PROSPECT AND RETROSPECT IN THE PHYSIOLOGY OF REPRODUCTION*

BY

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Sixty-one years ago, in November, 1900, a paper of great importance to the physiology of reproduction, and of special interest to Cambridge, was published in the *Quarterly Journal of Microscopical Science*. This paper was important because it laid the foundations of our present terminology of the reproductive cycle, and it was of special interest because it was written by a member of Trinity College working in the Department of Zoology. The author was Walter Heape. In this paper Heape redefined the terms reproductive period, sexual season, and oestrus, and introduced the other terms anoestrus, pro-oestrus, metoestrus, and dioestrus. This most constructive paper was the more notable in that Heape's earlier work had dealt with such scattered themes as the embryology of the mole, artificial insemination, transference of fertilized ova in the rabbit, and the cyclic changes in the uterus of monkeys. Heape, however, had evidently been influenced profoundly by the writings of the French biologist, Lataste. One of my more interesting possessions is the copy of Lataste's great monograph on the breeding habits of rodents presented to Heape by the author. To judge by the annotations, Heape seems to have pondered every word of the 676 pages and to have disagreed with not a few.

Heape's later papers, with one exception, were without much impact on the history of the subject, but the exception is a notable one. In 1905 he published the results of a study of ovulation in the rabbit and added one important fact and one illuminating idea to the literature. He showed that ovulation in the rabbit was dependent on, and happened about 10 hours after, mating, and he came to the conclusion that ovarian activity was conditioned by some extragonadal substance to which he gave the name "generative ferment." Heape's views on the hypothetical substance were never very clearly formulated, and it remained for John Hammond and F. H. A. Marshall, in their monograph on the rabbit published much later, to elaborate the idea in a meaningful way. Nevertheless, Heape's idea was essentially correct, as shown by the subsequent discovery of the pituitary gonadotrophins. Heape's contributions to the physiology of reproduction were the more remarkable because, in the words of his Royal Society obituary, written by Marshall, "He never had any systematic training in physiology and very little in zoology." He had started his working life in business,

and it is not certain from the Royal Society obituary that he graduated anywhere. However, after short periods at Manchester and Oxford he was firmly established by 1879 in the Department of Zoology in Cambridge, first with Balfour and then with Sedgwick, and it seems that he personally shot the elephant whose skeleton adorns the zoology museum. Heape received an honorary M.A. from Cambridge in 1885.

In spite of his inspiration about extragonadal regulation of the ovaries, Heape seems not to have realized that the ovaries might be performing a similar function for the female reproductive tract, and his pioneer work, which provided so much incentive in other directions, gave little to the study of the gonadal hormones. Yet even at this period some evidence was available because the effects of ovariectomy were well known. Moreover, the role of the corpus luteum in the maintenance of pregnancy had been demonstrated by Fraenkel in 1903, also by experiments with rabbits. It remained, however, for Marshall, then in Edinburgh, to visualize the role of the internal secretions of the ovary in the regulation of the oestrous cycle.

Marshall's Prescience

In 1905, in a paper with Jolly published in the *Philosophical Transactions of the Royal Society*, Marshall wrote:

"The mammalian ovary, in addition to its function of producing ripe ova, is an organ elaborating an internal secretion which reacts on the general metabolism of the animal, as is shown by the effects brought about by its removal. The secretion is probably formed in greater or less quantity at all times, but it is produced in greatest abundance at certain more or less regularly recurrent periods, when it brings about those conditions which characterize the pro-oestrus and oestrus. It is at these periods also in typical cases that the ova mature and the follicles discharge. . . . After ovulation, which takes place during oestrus, the corpus luteum is formed, and this organ provides a further secretion whose function is essential for the changes taking place during the attachment and development of the embryo in the first stages of pregnancy."

These words could hardly be improved upon to-day, but they were in fact written fifty-six years ago by a young man of 26, four years before Bouin, in Strasbourg, demonstrated that the progestational development of the uterus of the rabbit depended on the presence of the corpus luteum, ten years before definitely active extracts of the ovary were produced, and thirty years before the active substances were isolated from the ovary. We

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may well ask, therefore, about the background of this remarkable forecast of future discoveries.

