THE FOURTH HAMMOND MEMORIAL LECTURE

PUZZLES OF REPRODUCTIVE PHYSIOLOGY*

A. V. NALBANDOV

Even though I had met Professor Hammond and talked with him at length both at Cambridge and elsewhere, the meeting I cherish most occurred totally unexpectedly at Midway Airport which was then the international airport of Chicago. This was probably in 1958 or 1959. He had been a Visiting Professor at Washington State University and, when I met him at Midway, he was returning home, as was I. Both of us were 'weathered in' and we sat for some hours in the rather unattractive lounge at Midway and talked. Professor Hammond, who at that time had not yet been knighted, discussed at length the subject matter which had preoccupied him for years and which concerned ways and means of applying newly discovered scientific facts and methods to the improvement of domestic animals, their growth, productivity, and reproduction. Finally, he asked me about my own work. I was then in the process of revising my lecture notes and had put in a lot of thought on some problems pertaining to reproduction for which I saw no obvious answers. I presented these ideas to Professor Hammond and we discussed the problems in some detail. Since that time, considerably more thought has been applied to these questions but I am still unable to make obvious generalizations. Perhaps it might be of interest to restate the problems I posed to Professor Hammond and to see whether any of my listeners or readers will find it amusing to think about them and to come up with suggestions or answers.

I would like to call the first problem 'a myriad of ways of solving the problem of perpetuating the species.' First, let us agree that all physiological functions are vitally important for the perpetuation of a species. Obviously, the circulatory system, respiration, digestion etc., must all be functioning most efficiently in order to ensure the well-being of individual members of a species and hence, of the species as a whole. Yet, if we look at the variety of ways in which evolution has solved the problem of these different physiological functions, we find an amazing uniformity. For instance, if we look at the process of food gathering and digestion in the vertebrates, we find identically the same system. It always involves a mouth with teeth which are or are not used for mastication of the food. Next, we have a duct system for processing the food, which was built with some imagination in that birds have a crop for the predigestion of grains, while ruminants have four stomachs for the digestion of cellulose-rich foods, and omnivores or carnivores have only one stomach. Some single-stomach species have also evolved a caecum for the digestion of cellulose. With a few minor exceptions, basically identically the same enzyme systems are used for breaking down food components, and when an exception does occur, it usually involves

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the absence of one enzyme. There are no cases in which one 'set' of enzymes is used by one species, while a different 'set' is used by another.

We could go through the other physiological systems in the same manner and again emerge with the impression that the variations evolved are indeed minor. There are lungs versus gills, or combinations of the two; two-chambered versus four-chambered hearts, with the rest of the circulatory system being amazingly similar. The excretory system varies to the extent that in some species urine is filtered out, while in others it is concentrated to conserve water or concentrated and eliminated with faeces. It is as if the evolutionary process had found a good way to solve a specific problem and then used this method in all the descendants of a particular evolutionary branch.

This 'evolutionary monotony,' if you will pardon the expression, is in distinct contrast to the staggering variety of ways in which the problem of reproduction has been solved. It is the purpose of the next section of this paper to illustrate some but by no means all of this diversity. As you listen to me, I would like to caution you against adopting the attitude that the reproductive process is the most important control mechanism involved in the survival of the species. Consider the fact that well-functioning circulatory or digestive systems are equally, if not more, important because they must function optimally long before reproductive activity sets in.

First, let me call to your attention the generalization that variability exists mainly in the female. In contrast, those males which have been studied are rather standardized across the species and the major differences that come to mind are that in some species the testes are abdominal and in others scrotal; some have an os penis while others do not; some are seasonal breeders while others show continuous spermatogenesis.

