another possible origin of cleft palates. The existence of epithelial pearls in the line of fusion of the human palate (though not in lower mammals) and their presence in the shelves of a human fetus with cleft palate could be interpreted as evidence of midline breakdown of the fused palate. However, further evidence is needed.

Finally, the shelves may move to the horizontal at the right time, but fail to meet because the head is too wide. No proven case of this situation was cited. Possible examples may be the cleft palate following treatment with folic acid antagonists (D. G. Trasler) or associated with hypertelorism in man.

In summary, palate closure is a complicated process involving synchronized interactions of shelf, tongue, jaw, and head. Major interference at various points of the system either by specific environmental agents or by mutant genes can bring about failure to close. Study of these examples as well as further study of the normal embryology of closure may lead to further understanding of the process. It is likely that many "spontaneous" clefts in man may result from interaction of a large number of minor genetic and environmental factors which together cause delay in movement of the shelves to a point at which, when they do come up, they are too far apart to meet. Efforts to reduce the frequency of cleft palate in man should be directed not only to a better understanding of the process of closure and to the identification of specific environmental teratogens, but also to learning how to promote early shelf closure, thus reducing the probability that the embryo will reach the threshold of abnormality (F. C. Fraser). Much remains to be done.

This workshop was convened by the Oral-Facial Growth and Development Program, Extramural Programs, National Institute of Dental Research. (NIDR), Bethesda.

In attendance were: F. C. Fraser, McGill University, Montreal (chairman); R. Gerstner, New York University; M. C. Johnson, University of Toronto; B. Kraus, University of Pittsburgh (co-chairman); K.-S. Larsson, Karolinska Institute, Stockholm; I. Monie, University of California Medical Center, San Francisco; M. Pourtois and V. Vargas, University of Pittsburgh; A. J. Steffek, NIDR; D. G. Trasler, McGill University; A. C. Verrusio, National Institute of Neurological Diseases and Blindness; and B. E. Walker, University of Texas Medical Branch, Galveston. K. Hisaoka and R. C. Greulich (NIDR) attended as observers.

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Mammalian Oviduct: International Symposium

Progress has been made within the last decade in the biology of the oviduct. Such progress resulted from modern techniques and instrumentation in microanatomy, neurophysiology, genetics, endocrinology, biochemistry, pharmacology, and biophysics. In order to familiarize investigators in biological and health sciences with the application of techniques of these disciplines and the results to date, an international conference on the Mammalian Oviduct was held at Washington State University, Pullman, 31 July–4 August 1967. Participants were from England, the Netherlands, Puerto Rico, Scotland, Sweden, and the United States.

Oviductal organogenesis in many mammalian species is strikingly similar. During late fetal development, however, structural differences among species begin to become apparent. In in vitro studies, Dorothy Price and her co-workers showed that the fetal oviduct of the guinea pig fails to respond to testicular and adrenogenic hormones, and early morphogenesis does not depend upon specific hormonal stimulation.

The development of Müllerian ducts during fetal life is not dependent on maternal, placental, or fetal hormones for maintenance and early development. The oviductal segment of the primitive Müllerian duct can show much hormone-independent growth, coiling, and epithelial differentiation. The stage of development of the oviduct of the neonate at birth suggests prenatal hormone influence during late fetal life. The fetal ovaries and adrenals, which become metabolically active, may stimulate the fetal oviduct.

Improved methods of fixation for light and electron microscopic studies involve perfusion techniques for rapid fixation in 2.5 percent solution of glutan aldehyde in Sørensen phosphate buffer *p*H 7.4. *In situ* preservation is essential to establish the width of oviductal lumen or to evaluate the anatomical relationship between the oviductal epithelium and the developing embryo. Certain cyclical changes in the structure of the oviduct may or may not relate to function, but in some instances, such changes promise insight into some basic biologic processes. The elaborate lymphatic system of the oviduct seems to respond to steroid hormones. There is an increasing awareness that the uterus, and perhaps even the oviduct, plays an important role in the control of ovarian function. Both luteolytic and luteotrophic effects of uteri and of uterine contents (IUD or embryos) have been recorded. These effects are frequently local. Sometimes they do not seem to be mediated systemically. The possible local effect may be resolved by more thorough studies of the oviducto-uterine vascular, lymphatic, and nervous systems.