Marshall was born in 1878 into a family with a long legal tradition, and came up to Christ's College in 1896 to read geology, zoology, and chemistry. He records that, as at school, much of his work at Cambridge bored him and received little attention. He obtained a second class in Part I of the Natural Sciences Tripos and a third class in Part II. It would be difficult to maintain, therefore, that the study of the physiology of reproduction in Great Britain was based on scholastic distinction. After a few months' postgraduate work in Cambridge he heard that there might be an opportunity for him to go to work with Cossar Ewart in Edinburgh on certain problems connected with the physiology of reproduction, especially on telephony. Marshall's interest had earlier been aroused by this branch of biology, and before he left Cambridge he obtained an introduction to Walter Heape. Marshall's connexion with Heape, however, was only slight; the two men met on only a few occasions. Marshall, therefore, was not in any sense a pupil of Heape. His opportunity to work on the physiology of reproduction arose from the Edinburgh appointment, not from the Cambridge connexion. Marshall, in fact, was a pupil of no one but himself.

Marshall's Early Work

The work on telephony was designed to verify or refute the classic case of Lord Morton's mare, in which, it was said, an earlier mating of a pure-bred mare with a quagga had apparently resulted in the foal from a later mating with a pure-bred stallion being striped like the quagga. Marshall became involved in a detailed study of the hair of horses. This he found to be somewhat unprofitable, but at least it caused him to make visits to the Faroe Islands, Norway, and Sweden to study local races of Equidae. As might be expected, the unproductive task of proving a negative by experiments each taking a year did not absorb all Marshall's energies, and soon after his arrival in Edinburgh he started work on the oestrous cycle of sheep. The results formed the subject of his first important contribution to scientific literature. At about the same time he became interested in the ferret, publishing in the following year what was probably the first paper on the oestrous cycle of this animal. He then turned his attention to the dog and in 1905 published the paper already referred to, of which Part 1 dealt with the oestrous cycle of the dog and Part 2 with the ovary as an organ of internal secretion.

Marshall stayed in Edinburgh for nearly eight years, returning to Cambridge in 1908. Much of the time in Edinburgh was devoted to writing the *Physiology of Reproduction*, the first edition of which was published in 1910. In this book he gathered together for the first time in English, and probably in any language, all relevant knowledge concerning the breeding seasons, cyclic changes in the reproductive organs, gametogenesis, fertilization, embryology, chemical changes, co-ordination in reproduction, lactation, and the like. Against the background of contemporary knowledge this book was a masterpiece, and it formed a milestone in the history of biological literature. The second edition, published in 1922, unfortunately missed the rapid expansion of the subject which took place in the 1920s, and never achieved the pre-eminence of the first edition.

The third edition, of which the first volume to appear was published in 1952, can speak for itself, and does so at some length.

Marshall's perspicacity in deducing correctly the existence and respective roles of follicular and luteal hormones probably arose from his work on the ferret and dog, in which both phases of the cycle are well developed. This provides another interesting example of the way that opinions can be influenced by the peculiarities of the animal from which they are derived. Both Fraenkel and Bouin worked on the rabbit, in which the luteal phase is the dominating one. They concluded, therefore, that the corpus luteum was the essential regulator of the cycle. Later on, Edgar Allen and his colleagues worked on rats and monkeys. In the former the most obvious feature of the cycle is the cornification of the vaginal epithelium which occurs at oestrus; in the latter the length and overt characteristics of the cycle are not affected by whether or not ovulation takes place and a corpus luteum is formed. Edgar Allen therefore concluded that the complete cycle was in some way organized by the follicular hormone. Marshall's much broader knowledge of sexual cycles enabled him to avoid both these extremes.