The anatomical differences in the female duct system, however, are enormous. In some species the ovary is enclosed in a bursa ovarii, while others have an open fimbria. The argument that the bursa ensures against loss of eggs in polytocous animals does not hold because open fimbria are present in rabbits and pigs. The Didelphidae have two vaginal orifices and the male has a correspondingly forked penis. One vagina but two cervixes are present in rats, guinea-pigs, rabbits etc., completely separating the two uterine horns, while other animals have a single cervix with free intercommunication between the horns. It is interesting to note that it is not uncommon to find throwbacks in women, cattle, pigs and sheep in which two cervixes and two separate horns are present. Parenthetically, it might be mentioned that, in women, occasionally conception apparently may occur in the two horns at different times and, in a few cases, parturition of the twins has been separated by an interval of 30 days. Next, I remind you that, in some species, the uteri are capable of sloughing off the uterine endometrium which is accompanied by bleeding (menstruation), while, in all others, cyclic renewal of the endometrium occurs without bleeding. In none of these cases of anatomical difference, nor in those discussed anon, can the argument be made that one anatomical arrangement is more advantageous, thus making for greater reproductive efficiency. All the species with these peculiarities appear to be equally successful and none of the wild species appears to be in danger of extinction unless man intervenes.

Next we have species in which concupiscence is totally hormone-controlled. Thus, none of the domestic animals shows psychological oestrus in the absence of gonadal hormones. In some, oestrus appears to be induced by oestrogen alone; in others (the guinea-pig), interaction of oestrogen and progesterone is required. In women (and possibly other primates), it is still not clear whether sexual desire is totally hormone-independent or whether it is induced by the male sex hormone, androgen. In most mammalian species, sexual desire is closely linked to the time of ovulation so that mating is only permitted close to the time of ovulation. This maximizes the chance of a fresh young egg being fertilized by a young spermatozoon, apparently a very salutary evolutionary development because there is ample experimental evidence that syngamy of 'aged' gametes frequently leads, at best, to abortion, and at worst to physical deformities. It is not at all clear why in some higher primates the highly restricted mating period has been replaced by constant receptivity in which it is highly probable that young spermatozoa may fertilize old eggs or old spermatozoa freshly ovulated eggs. While there are few data for man and lower primates, it appears possible that the syngamy of aged gametes may result in debilities of the resulting young, such as increased susceptibility to infection, physical abnormalities and perhaps even mental defects.

When it comes to hormonal control mechanisms of vertebrate cycles, the variety of mechanisms employed is also startling. For instance, while testicular and ovarian function of all mammals totally depends on the dual action of FSH and LH (the gonadotrophic complex), in lower vertebrates this function is apparently fulfilled by FSH alone. In fact, it appears possible that lower vertebrates do not even make LH. When it comes to the mechanism of formation of the corpus luteum, the story becomes very complicated. Thus, in rats, guinea-pigs and in pigs, the corpus luteum of the cycle is formed in the absence of the pituitary gland while in women, sheep and cattle, hypophysial hormones are apparently required. These hypophysial hormones vary among species. In the first 12 days of pregnancy of the rat and mouse, prolactin is luteotrophic while LH is steroidogenic. In pregnant primates, cattle, sheep and pigs, LH appears to be the major luteotrophin although this statement still appears to be somewhat equivocal. The luteotrophic complex of the hamster is FSH, prolactin and a trace of LH. Most bizarre is the rabbit, which for some reason has chosen oestrogen as its luteotrophin.

It is understandable that for species with short cycles, such as rats, mice and hamsters, a pseudopregnant period following mating has evolutionary advantage. In these species, the egg remains in the oviduct about 3 days and does not reach the uterus until the 4th day. Thus, a new cycle would be well underway unless the cyclic activity is stopped. This is accomplished by automatic pseudopregnancy following mating. If this mechanism had not evolved, superovulation and litters of different ages could conceivably be present in the same uterus. However, the advantage of such a period of sexual inactivity in rabbits, guinea-pigs, cats or dogs is not obvious. In fact, it would seem detrimental to these species and perhaps this is a natural restraint built in by evolution to keep their populations from exploding. Animals with long cycles have evolved chemical or neural signalling mechanisms originating from the presence of the embryo

which cause prolongation of the life-span of the corpus luteum. Thus, they have no need for a period of pseudopregnancy.

