of the oviduct (Pauerstein and Eddy, 1979).

In the mouse, the muscularis is separated from the epithelium by a lamina propria two to three cells in depth. The epithelium and accompanying fibrous tissue form moderately high longitudinal folds, except at the tip of the colliculus tubarius. Reinius (1969) reported that the epithelial surfaces are usually in close contact, and follow the outline of the embryos, when embryos are present, but admitted that other authors have reported a wider lumen with different preparative procedures. Reinius also stated that no ciliated cells are present, confirming the report of Burdick et al., (1942). Greenwald (1961) reported that almost all the epithelial cells of the junctura of the rabbit are ciliated.

Beck et al., (1974) described ten different anatomical arrangements of the junctura (UTJ) in various mammalian species, based on the arrangement of the muscularis, the mucosa and the relationship between oviduct and uterus.

The oviducts are suspended between the uterine cornua and the tubal pole of the ovary by a derivative of the broad ligament, the Mesosalpinx. In the mouse, the duplication of the mesentery on the antimesosalpinxal border, is referred to as the mesotubarium superius. Together the mesosalpinx and the mesotubarium superius form a peritoneal fold which encloses the ovary and the infundibulum of the oviduct, known as the Bursa Ovarica. This peritoneal sac is described in the mouse by Agduhr (1927) and Wimsatt and Waldo (1945), and in the rat by Alden (1942A). Such a sac is not present in the rabbit or human oviduct.

The Epithelium of the Oviduct

The Tunica Mucosa, or mucosal layer of the oviduct can be subdivided into two layers, the outer Lamina Propria and the inner Lamina Epithelialias. Together these layers form the mucosal folds. The Lamina Propria is a loose connective tissue which forms a thin circular layer between the epithelium and the muscularis, and extends into the mucosal folds. It contains numerous small blood vessels and lymphatic spaces and consists mainly of collagen, into which are imbedded various types of cells, including fibroblasts, mast cells, macrophages and indifferent cells (a type of cell characteristic of the oviduct).

The microscopic anatomy of the Lamina Epithelialias has been examined by many authors (for reviews, see Beck and Boots, 1974; Brenner, 1969; and Nilsson and Reinius, 1969). Four distinct cell types are reported.

1. Ciliated Cells. The distribution of ciliated cells in the mouse oviduct has already been described (see General Introduction, the Anatomy of the Oviduct). Two types of cilia have been reported in oviduct epithelium. Nilsson (1957) reported the presence of stereocilia, that is cilia not capable of vibratile movement, found on

the surface of the secretory cells. Kinocilia or true vibratile cilia are restricted to those cells generally called ciliated. These cilia are of the typical 9+2 microfibril arrangement and have basal bodies (Brenner, 1969). The cilia of the rabbit are also reported to have anionic charged sites on the surface of the cilia (Anderson and Hein, 1977), particularly on the tip of the cilia, and these are suggested to have some unstated role in ovum transport.

Since the number of ciliated cells is much higher in the ampulla/preampulla, a role for ciliated cells in ovum pick-up and ampullary transit has been suggested, but this will be discussed more fully in a later section.

Changes in the numbers with cells of cilia during the menstrual cycle have been suggested. Brenner (1969) reported almost complete deciliation of the fimbrial ciliated cells in rhesus monkey oviducts on Day 2 of the menstrual cycle, increasing in number until Day 9, remaining constant to Day 21, and then deciliating, leaving only the occasional short, ciliated cell. This loss of cilia did not occur in the ampulla, but the height of the cells did cyclically change, growing smallest between Days 21 and 27. Brenner suggested that increasing blood levels of progesterone were responsible for the deciliation. This pattern is borne out in work on the pig-tailed Macaque by Rumery and co-workers (Rumery, Gaddum-Rosse, Blandau and Odor, 1978). In this case, cyclic deciliation was also seen, although to a lesser extent, throughout the ampulla. They also showed that ovariectomy in the monkey caused deciliation, which could be reversed by the administration of oestrogen. Verhage and co-workers (Verhage, Bareither, Jaffe and Akbar, 1979), working on human tissue, showed a 10% cyclic deciliation during the menstrual cycle, coupled with a decrease in cell height. The deciliation continued into pregnancy and the puerperium. This report is in conflict with the report of Critoph and Dennis (1977), who found no change in the mean % of

ciliated cells throughout the human menstrual cycle, although ciliation decreased slightly in the puerperium. Borell et al., (1956) and Kuhnel and Busch (1981) both reported no cyclic changes in the number of ciliated cells seen during the oestrus cycle, and preimplantation phase, respectively, of rabbits, as examined by electron microscope. However, Shipstone et al., (1974) reported an increase in the ciliation of the rabbit ampullary epithelium at 24 and 70 hours post-coitum, and an increase in isthmic ciliation at 70 hours post-coitum as compared by transverse electron microscope examination, to oestrus animals. The study of Shipstone et al., involved groups of four animals killed at 14, 24 and 70 hours post-coitum, and at least 100 ciliated cells examined in each region (ampulla/isthmus) of each rabbit. Thus the question of changes in ciliation of rabbit oviducts during embryo transport remains open. In mice, the work of Reinius, (1969; 1970) suggests that no such changes in ciliation take place during the embryo transport phase.

It is also possible that changes in the activity of cilia could be brought about by changes in the nature and consistency of the luminal contents of the oviduct. The work of Jansen (1978, 1980) suggests that in rabbit and man, there exists a column of tenacious mucus secretion around oestrus, which disappears after ovulation, and on treatment with progesterone. It is proposed that this mucus covers the cilia and prevents their correct functioning. The mucus is the product of a second cell type, the secretory cell.

2. Secretory cells. Some authors have only differentiated between ciliated and non-ciliated cells in the oviductal epithelium (Reinius, 1970; Critoph et al., 1977). However, most would agree that the secretory cells form a distinct group of epithelial cells. In most species, the proportion of secretory cells increases towards the uterus, as the number of ciliated cells decreases (cf). Beck and

Boots (1974) described a secretory cell as generally cylindrical with a large basophilic nucleus, and a more eosinophilic granular cytoplasm, which contains PAS positive granules in the apical region, when it is active. Usually they have well developed Golgi apparatus (Pauerstein and Eddy, 1979). Secretory cells contribute to the mileu of the oviduct lumen. Tubal fluid is also a product of transudation, follicular fluid, peritoneal fluid, uterine fluid and secretion from the bursa ovarica and from neighbouring areas of the oviduct. It is of diverse composition which probably varies along the length of the oviduct, and with the stage of the oestrus cycle. In the mouse, Borland et al., (1977) showed that the ionic composition of oviductal fluid varied in composition with time and position within the organ, when examined by electron probe micro-analysis, during the preimplantation phase of pregnancy.

Evidence that cyclic changes occur in secretory cells has come from several authors. Reinius (1970) found changes in non-ciliated cells in the mouse ampulla which showed evidence of increased secretory activity at the time ova were present in the ampulla. In mature cycling mice, Komatsu and Fujita (1978) reported an increase in width of secretory cells at oestrus, with accompanying increases in Golgi apparatus and rough endoplasmic reticulum, in comparison with dioestrus cells.

In the rabbit, the work of Lambert, reported by Stone and Hamner (1975), showed large secretory granules in the apical region of ampullary secretory cells at oestrus. After ovulation these granules were discharged into the lumen by exocytosis. By the fifth to seventh days of pregnancy, this exocrine secretion had diminished. In the work of Borell, Nilsson, Wersall and Westman (1956), a similar pattern was reported. They also reported that typical secretory cells were absent from castrated rabbits. However, cells containing solitary

secretory granules were found.

Odor, Gaddum-Rosse and Rumery (1983), in a complimentary study to that of Rumery, Gaddum-Rosse, Blandau and Odor of 1978, showed, in pig-tailed monkey, that changes in secretory cells structure occurred during the menstrual cycle. Using EM studies, they showed that, at mid-cycle, while variation between individual animals was quite large, most showed evidence of increased secretory activity. This activity was not seen in early follicular or late luteal phases. In long term ovariectomized animals, cell height was reduced, and secretory granules absent. Administration of oestradiol induced changes similar to a mid-cycle oviduct, although the response to a given dose of oestradiol varied from region to region, and animal to animal.

Verhage, Bareither, Jaffe and Akbar (1979) reported a complete cycle of a differentiation and dedifferentation of secretory cells in the fimbria and ampulla of the human. Cell height was at its greatest at mid-cycle, and restoration of secretory activity was associated with very low serum levels of progesterone.

Indirect evidence for cyclic changes in secretory cell function can be found in studies of the secretory rate of the oviduct.

Bishop (1956) investigated the rate of secretion of the fluids of the rabbit oviduct. He used a cannulation technique which involved the ligation of the utero-tubal junction and the insertion of a cannula into fimbriated end of the oviduct. This cannula was exteriorised. The animals were anaesthetised throughout the period of the experiment. He found secretion in the rabbit to be an active process, which was at its greatest at oestrus and lowest in late pregnancy. Castration reduced the secretory rate almost to zero and administration of oestrogen restored the rate to the level of the oestrus animal. Administration of pilocarpine to oestrus animals increased the rate of secretion. These observations were supported by

the work of Iritani et al., (1971) who found similar levels of secretion in oestrus unrestrained rabbits, using a technique similar to that of Bishop. Both of these techniques involved the sampling of fluid at discrete time intervals. The method of Clewe and Mastroianni (1969) allowed continuous volumetric collection of secretions from the fimbrial opening of the ligated rabbit oviduct. Using this system, the report of Mastroianni and Wallach (1961) showed that secretion rates were higher in oestrus, and fell by some 50% in the first 3 days of pregnancy. Further, Mastroianni, Beer, Shaw and Clewe (1961) examined the effects of exogenous steroid hormones and of castration on rabbit oviduct fluid production, using this method. They reported that, as in the report of Bishop (1956), castration reduced the secretory rate to about one-third of the precastration rate, and this effect is reversed by daily administration of the appropriate dose of oestrogen. The effects of exogenous steroid hormone administration will be discussed more fully later.

Thus it seems likely that secretory cells of the oviduct epithelium undergo cyclical changes during the menstrual cycle, which are possibly related to circulating or tissue steroid hormone levels. Two other cell types are recognized.

3. Indifferent or basal cells. Basal cells are so called because they are found along the base of the epithelium. Beck and Boots (1974) reported the presence of basal cells in all 41 species they examined, where, in such cases, they comprise less than 1% of the total number of cells. They reported the occurrence of basal cells as irregular among the segments of the oviduct. These cells are morphologically identical to the indifferent cells of the Lamina Propria previously mentioned. Pauerstein and Woodruff (1967) examined indifferent cells in human oviducts by light microscope and biochemical means. They came to the conclusion that the indifferent cells are the

cells from which mature epithelial cells are generated. This conclusion is also reached by Bullon, Merchan, Gonzales-Gomez, Furio and Poblete (1980) in their electron microscope study of rat oviduct epithelium. They laid down specific criteria for the differentiation of basal cells from other cellular elements, such as macrophages and lymphocytes. Basal cells can be identified by the presence of desmosomes in the cell contour, and less dense hyaloplasm compared to secretory or ciliated epithelial cells. For this reason, these are sometimes referred to as "clear cells". Bullon et al., also examined in some detail the fourth cell type, the peg cell.

4. Peg cells. Also known as the intercalary cell, the peg cell is so called because of its wedge-like shape, widest at the base, and narrowest at the apex. In the study of Beck and Boots (1974), peg cells were reported as being 0.5-1% of the total number of cells in most of the species studied. In some species, peg cells were not seen, but this is thought to be due to the time of examination, relative to the reproductive cycle. They suggest that, in the human, peg cells may represent exhausted secretory cells. This view is supported for the human oviduct by Bullon et al., (1980), but, in the rat, the peg cell is viewed as ciliated cells undergoing the process of necrobiosis. This apparent contradiction may be overcome if peg cells are the elimination step for any epithelial cell type in the oviduct, since it is suggested that ciliated cells have a shorter life-span in the rat than in the human, and are therefore more likely to appear as peg cells.

The evidence for cyclical changes in peg cells is tenuous, and more studies are required before a definitive answer is available.

In general, the epithelium of the oviduct comprises four cell types, which vary in number along its length, and vary in activity throughout the reproductive cycle. Increased activity occurs around

the time of ovulation, and activity is probably controlled by the levels of the various steroid hormones.

The Timing of Embryo Transport

As was stated in the introduction, the functions of the oviduct include the transport of ova and embryos, and the correct timing of all the events leading to the arrival of a properly developed embryo to the uterine lumen at the appropriate time. Alterations in the timing of the embryo transport will result in reduced fertility. The time course of embryo transport has been examined in several species.

In mouse, Burdick et al., (1942) reported that ova remained in the ampulla for 24 hours after ovulation, and took a further 20 to 24 hours to move the length of the isthmus to the junctura. Passage into the uterus was delayed at this point for a further 24 hours. Thus the whole process was reported to take 68 to 72 hours, from ovulation to passage into the uterus. The more detailed account of Humphrey (1968A) reported ova to be in the ampulla by 06:00h on Day 1 of pregnancy. Passage into the isthmus began as early at 18:00h Day 1, but most embryos were found in the isthmus by 09:00h Day 2 of pregnancy. Most embryos had reached the junctura by 18:00h Day 2, and paused there for 24 hours. The movement of embryos into the uterus started as early as 18:00h Day 3, and was 90% complete by 09:00h Day 4, some 81 hours after the presumed time of coitus.

In the rabbit, the work of Boling (1969) and Blandau (1969) indicates that ova reach the ampullary-isthmic junction within a few minutes after ovulation and are retained there for 15-18 hours. Transport through the isthmus to the uterus takes a further 24-48 hours.

The difficulties involved in measuring embryo transport in humans is clearly demonstrated in the report of Croxatto et al., (1972). Using the non-surgical technique of uterine flushing, they recovered

some 8 ova/embryos from 40 attempts. In general, embryos were found on Days 3 and 4 after ovulation.

A detailed knowledge of the time course of normal embryo transport is necessary for any study of oviduct function. This aspect of oviduct function for the R.G.I.T. colony of RGIT/SLAC mice is discussed in more detail elsewhere (see Chapter 1).

The Role of Steroid Hormones in the Control of Oviductal Embryo Transport

The role of steroid hormones in the control of embryo transport has not yet been fully elucidated. Several authors have reported the levels of oestradiol and progesterone (the two main sex steroids present) in plasma, during the oestrus cycle and pregnancy. It is not the purpose of this study to examine in depth the control of, and cyclic changes in, steroid hormones levels throughout the cycle or pregnancy. However, to reach any understanding of the functioning of the steroids in reproductive physiology, it is necessary to be familiar with the fundamental points. A review of steroid plasma levels and the sources of steroid secretion during the oestrus cycle in the rat has recently been reported (Feder, 1981). He reported the work of various authors, and concluded that a plasma peak of oestradiol exists in early proestrus of the cyclic rat, which falls off sharply to baseline levels prior to ovulation. During, and for three days after ovulation plasma oestradiol levels rise slowly. The absolute levels of oestradiol in the peripheral circulation are as yet unclear, and are probably affected by a number of factors including strain differences, sampling procedures, assay procedures, and relative contribution from ovarian and adrenal sources.

Feder also reported peak plasma levels of progesterone in the cyclic rat of the order of 25-50ng/ml, on the afternoon and evening of proestrus, falling sharply to baseline levels of 3-15ng/ml by the time

of ovulation. A second, smaller increase occurs during the first day of dioestrus.

Data on levels in mouse plasma are less common. Michael (1976) reported the level of progesterone in the peripheral plasma during the oestrus cycle of mice, using a radio-immunoassay technique. However, she found it necessary to combine plasma samples from several animals for assay. She reported a peripheral plasma peak of 60ng/ml on late proestrus, and a secondary peak of 30ng/ml in late metoestrus/early dioestrus. This pattern is similar to that reviewed in the rat by Feder (1981).

The pattern of post-ovulatory steroid secretion in the pregnant animal differs from that of the cyclic animal. Forcellado, Vera and Croxatto (1981) reported the day to day plasma levels of oestradiol and progesterone during the first five days of pregnancy in rats, comparing them to the levels occurring in cyclic and pseudopregnant animals. In pregnant rats, the plasma oestradiol level was not significantly changed over the five day period, at around 25-35pg/ml. (Although there was a slight non-significant rise between Day 2 and Day 3). Plasma progesterone rose from 15ng/ml on Day 1 to over 60ng/ml by Day 5. The ratio of oestradiol to progesterone fell consistently over the five day period.

The pattern of progesterone secretion reported in the rat by Forcellado et al., (1981) is similar to that reported for the pregnant mouse by Murr, Stabenfeldt, Bradford and Geschwind (1974). These authors examined plasma progesterone throughout pregnancy on a day to day basis, using a competitive protein binding method. They found that plasma progesterone rose from ca.10ng/ml on Day 1 to ca.45ng/ml on Day 4. Prior to the report of Murr et al., Atkinson and Hooker (1945) had shown this pattern of plasma progesterone in mouse in early pregnancy using a bio-assay technique. The gestation patterns of

steroid secretion (oestradiol, progesterone and testosterone) for several strains of mice were reported by Barkley, Geschwind and Bradford (1979). They confirm the pattern already stated, although absolute values vary from strain to strain. Testosterone levels remain constant throughout the embryo transport phase of pregnancy.

The relevance of plasma levels of steroid hormone to events at tissue and receptor level has been examined. Batra, Helm, Owman, Sjoberg and Walles (1980), examined the levels of oestradiol and progesterone in ampullary and isthmic tissue samples and plasma from humans in each of the follicular, ovulatory and luteal phases of the menstrual cycle. In general, they found poor correlation between tissue and plasma levels, and no significant difference between ampullary and isthmic levels at any stage. However, the correlation between tissue and plasma levels of progesterone in the "ovulatory phase" for the ampulla and isthmus were 0.81 and 0.91 respectively, indicating good correlation. At this time the coefficients for oestradiol were 0.39 and 0.40 respectively, indicating poor correlation. They also reported that tissue steroid levels were always higher than corresponding plasma levels, confirming that the oviduct is a target tissue for steroid hormones.

A more accurate indicator of steroid activity during the course of tubal embryo transport is the level of nuclear (translocated) receptor. Fuentealba, Vera, Nieto and Croxatto (1982) reported changes in nuclear oestrogen recepters in whole oviduct homogenates during embryo transport in the rat, and compared these to corresponding plasma oestradiol levels. They found an increase in the concentration of nuclear oestrogen receptor, starting at 07:00h on Day 4 of pregnancy, peaking at 10:00h and falling off sharply to reach baseline levels by 18:00h. This event was coupled with a transient plasma cestradiol peak, and occurred just prior to the start of embryo entry into the

uterus. The authors therefore hypothesised that oestradiol exerted a direct action at tubal level which stimulated entry of embryos into the uterus.

Puri and Roy (1980) reported changes in nuclear progesterone receptors in various portions of the rabbit oviduct during embryo transport. In particular, they noted a decrease in nuclear receptors at the AIJ at 24 hours post-coitum and an increase in nuclear receptors in the isthmus at 70 hours post-coitum. These, they suggest, are related to the passage of embryos, since at 24 hours post-coitum, embryos move into the isthmus, and this local decrease in progesterone activity, not reflected in plasma levels, may have a physiological role. At 70 hours post-coitum, in the rabbit, the embryos are within the isthmus, just prior to moving into the uterus, and the authors suggest that this increase in progesterone activity (correlated with increasing amplitude and decreasing frequency of tubal contractions) may be facilitatory to the passage of embryos into the uterus, in contrast to the suggestion by Fuentealba et al., (1982) that increased oestradiol activity was responsible.

The interaction of oestrogen and progesterone at the level of the receptor is complex and not completely understood. Reviewing the evidence, Leavitt, Evans, Okulicz, McDonald, Hendry and Robidoux (1982) suggest that in general (in uterine tissue) oestradiol action regulates the oestradiol and progesterone cytoplasmic receptor systems, by stimulating synthesis of receptor protein, in target cells, while progesterone action deactivates both oestradiol and progesterone cytoplasmic receptor mechanisms by unknown means. More specifically progesterone controls the retention of the oestradiol/receptor complex in the nuclear fraction of the target cell. How these mechanisms relate to the physiological events on-going during embryo transport is not known. West, Verhage and Brenner (1976) found that, using

ovariectomized cats, progesterone suppressed the oestradiol receptor in the oviduct, and this suppression correlated well with the atrophy of the oviduct reported viz percentage of epithelial ciliated cells, and cell height, both of which decrease in response to progesterone. These effects are, however, relatively long-term, in that measurements were made fourteen days after insertion of a progesterone filled silastic implant, into an ovariectomized cat already implanted with oestradiol. Resulting serum steroid levels were reported to be physiological.

The need for post-ovulatory ovarian steroid secretion for normal embryo transport is not certain. Wu, Dickman and Johnson (1971) found that ovariectomy on Day 1 of pregnancy had a delaying effect on embryo transport, when compared to intact control rats. However, they did not compare these results to sham-operated controls, and therefore the validity of their results is doubtful, particularly since the embryo recovery was much lower in the ovariectomized animals. Alden (1942B) suggested that post-ovulatory ovariectomy did not significantly alter the embryo transport in the rat, although his conclusions were based on a small number of replicates.

In the mouse, Kendle and Lee (1980) found that ovariectomy of Day 1 of pregnancy did not alter embryo position in comparison with sham-operated controls, measured on Days 2 to 4.

These results would tend to suggest that post-ovulatory ovarian steroid secretion is not necessary for normal transport. However, two further points should be noted. Firstly, the ovaries are not the only site of steroid production in the body, since the adrenal glands are capable of the production of progesterone and oestradiol (Shaikh and Shaikh, 1975). Secondly, removal of the steroid secreting organs need not necessarily immediately remove circulating steroid, or tissue bound steroids. Thus the removal of the ovaries alone is not sufficient

grounds to state that the post-ovulatory activity of the sex steroids is unnecessary for normal embryo transport. Indeed, Forcellado, Morales, Vera, Quijada and Croxatto (1982) reported that bilateral ovariectomy combined with adrenalectomy carried out on Day 1 of pregnancy in the rat caused a premature loss of embryos, and that this effect could be reversed by exogenous progesterone. These results are discussed more fully in the Results section (Chapter Five). The effects of exogenous hormones on the tubal embryo transport have been reported by several authors. Most work has been reported on the effects of exogenous oestradiol and progesterone.

The effects of exogenous, pharmacological doses of oestradiol on tubal embryo transport have been documented for the mouse (Burdick and Pincus, 1935; Burdick and Whitney, 1937; Greenwald, 1967; Humphrey, 1968B), for the rat (Greenwald, 1967; Wu, Dickmann and Johnson, 1971), and for the rabbit (Burdick and Pincus, 1935; Greenwald, 1967). These effects were reviewed by Chang (1976). The effects reported are dose, time and species related. The following generalisations can be made.

In the mouse, guinea-pig, hamster and rabbit, a sufficiently large dose of oestradiol given soon after mating (i.e. when the embryos are still in the ampulla) causes marked retention of embryos in the oviduct for a period of days, in excess of normal (Greenwald, 1967). The majority of embryos are retained in the ampulla while some are retained in the junctura. In the mouse Greenwald reported that higher doses of oestradiol (10-100ug) caused early loss of embryos. This is contrary to the pattern in guinea-pig, hamster and rabbit, in which increased doses increases the tubal retention. In the mouse the retentive effect of oestradiol given on Days 1-3 could not be reversed by concurrent administration of progesterone and was usually accompanied with a premature loss of some embryos (Humphrey and Martin, 1967; Humphrey, 1968B). If oestradiol is given to mice after the embryos have passed into the isthmus, rapid expulsion into the uterus occurs

(Humphrey, 1968B; Lee, 1979). In the rabbit, Pauerstein and Weinberg (1980) examined the role of the ovary in oestradiol induced tubal retention. Using 18 and 28 day castrates, they compared the effect of oestradiol on the transport of transferred ova from donor rabbits, and showed that ampullary retention was not significantly different in intact, sham-operated and ovariectomized rabbits treated with oestradiol but all were significantly greater than intact or ovariectomized rabbits not treated with oestradiol. They concluded that the ovary was not essential for the retentive effects of oestradiol to be seen in rabbits.

In the rat, administration of oestradiol results in premature loss of embryos via the uterus. Retention within the oviduct does not occur, regardless of dose (1-500ug oestradiol cyclopropionate on Day 1, reported by Greenwald, 1967, 0.5-25ug oestradiol on Day 1, reported by Ortiz et al., 1979, all caused acceleration). This appears to be a species specific effect, the nature of which is not known.

The effects of several so-called anti-oestrogenic drugs on tubal transport in mice have been reported (Humphrey and Martin, 1968; Humphrey 1976). Dimethylstilboestrol, Nafoxidine and others cause retention of embryos within the ampulla, when given on Days 1 to 3 of pregnancy, but without reducing the overall recovery of embryos, as is the case for oestradiol. Those anti-oestrogenic drugs which are considered to be more oestrogenic, such as U10997 and Erythro-M.E.A., show a similar spectrum of activity to oestradiol. These effects are borne out by the work of Bigsby, Lungu, Duby and Black (1983) who investigated the effects of anti-oestradiol immunoglobulin on P.M.S.G.-HcG stimulated mice. Daily administration of this immunoglobulin, starting 72h prior to HcG administration, resulted in ovum retention in the oviduct for at least 2 days longer than in controls. This

immunoglobulin was also able to reverse the retentive effect of 0.4ug oestradiol, when both were administered on Day 1 of pregnancy.

The effects of progesterone on tubal embryo transport are no less complicated than those of oestradiol. They have been reported in the rabbit by Pauerstein, Anderson, Chatkoff and Hodgeson (1974) and reviewed by Gonzalez de Vargas, Hodgeson and Pauerstein (1975). Treatment with progesterone earlier than 12 hours prior to ovulation, but not more than 60 hours prior to ovulation, induces acceleration of transport. A similar acceleration was reported in the mouse by Kendle and Lee (1980) when progesterone was administered in proestrus to animals subsequently mated. In this case, embryos were found in the isthmus late on Day 1 of pregnancy, and most were in the uterus by mid Day 3. Chang (1976) reviewed the evidence for post-ovulatory progesterone induced effects and concluded that transfer of embryos from oviduct to uterus was unaffected, although some slight acceleration along the tube might occur.

The possibility that progesterone exerts its effects by biotransformation into oestradiol can be discounted since the work of Kendle and Telford (1970) showed that megestrol acetate, a synthetic progestational agent which cannot be transformed into an oestrogen, could accelerate embryo transport in the rabbit, when administered three days prior to ovulation.

Mention has already been made to the effects of oestradiol and progesterone on ciliated cells, and on secretory cell function. The hormones may also affect tubal function through actions on muscular contractility, directly or indirectly. Lee (1979) reviewed the evidence for the role of steroid hormones in mouse oviduct smooth muscle contractility. She concluded that the effects of oestrogen and progesterone already noted could be the result of changes in calcium utilisation. Calcium ions play a vital role in the functioning of

smooth muscle, both in electrical and mechanical events. Disturbance of the availability of calcium, then, would alter contractility. Lee concluded that both oestrogen and progesterone acted mainly on tubal transport via this mechanism. This and other physiological/pharmacological mechanisms will be discussed further in a subsequent section.

Physiological Mechanisms involved in Embryo Transport

The physiological functions of the oviduct have been listed previously, and the process of ovum/embryo transport defined (see General Introduction, Embryo Transport in the Mouse). Mention has been made of the complex anatomy of the oviduct, and the timing of the events occurring in the tubal transport phase. The process of tubal transport is brought about by the co-ordination of muscular contractions, ciliary activity and possibly by variations in the rate of secretion and/or the composition of oviductal fluids. The relative contribution of each of these factors to the process of embryo transport is dependent upon the position of the embryos within the oviduct, upon hormonal and/or neural control, and are probably species specific.

Embryo transport can conviently be divided into 5 phases, although it must be remembered that it is a continuous process. Pick up of the ovum, transport through the preampulla/ampulla, transport of the fertilised ovum through the ampullary-isthmic junction (AIJ), transport through the isthmus and passage through the junctura (referred to by some authors as the utero-tubal junction (UTJ) cf.) make up the whole process, which has been reviewed by several authors (Aref and Hafez, 1973; Hafez 1973, Blandau, Bourdage and Halbert, 1979; Blandau and Verdugo, 1976). The complex nature of each step deserves individual examination.

Pick up of the ovum from the ovary is thought to be accomplished

in one of two different ways, which are species dependent. The anatomical configuration of the preampulla, and in particular the infundibulum, has already been described, and the presence of an enclosed ovarian bursal sac in rat and mouse, and absence in rabbit, cat and human has also been mentioned. At ovulation in those species possessing a bursa ovarica, it is dilated with fluid, and ova are released from the follicles into this fluid, surrounded by cumulus cells. Movement of the ovary within this fluid displaces the ova, bringing them under the influence of the infundibular cilla (Blandau, 1973; Aref and Hafez, 1973). In those species lacking a closed bursal sac, the finger-like projections of the infundibulum (cf) sweep over the surface of the ovary, bringing the ciliated epithelial surface into direct contact with the ruptured follicles. In these species the cumulus masses are referred to as "sticky" and are not released into the peritoneal fluid (Blandau, 1973; Aref and Hafez 1973; Eddy and Pauerstein, 1980). The ciliary beat in all mammalian species so far studied is in an abovarian direction. Anderson and Hein (1977) postulated that, in rabbit, (a species lacking a closed bursal sac), charged ionic sites on the crown of oviductal cilia could be associated with the cilia/ovum interaction. Mastroianni (1976) showed that infundibular pick up in rabbits was more effective for ova in cumulus, than for surrogates or denuded ova, suggesting a specific role for cumulus cells.

Transport of the ova, in cumulus masses, through the preampulla/ampulla is accomplished by a combination of muscular contractions and ciliary activity, the relative contribution of each being species dependent. At this time, the ampulla is distended with fluid and the dimension of the cumulus mass is relatively small in comparison to the diameter of the lumen. Aref and Hafez (1973) stated that transport through the preampulla was due to ciliary activity in

the rabbit, but that transport through the ampulla was due to muscular contractions, also postulated by Blandau (1973). However, the elegant in vivo study of Halbert, Tam and Blandau (1976) showed that ampullary transport of ova in cumulus was as rapid in oviducts in which muscular contractions were stopped by infusion of isoprenaline, as in control animals, although the pattern of movement in treated animals was more regular, not showing the peristaltic/antiperistaltic rushes seen in untreated animals. This suggests that ciliary activity alone is sufficient to transport ova through the ampulla of rabbits, as far as the ampullary-isthmic junction (AIJ). Halbert and Patton (1981) extended these observations, using surgical resection and anastamosis of rabbit ampullae to show that this procedure had no effect on the rate of transport of ova in cumulus, except when muscular contractions were inhibited by isoprenaline, when the ova were arrested at the anastamosis, suggesting that the role of the muscular contractions is to provide momentum to overcome barriers to ciliary transport. All the factors concerned were included in a proposed stochastic model for ovum transport in rabbit ampullae (Verdugo, Blandau, Tam and Halbert, 1976; Verdugo, Lee, Halbert, Tam and Blandau, 1980) in which it was proposed that ampullary transport could be looked upon as a one-dimensional random walk in a field of external force, analagous to a diffusion process. Ciliary activity is proposed as the external force, and the result of muscular contractions considered to be random in direction. The mathematical model derived proved to be a good representation of in vivo data, and application to other phases of ovum/embryo transport is possible. It showed that in rabbit, ampullary transport was primarily the result of ciliary activity, (in which all cilia beat in an abovarian direction). Mention has already been made (General Introduction, The Epithelium of the Oviduct) of the cyclic nature of oviductal ciliated cells in some species. Verdugo

(1980) and Verdugo, Rumery and Tam (1980) have proposed that ciliary activity may be increased by the presence of prostaglandins, acting as calcium ionophores. However, these are preliminary data based on the use of tissue cultures derived from rabbit fimbrial tissue, and as yet a physiological role has not been proposed.