Gonadal Hormones

In the fifteen years after the publication of Marshall's 1905 paper, little progress was made with the preparation of active extracts of the gonads. The years between the two wars, however, especially the middle decade, saw astonishing progress, which was greatly assisted by the proper selection of biological indicators and general improvement in assay techniques. In this period potent preparations of the oestrus-producing hormone were developed by Edgar Allen and Doisy, and crystalline oestrogens were isolated independently by Doisy, by Butenandt, and by Marrian. During the same period the classic work on the corpus luteum was brought to fruition by the preparation of active extracts by Corner and Willard Allen, and finally by the isolation of progesterone. At the same time, the male was not neglected; androgenic extracts of testis were prepared by Moore and Koch and crystalline testosterone was isolated in Laqueur's laboratory. The chemical constitution and relationship of the three gonadal hormones, and of derivatives thereof, were worked out with great speed because of the happy coincidence that decisive progress had just been made in related fields of steroid chemistry, notably, of course, in determining the constitution of cholesterol.

All this happened over a period of about six years and I had the good fortune to have a grandstand view of events, partly as a close associate of Marrian and partly as secretary to successive international conferences on the standardization of the newly discovered hormones. These were indeed stirring times, and the excitement of this endocrinological gold rush seemed to go to the heads of some of the diggers. One distinguished research worker wrote claiming "simultaneous priority" with someone else. The only possible answer seemed to offer him "prior simultaneity."

Nomenclature Problems

I remember well, also, the difficulties about nomenclature. In the course of its concentration and

isolation the oestrus-producing hormone had become thelin in the United States, progynon in Germany, folliculin in France, and oestrin in England. Fortunately, the nomenclature first mooted in 1932 for the three most important compounds, oestrone, oestradiol, and oestriol, was adopted almost everywhere within a few years.

Turning to the corpus luteum, the word progesterone has an interesting origin. When, in 1935, a conference on standardization was being organized, various names had come into use. Willard Allen and Corner were referring to their crystalline material as progestin, and Butenandt to his as luteosterone, and it seemed likely that much of the time of the conference would be spent in discussing this issue. Fortunately, the word "progesterone" had already been thought of by Willard Allen, though without enthusiasm, and when Marrian and I met him on his arrival in London for the conference we agreed after some discussion to recommend the name to the conference. On the following day, at the pre-conference party given to participants by Sir Henry Dale, the chairman, we tried out the word on Butenandt and others, with gratifying results. The name progesterone may thus be said to have been born, if not conceived, in a place of refreshment near the Imperial Hotel in Russell Square, where Willard Allen was staying. The female gonadal hormones were therefore fortunate in acquiring a terminology based on functional significance. The testicular hormone was less fortunate, because the obvious word, androsterone, had been pre-empted by Butenandt for a urinary excretion product, which at the time of its isolation was thought to be the essential testis hormone.

The wisdom of trying to avoid anatomical implications in the terminology of the hormones soon became apparent. Active substances supposedly characteristic of the other sex occur in the gonads and body fluids of both sexes. Thus there are testicular oestrogens and ovarian androgens, and it is not possible with certainty to distinguish a man from a woman by examination of the urinary androgens and oestrogens. As a result, the idea of maleness and femaleness as clearly defined endocrine states, which accompanied the discovery of the male and female hormones, has given way to the idea of sex as a balance of endocrine factors. In the light of present trends the most that can be said is that the endocrine balance usually comes down fairly clearly on one side or the other to produce a fairly well defined male or female. In this revision of ideas an important factor has been the demonstration that progestagens, androgens, and oestrogens appear as steps in a biosynthetic process, the biological effects of which depend on the stage at which the products reach the target organs.

Exciting Happenings

Simultaneously with the isolation of the gonadal hormones, exciting things had been happening in another direction. In 1927 Smith and Engel, in New York, followed up their observations on hypophysectomized rats by trying the effect of placing small pieces of anterior pituitary gland under the skin of immature animals. Almost simultaneously Zondek and Aschheim, in searching for the source of the extragonadal control of the gonads, carried out identical experiments. The results showed clearly that the anterior pituitary body contained substances which would cause follicular

maturation, ovulation, and corpus luteum formulation in the ovary, and that the anterior pituitary body was probably, therefore, the immediate seat of gonadal control. The control, however, was soon found to be a reciprocal one, because it became evident that the ovary was protected against overstimulation by what in this electronic age has become known as a feed-back mechanism, whereby the ovarian hormones inhibit pituitary activity if present in excess.