A most remarkable deviation from the sexual cyclicity found in most species has evolved in *Suncus murinus* (Asiatic shrew) as well as in some of the voles. In *Suncus*, there are no discernible cycles of either the ovary, the uterus or the vagina. However, sexual arousal occurs when a female meets a dominant male. A fierce battle between the sexes ensues at such meetings and the female may become sexually receptive after as little as 1 min of battle or not until after 40 or 50 min of vicious fighting. After an appropriate duration of mutual attacks, the female suddenly changes her raucous screams to a gentle chirp and her behaviour also changes remarkably in that she now actually solicits mating. If intromissions have been sufficiently frequent she ovulates 18 hr later. The identical behaviour pattern is exhibited by pregnant, pseudopregnant or non-pregnant females. All attempts to unravel the endocrinology of this intriguing and remarkable behaviour pattern have thus far failed.

Some species, like primates, sheep and cattle, use oestrogen as a signal for the release of ovulatory peaks of LH, while, in chickens, LH release is caused by progesterone. In rats, it is still not clear which of these two hormones has primary responsibility for the release of LH. In some very successful species, such as rabbits and cats, LH is released only in response to mating. In many other equally successful species, the release of the ovulation-inducing hormone is totally independent of copulation and cervical stimulation. Some species, like rats and mice, implant only in response to an oestrogen peak, while others require only progesterone. In rabbits, progesterone is mandatory for implantation and oestrogen only facilitatory. Paradoxically, the armadillo implants following castration, while the wallaby implants after hypophysectomy. This implies that in these two species no hormones are needed to trigger nidation; in fact, the presence of hormones seems to prevent implantation.

Primates make a placental gonadotrophin (HCG), which has the properties of LH and is apparently used as a luteotrophic substance to convert the corpus luteum of the cycle into the one of pregnancy. Equidae make their gonadotrophin (pregnant mare's serum, PMS) in the endometrial cups of the uterus and this hormone has both FSH and LH properties. For totally incomprehensible reasons, Equidae destroy the original corpus luteum of conception, re-ovulate in response to their own PMSG and form so-called accessory corpora lutea, which persist through part of pregnancy. Rats and guinea-pigs also make placental substances which are involved in the maintenance of corpora lutea of pregnancy but, as far as I know, nobody has been able to find such substances in sheep, cattle, pigs, cats or dogs. Such species as rats, rabbits and pigs, which do not make placental steroids, abort following castration at any time during gestation; others that do produce placental steroids tolerate castration at various stages of pregnancy. Again, there is no obvious explanation for these differences.

When it comes to hormonal mechanisms controlling maternal behaviour, the differences between species are again striking. Thus, in doves and rabbits, the primary hormone triggering maternal behaviour is progesterone while, in chickens and rats, it is prolactin. Not enough work has been done in other

species to allow conclusions. A remarkable adaptation in maternal behaviour has occurred in the Asiatic shrew (*Suncus murinus*). This animal has the highest metabolic rate of any mammal studied which, to be maintained, requires that the female forage almost constantly. In this species, maternal behaviour includes the mechanism of 'caravaning' in which the mother when leaving the nest, takes her young with her. One pup attaches itself to the mother's hair, the second grips the hair of the first young, the third that of the second, and so on. This allows the mother to drag her brood along with her as she goes about her activities of food gathering. Enormous variability also exists with regard to the self-dependence and maturity of young at birth. Rat pups are born blind, naked and totally dependent on the mother, while guinea-pig young are born haired, sighted and fully mobile. Such drastic differences in the maturity of young at birth are probably directly related to the habitats in which these species have evolved.