Having traversed the ampulla, the ova are retained for a time at the AIJ. This region provides a functional block to further passage, the nature of which is not yet known. Aref and Hafez (1973) and Hafez (1973) list several possible alternative mechanisms by which embryo retention might occur. These include localised tissue oedema, temporary ciliary inactivity, adovarian isthmic activity, constriction or inactivity of isthmic musculature, or locking due to some special sphincteric activity in the region. The anatomy of this area has already been described. Although the lumen of this region is narrow, Greenwald (1961) found no evidence in the rabbit of a specific sphincteric muscle. Brundin (1964) showed in oestrus rabbits that a functional occlusion of the AIJ occurred in oestrus, as shown by the difference in intraluminal pressure variations in the ampulla (low) and the isthmus (high). This study was carried out using intra-luminal catheters, the effect of which on the result cannot be estimated. Higgs and Moawad (1974) proposed that AIJ functioned as part of the isthmus, all of which was an adrenergic sphincter, released by rising blood levels of progesterone. The autonomic innervation of the oviduct has not yet been mentioned, and its role in tubal function is still very much in question. The role of adrenergic nerves has been reviewed by several authors (Brundin, 1976; Black, 1976; Hodgeson and Eddy, 1975; Helm, 1981). At present, it seems unlikely that adrenergic neurones have a major role to play in tubal embryo transport in the mouse, since Johns, Chlumeckly, Cottle and Paton (1976) found that chemical sympathectomy, using 6-hydroxydopamine did not alter subsequent fertility. Further, the work of Kendle (1969) showed

that the delaying effect of reserpine (a substance which depletes neurotransmitter stores in adrenergic neurones), on mouse oviductal embryo transport, was due to the lowering of body temperature and therefore not due to its activity on the sympathetic neurones.

Blandau, Bourdage and Halbert (1979) suggested that adovarian isthmic contractions were involved in sperm transport from uterus to ampulla, and, some time after ovulation, this pattern changed. This adovarian activity would therefore provide a barrier for the passage of ova/embryos at the AIJ. Manchanda, Choudhary, Sakhuta, Nayar and Sengupta (1979) suggested that isthmic activity reached a peak at 48 hours after HcG administration in rabbits, remaining high until 72 hours after HcG. This high activity is proposed as a mechanism for delaying transport of embryos into and through the isthmus, a theory also proposed by Marsafy and Hafez (1979). Talo (1980), examining mouse oviducts in vitro, suggested that passage of ova from the ampulla to the isthmus was the result of ampullary contractile activity, suggesting that no sphincteric activity was necessary, and the movement of ova being dependent only on the level of activity in the various regions.

The role of the oviductal secretions and ampullary dilation by fluid at this time is not clear. It is possible that ampullary distention is a result of fluid elaboration or transport from bursa, or isthmus, and may be an important factor in overcoming the blockade of transport at the AIJ. It seems likely that the true nature of the arrest of ova at the level of the AIJ is a combination of some or all of the factors mentioned, and possibly others as yet undiscovered, and that the whole process is under complex control by hormonal or neural changes.

Passage of the embryos through the isthmus has been considered by

many authors to be the result of muscular contractions (Aref and Hafez, 1973; Blandau, 1973; Hodgeson and Talo, 1978), although Hafez (1976) postulated that the role of the isthmic musculature was to delay the passage of the embryos, rather than assisting it. This basic conflict is as yet unresolved.

Jansen (1978) postulated that a column of tenacious mucus may block the isthmus of the rabbit oviduct, aiding sperm transport, thereafter being replaced by more fluid secretions, allowing pro-uterine isthmic transport of embryos. This theory would also account for the delay of passage at the AIJ, and is supported by similar data for the human (Jansen, 1980).

Talo and Hodgeson (1978), using rabbit oviducts in vitro postulated that the stochastic element of embryo transport in this phase was due to multiple pacemaker activity within the oviductal musculature, wherein embryos would be moved from areas of activity to areas of inactivity, progressing along the isthmus toward the uterus, using the area of the AIJ as a reflecting barrier to muscular contractions, and the area of the UTJ as an absorbing barrier, thus imposing a pro-uterine bias on contractions. Examining this hypothesis of a moving active/inactive border, Vedugo et al., (1980) noted that the concept of randomness could be applied to the same data, presuming ampullary ciliary activity to be the reflecting barrier and the uterine lumen as the absorbing area, (therefore the "field of external force" being tubal fluid movement, presumably) and the model thus derived fitted the physiological data. This suggests that isthmic transport in the rabbit may consist of random or pseudorandom short-range peristaltic movements. In the mouse, the in vitro study of Talo (1980) suggested that the situation is less complex, and three distinct pacemaker sites exist in the oviductal musculature, one in the ampulla, one in the distal isthmus and one in the proximal isthmus.

His results suggest that the concept of randomness might not apply in the mouse. However, these data were obtained from oviducts perfused in vitro having been freed of mesentery, and pinned by the bursa ovarica and uterine cornua to the floor of the organ bath, in a situation not dissimilar to that of Lee and Kendle (1979), in whose studies embryo transport in vitro through the isthmus did not occur. Therefore, further examination will be necessary before a stochastic model for isthmic transport in mouse can be dismissed.

Passage to embryos from the oviduct to the uterus occurs after a delay at the junctura. Hard data on this aspect of tubal physiology is scarce. Blandau (1973) suggested, on the work of Black and Asdell, the existence of an adrenergic sphincter, relaxed by increasing levels of progesterone. Talo and Hodgeson (1978) theorised that fluid pressure from increasing isthmic activity drives embryos from the oviduct into the uterus, or that the musculature of the junctura is activated at the appropriate time. Fuentealba, Vera, Nieto and Croxatto (1982) reported that, in the rat, levels of nuclear oestrogen receptor in the whole oviduct showed a peak a few hours before passage of embryos into the uterus, in conjunction with increased levels of oestradiol in the serum. The authors suggest that these data indicate a physiological role of oestradiol in the passage of embryos into the uterus.

Most authors suggest that passage of embryos into the uterus is a rapid process, described generally as a peristaltic rush.

Examination of the Oviduct in vitro

The transport of embryos through the oviduct in vivo is the result of a co-ordinated combination of several factors - ciliary activity, the hydrodynamics and rate of production of secretory products and muscular contractility, all of which may be under hormonal and/or neural control.

Methods for the study of oviduct function *in vivo* include direct observation of embryos or surrogates, intraluminal pressure monitoring devices, measurement of back pressure resulting from the forcing of fluid or gas through the oviduct and the use of strain gauge transducers attached to the surface of the oviduct (for reviews, see Blandau, Boling, Halbert and Verdugo, 1975; and Daniel, 1976). The examination of embryos in situ is a most useful technique, but the other methods mentioned above are invasive techniques, and the results of such experiments, in which unmeasurable contraints are placed on the oviduct, are at best equivocable.

The development of a suitable *in vitro* technique for the examination of embryo transport free of unknown *in vivo* controlling factors would be of value. Since the viability of any *in vitro* preparation is of vital importance to the study of embryo transport (a lengthy process *in vivo*) an examination of the methods used by previous workers is appropriate.

Most *in vitro* studies to date have examined a single component of oviduct function. Gaddum-Rosse et al., (1973, 1973 and 1976) have examined ciliary beat in opened excised oviducts from several species - rabbit, pig, human, macaque, sheep, cow, guinea pig and rat. The oviducts were slit open along their length and submerged in a balanced salt solution maintained at 37^{o}C, without gassing, to minimise muscular activity. The movement of particulate matter over the mucosal surfaces of the various regions (ampulla, isthmus, junctura) were examined for direction and force. This gives a measure of ciliary activity (although no quantitative data was reported), which is unrelated to the other components of embryo/sperm transport. Several species differences were reported.

Said Mounib and Chang (1965) used minced oviducts to investigate metabolic functions in estrous and pseudo pregnant rabbits, using

uptake of oxygen in a Warburg manometer as a measure. Sliced rabbit oviducts and scrapings of endosalpinx were used by Leese et al., (1981) to determine oxygen uptake, using a Clark electrode. This gave a measure of tissue viability, and indicated that the tissue preparations were viable for up to 40 minutes. However, although such methods would be of use in metabolic studies, no information directly related to embryo transport could be obtained. Leese et al., (1977, 1979, 1979, 1981) also used a luminally perfused rabbit oviduct preparation to provide evidence for facilitated diffusion of glucose into rabbit oviductal fluid, to monitor movement of pyruvate, and lactate and changes in lactate dehydrogenase, and transport of amino acids into the lumen. In this method, both ovarian and uterine openings of the oviduct were cannulated, and warmed normal saline solution perfused through the oviduct at a rate of 50ul/min. The whole preparation was suspended in an organ bath of Krebs-Ringer bicarbonate solution, gassed and maintained at 37^{o}C. At discrete time intervals, small (10ul) samples were withdrawn for analysis. This method gives an indication of the secretory activity of the endosalpinx.

Brunton and Brinster (1971) used an isolated rabbit ampullary sheet mounted on a special double chamber to measure chloride ion transport, (thereby suggesting that chloride ions are actively secreted into the oviductal fluid), and Leese et al., (1981) used a derivative of the isolated perfused oviduct already described to measure potential difference across the oviductal wall, in relation to oestrus and time after injection of hCG.

Most of the work carried out in vitro on oviducts have examined oviduct contractility, i.e. the ability of the smooth muscle component of the oviduct to contract under various hormonal, pharmacological and electrical influences. In general, most methods involve the isolation of a segment of the oviduct, (ampulla, isthmus, ring, sheet or strip)

in an organ bath where appropriate conditions of temperature, oxygenation and bathing medium are maintained.

Rodriguez-Martinez et al., (1982), and Howe (1976) used *in vitro* systems involving the placing of intraluminal pressure sensitive devices into the isolated oviduct, thus monitoring tubal motility. Rodriguez-Martinez et al., used sections of pig oviducts and examined the changes in motility due to oestrus cyclicity. Howe used complete rabbit oviducts to examine pressure changes brought about by the addition of cholinergic agents to the bathing medium. These methods, however, involve the disadvantages of similar *in vivo* techniques, in that the intrusion of pressure sensitive catheters into the lumen may induce changes not normally seen. They also suffer from the drawback of most such contractility studies - measurements are made at single points within the length of the oviduct and the direction of pressure change waves, and the effect on luminal contents cannot be measured.

By far the most common type of *in vitro* study involves the examination of single pieces of oviductal tissue, using tension recorders to measure muscular contractions. Rings of oviductal tissue have been used by several authors (Widdicombe et al., 1977; Higgs et al., 1973; Johns et al., 1976; Korenaga et al., 1981 and Paton, 1976) to record the activity of the circular smooth muscle layer of the oviduct. Such preparations use single pieces of tissue or chains of pieces suspended horizontally in an organ bath and attached to an isometic tension recording device above.

A similar method, but using a piece of tissue vertically orientated, has been used by many authors to examine the longitudinal muscular contractivity of the oviduct. Helm and co-workers in Lund have examined sympathetic and peptidergic control of human oviduct *in vitro* using rings and strips of muscle (Helm et al., 1981; Helm et al., 1982; Helm et al., 1982). Lindblom and collegues in Gotenburg have

isolated layers of longitudinal and circular smooth muscle from human oviductal isthmi and ampullary-isthmic junction in vitro in order to examine the effects of prostacyclin (Lindblom et al., 1979A) Prostaglandins E and F (Lindblom et al., 1978), adrenergic agonists and antagonists (Lindblom et al., 1979B) and the influence of prostaglandin synthetase inhibition on spontaneous activity (Tonpe et al., 1979). This group were also able to prepare similar muscle "ministrips" from human oviductal ampullae in order to examine the effect of prostaglandins on contractility (Caschetto et al., 1979).

Widdicombe et al., (1977) used isolated rings of oviductal tissue to examine the effects of various drugs and transmural electrical stimulation on monkey oviducts, while Johns et al., (1976) had reported the same technique using rabbit tissue to investigate the action of potassium ions on spontaneous contractility. Tissues were, however, maintained at 26°C, since the authors reported that spontaneous contractility could be maintained more successfully than at 37°C. This temperature was also employed (Johns et al., 1980) when the effects of ovulation and progesterone or oestrogen on the response of rabbit oviducts in vitro to nerve stimulation were examined. Transmural stimulation was employed to induce nerve stimulation, and release of noradrenaline.

Gimeno et al., (1976) employed a similar in vitro system to examine not only the contractility of isolated sections of guinea-pig oviduct, but also isolated strips of mesosalpinx, and reported responses to pharmacological agents which were different for the two smooth muscle structures.

It can be seen that a variety of modification of the basic isolated smooth muscle preparations are available. However, since the role of muscular contractility in the process of embryo transport is not year clear, interpretation of such studies is difficult.

Tissue culture of oviducts *in vitro* is another method for the examination of oviduct function. Early work by Biggers and co-workers (Biggers et al., 1962; Gwatkin et al., 1963) indicated that mouse embryos could develop normally in whole explanted cultured mouse oviducts, at least as far as the blastocyst stage. Oviducts were removed from mice on Day 1 of pregnancy and cultured on a chemicially defined medium (BGJb) maintained at 37°C for periods of up to four days. Oxygenation was encouraged by supporting each tissue on the surface of the medium. It was found, however, that normal muscular contractility ceased in culture for the first two days, but by the fourth day, all explants were active. It was suggested that this was simply indicative of degeneration of the tissue. Ciliary activity occurred throughout the culture period. Blastocysts developed in this way were collected and transferred into superovulated foster mice and allowed to develop until the seventeenth day of pregnancy. At autopsy the foetuses were found to be normal.

More recently, Menezo et al., (1981) has reported a preliminary study in which oviducts of the human, rabbit and rat could be cultured *in vitro* for periods of up to two days without evidence of degenerative changes upon histologic examination. This system involved the continuous perfusion of tissue culture medium TC199 through a chamber maintained at room temperature and continually gassed with 50% air/50% CO_2. It was also found that tissue integrity was maintained only in the presence of oestrogen (10ug 17beta-oestradiol/20ml culture). Addition of progesterone (100ug progesterone/20ml culture) resulted in weakness of epithelial cell attachment.

The use of such cultures seems to lie more in studies of *in vitro* fertilisation techniques than in the study of embryo transport. More useful information may be obtained from incubation, in a suitable Ringer solution, of whole oviducts with embryos in situ.

In vitro whole oviduct preparations have been reported by several authors. Hodgeson and Talo (Talo and Hodgeson, 1978; Hodgeson and Talo, 1978) examined the electrical activity of explanted rabbit oviducts at 18, 24 and 68 hours after an ovulation inducing dose of hCG. Seven or eight small suction electrodes were attached along the length of the oviduct, (maintained at 37.5°C in an organ bath containing oxygenated Tyrode Ringer which was replaced at 1ml/min.) inserted into the circular muscle layer. Origin, distance and direction of spread of each wave of contraction were recorded. The effects of in vivo administration of oestradiol and progesterone on in vitro electrical activity were also reported.

Natow et al., (1979) used whole rabbit oviducts to examine contractile events in a similar manner using suction electrodes, but coupled this with data on luminal pressure changes, after insertion of an open ended saline filled catheter into the isthmic lumen. Neuronal stimulation of the tissue was achieved by threading the ovarian vessels and perivascular nerves through a set of platinum ring electrodes. Stimulations of 5.0mA/0.5ms at 20 or 40Hz for 1 sec. resulted in a measurable contraction, but such levels of stimulation are well above the normal physiological range of neuronal activity.

Talo (1980) reported the examination of whole mouse oviducts in vitro using suction electrodes to determine myoelectrical activity. Also, he chose to use animals in which unfertilised ova were present in the oviduct, and coupled his electrical investigations with observations of the ova in situ. Since the oviducts had been freed of mesentery, rough measurements of position were noted. Since the experiments were run only over relatively short periods of time, no changes in ova position within the tract were reported.

A more complete in vitro system was reported in 1973 by Tojo et al. The utero-tubo-ovarian unit of the human was perfused through

the uterine arteries/veins using an artificial heart pumping a perfusate containing 20% patient or preserved blood, 6% dextran, and 10% Hank's solution in aseptic distilled water, to which was added 5,000 units of heparin and 5,000 units urokinase per 300mls and pH adjusted to 7.4 with sodium bicarbonate. The perfusate was gassed with 95% O_2 + 5%CO_2, although the supply of gas and proportion of O_2/CO_2 altered as the PO_2, PCO_2 and pH of the perfusate varied. The tissue was placed in a chamber maintained at 36.5-37°C with 100% humidity. Extensive testing of perfusate composition were carried out over time periods up to six hours. They included RBC count, Hb content, haematocrit, PO_2, PCO_2, pH, lactate, hCG and progesterone. Tissue samples of units containing normal or neoplastic trophoblastic material and ovarian tissue were collected to study nucleic acid synthesizing activity. Uterine and ovarian electromyograms were obtained using an electrode inserted into the smooth muscle of the UTJ, and another into the paraluteal area of the ovary. The results of this battery of tests showed that it was possible to maintain the utero-tubo-ovarian units in some cases (but not all) in a state similar to in vivo situation, and to use this system to investigate normal and pathologic endocrine conditions, for periods of up to six hours in vitro.

Raess et al., (1980) reported an isolated perfused rabbit oviduct system. The oviduct was perfused through the ovarian artery/vein with a perfusate containing defibrinated rabbit blood/oxygenated Earles medium in the ratio of 1:2, with a hydrostatic pressure head of 65mmHg. The organ was placed in a chamber containing oxygenated Earles medium at 35°C, which was constantly replaced at the rate of 2-3ml/min. The authors measured various outflow and oviduct motility using pressure transducer and mutual induction coils/optical cuff transducers respectively. The preparation was able to autoregulate.

That is, if temperature pH etc., are maintained, the tissue will respond to changes in perfusion pressure with vascular changes maintaining constant flow through the organ. Further, the authors reported that such oviducts *in vitro* were able to transport stained rabbit ova in cumulus masses through the ampulla, at a rate consistent with reported *in vivo* work (Boling and Blandau, 1971).

The work of Lee and Kendle (Lee and Kendle, 1979; Lee, 1979) provided evidence that an *in vitro* mouse oviduct preparation could be developed specifically to examine embryo transport *in vitro*. In this method a mouse oviduct, freed of mesentery was placed in an air-tight optically transparent chamber, through which a gassed Ringer solution at 33^{o}C was perfused. The chamber allowed continuous microscopic examination of the oviduct and therefore calculation of the mean percentage oviduct traversed by the embryos (see Materials and Methods, Section 2). Motility of the oviduct could be assessed visually. Oviducts were incubated for periods of up to 24 hours, from early on Day 2 of pregnancy to Day 3 of pregnancy. Such a preparation maintained the viability of the oviduct musculature which, although diminished in frequency, continued to contract rhythmically for the 24 hour period. However, no change in the mean % oviduct traversed over the incubation period was found. It was suggested that the method might have impaired the physiological integrity of the muscosal layers of the oviduct, thereby removing some important controlling factor (Lee, 1979). This brief review of the methods available for the examination of oviduct function *in vitro* indicates that the development of a system in which embryo transit could be examined *in vitro*, would be advantageous. In such a system the tissue would be viable for a reasonable length of time, free of normal *in vivo* controlling factors, (both those already documented, and any unknown factors) and be capable of pharmacological manipulation.

Aim of the Investigation

The proposed study is designed to examine the factors controlling embryo transport through the oviduct of the mouse. It is suggested that an *in vitro* model of embryo transport may be developed to assess the extent of normal function possible in the absence of *in vivo* hormonal and/or neural fluxes in comparison to normal *in vivo* data. This system may then be used to examine the effects of hormones or drug substances on tubal embryo transport. *In vivo* work will examine three aspects of oviduct function. Firstly, the passage of fluid from oviduct to uterus during the passage of embryos will be examined. Secondly, local administration of drugs into the fluids of the oviduct via the bursa ovarica will be assessed. Thirdly, the role of the steroid secreting organs, the ovary and the adrenal gland, will be examined by removal of such organs on Day 1 of pregnancy, and subsequent examination of embryo transport, and determination of resulting serum progesterone levels.

This latter examination is suggested because it is hypothesised that continued progesterone activity is required throughout the embryo transport phase for normal tubal transport in the mouse, as was suggested previously (Kendle and Lee, 1980). To examine this hypothesis in more depth, the effects of several drug substances, particularly androgens, will be investigated.

An extensive examination of the pattern of embryo transport *in vivo* in the RGIT/SLAC colony of mice is planned.

GENERAL METHODS

METHODS

1. Animal Husbandry

In all experiments, mice of the RGIT/SLAC strain were used. Animals were housed in constant conditions - 9 hours light, 09:00h to 18:00h; 15 hours dark, 18:00h to 09:00h, (intensity 30 lumens), temperature of 21+/-2^{o}C (except where experimental conditions state otherwise), humidity 50% + RH and diet of Oxoid pasturised breeding diet for rats and mice and water were freely available.

For mating purposes, mature virgin females of between 5 and 12 weeks of age were caged with mature males of between 6 and 14 weeks of age, in the ratio of 2:1. The females were examined at or soon after 09:00h each morning for the presence of a vaginal plug, which was taken as evidence of successful copulation. The day on which a plug was found was defined as Day 1 of pregnancy. At the end of each week the females were replaced, and the males replaced every 2-3 months.

For examination of androgen activity, male mice, 3-4 weeks old, which had been weaned that week, were used.

2. Measurement of the position of the embryos within the reproductive tract

Mice were killed by cervical dislocation and the ovaries, oviducts and uterine cornua exposed by a mid-ventral abdominal incision. The oviduct, ovary and associated fatty tissues were freed from the uterus by sectioning the uterine cornua 1-2mm from its tip.

The ovary/oviduct unit was transferred onto fine filter paper under a stereo dissection microscope (Nikon SMZ, magnification 10-15X). Under trans-illumination, straight, iridectomy forceps were used to hold the uterine attachment, and the mesosalpinx, bursa ovarica and fatty tissue removed using pointed, straight iridectomy scissors. The mesotubarium superius was then carefully removed, starting at the uterine end, thereby, gently straightening the coils of the oviduct. Finally, the ovary was removed. The straightened oviduct was then

immediately placed on a microscope slide and covered with a heavy coverslip, under which was pipetted normal saline (0.9% w/v sodium chloride in aqueous solution) at room temperature, using a Pasteur pipette. This slide arrangement was placed on a Vickers microscope (magnification 100X) with mechanical stage and Vernier scale. The exact position of the ovarian end, the uterine end and each embryo was then read off the Vernier scale and recorded.

To locate embryos within the uterus, the uterine horn was freed by cutting the mesometrium. A blunted 23g hypodermic needle was inserted into the lumen of the distal end and held in place by a Dieffenbach bulldog clamp. The proximal end was sectioned and the uterine horn flushed with 0.1ml normal saline solution into a cavity microscope slide. The needle was then removed from the distal end, and clamped into the proximal end, and the uterine horn flushed as before, in the reverse direction. The collected fluid was then examined under a stereo dissection microscope (Nikon, magnification 40X). The number and developmental state of any embryos were noted.

Embryos found in the uterine lumen were defined as having traversed the whole oviduct i.e. 100%, and using this data and the position of the embryos within the oviduct, the mean percentage oviduct traversed was calculated.

These procedures were carried out on both sides of the reproductive tract.

3. Surgical procedures

The procedures of ovariectomy, adrenalectomy, ligation of the uterine cornua and injections into the bursa ovarica were all carried out under pentobarbitone anaesthesia. Surgical anaesthesia was obtained by intravenous administration, into the tail vein, of pentobarbitone sodium ("Sagatal", May and Bayer; 60mg/ml, diluted 1 in 10 with normal saline just prior to administration; dose volume of

10ml/kg). All instruments were kept throughout the operations in a solution of 1% w/v cetrimide and 0.1% w/v chlorhexidine gluconate in 70% aqueous ethanol. This solution was also used as a skin swabbing solution, and general cleaning solution for working surfaces.

The skin was prepared by removal of fur, using a small animal shaver, and swabbing the exposed skin. Bilateral skin and peritoneal incisions were made using a scalpel fitted with a No. 12 blade and curved iridectomy scissors. Excess subcutaneous fat was trimmed off.

For adrenalectomy, the adrenal was exposed and gently brought to the body surface using curved iridectomy forceps where it was cut free.

For ovariectomy, the ovary was exposed, and gently brought to the body surface, where the bursa ovarica was opened, using curved iridectomy scissors, the ovarian attachments grasped between two pairs of forceps and the ovary pulled free. Any slight haemhorrage was curtailed by lightly blotting the tissue with a sterile gauze swab.

The detailed methods for uterine ligation and intra-bursal injections are described elsewhere.

Once the procedure was complete, the peritoneum was closed using individual cotton sutures, and the skin with 7.5mm Michel suture clips. The animals were maintained at 30+/-2^{o}C in a heated cabinet until recovery from anaethesia, unless otherwise stated.

The procedures of orchidectomy and collection of blood by cardiac puncture were carried out under ether anaesthesia.

For orchidectomy, instruments were stored as previously described. The scrotal skin was cleansed with swabbing solution, and a mid-line incision made with a scalpel fitted with a No. 10 blade. Each testicle was gently exposed by light pressure on the lower abdomen, and removed by gently pulling free with forceps. Haemhorrage was slight or absent. The wound was closed with a single cotton suture.

For collection of blood by cardiac puncture, the wall of the thorax of the anaesthetised animal was pierced on the left side and a chamber of the heart entered using a 23 gauge hypodermic needle attached to a 2ml syringe. Samples of 1 to 2ml of blood were taken, using gentle suction. After collection, the animal was immediately killed by cervical dislocation, and the success of any surgical procedure previously carried out was confirmed by autopsy. Serum was obtained from the blood by allowing the blood to stand at room temperature for 15 minutes, and then centrifuging for 15 minutes at 2500rpm. Serum was aspirated from each sample and stored at $-20^{o}C$ until analysis.

4. Serum Progesterone Determination

Serum obtained as described above was analysed for progesterone using a commercially available radioimmunoassay kit for the determination of serum or plasma progesterone, using (^{3}H) progesterone. The kit was purchased from Radioassay Systems Laboratories Inc., Carson, California. Standards ranged from 0.05ng/ml to 2.0ng/ml, and the specificity of the antiserum was as follows:-

Steroid	% cross reaction
Progesterone	100
Desoxycorticosterone	2.1
Pregnenolone	0.8
20 Alpha-Dihydroprogesterone	0.3
Testosterone	0.09
Androstenedione	0.07
5 Alpha - Dihydrotestosterone)	
17 Beta - Oestradiol)	
Oestrone)	<0.01
Oestriol)	

5. In Vitro Mouse Oviduct Preparation

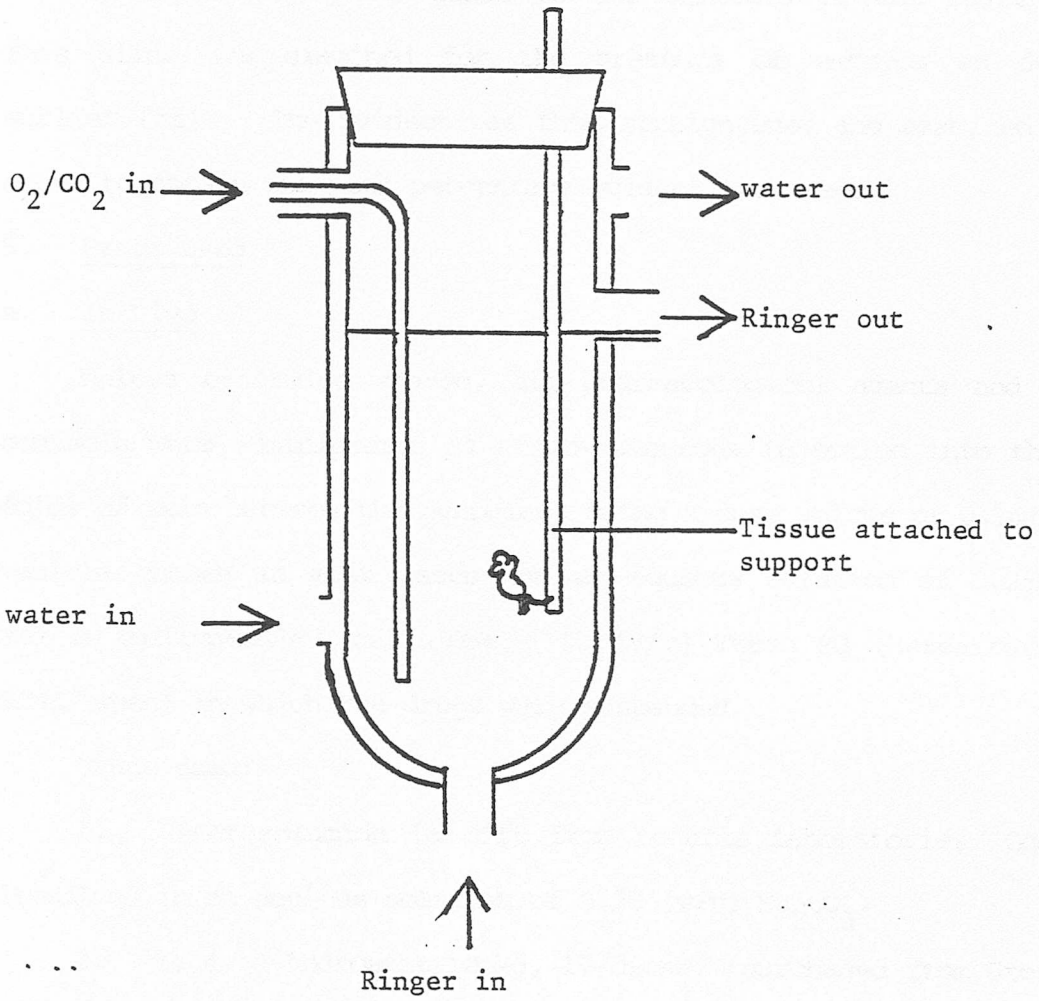
In order to examine embryo transport uninfluenced by normal *in vivo* controlling factors, a system for the incubation of oviducts *in vitro* was developed, using the organ chamber shown in Figure 1.

Ringer solution was pumped into the chamber from below and passed out through a side-arm, thus maintaining the level of fluid within the chamber. $95\%O_2/5\%CO_2$ was passed into the chamber by a polypropylene air-line through a second side-arm. The tissue was attached to a polypropylene support, which was held in place by a bung which closed the top opening of the chamber, thus minimising loss of fluid by evaporation. The whole chamber was surrounded by a water jacket.