A most interesting use of this feed-back mechanism is now being made, based on the availability of artificial progesterone-like substances, the artificial progestagens. Some of these substances are not only active by mouth but are also especially effective in inhibiting the gonadotrophic activity of the anterior pituitary body. By virtue of these two properties the substances are now being used for the suppression of ovulation and, therefore, of fertility in the human female. With the population of the world increasing in a geometric fashion, there is certainly room for a simplified method of controlling human fertility. But I doubt whether twenty pills a month taken to a set regime, quite apart from unknown ultimate effects, is the final answer on a global scale. Nevertheless, I feel that Marshall would have been pleased with this development. He once told me with considerable satisfaction that he had become the chairman of the executive committee of one of the birth-control organizations. History does not relate how he got on with this assignment, but at least it showed that his interests were not entirely academic or even agricultural.

An Unsolved Problem

In spite of tremendous progress in the study of the endocrinology of reproduction, one problem remains obstinately unsolved. It is, by what means do events in the uterus exert their effects on the reproductive cycle? This problem can best be posed as a series of questions. First, by what means is the anterior pituitary body, and thence the ovary, informed that implantation of a fertilized egg has taken place, in time to prevent the degeneration of the corpus luteum? In some species, including man, a tight schedule is indicated. Second, in most species superovulation induced by the use of exogenous gonadotrophic substances does not lead to a superpregnancy; there is a very large loss of embryos before they begin to make any obvious excessive calls on the mother. What is the reason for this excessive mortality among an excessive number of embryos? Third, removal of the uterus prolongs the life of an existing corpus luteum in some animals. What is the mechanism of this action? It may well be that there is some common denominator, possibly an endocrinological one, to these apparently unrelated observations.

Later Work at Cambridge

I have devoted most of this lecture to endocrinology because of Marshall's special interest in it. Endocrinology, however, is only one facet of the physiology of reproduction; and the study of other aspects of sexual physiology, and of spermatozoa and ova, has formed a very important part of the work at Cambridge. In more recent years this study has received a great impetus from the application to it by Dr. T. Mann of modern biochemical thought and technique. Over the years much of the work in this field has had very definite applications, and

I need hardly say that here Sir John Hammond, with his first-hand knowledge of practical matters and his unquenchable enthusiasm, has played a leading part. I asked Hammond a few days ago what he considered to be his most important single application of science to the practice of stock-breeding in this country. He answered, without hesitation, the introduction of artificial insemination in cattle-breeding, and he went on to recall some of the extraordinary adventitious objections with which he had been faced. The fact that technical difficulties were overcome comparatively easily was due largely to the long and devoted labours of Arthur Walton, and it is fitting to-day that we should recognize the great part played by Walton over more than thirty years in the development of our knowledge of the physiology of reproduction.

Later developments in artificial insemination also owe much to Cambridge, where, following laboratory experiments in London, the pioneer experiments in the practical use of deep-frozen bull semen were carried out. I may add that still more recently, and from other sources, there have been reports of the successful use of freeze-dried bull spermatozoa for insemination, and there is urgent need for these reports to be confirmed or refuted. In rather the same way there have been reports of the separation of X- and Y-spermatozoa, as indicated by the production of offspring wholly or mainly of one sex. These reports, also, are in urgent need of confirmation, or more likely refutation. I doubt if such a separation has so far been achieved, but I have no doubt that some time, somewhere, such separation will be effected, either as the result of advances in relevant knowledge or by a stroke of inspiration. It may even be effected, as was the freezing of bull sperm, by people who in their innocence are unaware that what they are doing is well known to be impossible.