The milk-producing apparatus and the hormonal mechanisms involved in the control of lactation seem to be an exception to the apparent rule of diversity discussed above. Microanatomically, the breasts of all species studied appear to be remarkably similar. The only diversity occurs in the number of ducts in the nipple and in the number of mammary glands, which is correlated with the number of young born; and the location of the glands in that, in elephants and primates, the paired glands are pectoral, while in all other monotocous species they are inguinal. The hormones involved in the initiation and maintenance of lactation also appear to be very similar across the species studied and the only diversity that occurs involves the chemical composition of the milk produced by different species.

I feel that the examples given so far are sufficiently convincing to allow us to restate the initial query. Why is it that such amazing diversity has evolved in the mechanisms which basically have the aim of shedding ova and maximizing the chances of their getting fertilized, implanted and carried to term? Furthermore, why is this diversity restricted to the female of the species, while all the males have solved their reproductive problem with amazing uniformity?

It is, of course, true that the task of the male is infinitely more simple than that of the female. All the mammalian male has to do is to manufacture sperm and ejaculate it at the appropriate time. His part in reproduction is thus ended while that of the placental female is just beginning, in that she must shelter the zygote and nurture it through gestation and nourish it after parturition. The closest counterpart to the rôle of the vertebrate male in reproduction is that of the egg-laying vertebrate female. Unfortunately, only the chicken has been studied in any detail as far as its endocrine control systems are concerned. However, if one includes the behavioural aspects of these egg-laying species, one again finds enormous diversity. Thus, some species bury their eggs in mouldy leaves and let them serve as an incubator, in others only the female sits on eggs, in still others both sexes brood the nest and there are also some in which only the male assumes the task of hatching the eggs. Thus, it appears that the original statement that diversity is restricted to the female is still valid and the question why this is so remains.

As I said at the start, I have no answer to this question. It is interesting that

only one other essential problem of survival has been solved by evolution with as much imagination as has that of reproduction and that is the one of locomotion. Here we have creatures which swim, fly, crawl, walk on twos or fours, or hop. As far as adaptation of the appendages is concerned, variation is also great in that some get along on one toe, others have two and still others get along just as well on three, four or five digits. There is, however, a distinct and important difference between the evolution of the reproductive process and that of locomotion. The variability of the latter can be understood as an adaptation to the environment, be it air, water, trees, solid earth or swamp. No such apparent interaction between environmental conditions and the reproductive process appears to exist.

There may be one major flaw in this argument. The trouble is that we are looking at diversity or uniformity as it exists in our age. We have no real idea what the environmental conditions were when these evolutionary adaptations of the reproductive system of females occurred. Thus, for instance, the roe deer is the only member of the deer family, in fact the only ruminant, which developed delayed implantation. It could be postulated that unless members of the roe deer family mated in the fall, the chances of a meeting of the two sexes later on would be minimal, perhaps because of deep snow and dispersion of the group in search of feed. Delayed implantation also allows the young to be born in the spring when the chances of survival are maximal. Perhaps other ruminants were evolving under less stringent climatic conditions and thus there was no need for delayed implantation.

It has been proposed that the constant concupiscence of women has evolved because it was beneficial since a hunting male would more readily return with food for her and her young and could always expect to be rewarded by a sexually receptive partner. However, in many species in which sexual receptivity is temporally restricted, the male participates in providing food and in caring for the young. Similar rationalizations have been proposed for the evolution of copulation-induced versus spontaneous ovulation. None of them sounds convincing and exceptions to the reasons proposed abound.

Finally, I would like to take up one more problem which I discussed with Sir John Hammond and which I would like to share with you. I would like to call this part of my discussion 'the case of the talented hormone.' It concerns the distinct specialization of the physiological activity of the great majority of hormones. The number of physiological tasks performed by each of these hormones is very small. Thus, for instance, the gonadotrophins are only concerned with gametogenesis and steroidogenesis; the corticosteroids with mineral and glucose metabolism. If you think of prolactin, testosterone, thyroxin or progesterone, you will find their field of physiological influence and control is very limited. Not so with oestrogen.

