

*This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.*

WORLD HEALTH ORGANIZATION  
TECHNICAL REPORT SERIES

No. 280

**BIOLOGY OF  
HUMAN REPRODUCTION**

**Report of a WHO Scientific Group**

	Page
1. Introduction . . . . .	3
2. Comparative aspects of reproduction . . . . .	3
3. Neuro-endocrine aspects of reproduction . . . . .	5
4. Biology of the gonads and gametes . . . . .	10
5. Gestation . . . . .	15
6. Biochemistry of the sex steroids. . . . .	23
7. Immunological aspects of reproduction . . . . .	26
8. Pharmacological aspects of reproduction . . . . .	27
9. Recommendations . . . . .	29

WORLD HEALTH ORGANIZATION

GENEVA

1964

WHO SCIENTIFIC GROUP  
ON THE BIOLOGY OF HUMAN REPRODUCTION

Geneva, 2-8 April 1963

*Members \* :*

Professor R. Caldeyro-Barcia, Servicio de Fisiología y Obstétrica, Facultad de Medicina, Montevideo, Uruguay (*Rapporteur*)

Dr R. I. Dorfman, Worcester Foundation for Experimental Biology, Shrewsbury, Mass., USA

Professor J. Ferin, Département de Gynécologie et Obstétrique, Université de Louvain, Belgique

Dr R. O. Greep, Harvard School of Dental Medicine, Boston, USA (*Rapporteur*)

Professor G. W. Harris, Department of Human Anatomy, University of Oxford, England (*Chairman*)

Professor A. St. G. Huggett, Department of Physiology, St. Mary's Hospital Medical School, London, England

Dr A. B. Kar, Division of Endocrinology, Central Drug Research Institute, Lucknow, India (*Vice-Chairman*)

Dr Takashi Kobayashi, Department of Obstetrics and Gynaecology, Tokyo University, Japan

Professor S. R. Mardachev, Academy of Medical Sciences, Moscow, USSR

Professor A. Tyler, Division of Biology, California Institute of Technology, Pasadena, USA

*Secretariat :*

Professor A. S. Parkes, Physiological Laboratory, University of Cambridge, England (*Consultant*)

Dr W. Winnicka, Chief Medical Officer, Maternal and Child Health, WHO (*Secretary*)

---

\* Unable to attend :

Dr A. Jost, Laboratoire de Biologie animale, 7 rue Cuvier, Paris, France

# **BIOLOGY OF HUMAN REPRODUCTION**

## **Report of a WHO Scientific Group**

### **1. INTRODUCTION**

A WHO Scientific Group on the Biology of Human Reproduction was convened in Geneva from 2 to 8 April 1963 for the purpose of advising the Director-General on developments and major research needs in that field. The meeting was opened by Dr Grundy, Assistant Director-General, on behalf of the Director-General; he stressed the growing importance of knowledge of the reproductive processes in human health and welfare.

Professor Harris was elected Chairman, Dr Kar Vice-Chairman, and Dr Caldeyro-Barcia and Dr Greep Rapporteurs.

The biology of human reproduction is an extremely broad scientific topic, which impinges to some degree on virtually all the basic medical disciplines. The development of this field derives from a very large literature currently expanding at the rate of approximately 7000 papers each year. Clearly, in a report of this length, no topic can be treated in other than its most essential and currently important features. Some topics have necessarily been excluded deliberately from consideration. These include the relation between nutrition and reproduction, which might well be the subject of a study by WHO; the genetical and cytogenetical aspects of reproduction, which also involve other aspects of the work of WHO; galactopoiesis; sexual behaviour; and the biochemistry of the gonadotrophins.

### **2. COMPARATIVE ASPECTS OF REPRODUCTION**

Many of our ideas of the biology of human reproduction are based on information obtained by experiments and observations on lower animals. It is important to realize, therefore, that the information in question has very narrow foundations.