Ringer solution of the following composition was used, unless otherwise stated, g/l (mM):- NaCl, 9.00 (154.0); KCl, 0.42 (5.6); $CaCl_2$, 0.24 (2.2); $NaHCO_3$, 0.50 (6.0); Glucose, 1.00 (5.6). This solution was gassed with 95% O_2 + 5% CO_2, as described above and the chamber temperature maintained at 30+/-1^{o}C. Using a Corning multi-channel peristaltic pump, the solution was passed into the chamber at the rate of 1ml/min. Up to six chambers could be in operation at any one time.

In animals used for *in vitro* experiments, the ovaries, oviducts and uterine cornua were exposed as described earlier. The oviduct, ovary, distal 1/3 of the uterine cornua and associated fatty tissues were removed and placed on fine filter paper, under a stereo dissection microscope (cf). The mesometrium, mesosalpinx, bursa ovarica, ovary and fatty tissues were removed. A ligature was passed round the uterine cornua 4-5mm from its distal tip, closing off the uterine lumen, and the excess, proximal, tissue removed. This ligature was used to tie the tissue to the tissue support, and the tissue then transferred to the organ chamber.

Figure 1. In vitro oviduct chamber.



After incubation in vitro, the tissue was removed from the chamber, and lightly blotted dry. The section of uterine cornua was cut, using fine, pointed iridectomy scissors, and the fluid from the lumen collected in the well of a cavity slide. The tissue was washed with one or two drops of normal saline solution at room temperature, and these washings were added to the contents of the cavity slide. This slide was examined for the presence of embryos as described earlier (cf). The oviduct was then straightened and examined (cf) in order to obtain the mean percentage oviduct traversed.

6. Drugs used

A. In Vivo

Unless otherwise stated, all pharmacological agents and vehicle controls were administered as a sub-cutaneous injection into the loose folds of skin between the scapulae, using a dose volume of 0.1ml. The vehicle chosen in most cases was an aqueous solution of 0.25% (w/v) sodium carboxymethyl cellulose + 1% (v/v) Tween 80 (hereafter called SCMC/Tween) in which the drugs were suspended.

Drugs used:

1. Acetazolamide (a gift from Lederle Laboratories, Gosport), dissolved in an aqueous solution of 0.2% (w/v) Na_2CO_3.

2. 1, 4, 6-Androstatrien-3, 17-dione, (purchased from Steraloids UK Ltd., Croydon).

3. 5 Alpha Dihydrotestosterone, (purchased from Sigma Chemical Co., Poole).

4. 17 Beta-Oestradiol, (purchased from Sigma Chemical Co., Poole).

5. Progesterone, (purchased from BDH Chemicals Ltd., Poole).

6. RMI 12,936, (a gift from Richardson-Merrell Ltd., Egham).

7. Tamoxifen Citrate, (a gift from ICI Pharmaceuticals Division, Macclesfield).

8. Testosterone Propionate, (purchased from Sigma Chemical Co., Poole).

B. In Vitro

Wherever possible pharmacological agents were dissolved directly into Ringer solution (cf).

Drugs used:-

1. Isoprenaline Sulphate, (a gift from Riker Laboratories, Loughborough).

2. Noradrenaline Acid Tartrate, (purchased from BDH Chemicals, Poole).

3. Oestradiol (cf) (prepared as a stock solution in ethanol. The volume of ethanol added to Ringer never exceeded 1ml/L).

4. Phentolamine Mesylate, (a gift from Ciba Laboratories, Horsham).

5. Progesterone, (cf), (prepared as a stock solution in ethanol. The volume of ethanol added to Ringer never exceeded 1ml/L).

6. Propranolol Hydrochloride (purchased from ICI Pharmaceutical Division, Macclesfield).

Experimental Results

Chapter One - The Normal Pattern of Embryo Transport In Vivo

Introduction

The time course of embryo transport through the oviduct of the mouse has been briefly discussed in the General Introduction. The reports of Burdick et al., (1942) and Humphrey (1968A) gave accounts of the timing and pattern of embryo transport in the mouse colonies of their own laboratories. They described in broad terms the areas within the oviduct/reproductive tract in which embryos were found at various times during the tubal transport phase. Since the timing of ovulation and therefore subsequent events will be affected by alterations in enviromental factors such as the light/dark ratio and temperature, a more detailed study of the time course of normal tubal embryo transport in the RGIT/SLAC strain of mice would be useful. The method used in this study for the determination of mean % oviduct traversed (see General Methods, Section 2) incorporates the position of all the embryos for each oviduct into a single figure and the mean for a group of these results is therefore a mean for a group of means, distributed normally, allowing statistical analysis by Students 't' test and the Aspen-Welch test (Welch, 1947).

Previous work (Kendle, unpublished observations) has indicated that the within animal variation in mean % is as great as the between animal variation, and therefore each oviduct can be presumed to be an independent observation. This detailed study is necessary for the subsequent study of the effects of drug substances, hormones, surgical procedures and *in vitro* perfusion on tubal embryo transport.

Materials and Methods

At various times throughout the four days of embryo transit through the oviduct, groups of animals were sacrificed and the mean % oviduct traversed determined by the method described (see General Methods, Section 2). Ten animals (that is, a group size of 20 oviducts) were sacrificed at 12:00h (+/- 30 minutes) on Day 1 of

pregnancy, and thereafter groups of five animals were sacrificed at six hourly intervals until 09:00h on Day 2, (an extra group of five animals was examined at 17:00h on Day 1). From 09:00h on Day 2 to 21:00h the same day, groups of five animals were sacrificed at hourly intervals (+/- 10 minutes). From 21:00h on Day 2 to 09:00h on Day 3, groups of five animals were sacrificed at three hourly intervals (+/- 30 minutes), and from 09:00h on Day 3 to 21:00h the same day, groups of five animals were sacrificed at hourly intervals (+/- 10 minutes). Thereafter, groups of five animals were sacrificed at three hourly intervals (+/- 30 minutes) until 12:00h on Day 4, when a group of ten animals was examined.

From 18:00h on Day 3 onwards, the number and distribution of embryos (i.e. oviductal or uterine) were also examined.

To determine the time interval which must occur before a change in mean % becomes statistically significant, the more detailed data from Days 2 and 3 were examined. Each 09:00h value was compared to the values four, eight and twelve hours later, by multiple 't' test.

Results

The group mean % oviduct traversed (mean %) at the time stated in the methods section are shown in Table 1.1, and graphically in Figure 1.1.

In the earliest group examined, 12:00h on Day 1 of pregnancy, the embryos were found in the ampulla as a group. Most embryos remain in this position until late on Day 1 or early on Day 2. In these early Day 1 groups, the mean % remained relatively static, and the standard error of the mean (SEM) small (<2). Between Day 1 and Day 2 the mean % increased as embryos move into the isthmus. Throughout Day 2, the mean % increased and the associated SEM's were larger (>2). A sudden increase in mean % occurred between 20:00h and 21:00h, when embryos were found in the distal junctura. Throughout Day 3, the mean % gradually increased and the SEM decreased as embryos progressed through

the junctura, until passage into the uterus began, by late Day 3. By early on Day 4 of pregnancy, the process is essentially complete.

The analysis of embryo distribution, between 18:00h on Day 3 and 12:00h on Day 4 is shown in Table 1.2, and graphically in Figure 1.2. The passage of embryos into the uterus began in early evening of Day 3, and was complete by the next morning (the process was 50% complete between 24:00h Day 3 and 03:00h Day 4). Examination of the raw data (not shown) indicates that, at any chosen time, individual sides of the reproductive tract may have embryos in the oviduct and the uterine cornua.

The comparison of Day 2 and Day 3 data to establish the minimum time necessary for a significant change in mean % to occur is shown in Table 1.3. On Day 2, comparison of the 09:00h value to each of the 13:00h, 17:00h and 21:00h values shows that significant difference is not seen eight hours later (i.e. 17:00h) but is seen by twelve hours later (i.e. 21:00h). Similarly, comparison on Day 3 reveal that no significant difference can be seen after four hours (i.e. 13:00h) but can be seen after eight (i.e. 17:00h). For a more detailed analysis, see Appendix 1.

Discussion

One of the difficulties in reporting a study such as this, is the choice of a reference point from which timing can be measured. Burdick et al., (1942) chose to report their data as hours "after the vaginal plug". Since the vaginal plug may remain in situ for several hours after coitus (the basis upon which this method is used), it cannot be an accurate measure. The authors also reported times "after ovulation" in the same study, but the time of ovulation was not reported.

Humphrey (1968A) chose to report his data as hours "after coitus", assuming coitus to have occurred at the preceding midnight,

although this cannot be presumed to be the case.

In this study, in order to avoid the use of such an arbitrary zero, mean percentages were reported at the time of day, on each day of pregnancy.

The pattern of embryo transport reported here corresponds well with the data on mice reported by Burdick et al., (1942) and Humphrey (1968A). Humphrey found ova in the ampulla as early as 06:00h on Day 1 of pregnancy. In this study, the earliest group was examined at 12:00h on Day 1, at which time all the embryos were in the dilated loop of the ampulla, usually clustered together, in a mass of cumulus cells. Since the proportion of the oviduct which makes up the preampulla and ampulla seems constant in the mice examined, the mean % reported for each oviduct is very similar, and the SEM is correspondingly low. This is true for all the groups in which the majority of embryos were found in the ampulla.

The embryos were found in the ampulla until late Day 1/early Day 2. This is in agreement with the 24 hours for which ova remain in the ampulla, reported by Burdick and co-workers, and the data of Humphrey, who reported some ova in the isthmus by 18:00h Day 1, and most by 09:00h Day 2. This pause at the ampullary-isthmus junction is in common with other species, although timing can be different. Greenwald (1961) reported that in rabbits ova arrived in the ampulla by 12 hours post-coitus, and remained there until up to 48 hours post-coitus. The study of the rat by Alden (1942A) is in general agreement. However, to examine transport beyond the ampulla, Alden found it necessary to inject a vital dye, Janus Green, into the periovarian space soon after ovulation, and therefore the value of the results are questionable, at least in relation to normal physiological events. Indeed, Alden reported that embryo transport in the rat took

some 95-100 hours, and that arrival in the junctura usually took 72-80 hours, figures which are much higher than the data for the mouse, of Burdick et al., Humphrey and this study.

Transport through the isthmus, in this study, took from early on Day 2 to late Day 2/early Day 3. During this time embryos were found spread throughout the isthmus, typically in groups of 2 to 3. The lumen of the oviduct was dilated around the embryos, but not sufficiently to allow anything but single file. Since the start of the isthmic transport phase is the opening of the ampullary-isthmic junction, and this is probably linked to ovulation or the events controlling ovulation the group mean %'s have a large SEM, due to the large between animal and within animal variation during this phase.

Humphrey (1968A) described the movement of embryos within the isthmus of oviducts, maintained at 37°C in Krebs-Ringer phosphate solution in vitro for periods up to 30 minutes, as pendular, consisting of peristaltic and antiperistaltic contractions. This was also the view of Lee and Kendle (1979), who reported such peristaltic/antiperistaltic movements in mouse oviducts maintained in vitro in specially constructed organ chambers, allowing continuous observation and measurement of mean % oviduct traversed. However, no net forward movement was observed in either case.

Passage of embryos through the junctura, between Day 2 and Day 3, was slow. This period was described by Humphrey (1968A) as sphincteric in nature, suggesting that the embryos remained static outside the intramural section of the oviduct for about 30 hours. The data reported here (Table 1.1, Day 3 09:00h onwards) indicates a more gradual forward progression during this time. Since, anatomically, this region is found at a similar position in all animals, and the embryos became grouped together for this slow phase, the associated SEM is lower than the isthmic phase.

In this report, embryos began to pass out of the oviduct into the uterine lumen by 19:00h Day 3, and the process continued until, by 12:00h Day 4, almost all embryos were found in the uterine flushings. Examinations of the raw data for the distribution of embryos within each side of the reproductive tract showed that many contained both oviductal and uterine embryos, suggesting that this is a gradual transition, not a rapid all-or-none process. At this time i.e. Day 4 of pregnancy, the total number of embryos recovered fell, indicating that the recovery of uterine embryos was less certain, particularly when embryos were situated in the distal uterine cornua. Recovery increased on Day 5 of pregnancy (Kendle, unpublished observations) when the embryos are evenly spread throughout each uterine horn, prior to implantation.

Since the position of all embryos within the uterine lumen must be reported as being 100% of the oviduct traversed, as more embryos enter the uterus, the within and between animal variations fall, and the standard error associated with the group mean becomes less.

The work of Kendle (unpublished observations) and Kendle and Lee (1980) have suggested a period of four hours or less might produce a significant change in the mean % oviduct traversed in any group of animals examined. The analysis of the detailed data shown here for Days 2 and 3 of pregnancy suggest that this is not the case. Inspection of all the data reported here, shown graphically in Figure 1.1, shows that embryo transport through the oviduct is a discontinuous process, consisting of several distinct phases, during which the rate of passage of the embryos varies. Therefore this single figure for a minimum time by which a significant change in mean % will occur is not appropriate. This is borne out in the analysis present in Table 1.3, which shows that during Day 2 (considered to be the fast, isthmic transport phase) between eight and twelve hours are necessary for a

significant change to occur, while on Day 3, (considered by Humphrey (1968A) to be a static phase, but shown here to be slow progressive forward movement) a significant change occurs between four and eight hours after the initial measurement. It should be remembered, however, that the isthmic transport phase is associated with a high SEM, and this would account for the increased time interval required for a statistically significant change in mean % in what is considered to be a rapid phase.

Further evaluation of the data by, for example, mathematical transformations with evaluation of regression models is to be undertaken to determine whether a mathematical model of the embryo transport process in RGIT/SLAC mice can be developed. Further details of this approach are given in Appendix 1.

Table 1.1 The mean % oviduct traversed by embryos *in vivo* at various times during the course of tubal embryo transport.

Day	Time	Mean %
1	12:00h	22.38 +/- 1.86 (20)
	17:00h	19.55 +/- 1.67 (10)
	18:00h	19.49 +/- 1.53 (10)
	24:00h	25.56 +/- 2.24 (10)
2	06:00h	30.16 +/- 2.80 (10)
	09:00h	39.92 +/- 3.71 (10)
	10:00h	36.82 +/- 3.21 (10)
	11:00h	36.49 +/- 3.47 (10)
	12:00h	44.52 +/- 3.05 (10)
	13:00h	44.44 +/- 4.72 (10)
	14:00h	43.99 +/- 2.42 (10)
	15:00h	46.57 +/- 3.36 (10)
	16:00h	49.41 +/- 3.79 (10)
	17:00h	46.38 +/- 4.16 (10)
	18:00h	53.38 +/- 4.77 (10)
	19:00h	43.09 +/- 2.48 (10)
	20:00h	48.39 +/- 4.80 (10)
	21:00h	76.02 +/- 3.92 (10)
	24:00h	71.66 +/- 4.18 (10)
3	03:00h	75.94 +/- 2.13 (10)
	06:00h	81.78 +/- 2.61 (10)
	09:00h	80.82 +/- 2.40 (20)
	10:00h	74.54 +/- 3.42 (10)
	11:00h	81.95 +/- 4.62 (10)
	12:00h	86.66 +/- 1.38 (10)
	13:00h	86.44 +/- 1.18 (10)
	14:00h	87.46 +/- 1.64 (10)
	15:00h	88.16 +/- 1.33 (10)

Table 1.1 (continued)

Day	Time	Mean %
3	16:00h	88.94 +/- 1.38 (10)
	17:00h	90.91 +/- 0.55 (10)
	18:00h	87.91 +/- 2.27 (10)
	19:00h	91.60 +/- 0.69 (10)
	20:00h	91.58 +/- 0.68 (10)
	21:00h	94.37 +/- 0.78 (10)
	24:00h	96.89 +/- 1.05 (10)
4	03:00h	97.81 +/- 1.11 (10)
	06:00h	99.58 +/- 0.42 (10)
	09:00h	99.80 +/- 0.20 (10)
	12:00h	99.93 +/- 0.07 (10)

Values are mean % oviduct traversed +/- SEM (n = No. of ovidcuts)

Figure 1.1 Graphic representation of the time course of tubal embryo transport, from the data presented in Table 1.1.

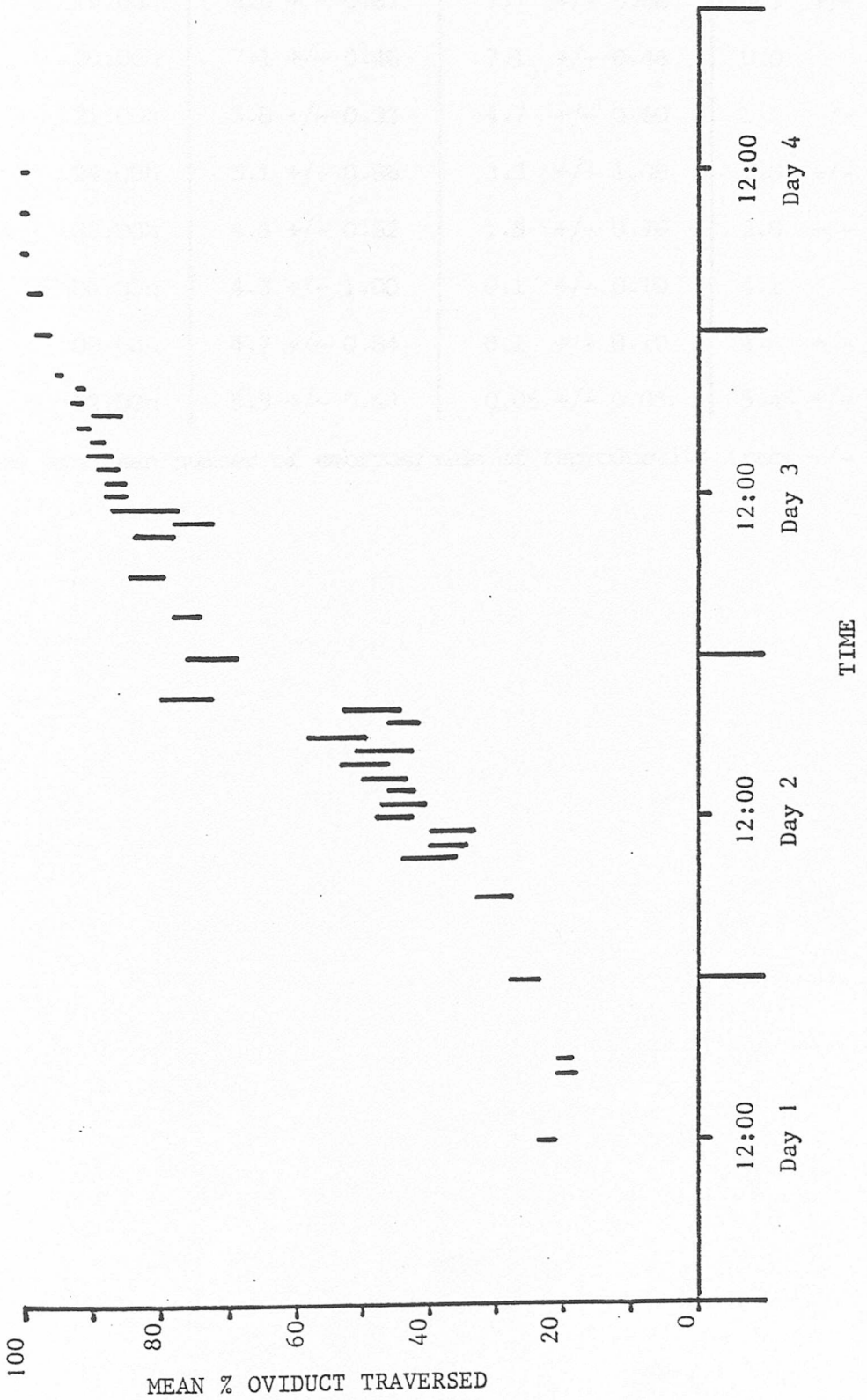


Table 1.2 Analysis of the distribution of embryos recovered in groups between Day 3 18:00h and Day 4 12:00h.

Time		Embryo recovery: Total	Oviductal	Uterine
Day 3	18:00h	7.5 +/- 0.89	7.5 +/- 0.89	0.0
	19:00h	8.0 +/- 0.67	7.7 +/- 0.68	0.3 +/- 0.30
	20:00h	7.1 +/- 0.46	7.1 +/- 0.46	0.0
	21:00h	5.8 +/- 0.33	4.7 +/- 0.60	1.1 +/- 0.48
	24:00h	5.1 +/- 0.86	3.3 +/- 1.08	1.8 +/- 0.55
Day 4	03:00h	4.3 +/- 0.52	1.5 +/- 0.76	2.8 +/- 0.68
	06:00h	4.3 +/- 1.00	0.1 +/- 0.10	4.1 +/- 1.04
	09:00h	4.7 +/- 0.84	0.1 +/- 0.10	4.6 +/- 0.87
	12:00h	5.5 +/- 0.43	0.05 +/- 0.05	5.45 +/- 0.41

Values are mean number of embryos/side of reproductive tract +/- SEM.

Figure 1.2 Graphic representation of the changes in embryo recovery and distribution between Day 3 18:00h and Day 4 12:00h, from data presented in Table 1.2.

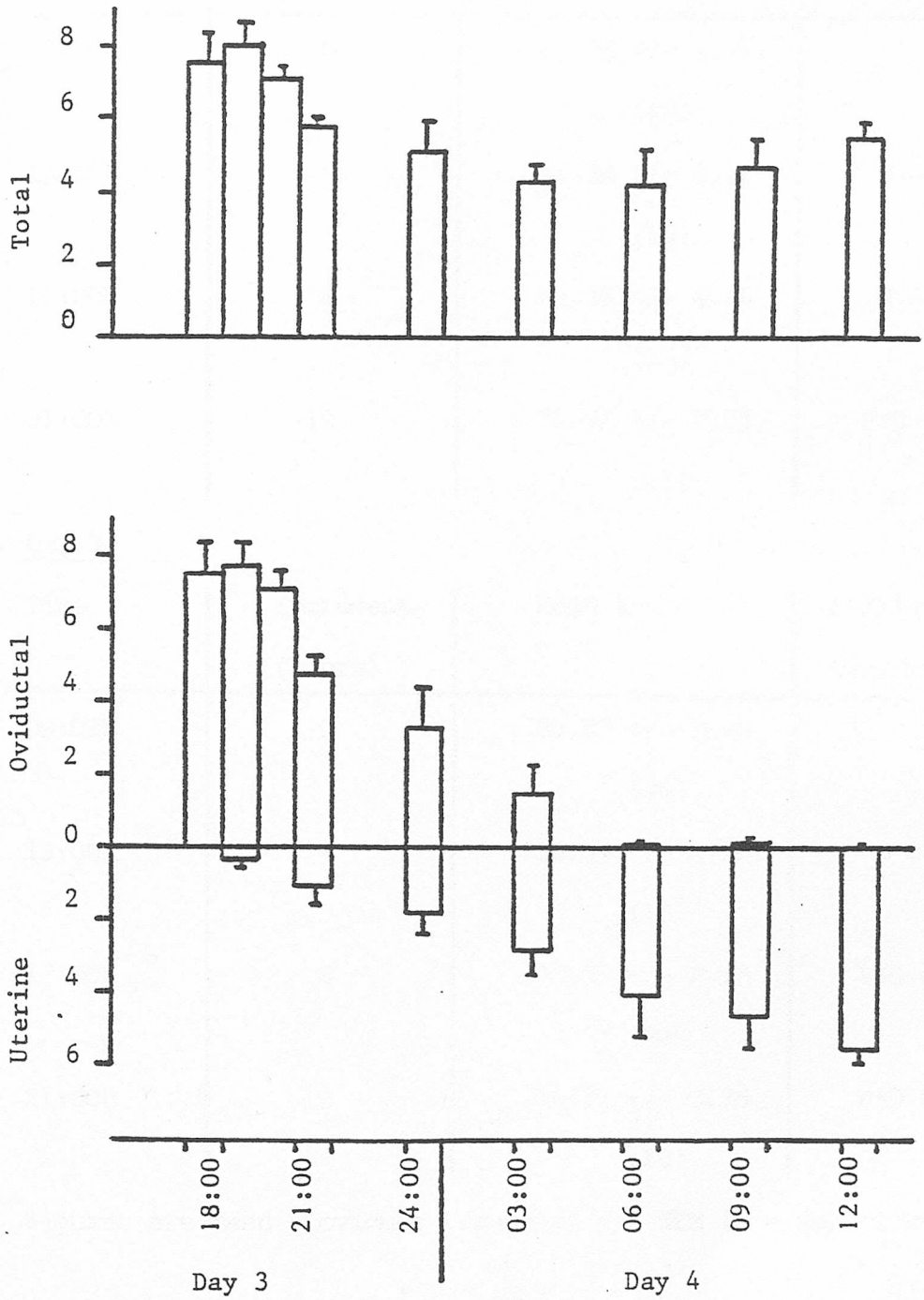


Table 1.3 Comparison of the Day 2 and Day 3 09:00h mean % oviduct traversed to values four, eight and twelve hours later.

Day 2

Time	Increment (hours)	Mean %	Statistical evaluation
09:00h	0	39.92 +/- 3.71 (10)	
13:00h	4	44.44 +/- 4.72 (10)	N.S.
17:00h	8	46.38 +/- 4.16 (10)	N.S.
21:00h	12	76.02 +/- 3.92 (10)	P<0.01

Day 3

Time	Increment (hours)	Mean %	Statistical evaluation
09:00h	0	80.82 +/- 2.40 (20)	
13:00h	4	86.44 +/- 1.18 (10)	N.S.
17:00h	8	90.91 +/- 0.55 (10)	P<0.01
21:00h	12	94.37 +/- 0.78 (10)	P<0.01

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts).

Chapter Two - The effects of miscellaneous Pharmacological Agents - Pentobarbitone, Oestradiol, Tamoxifen and Acetazolamide - on Embryo Transport in vivo

Introduction

During the course of these studies several preliminary investigations have been undertaken for various reasons. The effects of pentobarbitone has been investigated, because it is the general anaesthetic used in most of the surgical procedures in this work; Acetazolamide, to investigate the possible role of carbonic anhydrase; Oestradiol; and Tamoxifen, which is an oestrogen antagonist in man, but considered to be an agonist in mice.

These studies were peripheral to the main investigation but may warrant more detailed investigation at some future date. The preliminary data are therefore reported together in this chapter with a short Introduction, Materials section, Results and Discussion for each of the drugs examined.

A. The Effect of Pentobarbitone

Introduction - The use of barbiturates in medicine date to the early 1900's, and the use of pentobarbitone as an anaesthetic agent for small mammals is well established. The exact mode or molecular site of action of barbiturates is not yet clearly understood. In man, pentobarbitone is classed as a short-to-intermediate acting barbiturate, and in mouse produces surgical anaesthesia for periods of up to 10-20 minutes. Recovery from anaesthesia can take 2-3 hours, but the plasma half-life, in man, is thought to be of the order of twenty to forty hours. It has been shown that surgical procedures in mice, involving pentobarbitone general anaesthesia (even sham-operated control procedures), induced effects on tubal transport of embryos. The role of pentobarbitone in these effects is worthy of examination. It is known that hypothermia in mice, induced centrally by reserpine,

can retard embryo transport, and that correct maintenance of body temperature of reserpine treated animals reverses this effect (Kendle, 1969). Since hypothermia is a problem in the maintenance of general anaesthesia in small mammals, it is necessary to maintain the body temperature of pentobarbitone treated mice until recovery from anaesthesia is complete.

Materials and Methods - Two groups of five animals were anaesthetised with pentobarbitone (as described in General Methods, Section 3, Surgical Procedures) at 09:30h on Day 1 of pregnancy. One group was sacrificed at 12:00h on Day 2 of pregnancy, and the other group at 12:00h on Day 3 of pregnancy. A further group of five animals were anaesthetised at 13:00h on Day 1 of pregnancy, and sacrificed at 12:00h on Day 2. After induction of anaesthesia, all animals were caged in a heated cabinet (30+/-2^{o}C) until recovery was complete. In each case the mean % oviduct traversed was calculated by the procedures described earlier, and compared statistically to appropriate in vivo control data (obtained from Chapter 1), using a Student 't' or Welch test.

Results - The results are shown in Table 2.A.1. Comparison of the mean % oviduct traversed of animals anaesthetised at 09:30h on Day 1 of pregnancy with appropriate in vivo controls, all sacrificed at 12:00h on Day 2, shows that the treated group exhibits significant retardation of tubal transport ($P<0.05$). Examination of the mean % oviduct traversed from those animals sacrificed at 12:00h on Day 3 shows no significant difference from the position of the in vivo control group.

The mean % oviduct traversed by the group anaesthetised at 13:00h on Day 1 and sacrificed at 12:00h on Day 2 is significantly less than the appropriate control value ($P<0.005$).

Discussion - Embryo position measured some 26 hours after

induction of pentobarbitone anaesthesia is retarded significantly, and this retardation is no longer apparent some 50 hours after induction. Thus the delay, (but not arrest) induced by pentobarbitone is still seen 26 hours after induction, but is followed by a period in which embryo transport "catches up", to reach a position which can be considered normal by or before 50 hours after induction.

Previous work (Kendle, unpublished observations) has indicated that surgical procedures involving ablation or trauma to the ovary early on Day 1 of pregnancy may cause loss of embryos on subsequent measurement of embryo position, thus surgery at the later time of 13:00h was recommended. To determine the effect of anaesthesia at this time the second part of the experiment was carried out. Again, on examination, retardation was seen some 11 hours later, confirming the fact that pentobarbitone causes retardation.

Since the effect of pentobarbitone on body temperature has effectively been removed, the mode of action must be by some other means. The nature of the effect is unknown, but could be one or more of several sites - directly on any of the components of the oviduct, fluid, cilia or musculature, or at a higher level, controlling via hormonal or neural means via the CNS. It is known that pentobarbitone, in less than anaesthetic doses, is sufficient to block the proestrus luteinizing hormone surge necessary for ovulation (Everett, 1961).

It should be noted that in this study, only anaesthesia on Day 1 of pregnancy was examined. Since the embryos pass from the ampulla to the isthmus between Days 1 and 2, the ampullary-isthmic junction is a possible site of action for pentobarbitone. To confirm that the drug has a general retarding effect, it would be necessary to examine the effects of anaesthesia on different days during the time of tubal embryo transport, and compare the effects of other general anaesthetics.

Table 2.A.1 - The effect of pentobarbitone on tubal embryo transport.

Anaesthesia induced at 09:30h on Day 1 of pregnancy

	Mean % oviduct traversed		
Animal sacrificed	Anaesthetised	Intact control	Statistical evaluation
12:00h on Day 2 of pregnancy	35.26+/-3.19 (10)	44.52+/-3.05 (10)	Significant P<0.05
12:00h on Day 3 of pregnancy	88.77+/-1.37 (10)	86.66+/-1.38 (10)	Not significantly different

Anaesthesia induced at 13:00h on Day 1 of pregnancy

	Mean % oviduct traversed		
Animal sacrificed	Anaesthetised	Intact control	Statistical evaluation
12:00h on Day 2 of pregnancy	31.01+/-1.54 (10)	44.52+/-3.05 (10)	Significant P<0.005

Figures are mean +/- SEM (n = No. of oviducts)

B. The effect of Oestradiol

Introduction - The role of steroid hormones in tubal transport of embryos has been discussed in the General Introduction. The purpose of this short study was to examine the effects on embryo transport in this colony of a single administration of oestradiol early on Day 1 of pregnancy. The effects of such a regimen have previously been examined by other workers (Greenwald, 1967; Humphrey, 1968B). However, the species, time and dose dependant nature of the effects of exogenous oestradiol is well known, and work reported in Chapter 4 depends on a knowledge of the effects of such a dosage regimen in this colony. Dosages of 2 and 4ug of oestradiol were chosen, since the previous work of Lee (1979) has examined the effect of a single does of 2ug oestradiol at 09:00h on Day 2 of pregnancy on subsequent embryo transport and oviduct calcium levels, in this colony of mice.

