THE ENDOCRINOLOGY OF REPRODUCTION

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Dedicated to Our Inspiring Scholars

FREDERICK L. HISAW
and
SOMERS H. STURGIS

and Inscribed in the Memory of

EDGAR ALLEN
and
GEORGE B. WISLOCKI

THEIR RESEARCHES HAVE SHOWN US THE WAY AND HAVE PROVIDED THE IMPETUS FOR THE PROGRESS MADE IN THIS EXPANDING AREA OF THE ENDOCRINOLOGY OF REPRODUCTION

Preface

THE INITIAL OBSERVATIONS of Frederick L. Hisaw, George Corner, Carl G. Hartman, Herbert M. Evans and Edgar Allen were most illuminating in establishing the endocrinology of reproduction as an important aspect of the medical sciences. The recent advances made in the basic endocrinologic laboratories and gynecologic clinics have achieved great strides of progress. These have led to a more scientific understanding of the important action of the hormones on the physiologic career of the reproductive organs. The progress made in this area has been so meaningful and of such rapid balloon-raising speed that the endocrinology of reproduction has gained an intellectual dividend not experienced too often in the sciences. Witness the numerous scientific papers with their rich contributions and the large number of award-winning scientists in the laboratories and clinics concerned with an elucidation of reproductive endocrinology. Scientists during the second half of the twentieth century not only presented some interesting discoveries as regards reproductive endocrinology, but stimulated enough interest for the labors of a whole generation of basic and medical scientists.

The current interest in the endocrinology of reproduction may well be said to be due to a combination of the numerous recent discoveries and the stimulation provided by the information derived therefrom. Perhaps the best justification for a book of this kind is the eager desire of many people to ascertain basic and clinical knowledge pertaining to the endocrinology of reproduction. Furthermore, it has been almost two decades since an analytical statement of this subject has been placed before students, investigators, and practitioners. The future awaits the clarification of numerous endocrine complexities associated with processes of reproduction, and it is hoped that the present account, although brief in nature, will provide an analysis of available information and existing problems in the area of reproduction. Therefore, the first purpose of this book is to provide a statement of our knowledge concerning the endocrinology of reproduction.

To study the endocrinology of reproduction it is important to know something of the fundamental considerations, which include genetics, embryology, endocrine trigger mechanisms, and the functional potentiality of the gonads. Such knowledge provides a basic framework upon which one could build a more comprehensive understanding of the sequence of events of male and female endocrinology and that of pregnancy as well. This is essentially the approach used throughout this volume (I) fundamental considerations, (II) female endocrinology, (III) male endocrinology, and (IV) aging.

This book utilizes research reports and observations from numerous and diverse parts of the world. The contributors to this volume are most thankful to all of those people who made their information available for inclusion here. Grateful acknowledgment is here recorded for the number of illustrations borrowed from other authors and publishers. Special thanks are due Dr. Frank N. Netter for the colored plates here included. The wise counsel and friendly assistance of Professors W. U. Gardner and J. F. Fulton were most beneficial in the editing of the present volume. The editor wishes to express his sincerest thanks to all of the aforementioned. The contributors have worked far beyond all expectations, and have given most unselfishly of their time and scholarly efforts. Their style has remained editorially intact save for slight modifications. Messrs. James W. Zarbock and Douglas C. Ross, Jr., Miss Leona Capeless, and the entire staff of the Oxford University Press have made our present literary task most enjoyable. Finally, the editor expresses his wholehearted appreciation to his very patient wife, Foresta Monica, and his family, Rose Marie, Arthur, and Julia, for the understanding and constant help he has received during the development, writing, and editing of the present project.

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Dynamics of Endocrinology

Endocrinology as a science is comparatively new. Although Berthold in 1849 observed that transplanted testes were capable of maintaining the secondary male characteristics of the cockerel, and Claude Bernard in 1855 expressed the concept that there are internal secretions which maintain the internal environment, it was not until 1889 when the controversial report of Brown-Séquard appeared that endocrinology as an area of specialization received sufficient impetus to excite attention. Brown-Séquard claimed to have achieved marked rejuvenating effects by injecting himself with testicular extracts at the age of seventy-two. Although his experiment was one of the first attempts at endocrine substitution treatment, his claims of 'rejuvenation' were primarily due to autosuggestion rather than to any hormone present in his injected extracts. It is interesting to note that the year of 1889 also saw the report of von Mering and Minkowski on the experimental production of hypoinsulinism.

More than six and one-half decades have passed since Brown-Séquard performed his experiment, the results of which have been repeatedly questioned. Both his experiment and his interpretation of the effects of testicular extracts were not entirely without a positive effect on the future of medicine and particularly endocrinology. If his interpretation has taught us that it is well-nigh impossible to measure the feeling of well-being, then it has made us cautious of the subjective signs of symptomatology. We will undoubtedly profit from a knowledge of the early trials and errors, as well as from the experiences of scientists who labored before the dawn of modern technology and measuring devices.