Of the several thousands of species of mammals that exist, we have extensive knowledge of the reproductive processes of only about a dozen. We have some knowledge of about another 100; of the rest, we know little or nothing. This restriction of knowledge to a handful of laboratory and domestic animals has serious implications. The reproductive processes vary from species to species to a greater extent than any other bodily

function, and the species we know about cannot be considered as representative of mammals as a class. Especially, they cannot be considered as adequate prototypes for the study of human reproduction. Even apes and some monkeys, which have a menstrual cycle superficially similar to that occurring in man, differ markedly in other ways; they do not, for example, have the comparatively enormous output of gonadotrophin and oestrogen characteristic of human pregnancy. Every effort should be made, therefore, to broaden the basis of our knowledge of mammalian reproduction so as to increase the support it can give to the study of human reproduction.

What is true of mammals as a whole is true to a lesser extent of the human race. Our knowledge of geographical and ethnic variation and secular trends in the reproductive processes in man is quite inadequate. We need to know how the age of onset of puberty and of the menopause, the time of the return of the cycle during lactation, the occurrence of anovular cycles, etc. differ in different places with different peoples and under different conditions. Especially, we need to know to what extent and in what way such indices are changing under the impact of modern life.

With both the lower mammals and man, therefore, the need is to promote the study of the comparative biology of reproduction. This applies to the physiological and biochemical as much as to the morphological aspects. There is urgent need for further study of the comparative biochemistry of the steroid and protein hormones. For instance, analogous hormones obtained from different species may show structural and immunological differences that limit their use in a different species, e.g., man. In another field, it is known that basic differences exist in the way in which the foetus and placenta metabolize carbohydrates. At present methodology is a limiting factor, but further comparative studies are urgently needed.

Such comparative studies will require the introduction of new laboratory animals. For instance, the most interesting group of South American rodents, with long periods of gestation in relation to their size, is represented in laboratories only by the guinea-pig, which differs reproductively in almost every way from other laboratory animals. Again, a laboratory mammal regularly producing identical twins or quadruplets would be invaluable for certain studies.

Another example of the need for broadening the basis of our knowledge is that in two wild species there is good evidence of unilateral endocrine effects, in that progesterational development of the endometrium takes place only in the uterine horn on the same side as the single corpus luteum. Here is a condition otherwise known only in the foetus. Evidently, systematic efforts should be made to collect and review information relating to special features of little known animals and, where promising, to secure their introduction into laboratories. An obvious starting point would

be to draw upon the knowledge and experience of zoological societies and national organizations concerned with laboratory animals.

It may be added that in the past new laboratory animals have proved valuable in many ways, e.g., the cotton-rat and the gerbil, which have been of great assistance in work on typhus, filariasis, and atherosclerosis.

### 3. NEURO-ENDOCRINE ASPECTS OF REPRODUCTION

#### Effect of external factors on reproductive functions

The factors considered here are climatic and social. Climatic factors can be studied in different parts of the world under natural conditions or in laboratories under artificial conditions. Light appears to be one of the most generally important, and to depend for its effect essentially on a change in the light ration. The direction of change is not always the same; initiation of the breeding season may follow an increase of the light ration, as in ferrets, or a decrease of the light ration, as in sheep. Moreover, clearly demarcated breeding seasons may occur under virtually constant climatic conditions and in such cases breeding seasons are not necessarily synchronized in different species. There is good evidence, therefore, of internal reproductive clocks as well as of responsiveness to environment.

Among social factors, the most important among mammals appears to be smell, which is concerned with the recognition of sex and sexual condition and the evocation of sexual behaviour, and which is now also known to be a potent exteroceptive factor working through slowly-acting neuro-humoral mechanisms. This latter effect of smell has been studied most carefully in mice. In these animals, the crowding together of females results in an increase in spontaneous pseudopregnancy or even in anoestrus, both of which conditions respond quickly to the introduction of males, so that there is a peak mating about three days later (the Whitten effect). An even more interesting social effect on reproduction in mice is seen in the fact that about 80% of newly mated females fail to become pregnant if exposed during the pre-implantation stages to the smell of males of a different strain from the stud male (the Bruce effect). Both these social effects are mediated through smell, and in the case of the Bruce effect failure of prolactin secretion by the adenohypophysis, which would normally follow even sterile mating, is implicated.