Materials and Methods - Six groups of five animals were treated with oestradiol at 09:30h on Day 1 of pregnancy. Three groups were treated with 2ug and three with 4ug, each subcutaneously in 0.1ml SCMC/Tween as previously described, one group from each dose was killed at 12:00h on each of Days 2, 3 and 4. The mean % oviduct traversed was calculated and compared to groups which had been vehicle treated.

Results - These are shown in Table 2.B.1. There is no significant difference between groups examined on Day 2, although both treated groups show a non-significant increase in mean % oviduct traversed and increase in SEM. The 2ug/mouse group examined on Day 3 showed a significant decrease in mean % when compared to either control or the 4ug/mouse group ($P<0.001$ and $P<0.05$ respectively) which were not significantly different from each other. On Day 4, both treated groups were highly significantly decreased ($P<0.001$) in comparison to the control value.

Discussion - The results confirm the studies of Greenwald (1967)

and Humphrey (1968B) in that the single dose of oestradiol, given when embryos are still within the ampulla, causes retention of embryos within the oviduct for period longer than that of control animals. The retention seen in this study was primarily in the ampulla, although some embryos were present in the junctura on Days 3 and 4 (data not shown). Whether this represents a second site of retention, or merely the positions of embryos which had passed into the isthmus later than normal, is not known. The results of Lee (1979) suggest that this latter case is more likely since embryos within the isthmus when treated with oestradiol are rapidly expelled into the lumen of the uterus. Examination of the raw data (not shown) for the 4ug/mouse group examined on Day 3, shows that a number of embryos were recovered from the uterine lumen, and the total number of embryos recovered was less than control values. This suggests a significant acceleration in this group which is not seen in the group sacrificed on Day 4. This difference indicates the very individual nature of the response to oestradiol, and also confirms the view of Greenwald (1967) that higher doses of oestradiol could cause acceleration rather than retention. A fuller discussion of the effects of administration of exogenous oestradiol in vivo will be presented in Chapter 4.

Table 2.B.1 The effects of sub-cutaneous injection of 2 or 4ug oestradiol at 09:30h on Day 1 of pregnancy on subsequent embryo transport, compared to vehicle treated controls.

Mean %

Animal sacrificed	Vehicle controls	Dose of oestradiol ug		Statistical evaluation
		2.0	4.0	
12:00h Day Two	48.76+/-5.54 (10)	70.62+/-7.18 (10)	62.36+/-8.72 (10)	Cvs2.0 N.S. Cvs4.0 N.S. 2.0vs4.0 N.S.
12:00h Day Three	90.57+/-0.49 (10)	48.47+/-6.33 (10)	76.40+/-8.07 (10)	Cvs2.0 P<0.001 Cvs4.0 N.S. 2.0vs4.0 P<0.05
12:00h Day Four	97.98+/-1.04 (10)	51.16+/-5.99 (10)	43.72+/-8.68 (10)	Cvs2.0 P<0.001 Cvs4.0 P<0.001 2.0vs4.0 N.S.

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts)

C. The effect of Tamoxifen

Introduction - The effects of anti-oestrogenic drugs has already been mentioned (see General Introduction, The Role of Steroid Hormones in the control of Oviductal Embryo Transport). Tamoxifen (ICI-46474) is a non-steroidal anti-oestrogen used clinically in the treatment of oestrogen dependant breast carcinoma. Harper and Walpole (1967) reported that Taxoxifen was, in fact, an oestrogen agonist in mice. This view was based on the effects of Tamoxifen on vaginal cornification, and the ratio of the dose required to terminate pregnancy, and to the dose required to produce vaginal cornification. Tubal embryo transport was not examined. The purpose of this short study is to examine the effects of Tamoxifen on embryo transport in the mouse oviduct, in order to compare and contrast any effects with those of oestradiol already known.

Materials and Methods - The effects of two dose levels in two different vehicles were examined. 9 and 90ug of Tamoxifen in 0.1ml of each of arachis oil or SCMC/Tween (cf) were given as a subcutaneous injection to groups of 5 animals at 09:30h on each of Days 1, 2 and 3 of pregnancy, and the mean % oviduct traversed determined at 12:00h on Day 3. Appropriate vehicle treated controls were also carried out. Statistical evaluation used the Welch test.

Results - When administered in arachis oil, no significant difference in mean % was seen at 9.0 ug/animal/day compared to vehicle treated controls. Administration of 90ug/animal/day resulted in a significant decrease in mean %. When administered in SCMC/Tween, the mean % for both 9.0 and 90ug/animal/day groups were significantly reduced ($P<0.001$), and were not significantly different from each other. Comparison of each dose group for the two vehicles indicates that in the 9.0ug and 90ug/animal/day the mean %'s were significantly less ($P<0.01$) for the SCMC/Tween groups compared to the arachis oil

groups. However, the two vehicle treated control groups were also significantly different ($P<0.05$), the SCMC/Tween group having the higher mean % oviduct traversed.

Discussion - Tamoxifen has marked retarding effects on tubal embryo transport, which are dose and route of administration dependant. Tamoxifen administered in SCMS/Tween has significantly more effective then Tamoxifen administered in arachis oil. However, the need for a wide bore needle (21g) for the administration of the arachis oil induced leakage from the needle track and this must be borne in mind when comparing the effects of the two vehicles. ICI recommend the use of vegetable oils for injection of Tamoxifen, but, in this case, the aqueous suspension has proved the more useful vehicle. The increase in mean % in the SCMC/Tween vehicle group compared to the arachis oil group, although significant, is more likely to be a reflection of the inherent degree of error present in the data, and discussed in Chapter 1, and Appendix I. Considerations of the availability of Tamoxifen from the vehicle aside, the effects on tubal transport are marked. This study was designed to detect retention or acceleration of embryos by examining on Day 3 of pregnancy. It is also useful for the examination of oestrogens or anti-oestrogens, since the work of Humphrey et al., (1967) suggested that continued administration of oestradiol on Days 1 to 3 of pregnancy resulted in retention in the ampulla, coupled with loss of up to 50% of the embryos, while the anti-oestrogenic substances MER-25 and MRL 37, showed retention without loss. The extended work of Humphrey (1976) confirmed these results. In this short study, embryo recovery (data not shown) was not reduced, and many embryos were found within the isthmus/junctura. Thus it remains possible that the effects seen here in response to Tamoxifen may be of an anti-oestrogenic nature. Further studies are required to confirm this.

Table 2.C.1 The effects of Tamoxifen administered as a subcutaneous injection in each of two different vehicles on each of Days 1, 2 and 3 on the mean % oviduct traversed, in comparison to vehicle treated controls.

	Dose of Tamoxifen ug/animal/day			Statistical
Vehicle used	(1) 0.00	(2) 9.0ug	(3) 90ug	evaluation
Arachis oil	86.70+/-1.29 (10)	83.99+/-1.78 (10)	68.92+/-7.25 (10)	1vs2 N.S. 1vs3 P<0.05 2vs3 N.S.
SCMC/Tween	90.57+/-0.49 (10)	51.10+/-7.83 (10)	39.28+/-8.58 (10)	1vs2 P<0.001 1vs3 P<0.001 2vs3 N.S.
Statistical evaluation	P<0.05	P<0.01	P<0.01	

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts)

D. The effect of Acetazolamide

Introduction - Blandau, Jensen and Rumery (1958) reported the pH value of fluids collected from the dilated ampullae of rats in oestrus and found them, at 8.04 (standard deviation - 0.25) significantly higher than the pH of fluid taken from the uterine cornua, at 7.76 (standard deviation - 0.12). In studying the secretory rate and composition of oviductal fluid from rabbits, Iritani, Nishikana, Gomez and Vandemark (1971) reported that the pH of fluid from oestrus was higher than that of pseudopregnant animals. Also in rabbit, Vishwakarma (1962) reported that tubal pH was higher at 7.9 than plasma values at 7.52. He also reported tubal fluid levels of bicarbonate, and found a ratio of tubal fluid bicarbonate to that of plasma to be of the order of 2.17. The author suggested that this was due to active transport of bicarbonate into the oviductal lumen, and such levels of bicarbonate were able to prevent the degeneration of spermatozoa in oviducts.

The enzyme responsible for the production of bicarbonate from carbon dioxide is carbonic anhydrase. Lutwak-Mann (1955) reported the presence of carbonic anhydrase in the oviduct of the rabbit during early pregnancy, and following progesterone treatment, although none was found in non-pregnant animals. The uterine third of the oviduct of the sheep was reported to show carbonic anhydrase activity, whilst the fimbrial region was reported to give high activity in the cow. Maren (1967) reported carbonic anhydrase activity in the mouse uterus, but no reference was made to the oviduct in this review. The purpose of this short experimental series is to examine the effects of acetazolamide, an inhibitor of carbonic anhydrase, on tubal transport of embryos in mice.

Materials and Methods - A group of 5 animals were injected with acetazolamide 25mg/kg SC in 0.1ml 0.2% w/v Na_2CO_3 aqueous solution

at 09:30h and 17:00h on Day 1, 09:30h and 17:00h on Day 2 and 09:30h on Day 3 of pregnancy. The animals were sacrificed at 12:00h on Day 3 and the mean % oviduct traversed calculated (cf). Two groups of 5 animals were treated with 50mg/kg acetazolamide. In one group, the protocol was as described above, while in the other, the animals were sacrificed at 12:00h on Day 2 of pregnancy. The mean % oviduct traversed were calculated and in all cases compared to the values of untreated control animals (obtained from Chapter 1) by Students 't' test.

Results - The effect of 25mg/kg twice daily on tubal embryo transport is shown in Table 2.D.1 No significant difference between drug treated and untreated values is found. The effect of 50mg/kg twice daily is shown in Table 2.D.2. For each group (examined on Days 2 and 3 of pregnancy) there is no significant difference between treated and control untreated values.

Discussion - Although the LD_{50} of acetazolamide in mice is between 3000 and 6000mg/kg, dosage levels of 25-100mg/kg have been found to be a suitable dosage range to examine pharmacological effects (Lederle Laboratories, unpublished observations). Since the biological half-life of acetazolamide is probably short in mice, a twice daily dosage regimen was chosen. However, at neither of the two dose levels examined were any effects on the time course of tubal embryo transport seen. It remains possible that higher doses, or more frequent or prolonged administration could produce effects. It was, however, noted that in treated groups, the development of some embryos seemed delayed, in that at high dose, examined on Day 3 of pregnancy, some one celled embryos were seen. (It should be noted, however, that complete data was not obtained, as it is outwith the scope of this project). Bicarbonate is known to be a factor important for the correct development of embryos being the factor responsible for

dispersing the corona radiata cells in rabbit (Aitken, 1979, a review). Noreiga and Mastroianni (1969) showed that administration of acetazolamide soon after copulation, in a complex multiple dosage regimen, delayed embryo development possibly due to changes in sperm penetration timing, modification of fertilisation, or decelerated cleavage. Since analyses of this type are outwith the scope of this investigation, and because of supplies of drug were limited, the investigation was not taken further.

Table 2.D.1. - The effect of acetazolamide 25mg/kg administered twice daily by subcutaneous injection on mean embryo position in comparison to untreated controls.

Sacrificed	Treated	Untreated	Statistical evaluation
12:00h Day 3	82.72+/-5.86 (10)	86.66+/-1.38 (10)	N.S.

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts)

Table 2.D.2. - The effect of acetazolamide 50mg/kg administered twice daily by subcutaneous injection on mean embryo position in comparison to untreated controls.

Sacrificed	Treated	Untreated	Statistical evaluation
12:00h Day 2	47.87+/-3.12 (10)	44.52+/-3.05 (10)	N.S.
12:00h Day 3	86.04+/-1.28 (10)	86.66+/-1.38 (10)	N.S.

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts)

Chapter Three - Measurement of the passage of fluid from the oviduct into the uterus using uterine ligatures, and the effect of such procedures on embryo transport in vivo

Introduction

The cyclic nature of secretory activity during the oestrus/menstrual cycle has been discussed earlier (see General Introduction, The Epithelium of the Oviduct). Secretory activity is at its highest around oestrus (for reviews, see Hamner, 1973 and Perkins, 1974).

The composition of the fluid is known to be critical for fertilisation and subsequent development of the embryo (Stone and Hamner, 1975). However, the role of tubal fluids in the transport of embryos along the length of the oviduct is less certain. In the rabbit, ewe and cow, fluid flow is reported to be in an adovarian direction, except for three to six days after ovulation (Perkins, 1974).

Early studies on oviduct secretion rates involved ligation of ovarian and uterine ends of the rabbit oviduct, and subsequent aspiration of fluid (Blandau, Jensen and Rumery, 1958). More sophisticated techniques involved the catheterisation of the ovarian end of the oviduct, coupled with ligation of the uterine end of the rabbit oviduct (Bishop, 1956; Clewe and Mastroianni, 1960), or simply by fixing a balloon-tipped catheter into the lumen, of the human oviduct (Lippes, Krasner, Alfonso, Dacalos and Wcerdo, 1981). Kendle and Telford (1970) ligated the ostium and AIJ of the rabbit oviduct and aspirated fluid as a measure of secretory rate. These techniques are unable to determine direction of fluid flow, or its role in embryo transport.

It was therefore decided, in this study, to examine the amount of

fluid collected in a small section of distal uterine cornua, ligated to act as a collecting vessel. Thus, the amount of fluid passing in an abovarian direction during selected intervals within the course of tubal embryo transport could be measured by weight on autopsy and related to the position of the embryos within the reproductive tract. Therefore, unlike previous cannulation techniques, the oviduct remains relatively undisturbed, and fluid is free to pass in an adovarian direction, so that only fluid moving in an abovarian direction is measured.

Materials and Methods

Anaesthesia and general preparation were as described in General Methods (Section 3). To investigate the amount of fluid collected by a uterine ligature the ovary, oviduct and distal uterine cornua were exposed and a ligature placed round the uterine cornua, some 4-5mm from the junctura. A sham procedure consisting of all of the above steps, except the ligature, was carried out on every animal on the contralateral side. In half of each experimental group of ten animals the ligature was on the left side, and half on the right.

As further controls, groups of five animals in which two ligatures were placed on each uterine cornua were examined. One ligature was passed round the junctura, and the other 4-5mm proximally.

Groups of animals were operated at 13:00h on each of Days 1, 2 and 3 of pregnancy. The animals were sacrificed 20 hours later, viz at 09:00h on each of Days 2, 3 and 4.

Upon sacrifice, the abdomen was opened and the ovary, oviduct and distal uterine cornua removed. Under a dissection microscope (cf) the ovary and surrounding fatty tissue was removed. The organ was then weighed to the nearest 0.1mg, the ligatured cornua cut, to allow the trapped fluid to spill out, the tissue lightly blotted on filter paper, and reweighed. The difference between these two measurements is

therefore the amount of fluid collected in the ligatured section of uterus over the time period stated. For those animals killed on Day 4 of pregnancy, the fluid was collected in a cavity slide and examined for the presence of embryos. The mean % oviduct traversed was then calculated as described earlier (see General Methods, Section 2).

The amount of uterine fluid trapped by the ligature at the time of the operation was examined by sacrificing groups of five animals at 13:00h on each of Days 1, 2 and 3, immediately after a ligature had been tied 4-5mm proximal to the junctura of both uterine cornua, as described earlier. The amount of fluid was measured by weight difference as described above.

Results

The mean % oviduct traversed for each group of animals ligated on one side, using the contralateral side as a sham operated control, are shown in Table 3.1. Comparison of the mean % oviduct traversed in the real side vs the sham side by paired Student 't' shows that, for each experimental group, there is no significant difference between real and sham operated sides ($P>0.05$).

Therefore, for comparison of the mean % oviduct traversed in surgically operated animals and intact in vivo control animals, killed at the appropriate time, (results are taken from Chapter 1), the results for real and sham-operated animals have been combined and are shown in Table 3.2. Comparison of operated vs. intact control animals show significant retardation of tubal transport in each case (Day 2, $P<0.005$; Day 3, $P<0.05$; Day 4, $P<0.01$; using Student 't' test or Welch test).

The amount of fluid obtained from a ligature placed at 13:00h on Day 1, 2 or 3, immediately before sacrifice, are shown in Table 3.3. There is significantly more fluid in the ligated section of uterus on Day 1, compared to Day 2 or Day 3, by multiple 't' test ($P<0.01$ in each

case) whereas the amount of fluid on Days 2 is not significantly different from the Day 3 value (there is, however, a slight non-significant increase on Day 3).

Table 3.4 shows the mean values for the weight of fluid collected over the three 20 hour periods, in single ligature and double ligature preparations. In the single ligature experiment, significantly more fluid is found on Day 1 to 2 than on Day 2 to 3 or Day 3 to 4 ($P<0.01$ in each case) and the amount of fluid collected on Day 2 to 3 is not significantly different from the Day 3 to 4 result. In the double ligature experiment, significantly more fluid is found on Day 1 to 2, than on Day 2 to 3 or Day 3 to 4 ($P<0.05$, $P<0.01$, respectively) and the amount of fluid collected on Day 2 to 3 is significantly greater than on Day 3 to 4 ($P<0.05$).

Comparison of the single ligature results with the double ligature results by Student 't' test, shows no significant differences in the Day 2 to 3, and Day 3 to 4 groups, but significantly more fluid is found in the single ligature group on Day 1 to 2 ($P<0.05$).

Discussion

The results shown on Table 3.1 indicate that the mean % oviduct traversed in oviducts with ligated uterine cornua is not different from the value for the contralateral, sham-operated sides. Bishop (1956) found that double ligation and cannulation of the rabbit oviduct caused the lumen to balloon and become highly distorted, resulting in a turgid tube, with a flattened epithelial lining. He used this phenomenon to produce a measure of the pressure against which the oviduct would continue to secrete, but added that this would contain a contribution from the distensibility of the oviduct. It is obvious that, under those conditions, normal tubal transport of embryos would not be possible. The results in Table 3.1 show that this problem has not occurred in this experiment, and therefore the presence of ligature has

not affected embryo transport significantly. So, to determine the effect of the whole surgical procedure on tubal transport, real and sham-operated values have been combined, for comparison to intact, unoperated control values, using a Student 't' or Welch test. The results in Table 3.2 indicate that, in each group, the surgical procedure retards the movement of embryos significantly. It can be suggested that, from the results shown in Chapter 2, Section A, that surgical anaesthesia using pentobarbitone sodium, is sufficient, without surgery, to delay embryo transport over at least a 24 hour period, although a contribution from the surgery (handling, trauma, and tissue exposure to room temperature) is probably also present.

Examination of the data in Table 3.3 shows that there is significantly more fluid in the ligated section of uterine cornua on Day 1 of pregnancy than on either of days 2 or 3 (between which there is no significant difference when examined with a multiple 't' test). this pattern is borne out in Table 3.4. That is to say, that more fluid is found in the Day 1 to 2 groups, than the others.

The use of a double ligature separates a section of uterine cornua and examines its secretory capacity over the three time periods, free of any contribution from the oviduct. Bishop (1956) reported that double ligated uterine sacs did not accumulate fluid in rabbits, but comparison of the results in Table 3.4, with those of Table 3.3, between which there are an order of magnitude of difference, indicates that this does not hold for the mouse.

Significantly more fluid appears in the double ligated uteri on Day 1 to 2 than Days 2 to 3, and Day 3 and 4, and the Day 2 to 3 value is greater than the Day 3 to 4 value. This suggests that the capacity of the uterine endometrium to elaborate fluid is greater around Day 1, falling off toward Day 3, and further suggests that the measurements made here are not simply inflammatory exudate, which would be the same

in all groups. The increased amount of fluid trapped by a single ligature on the Day 1 to 2 group, compared to the appropriate double ligature group in Table 3.4, reflects this increased endometrial activity, but also shows a contribution of fluid from the oviduct to the uterus, which is not seen at either of the other time periods examined.

The slight but not significant increase in fluid found in the Day 3 to 4 single ligature group compared to the appropriate double group, and the non-significant increase in fluid found in utero on Day 3, shown in Table 3.3, compared to the Day 2 figure, suggest that there may be a slight oviductal contribution at this time, the magnitude of which is too small for this method to observe fully. The use of multiple comparison 't' tests in this way will tend to lose smaller changes as the number of comparisons increases, particularly if one group has a larger standard error, as in the case of Table 3.3.

Increases in oviductal secretory rates around the time of ovulation have been reported by several authors, usually using catheterisation techniques. In rabbits, Mastroianni and Wallach (1961) used such a technique for the continuous volumetric collection of oviductal fluid around ovulation and early gestation, using the contralateral oviduct/uterine horn as a control by which success of fertilisation and implantation could be monitored. They reported that secretory rates were high in oestrus (mean = 1.57ml/24 hours), falling on the second or third day following coitus (mean = 1.08 and 0.81ml/24 hours respectively), thereafter falling again to approximately 50% of the oestrus rate, and stabilising at that level. Riddick (1975) using a similar procedure, also found rabbit secretory rate to fall by three days after mating (from mean = 1.24ml/24 hours to 0.77ml/24 hours). Kendle and Telford (1970) reported similar findings using double ligated rabbit ampullae in artificially inseminated rabbits. Fluid

measured by weight difference on autopsy was converted to volume by measurement of density. Values were grouped into two-day groups, and over the first four days of pregnancy, ampullary secretion rates fell from 0.9ml/24 hours to 0.6ml/24 hours.

Thus the data presented here accords with previous workers, although the previous reports were unable to ascribe a direction of flow to the fluid. One of the problems of this system, however, as with others, is that a single determination over a period of some 20 hours is used to provide a figure for secretory rate, and, therefore, variations within this time period cannot be detected. Further, this measurement is begun immediately post-surgery, and the effect of surgery on secretory activity (marked on tubal embryo transport) cannot be determined. No analysis of collected fluid is possible because tubal and uterine fluids are mixed.

The purpose of this study was to determine if there existed an abovarian flow of fluid from oviduct to uterus, during, and in concert with, tubal embryo transport. Although the results cannot be considered a reflection of the physiological process, they are probably indicative of the trend i.e. that fluid passes from the oviduct to the uterus, particularly between Days 1 and 2 of pregnancy, a fact as yet unreported. It is known that the ampulla of the mouse is dilated on Day 1 of pregnancy (Humphrey, 1968A; Reinius, 1969), and full of fluid. This fluid disappears by Day 2 of pregnancy, and it seems likely that this may be the source of the fluid recovered in utero. Whether the role of this fluid is active or passive cannot be determined at this stage.

A further use of the data obtained in this study will be in two further studies in this work. The fact that an abovarian current exists in the oviduct between Days 1 and 2 of pregnancy will be used in Chapter 4, when the use of the bursa ovarica as a novel site of

injection is discussed, and in Chapter 7, where in vitro techniques investigating embryo transport uses cannulation and positive pressure fluid to simulate the secretory activity of the oviduct.

Table 3.1 The effect of uterine ligation at various times during the course of tubal embryo transport on the mean % of oviduct traversed, compared to the contralateral sham-operated control side.

Ligation	Sacrificed	Mean % oviduct traversed		Statistical Evaluation
		Real	Sham	
Day 1 13:00h	Day 2 09:00h	24.97 +/- 3.53 (10)	24.45 +/- 1.70 (10)	N.S.
Day 2 13:00h	Day 3 09:00h	66.77 +/- 7.29 (10)	71.60 +/- 5.63 (10)	N.S.
Day 3 13:00h	Day 4 09:00h	94.92 +/- 1.73 (10)	97.13 +/- 1.01 (10)	N.S.

Values are mean +/- SEM (n = No. of oviducts)

Table 3.2 The effect of the surgical procedure, combined uterine ligation with contralateral sham-operation, at various times during the time course of tubal embryo transport on the mean % oviduct traversed, compared to intact *in vivo* control animals.

Ligation (where appropriate)	Sacrificed	Mean % oviduct traversed		Statistical evaluation
		Operated	Control	
Day 1 13:00H	Day 2 09:00h	24.72 +/- 1.91 (20)	39.92 +/- 3.71 (10)	P<0.005
Day 2 13:00h	Day 3 09:00h	69.19 +/- 4.52 (20)	80.82 +/- 2.40 (20)	P<0.05
Day 3 13:00h	Day 4 09:00h	96.03 +/- 1.01 (20)	99.80 +/- 0.20 (10)	P<0.01

Values are mean +/- SEM (n = No. of oviducts)

Table 3.3 The weight of fluid (mg) trapped by bilateral single uterine ligatures, tied just prior to autopsy, at various times during the course of tubal embryo transport.

Animal sacrificed	Fluid accumulation
Day 1 13:00h	2.15 +/- 0.46 (10)
Day 2 13:00h	0.38 +/- 0.05 (10)*
Day 3 13:00h	0.62 +/- 0.08 (10)*

Figures are mean weight (mg) +/- SEM (n)

* Significantly different from the Day 1 value ($P<0.01$ in both cases) and not significantly different from each other.

Table 3.4 Comparison of the weight of fluid (mg) trapped by a single uterine ligature compared to double ligatured control uteri, and to similar measurements at different times during the course of tubal embryo transport.

Title	Ligature placed	Animal sacrificed	Ligature		Statistical evaluation
			Single	Double	
Day 1 to Day 2	Day 1 13:00h	Day 2 09:00h	12.74+/-2.16 (10)	7.74+/-1.55 (10)	P<0.05
Day 2 to Day 3	Day 2 13:00h	Day 3 09:00h	3.76+/-0.77[2] (10)	3.33+/-0.33[3] (10)	N.S.
Day 3 to Day 3	Day 3 13:00h	Day 4 09:00h	2.61+/-0.46[2] (10)	1.78+/-0.27[4] (10)	N.S.

Figures are mean weight (mg) +/- SEM (n)

[2]Significantly different from Day 1 to Day 2 single value (P<0.01) in each case but not significantly different from each other

[3]Significantly different from Day 1 to Day 2 double value (P<0.05)

[4]Significantly different from Day 1 to Day 2 double value (P<0.01) and significantly different from Day 2 to Day 3 double value (P<0.05)

Chapter Four - The use of the Bursa Ovarica as a novel site for drug administration, and the effects of Oestradiol administered by this route on Embryo Transport in vivo

Introduction

The presence of a peritoneal sac, covering the ovary and ostium of the oviduct has been mentioned in the General Introduction (The Anatomy of the Oviduct). Such a structure is found in the rat and mouse, but is not present in rabbit, man and other non-human primates. The functional significance of the bursa ovarica and its role in reproduction are as yet unclear.

The structure of the bursa ovarica of the mouse was described in some detail by Agduhr (1927). He reported that the bursa ovarica completely separates the tubal ostium from the peritoneal cavity, and that the structure of the tissue changes with the reproductive state of the animal, becoming thinner around ovulation, when the cavity is very full of fluid. Wimsatt and Waldo (1945) noted that, in the mouse, as is the case in the rat, a communicating channel between the periovarian and peritoneal spaces exists. They further showed that this channel permits the passage of Indian ink particles from the peritoneum to the periovarian space, and although this function is not related to the stage of the oestrus cycle, the amount of ink found is greater at those times when ova are present in the oviducts. Recovery of ink particles from the oviducts follows a similar pattern. Thus, soon after the particles of ink have entered the periovarian space, they are transported through the tubal ostium into the oviduct.

It is suggested, therefore, that the bursal sac could be used as a novel site for the injection of drug substances, in particular those drugs which might otherwise be rapidly inactivated by metabolism if given systemically. To establish the effectiveness of this method, the effect of the injection of 1ul of fluid into the bursal space is

reported, and the effect of various amounts of oestradiol in 1ul of fluid examined. Oestradiol has been chosen as the test drug, since its effects on the oviduct, although complex and dose and time related, are of long duration after single dose and are well documented (see General Introduction, The role of steroid hormones in the control of oviductal embryo transport; also, Chapter 2, Section B).

Materials and Methods

Anaesthesia and general preparation were as described in the General Methods (Section 3). The ovary, surrounded by the bursa ovarica, was exposed, and 1ul of fluid injected into the fluid filled space surrounding the ovary using a 10ul Hamilton syringe.

As controls, groups of five animals were injected with 1ul of SCMC/Tween vehicle between 09:30h and 11:00h on Day 1 of pregnancy and sacrificed at 12:00h on each of Days 2, 3 and 4. The mean % oviduct traversed in each case was compared to appropriate untreated controls.

In examination of the effects of oestradiol, each animal acted as its own control. A suspension of oestradiol was injected into one bursal space and the contralateral side treated similarly with vehicle. Doses of 0.02, 0.04, 0.08 and 0.2ug/animal were investigated, animals were sacrificed at 12:00h on Day 4 of pregnancy and the mean % oviduct traversed calculated as described previously. It is possible to examine for unilateral and bilateral effects by side to side and to vehicle treated control value comparisons. In those groups in which a treated to control (side to side) paired 't' test shows no significant difference, the values were combined and compared to vehicle treated control values by 't' test. In those groups with a significant side to side difference each side is compared to vehicle control values, using a Welch test.

One group of 5 animals were injected sub-cutaneusly with a suspension of 0.2ug oestradiol in 0.1ml SCMC/Tween vehicle at 09:30h on

Day 1 of pregnancy. The mean % oviduct traversed was calculated on autopsy at 12:00h on Day 4 of pregnancy, and compared to the appropriate intact in vivo control value, using a Welch test.

Results

The effect of the injection of 1ul of SCMC/Tween vehicle into the bursa ovarica on Day 1 of pregnancy, in comparison to intact control data (taken from Chapter 1) is shown in Table 4.1. Comparison using a Student 't' test indicates that the procedure does not significantly alter the mean % oviduct traversed at any of the three times examined.

The effects of varying doses of oestradiol injected intrabursally are compared with the contralateral vehicle injected side in Table 4.2, using a paired Student 't' test. At dose levels of 0.02, 0.04 and 0.08ug there is no significant difference between the drug treated and contralateral vehicle treated side. Therefore, in each group, drug treated and vehicle control sides have been combined and compared to the vehicle alone figure shown in Table 4.1 for Day 4. No significant difference is seen in each case.

When 0.2ug oestradiol is administered a significant difference ($P<0.005$) between drug treated and vehicle control sides is seen, in which the mean % for the drug treated side is significantly higher than the vehicle control side. Both drug treated and vehicle control values are significantly less than the vehicle only control from Table 4.1 ($P<0.05$ and $P<0.01$, respectively). Although the data is not shown, the mean number of embryos recovered in each group is not significanatly changed from normal intact animal values (see Chapter 1). The effect of SC administration of 0.2ug oestradiol is shown in Table 4.3 Compared to the intact, untreated control result, SC administration results in a significant retardation ($P<0.005$).