While we are living in an age of earth satellites, nuclear devices and nuclear energy, numerous frontiers of human biology await exploration. This is particularly true of endocrinology, the science of the ductless glands. Admittedly, the definition of endocrinology is vague, and perhaps from the standpoint of due caution one should be careful not to attempt to propose a definition of a dynamic subject which has not yet received sufficient critical investigation. The future will undoubtedly tell us more of what endocrinology is than what it is not. The internist receives numerous patients who believe that they have 'glandular disturbances,' whereas his wise clinical judgment and battery of endocrine diagnostic tests tell him otherwise. Modern-day endocrinology has advanced to the point where it is regarded as a science, as well as a reliable field of specialization to care for something more than the side-show 'freaks' and the circus midget, fat boy, and giant. Endocrinology focuses its aims on the integrative mechanisms of the human machine during health and aberrant and pathologic states.[18] The field of endocrinology has expanded so diversely and has become so specialized that it might well be compared to a military tactical operation. For every endocrinologist (General) there are numerous and highly specialized technicians (enlisted men and women) behind the scenes. Endocrinology perforce must depend upon multiple laboratory analyses and laboratory diagnostic procedures to achieve its objectives: diagnosis and treatment. The past three decades have witnessed the perfection of numerous biological, chemical, and biophysical tests that supplement the findings of the physical examination.[10, 11] All of these factors are the 'handy tools' of the endocrinologist working in the area of endocrinology. Both the front-office physician and the senior surgeon have become familiar with certain biochemical and biophysical facts and are importantly concerned with the whole body composition before an attempt is made to correct or remove the endocrinopathy at its source. Within the scope of our present-day knowledge of the endocrines has evolved a much sharper definition of the 'wisdom of the body.' Today, knowledge is available concerning the use of a number of substances that can replace the naturally occurring glandular secretions. More important, information is available concerning the anabolic and catabolic actions and pathways of certain endocrine products.

From an anatomical and physiologic viewpoint, an endocrine gland might be defined as a specialized epithelial or epithelioid structure, ductless in form, that produces by normal physiologic processes specific chemical compounds. These compounds, called hormones, are transported usually by the blood stream, and exert both generalized and specialized effects in other tissues or organs of the body. This definition is implied in the Greek origin of the word endocrinology (*endon*, within; *krinen*, to separate). Definitions are always self-limiting; exceptions will be found in regard to the action(s) of endocrine products on target organs which produce them. Furthermore, it will not be surprising to discover that ovarian substances might well react on the organ that produces them. What are these endocrine products, or endocrine glandular substances? Are they both precursors of hormonal substances and hormones? Yet, another question is, does the endocrine gland transform the precursor substance, or first secrete the precursor substance and then transform it into the native hormone of the human being, and then secrete it into the blood stream for activation at another site, thus allowing it to accomplish its task, and then return it to the liver or elsewhere for inactivation? This is a difficult series of questions to answer, but it is equally important to know that these questions exist.

Of additional importance is the consideration of the role of the hormones. Starling defined the term hormone as 'any substance normally produced in the cells of some part of the body and carried by the blood stream to distant parts which it affects for the good of the body as a whole.' This definition is more than forty years old and the advances made during the past half-century point to the usefulness of Starling's concept of a hormone. More precisely, evidence at hand reveals that *hormones and their metabolites* may be stimulatory as well as inhibitory. Therefore, it is important to discuss the response of the endocrine receptor organs in terms of the actions and interactions of the hormones and their metabolites. In this regard, it is impossible to discuss the action of one hormone in the classical sense; as a matter of fact, it would be more nearly correct to relate the expression of the endocrine receptor organ to the sum total of all of the hormones in the biological system at a specific time. There is considerable evidence favoring this concept. It is firmly established that the hormones and their metabolites act back on the adenohypophysis, and that numerous hormones and their related substances are in the blood stream at the same time (Fig. 1). This emphasizes the fact that numerous interactions or interrelationships may exist in the human body.[13]

Since hormones travel in the blood stream, by necessity one should give more attention to the vasculature associated with the endocrine glands. The vascular system is an important part of the endocrine effector system. Reynolds [12] indicated the importance of the consideration of the vascular system, and at the turn of the second half of this century he concluded that 'we generally overlook the fact that an essential concomitant condition of endocrine function is an adequate and ample vasculature of the endocrine gland.' Neither transplanted endocrine glands nor tissue-culture explants of endocrine tissues negate the importance of the vascular system. One has only to look at the vascular supply of the reproductive organs, suprarenal cortex, thyroid and parathyroid glands to become impressed with the lavish supply of blood vessels with which each of these endocrine organs are supplied to ensure the various parts of the body of receiving their hormones. Endocrinologists must be concerned with the hemodynamics of the endocrine glands as well as the histology, biochemistry, and clinical evaluations. No student of the endocrinology of reproduction should be denied the opportunity to read Reynolds' essay on the vasculature of the ovary and ovarian function.[12]

One of the most perplexing aspects of endocrinology is the subject of hormone actions. Hormonal actions and interactions are most difficult to understand. Numerous researches have been focused on hormone action, and it is disappointing to state that very little is known concerning the *modus operandi* of a single hormone. Certain of the newer biophysical techniques and the incorporation of radio-active labeled hormones will undoubtedly help clarify our understanding of the physiologic career of the hormones through their anabolic and catabolic phases in the human body.

While endocrinologists have become concerned with biochemical analyses, this approach is insufficient alone; it must be coupled with some descriptive physiologic experiments and/or analyses of hormones in blood during *normal* conditions as well as during the several pathological variants or abnormal conditions free of pathological entities.