The source and nature of the odorous substances produced by male mice that affect females are not known with certainty, but it seems very probable that they are excreted with the urine. Whatever their source, the substances in question clearly belong to a group now coming into prominence—externally secreted substances having an effect on other individuals. Evidently, wide prospects are appearing in this comparatively new field of

research, which may conveniently be regarded as a major branch of exocrinology.

Among other external social factors affecting reproduction is the neonatal maternal environment, which may affect the development of sexual behaviour and of the reproductive organs and is an important field of study likely to expand in the near future.

### **Central nervous system and reproduction**

The central nervous system is related to the reproductive processes in two main ways. Firstly, it controls directly, or by mediating reflex activities, the secretion of oxytocic hormone from the posterior pituitary gland and of gonadotrophic hormones from the anterior pituitary gland. And secondly, it mediates the feedback action of ovarian and testicular hormones, which are probably concerned with the organization and development of the foetal central nervous system<sup>1</sup> and with the regulation of anterior pituitary activity and sexual behaviour in the adult.

Environmental stimuli (discussed above) affect reproductive processes by acting through reflex nervous pathways involving many parts of the central nervous system. The nervous effects are focused eventually through the hypothalamus and pituitary gland. Little is known regarding the nerve tracts and neural mechanisms involved, although data are beginning to accumulate on an important gonadotrophic function of limbic lobe structures such as the hippocampus and amygdaloid nuclei.

The nervous control of the posterior pituitary gland seems important in at least three reflex responses. It has been clearly shown that during suckling reflex activation of oxytocic secretion is concerned with the contraction of the mammary tissue and thus ejection of milk to the suckling child. Knowledge of this mechanism is now being applied therapeutically in obstetric clinics. During parturition afferent stimuli arising from the uterus and vagina reflexly excite oxytocic secretion which in turn stimulates uterine contractions. It is probable that during mating the reflex release of oxytocic hormone causes an increase in uterine motility which may be important for the transport of spermatozoa in the female tract. The neuro-anatomical details concerned in these reflexes are largely unknown. Conditioned reflexes also play a large part in these responses in the human.

The hypothalamic control of anterior pituitary secretion of gonadotrophic hormones has been investigated in many experiments involving lesions or electrical stimulation of different neural areas. The central nervous system seems to regulate gonadotrophic secretion by a balance of excitatory and inhibitory influences. Although different areas of the hypothalamus can now be demarcated with reference to various anterior

---

<sup>1</sup> See page 17.

pituitary hormones, the details of the neural mechanisms involved in this control are, again, largely unknown. The pathway by which the hypothalamus affects anterior pituitary secretion involves the hypophysial portal vessels of the pituitary stalk and probably involves the release of humoral transmitter agents by nerve tracts in the hypothalamus into these vessels. Various groups of workers in different countries are studying this mechanism by applying crude hypothalamic extracts to anterior pituitary tissue by a variety of techniques and studying the release of luteinizing hormone (LH). *In vitro* techniques seem to hold much promise for future work. Much evidence has accumulated that these hypothalamic extracts (but not extracts of other areas of the nervous system) contain a substance that is active in causing secretion of LH, but the nature of this substance is uncertain and requires active investigation by modern chemical procedures. The mechanism underlying the secretion of follicle-stimulating hormone (FSH) appears to be similar in nature, but awaits investigation.

A feedback action of gonadal hormones on the central nervous system has been clearly implicated in the control of gonadotrophic secretion. Many types of study have substantiated this, but the neural mechanism involved is far from clear. Further study is required on all aspects of this problem, including the permeability of the "blood-brain barrier" to steroid hormones, the mechanism by which steroids affect neuronal activity, and the neurone groups implicated in feedback effect. The action of various steroids on the brain in evoking oestrus in many lower mammals has recently been studied by methods involving direct implantation of such substances in the brain and the use of radioactive hormones. It is clear that ovarian steroids act on some integrating mechanism in the hypothalamus to regulate patterns of sexual behaviour. Work in this field is only just beginning but has great implications for man. For example, since it has been shown that oestrogens accumulate on, or in, specific nerve cells, the possibility exists that injection of large amounts of radioactive oestrogens could result in the hormonal "dissection" and destruction of the nervous mechanism associated with sexual behaviour.