Discussion

Examination of the data shown in Table 4.1 shows that injection of

1ul of SCMC/Tween vehicle into each bursa ovarica early on Day 1 of pregnancy does not affect the mean % oviduct traversed by embryos at each of the three times examined, in comparison to the expected normal in vivo positions. It is of interest that there is no significant difference between operated and intact control animals examined on Day 2 of pregnancy, since the results presented in Chapter 2, Section A indicate that anaesthesia induced by pentobarbitone, without surgery, on Day 1 of pregnancy, at a time similar to that used for surgery in these experiments, caused a significant delay in embryo transport when examined on Day 2 of pregnancy, and this delay was not seen on Day 3 of pregnancy. This would suggest that the procedure used here has prevented this delay. It is therefore possible that injection of 1ul of fluid into the bursal sac has a slight accelerating effect on embryo transport. Another possible explanation is that the stress placed on the animal by the surgery has caused this slight acceleration, possibly by increased levels of circulting hormones, probably adrenal in origin (evidence for an immediate post-operative increase in circulating progesterone is presented in Chapter 5). It is also possible that the effect of pentobarbitone is seasonally variable, since the block of ovulation using pentobarbitone on pro-oestrus varies with the time of year, due to some unknown factors (Kendle, unpublished observations).

However, from the results in Table 4.1, it is possible to say that injection of 1ul of vehicle does not significantly alter the subsequent embryo transport pattern, and the technique could be of value for the investigation of labile drug substances, whose effects would be lost due to systemic metabolism, or whose systemic effects are too toxic.

Takahashi, Kogo and Aizawa (1979) administered indomethacin directly into the lumen of the uterus in doses much less than normally given systemically, and found a significant change in ovarian steroid content compared to vehicle treated controls. This, they postulated,

is evidence for a local effect of indomethacin at the level of the uterus. Thomas, Bastiaans and Rolland (1980) administered PGF_{2alpha} to guinea-pigs on Days 1 or 2 or pregnancy as either a sub-cutaneous or intra-peritoneal injection. The found that sub-cutaneous administration was without significant effect on embryo transport, while intra-peritoneal injections of identical doses of PGF_{2alpha} significantly increased the rate of embryo transport, particularly when administered on Day 2 of pregnancy, (which compared to vehicle treated control animals). Thomas et. al., suggested that the IP route of administration allowed direct contact between the PGF_{2alpha} and the peritoneal surface of both oviducts, resulting in higher tissue concentrations than would be achieved by administration using another route.

Administration of oestradiol into one bursal sac in doses of 0.02 to 0.08ug is without significant effect, although the magnitude of the standard error in some groups shows the spread of results to be quite wide, for both drug treated and vehicle control sides. When 0.2ug oestradiol is administered, a bilateral effect is seen, in which retardation is seen in both sides, although the degree of retardation is greater in the vehicle control side, than the oestradiol treated side. This shows that the oestradiol has acted systemically, or has passed from one bursa to the other, possibly via the peritoneal cavity, and has acted on both oviducts at a level sufficient to cause retention, without embryo loss. The effect of subcutaneous injection of 0.2ug oestradiol indicates that retention of embryos occurs with this low dosage (the effects of 2 and 4ug are reported in Chapter 2). The extent of retention is less than in those animals in which oestradiol was administered intrabursally, and occurs evenly in both sides of the reproductive tract, as evidenced by the relatively small SEM. This would suggest that potentiation of the oestradiol effects occur when it is administered by this route.

Greenwald (1967) reported that, in the rabbit, low doses of oestradiol given on Day 1 caused rapid expulsion of ova through the oviduct into the uterus, while higher doses cause retardation. It seems likely, however, that in the mouse, the converse is true, since Greenwald also reported that high doses of oestradiol (10-100ug) caused early loss of embryos in the mouse. Further, Humphrey (1968B) reported that repeated doses of oestradiol on Days 1-3 of pregnancy resulted in a combination of retention and acceleration. It is possible, then, that the results presented here indicate a retention in the vehicle control sides due to a lower level of oestradiol than on the drug treated side, which is exhibiting a mixed retention/acceleration due to higher, more prolonged oestradiol exposure. The potentiation of activity when given intrabursally, compared to subcutaneously, could be more fully explained by an investigation of the effects of intraperitoneal administration of a similar dose of oestradiol to intact and sham-operated animals (that is, animals in which the bursa ovarica is punctured under pentobarbitone anaesthesia, as is the case for intrabursal drug administration).

Despite the oestradiol effects reported here, it remains possible that intrabursal administration of drugs would be a useful technique for the investigation of labile drug substances, but the possibility of bilateral effects due to trans-peritoneal transfer must be borne in mind, and so the use of this technique for unilateral administration is probably limited.

Table 4.1. The effect on embryo transport of injection of 1ul of vehicle into each bursa ovarica early on Day 1 of pregnancy, compared to the normal *in vivo* positions on each of Days 2, 3 and 4 of pregnancy.

Animal sacrificed	Real operated	Intact * controls	Statistical evaluation
Day 2 12:00h	39.31 +/- 4.59 (10)	44.52 +/- 3.05 (10)	N.S.
Day 3 12:00h	86.86 +/- 1.07 (10)	86.66 +/- 1.38 (10)	N.S.
Day 4 12:00h	97.84 +/- 1.56 (10)	99.93 +/- 0.07 (10)	N.S.

Figures are mean % oviduct traversed +/- SEM (N)

* Data obtained from Chapter 1

Table 4.2. The effect on embryo transport of injection of various doses of oestradiol into one bursa ovarica at 09:30h on Day 1 of pregnancy.

Dose of oestradiol (ug)	Mean % on Day 4			Statistical evaluation
	Control	Treated	Combined	
0.02	91.50+/- 2.86 (10)	89.45+/-6.60 (10)	90.47+/-3.41 (10)	Control vs treated N.S. Combined vs vehicle * N.S.
0.04	87.24+/-12.24 (5)	96.62+/-1.98 (5)	91.93+/-6.28 (10)	Control vs treated N.S. Combined vs vehicle * N.S.
0.08	91.11+/- 5.49 (5)	98.47+/-1.04 (5)	95.79+/-2.78 (10)	Control vs treated N.S. Combined vs vehicle * N.S.
0.2	43.10+/- 7.47 (10)	76.05+/-8.14 (10)		Control vs treated P<0.005 Treated vs vehicle * P<0.05 Control vs vehicle * P<0.001

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts)

* Data obtained from Table 4.1, Day 1, real operated

97.84+/-1.56 (10)

Table 4.3. The effect on embryo transport of a sub-cutaneous injection of 0.2ug oestradiol early on Day 1 of pregnancy, compared to the normal *in vivo* position on Day 4 of pregnancy.

Animal sacrificed	Oestradiol treated	Intact control	Statistical analysis
12:00h Day 4	93.36+/-1.96 (10)	99.93+/-0.07* (20)	P<0.005

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts)

* Data obtained from Chapter 1

Chapter Five - The effect of Ovariectomy, Adrenalectomy or Ovariectomy and Adrenalectomy on Tubal Transport in vivo, and on Serum Progesterone levels

Introduction

Previous work by Kendle and Lee (1980) has shown that in the mouse, post-ovulatory ovariectomy has no significant effect on subsequent embryo transport, (confirming the study in the rat by Alden (1942B)). Kendle and Lee also showed that post-ovulatory administration of progesterone did not substantially affect embryo transport. However, administration of the anti-progestational steroid RMI 12,936, caused arrest of tubal transport, which could be reversed, after a latent period of 24 hours, by administration of progesterone. RMI 12,936 is an inhibitor of progesterone biosynthesis, which, in the rat, is metabolised to 7-alpha methyltestosterone, and thereafter to further unknown compounds, one of which probably antagonizes the effect of progesterone at the receptor level (Kendle, 1978).

These results suggest that tubal embryo transport requires the continued activity of progesterone, and in ovariectomized animals, this requirement could be met by progesterone of extra-gonadal origin. It has been suggested (Kendle and Lee, 1980) that the adrenal gland could be the source of this progesterone. This hypothesis is supported by the work of Forcellado, Morales, Vera, Quijada and Croxatto (1982), who examined embryo transport in rats, (ovariectomized, adrenalectomized or both adrenalectomized and ovariectomized), the effect of administration of progesterone and the subsequent serum progesterone levels was examined. They found that ovariectomy on Day 1 resulted in a loss of embryos when measured on Days 4 and 5, and a drop in serum progesterone from Day 2 onward, while ovariectomy and adrenalectomy resulted in a significant loss of embryos from Day 2, and was accompanied by lower serum levels of progesterone, and these early embryo losses were not

found in animals concurrently treated with progesterone.

Since treatment with RMI 12,936 in rats causes acceleration of embryo transport (Kendle 1975), the hypothesis that continued progesterone activity is required for normal tubal transport is worthy of examination, although the nature of progesterone effect seems species specific.

To test this hypothesis, the following series of experiments have investigated the role of adrenal secretion in embryo transport in the mouse by surgical removal of ovaries and/or adrenal glands, and administration of progesterone. Resulting changes in serum progesterone have also been measured.

Materials and Methods

Effects on embryo transport

All surgical procedures were carried out as described in the General Methods (Section 3, Surgical Procedures). 7 groups of 5 animals were examined for the effects on tubal transport of embryos. Group A were adrenalectomized, Group B ovariectomized and Group C ovariectomized and adrenalectomized. Groups D, E and F were appropriate sham-operated controls for groups A, B and C respectively. Group G were ovariectomized, adrenalectomized and treated with progesterone, 0.8mg daily at 09:30h subcutaneously in 0.1ml SCMC/Tween vehicle.

All surgical procedures were carried out between 09:30h and 11:00h on Day 1 of pregnancy. The animals were killed and the mean % oviduct traversed calculated at 12:00h on Day 4 of pregnancy, and the success of the surgical procedures confirmed. During the course of the experiments, all animals were maintained in a specially heated cabinet at 30+/-2^{o}C and all animals which had been adrenalectomized were given saline (0.9% w/v NaCl in water) in place of drinking water. Each group was compared to its sham-operated control using the Welch test.

Effects on serum progesterone levels

For the determination of serum progesterone levels, 17 groups of 2 animals were examined. All surgical procedures were carried out as detailed above and in the General Methods section. Blood samples were obtained at 12:00h on each of Days 1, 2, 3 and 4 of pregnancy by cardiac puncture (cf) just prior to sacrifice at which time the success of the procedures was confirmed. Ovariectomized, adrenalectomized, ovariectomized and adrenalectomized, and intact, untreated control animals were examined. Further, one group of 2 animals which had been ovariectomized and adrenalectomized and treated with progesterone as described above, were examined on Day 4 of pregnancy. Due to the small group size and large variation, no statistical evaluation is possible.

Results

Effects on embryo transport

The effects of adrenalectomy, ovariectomy or a combination of both procedures (groups A to C) are compared to the appropriate sham-operated control animals (groups D to F), and the effect of adrenalectomy and ovariectomy with daily progesterone administration (group G) are shown in Table 5.1.

Examination of the mean % oviduct traversed in each case shown that neither adrenalectomy or ovariectomy has a significant effect on embryo position, compared to appropriate controls using a Welch test, although the figures for ovariectomy and sham-ovariectomy are, in general, lower. However, combination of ovariectomy and adrenalectomy results in a mean % significantly less than the appropriate sham-operated control or adrenalectomized, ovariectomized animals treated with progesterone ($P<0.05$ in each case) while these two groups are not significantly different.

Effects on serum progesterone levels

Due to the limited numbers of animals/group and the range of values examinable by the commercially purchased RIA kit, no statistical analysis of the results, presented in Table 5.2, can be made. However, the following observations are possible.

In intact, untreated control animals serum progesterone levels are below 1.25ng/ml on Day 1, around 15-20ng/ml on Day 2, and above 50ng/ml on each of Days 3 and 4.

A similar pattern is seen in animals adrenalectomized on Day 1, although absolute levels are different, (in 1 animal 5.4ng/ml was found on Day 1, and in another 16.4ng/ml on Day 3).

In ovariectomized animals, levels on Day 1 and 2 were >50ng/ml, falling on Day 3 to around 10ng/ml, and on Day 4 to between 1-10ng/ml.

In adrenalectomized and ovariectomized animals, levels on Day 1 are below 1.25ng/ml, and range from 3-12ng/ml on Days 2 to 4.

Adrenalectomized, ovariectomized animals, treated daily with 0.8mg progesterone were only examined on Day 4 at which time serum levels were >50ng/ml.

Discussion

The findings presented here confirm in part the study of Kendle et. al., (1980), in that post-ovulatory ovariectomy shows no more effect on mean % oviduct traversed than does a sham-operated control. However, the large SEM associated with both real and sham-operations indicates a wide spread of results, not seen in the previous study. It is possible that the earlier time of ovariectomy in this study (09:30-11:00h) compared to that of Kendle and Lee (12:00-14:00h) may adversely affect subsequent embryo position in some animals, since previous work (Kendle, unpublished observations) indicated that early surgery resulted in some loss of embryos and disturbance of transport which was not seen in later surgery, confirming the results of Wu, Dickman and

Johnson (1971). Although loss of embryos was not seen in this study, rupture of the bursa ovarica and handling of the ovary early on Day 1 could explain the spread of results in the ovariectomy/sham ovariectomy groups. However, in the sham ovariectomy and adrenalectomy group, no such spread is seen, although this procedure contains all the steps involved in sham ovariectomy, but includes handling of the adrenal. It is possible that this component may influence subsequent tubal events, and so modify the effects induced by sham ovariectomy. It remains possible, however, that the effects of sham ovariectomy are simply the result of between animal variation, and a more accurate reflection of the effect of the sham procedure would be a combination of both the sham ovariectomy and sham ovariectomy and adrenalectomy, both of which involve rupture of the bursa ovarica, and exposure of the ovary and oviduct to the external environment.

Previous work on the effects of post-ovulatory ovariectomy on subsequent tubal embryo transport has been conflicting. Alden (1942B) found that the presence of the ovary was not necessary for normal tubal transport. However, his conclusions were based on a relatively small number of animals (11 in total, of which 2 were not pregnant) which were ovariectomized between 7 and 20 hours after finding a vaginal plug, and the results were not compared to sham-operated or intact control animals, but were based on the position and developmental state of embryos recovered. Also, embryo numbers were not reported. However, in his conclusions, Alden stated that if any ovarian secretion were necessary for tubal transpot and development, it could be present in sufficient quantity in some other organ.

Wu, Dickman and Johnson (1971) reported that ovariectomy in rats between 09:30 and 10:30h on Day 1 of pregnancy resulted in some 30% of embryos recovered retained within the oviduct, when examined on Day 5

of pregnancy. However, they compared these figures only with intact control values, and therefore the effect of the surgical procedure was not accounted for. Further, they reported a mean recovery in intact animals of 11.3+/-0.54 embryos/animal compared to 4.2+/-0.95 for ovariectomized animals, indicating a loss of embryos for which no explanation is given. Indeed, in 4 of 16 animals ovariectomized, no embryos were recovered.

In the mouse, Roblero and Garavagno (1979) found that ovariectomy in mice, carried out between 10:00 and 12:00h on Day 2 of pregnancy resulted in retention of a proportion of embryos within the oviduct when examined on Day 4 of pregnancy, although this was in comparison to intact control animals. Treatment with progesterone or progesterone and oestradiol resulted in a more normal pattern, while oestradiol alone, (0.6 or 6.0ng/day on Days 2, 3 and 4) resulted in some embryo loss. The authors concluded that the results showed retention of embryos, analagous to that of Wu et. al., (1971), and this effect could be reversed by progesterone, or, synergistically, with oestradiol and progesterone. Although they stated in their conclusions that laparotomy on Day 2 of pregnancy had no effect on tubal transport, no data was provided, and the nature of the laparotomy (i.e. handling of ovaries, or opening of ovarian bursa, etc.) was not described.

The most detailed study of the effect of post-ovulatory ovariectomy on subsequent tubal transport in the rat is that of Forcellado, Morales, Vera, Quijada and Croxatto (1982), who examined the effect of ovariectomy on each of Days 1, 2, 3 or 4 of pregnancy on embryo position (oviductal or uterine), on each of the days following surgery up to Day 5 of pregnancy and compared these values to sham-operated control animals. They showed that sham-ovariectomy, including opening of the bursa ovarica, had no effect on tubal transport when compared to intact controls, and ovariectomy resulted in

premature loss of embryos, becoming significant within 48 hours after surgery. In a further experiment they examined tubal transport in animals adrenalectomized and/or ovariectomized on Day 1 of pregnancy, and found a loss of embryos measured on Days 4 and 5 in ovariectomized and ovariectomized and adrenalectomized animals, but not in adrenalectomized only animals, and that this loss could be prevented by a progesterone implant at the time of surgery. At the same time, serum progesterone levels were reported, and found to be much lower in the ovariectomized, and ovariectomized and andrenalectomized groups than all other groups, throughout the five day period, although progesterone remained detectable throughout. This allowed the authors to conclude that post-coital secretion of progesterone was necessary for normal tubal transport in the rat, without which acceleration and premature loss of most embryos occurred, (as was probably the case for Wu et al., 1971).

The results presented in this study are in general agreement with the conclusions reached by Forcellado et. al., in that ovariectomy and adrenalectomy on Day 1 in the mouse disrupts subsequent embryo transport, and this disruption can be prevented by the administration of progesterone. In the case of the mouse, this distruption is shown as oviductal embryo retention, as reported by Kendle and Lee (1980) to be the effect induced by anti-progestational steroids. The serum levels of progesterone shown here indicate that the ovary is the major source of progesterone in the pre-implantation stage of pregnancy in the mouse, since adrenalectomy does not markedly affect subsequent levels, while ovariectomy and adrenalectomy results in low levels throughout this phase, although measureable serum levels are still seen on Day 4 (confirming the results of Forcellado et al., (1982)). Ovariectomy, on the other hand, shows an increase in serum progesterone on Days 1 and 2 compared to intact controls which lowers over Days 3

and 4 to levels similar to that of the ovariectomized and adrenalectomized animals. Since this increase does not appear in the other operated groups, the adrenal gland seems to be the likely source. Feder, Resko and Goy (1968) found that progesterone was present in rat plasma in ovariectomized animals for at least 25 days after surgery, but reported that in ovariectomized and adrenalectomized animals, progesterone disappeared from the plasma within 8 hours. Surgery was carried out under ether anaesthesia on Day 2 of the oestrous cycle and progesterone measured by gas chromatography measuring down to 2ng/ml using electron capture detection. Resko (1968) found that progesterone secretion by the adrenal of the rat could be suppressed by dexamethasone and increased by exogenous ACTH, and suggested therefore that adrenal progesterone secretion was under the control of ACTH, and was elevated in times of stress. This confirms the observation of elevated serum progesterone following ovariectomy in this study, pointing to surgical stress as the likely causitive agent. It is possible then that any true effect of ovariectomy on tubal transport is masked by the stress-induced increase in serum progesterone following surgery, which combined ovariectomy and adrenalectomy removes. The presence of progesterone in the serum for at least 4 days following surgery is as yet unexplained. Since the major route of steroid inactivation is probably hepatic, a decrease to a level below detection relatively rapidly, as was the case for Feder et. al., (1968) would be expected. However, it is possible that tissue levels are high enough to allow a low serum level to persist for some days. Alternatively, it is possible that any adrenal tissue remaining within the body is activiated by ACTH to produce progesterone or some other substance which will cross react in the radio-immunoassay system (see General Methods, Section 4), since, at least in man, besides the main adrenal

gland, there may exist some small islets of adrenal tissue containing both cortex and medulla and probably capable of hyperplasia in the absence of the main gland. Indeed, Shaikh and Shaikh (1975) reported that oestradiol levels in the peripheral plasma of ovariectomized and adrenalectomized rats were less than 10pg/ml when animals were sacrificed within 4 days of surgery, but were 25pg/ml when sacrificed after one week after surgery.

Since progesterone administered after ovariectomy and adrenalectomy can reverse the effect of the surgery alone, this indicates that continued progesterone activity at a level higher than in the ovariectomized and adrenalectomized animals is required for normal tubal embryo transport, which is supplied by the adrenal gland in ovariectomized animals, and absence of which, in the mouse, causes retardation of embryo transport. The nature of this progesterone dependent control is as yet unclear.

Table 5.1. The effect of ovariectomy, adrenalectomy or ovariectomy and adrenalectomy on tubal transport of embryos, in comparison to appropriate sham-operated controls.

Surgical procedure	Group	Real	Group	Sham	Statistical evaluation
Adrenalectomy	A	91.37+/-5.14 (10)	D	98.67+/-0.94 (10)	AvsD N.S.
Ovariectomy	B	88.18+/-3.56 (10)	E	89.23+/-2.72 (10)	BvsE N.S.
Ovariectomy/ Adrenalectomy	C	83.60+/-5.65 (10)	F	98.41+/-1.04 (10)	AvsB P<0.05 AvsC P<0.05 BvsC N.S.
Ovariectomy/ Adrenalectomy with Progesterone	G	99.07+/-0.93 (10)			

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts)

Table 5.2. Serum progesterone levels on Days 1 to 4 of pregnancy in intact, adrenalectomized, ovariectomized and adrenalectomized/ovariectomized animals.

Procedure	Day of pregnancy 1	2	3	4
Intact	<1.25	15.93	>50	>50
	<1.25	18.60	>50	>50
Adrenalectomized	<1.25	10.77	>50	>50
	5.40	10.77	16.4	>50
Ovariectomized	>50	>50	8.57	8.11
	>50	>50	12.26	2.10
Adrenalectomized/	<1.25	2.94	4.24	11.90
Ovariectomized	<1.25	11.30	3.35	5.05
Adrenalectomized/				
Ovariectomized/	—	—	—	>50
Progesterone treated	—	—	—	>50

Figures are ng/ml progesterone in serum. Each group is shown as individual animal results.

Chapter Six - The effects of Testosterone and 5-alpha Dihydrotestosterone on Tubal Transport in vivo, and the modification of these effects by Progesterone and 1,4, 6-Androstatrien-3,17-dione

Introduction

Studies of the activity and mode of action of the steroid RMI 12,936 have suggested that the compound is anti-progestational. When RMI 12,936 is administered at any time during tubal transport of embryos in the mouse, transport is arrested, and this arrest can be reversed by concurrent administration of progesterone (Kendle and Lee, 1980). However, the compound has been reported to possess weak oestrogenic and androgenic activities (for a review, see Kendle, 1979). In the mouse, Bullock, Feil, Gupta, Demers and Bardin (1978) compared the androgenicity of RMI 12,936 with the standard compound, testosterone, using a battery of *in vivo* and *in vitro* test procedures, and concluded that RMI 12,936 possessed androgenic activity similar in potency to that of testosterone, but might also demonstrate oestrogenic activity *in vivo*.

Burdick, Emerson and Whitney (1940) reported that testosterone propionate could exert a retentive effect on tubal transport in the mouse. In the light of these studies, it can be suggested that RMI 12,936 could exert its effects via the androgen receptor, and, in this case, progesterone would act as an anti-androgen, which has been shown to occur in other systems (Giacomini and Wright, 1979).

In this study, the effects of the androgens testosterone propionate and 5-alpha dihydrotestosterone on tubal embryo transport were investigated, and the nature of the testosterone effect examined.

Materials, Methods and Results

Eight separate experiments are reported. To clarify presentation and prevent repetition for each experiment, the methods and results are reported together, although a combined discussion is presented in the subsequent section.

Experiment 1 - Comparison of the androgenic effects of testosterone propionate and the anti-progestational steroid RMI 12,396

Four groups of male weanling mice were orchidectomized as detailed in the General Methods (Section 3, Surgical Procedures). Each group was treated with either RMI 12,936 or testosterone propionate (TP), 0.1mg or 0.3mg as a daily sub-cutaneous injection in 0.1ml SCMC/Tween vehicle (cf), for ten days. On the morning of the eleventh day, all the animals were sacrificed. The seminal vesicles were removed and weighed to the nearest 0.1mg. The group mean weights were compared by 2 + 2 parallel line assay, with accompanying Analysis of Variance, and the potency ratio of RMI 12,936 to TP calculated.

The results and statistical analyses are shown in Table 6.1. All four groups showed an increase in seminal vesicle weight, (control data not shown), which was dose related. The Analysis of Variance table indicates that the assay is valid, since there is significant linear regression and no significant deviation from parallelism. The potency ratio of RMI 12,936 to TP in this assay is 1.71, with fiducial limits (95%) of 1.02 and 3.39.

Experiment 2 - The effects of daily administration of 5-alpha dihydrotestosterone on tubal embryo transport

Six groups of five mice were examined. Two dosages of 5-alpha dihydrotestosterone (DHT) were selected, 0.6mg/animal/day and 1.2mg/animal/day. Groups were given the chosen dose as a sub-cutaneous injection in 0.1ml SCMC/Tween (cf) at 09:30h on Days 1 and 2 and sacrificed on Day 2, on each of Days 1, 2 and 3 and sacrificed on Day 3, or on each of Days 1, 2, 3 and 4 and sacrificed on Day 4. All autopsies were carried out at 12:00h, and the mean % oviduct traversed was calculated as described earlier and compared to vehicle treated controls using a multiple 't' test or Welch test.

The results are shown in Table 6.2. When compared to vehicle

treated controls, no statistically significant difference is seen at either dose level on Day 2 or Day 4. On Day 3, the 0.6mg DHT result is significantly less than either the control value or the 1.2mg DHT group ($P<0.05$, in each case). There is also a non-significant decrease in the Day 4 1.2mg DHT value.

Experiment 3 - The effect of daily administration of 2.5mg 1, 4, 6-androstatrien-3, 17-dione (ATD) on tubal embryo transport

Three groups of five animals were examined. Each group received a daily sub-cutaneous injection of 2.5mg ATD in 0.1ml SCMC/Tween (cf) at 09:30h. One group was treated on Days 1 and 2 and sacrificed on Day 2, a second group treated on Days 1, 2 and 3 and sacrificed on Day 3 and the third group treated on Days, 1, 2, 3 and 4 and sacrificed on Day 4. All animals were sacrificed at 12:00h, and the mean % oviduct traversed was calculated and compared to the vehicle treated controls using the Welch test.

The results are shown in Table 6.3. There is no significant difference in mean % oviduct traversed when comparing ATD treated to vehicle treated controls on either Day 2 or Day 4. However, examination on Day 3 shows a slight but significant decrease in mean % in the ATD treated group ($P<0.05$).

Experiment 4 - The effect of daily administration of testosterone propionate on tubal embryo transport, and the modification of these effects by concurrent administration of progesterone or 1,4,6-androstatrien-3, 17-dione, or by removal of ovaries and adrenal glands

Groups of five animals were examined throughout. One group was treated with a sub-cutaneous injection of 1mg testosterone proprionate (TP) in 0.1ml SCMC/Tween at 09:30h on each of Days 1, 2, 3 and 4 of pregnancy and sacrificed at 12:00h on Day 4 when the mean % oviduct traversed was calculated. A second group was treated similarly with 5mg TP daily. These were compared to vehicle treated controls using

the Welch test. Two further groups were treated as above, but were also treated concurrently with a daily sub-cutaneous injection of 0.8mg progesterone (P) in 0.1ml SCMC/Tween. These were compared to each other, and with TP alone groups using the Welch test.

A further group was treated as above with 1mg TP daily, and was also treated with daily sub-cutaneous injections of 2.5mg ATD in 0.1ml SCMC/Tween, and was compared to TP treatment alone, to vehicle treatment alone and ATD alone (from Experiment 3) using the Welch test.

Lastly, two groups were treated with 1 and 5mg TP as above but were also ovariectomized and adrenalectomized between 09:30h and 10:00h on Day 1 of pregnancy, and the mean % measured was compared to ovariectomized and adrenalectomized animals (data from Chapter 5), using the Welch test.

The results are shown in Table 6.4. Both 1 and 5mg TP administered daily resulted in a significant retardation of embryo transport ($P<0.01$ in each case and no significant difference between 1 and 5mg). Concurrent administration of progesterone reverses the effect of 1mg TP (not significantly different from vehicle control, significantly greater than TP alone, $P<0.01$), whereas concurrent administration of P does not reverse the effect of 5mg (significantly less than vehicle control, $P<0.05$; significantly less than 1mg TP + P, $P<0.05$; not significantly different from 5mg TP alone). Concurrent administration of ATD reverses the effect of 1mg TP daily (not significantly different from vehicle or ATD alone; significantly greater than 1mg TP along, $P<0.01$). Administration of 1 or 5mg TP to animals ovariectomized and adrenalectomized (ovax/adrx) on Day 1 of pregnancy, results in mean %'s not significantly different from animals operated but not drug treated, and not significantly different from each other. Treatment of ovax/adrx animals with 1mg TP daily resulted in a mean % not significantly different from the effect of 1mg TP in

unoperated animals, while treatment of ovax/adrx animals with 5mg TP daily resulted in a mean % significantly greater than animals treated with 5mg TP along ($P<0.05$).

Experiment 5 - Comparison of the effects of a single dose of testosterone propionate or 5-alpha dihydrotestosterone given early on Day 1 of pregnancy on tubal embryo position measured on Day 4 of pregnancy

Three groups of five animals were examined. One group was given a sub-cutaneous injection of 1mg TP in 0.1ml SCMC/Tween at 09:30h on Day 1 of pregnancy. The second group was treated similarly with 5mg TP, and the third with 1.2mg DHT. All animals were sacrificed at 12:00h on Day 4 of pregnancy and the mean % oviduct traversed calculated and compared to the normal in vivo position of embryos at this time (data from Chapter 1), using the Welch test.

The results are shown in Table 6.5. Comparison of the effects of 1mg and 5mg TP and 1.2mg DHT with untreated controls shows no significant difference in mean % for any group. A slight but not significant decrease was seen in the group treated with 5mg TP. Some embryos were found in the ampullae of two oviducts in this group (data not shown).

Experiment 6 - The effect of daily administration of 1mg testosterone propionate from Day 2 of pregnancy onward

Two groups of five animals were examined. The first group were treated with a sub-cutaneous injection of 1mg TP in 0.1ml SCMC/Tween at 09:30h on each of Days 2 and 3 of pregnancy and were sacrificed at 12:00h on Day 3. The second group were treated similarly on each of Days 2, 3 and 4 and sacrificed at 12:00h on Day 4. On autopsy, the mean % oviduct traversed was calculated and compared to the normal in vivo position by Students 't' test.

The results are shown in Table 6.6. The effect of administration of 1mg TP on each of Days 2 and 3 is to produce a slight but significant retardation ($P<0.05$), as is also the case for administration on each of Days 2, 3 and 4 ($P<0.05$).

Experiment 7 - The effect of 1mg testosterone propionate given on each of Days 3 and 4 of pregnancy, and its modification by concurrent administration of progesterone or 1,4,6-androstatrien-3,17-dione

Three groups of five animals were examined. Each group was treated with a sub-cutaneous injection of 1mg TP in 0.1ml SCMC/Tween at 09:30h on Days 3 and 4 of pregnancy. One group received no other treatment. The second received concurrent administration of 0.8mg progesterone in a similar form, and the third received 2.5mg ATD instead of progesterone. All animals were sacrificed at 12:00h on Day 4, the mean % oviduct traversed calculated, and compared to intact control animals and to each other, using the Welch test.

The results are shown in Table 6.7. The effect of administration of 1mg TP on each of Days 3 and 4 is to significantly retard transport, in comparison to intact control animals, or to animals treated with TP + P or TP + ATD ($P<0.001$, in each case), while administration of TP + P results in a mean % not significantly different from the intact control group. Administration of TP + ATD results in a mean % significantly lower than control animals, ($P<0.05$) but not significantly different from the TP + P treated group.