The use of two atomic bombs in World War II in Japan, and the 'fall-out' associated with the experimental development of newer nuclear explosives, will undoubtedly complicate our understanding of the normal endocrine expression. While the effects of 'fall-out,' cosmic rays, and changing environmental conditions are difficult to ascertain, only the future will provide an answer to these effects on endocrine mechanisms.

The methods of endocrinology have recently included tissue culture, electronmicroscopy, and enzymatic determinations.[4] Each of these disciplines provides newer fragments of information. The caution to be stressed here is that each piece of information should be interpreted in its proper perspective, and

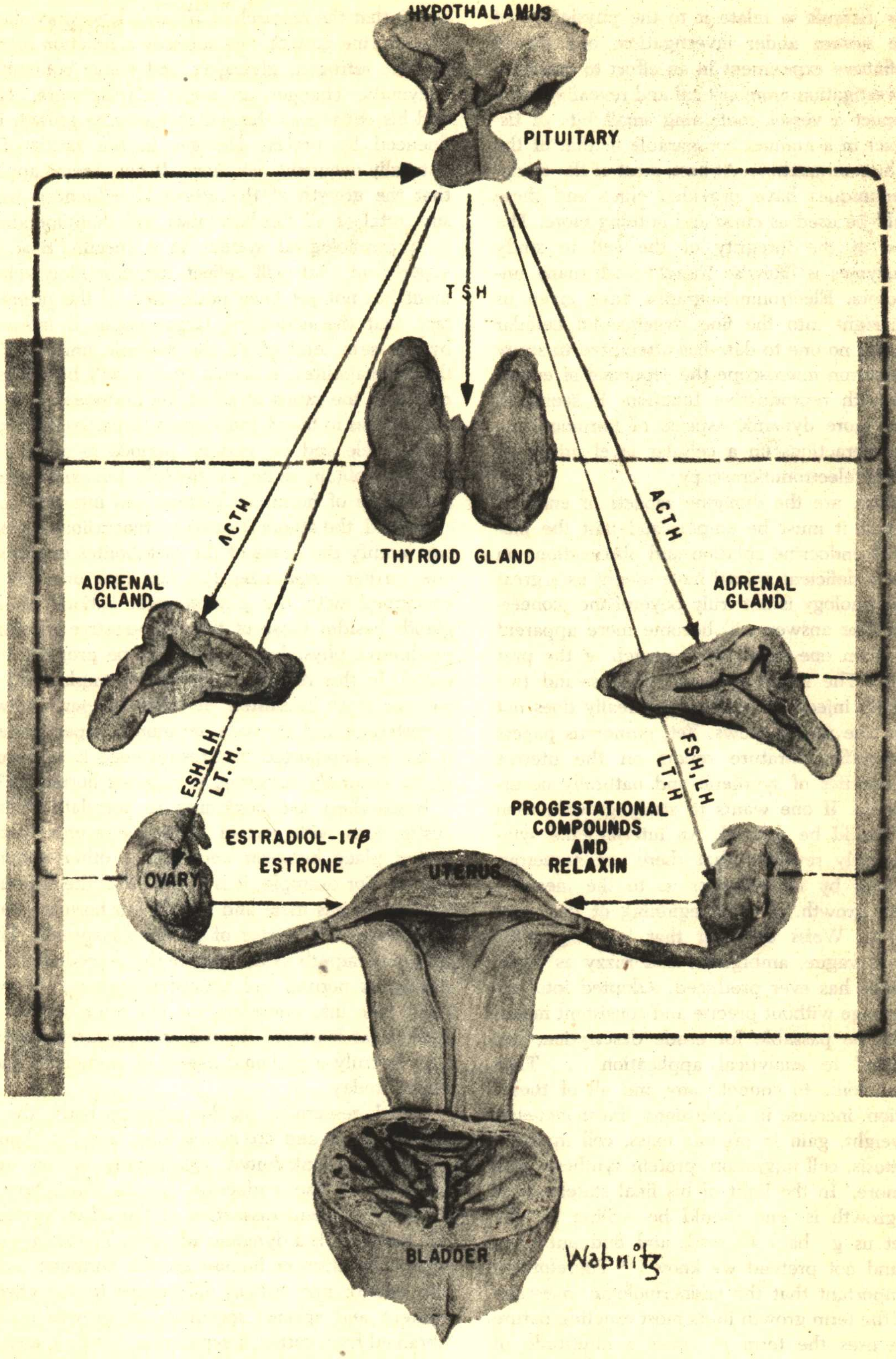

Fig 1. ENDOCRINE INTERRELATIONSHIPS IN THE FEMALE.

while it is difficult to relate it to the physiology of the whole system under investigation, one should plan a definitive experiment in an effort to make his plan of investigation more critical and revealing. One cannot expect a vessel containing small bits of tissues to react in a manner comparable to that of the dynamic, human machine. At best, most of the tissue-culture techniques have provided clues and these facts should be used as clues and nothing more. The destruction of the integrity of the cell to study cellular enzymes is likewise fraught with many unknown factors. Electronmicrographs have given us a better insight into the fine structure of cellular processes, but no one to date has attempted to study with the electron microscope the sequence of events associated with reproductive function. It seems as though the more dynamic aspects of hormonal actions and interactions on a cellular level might be revealed with electronmicroscopy.