In human beings social behaviour as well as sexual behaviour can be affected by sex steroids.

#### **Effects of gonadotrophins**

In most of the mammals studied hypophysial control of the gonads appears to be brought about through the action of two gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH, also termed interstitial-cell-stimulating hormone, ICSH). A third anterior pituitary hormone, prolactin (lactogenic hormone, luteotrophic hormone), has a luteotrophic action in rats and mice, stimulating the secretion of progesterone by pre-formed corpora lutea. FSH is responsible for the

growth and maturation of ovarian follicles, LH along with FSH for ovulation, and LH for the conversion of the ruptured follicle to a corpus luteum. By what stimulus the corpus luteum is induced to secrete progestogens is, however, largely unknown except possibly in the rat, mouse, hamster, sheep, and man. The fate of the fertilized ovum and the success of the pregnancy rest heavily on the progestogen output by the corpus luteum, and there is urgent need for a better understanding of the control of this organ. In many animals, removal of the luteal body (or bodies) at any time during gestation results in abortion; in others, including man, the functions of the corpora lutea are shared, or entirely taken over, by the placenta at the very early stage. The secretion of progesterone by the corpora lutea is clearly responsible for the inhibition of ovulation that occurs in most mammals during pregnancy.

Recently it has been demonstrated that the addition of LH to incubated slices of bovine corpora lutea brings about an increased net synthesis of progesterone. The effect is not specific, as the same result was obtained with FSH or even the co-factors triphosphopyridine nucleotide (TPN) or reduced TPN (TPNH). No effect was observed with inactivated LH, adrenocorticotrophic hormone (ACTH), the posterior pituitary hormones, and albumins.

Observations based on the collection and analysis of ovarian venous effluent in rabbits indicate that injection of LH produces a prompt outpouring of progestogens. The response does not require the presence of corpora lutea or even follicles, but only ovarian interstitial cells. The latter are an especially prominent feature of the rabbit ovary and the effect observed may not apply generally to mammals.

Corpora lutea are capable of secreting oestrogen, and under suitable experimental conditions this may become the dominant secretion and thereby mask the effect of simultaneously produced progesterone.

Progesterone and many orally active synthetic progestogens are effective in inhibiting ovulation in the human female. The mechanism of their action has not been determined. The possibilities are that the site of action of such compounds may be the hypothalamus, the anterior pituitary, or the ovary. It is presumed that the progestogens now in wide-scale use act at the level of the hypothalamus to inhibit the neurohumoral mechanisms responsible for the release of LH. Study of the mechanism of action of ovulation-inhibiting progestogens requires the use of pure compounds alone, yet in practice these are almost invariably administered with an effective quantity of oestrogen. The need for additional investigation of the mode and mechanism of action of these compounds is of urgent importance.

It has been demonstrated that the administration of synthetic progestogens may produce severe involution of the reproductive organs and induce hyperthermia.

Urine collected from children, men, and women contains a substance (gonadotrophic inhibiting material—GIM) that may be obtained by alcohol precipitation, is relatively heat stable, appears to have a molecular weight of about 50 000, and inhibits the action of LH on the ovary and testis. The action of FSH is not inhibited by GIM-like material, nor is this material capable of interfering with the activity of androgens or oestrogens on their target tissues. Neither casein or purified albumin exhibit any of the properties of GIM. A GIM-like material, if established as a physiological entity, would obviously have many important implications in reproduction.