Experiment 8 - The cross-reactivity of 1,4,6-androstatrienal-3,17-dione for the progesterone anti-serum from the (^{3}H) progesterone radioimmundassay kit

Using the radio-immunoassay kit used in Chapter 5 (see General Methods, Section 5) the cross-reactivity of the aromatization inhibitor ATD was determined by comparing the % binding of a series of standard solutions of known concentrations of progesterone and of ATD. In each

case, the concentration producing 50% binding was found and the cross-reactivity determined by 100X (concentration of progesterone producing 50% binding/(concentration of ATD producing 50% binding). This was compared to the cross-reactivities stated by the manufacturer. The range of progesterone standard used was 0.05-2.0ng/ml and ATD standards 0.01-10.0ug/ml.

The concentration of progesterone producting 50% binding was 0.152ng/ml. The concentration of ATD producing 50% binding is 0.41ug/ml. This produces a cross-reactivity of 1,4,6-androstatrien-3,17-dione for the progesterone antiserum of 0.04%. The stated value for cross-reactivity of testosterone in 0.09% and for androstendione 0.07% (for others, see General Methods, Section 4, Serum progesterone determination).

Discussion

Daily administration of TP from Day 1 of pregnancy has been shown in this study to delay the passage of embryos along the oviduct, confirming the finding of Burdick, Emerson and Whitney (1940). Burdick et al., however, proposed that this activity was anti-oestrogenic in nature, and considered TP to be acting as a progestogen. The finding that RMI 12,936 is as potent as TP as an androgen in the male castrate study, coupled with the activity of RMI 12,936 on tubal embryo transport in mice, causing an arrest (Kendle and Lee, 1980) suggests that this activity might be due to androgenic action. It is possible that the reversal of the TP delay by concurrent administration of progesterone is due to the anti-androgenic activity of progesterone, since progesterone is known to inhibit 5alpha-reduction of testosterone to 5alpha-dihydrotestosterone (DHT) (Giacomini and Wright, 1980), and DHT is considered to be the active species acting at the level of the androgen receptor (reviewed for the rat prostate by Tenniswood, Bird and Clark, 1982). However, the finding of neglibible activity of DHT

on the mouse oviduct, in doses calculated to have similar androgenic potency to the doses of TP employed (data not shown) indicates that either DHT is not the active species at any androgen receptor in the oviduct or that the activity of TP is not mediated via the androgen receptor. A physiological role for androgen receptor mediated activity in tubal transport can be argued against by the finding that daily administration of cyproterone acetate, an antiandrogen, from Day 1 of pregnancy, in the mouse, did not affect the number of implanted foetuses measured on Day 21 (Ellendorff, Rover and Schmidt, 1973). These results then suggest that the activity of TP is not mediated via an androgen receptor system in the oviduct.

Oestradiol is known to delay the passage of embryos along the oviduct when administered on Day 1 of pregnancy in the mouse (Humphrey, 1968A; Greenwald, 1967; this study, Chapter, 2 Chapter 4). Testosterone is a biological precursor of oestradiol in vivo, and it is possible that the activity of TP is manifestation of the conversion of sufficient testosterone to oestradiol to produce "tube-locking" when administered from Day 1 of pregnancy. This hypothesis can be examined in several ways. 1,4,6-androstatrien-3,17-dione (ATD) is an inhibitor of the aromatization enzyme system required for the production of oestradiol from testosterone. While administration of ATD alone shows only a slight delaying in tubal transport, seen only on Day 3, and probably a reflection of the high value of the whicle treated control and the size of the inherent error (see Chapter 1), administration of TP and ATD concurrently shows that retardation does not occur, reinforcing the hypothesis that TP is active by virtue of metabolic transformation to oestradiol, and subsequent activation of oestrogen receptors. If this is the case, removal of the ovaries and adrenals, the sources of oestradiol production should also inhibit the activity of TP. The results from this experiment, however, are inconclusive,

since administration of 1mg TP to operated animals results in a mean % measured on Day 4 not significantly different from untreated operated animals and also not significantly different from TP (1mg daily) treated animals. In the case of daily treatment with 5mg TP, treated operated animals showed no significant difference from untreated operated animals but was significantly greater than TP (5mg daily) treated animals ($P<0.05$). However, these groups are not strictly comparable, since the effects of surgery on the actions of TP are not accounted for. The fact that treatment with TP (1 or 5mg daily from Day 1) does not alter the retardation of tubal transport due to ovariectomy and adrenalectomy suggests that the full activity of TP requires the activity of either the ovaries or adrenal glands. This is confirmatory to the hypothesis that TP activity is the manifestation of biotransformation to oestradiol. However, several other avenues of investigation argue against this. Firstly, concurrent administration of progesterone with TP (1mg daily) reverses the delaying effect, (although this is not the case with 5mg TP daily), suggesting dose dependent reversal, due perhaps to competitive inhibition at same receptor. Humphrey (1968B) found that the "tube-locking" effect of oestradiol could not be reversed by the concurrent administration of progesterone (P). It is possible that P administration somehow prevents the biotransformation of TP to oestradiol, possibly by saturation of the enzyme systems involved, although this seems unlikely, since progesterone itself is a biological precursor of oestradiol, after biotransformation to testosterone.

Secondly, administration of a single dose of TP (1 or 5mg) early on Day 1 of pregnancy (Table 6.5) does not produce the long lasting retardation typical of oestradiol. If the hypothesis is correct, sufficient of the TP administered daily must be converted to oestradiol before the embryos enter the isthmus, since administration of

oestradiol after this time results in acceleration and early loss of embryos (for a review, see General Introduction, The role of steroid hormones in the control of oviductal embryo transport; Chapter 2, Chapter 4). Therefore, if sufficient of the first dose of daily administration is converted to sufficient oestradiol to retain the embryos, at least until administration of the second dose, this retention should be seen as a delay on Day 4, after a single Day 1 administration. This does not occur, although a non-significant retention is seen with a single administration of 5mg on Day 1. However, more conclusive evidence that the effects of TP are not manifest via biotransformation are shown by administration of TP after the embryos have entered the isthmus, from Day 2 onwards (Table 6.6) which shows retardation in comparison to intact control animals. Although it is possible that the retardation would not be seen if comparisons were made to vehicle treated controls, it is important to note that acceleration and early loss of embryos is not seen, which is the result reported for oestradiol administration at a similar time (Lee, 1979; Humphrey, 1968B). More importantly, the retardation seen if 1mg TP is given on each of Days 3 and 4 (Table 6.7) is not typical of oestradiol activity. It is reversed by concurrent administration of progesterone, and partly reversed by concurrent administration of ATD.

In the light of these results, it can be hypothesised that the activity of TP is anti-progestational in nature. Kendle and Lee (1980) proposed that progesterone activity initiated and continued activity was required for normal embryo transport in the mouse, as evidenced by the arrest of transport induced by the anti-progestational steroid RMI 12,936, an effect which could be reversed after a 24 hour lag time by concurrent administration of progesterone. It was suggested that RMI 12,936 was active by two mechanisms, the parent

compound inhibiting the synthesis of progesterone, and a metabolite acting as a competitive inhibitor at the level of the receptor. R2323, also considered to be a competitive progesterone receptor inhibitor, showed similar results, but showed a delay rather than arrest, (which was not reversed by the dose of progesterone employed, but the effect of R2323 was only apparent for 24 hours, and the reversal of the RMI 12,936 effect by progesterone occurred after 24 hours indicating that progesterone required 24 hours to manifest its effects). The hypothesis that TP is acting as an anti-progestational agent is supported by the reversal of its delaying activity when 1mg is given concurrently with 0.8mg progesterone daily from Day 1 to Day 4 and Days 3 and 4. Further the delay in transport seen when TP is administered after the embryos have entered the isthmus is similar to that found with R2323 or RMI 12,936, but much less profound, and the opposite effect can be seen after oestradiol administration. (This suggests that the block to embryo transport is manifest mainly at the level of the AIJ and UTJ). However, administration of 1mg TP daily from Day 1 to Day 4, can be reversed and the effect of 1mg TP on each of Days 3 and 4 partly reversed by concurrent administration of 2.5mg ATD. ATD is known to inhibit the biotransformation of testosterone to oestradiol (Christensen and Clements, 1975) but does not affect the uterotrophic effect of RMI 12,936 in immature rats (Kendle, 1978), which Kendle suggested was evidence to suggest the active metabolite of RMI 12,936 was not oestrogenic. If similar criteria are applied to these data, it would be suggestive of an oestrogenic activity, not anti-progestational. However, the cross-reactivity of ATD for the progesterone anti-serum from the radio-immunoassay kit used in Chapter 5 (cf) is of a similar order to that quoted for testosterone. An anti-serum is raised to be as specific for a compound as possible and is not a true reflection of receptor specificity, but cross-reactivity

does show the similarities in structure and affinities of the compounds, (but cannot, however, distinguish between agonists and antagonists). From the results it can be concluded that it is possible that ATD is also progestational in activity, although further testing for *in vivo* biological activity is necessary to confirm this hypothesis. The limited supplies of ATD have precluded this. If it is the case that ATD is a progestational agent, this would account for the results reported, and support the hypothesis that that continued progesterone activity is required for normal tubal transport.

Table 6.1 2 + 2 assay comparing the androgenicity of RMI 12,936 with testosterone propionate on seminal vesicle weight in castrate male weanling mice.

	Seminal Vesicle weight	
Daily dose of steroid	Testosterone propionate	RMI 12,936
0.1mg	45.3+/-2.41 (5)	62.9+/- 7.10 (5)
0.3mg	85.5+/-5.18 (4)	112.8+/-16.82 (4)

Figures are mean weight (mg) +/- SEM (n = No. of animals)

Analysis of Variance

Source	DF	SS	MS	F	
Prep	1	2158.3	2158.3	6.24	P<0.05
Reg	1	9025.0	9025.0	26.11	P<0.01
Para	1	104.0	104.0	0.36	N.S.
Dose	3	11287.3			
Error	14	4840.0	345.7		
Total	17	16127.3			

Potency of RMI 12,936 vs Testosterone propionate = 1.71 with fiducial limits 1.02-3.39

Table 6.2 The effect of daily doses of 5 alpha-dihydrotestosterone on mean % oviduct traversed measured on Days 2, 3 and 4 of pregnancy, compared to vehicle treated controls.

Sacrificed	Daily steroid dose (mg) 0.0 (A)	0.6 (B)	1.2 (C)	Statistical evaluation
12:00h Day Two	48.76+/-5.54 (10)	44.83+/-5.54 (10)	49.25+/-5.69 (10)	A vs B N.S. A vs C N.S. B vs C N.S.
12:00h Day Three	90.57+/-0.49 (10)	81.91+/-3.49 (10)	92.45+/-2.64 (10)	A vs B P<0.05 A vs C N.S. B vs C P<0.05
12:00h Day Four	97.98+/-1.04 (10)	97.18+/-1.14 (10)	89.77+/-8.04 (10)	A vs B N.S. A vs C N.S. B vs C N.S.

Figures are mean % oviduct traversed +/- SEM (n = No. ov oviducts)

Table 6.3 - The effect of daily injections of 2.5mg 1,4,6 Androstatrien-3,17-dione (ATD) on the mean % oviduct traversed, measured on Days 2, 3 and 4 of pregnancy, compared to vehicle controls.

Sacrificed	Control	ATD	Statistical evaluation
12:00h Day Two	48.76+/-5.54 (10)	48.89+/-8.03 (10)	N.S.
12:00h Day Three	90.57+/-0.49 (10)	87.32+/-1.21 (10)	P<0.05
12:00h Day Four	97.98+/-1.04 (10)	100+/-0.0 (10)	N.S.

Table 6.4 The effects of daily administration of testosterone propionate (TP) on mean % oviduct traversed measured on Day 4 of pregnancy, and the modification of these effects using progesterone (P),1,4,6-androstatrien-3,17-dione (ATD), or in animals ovariectomized and adrenalectomized on Day 1 of pregnancy (ovax/adrx).

	Dose of TP (mg/day)			Statistical evaluation
	0 (A)	1 (B)	5 (C)	
TP alone (D)	97.98+/-1.04* (10)	56.02+/-9.50 (10)	48.75+/-10.70 (10)	AvsB P<0.001 AvsC P<0.01 BvsC N.S.
TP + P (E)	———	94.27+/-3.00	61.22+/-12.10	BvsC P<0.05
TP + ATD (F)	100+/-0.0 * (10)	97.67+/-1.01 (10)	———	AvsB N.S.
TP in ovax/adrx (G)	83.60+/-5.65* (10)	69.87+/-11.02 (10)	77.88+/-7.53 (10)	AvsB N.S. AvsC N.S. BvsC N.S.
		D vs E P<0.01 D vs F P<0.02 D vs G N.S.	D vs E N.S. DvsG P<0.05	ADvsBE N.S. ADvsBF N.S. ADvsCE P<0.05

Figures are mean % oviduct traversed +/-SEM (n = No. of oviducts)

* Data taken from previous chapters/tables

Table 6.5 Comparison of the effects of various doses of testosterone propionate, of 5alpha-dihydrotestosterone (DHT) given on Day 1 of pregnancy, vs untreated controls on the mean % oviduct traversed measured on Day 4.

Treatments on Day 1	Mean % on Day 4	Statistical evaluation
None *	99.93+/-0.07 (20)	——
1mg TP	98.58+/-1.43 (10)	N.S.
5mg TP	86.02+/-8.20 (10)	N.S.
1.2mg DHT	99.10+/-0.48 (10)	N.S.

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts)

* Data from Chapter 1

Table 6.6 The effect of 1mg testosterone propionate given daily from Day 2 of pregnancy.

Animal sacrificed	TP treated	In vivo control *	Statistical evaluation
12:00h Day 3	80.31+/-1.86 (10)	86.66+/-1.38 (10)	P<0.05
12:00h Day Four	97.26+/-0.98 (10)	99.93+/-0.07 (10)	P<0.05

Values are mean % oviduct traversed +/- SEM (n = No. of oviducts)

* Data from Chapter 1

Table 6.7 The effect of 1mg testosterone propionate (TP) given daily on Days 3 and 4 of pregnancy on mean % oviduct traversed on Day 4 of pregnancy, and the modification of this effect by concurrent administration of 0.8mg progesterone (P) or 2.5mg 1,4,6-androstrien-3,17-dione (ATD).

Treatment	Mean %	Statistical analyses
Untreated * (A)	99.93+/-0.07 (20)	A vs B P<0.001 A vs C N.S. A vs D P<0.005
TP (B)	92.18+/-1.22 (10)	B vs C P<0.001 B vs D P<0.001 C vs D N.S.
TP + P (C)	98.43+/- 0.43 (10)	
TP + ATD (D)	97.73+/-0.61 (10)	

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts)

* Date from Chapter 1

Chapter Seven - Examination of Embryo Transport in Oviducts maintained In Vitro

Introduction

The value of an *in vitro* system for the examination of oviduct functions lies in the removal of uncontrolled factors such as anaesthesia, circulatory changes, hormone fluxes and extrinsic nervous activity (Marshall, 1976). Further, the ease with which the action of drugs and hormones may be examined without the problems of *in vivo* biotransformation and degradation, and the control of external enviromental factors such as extra-cellular fluid composition and temperature, mean that an *in vitro* system would be very useful. Previous work, reviewed in the General Introduction, has centred on isolating the various factors responsible for tubal embryo transport, such an ciliary activity, muscular contractility and secretory rate, etc. A more composite approach is to examine the position of the embryos in situ.

The development of a perfused system for continued embryo transport *in vitro* has been examined by Lee (1979) and Lee and Kendle (1979). They developed a technique for the continuous monitoring of embryo position and visual assessment of tubal contractility *in vitro*, for periods of up to 20 hours. The oviduct was contained in a specially constructed organ chamber, having been previously freed of mesentery, and perfused with a Ringer solution. From Day 2 of pregnancy continued embryo movement was seen, in the form of peristaltic and anti-peristaltic rushes within the isthmic lumen, but no net forward movement of embryos occurred. Kendle (1969) examined oviducts, intact and freed of mesentery, perfused in Ringer solutions or tissue culture media, for 48 hours from Day 1 of pregnancy, but found no net forward movement of embryos in any medium used.

A full description of the pattern of embryo transport in the mouse

has been given (General Introduction, The Timing of Embryo Transport, and Chapter 1). Several key events can be examined at specific time periods during the embryo transport phase. In vivo between Day 1 and 2 of pregnancy, embryos pass from the ampulla to the isthmus. Between Days 2 and 3 the rapid isthmic transport phase is completed and between Days 3 and 4, embryos pass from the oviduct into the uterus. These three time periods have therefore been selected for examination in vitro, using a system in which the oviduct and mesenteries are perfused intact, in an organ chamber which permits three dimensional movement of the organ. Use of appropriate in vivo control data for statistical comparison allows examination of the progression of embryo in vitro.

Materials and Methods

Unless otherwise stated, the procedures used were as described in the General Methods (Section 5, In vitro mouse oviduct preparation). The experimental work is divided into 3 distinct sections.

Section 1 - All animals were sacrificed at 17:00h on Day 1 of pregnancy, at which time the oviducts were set up in vitro as previously described. The following experimental groups were examined. 12 oviducts in group A were perfused with Ringer solution alone. Group B (12 oviducts) were perfused with the Ringer solution described, but prepared without calcium chloride (calcium-free Ringer). Groups C and D (12 oviducts each) were perfused with Ringer solution containing progesterone (0.1 and 1.0ug/ml respectively). Group E (12 oviducts) were perfused with Ringer solution containing oestradiol 0.001ug/ml and group F (12 oviducts) were perfused with Ringer solution containing $1x10^{-4}$M noradrenaline, and $1x10^{-4}$M ascorbic acid. Using cannulae prepared from blunted 27g hypodermic needles, one oviduct from each of 8 animals in group G were internally perfused with 0.9% aqueous saline solution through the infundibular ostium, at 0.55 ul/hour and the contralateral oviducts cannulated, but not internally

perfused, while being perfused in Ringer solution as described above.

All oviducts were examined at 09:00h the following morning (i.e. comparable to Day 2 of pregnancy) and the mean % oviduct traversed calculated. These values were compared to appropriate in vivo control values for Day 1 17:00h and Day 2 09:00h, taken from Chapter 1, using a multiple 't' test or Welch test. In the case of internally perfused oviducts, these were compared to the contralateral cannulated but not perfused control oviducts by paired 't' test, and combined for comparison to Day 1 17:00h in vivo control data.

Section 2 - All animals were sacrificed at 17:00h on Day 2 of pregnancy, at which time the oviducts were set up in vitro as previously described. The following experimental groups were examined. 12 oviducts in group H were perfused with 0.9% aqueous saline, which was not gassed with 95% O_2 + 5% CO_2. Group I (12 oviducts) were perfused with calcium-free Ringer (cf) and group J (12 oviducts) were perfused with Ringer solution.

In group K, 10 oviducts were perfused with Ringer solution containing $1x10^{-4}$M ascorbic acid. Group L (10 oviducts) were perfused with Ringer solution containing $1x10^{-5}$M noradrenaline (as acid tartrate) and $1x10^{-4}$M ascorbic acid. Group M (12 oviducts) were perfused with Ringer solution containing $1x10^{-6}$M phentolamine (as mesylate) and $1x10^{-4}$M ascorbic acid, while group N were perfused with Ringer solution containing both $1x10^{-5}$M noradrenaline and $1x10^{-6}$M phentolamine, as well as $1x10^{-4}$M ascorbic acid.

In groups P to T (12 oviducts in each), the oviducts were perfused with Ringer solutions containing isoprenaline (as the sulphate, $1x10^{-6}$M, $5x10^{-6}$M, $8x10^{-6}$M and $1x10^{-5}$M respectively) and $1x10^{-4}$M ascorbic acid.

All oviducts were examined at 09:00h the following morning (i.e. comparable to Day 3 of pregnancy) and the mean % oviduct traversed

calculated. For groups K to N, the embryo distribution (i.e. oviductal or uterine) was also noted. Groups H, I and J were compared to appropriate *in vivo* control values for Day 2 17:00h and Day 3 09:00h, taken from Chapter 1, using a multiple 't' test or Welch test. Groups L to N were compared to Group K using a multiple 't' test. Embryo distribution in these groups were compared by Fisher exact test. Groups P to T were compared to group K using the Welch test.

Section 3 - All animals were sacrificed at 17:00h on Day 3 of pregnancy, at which time the oviducts were set up *in vitro* as previously described. The following experimental groups were examined. 12 oviducts on Group V were perfused with Ringer solution alone. Group W (10 oviducts) were perfused with 0.9% aqueous saline, which was not gassed with 95% O_2 + 5% CO_2. Group X (12 oviducts) were perfused with calcium-free Ringer (cf).

All oviducts were examined at 09:00h the following morning (i.e. comparable to Day 4 of pregnancy) and the mean % oviduct traversed calculated. In each group the distribution of embryos was also recorded, the mean % for each group was compared to appropriate *in vivo* control values. Using a multiple 't' test, the mean number of embryos recovered (from each side of the reproductive tract) and the mean number of oviductal (retained) embryos for each of Groups V, W and X were compared to appropriate *in vivo* control data, using the Welch test.

Results

Section 1

The results for *in vitro* groups A to F are shown in Table 7.1. Each of the *in vitro* perfused groups, A to F, were compared to Day 1 17:00h *in vivo* control data and were not significantly different in any of case. Comparison to Day 2 09:00h *in vivo* control data showed that each group mean % oviduct traversed was significantly less than the

control ($P<0.005$, $P<0.01$, $P<0.005$, $P<0.01$, $P<0.05$ and $P<0.05$, respectively). Comparison of the two *in vivo* control groups showed that a significant difference exists ($P<0.005$).

Data for the luminally perfused oviducts, group G, are shown in Table 7.2. When the mean % for luminally perfused oviducts are compared to the mean % for the contralateral cannulated but not luminally perfused oviducts, no significant difference is seen. Combination of both luminally perfused and cannulated control data show no significant difference from Day 1 17:00h *in vivo* control data.

Section 2

The results for *in vitro* groups H to J are shown in Table 7.3. Each of groups H to J were compared to *in vivo* control data from 17:00h Day 2 and 09:00h Day 3. Group H (saline perfused) was not significantly different from the Day 2 control value, but was significantly less ($P<0.01$) than the Day 3 value. Group I (calcium-free Ringer perfused) was not significantly different from the Day 2 value, but was significantly less ($P<0.05$) than the Day 3 value. However, group J (Ringer perfused) was significantly greater ($P<0.01$) than the Day 2 value, and was not significantly different from the Day 3 value. Comparison of the two control values showed a significant difference ($P<0.01$).

The mean % oviduct traversed for *in vitro* groups K to N are shown in Table 7.4, and the distribution of embryos (oviductal or uterine) shown in Table 7.5. The mean % for each of groups L, M and N are not significantly different from group K (that is, Ringer with 10^{-5}M NA, Ringer with 10^{-6}M phentolamine Ringer with both NA and phentolamine and Ringer alone, respectively). Examination of the embryo distribution (Table 7.5), however, shows that although a normal number of embryos are recovered in each case (data not shown) the presence of uterine embryos is seen only in group L, (10^{-5}M NA), which is

significantly different from each of the other groups (Ringer 10^{-6} M phent and 10^{-5}M NA with 10^{-6}M phent, P<0.02 in each case).

The mean % oviduct traversed for *in vitro* groups P to T, in comparison with group K, is shown in Table 7.6, in which the effects of various concentrations of isoprenaline are compared to Ringer perfused control oviducts (group K). In each case, no significant difference is seen.

Section 3

The mean % oviduct traversed for groups V, W and X are shown in Table 7.7. Each of the groups were compared to *in vivo* control data from 17:00h Day 3 and 09:00h Day 4. Group V (Ringer perfused) was significantly greater than the Day 3 value (P<0.005) and not significantly different from the Day 4 value. Group W (saline perfused) was not significantly different from either the Day 3 or the Day 4 value. Group X (calcium-free Ringer) was not significantly different from the Day 3 value, but was significantly less than the Day 4 value (P<0.001). Comparison of the two control values showed a significant difference (P<0.001). Examination of the embryo distribution for these groups, in comparison to *in vivo* control data, is shown in Table 7.8. Total embryo recovery is lower in the saline perfused group than any other (P<0.05, in each case). Oviductal (retained) embryos were significantly higher in Day 3 *in vivo* animals than in Day 4 *in vivo* animals, or animals in groups V (Ringer) and W (saline) (P<0.001, in each case), but was not significantly different from group X (calcium-free Ringer). Group X (calcium-free Ringer) had singificantly more retained embryos than each of groups V, W and Day 4 *in vivo* (P<0.01, P<0.01 and P<0.001, respectively). The Day 4 *in vivo* group had significantly less retained embryos than group W (saline) (P<0.05), but was not significantly different from group V (Ringer). Groups V and W were not significantly different.

Discussion

The distinct and discrete nature of the phenomena examined in each of Sections 1, 2 and 3 of this chapter warrant individucal discussion, but must also be viewed, overall, as an *in vitro* system designed to simulate or stimulate embryo transport *in vitro* in a manner similar to the continuous *in vivo* process.

In Section 1, the passage of embryos from the ampulla into the isthmus was examined. Perfusion of oviducts in Ringer solution did not result in movement of embryos *in vitro*. This mirrors the earlier work of Kendle (1969) and suggests that some factor (or factors) is required for the passage of embryos into the isthmus, which is not present in the *in vitro* system. The nature of the block to transport of the AIJ has been discussed in the General Introduction (Physiological Mechanisms involved in Embryo Transport). After perfusion *in vitro* in this system, it has been observed that ampullary ciliated cells are still active. (However, the rate or direction of such activity was not investigated). Having noticed from results in Section 2, using calcium-free Ringer, that ciliary activity in the ampulla was still observed after perfusion, this medium was examined on oviducts perfused from Day 1. No net forward movement occurred, but ciliary activity was observed at the end of the perfusion period. The experiment was designed to investigate whether the combination of ciliary activity and muscular relaxation induced by lack of calcium might result in movement of embryos through the AIJ into the isthmus since Lee (1979) previously reported that cessation of muscular contractility in oviducts perfused *in vitro* with calcium-free Ringer occurred after about four hours.

The effect of two concentrations of progesterone (0.1ug/ml and 1.0ug/ml) were investigated. Higgs and Moawad (1974) hypothesised that the isthmus was functioning as an adrenergic sphincter, in which

rising plasma levels of progesterone activited or increased the number of beta adrenergic receptors, leading to relaxation. While the concept of an adrenergic sphincter seems unlikely, (since treatment with 6-hydroxydopamine does not adversely affect fertility (Johns et. al., 1975)), it is known that levels of progesterone rise in the early stages of pregnancy (Murr et. al., 1974, and see General Introduction) and so the effects of progesterone were worthy of investigation. In both cases, no net forward movement of embryos was seen. Similarly, the effect of 0.001ug/ml oestradiol was investigated, since oestradiol is suggested to be stimulatory to oviductal musculature (Coutinho, De Mattos and Rita de Silva, 1971). Once more no net forward movement was seen. It remains possible that a combination of physiological levels of oestradiol and progesterone would provide the missing factor. One further compound was investigated. Previous work in this laboratory, (during an Honours student research project), suggested that noradrenaline in vitro could stimulate the passage of embryos from the ampulla into the isthmus since 10^{-5}M noradrenaline caused a non-significant increase in mean %. (K. M. Manson, K. E. Kendle, unpublished observations). However, 10^{-4}M noradrenaline added to the perfusing medium (protected from degradation by 10^{-4}M ascorbic acid) did not produce net forward movement of embryos. Finally, the effect of luminal cannulation and positive pressure perfusion has been examined. The level of luminal perfusion selected was derived from the data presented in Chapter 3. Although preliminary in nature, the study indicates that the process of insertion of a cannula into the lumen of the preampulla does not affect embryo position in this in vitro system. Also, perfusion with 0.55ul/hour of saline solution does not stimulate movement of embryos. It is possible that a higher rate of perfusion would result in passage of embryos into the isthmus, since the perfusion rate chosen is based on the amount of fluid found

in ligated sections of uterus in animals in which the movement of embryos was retarded due to the surgical procedure. It is therefore possible that normal transport requires the presence of a greater secretory pressure, which is missing in the animals surgically treated to introduce uterine ligatures. Further, the measurements of secretory rate made in Chapter 3 were derived from results obtained over a 20 hours period, and fluctuations within that period may have occurred. Therefore it is possible that a higher perfusion rate, over a shorter period, would propel the embryos into the isthmus. Also, the composition of the fluids found in the ampulla at this time is not fully known. The ionic composition has been reported by Borland, Hazra, Biggers and Lechene (1977), but the viscosity of the fluid has not been reported. An increase in perfusing fluid viscosity could also be a factor required to initiate movement, in that the low viscosity of saline could result in the passage of a column of saline through the ampulla and ampullary-isthmic junction, leaving the fertilised ova within the ampulla.

The results presented for this section show that the nature of the ampullary-isthmic junction is complex, and the factors required to initiate passage of the fertilised ova into the isthmus are not all met by this system. The results presented in Section 2 show that embryo transport through the isthmus can occur in vitro in oviducts perfused with Ringer solution alone. The use of a saline perfused control shows that non-specific stimuli, such as tissue movement in the organ bath or degradation of the tissue, does not account for the movement observed. (Biggers, Gwatkin and Brinster (1962) observed segmental contracts in cultured oviducts after, but not before, two days of culture, and suggested that these were the result of spontaneous discharges due to adverse conditions, indicating degeneration of the tissue).

The use of a calcium-free medium, as previously described, indicates the tissue requirement for calcium for embryo transport (since transport did not occur in oviducts perfused in calcium-free Ringer) although a non-significant increase in mean % was seen, over the Day 2 in vivo control value. It is interesting to note that after perfusion in this medium, ampullary cilia are still active, and, although, as stated previously, rate and directions of beat cannot be determined, this suggests that the sole driving force for isthmic transport is not ampullary ciliary activity, providing a column of fluid moving in an abovarian direction, but that isthmic (or ampullary) muscular contractility is also required. The role of secretory products will be discussed later.

It is known that the oviduct possesses autonomic innervation. As was briefly stated earlier, it has been suggested that the isthmus functions an an adrenergic sphincter (Higgs et. al., 1974). The nature of the adrenergic innervation of the oviduct has been reviewed by several authors (Hodgeson and Eddy, 1975; Marshall, 1981; Paton, 1976; Black, 1976). Adrenergic innervation is greatest at the AIJ, and possibly junctura, and consists of both long and short post-ganglionic sympathetic neurones, a unique situation, in which the 2 types of neurones differ physiologically as well as anatomically (Black, 1976). Both alpha and beta adrenergic receptors have been demonstrated in several species (with alpha receptors mediating increased muscular contractility, and beta receptors relaxation) and the sensitivity of the receptor systems vary with steroid hormone background (Paton, 1976; Marshall, 1981, Helm, 1981). The results of the denervation studies using 6-hydroxydopamine (Johns et. al., 1974; Eddy and Black, 1973, 1974) and the work of Kendle (1969) who used reserpine to deplete peripheral noradrenergic terminals of neurotransmitter, and found that if the body temperature of the mice

was maintained, no effect on tubal transport was seen, suggest that the role of the adrenergic neurones of the mouse and rabbit do not have a major role to play in oviduct function in these species. However, several notes of caution must be made regarding these studies. Firstly, although 6-hydroxydopamine causes irreversible degeneration of peripheral adrenergic nerve terminals, the studies cited indicate that denervation was not complete, and a small proportion of neurones remained intact after luminal perfusion (Eddy and Black, 1973) or I.P. administration (Johns et. al., 1974). The effect of reserpine is also considered to be incomplete (Paton 1976). It is possible, then, that sufficient adrenergic activity remained to ensure reasonably normal embryo transport, particularly if post-junctional receptor super-sensitivity was induced, by decreasing levels of noradrenaline (Polidoro, Heilman, Culver and Reo, 1976). Secondly, the oviduct is known to be a system controlled by several factors, and it is possible the alteration of one factor simply results in a compensatory shift in others, arriving at the same net result (Greenwald, 1976).