What, then, are the dynamic aspects of endocrinology? First, it must be emphasized that the pioneer days of endocrine ablation and observations on a hormonally deficient animal have taught us a great deal. Endocrinology is not truly beyond the pioneering stages. The answer will become more apparent than real when one views the research of the past half-century. The removal of the ovaries—and two or three daily injections of estrogen—really does not tell us how the uterus grows. Yet, numerous papers in the scientific literature report on the uterine growth properties of synthetic and naturally occurring estrogens. If one wants to study growth, then the term should be defined. An international symposium recently revealed that there are numerous concepts held by investigators as to the meaning of the term growth. At the beginning of this symposium, Paul Weiss declared that '. . . "growth" is a term as vague, ambiguous and fuzzy as every day language has ever produced. Adopted into scientific language without precise and consistent meaning, it may be passable for crude description, but it is ill-fitted to analytical application . . . Thus, growth has come to connote any and all of these: reproduction, increase in dimensions, linear increase, gain in weight, gain in organic mass, cell multiplication, mitosis, cell migration, protein synthesis, and perhaps more.' In the light of his final statement as to what growth is, one should be willing to take heed: 'Let us go back to work and find out more about it and not pretend we know.'[14] Therefore, it is most important that the endocrinologic investigator define the term growth in its most exacting nature before he uses the term to cover a multitude of features indicated in his interpretation. The same holds true not only for the term growth but for other ambiguous terms employed in oversimplifications of endocrine expressions. It was not until recently that the research of Hisaw's laboratory showed that uterine growth was not only a function of phosphorus, nitrogen, glycogen, and water content, but enzymatic changes as well.[8] Furthermore, Hisaw and his colleagues showed that uterine growth is influenced by precise changes in the ratios of the naturally occurring estrogens. It now seems apparent that the growth of the uterus is influenced by the sum total of all the hormones and their metabolites in a physiological system at a specific time. The experiment that will reflect the crowning achievement has not yet been performed. If the premise is true that the endocrine target organ is influenced by the sum total of all the biologic hormones and their metabolites, it seems that it will be necessary to obtain the ratios of all of the hormones and their metabolites in blood and excreta throughout the menstrual cycle and for varying periods in males in an effort to obtain some worthwhile information as to the nature of hormonal actions and interactions.

Second, the demonstration[4,7] that triiodothyronine can modify the ratios of the metabolites of testosterone further emphasizes the fact that one must be concerned with the physiological actions of other glands besides those of the reproductive tract if reproductive physiology is going to be properly elucidated. In this regard, it should be emphasized that we are most interested in what happens between testosterone and thyroidal hormones, especially since it has been reported that testosterone is a precursor of the naturally occurring female sex hormones.[2]

Researchers are beginning to correlate their investigations with certain endocrine events that are taking place between and among other endocrine glands. For example, it is known that the suprarenal cortex secretes male and female sex hormones as do the ovaries and testes of numerous species. Therefore, any superb evaluation of the reproductive tract during its normal and abnormal sequence of events must take into consideration the roles of the other glands (at least the thyroid and suprarenal cortex). This is truly a dynamic aspect of endocrinologic research today.

Third, researches on the pituitary body, the adenohypophysis and the neural lobe, and the hypothalamus have contributed significantly to our understanding of the endocrine glands throughout the body. The recent dissection of the adrenocorticotropin molecule is a dynamic advance. The isolation and characterization of human growth hormone indicate the minute but critical differences in the chemical content and species specificity of growth hormone obtained from cattle, sheep, swine, monkey, and man. The recent impetus on the purification of the gonadotropins is likewise extending our appreciation of the so-called trigger mechanisms of endocrine reactions. Likewise, the synthesis of oxytocin and vasopressin represents tremendous advances in our knowledge.

Fourth, the isolation of progesterone, Δ^4, 3-ketopregnene-20α-ol, and Δ^4, 3-ketopregnene-20β-ol, all active progestational compounds, from human ripe ovarian follicles, corpora lutea, blood and placental tissue helps to emphasize the dynamic achievements of endocrinology during the past decade. In this same period of time we have witnessed the isolation and characterization of aldosterone.

Fifth, although relaxin was discovered more than thirty years ago by Hisaw, it has just found its way into the obstetrical clinic for evaluation. Future research will undoubtedly discover new hormones, and elucidate some of the hitherto unexplained endocrine complexities.

Sixth, while we are aging, we are gaining a considerable amount of information on the aging process because endocrinologists have a better insight into the aging problem. Witness the scholarly essays on hormones and the aging process edited by E. T. Engle and G. Pincus.[6]

Seventh, endocrinologists have become very much interested in the diseases of the endocrine glands and have advanced our knowledge tremendously in this area. Recently Soffer, Gabrilove, and Sohval have produced a most extensive survey of our knowledge pertaining to the diseases of the endocrine glands.[15]

Lastly, modern medicine has utilized well the numerous advances of endocrinology and has applied this information not only to sickness and disease but specifically to the cancer problem as well.[1]

Thus from what has been said above, it is readily apparent that the frontiers of human knowledge and skill, the incorporation of mechanical devices, and the recently available radio-active tracer substances have all aided basic and clinical scientists to make endocrinology a dynamic science. The population of the world is expanding so rapidly that it appears essential for us to expand our medical horizons to meet the demands of a larger and closer world society.[8] In this aim, help from endocrinology has already been identified.