First, let me remind you that oestrogen or oestrogen-like substances are very widely spread in nature. Thus, an oestrogenic substance was extracted by Zondek from the mud on the bottom of the Dead Sea. Oestrogen-like substances occur in many plants, such as pussy-willows, lettuce and most legumes including the notorious subterranean clover. It has been found that many moulds, most specifically those growing on corn and certain other grains, produce oestrogenic

substances which when consumed by domestic animals or man can seriously impair reproduction, just as does subterranean clover.

Some years ago, I began a list of all the physiological systems oestrogen affects and came to the conclusion that oestrogen, in contrast to the other hormones, is indeed a 'talented' hormone. This list of the diverse effects of oestrogen, which is probably incomplete, will be discussed briefly.

Oestrogen, in an as yet totally unknown manner, acts on the central nervous system and induces oestrous behaviour in the majority of mammals. It is a growth hormone in that it causes spectacular increases in weight and size of uterus, vagina and breasts, even without the help of progesterone. In addition to causing protein anabolism in these tissues, it may also act directly on somatic cells causing hypertrophy and hyperplasia, although this may be due to the possible ability of oestrogen to release hypophysial growth hormone. This effect is, of course, the basis for the use of oestrogenic substances for fattening livestock. In some species (birds and mice), oestrogen has pronounced effects on both calcium and fat metabolism: it acts at the level of gut, liver, depot fat or bones causing mobilization of calcium or fat from these structures. In other species, this effect is present but it is not nearly so dramatic as it is in mice and chickens. The late William Bloom and I found a paradoxical action of oestrogen in the hypophysectomized chicken in that it affects both the osteoblasts and osteoclasts, thus causing either Ca deposition or its mobilization from bones.

It is now well established that mammalian females under oestrogen domination are much less prone to uterine infections than are females under the influence of progesterone. This phenomenon is well understood and is now known to be due to the ability of oestrogen to mobilize leucocytes into the uterine secretions. Such secretions become bacteriostatic. The late Professor Karl Koch told me that cows with positive tuberculin reactions and active lung lesions show negative TB reactions after treatment with oestrogen and their lung lesions become calcified. During the last world war, this method was used in Germany to retain TB-reactors for milk production.

Oestrogen injected into rats causes an increase in plasma riboflavin but it is not clear whether it actually induces synthesis of this vitamin or has a sparing action on it. In chickens, it markedly increases plasma folic acid by increasing its synthesis. Oestrogen dramatically increases mitotic rates in the uterus and vagina and perhaps in non-target tissues as well. It increases vascularization of all target tissue, as well as other tissues such as the skin, kidney, liver, etc. The number and size of mitochondria in the uteri of oestrogen-treated intact or castrated rats is greatly increased, as is the oxygen consumption of uterine tissue. Finally, the contractility of the muscles of both uterus and oviduct is controlled by oestrogen.

Thus, oestrogen is apparently a general hormone which affects not only tissues concerned with reproductive functions but also many tissues not directly concerned with it. The question why substances with oestrogenic properties are common to both plants (in which their rôle is totally unknown) and animals (both vertebrates and invertebrates), I find intriguing, especially if one considers that substances with progestational, androgenic, or corticosteroid-like activities are largely restricted to vertebrates and, as far as I know, do not occur

in plants. It should be kept in mind that all the hormonal steroids resemble each other closely chemically, and that oestrogen is chemically totally different from the oestrogen-like compounds produced by plants or by mouldy corn. The questions are posed and I will leave it to you to speculate on this problem if you are so inclined.

These problems I discussed with Sir John who agreed that answers to them may provide clues to a better understanding of the reproductive processes and especially for devising better control of these systems. He also said that our discussion lent further support to his growing conviction that each of the species is an entity unto itself and that it is highly improbable that information on one species would be necessarily applicable to another. I agreed.

I consider it a great privilege to have known Sir John Hammond and to have had an opportunity to have him all to myself for a few hours at a weathered-in airport. His penetrating wisdom and insight were indeed impressive.