Lee (1979), examining mouse oviduct contractility in vitro, observed that adrenaline, 10uM, resulted in an increased frequency of contraction, and postulated that this indicated the presence of alpha adrenergic receptors in the mouse oviduct. The extension of this observation to this in vitro system indicates that noradrenaline is facilitatory to embryo transpot in vitro, aiding the passage of embryo through the isthmus and junctura, into the uterine lumen. The blockage of this effect using phentolamine, (which alone has no effect) shows that this is indeed an alpha-adrenergic receptor mediated effect. Examination of the effect of various concentrations of isoprenaline confirms the initial report of Lee (1979) who observed that isoprenaline did not affect the contractility of mouse oviducts in vitro. These in vitro results are complementary to the in vivo work

of Polidoro, Heilman, Culver and Reo (1976), who found an increase in rabbit embryo transport rate using adrenaline, noradrenaline and phenylephrine, and found isoprenaline to be without major effect. They also reported an increase in the number of uterine embryos 72 hours post-coitum as a result of adrenaline noradrenaline, phenylephrine and isoprenaline. This latter finding was unexplained, and was not seen in this in vitro study. It is possible that at the very high doses used, isoprenaline may exert a feeble alpha adrenergic agonist activity. It should be remembered, also that mortality rates of 47 and 52% were found in the isoprenaline treated groups, and general, non-specific, stress factors may affects results. The use of an in vitro system circumvents these problems and it remains possible that higher concentrations of isoprenaline in vitro would result in effects on tubal transport. Indeed, the higher concentrations of isoprenaline used in this study resulted in a final mean % oviduct traversed significantly less than the normal 09:00h Day 3 in vivo position, but in comparison to Ringer perfused in vitro controls, this significance disappeared, due to the large standard error of the control group, (which will be discussed later), indicating a possible beta-adrenegric inhibition of transport.

Section 3 examined the passage of embryos through the utero-tubal junction into the uterus. Perfusion of oviducts in vitro with Ringer solution resulted in passage of most of the embryos recovered into the uterus. However, the significance of this result was initially in doubt since perfusion of oviducts with saline also allowed passage into the uterus to occur in vitro. This suggested that a degenerative process could, either by relaxation of the musculature, or by random contractions, already described (see Biggers et. al., 1962), allow passage of embryos into the uterus. However, the significant loss of embryos in the saline perfused group meant that it was difficult to

interpret the result, as degeneration and destruction of the embryos could have occurred in the oviduct or the uterus, and the opacity of the saline perfused oviduct and the amount of debris found in the uterine fluid made observations difficult. To examine this more closeley, the effect of perfusion in calcium-free Ringer was noted. Significant oviductal retention of embryos was found (not significantly different from the Day 3 17:00h in vivo control value). This suggests that the process of passage of embryos into the uterus is an active process requiring the presence of calcium, probably for muscular contractility, and also confirms that the process observed in Ringer perfused oviducts is unlikely to be the result of a degenerative change. The nature of this calcium dependent process is not known. Blandau (1973) suggested the presence of an adrenergic sphincter at the UTJ, relaxed by increasing levels of progesterone, while Talo and Hodgeson (1978) believed that increasing isthmic or junctural muscular activity could drive the embryos into the uterus. The results presented here, (coupled with the results in Section 2), indicate that an adrenergic sphincter mechanism is not likely, since noradrenaline (via an alpha-adrenergic mediated pathway) increases the number of embryos passing through the UTJ, at a time at which the junction should be closed. It is possible that activation of isthmic musculature via alpha-adrenergic receptors, coupled with relaxation of the junctural region via beta-adrenergic receptors (both of which can be stimulated by noradrenaline) resulted in passage of embryos into the uterus. However, the result of perfusion with calcium-free Ringer in Section 3 suggest that relaxation of the junctural region (or at least quiesence) is not sufficient to allow passage. Therefore the suggestion of Talo and Hodgeson (1978), seems to be the most likely.

The advantages of an in vitro system for the examination of embryo transport have already been discussed in the introduction of this

chapter, and in the General Introduction (Examination of the oviduct *in vitro*). The system presented here, under appropriate conditions, allows embryo transport to occur *in vitro* from Days 2 and 3 of pregnancy for period of up to sixteen hours. However, the results must be regarded as preliminary in nature, as the system can be developed further, in order to produce results more similar to the *in vivo* process. A working temperature of 30°C has been employed throughout. Although obviously less than the physiological level, work by Lee (1979) showed that lower temperature *in vitro* slightly aided the long term viability of the tissue. This was also the view of Johns et al., (1976, 1980) who examined oviductal tissue *in vitro* at 26°C, since the authors reported that spontaneous contractility could be better maintained at this low temperature. The Ringer solution employed is isotonic with normal body fluids, but is not iso-osmotic, and exposure of the tissue to this environment rapidly leads to accumulation of fluid within the tissue. All tissues examined after perfusion *in vitro* showed evidence of such "water-logging", and in the oviducts examined from Day 2, many showed an enlarged, fluid-filled lumen, both in the ampulla and isthmus, which is not found in the freshly prepared tissues. The resulting distortion may be responsible for the proportion of oviducts which did not transport embryos correctly, as evidenced in the large SEM associated with some Ringer perfused groups. This distortion has obvious implications for the functioning of the oviductal epithelium. The presence of large volumes of transudate in the lumen argues against necrosis of the epithelium due to lack of nutrients but *in all cases*, development of the embryos was distorted or arrested *in vitro*, and although no quantitative data is presented, it seems likely that damage to the oviductal secretory apparatus, or dilution of luminal fluids by osmotic infiltration/transudation is sufficient to affect development of the

embryos. Pyruvate and lactate are known to be elaborated in high concentrations within the lumen of the oviduct and are required for the correct development of the embryo. Addition of these substances to the perfusing medium might supply the needs of the developing embryo (H. J. Leese, personal communication).

Unlike the method devised by Lee (1979), the in vitro method used here does not allow continuous direct observation of embryos in situ, nor does it allow measurements of contractility to be made. Further, the need to use in vivo control data, rather than a before/after comparison of each oviduct, is somewhat artificial, involving complex statistical analyses. A mathematical model for the prediction of mean embryo position, at a given time, is discussed in Appendix I. The use of such a model would remove the need for the use of in vivo control animals, and allow more accurate definition of acceleration and retardation in vitro i.e. retarded by 4 hours, accelerated by 6 hours, etc. A combination of some of the factors used with this method, i.e. unconstrained three dimensional movement of the tissue, collection of uterine fluids, with a previously straightened oviduct, in which the mean % oviduct traversed could be determined both prior to and after perfusion is the logical extension of this method.

It is likely that methods such as are reported here are of value only in the mouse, since the requirements of larger tissues for oxygen and nutrients are probably too great to be met by diffusion, and a system of vascular perfusion of a nutrient solution is the most likely approach to maintenance of larger tissues for the periods of time required for the examination of embryo transport.

Table 7.1 - Mean % oviduct traversed, after perfusion in vitro under various experimental conditions, from 17:00h on Day 1 of pregnancy, for a period of 16 hours, and comparison to in vivo control data.

Group	In vitro	Day 1 17:00h	Day 2 09:00h	
In vivo controls	———	19.55+/-1.67 (10)	39.92+/-3.71 (10)	Day 1 17:00h vs Day 2 09:00h P<0.005
A (Ringer)	22.81+/-3.91 (12)	N.S.	P<0.005	
B (Calcium-free Ringer)	23.42+/-4.44 (12)	N.S.	P<0.01	
C (Progesterone 0.1ug/ml)	16.35+/-0.52 (12)	N.S.	P<0.005	
D (Progesterone 1.0ug/ml)	18.91+/-1.33 (12)	N.S.	P<0.01	
E (Oestradiol 0.001ug/ml)	25.24+/-5.55 (10)	N.S.	P<0.05	
F (NA 10^{-4}M + Ascorbic acid 10^{-4}M)	27.03+/-5.53 (12)	N.S.	P<0.05	

Values are mean % oviduct traversed +/- SEM (n = No. of oviducts).

Table 7.2 - The effect of cannulation and internal perfusion with saline (0.55ul/hour) on mean % oviduct traversed after *in vitro* perfusion from 17:00h on Day 1 of pregnancy.

Group G

Control	Perfused	Statistical analysis
17.93+/-0.77 (8)	24.33+/-4.18 (8)	N.S.

Day 1 17:00h

Group	Combined cannulated	*In vivo* control	Statistical analysis
G	21.13+/-2.20 (16)	19.55+/-1.67 (10)	N.S.

Values are mean % of oviduct traversed +/- SEM (n = No. of oviducts)

Table 7.3 - Mean % oviduct traversed after perfusion in vitro under various experimental conditions, from 17:00h on Day 2 of pregnancy, for a period of 16 hours, and comparison to in vivo control data.

Group	In vitro	Day 2 17:00h	Day 3 09:00h	
In vivo controls	———	46.38+/-4.16 (10)	80.82+/-2.40 (10)	Day 2 17:00h vs Day 3 09:00h P<0.01
H (Saline)	44.24+/-4.13 (12)	N.S.	P<0.01	
I (Calcium-free Ringer)	58.67+/-4.36 (12)	N.S.	P<0.05	
J (Ringer)	74.39+/-4.67 (12)	P<0.01	N.S.	

Figures are mean % oviduct traversed + SEM (n = No. of oviducts)

Table 7.4 - The effects of noradrenaline (10^{-5} M) and phentolamine (10^{-6}M), alone and together, on mean % oviduct traversed in oviducts perfused *in vitro* from 17:00h on Day 2 of pregnancy.

Group	In vitro	Comparison to Group K
K (Ringer)	70.24+/-6.50 (10)	———
L (10^{-5}M NA)	74.18+/-9.99 (10)	N.S.
M (10^{-6}M Phent)	66.96+/-7.72 (10)	N.S.
N (10^{-5}M NA +10^{-6}M Phent)	70.32+/-3.09 (12)	N.S.

NA = Noradrenaline

Phent = Phentolamine

Figures are mean % oviduct traversed +/- SEM (n = No. of oviducts)

Table 7.5 - The distribution of embryos (oviductal or uterine) in oviducts perfused in vitro from 17:00h on Day 2 of pregnancy, and the effects of noradrenaline and/or phentolamine

Group	Distribution of embryos		
	Oviductal	Uterine	
K (Ringer)	6.1 +/- 0.7	0.0	
L (10^{-5}M NA)	3.8 +/- 1.1	1.1 +/- 0.05	P<0.02 Uterine embryo recovery is significantly different from all other groups.
M (10^{-6}M Phent)	7.1 +/- 0.4	0.0	
N (10^{-5}M NA + 10^{-6}M Phent)	6.8 +/- 0.6	0.0	

NA = Noradrenaline

Phent = Phentolamine

Figures are mean number of embryos/oviduct or uterine cornua +/- SEM

Table 7.6 - The effect of various concentrations of isoprenaline on mean % oviduct traversed in oviducts perfused in vitro from 17:00h on Day 2 of pregnancy.

Group	In vitro	Comparison to Group K
K (Ringer)	70.24+/-6.50* (10)	——
P ($1x10^{-6}$M ISO)	71.55+/-2.48 (12)	N.S.
R ($5x10^{-6}$M ISO)	68.03+/-2.40 (12)	N.S.
S ($8x10^{-6}$M ISO)	64.22+/-5.13 (12)	N.S.
T ($1x10^{-5}$M ISO)	64.24+/-2.37 (12)	N.S.

ISO = Isoprenaline

* Data from Table 7.4

Values are mean % oviduct traversed +/- SEM (n = No. of oviducts)

Table 7.7 - Mean % oviduct traversed, after perfusion in vitro under various experimental conditions, from 17:00h on Day 3 of pregnancy, for a period of 16 hours.

Group	In vitro	Day 3 17:00h	Day 4 09:00h	
In vivo controls	———	90.91+/-0.55 (10)	99.80+/-0.20 (10)	Day 3 17:00h vs Day 4 09:00h P<0.001
V (Ringer)	99.19+/-0.46 (12)	P<0.005	N.S.	
W (Saline)	94.18+/-2.64 (10)	N.S.	N.S.	
X (Calcium-free Ringer)	90.11+/-2.09 (12)	N.S.	P<0.001	

Values are mean % oviduct traversed +/- SEM (n = No. of oviducts)

Table 7.8 - The distribution in the reproductive tract of embryos in oviducts perfused *in vitro* under various experimental conditions, from 17:00h Day 3 of pregnancy, for a period of 16 hours, and comparison to *in vivo* control data.

Group	Mean number embryos/side			
	Total	Uterine	Oviductal	
In vivo Day 3 17:00h	5.80+/-0.61 (10)	0.00	5.80+/-0.61[a]	a vs b P<0.001 a vs c P<0.001 a vs d P<0.001 a vs e N.S. d vs c N.S. b vs d P<0.005 b vs e P<0.001 c vs d N.S. c vs e P<0.01 d vs e P<0.01
Day 4 09:00h	4.70+/-0.84 (10)	4.60+/-0.87	0.10+/-0.10[b]	
In vitro V (Ringer)	5.67+/0.58 (12)	4.67+/-0.67	1.00+/-0.55[c]	
W (Saline)	2.2+/-0.39* (10)	1.2+/-0.33	1.0+/-0.39[d]	
X (Calcium-free Ringer)	6.67+/-0.60 (12)	1.50+/-0.63	5.17+/-0.98[e]	

Figures are mean No. of embryos/side of the reproductive tract (n = No. of sides examined)

* Significantly lower than other groups (P<0.05 in each case)

General Discussion

In the General Introduction, the aims of the proposed study were stated, (namely an investigation of the factors controlling embryo transport in the mouse). To this end, the normal *in vivo* pattern of embryo transport in the mouse, from ampulla to uterus, has been examined in some detail. Although no study of the pick-up of ova or ampullary transit were made, the results confirm earlier studies, in particular Burdick et. al., (1940), Humphrey (1968A) and Lee (1979). This data has been used throughout the subsequent studies in both *in vivo* and *in vitro* work for comparative purposes, and has been analysed mathematically. This novel approach indicates the possibility of the use of such models in the future for the accurate prediction of the rate of transport and position of embryos, a method which systematically takes into account the biological error inherent in the data, and is of value in the type of *in vitro* approach reported here, in which a single post-incubation measurement is taken and compared to appropriate *in vivo* control data i.e. no direct "before and after" comparisons on the same tissue are possible, as was previously the case (Lee, 1979; Lee and Kendle, 1979).

At the outset, a major objective of this work was the examination of oviduct function *in vitro*, and determination of the extent to which normal tubal transport would occur, or could be simulated, in such a system, free of normal innervation and hormonal fluxes. The *in vitro* approach employed here has resulted in a system in which embryo transport occurs *in vitro* from Day 2 for a period of 16 hours and from Day 3 for a similar period, resulting in movement not dissimilar from the normal *in vivo* transport at these times, a finding which has not previously reported by other workers. However, incubation *in vitro* from Day 1 of pregnancy in a similar manner does not result in embryo transport, regardless of the manipulations carried out, confirming the

study of Kendle (1969). It is suggested that further improvements in the system may overcome these difficulties, and produce conditions more like those required for the physiological process while retaining the advantages of the in vitro approach, allowing manipulation of the extracellular fluid composition, temperature and allowing direct access for pharmacological and physical manipulations. Indeed, addition of noradrenaline to oviducts perfused in vitro from Day 2 of pregnancy resulted in acceleration and early passage of embryos into the uterine lumen via an alpha-adrenergic receptor mediated pathway, confirming the results of the in vivo study of Polidoro et al., (1976), indicating a possible role for the sympathetic nervous system, perhaps in passage of embryos modified by oestradiol (Pauerstein, Fremming and Martin, 1970) and a possible physiological role in normal isthmic transport, despite the conflicting data of denervation studies (Eddy and Black, 1973, 1974; Johns et al., 1974; Kendle, 1969), as denervation by the means reported is incomplete, and receptor sensitivity may vary as changes in neurotransmitter levels occur. It remains possible, also, that other neuronal controlling factors may be found to have a complementary role with the sympathetic neurones, in the control of oviduct function, in which changes in the level of activity of one system could be balanced out by changes in the other. Recently, Martin Del Rio (1981) reported the presence of relatively large amounts of gamma-aminobutyric acid (GABA), considered to be a neurotransmitter which produces an inhibitory effect on post-synaptic cells, in the oviduct of the rat (but not in the oviducts of the mouse, guinea-pig, rabbit, cat, dog, and macaque), associated with an enzyme system similar to that found in GABA containing neurones of the CNS. Ferandez, Azuara and Orensanz (1981) reported the existence of 3HGABA high affinity binding in the rat oviduct, typical of the so-called $GABA_b$ receptor (Bowery, 1982).

In the human, the presence of neurones containing Vasoactive

Intestinal Polypeptide (VIP) has been reported (Helm, Otteson, Fahrenkrug, Larsen, Owman, Sjoberg, Stolberg, Sundler and Walles, 1981; Helm, Hakanson, Leander, Owman, Sjoberg and Sporrong, 1982; reviewed by Helm, 1981). The authors suggest that VIP containing neurones are associated with the oviductal isthmus and induce relaxation of the isthmic musculature. Thus the presence of non-adrenergic/non-cholinergic neurones (NANC neurones) has been established in two species, and the possibility that these or other systems exist in other species is likely.

The initial working hypothesis of this work, stated in the Aims in the General Introduction, has been that continued progesterone activity throughout the embryo transport phase is required for normal tubal transport. At first sight, the in vitro work reported would seem to contradict this hypothesis, since transport has been shown to occur, at least from Days 2 and 3 for period of up to 16 hours in the absence of any circulating steroid. However, the binding of the steroids, both specific and non-specific, may be sufficient to allow continued activity throughout the time period examined. To examine this hypothesis more fully, measurement of tissue progesterone levels, and levels of progesterone receptor, would need to be undertaken. It is also possible that the lack of forward progression of embryos when oviducts are perfused in vitro from Day 1 of pregnancy, is a reflection of the lack of progesterone activity, since the levels of progesterone in the serum are known to be low at this time, and sufficient bound steriod might not be present at the time of initiating the perfusion. This would seem unlikely in view of the fact that addition of progesterone to the perfusing medium did not result in net forward movement, but penetration of the steroid into the tissue was not measured. Also, it is possible that some oestrogenic activity is required to elaborate progesterone receptor tissue, and that a

combination of oestrogen and progesterone, in appropriate concentrations, might be the appropriate stimulus.

The actions and interactions of the steroids at the level of the receptor is complex and not clearly understood. It is known that oestrogen priming is required for the full manifestation of progesterone activity. Leavitt et al., (1982) suggested that oestradiol acted as a regulating mechanism for the amount of oestrogen and progesterone receptor present in uterine cytosol. Their work suggested that activation of oestradiol receptors increased the amount of both oestrogen and progesterone receptor in the target tissue, and that activation of progesterone receptor down regulated both oestrogen and progresterone receptor by an unknown mechanism. This confirms the work of West et al., (1976) who suggested that in the cat oviduct, progesterone suppressed the oestradiol receptor, and thus progesterone activity was, in fact, the result of loss of oestrogenic activity. These controlling mechanisms of receptor concentration makes understanding of steroid activity difficult, particularly when based on serum or plasma levels of the steroid.

Bearing these problems in mind, the working hypothesis that continued progesterone activity is required for normal tubal transport is supported by the results presented in Chapters 5 and 6 of this work. In Chapter 5, the results suggest that ovariectomy and adrenalectomy early on Day 1 of pregnancy results in retardation of tubal transport, which is associated with low serum levels of progesterone, and which can be reversed by exogenous progesterone. Since progesterone is thought to lower the levels of oestrogen receptor tissue, and it is unlikely that biotransformation of progesterone to oestradiol can occur in the absence of the ovaries and adrenal glands, it does not seem likely that this activity is oestrogenic in nature. These findings are supported by the study of Forcellado, Morales, Quijada

and Croxatto (1982) who showed that in the rat, removal of the ovaries and adrenal glands resulted in accelerated embryo transport and early loss of embryos, which could be prevented by concurrent administration of progesterone. The discrepancy between the effects of the surgery in rat and mouse is also seen in the activity of the anti-progestational steroid RMI 12,936, which causes acceleration and early loss in the rat, (Kendle, 1976) and arrest of transport in the mouse, (Kendle and Lee, 1980), and is indicative of the species differences in the actions of drugs and hormones on tubal activity in mammals.

Further light on the nature of the progestational dependence of oviductal embryo transport has been presented in Chapter 6. The novel finding that the retardation caused by testosterone administration can be reversed using appropriate dosages of progesterone advances knowledge of the control of tubal function by suggesting that the androgenic compound is acting in this system as an anti-progestational agent, possibly acting as a competitive inhibitor at the level of the receptor, and that this activity is manifest mainly at the level of the AIJ and UTJ. It remains possible that the effects of testosterone, at least in part, are due to its oestrogenic activity, since the retardation is also reversed by ATD, an aromatization inhibitor, and may be affected by removal of ovaries and adrenal glands. However, the suggestion that ATD could also be progestational in activity further reinforces the idea that progesterone activity is required. Reviewing the molecular make-up of compounds which have activity (agonist or antagonist) at the progesterone receptor, Kendle (1982) found that norethisterone and particulary 5 alpha dihydronorethisterone, which are testosterone derivatives, were found to be antagonists of progesterone.

The results confirm the hypothesis that continued progesterone activity is required for normal tubal transport, but further

investigation into the nature of this activity is required, particularly at the level of the receptor and post-receptor events. If progesterone activity is manifest by the loss of oestrogen receptor, (and so an anti-oestrogenic activity) changes in muscular contractility due to depletion of alpha-adrenergic receptors within the oviductal musculature may occur, oviductal secretion fall, and tissue oedema lessen. Further the level of oviductal calcium is lowest at the time embryos pass into the isthmus (Lee, 1979) and this fall is also seen when transport is accelerated by pre-ovulatory progesterone. This would suggest that progesterone activity is manifest at the level of the AIJ and isthmus, and tubal transport, considered to consist of a fast (ampullary transport) phase and a slow (isthmic transport) phase, could be initially the result of oestrogenic activity, with a change over to progesterone activity at the ampullary-isthmic junction.

In the final analysis, it is likely that the control of oviduct function in the mouse is multi-factorial and changes in the activity of some controlling factor, such as the autonomic nervous system, may not give apparent changes in overall activity, while changes in a more fundamental controlling system, the steroid hormones, produces marked effects. To examine these controlling systems more fully, analysis of hormone receptor levels (both cytoplasmic and nuclear), post-synaptic neurotransmitter receptor levels, coupled with steroid hormone levels in serum and tissue could provide more evidence for a post-ovulatory role, which could then be examined <u>in vivo</u> and <u>in vitro</u> by pharmacological and surgical manipulation.

Bibliography

AGDUHR, E. (1927). Studies on the structure and development of the bursa ovarica and the tuba uterina in the mouse. Acta Zool. 8, 1-41.

AITKEN, R. J. (1979). Tubal and uterine secretions; The possibilities for contraceptive attack. J. Reprod. Fert. 55, 247-254.

ALDEN, R. H. (1942A). The oviduct and egg transport in the albino rat. Anatomical Record. 84, 137-169.

ALDEN, R. H. (1942B). Aspects of the egg-ovary-oviduct relationship in the albino rat. J. Exp. Zool. 90, 159-168.

ANDERSON, R. G. W. and Hein, C. E. (1977). Distribution of anionic sites on the oviduct ciliary membrane. J. Cell Biol. 72, 482-492.

AREF, I. and HAFEZ, E. S. E. (1973). Utero-oviductal motility with emphasis on ova transport. Obstet. Gynecol. Surv. 28 (10), 679-703.

ATKINSON, W. B. and HOOKER, C. W. (1945). The day to day level of estrogen and progestin throughout pregnancy and pseudopregnancy in the mouse. Anat. Record. 93, 75-89.

BARKLEY, M. S., GESCHWIND, I. I. and BRADFORD, G. E. (1979). The gestational pattern of estradiol, testosterone and progesterone secretion in selected strains of mice. Biol. Reprod. 20, 733-738.

BATRA, S., HELM, G., OWMAN, C., SJOBERG, N. O. and WALLES, B. (1980). Female sex steroid concentrations in the ampullary and isthmic regions of the human Fallopian tube and their relationship to plasma concentrations during the menstrual cycle. Am. J. Obstet. Gynecol. 136 (8), 986-991.

BECK, L. R., and BOOTS, L. R. (1974). The comparative anatomy, histology and morphology of the mammalian oviduct. In: The Oviduct and Its Functions, Eds. Johnson, A. D. and Foley, C. W. Academic Press, New York.

BIGGERS, J. D., GWATKIN, R. B. L., and BRINSTER, R. L. (1962). Development of mouse embryos in organ cultures of Fallopian tubes on a chemically defined medium. Nature 194, 747-749.

BIGSBY, R. M., LUNGU, J. C. N., DUBY, R. T. and BLACK, D. L. (1983). Effects of passive immunisation against oestradiol on mouse ovum transport. J. Reprod. Fert. 67, 281-289.

BISHOP, D. W. (1956). Active secretion in the rabbit oviduct. Am. J. Physiol. 187, 347-352.

BLACK, D. L. (1976). Autonomic mechanisms in embryo transport. In: Ovum Transport and Fertility Regulation, Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

BLANDAU, R. J. (1969). In: The Mammalian Oviduct - Comparative Biology and Methodology. Eds. Hafez, E. S. E. and Blandau, R. J. Univ. Chicago Press, Chicago.

BLANDAU, R. J. (1973). Gamete transport in the female mammal. In: Handbook of Physiology. Eds. Greep, R. O. and Astwood, E. B. American Physiol. Soc., Washington D.C.

BLANDAU, R. J., BOLING, J. L., HALBERT, S. and VERDUGO, P. (1975). Methods for studying oviductal physiology. Gynecol. Invest. 6, 123-145.

BLANDAU, R. J., BOURDAGE, R. J. and HALBERT, S. (1979). Tubal transport. In: The Biology of the Fluids of the Female Genital Tract. Eds. Beller, F. K. and Schumacher, G. F. B. Elsevier/North Holland.

BLANDAU, R. J., JENSEN, L. and RUMERY, R. (1958). Determination of the pH values of the reproductive tract fluids of the rat during heat. Fertil. Steril, 9, 207-214.

BLANDAU, R. J. and VERDUGO, P. (1976). An overview of gamete transport. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M. Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

BOLING, J. L. (1969). In: The Mammalian Oviduct - Comparative Biology and Methodology. Eds. Hafez, E. S. E. and Blandau, R. J. Univ. Chicago Press, Chicago.

BOLING, J. L. and BLANDAU, R. J. (1971). Egg transport through the ampullae of the oviducts of rabbits under various experimental conditions. Biol. Reprod. 4, 174-184.

BORELL, U., NILSSON, O., WERSALL, J. and WESTMAN, A. (1956). Electron-microscope studies of the epithelium of the rabbit Fallopian tube under different hormonal influences. Acta. Obstet. et Gynec. Scand. 35, 35-41.

BORLAND, R. M., HAZRA, S., BIGGERS, J. D. and LECHENE, C. P. (1977). The elemental composition of the environments of the gametes and pre-implantation embryo during initiation of pregnancy. Biology of Reproduction 16, 147-157.

BOWERY, N. G. (1983) Baclofen : 10 years on. Trends in Pharmacological Science. 3 (10), 400-403.

BRENNER, R. M. (1969). In: The Mammalian Oviduct - Comparative Biology and Methodology. Eds. Hafez, E. S. E. and Blandau, R. J. Univ. Chicago Press, Chicago.

BRUNDIN, J. (1964). A functional block in the isthmus of the rabbit Fallopian tube. Acta. Physiol. Scand. 60, 295-296.

BRUNDIN, J. (1976). Adrenergic mechanisms in ovum transport. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

BRUNTON, W. J. and BRINSTER, R. L. (1971). Active chloride transport in the isolated rabbit oviduct. Am. J. Physiol. 221, 658-661.

BULLOCK, L. P., FEIL, P. D., GUPTA, C., DEMERS, L. M. and BARDIN, C. W. (1978). Androgenic effects of the anti-progestagen RMI 12,936. J. Reprod. Fert. 52, 365-371.

BULLON, F., MERCHAN, J. A., GONZALEZ-GOMEZ, F., FURIO, V. and POBLETE, E. G. (1980). Ultrastructure of the oviductal mucosa of the rat III. Basal and peg cells. Int. J. Fertil. 25 (4), 293-297.

BURDICK, H. O., EMERSON, B. B. and WHITNEY, R. (1940). Effects of testosterone propionate on pregnancy and on passage of ova through the oviducts of mice. Endocrinol. 26, 1081-1086.

BURDICK, H. O. and PINCUS, G. (1935). The effect of oestrin injections upon the developing ova of mice and rabbits. Amer. J. Physiol. 111, 201-208.

BURDICK, H. O. and WHITNEY, R. (1937). Acceleration of the rate of passage of fertilised ova through the Fallopian tubes of mice by massive injections of an estrogenic substance. Endocrinology 21, 637-643.

BURDICK, H. O., WHITNEY, R. and EMERSON, B. (1942). Observations on the transport of tubal ova. Endocrinology 31, 100-108.

CASCHETTO, S., LINDBLOM, B., WIQVIST, N. and WILHELMSSON, L. (1979). Prostaglandins and the contractile function of the human oviductal ampulla. Gynecol. Obstet. Invest. 10, 212-220.

CHANG, M. C. (1976). Estrogen, progesterone and egg transport. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

CHRISTENSEN, L. W. and CLEMENS, L. G. (1975). Blockade of testosterone-induced mounting behaviour in the male rat with intracranial application of the aromatization inhibitor, Androst-1,4,6,-Triene,-3,17-dione. Endocrinol. 97, 1545-1551.

CLEWE, T. H. and MASTROIANNI, L. (1960). A method for continuous volumetric collection of oviduct secretions. J. Reprod. Fertil. 1, 146-150.

COUTINHO, E. M., DE MATTOS, C. E. R. and RITA DA SILVA, A. (1971). The effect of ovarian hormones on the adrenergic stimulation of the rabbit Fallopian tube. Fertil. Steril. 22 (5), 311-317.

CRITOPH, F. N. and DENNIS, K. J. (1977). The cellular composition of the human oviduct epithelium. Brit. J. Ob. Gyn. 84, 219-221.

CROXATTO, H. B., DIAZ, S., FUENTEALBA, B., CROXATTO, H. B., CARILLO, D. and FABRES, C. (1972). Studies on the duration of egg transport in the human oviduct. The time interval between ovulation and egg recovery from the uterus in normal women. Fertil. Steril. 23 (7), 447-458.

DANIEL, E. E. (1976). A critical introduction to analysis of the role of oviductal motility in ovum transport. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

DEANE, H. W. (1952). Histochemical observations on the ovary and oviduct of the albino rat during the estrous cycle. Amer. Jo. Anat. 91, 363-413.