Histochemical techniques have been used to study the distribution of metabolites and enzymes, and the metabolic patterns of different regions of the oviduct. Man has more glycogen in the inner circular muscle than in the outer longitudinal layer of the oviduct. The amount of glycogen shows cyclical changes, being low postmenstrually, rather high postovulatory, and particularly high during pregnancy and in the early puerperium. These variations are very similar to those in the human myometrium. Succinicdehydrogenase, DPN-diaphorase, amylophosphorylase, and other enzymes have been demonstrated in oviductal muscle but evidence of a correlation to the sexual cycle is lacking. Histochemical techniques have been used to study the nervous regulation of the oviductal musculature.

In the rhesus monkey, ciliogenesis occurs in the oviduct during the normal menstrual cycle, particularly in the fimbriae. Deciliation of the fimbriae in the luteal phase depends on the presence of a functional corpus luteum. The ampulla shows no such dramatic deciliation. R. M. Brenner showed that after ovariectomy, the ampulla loses most of its cilia. Thus, the real difference between the fimbriae and ampulla is one of degree of responsiveness to changes in blood levels of estrogen and progesterone. Ovariectomy in the rabbit does not cause deciliation of the oviduct. Comparative studies on the uptake of micromolecules during ciliogenesis may use electron microscopy, autoradiography, microbiochemical analysis and the isolated fimbriae in an abdominal well.

Brenner raised several questions: Is the rate of ciliogenesis dose-dependent? What minimal dose provokes ciliogenesis? Will this minimal dose be different for the fimbriae versus the ampulla? Will an extremely high dose cause each cilium to grow longer, or will more ciliated cells regenerate? How do cilia grow; do they add new proteins at their growing tips or at their bases? If they add new proteins at the base, how do the old proteins migrate up the growing shaft? What is the role of the old centriole in the replication of the new crop of basal bodies? Will inhibitors of RNA synthesis and protein synthesis prevent cilia regeneration in mammalian oviducts, as they do in protozoa? Is there a way of specifically inhibiting ciliogenesis without interfering with other cell functions? If so, would it be of any contraceptive value? What are the biochemical changes associated with each phase of ciliogenesis? Are some key enzymes synthesized at specific times during growth? Is there a characteristic pattern of enzymogenesis?

The structure of the uterotubal junction (UTJ) varies remarkably between species. Among litter-bearing species, four types of UTJ are found. In the rabbit and the pig, rather complicated projections into the uterine lumen form a rosette-like structure. In the hamster, the lumen of the UTJ resembles a corkscrew. The rat and the mouse have a papilla and an arched coil. The dog has a mound with a long slit ostium. In single-bearing species, two variations are found. In cattle and sheep there is a flexure, but no projecting folds from the isthmus. Primates have a long intramural portion without projecting folds.

The role of the UTJ in normal reproduction remains obscure. Some evidence suggests that it controls the entry of eggs into the uterus and the transport of sperm into the oviduct.

To elucidate the function of the UTJ, areas of comparative research that may be beneficial are: (i) neural, vascular, and lymphatic supply of the UTJ; (ii) histological and histochemical changes following copulation at the time of egg transport to the uterus and following ovariectomy or administration of exogenous steroids; (iii) the architecture and fine structure of the muscle fibers in the circular and longitudinal musculature in relation to that of the mucosal folds and the myometrium; (iv) anatomical and physiological function of the ligaments attaching the oviduct to the uterus; the endocrine and pharmaco-physiological aspects of these ligaments; (v) relative importance of UTJ, in comparison with other parts of the oviduct, in controlling entry of eggs into the uterus; (vi) mechanisms involved in transport of sperm and eggs through the UTJ; (vii) effects of sexual cycle stages, ovarian function, and exogenous hormones on the opening and closure of the UTJ; and (viii) neural control and neuro-endocrine control of the UTJ.

The functions of the oviduct include the reception of the egg from the site of ovulation into the oviductal lumen, conditioning of the sperm before fertilization, the fertilization process, cleavage of the zygote through several cell divisions, and the transport of the developing zygote into the uterus after a discretely timed interval. These functions may be conveniently divided into two categories: oviductal transport of gametes, and provision of a suitable environment for the gametes and the early development of the embryo.

The oviduct functions periodically and only relatively briefly the first three post-ovulatory days. It is usually assumed that estrogens are directly and primarily responsible for increased muscle activity. In the normal preovulatory animal, estrogens are present at physiological levels and the oviduct is quiescent. At ovulation, the mesotubarium and oviduct contract vigorously. The administration of progesterone causes oviductal activity similar to that which occurs at the time of an induced ovulation.