### Lactation

The nervous system and pituitary gland are associated with the process of lactation in two ways :

Anterior pituitary gland - Milk secretion	}	Lactation
Posterior pituitary gland - Milk ejection		

Milk formation and secretion are under the direct and indirect control of the anterior pituitary. The transfer of milk (already formed) from the mother to the suckling young involves an active contraction of breast tissue stimulated by the oxytocic hormone. It is likely that the act of suckling stimulates secretion of both the lactogenic hormone from the anterior pituitary and the oxytocic hormone from the posterior pituitary gland. Only the milk ejection aspect of lactation is considered below; the wide field of research dealing with milk secretion is omitted.

For many years now it has been known that the mammary gland could not be emptied by the action of suckling alone, but that a positive reaction of the breast or udder was involved in the transference of milk from mother to young. It is now established that the sensory stimuli associated with the act of suckling and conditioned stimuli act by nervous reflex pathways, via the hypothalamus, to stimulate oxytocin secretion. The oxytocin hormone is in turn carried by the general circulation to the breast where it excites contraction of the myoepithelial cells and squeezes milk from the depth of the glandular tissue into the ducts. This mechanism has been clarified by much work since direct evidence was first obtained, in rabbits and goats, that electrical stimulation of the hypothalamus results in oxytocic secretion and milk ejection.

Milk ejection from a lactating mammary gland is now regarded as a specific indication of the presence of oxytocic hormone in the blood; it is evoked by sexual excitement and coitus in both experimental animals and man.

The recording of intramammary pressure in puerperal women shows that suckling of one breast elicits rhythmic contractions of the myoepithelium of the other breast; similar contractions can be produced by the intravenous.

infusion of oxytocin at rates of 4 to 8 mU/min/60 kg. Release of oxytocin has also been obtained by conditioned reflex mechanisms elicited by stimuli such as seeing the baby.

With or without lactation, there exists after delivery a phase of amenorrhoea and non-ovulation. The duration of this phase varies greatly in different women, and menstruation often returns before ovulation. Various factors such as age, parity, nutrition, race and, most of all, length of lactation are involved. Infertility tends to persist if lactation is prolonged, but the exact mechanism is unknown. Administration of ovarian inhibitors could be contemplated in order to reinforce this physiological mechanism and so protect lactation against a premature conception.

#### 4. BIOLOGY OF THE GONADS AND GAMETES

The gonads are characterized by being organs of both internal and external secretion and their endocrine and gametophoric activities may be affected very differently by different conditions and at different stages of the life cycle. The endocrine activity of the gonads differs in the two sexes in that the testis produces mainly androgenic substances and the ovary mainly oestrogenic and progestogenic substances, but all these are produced as the result of a continuous biosynthetic process<sup>1</sup>. The production of gametes, however, is entirely different in the two sexes, in that the adult testis maintains a continuous production of enormous numbers of spermatozoa, while the adult ovary periodically sheds small numbers of ova drawn from a limited population of metabolically inert oocytes, which is laid down very early in life and is depleted far more by atresia than by ovulation.

##### Ovary and ovum

The causes of follicular atresia and the reasons why one particular follicle matures and ovulates, while apparently similar ones regress, are unknown. This continuous wastage of a limited oocyte population laid down before or soon after birth has the significant implication that offspring born towards the end of reproductive life are derived from oocytes that may be 40 years old in the case of the human female, which are the survivors of an aging population and may well have developed genetic defects.

This hazard may account at least in part for the observed fact that older mothers produce a larger proportion of congenitally defective children than do those in the prime of reproductive life. The competence of oocytes maturing at different stages of the life cycle and the relation to early

---

<sup>1</sup> See page 23.

embryonic mortality and later defect is thus an important question and one that should be susceptible to experimental attack as well as to ultra-structural studies. In this connexion, techniques recently worked out for routine orthotopic grafting, which permit the functional transfer of ovarian tissue between old and young animals, may be extremely useful.