Artificial insemination enables the stock-breeder to exploit the reproductive potential of superior males. What can be done about the superior female? So far, the only practical approach has been to collect fertilized ova from such females, possibly following a gonadotrophin-induced superovulation—and distribute them by surgical means among “incubator” females. The latest manifestation of this technique—another Cambridge enterprise—is the transportation of fertilized sheep ova to South Africa in the Fallopian tubes of live rabbits, used not as incubator females but as handy thermostated containers, cheaper to send by air than sheep and less likely to arouse the interest of veterinary officers at the port of entry.

Not all experiments on ova carried out in Cambridge have been so successful or appropriate. Some twenty-five years ago Gregory Pincus, a visiting worker from the United States, reported several series of experiments which appeared to show that he had caused female rabbits to produce young parthenogenetically. This report has never been confirmed, but it is certainly curious that the one serious claim to have abolished the biological need for the male mammal should have originated in a comparatively monastical University.

Study of spermatozoa, seminal fluid, and ova, however, is only one part of the modern Cambridge contribution. At the present time the traditional morphological approach to the reproductive cycle and the action of the gonadal hormones has largely given

way, so far as laboratory and farm animals are concerned, to biochemical studies of the changes appearing naturally or induced by treatment. Here, again, the swing towards the biochemical approach has been largely due to the work and influence of Dr. Mann, and there can be little doubt that much of future progress with well-known species will be along biochemical lines. This does not mean, however, that the morphological approach no longer has a place in the study of reproduction; far from it. With the exception of laboratory, farm, and a few wild animals, we know very little about the reproductive processes of mammals.

Of the thousands of different species of mammals, we have substantial knowledge of the reproductive processes of fewer than 100. Nor can we assume that the known types give us a fair cross-section of the world of mammals. Almost every new species which is to be investigated presents something new and significant. There is no limit to the variations which are being found in what was once thought to be the typical pattern of the reproductive cycle. I sometimes wonder how our views on the physiology of reproduction in mammals would have developed had they been based not on domestic and laboratory animals but on seals, bats, elephants, and the like. There can be no doubt that new and exciting discoveries await in the unexplored territories of mammalian reproduction, and the basic study, especially with field material, is preferably or even necessarily morphological.

Neurohumoral Mechanisms

I have said a good deal about the endocrinology of reproduction. But another type of crinology is now becoming important in this field. External factors which set up neurohumoral mechanisms working through the hypothalamus and the anterior pituitary body are well known in mammalian reproduction. For instance, the effects of light on the anoestrous ferret, originally investigated by Bissonnette in Cambridge, aroused Marshall's interest, and external factors entered largely into his Croonian Lecture given to the Royal Society in 1936. Marshall, moreover, was greatly interested in the breeding habits of birds, as influenced by external stimuli of social origin. He would have been delighted to know that in mammals, also, social stimuli may have profound neurohumoral effects, quite apart from the well-known effects on aggressive and sexual behaviour. Thus the oestrous cycles of female mice kept together in a large group become irregular, but are restored to normal by the introduction of even a castrated male. More surprisingly, pregnancy fails in a large proportion of newly mated female mice exposed during the pre-implantation stage to males of a different strain from the stud males. Experimental analysis has clearly indicated smell as the operative factor in these social effects in mice, but the source of the odorous substances involved is not yet known. Evidently, however, they belong to a group of substances now coming into prominence, substances which in distinction to hormones secreted internally for the integration of the individual are secreted externally by individuals to integrate a population of individuals. Such substances have recently been called “pheromones,” and their study can properly be called exocrinology. In the light of recent work we may look forward to the exocrinology of

reproduction as yet another happy hunting-ground for the experimental biologist.

Conclusion

In this lecture I have tried to look back on 60 years of the physiology of reproduction, especially on the decisive Cambridge contribution, and I have tried to visualize some future trends of work. In doing so I have aimed to give an impression of Marshall's work and influence. Marshall was indeed a great man, and I am proud to hold the Chair which he founded and proud to inaugurate it in the presence of so many of his

old friends and colleagues and in this room where so many distinguished scientists, including Marshall, have given of their knowledge.