The isthmus is endowed with a rich supply of noradrenergic nerve endings that decrease in number following section of the hypogastric nerve. Norepinepherine increases muscular tone in the region. The kymographic technique used by J. Brundin has allowed evaluation of the effect of various pharmacological agents on the isthmus. The exact mechanism for the temporary closure of the isthmus deserves further exploration. The changes induced by alterations in the endocrine environment may affect the activity of the adrenergic nerves to the oviduct, as well as the effect of adrenergic nerve impulses on the smooth muscles. There is evidence that it comes under endocrine control but what is the relationship between neural influences and the hormonal status of the ovary? What influences the accurately timed process by which eggs are finally transported into the uterus?

Pathologic syndromes of the oviduct such as tubal pregnancy, congenital anomalies, and tumors are very common in women, but rare in animals. Venereal diseases are spreading faster in man than in domestic and wild animals. While inflammatory lesions may occur more often in animals than hitherto thought, neoplasms are presumably less common, since most animals are not allowed to attain their full life span.

The mechanism by which eggs are transported into the oviductal ostium varies considerably among different species. At ovulation, the fimbriae contact the ovary. They move back and forth over the ovulating follicles, practically assuring egg pickup. R. J. Blandau documented this action with cinematography. Eggs, as they are released with their accompanying granulosa cells, remain adherent to the ruptured follicle unless they are swept free by the fimbriae. That a suction mechanism is not essential to egg pickup in the rabbit has been demonstrated by the ability of the oviducts, ligated just beyond the fimbriae, to pick up eggs. The activity of the mesovarium and mesosalpinx is responsible for the movement of the fimbriae over the surface of the ovary. The muscular elements in these structures contract vigorously and rhythmically. The sequence of events that cause this movement is unknown.

Once the egg is safely within the oviductal ostium, it passes within a few minutes to the ampullary-isthmic junction. During the transport, muscular contractions are vigorous. J. L. Boling suggested that increased contractibility of the rabbit's oviduct is due to estrogen withdrawal rather than estrogen, as previously supposed. Administering progesterone augmented oviductal contractions. In Ruth Rumery's studies of the fetal mouse oviduct, estradiol suppressed muscular activity in organ culture.

Fertilization occurs within the ampulla of the oviduct and the egg is retained within the oviduct for about 3 days. There are exceptions to this rule. For example, in marsupials and monotremes, fertilization occurs at the ovarian level. In some species, such as swine, eggs are transferred more rapidly from the oviduct. Interest in the mechanism for retention of eggs has focused attention on the isthmus and uterotubal junction. Several mechanisms are responsible for the transport of ova and embryos. Examples include the muscular contractions of the tunica muscularis and of the muscularis mucosa, volume changes of the lamina propria, the beating of the kinocilia, and the secretory activity of the lamina epithelialis.

The ampullary-isthmic junction and the uterotubal junction play a major role in regulating the rate of egg transport. Since the isthmus has a narrow lumen, the ampullary-isthmic junction has to widen by relaxing the muscular coat or by diminishing the mucosal folds to allow the transport of the egg.

The sphincter-like structure of the uterotubal junction may depend on one or more of four characteristics: (i) the connecting ligaments may cause flexure of the UTJ in some species, such as ruminants; (ii) the extent of the intramural portion and surrounding uterine muscle; (iii) the arrangements of circular and longitudinal musculature at and near the junction; and (iv) the presence of muscle spirals.

The quantity and quality of the oviductal secretion vary with the reproductive cycle. Species differ in the degree of cyclical changes. The peak of fluid secretion occurs at ovulation. The increase in oviductal fluid following the administration of estrogen may be a refection of the pronounced changes in growth and differentiation of the oviduct. Hence, comparative biochemical studies of the changes in the composition of oviductal fluid—and the oviduct itself—are important in elucidating the functions of oviductal secretions.

Experimental evidence supports the hypothesis that transudation contributes much to oviductal secretions: (i) the dry matter content and osmolarity of estrogen-treated rabbit fluid is low; (ii) estrogen greatly enhances secretion rates; (iii) the oviduct is edematous with the lymphatic system greatly expanded at estrus.