There are many ways to tell a story, particularly that of the development and growth of the endocrinology of reproduction. One might peer into the papyrus reeds of the Egyptians or at the clay tablets of the Babylonians wherein one could pick up the ancient threads of our science. It will be recalled that the ancient peoples had great concern for their fertility status. Perhaps this view is by far too retrospective. In this regard, it is impossible to introduce the subject of endocrinology, owing largely to a lack of information as to where it actually started. Perhaps the next half-century will find it appropriate to use present-day information as its introduction. In this regard, and with a sense of academic humility, the following sections are here presented with a view of relating our information on the endocrinology of reproduction.

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II | S. A. Asdell, M.A., Ph.D.

The Genetics of Reproduction

The theory of the connection between genetic factors and the chromosomes gave biology a satisfactory explanation for the simple numerical ratios that form the basis of inheritance as these were observed by Mendel. This theory was one of the major advances in science and it has, like all valid generalizations, given rise to much fruitful work. It was obvious to some that the production of the two sexes, male and female, in approximately equal proportions, must fit fairly easily into this chromosomal-genetic framework and by 1905 the basic explanation was forthcoming. The theory as developed by McClung [33] and others was that the sexes differed in their chromosomal make-up only in regard to one pair of chromosomes, the members of which were alike in one sex and unlike in the other. In mammals, it was suggested that the female possesses like 'sex' chromosomes while the male has an unpaired set. The female pair has been designated XX, while the male pair has been indicated by XY. The X and Y chromosomes usually differ in size, the Y chromosomes being the smaller. Proof of these views came with the realization that they explained the phenomena of sex linkage, or the transmission of characters by parents to offspring of the opposite sex. A later development was the recognition of the fact that the chromosomal mechanism was not identical in all species but that between certain groups the plan differed considerably.

A new consideration was introduced by Morgan and the *Drosophila* school,[35] who were able to show that the genes causing the development of maleness or of femaleness, i.e. the sex-determining genes, are not merely qualitatively different in each sex in a given species but must be interpreted as quantitatively different. If the strength of male determiners is greater than that of female determiners the embryo develops with testes; if that of the female determiners is the greater the resulting development is that of a female, with ovaries.

Some forms of lower animals are hermaphroditic. Some, such as the sponges, are simultaneously hermaphroditic: the gonads of both sexes are present and functional in the same individual at the same time. On the other hand, in oysters the animal passes through a male stage before it becomes a female. In both these types it may be concluded that the genes for maleness and femaleness are present in equal strengths so that the one individual may develop ambisexually, that is, be produced as an hermaphroditic animal.

As evolution proceeded the genes for the determination of one of the two sexes became concentrated upon one pair of chromosomes. These, together with the Y chromosome which often contains but little recognized genetic material, are known as the sex chromosomes. Apart from these the other chromosomes in the cell nucleus are designated autosomes. The genetic material for determining the opposite sex is diffused among these. Two types of sex determination of this nature are known, one with the genes for femaleness concentrated in the sex chromosomes, and the other with the genes for maleness similarly concentrated. These may be called for simplicity the mammalian and the avian types, respectively, as these groups of animals are characterized by the two methods. A third type of sex determination, found in ants, bees, and wasps, appears to be a modification of the mammalian type. It will be described briefly after the first two major types have been dealt with.

The Major Types of Sex Determination

In the mammalian type the genes for femaleness are concentrated upon the X pair of chromosomes and those for maleness remain distributed throughout all the autosomal chromosomes, usually designated by the letter A. Thus all embryos have the same amount of maleness genes, but if each cell of the embryo has only one X chromosome there is only one dose of femaleness and this is insufficient to cause development as a female, so the embryo becomes a male. But if two X chromosomes are present the dose of femaleness is doubled and this is sufficient to override the maleness so that development is as a female. This form of sex determination is widespread and is found among mammals, most frogs, some fishes, and in two-winged flies (Diptera).

In some groups, e.g. in birds, butterflies, and moths, it is the maleness that becomes concentrated in certain chromosomes while the femaleness remains dispersed. The result is that an individual with the one sex chromosome, designated in these cases as Z, is a female, while the one with two sex chromosomes (ZZ) is a male. The method of sex determination is the same as in the XX, XY type but the mechanism differs in that the 'heterozygous' (XY) sex in mammals and other species of similar behavior is the male, while in birds heterozygosity is in the female. In the avian group the small, relatively inert chromosome equivalent to the Y in mammals is denoted by the letter W. This method of sex determination is followed by birds, some fishes, toads, most salamanders, reptiles, and Lepidoptera. In Europe the WZ-ZZ nomenclature has not been adopted. The symbols XY and XX are used, as in mammals, which is confusing to the student.