DIRKSEN, E. R. (1974). Ciliogenesis in the mouse oviduct - A scanning electron microscope study. J. Cell Biol. 62, 899-904.

DIRKSEN, E. R. (1975). In: Scanning Electron Microscopic Atlas of Mammalian Reproduction. Ed. Hafez, E. S. E. Georg Thieme Verlag, Stuttgart.

EDDY, C. A. and BLACK, D. L. (1973). Chemical sympathectomy of the rabbit oviduct using 6-hydroxydopamine. J. Reprod. Fert. 33, 1-9.

EDDY, C. A. and BLACK, D. L. (1974). Ovum transport through rabbit oviducts perfused with 6-hydroxydopamine. J. Reprod. Fert. 38, 189-191.

EDDY, C. A. and PAUERSTEIN, C. J. (1980). Anatomy and physiology of the Fallopian tube. Clin. Obstet. Gynec. 23, 1177-23.

ELLENDORFF, F., ROVER, H. F. and SMIDT, D. (1973). The effect of cyproterone acetate on the duration of pregnancy and parturition in mice. J. Reprod. Fert. 32, 299-303.

'ESPINASSE, P. G. (1935). The oviductal epithelium of the mouse. J. Anat. (Lond.) 69, 363-368.

EVERETT, J. W. (1967). Physiology of gonads. In: Sex and Internal Secretions, 3rd Edition. Ed. Young, W. C. Williams and Wilkins, Baltimore.

FEDER, H. H. (1981). Estrous cyclicity in mammals. In: Neuroendocrinology of Reproduction. Ed. Alder, N. T. Plenum Press, New York.

FEDER, H. H., RESKO, J. A. and GOY, R. W. (1968). Progesterone levels in the arterial plasma of pre-ovulatory and ovariectomized rats. J. Endocr. 41, 563-569.

FERNANDEZ, I. F., AZUARA, M. C. and ORENSANZ, L. M. (1981). ^{3}H-GABA binding in rat oviduct. Neuroscience Letters. Suppl. 7, S35.

FORCELLADO, M. L., MORALES, P., VERA, R., QUIJADA, S. and CROXATTO, H. B. (1982). Role of ovarian and adrenal progesterone in the regulation of ovum transport in pregnant rats. Biol. Reprod. 27, 1033-1041.

FORCELLADO, M. L., VERA, R. and CROXATTO, H. B. (1981). Ovum transport in pregnant, pseudopregnant and cyclic rats and its relationship to estradiol and progesterone blood levels. Biol. Reprod. 24, 760-765.

FUENTEALBA, B., VERA, R., NIETO, M. and CROXATTO, H. B. (1982). Changes in nuclear estrogen receptor level in the rat oviduct during ovum transport. Biol. Reprod. 27, 12-16.

GADDUM-ROSSE, P. and BLANDAU, R. J. (1973). In vitro studies on ciliary activity within the oviducts of the rabbit and pig. Am. J. Anat. 136, 91-104.

GADDUM-ROSSE, P. and BLANDAU, R. J. (1976). Comparative observations on ciliary currents in mammalian oviducts. Biol. Reprod. 14, 605-609.

GADDUM-ROSSE, P., BLANDAU, R. J. and THIERSCH, J. B. (1973). Ciliary activity in the human and macaca nemestrina oviduct (1). Amer. Jo. Anat. 138, 269-275.

GIACOMINI, M. and WRIGHT, F. (1980). The effects of progesterone and pregnanedione on the reductive metabolism of dihydrotestosterone in human skin. J. Steroid Biochem. 13, 645-651.

GIMENO, M. F., BORDA, E. S., STERIN-BORDA, L., STERIN-SPEZIALE, N. and GIMENO, A. L. (1976). Contractile activity of the oviduct and the mesosalpinx isolated from guinea-pigs in different phases of the sex cycle. Int. J. Fertil. 21, 31-41.

GONZALES DE VARGAS, M. I., HODGESON, B. J. and PAUERSTEIN, C. J. (1975). Temporal relationships critical to progesterone-induced acceleration of ovum transport. Obstet. Gynecol. 46, 299-301.

GOULD, K. G. (1974). Fertilisation : A function of the oviduct. In: The Oviduct and Its Functions. Eds. Johnson, A. D. and Foley, C. W. Academic Press, New York.

GREENWALD, G. S. (1961). A study of the transport of ova through the rabbit oviduct. Fertil. Steril. 12 (1), 80-95.

GREENWALD, G. S. (1967). Species differences in egg transport in response to exogenous estrogen. Anat. Rec. 157, 163-172.

GREENWALD, G. S. (1976). Effects of estrogen and progesterone on egg transport. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

GWATKIN, R. B. L. and BIGGERS, J. D. (1963). Histology of mouse Fallopian tubes maintained as organ cultures on a chemically defined medium. Int. J. Fert. 8, 453-457.

HAFEZ, E. S. E. (1973). Endocrine control of the structure and function of the mammalian oviduct. In: Handbook of Physiology. Eds. Greep, R. O. and Astwood, E. B. American Physiol. Soc., Washington D.C.

HAFEZ, E. S. E. (1976). Anatomical and physiological mechanisms of ovum transport. In: Ovum Transport and Fertility regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

HALBERT, S. A. and PATTON, D. L. (1979). Egg transport in the rabbit oviduct following ampullary resection and microsurgical end-to-end anastamosis. Fertil. Steril. 32, 687-690.

HALBERT, S. A., TAM, P. Y. and BLANDAU, R. J. (1976). Egg transport in the rabbit oviduct : The roles of cilia and muscles. Science 191, 1052-1053.

HAMNER, C. E. (1973). Oviductal fluid:composition and physiology. In: Handbook of Physiology. Eds. Greep, R. O. and Astwood, E. B. American Physiol. Soc., Washington D.C.

HARPER, M. J. K. and WALPOLE, A. L. (1967). A new derivative of triphenylethylene : Effect on implantation and mode of action in rats. J. Reprod. Fert. 13, 101-119.

HELM, G. (1981). Adrenergic and peptidergic neuromuscular mechanisms in the human Fallopian tube, with special regard to cyclic influences. Acta. Obstet. Gynecol. Scand. Suppl. 104.

HELM, G., HAKANSON, R., LEANDER, S., OWMAN, C., SJOBERG, N. O. and SPORRONG, B. (1982). Neurogenic relaxation mediated by vasoactive intestinal polypeptide (VIP) in the isthmus of human Fallopian tube. Regulatory Peptides 3, 145-153.

HELM, G., OTTESEN, B., FAHRENKRUG, J., LARSEN, J. J., OWMAN, C., SJOBERG, N. O., STOLBERG, B., SUNDLER, F. and WALLES, B. (1981). Vasoactive intestinal polypeptide (VIP) in the human female reproductive tract : Distribution and motor effects. Biol. Reprod. 25, 227-234.

HELM, G., OWMAN, C., SJOBERG, N. O. and WALLES, B. (1982). Quanitative pharmacological characterization of beta-receptors and two types of alpha-receptors mediating sympathomimetic smooth muscle response in the human Fallopian tube at various cyclic stages. Acta. Physiol. Scand. 114, 425-432.

HIGGS, G. W. and MOAWAD, A. H. (1974). The effect of ovarian hormones on the contractility of the rabbit oviductal isthmus. Can. J. Physiol. Pharmacol. 52, 74-83.

HODGESON, B. J. and EDDY, C. A. (1975). The autonomic nervous system and its relationship to tubal ovum transport - A reappraisal. Gynecol. Invest. 6, 162-185.

HODGESON, B. J. and TALO, A. (1978). Spike bursts in rabbit oviduct. II. Effects of estrogen and progesterone. Am. J. Physiol. 234, E439-443.

HOWE, G. R. (1976). Cholinergic mechanisms, oviductal motility and ovum transport. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

HUMPHREY, K. W. (1968A). Observations on transport of ova in the oviduct of the mouse. J. Endocrinol. 40, 267-273.

HUMPHREY, K. W. (1968B). The effects of oestradiol-3,17beta on tubal transport in the laboratory mouse. J. Endocrinol 42, 17-26.

HUMPHREY, K. W. (1976). Influence of estrogen and progesterone on oviductal function. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

HUMPHREY, K. W. and MARTIN, L. (1968). The effect of oestrogen and anti-oestrogens on ovum transport in mice. J. Reprod. Fert. 15, 191-197.

IRITANI, A., NISHIKAWA, Y., GOMES, W. R. and VANDEMARK, N. L. (1971). Secretion rates and chemical composition of oviduct and uterine fluids in rabbits. Jo. Anim. Sci. 33, 829-835.

JENSEN, R. P. S. (1978). Fallopian tube isthmic mucus and ovum transport. Science 201, 349-351.

JANSEN, R. P. S. (1980). Cyclic changes in the human Fallopian tube isthmus and their functional importance. Am. J. Obstet. Gynecol. 136 (3), 292-308.

JOHNS, A., CHLUMECKY, J., COTTLE, M. K. W. and PATON, D. M. (1975). Effect of chemical sympathectomy and adrenergic agonists on the fertility of mice. Contraception 11, 563-570.

JOHNS, A., MOLNAR, S. and PATON, D. M. (1976). Inhibition of spontaneous contractility of the human and the rabbit Fallopian tube by potassium. Am. J. Obstet. Gynecol. 124, 506-509.

JOHNS, A., RADICKE, D. A. and PAUERSTEIN, C. J. (1980). Effect of ovulation and progesterone or estrogen on the responses of the rabbit oviduct to nerve stimulation. Biol. Reprod. 22, 772-776.

KENDLE, K. E. (1969). Some pharmacological studies upon the factors controlling egg transport through the mouse oviduct. Ph.D. Thesis, London University.

KENDLE, K. E. (1976). Some biological properties of RMI 12,936, a new synthetic antiprogestational steroid. J. Reprod. Fert. 43, 505-513.

KENDLE, K. E. (1978). Further investigation of the antifertility effects in the rat of the antiprogestational steroid, RMI 12,936, and related compounds. J. Reprod. Fert. 52, 373-377.

KENDLE, K. E. (1979). Current investigations of antiprogestational steroids. In: Antihormones. Ed. Agarwal, M. K. Elsevier/North Holland Biomedical Press.

KENDLE, K. E. (1982). Advances in the study of antiprogestational agents. In: Hormone Antagonists. Ed. Agarwal, M. K. Walter De Gruyter, Berlin.

KENDLE, K. E. and LEE, B. (1980). Investigation of the influence of progesterone on mouse embryo transport by using anti-progestational steroids. J. Reprod. Fert. 58, 253-258.

KENDLE, K. E. and TELFORD, J. M. (1970). Investigations into the mechanism of the anti-fertility action of minimal doses of megestrol acetate in the rabbit. Brit. J. Pharmacol. 40, 759-774.

KOMATSU, M. and FUJITA, H. (1978). Electron-microscopic studies on the development and aging of the oviduct epithelium of mice. Anat. Embryol. 152, 243-259.

KORENAGA, M. and KADOTA, T. (1981). Changes in mechanical properties of the circular muscle of the isthmus of the human Fallopian tube in relation to hormonal domination and postovulatory time. Fertil. Steril. 36, 343-350.

KUHNEL, W. and BUSCH, L. C. (1981). Functional morphology of the oviductal mucosa and the endometrium as viewed by S.E.M. Biomed. Res. 2 (Supplement), 341-353.

LEAVITT, W. W., EVANS, R. W., OKULICZ, W. C., McDONALD, R. G., HENDRY, W. J. and ROBIDOUX, W. F. (1982). Progesterone inhibition of nuclear estrogen receptor retention. In: Hormone Antagonists. Ed. Agarwal, M. K. Walter De Gruyter, Berlin.

LEE, B. (1979). Investigation of the role of smooth muscle function in egg transport in the mouse oviduct. Ph.D. Thesis, C.N.A.A.

LEE, B. and KENDLE, K. E. (1979). The effect of reserpine, oestradiol and *in vitro* perfusion on oviduct calcium levels in the mouse during egg transport. J. Reprod. Fert. 55, 489-493.

LEESE, H. J. and ALDRIDGE, S. (1979). The movement of pyruvate, lactate and lactate dehydrogenase into rabbit oviductal fluid. J. Reprod. Fert. 56, 619-622.

LEESE, H. J., ALDRIDGE, S. and JEFFRIES, K. S. (1979). The movement of amino acids into rabbit oviductal fluids. J. Reprod. Fert. 56, 623-626.

LEESE, H. J., HUMPHERSON, P. G. and JEFFRIES, K. S. (1981). Two preparations for the study of the isolated rabbit oviduct. J. Reprod. Fert. 63, 249-255.

LEESE, H. J. and JEFFRIES, K. S. (1977). Evidence for the facilitated diffusion of glucose into rabbit oviductal fluid. J. Reprod. Fert. 51, 93-97.

LINDBLOM, B., HAMBERGER, L. and WIQVIST, N. (1978). Differentiated contractile effects of prostaglandins E and F on the isolated circular and longitudinal smooth muscle of the human oviduct. Fertil. Steril. 30, 553-559.

LINDBLOM, B., LJUNG, B. and HAMBERGER, L. (1979). Adrenergic and novel non-adrenergic neuronal mechanisms in the control of smooth muscle activity in the human oviduct. Acta. Physiol. Scand. 106, 215-220.

LINDBLOM, B., WILHELMSSON, L. and WIQVIST, N. (1979). The action of prostacyclin (PGI_2) on the contractility of the isolated circular and longitudinal muscle layers of the human oviduct. Prostaglandins 17, 99-104.

LIPPES, J., KRASNER, J., ALFONSO, L. A., DACALOS, E. D. and LUCERO, R. (1981). Human oviductal fluid proteins. Fertil. Steril. 36, 623-629.

LUTWAK-MANN, C. (1955). Carbonic anhydrase in the female reproductive tract. Occurrence, distribution and hormonal dependence. J. Endocrin. 13, 26-38.

MANCHANDA, S. K., CHOUDHARY, R. R., SAKHUJA, D., NAYAR, U. and SENGUPTA, J. (1979). Receptive relaxation and post-ovulatory motility pattern of the oviduct on concious rabbits. Ind. J. Physiol. Pharmacol. 23, 185-192.

MAREN, T. H. (1967). Carbonic anhydrase : Chemistry, physiology and inhibition. Physiological Reviews 47, 749-781.

MARSAFY, Y. M. and HAFEZ, E. S. E. (1979). Post-coital changes in isthmic contractility during oviductal sperm transport in the rabbit. Archives of Andrology 3, 211-217.

MARSHALL, J. M. (1976). Studies of oviductal contractility. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

MARSHALL, J. M. (1981). Effects ovarian steroids and pregnancy on adrenergic nerves of uterus and oviduct. Am. J. Physiol. 240, C165-C174.

MARTIN DEL RIO, R. (1981). Gamma-animobutyric acid system in rat oviduct. J. Biol. Chem. 256, 9816-9819.

MASTROIANNI, L. (1976). Potentials for fertility regulation at the tubal level. Contraception 13 (4), 447-459.

MASTROIANNI, L., BEER, F., SHAH, U. and CLEWE, T. H. (1961). Endocrine regulation of oviduct secretions in the rabbit. Endocrinology 68, 92-100.

MASTROIANNI, L. and WALLACH, R. C. (1961). Effect of ovulation and early gestation on oviduct secretions in the rabbit. Amer. J. Physiol. 200, 815-818

MENEZO, Y., BREMOND, A., KHATCHADOURIAN, C., ORGIAZZI, A. and RUDIGOZ, R. (1981). Culture "in vitro" de l'oviducte de femme, de rate et de lapine. Resultats preliminaries. (French language). J. Gyn. Biol. Repr. 10, 651-654.

MICHAEL, S. D. (1976). Plasma prolactin and progesterone during the oestrus cycle in the mouse. Proceedings of the Society for Experimental Biology and Medicine 153, 254-257.

MURR, S. M., STABENFELDT, G. H., BRADFORD, G. E. and GESCHWIND, I. (1974). Plasma progesterone during pregnancy in the mouse. Endocrinology 94, 1209-1211.

NATOW, A. J. and MARSHALL, J. M. (1979). Effects of PGE_2 on neurogenic activity in oviducts from estrous and hCG treated rabbits. Biol. Reprod. 21, 787-792.

NILSSON, O. (1957). Observations on a type of cilia in the rat oviduct. J. Ultrastructure Research 1, 170-177.

NOREIGA, C. and MASTROIANNI, L. (1969). Effect of carbonic anhydrase inhibitor on tubal ova. Fertil. Steril. 20, 799-804.

ODOR, D. L. (1953). Electron microscopy of the rat oviduct. Anatomical Record 115, 434-435.

ODOR, D. L., GADDUM-ROSSE, P. and RUMERY, R. E. (1983). Secretory cells of the oviduct of the pig-tailed monkey, macaca nemestrina, during the menstrual cycle and after oestrogen treatment. Am. J. Anat. 166, 149-172.

ORTIZ, M. R., VILLALON, M. and CROXATTO, H.B. (1979). Ovum transport and fertility following postovulatory treatment with estradiol in rats. Biol. Reprod. 21, 1163-1167.

PATON, D. M. (1976). Summary of discussions on autonomic mechanisms in ovum transport. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

PAUERSTEIN, C. J., ANDERSON, V., CHATKOFF, M. L. and HODGESON, B. J. (1974). Effect of estrogen and progesterone on the time-course of tubal ovum transport in rabbits. Am. J. Obstet. Gynec. 120, 299-308.

PAUERSTEIN, C. J. and EDDY, C. A. (1979). Morphology of the Fallopian tube. In: The Biology of the Fluids of the female genital tract. Eds. Beller, F. K. and Schumacher, G. F. B. Elsevier/North Holland.

PAUERSTEIN, C. J., FREMMING, B. D. and MARTIN, J. E. (1970). Influence of progesterone and alpha-adrenergic blockade upon tubal transport of ova. Gynec. Invest. 1, 257-267.

PAUERSTEIN, C. J. and WEINBERG, L. C. (1980). Failure of castration to modify estradiol-induced tube-locking of rabbit ova. Fertil. Steril. 34, 588-592.

PAUERSTEIN, C. J. and WOODRUFF, J. D. (1967). The role of the indifferent cell of the tubal epithelium. Am. J. Obstet. Gynec. 98 (1), 121-125.

PERKINS, J. L. (1974). Fluid flow of the oviduct. In: The Oviduct and Its Functions. Eds. Johnson, A. D. and Foley, C. W. Academic Press, New York.

POLIDORO, J. P., HEILMAN, R. D., CULVER, R. M. and REO, R. R. (1976). Effects of adrenergic drugs or denervation on ovum transport in rabbits. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M. Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

PURI, R. K. and ROY, S. K. (1980). Progesterone nuclear receptors in different parts of rabbit Fallopian tube and uterus during ovum transport. Ind. J. Exp. Biol. 18, 553-556.

RAESS, B. U. and VINCENZI, F. F. (1980). The isolated-perfused rabbit oviduct : A simple experimental procedure. Journal of Pharmacological Methods 3, 267-273.

REINIUS, S. (1969). Morphology of oviduct, gametes and zygotes as a basis of oviductal function in the mouse. Ph.D. Thesis, University of Uppsala.

REINIUS, S. (1970). Morphology of oviduct, gametes, and zygotes as a basis of oviductal function in the mouse. I. Secretory activity of oviductal epithelium. Int. J. Fertil. 15, 191-209.

RESKO, J. A. (1969). Endocrine control of adrenal progesterone secretion in the ovariectomized rat. Science 164, 70-71.

RIDDICK, D. H. (1975). The composition of rabbit oviducal fluid collected continuously throughout gestation. J. Reprod. Fert. 43, 563-566.

ROBELERO, L. S. and GARAVAGNO, A. C. (1979). Effect of oestradiol-17beta and progesterone on oviductal transport and early development of mouse embryos. J. Reprod. Fertil. 57, 91-95.

RODRIGUEZ-MARTINEZ, H., EINARSSON, S., LARSSON, B., AKUSU, M. and SETTERGREN, I. (1982). Spontaneous motility of the pig oviduct in vitro. Biol. Reprod. 26, 98-104.

RUMERY, R. E., GADDUM-ROSSE, P., BLANDAU, R. J. and ODOR, D. L. (1978). Cyclic changes in ciliation of the oviductal epithelium in the pig-tailed macaque (macaca nemestrina). Am. J. Anat. 153, 345-366.

SAID MOUNIB, M. and CHANG, M. C. (1965). Metabolism of endometrium and Fallopian tube in the estrous and the pseudopregnant rabbit. Endocrinology 76, 542-546.

SHAIKH, A. A. and SHAIKH, A. S. (1975). Adrenal and ovarian steroid secretion in the rat estrous cycle temporally related to gonadotropins and steroid levels found in peripheral plasma. Endocrinology 96, 37-44.

SHIPSTONE, A. C., BAJPAI, V. K., GUPTA, D. N. and KARKUN, J. N. (1974). Studies on the ultrastructure of the Fallopian tube : Part One - Changes in the ciliated cells of the rabbit Fallopian tube during ovum transport. Ind. J. Exp. Biol. 12, 115-122.

STONE, S. L. and HAMNER, C. E. (1975). Biochemistry and physiology of oviductal secretions. Gynecol. Invest. 6, 234-252.

TAKAHASHI, M., KOGO, H. and AIZAWA, Y. (1979). Effect of intra-uterine administration of indomethacin on ovarian steroids. Japan. J. Pharmacol. 29, 684-686.

TALO, A. (1980). Myoelectrical activity and transport of unfertilised ova in the oviudct of the mouse in vitro. J. Reprod. Fert. 60, 53-58.

TALO, A. and HODGESON, B. J. (1978). Electrical slow waves in oviductal smooth muscle of the guinea-pig, mouse and immature baboon. Experientia 34 (2), 198-200.

TALO, A. and HODGESON, B. J. (1978). Spike bursts in rabbit oviduct. 1. Effect of ovulation. Am. J. Physuiol. 234, E430-438.

TENNISWOOD, M., BIRD, C. E. and CLARK, A. F. (1982). The role of androgen metabolism in the control of androgen action in the rat prostate. Molecular and Cellular Endocrinology 27, 89-96.

THOMAS, C. M. G., BASTIAANS, L. A. and ROLLAND, R. (1980). Effects of prostaglandin F_2alpha, indomethacin and estradiol on ovum transport and pregnancy in the golden hamster. Biol. Reprod. 23, 687-698.

TOJO, S., MOCHIZUKI, M., KANAWA, S., MIKAMI, K., TSUCHIHASHI, T. and SHIMURA, T. (1974). In vitro perfusion of the human utero-tubo-ovarian unit. Am. J. Obstet. Gynecol. 118, 119-129.

TONPE, N. and LINDBLOM, B. (1979). The influence of prostaglandin synthetase inhibition of the spontaneous contractile activity and induced responses of the human oviduct. Acta. Physiol. Scand. 107, 181-183.

VERDUGO, P. (1980). Ca^{++} dependent hormonal stimulation of ciliary activity. Nature 283, 764-765.

VERHAGE, H. G., BAREITHER, M. L., JAFFE, R. C. and AKBAR, M. (1979). Cyclic changes in ciliation, secretion and cell height of the oviductal epithelium in women. Am. J. Anat. 156, 505-522.

VEROUGO, P., BLANDAU, R. J., TAM, P. Y. and HALBERT, S. A. (1976). Stochastic elements in the development of deterministic models of egg transport. In: Ovum Transport and Fertility Regulation. Eds. Harper, M. J. K., Pauerstein, C. J., Adams, C. E., Coutinho, E. M., Croxatto, H. B. and Paton, D. M. Scriptor, Copenhagen.

VERDUGO, P., LEE, W. I., HALBERT, S. A., BLANDAU, R. J. and TAM, P. Y. (1980). A stochastic model for oviductal egg transport. Biophysical Journal 29, 257-270.

VERDUGO, P., RUMERY, R. and TAM, P. Y. (1980). Hormonal control of oviductal ciliary activity : Effects of prostaglandins. Fertil. Steril. 33 (2), 193-196.

WELCH, B. L. (1947). The generalisation of "Students" problem when several different population variances are involved. Biometrika 34, 28-34.

WEST, N.B., VERHAGE, H. G. and BRENNER, R. M. (1976). Suppression of the estradiol receptor system by progesterone in the oviduct and uterus of the cat. Endocrinology 99, 1010-1016.

WIDDICOMBE, N. B., JOHNS, A. and PATTON, D. M. (1977). Responses of the monkey oviduct to transmural electrical stimulation and to drugs. J. Reprod. Fert. 50, 141-144.

WIDDICOMBE, J. H. and PATON, D. M. (1979). Fine structure and innervation of smooth muscle in the monkey oviduct. Gynecol. Obstet. Invest. 10, 221-230.

WIMSATT, W. A. and WALDO, C. M. (1945). The normal occurrence of a peritoneal opening in the bursa ovarii of the mouse. Anat. Rec. 93, 47-57.

WU, J. T., DICKMANN, Z. and JOHNSON, D. C. (1971). Effects of ovariectomy or hypophysectomy on day one of pregnancy on development and transport of fertilised rat eggs. J. Endocr. 49, 507-513.

APPENDICES

APPENDIX I

The basis of statistical analysis throughout this work has been the assumption that left and right oviducts from the same animal can be treated as independent samples. This was based on the work of Kendle and Lee (Unpublished Observations) in which inspection of the data seemed to show that within animal variation was as great as between animal variation. The availability of the large volume of data prepared for Chapter 1 of this work presents an opportunity to examine this hypothesis and to analyse further in the light of any conclusions reached.

The data was analysed using the Analysis of Variance for a nested design, presentation Table I, using a comparison of left and right oviducts (as within animal) and between animal, removing the time effect. The contribution of within animal and between animal variances to the total are significantly different ($P<0.001$). This therefore means that the variance within animals (i.e. left or right) is less than between animals (i.e. animals within each time group) and so there would seem to be a possible correlation in position between the two oviducts in each in each animal (with left/right differences not being representative of random error). However, the anatomical constraints of the ampullary-isthmic and utero-tubal junctions, which retain embryos at locations relatively constant for each animal and each oviduct for long periods of time, could create difficulties in interpretation of results. Transport of embryos along the isthmus is a rapid phenomenon, during which the mean position of embryos is free from the constraints mentioned. Therefore, a nested Analysis of Variance was prepared for only those groups involved in the rapid isthmic transport phase - 09:00h Day 2 to 20:00h Day 2 inclusive, and the table is presented in Table II. Again the contribution to the

variance of the within animal source is significantly less than from the between animal source ($P<0.001$) showing that a significant correlation exists between left and right oviducts of the same animal, at all times during the embryo transport phase.

This conclusion must therefore modify future presentation of data. Since left and right oviducts from the same animal may not be called independent samples, combination of the two figures to produce a single mean figure for each animal would be advantageous. Re-examination of the control data presented in Chapter 1 and used in this appendix has also been undertaken. Production of a single mean figure for each animal halves the group size, with consequent effects on standard errors. This newly generated data has been further examined. Presented in Table III is the Analysis of Variance table obtained using a regression analysis program developed by Mr. A. W. Wilson, School of Mathematics, R.G.I.T. The regression analysis of the raw data shows a significant straight line model with a positive slope, as would be expected. However, examination of the residuals shows that these do not form a random sequence but alternate in groups of positive and negative sign, with a few exceptions. This demonstrates the inadequacy of the straight line model and is consistent with a sigmoid shaped model. A transformation of the dependent variable to produce a straight line regression model on the transformed scale is therefore required. For this type of data, involving proportions (%'s), the arcsin or angular transformation is recommended (A. W. Wilson, personal communication; Snedecor and Cochrane, 1967). Table IV shows the Analysis of Variance for the data after all group means have been converted by an arcsin transformation. Again a significant linear model is seen, but in this case the residuals are closer to forming a random sequence, suggesting the model provides a good fit. Additionally, the percentage variation explained by the model has

increased from 87.8% to 90.8%. It is therefore possible to produce a mathematical representation of the relationship between mean % oviduct traversed by embryos and time. From this data, the formula, with appropriate 95% confidence limits, is given in Table V. Further manipulation of this data will involve the production of a formula for the prediction of time from the mean % with appropriate confidence limits, and further refining the relationship, involving the generation of new data.

I am indebted to Mr. A. W. Wilson of the School of Mathematics R.G.I.T. for his expert guidance on the statistical procedures used here.

Reference

Snedecor, G. W. and Cochrane, W. G. (1967)
"Statistical Methods"
Iowa State University Press

Table I - Analysis of Variance table for control data presented in Chapter 1, breaking down between animal and within animal variation.

Source	DF	SS	MS	F	P
Time	38	301833	7942	186	P<0.001
Between animals (within time)	171	19765	116	2.71	P<0.001
Within animals	210	8952	43		
Total	419	330551			

Table II - Analysis of Variance table for control data from 09:00h Day 2 to 20:00h Day 2 - the isthmic transport phase - breaking down between animal and within animal variation.

Source	DF	SS	MS	F	P
Time	11	2721	247	2.81	P<0.001
Between animals (within time)	48	9877	206	2.34	P<0.001
Within animal	60	5281	88		
Total	119	17880			

Table III - Analysis of Variance table for the untransformed control data, converted to single animal mean figures.

Source	DF	SS	MS	F	P
Regression	1	141187	141187	1497.3	P<0.001
Residual	208	19613	94.3		
Total	209	160799			

Table IV - Analysis of Variance table for the arcsin transformed control data, previously converted to single animal mean figures.

Source	DF	SS	MS	F	P
Regression	1	22.9	22.9	2060.1	P<0.001
Residual	208	2.3	0.011		
Total	209	25.2			

Table V - Mathematical representation of the relationship between mean % oviduct traversed by embryos, and time, using control data previously converted to single animal mean figures.

$$Y = 100\ SIN^2\ (0.01723X + 0.1293)$$

where Y = mean %, X = time (hours)

95% confidence limits for the mean Y_K given X_K :-

$$100\ SIN^{-2}\left[(0.01723X+0.1293+/-1.97\sqrt{0.011}\sqrt{\frac{1+(X_K-50.55)^2}{210\ (209\times19.2157^2)}}\right]$$

95% confidence limits for individual Y_K given X_K :-

$$100\ SIN^{-2}\left[0.01723X+0.1293+/-1.97\sqrt{0.011}\sqrt{1+\frac{1+(X_K-50.55)^2}{210\ (290\times19.2157^2)}}\right]$$

APPENDIX II

Post-Graduate Courses

A programme of guided reading, lectures and tutorials was undertaken.

Lecture and tutorial times were:-

Reproductive physiology/pharmacology	20 hours
Principles of experimental design and analysis of results	12 hours
Computer programming and use of advanced statistical packages	12 hours

In addition, a period of 3 weeks was spent in the industrial environment of Beecham Pharmaceuticals, during which time the principles and practice of industrial research and development were examined in general, and specific expertise in the process of radio-immunoassay was gained.

APPENDIX III

Communications

VIIIth Dutch-British Endocrine meeting, August, 1982, Noordwijkerhout, The Netherlands. A poster presentation, "Investigation of the Role of Gonadal and Extra-Gonadal Progesterone Secretion in Tubal Transport of Eggs in Mice", K. L. Grieve and K. E. Kendle.

Summer meeting of the Society for the Study of Fertility, July 1983, Manchester. An oral communication, "Embryo transport in Mouse Oviducts in vitro", K. L. Grieve, K. E. Kendle and K. M. Manson.