The functional significance of oviductal secretion should be considered in relation to the gametes and the developing embryo. Oviductal secretions may function in several ways. They may be necessary for the final maturation of sperms and eggs, for the essential preludes to fertilization. Oviductal secretions may provide nutrients for the oviductal embryos. The effects of oviductal secretions may extend to the endometrium rather than exclusively influence the environment of the oviduct. Perhaps the oviductal secretions are unessential for embryonic development and merely represent changes in a secondary target organ to estrogen and progesterone. Further investigations should reveal which parameters of oviductal secretions are most important.

Methods are now available for the continuous collection of oviductal fluid over prolonged periods, species comparisons of oviductal fluid composition, and the use of oviductal secretions for in vitro fertilization, embryo development, and metabolic studies of both sperm and eggs.

Speculation is rampant but we do not know how sperm are transported through the oviduct. More precise observations must be made in a variety of animals to resolve the conflicts arising from species variations in the mechanisms involved.

Blandau raised several questions for descriptive and quantitative studies. What is the role of the segmental peristaltic and antiperistaltic muscular contractions in sperm transport? What controls the specific types of contraction observed in the ampullary and isthmic zones? Are sperm transported by different mechanisms in the isthmic and ampullary segments of the oviduct? What is the arrangement and significance of the oviductal folds in the rotation of eggs and their transport in different animals? What is the arrangement of the ciliated cells, and how does their beat affect sperm movement? Does sperm motility itself play any role in transport or is it effective only at various barriers such as the cervix, uterotubal junction, or ampullary-isthmic junction? Do local physical or chemical factors determine the directional movements of sperm within the oviducts? Do the chemical and physical characteristics of the oviductal fluids vary at different times of the cycle and may these differences affect sperm transport? Does the ampullary-isthmic junction control the number of sperm that reach the site of fertilization? Do rhythmic contractions of the mesosalpinx or other membranes attached to the oviduct play any role in sperm transport? What are the specific effects of various hormones and other pharmacologic agents on the rate, amplitude, and pattern of oviductal contractions?

The sperm are trapped temporarily in pocket-like diverticulae at the uterotubal junction of some species. Hafez emphasized possible physiological significance of these pockets in sperm capacitation and gamete transport. Factors necessary for capacitation of sperm are present in the oviduct and are specific for the female reproductive tract. In vitro capacitation of rabbit sperm may be prohibited in female tract secretions because the factors necessary deteriorate rapidly or because the sperm cannot capacitate without intimate contact with the epithelial cells of the female genital tract. The factors in oviductal fluid essential for in vitro fertilization may degenerate rapidly when exposed to an external environment.

The egg is structurally and metabolically more complex than the somatic cell. Much more is known about the physiology of the egg than about the specific causes of its morphogenesis and differentiation, and the constitution of the oviductal egg at the molecular level. The precise arrangement of the constituent proteins and lipids is not known. Little is known about the energy sources in mammalian oviductal eggs, which contain very small amounts of yolk material.

Comparative biochemical studies are needed in: techniques for identifying metabolites and enzymes; electron microscopy for the study of the physical and biochemical properties of the egg vitellus; the mechanisms by which the blastomeres differentiate biochemically and morphologically; the role of biochemical inhibitors in the unfertilized egg, and in eggs with retarded cleavage rate; how the differentiating cells become properly adjusted to the *milieu intérieur*; how their spatial distribution is determined, and how they differentiate into tissues and organs characteristic of the species.

Cleavage is the most characteristic activity of oviductal eggs. There are breed and strain differences in cleavage rate. Reciprocal crosses between slow and fast cleaving strains have shown that genes operate during cleavage. Fluorescence studies have shown that serum antigens are taken up by one-celled eggs, and can be demonstrated in all subsequent preimplantation stages. The oviduct is a favorable site for the cleaving egg, which should not stay there too long.

Several procedures have been used to interfere with the normal process of prenatal development. Some are: alteration of embryonic development by irradiation during cleavage, injection of macromolecules in the eggs with a micropipette, destruction of blastomeres, and the augmentation of blastomere number by fusing two embryos to form a single chimeric individual.

Several preparations of gonadotrophins (FSH, LH, PMS, and HCG) have been used to induce superovulation and produce many fertilized eggs from the donor females. Hormonal methods have also been used to synchronize the estrous periods of recipient females. Investigations of egg transfer in different species of laboratory animals provided a useful background for practical applications in the improvement of livestock and perhaps also in human gynecological practice. Eggs have been transferred successfully in pigs, sheep, and cattle by surgical and non-surgical methods. These methods will be refined in the future to increase their usefulness. The application of egg transfer in research is not limited to studies on egg maturation, fertilization, and development. The range of the subject already includes such wider aspects of general biological interest as interactions between the embryo and uterus, immunological relationships between the conceptus and mother, and developmental genetics.