The means by which sex determination by union of sexual cells, or gametes, becomes possible resides in the special type of cell division that occurs during spermatogenesis and oögenesis. At the division between the formation of secondary spermatocytes from primary spermatocytes the number of chromosomes is halved. The pairs are brought together at the equatorial plate of the dividing nucleus as is usual in cell division, but, instead of each chromosome splitting in the usual manner at mitosis, the pairs divide, one whole member of each pair going to the corresponding pole of the nucleus. In this way, in mammals, each of the newly formed nuclei receives one quantum of maleness in the autosomes. One nucleus receives the X chromosome and thus one quantum of femaleness, while the other receives the Y chromosome with no sex determiners so far as is known. The secondary spermatocytes undergo further mitotic division and the spermatids so formed ultimately become spermatozoa. Thus there are two kinds of spermatozoa, X-bearers and Y-bearers. In consequence the male is described as the heterogametic sex.

In the development of the egg, events are very similar. At the division corresponding to that of secondary spermatocyte formation in the male the secondary oöcyte and the first polar body each receive one set of autosomes (giving one quantum of maleness) and one X chromosome (giving one quantum of femaleness). The polar body is an abortive cell; the secondary oöcyte undergoes a further division with the extrusion of another abortive polar body. The ova are all of the same kind in mammals, X-bearers, so the female is described as the homogametic sex.

At the time of fertilization, when spermatozoön and egg unite, the latter may be fertilized by an X-bearing spermatozoön or by a Y-bearing one. Since each kind of spermatozoön is produced in equal numbers there is an equal chance that an X-bearing spermatozoön or a Y-bearer will fertilize the egg. Accordingly, the sexes are produced in essentially equal numbers because, if the X-bearing egg is fertilized by an X-bearing spermatozoön the resulting zygote contains two sets of autosomes and two X chromosomes. Two quanta of maleness and two quanta of femaleness are present, and since the latter are the stronger a female results. On the other hand if the egg is fertilized by a Y-bearing spermatozoön the resulting zygote contains two sets of autosomes and but one X chromosome. Two quanta of maleness and only one of femaleness are present in the zygote and, since the two of maleness outweigh the one of femaleness, the resulting embryo is male. The mechanism is a remarkably simple one.

In the WZ type of sex determination the method is the same but the male, being ZZ, is the homogametic sex. All the spermatozoa contain one set of female-bearing autosomes and one male-bearing Z chromosome. The female is the heterogametic sex and produces two kinds of eggs. All of these contain one set of female-bearing autosomes but half the eggs contain the male-bearing Z chromosome while the other half contain the sexually inert W chromosome (if it is present, otherwise this position in the nucleus is void). When a spermatozoön enters a Z-bearing egg a male results because the two male bearing Z chromosomes have a greater sex-determining value than the two sets of female-bearing autosomes. If, however, a W-bearing egg is fertilized there are two quanta of autosomal femaleness and only one of Z chromosomal maleness so that the resulting embryo is a female.

In many species the inert Y or W chromosome has disappeared so that the mechanism may be described as XO or ZO (better as X- or Z-) instead of XY or ZW. The Japanese mouse is a species in which the Y chromosome is no longer present, while in birds there is no W chromosome. The essential method is not altered by the disappearance of the inert chromosome.

The two methods, control by concentration of maleness and of femaleness, seem to have arisen at much the same time in evolution since, in fishes they are found side by side even in closely related species, so closely related, in fact, that they hybridize.[19] By bringing X and Z chromosomes together in such hybrids it has been possible to show that in X chromosomes the concentration is one of femaleness and in the Z one of maleness. One can only speculate on the cause of concentration and on the generalized way in which it acted when it came. Living material seems to reach a point where a certain type of change is inevitable. The details of the change may vary but it has to occur.

In the Hymenoptera (ants, bees, and wasps), in certain other insects, and in rotifers the male is normally haploid, i.e. he has half the full complement of chromosomes, while the female is diploid. This makes the male 1A1X, and the female 2A2X. At first sight this does not fit into the genic balance pattern as the A:X ratio is the same in each case. The female produces haploid eggs. If these are fertilized they develop as females (diploids) but if they are not fertilized they develop as males (haploid). But not all X chromosomes are the same; there are at least two types, X^1 and X^2, which have been identified by sex-linked genes borne upon them.[44] Normally a kind of self-sterility exists so that an egg containing, say, an X^1 chromosome cannot be fertilized by an X^1-bearing spermatozoön. If, however, like X chromosomes happen to be brought together exceptionally the resulting diploid is a male. This condition has been found in certain hybrids. When X^1 and X^2 chromosomes come together the zygote develops as a female. The male, therefore, is either hemizygous or homozygous for certain sex-determining genes while the female is heterozygous for them. The basic pattern is of the mammalian type.

Factors Modifying Sexual Development

Enough has been said to indicate that sex determination, i.e. the decision whether the gonads are to be male or female, depends upon genes that are carried in the chromosomes. The chromosomes themselves do not determine sex; that is the function of the genes. In the view of the writer the sex-determining genes represent a very ancient mechanism from the evolutionary point of view. They have taken part in the arrangement of hereditary material into the form of chromosomes and have done so in a variety of ways so that there is apparent variety in sex-determining patterns. In the more primitive animals the ratio between male-determining genes and female-determining genes is so close that a species may be functionally hermaphroditic, either simultaneously male and female, as in sponges, or successively so, as in oysters. Occasionally sex may be determined largely by factors outside the organism, as in the worm, *Bonellia,* discussed later in this chapter. During the course of evolution the ratio between the two kinds of sex-determining genes has become wider so that under normal conditions hermaphroditism no longer exists, though it is possible in some species when the normal sequences of development are upset. It follows that sex determination may be implemented or upset by a number of influences. The relative values of the male and female determiners themselves may vary within a species; their relative concentrations may change if the usual pattern of chromosome distribution is disturbed in any way; additional specific genes (modifiers) may alter the ratio, either directly by the addition of their own values on one side or other of the ratio, or indirectly by modifying the usual chromosomal distributions. Or factors external to the organism may override the normal developmental patterns either by modifying normal chromosomal distribution or by altering the nature of the genic action.