One of the main difficulties in studying the human ovarian cycle lies in determining the time of ovulation. Most lower mammals and some primates have changes associated with the time of ovulation, which can be detected externally and by which, therefore, the time of ovulation can be diagnosed with some accuracy. In most lower mammals, this phase of ovulation and sexual receptivity (oestrus) constitutes the overt focal point of the cycle. In most primates, however, and notably man, menstruation resulting from the breakdown of the endometrium at the end of a cycle that has not resulted in pregnancy is the overtly focal point, and there is no obvious change associated with ovulation. On general principles ovulation can be assumed to occur about 12-16 days before the next menstruation is due (itself an uncertain factor), but it can be diagnosed only retrospectively, by pregnanediol excretion or temperature rise. The development of a simple method of detecting accurately the time of ovulation in women, especially its detection in advance, would warrant almost unlimited research effort. Unfortunately at the present time no clear lead exists, and progress will probably depend on developments in other related work.

Much of our basic knowledge of the endocrinology of the ovary has been derived from a study of ovarian grafts, which also provides a good demonstration of the reciprocal control between the gonad and the hypophysis. Orthotopic grafts become well vascularized and function as normal ovaries unless the supply of oocytes fails. Ectopic grafts, by contrast, are often poorly vascularized and sooner or later become morphologically or endocrinologically abnormal. The type of abnormality and the speed with which it appears seems to depend on the extent to which the feedback mechanism to the hypophysis is interfered with by inadequate vascularization, which affects the transfer of steroid hormones from the graft more than it does the transfer of gonadotrophins to it. An extreme case of the breakdown of the feedback mechanism is seen with intra-splenic ovarian grafts, the steroid hormones from which are destroyed in the liver system before reaching the pituitary.

### **Testis and spermatozoon**

A spurt of growth of the testis occurs with the onset of puberty in all known mammalian species from rat to man, but the nature of pituitary control of this phenomenon has not been properly elucidated. Nor is anything known about the identity of the testicular hormone that activates the hypothalamic-pituitary mechanism at puberty. By analogy with

females, this activating hormone may be oestrogen, which is known to be elaborated in the adult testis, possibly in the seminiferous tubules.

It is not known with certainty when the testis acquires its initial sensitivity to gonadotrophins. Scrotal grafts of the testis of new-born males into castrated adults might give useful information. In this connexion, it will be necessary to know the biochemical basis of the development of the initial sensitivity to gonadotrophins and the influence, if any, on the process of other endocrine organs such as the thyroid and the adrenal cortex, which have been shown to influence the sensitivity of the adult testis to exogenous gonadotrophins.

Little is known about the metabolism of the primary spermatogonia which, together with the Sertoli cells, are the only elements present in the tubules of the early immature testis, but the spermatogonia of the adult mouse testis have been reported, for instance, to have an unusually long deoxyribonucleic acid (DNA) synthesis pattern. The time lag between ribonucleic acid (RNA) and DNA synthesis is also appreciably greater than in the somatic tissues. It would be useful to know whether the same is true for spermatogonia in the immature testis and whether any change occurs in the rate and pattern of nucleic acid synthesis with puberty. The extent of the influence of gonadotrophins and androgens on the nucleic acid metabolism of these cells would be worth exploring, especially in view of the fact that spermatogenesis can be maintained for a time after hypophysectomy by administration of androgen. In adults, the spermatogenic cycle is known to begin at the moment when a type A spermatogonium embarks on the process of differentiation, but precisely when and why this happens remains obscure, as does the mechanism that initiates the first spermatogenic cycle at puberty.

It is known for the rat, mouse, ram, and bull, among other species, that some of the seminiferous epithelial elements undergo spontaneous degeneration. The most susceptible ones are the primary spermatogonia of the foetal and early post-natal testis and spermatogonia of the intermediate type, early spermatocytes, and spermatids in adults. Little is known about any analogous spontaneous degeneration of seminiferous epithelial elements in monkeys and man, and the factors responsible are obscure.

In contrast to the wealth of information about the ultra-structure of spermatozoa, the electron-micrographic details of the testis have received relatively little attention. There remain to be investigated, for instance, the ultra-structure of the immature testis and the nature of the changes that occur with the onset of puberty, and, in the evolution of the cytoplasmic bridge system, the organization of Sertoli cells, and the differentiation of Leydig cells from mesenchymal precursors.