Brinster has indicated that the basic medium for the cultivation of oviductal mammalian embryos might consist of: (i) the salts in blood plasma, or Krebs-Ringer bicarbonate; (ii) a sodium bicarbonate buffer with a concentration of approximately 25 mmole; (iii) an atmosphere containing 5 percent CO_2 in the gas phase; (iv) a pH of approximately 7.4 which would be the result of items (ii) and (iii); (v) a protein concentration of 1 to 10 mg/ml; (vi) a glucose concentration of 1 mg/ml; (vii) a pyruvate concentration of about $5 \times 10^{-4}M$. Supplementary compounds such as serum, embryo extract, lactate, amino acids, vitamins, and cofactors could be added. The use of complex natural or undefined substances in the medium makes it hard to determine the exact effect of other omissions or additions to the medium.

If the current rate of increase continues, the world's population will double between 1960 and 1995, and reach 6 billion before 2000 A.D. The predicted increase is based on the UN-calculated world growth rate of 2 percent per year. Southam of the Ford Foundation has shown that many countries began family planning programs in the last few years. In the 13 developing countries with populations of 25 million or more, at least 11 have national programs or family planning activities in government facilities. Many men and women in developing countries generally accept the concept of family planning. A clear understanding of oviductal physiology is needed for clinical treatment of sterility and for contraception because of the critical early phases of reproduction in the oviduct and the influence that substances transported to the oviductal lumen could have on conception.

Ligation of the oviduct is a fine procedure for sterilization rather than family spacing. The surgery requires a 6- to 7-day hospitalization—an economic setback, and it is reversible in no greater than 30 percent if the mother changes her mind. The mortality rate is less than 1 percent. The morbidity is considerably higher than one expects, and some of the symptoms that follow are congested pelvic pain and hypermia in a small percentage of cases. This procedure is very important in some parts of the world. A refinement of the traditional procedure does not require an abdominal incision. The tubal ligation or cauterization is carried out by cautery inserted through a laparoscope, requiring only 24-hour hospitalization.

Other oviductal procedures have been studied for effectiveness in birth control. A surgical glue (methyl 2-cyanoacrylate nonomere) injected into the oviduct binds well, but is very difficult to apply without attaching segments of the bowel. More importantly, the substance may be carcinogenic. Also studied was a silastic plug, injected into the oviduct in fluid form. When solidified, the plug would be relatively easy to remove when desired. However, peristalsis of the oviduct may eject the silicone plug, permitting the transport of the eggs and sperm.

Steroid hormones are being used extensively in fertility control without basic knowledge of the way they function. A thorough understanding of the proper hormonal balance needed for preparing the sperm for fertilization in the female reproductive tract would greatly enhance sterility treatment. Likewise, the mechanism of inhibition of sperm fertilizing ability by progesterone would aid contraceptive approaches.

Using fluorescent techniques, Glass showed that the egg can take up both native and foreign serum antigens. This and other work raises the possibility of influencing the development of the egg at the oviductal level. Little is known about oviductal influence on capacitation of sperm and the effect of oviductal fluid on fertilization. S. J. Behrman estimated that at least 10 percent of all unexplained infertility in man is due to a naturally occurring immune phenomenon. Positive sperm agglutinating antibodies are a very rare occurrence in the normal population, but common in prostitutes possibly due to excessive exposure to the sperm antigen.

The proceedings of the symposium, edited by E. S. E. Hafez and R. J. Blandau, are being published by the University of Chicago Press.

This monograph has a twofold purpose: to stress the need to study the entire oviduct in mammalian species at all levels of evolution in order to gain a full understanding of its function, and to explore how far such studies in laboratory animals can clarify similar problems in domestic animals and man.

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E. S. E. Hafez
Washington State University, Pullman

Calendar of Events—January

National Meetings

4–6. **Human Factors in Automotive Engineering** Design, Ann Arbor, Mich. (Society of Automotive Engineers, Continuing Education Program, 485 Lexington Ave., New York 10017)

7–12. American **Chemical** Soc., New Orleans, La. (Meetings Manager, 1155 16th St., NW, Washington, D.C. 20036)

8–9. National Specialists Symposium on **Orbital Resonance**, Redondo Beach, Calif.