This genic action as expressed in sex differentiation appears to be relatively simple. If one invasion of yolk-sac cells reaches the germinal ridges, development proceeds in the male direction; if a second invasion follows the first, development proceeds in the female direction. Consequently, for an individual to become male only one action is required; if a further action happens a female develops. If a modifying mechanism follows the original one immediately, that is, if a second invasion is induced without delay in a genetic male, complete reversal to female may result. If the induced invasion is delayed the result may be a mixture of male and female. The duration of time during which these types of change, i.e. modification of a genetic male, may occur seems to be rather limited. On the other hand the genetic female has always the possibility that the second invasion of yolk-sac cells may fail, or its effects die out, increasing or uncovering the male potentialities of the first invasion. Accordingly, this type of change, namely, reversal from female to male, may happen at any time during life. The result is that far more reversals are found in the direction from femaleness to maleness than from maleness to femaleness.

It must be pointed out that this embryonic pattern of sex differentiation, the first yolk-sac invasion producing male and the second one, female, is general. It does not depend upon the concentration of maleness or of femaleness into specific chromosomes.

These generalizations require some justifications which follow. The marine worm, *Bonellia,* was cited as an example in which external factors have a major influence in determining sex.[8] The males and females differ widely in form. The female has a bean-shaped body, about two inches long, and a proboscis a foot or more long. The male is a minute organism that lives as a parasite attached to the proboscis of the female. If the larvae are allowed to develop in an aquarium in which mature females are present the former may settle upon or remain close to a female, whereupon they develop as males. Larvae developing in an aquarium without females became females. Development in the male direction is also induced if the larvae are exposed to an extract of female proboscis or to a change in the pH of the water. If larvae that have been developing as males upon the proboscis of the female are removed from contact with the female they continue development as females with the result that they are intersexual forms. However, a few larvae develop as males even though they are not exposed to the influences that are usually necessary for this to happen. But, in the main, external influences determine the sex; the genetic constitution must be evenly balanced to allow this to be so.

Values of male and female determiners differ within the species of the gypsy moth, *Lymnantria dispar*.[17] This species has a very wide geographic distribution. Males have pointed, narrow abdomens and dark wings, females have blunt, wide abdomens and lighter wings. Moths taken in Germany breed normally among themselves and the offspring are normal. So, also, do moths captured in Japan. But if the European and Japanese races are crossed intersexes result when the mating, Japanese male x European female, is made. These intersexes are genetic (chromosomal) females but they display a mixture of male and female characters. With other crosses, intersexes result in crosses involving generations subsequent to the first. The results may be explained by assuming numerical variation in the values of maleness and of femaleness between the two races. The Japanese race is given a higher value for maleness and femaleness than is the European race; it is described as 'stronger.' Within each race the relative values of male genes and of female genes are such that sex determination is normal. But when the races are mixed the normal relationships are upset and close approximations of male and female values in the same individual lead to labile states in which intersexes result. Other races than those mentioned have also been tested and given numerical values for the sex-determining genes. In some instances it has been possible, on the basis of these allotted values, to predict the degree of intersexuality that would result from a particular cross. It may be noted in passing that this situation leads to sterility in the racial crosses. It may be a step in the evolution of new species.

An example of faulty chromosomal distribution as a cause of intersexuality is found in the fruit fly, *Drosophila melanogaster*.[4] In certain strains of this fly, which normally follows the mammalian type of sex determination, zygotes have occurred in which the number of autosomes has become greater than usual. This may bring the ratio of maleness in the female (XX) to a value too narrow for determination as a female, so that intersexes result.

In the aphids, sex determination is regularly brought about by extrusion of an X chromosome from the zygote in certain cases. Spermatozoa that do not contain an X chromosome degenerate so that all eggs are fertilized by X-bearing spermatozoa. This should lead to the production of all females (XX) but an X chromosome is extruded from the nucleus in some zygotes. These X zygotes then develop as males. A subsidiary genetic mechanism apparently causes this extrusion of part of the female-producing genes.

Instances of specific genes that modify the normal course of sex determination are fairly numerous, though in any one species they are rare. They have been found in some species of the fruit fly, *Drosophila*,[30] and also in goats and man. They are usually autosomal recessives. In goats the gene is closely linked with the dominant one that causes hornlessness, a character desired by the breeders and consequently selected for. This selection has increased the incidence of intersexuality so that it has become a matter of economic importance.[1] In one strain of Saanen goats the percentage of intersexes born was 14.3, in another 11.1. Sex-ratio figures for the second group showed 49.3 per cent males, 39.6 per cent females, and 11.1 per cent intersexes, so that the intersexes are most probably genetic females. Figures from other strains support this conclusion.