I will conclude by quoting some words which I wrote in 1949 at the time of Marshall's death. "Scientists are of many kinds, but inspiration flows most fruitfully from those who are able, by some gift withheld from lesser men, to divine the richness of uncharted country and sense the vital landmarks. Thus do they avoid the barren places and the morasses of unimportant detail which engulf so many. To these, discovery is an art rather than a science; a matter of instinct rather than of intellectual machinery. Such was Marshall."

"ANOVLAR" AS AN ORAL CONTRACEPTIVE

BY

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The two most promising and most extensively tested progestational compounds to be used as oral contraceptives are norethynodrel and norethisterone. Norethynodrel was the first to be used in large-scale trials in this country under the auspices of the Council for the Investigation of Fertility Control in Birmingham, Slough, and London, and some of the results have already been published (Eckstein *et al.*; 1961; Mears, 1961). The 5-mg. tablet of norethynodrel containing 0.075 mg. of ethinyloestradiol-3-methyl ether ("conovid") was also the first oral contraceptive to be approved by C.I.F.C. for use in Family Planning Association clinics.

Norethisterone (17 α -ethinyl-19-nortesterone; "primolut N," "norlutin") was given as an oral contraceptive by Tyler *et al.* (1961) to 570 patients for 7,194 cycles over a period of four years. Norethisterone acetate was later used, and 536 women had received this preparation with varying amounts of oestrogen for 2,292 cycles (Peeters *et al.*, 1960, 1961; Margulis *et al.*, 1961; Matsumoto *et al.*, 1961; Grant, 1961; Swyer, 1962) when this trial began.

Two years ago small carefully controlled preliminary trials of norethisterone, norethisterone acetate, with and without oestrogen, were begun as outlined in previous papers (Jackson, 1961; Mears, 1961) to assess the ability to postpone menstruation, to inhibit ovulation, and to produce secretory changes in the endometrium. The first large-scale trial began in London in May, 1961, using the dose which seemed to be most satisfactory—4 mg. of norethisterone acetate plus 0.05 mg. of ethinyloestradiol ("anovlar").

Large-scale Clinical Trial

The efficiency of the tablet to be used had already been proved in the 536 women mentioned above. The objects of the present trial were to confirm this, to compare the incidence of side-effects with other available oral contraceptives, and to test the acceptability of this tablet to women in Britain. The organization of large-scale trials under the auspices of C.I.F.C. has already been described in detail (Eckstein *et al.*, 1961; Mears, 1961).

The volunteers had to satisfy the following criteria: to be under 40 years at the time of beginning the trial, to have proved their fertility, to have at least one living child of the present marriage, to have had not more abortions than children, to have a body weight not exceeding 11 stone (70 kg.), to reside within easy reach of the clinic, to be prepared to come regularly for examination, and to remain in the trial for a minimum of six months (and to use no other form of contraceptive). In every case the patient and her husband signed a consent form and the general practitioner was informed of the patient's participation in the trial and of the tablet to be used. The patient's first visit was arranged wherever possible for the premenstrual period so that the examination could include a routine endometrial biopsy. At the first visit a full gynaecological and obstetric history was obtained. She was given a general examination which included breast examination, blood-pressure, haemoglobin, weight, pelvic examination, routine cancer smear, and where possible endometrial biopsy.

Every patient was given two bottles, each containing 20 tablets, and was told to start, after the next monthly period, taking one tablet every night from the fifth day of each menstrual cycle for 20 days (counting the first day of bleeding as day 1). They were told that if slight staining or spotting of blood occurred while taking the tablets they were to continue to take them (marking "spotting" on the record card), but if as much bleeding as in a normal period occurred before the 20 tablets had been finished they were to stop taking them and resume the next course of 20 in the usual way on the fifth day. They were warned that amenorrhoea occasionally might occur, and if so they should allow five clear days and start the next course of tablets. They were instructed to take the tablets regularly, but that if they forgot the tablet at night they should take it the next morning. All patients were given a record card on which to note days of the menstrual period, of tablet-taking, and any side-effects which occurred, as well as to record when intercourse took place. They were instructed to return to the clinic at the beginning of the second cycle and three-monthly thereafter. An examination of each patient was made after three cycles