In man, intersexes are moderately frequent (see p. 16). There is ample evidence that inheritance accounts for many of the cases as they tend to occur in certain family lines.[16] Sex-ratio data from most of these lines indicates that the majority of them are genetic males (XY), and that the modifying gene is carried in the X chromosome. Limited cytoplasmic evidence supports the view that these intersexes are XY, though one case has been described that may have been XX.

An example of an external factor that overrides the normal chromosomal distribution and thus influences sex determination is found in the rotifer, *Hydatina senta*.[32] If the food supply is adequate, sexual reproduction of the haploid-diploid type is the rule. If it is inadequate the females produce diploid eggs that develop without fertilization as females. When the food supply is restored to normal, haploid eggs are produced that develop as males, after which fertilization again becomes possible.

Genic action modified by external factors is found in a number of instances. Parasitization of the male crab of the genus, *Inachus*, by another crustacean, *Sacculina*, causes the gonads of the former to produce ova instead of spermatozoa, a reversal the mechanism of which is still unexplained.[42] In poultry the right ovary is normally rudimentary while the left is functional. If the latter is damaged by disease or is surgically removed the rudimentary right ovary begins to develop as a testis or ovotestis.[6] This situation holds for all birds except birds of prey in which both ovaries are functional. In cattle, by far the majority of females born twin to a bull calf are sterile They possess rudimentary testes. The condition has been explained on the grounds that an anastomosis of the placental blood vessels causes an interchange of blood to occur between the twins.[31] The testis of the bull calf, developing before the ovary of the female, secretes hormones that modify the ovarian development in the female twin, causing it to assume a testis-like character. Suppression of the second invasion of yolk-sac cells may be the cause. An objection to this view is that vascular anastomoses have been shown to be present in other species without causing a change in sex. But there may be something unusual in the quantitative relationship between hormone and ovary in cattle that predisposes to this condition. Whatever the explanation may be, the fact remains that in some way the presence of a bull calf *in utero* with a heifer calf suppresses the normal genetic female development. The abnormal calf is known as a 'freemartin.'

Secondary Sexual Characters

The term 'secondary sexual characters' includes those physical characteristics that are not directly concerned in the reproductive processes but that differ in the two sexes. Typical of the group are sex differences in body build, size and width at shoulders and hips, hair and pigment distribution, voice, i.e. a physical difference in the larynx, and, in birds, differences in feathering. The teleologist would describe them as the characters most concerned in sexual display. As one surveys the causes of these differences, or the influences that modify the characters in question, one notices that these causes differ from group to group. In some the control is genetic, in others, hormonic. In still others there is a mixture of genetic and hormonic control. At base it is always genetic because the type of control inherent in the individual is governed by the genetic constitution of the species. It is in the immediate causes that the differences occur.

In many species of insects the males and females differ markedly in their appearance, so much so in some instances that it is difficult to recognize the two sexes as members of one and the same species. In butterflies castration or ovariectomy in the caterpillar stage does not affect the appearance when the adult stage is reached,[34] nor do transplantations of gonads or injections of sex hormones. The control is entirely genetic in immediate action and is fixed by the sex-gene constitution of the individual.

In the domestic fowl the males and females of certain breeds differ in the size and shape of their combs and in the color and shape of the feathers. Conditions in the Brown Leghorn have been closely investigated as, in this breed, the distinctions are very marked.[12] The comb of the rooster is larger than that of the hen; the feathers are long and pointed and are mainly colored brightly with reds and blacks. The hen's feathers are shorter and blunt tipped while the colors are more subdued, with browns and grays as the prevailing tints Castration of the rooster causes the comb to retrogress until it is quite small; bilateral ovariectomy of the hen also produces some retrogression so that the condition is similar in the gonadectomized bird of either sex. On the other hand, castration causes no change in feather characteristics when new ones grow after a molt or after some have been plucked. Ovariectomy, however, is followed by the growth of new feathers, pointed and brightly colored like those of the rooster. The neutral condition, therefore, is one in which the comb is small and the feathers are of the rooster type. Male hormones, androgens, when they are injected or implanted in tablet form promote comb growth until it is as large as that of the rooster, but the feathers are not affected. Female hormones, estrogens, do not promote comb growth but new feathers growing during the period of hormone administration are typically 'henny' in shape and color These results do not differ if the injections are made into castrated or ovariectomized birds, and androgens cause comb growth in the hen while estrogens produce henny plumage in the rooster. A mixture of the two types of hormones produces both comb growth and henny plumage; each type of hormone produces its own effect. It is evident that, in the Brown Leghorn, sex hormones are important as determiners of the secondary sexual characters. At the same time other hormones are involved, at least in the development of henny plumage, since estrogens do not produce their full 'bleaching' effect in the absence of the thyroid gland.

In the Sebright Bantam, the rooster is naturally henny feathered with feather shapes and hues resembling these of the female Sebright and the Brown Leghorn hen. The bright, pointed feathering does not occur. But both castration and ovariectomy produce, after a molt, the bright, pointed feathers of the neutral type. Hormone injections, whether they are estrogens or androgens, cause the development of henny plumage. The feather embryos are genetically conditioned to respond to both types of hormones while in the Brown Leghorn they can respond only to estrogens.

In a third breed, the White Leghorn, there is no plumage difference between the sexes and neither castration nor ovariectomy has any effect. Hormone injections, too, do not affect plumage development