The nature of the hormonal or other mechanisms involved in the descent of the testis remains to be elucidated, with particular reference to the gubernaculum, which is known to play some role in the descent of the

testis. The biochemical changes in the testis in different stages of descent would be worth consideration in comparison with those in the undescended testis. The latter is known to produce hormones, but their true identity and metabolism are poorly understood.

A basic structural similarity exists in spermatozoa throughout the animal kingdom; for instance, all spermatozoa exhibit an extreme cytoplasmic reduction signifying perhaps a loss of the mechanism of synthesis. The factors initiating motility in spermatozoa are largely unknown, as is the mechanism whereby the necessary energy is transferred to motion. Whether primordial germ cells will differentiate into spermatozoa or into eggs is determined not by their own chromosomal constitution but by that of the surrounding cells located in the genital ridge.

While some evidence is available concerning the life span of mature spermatozoa in the reproductive tract of the male, little is known of factors that influence their life span in the reproductive tract of the female, though in most mammals it is thought to be one to three days at the most. The aging of sperm and the factors that influence capacity for fertilization are worthy of additional study. There is evidence that human spermatozoa acquire from the male tract substances that may be detected by immunological means, but it is not clear how this may be related to their fertilizing capacity. Dilution of semen affects spermatozoa adversely, due to the binding of trace metals and also probably to the loss of essential components of the energy-supplying system.

Metabolically, spermatozoa are able to utilize food from both endogenous and exogenous sources. Although they glycolyse fructose, glucose, and many other sugars under either aerobic or anaerobic conditions, their metabolic functions under the latter circumstances are quite limited. Existing knowledge provides a firm basis for determining the metabolism of the endogenous substrate—such further study of energy stores being relevant to their capacity to ascend the female genital tract.

Much work has been done on the spermatozoa of various animals with respect to chemical and physical factors that influence fertilizing capacity. Among these factors the action of ionizing radiation—at present a matter of great importance—has been studied to an insufficient extent, and the findings are inconsistent. In mammals, there have been experiments on rabbits and mice showing that very large doses to mature spermatozoa are required to prevent their penetrating the egg, but both their motility and their genetic properties are adversely affected by comparatively small doses. In marine animals all these properties of the spermatozoa are affected. Large-scale critical experiments need to be done to ascertain the extent to which spermatozoa differ, if they do differ, from other kinds of cells in their sensitivity to ionizing radiation, and to settle important questions concerning the extent to which the genetic and vegetative functions of spermatozoa can be differentially affected by ionizing radiation.

### Fertilization

There are a number of basic problems about fertilization and early development. The first relate to the factors responsible for bringing spermatozoa and eggs together, including the liberation of the gametes, their life span, the motility of spermatozoa, the role of the genital ducts in the transport of gametes, their further maturation, and the possibility of chemotaxis between them. Then there are the problems of species-specific and tissue-specific adherence of spermatozoa to the egg, and of the penetration of the spermatozoa through the extraneous coats and the surface of the egg proper—a surface that has no openings large enough to admit a spermatozoon. There is the important problem of the block to polyspermy and those raised by the phenomenon of activation of the egg, whereby a chain of biosynthetic events is set in motion.

These problems, studied mainly in lower animals, are common to all animals. So results obtained on one kind of organism are likely to hold for others when due allowance is made for special features and adaptations. Nevertheless, there is need for investigation in mammals with respect to *in vitro* fertilization, which has not been readily demonstrated. Also, chemotactic response of spermatozoa to egg materials has not been demonstrated in animals.

*Specific interacting substances of the gametes.* Specific adherence of spermatozoa to the egg involves the interaction of receptor substances (fertilizin and antifertilizin) on the surface of spermatozoa and eggs respectively, but not on other tissue cells. Interaction of the receptors resembles that of antigen with antibody. Penetration of egg coats involves the action of enzymes, like hyaluronidase in mammals. There is also antifertilizin located below the surface of the egg. Knowledge is accumulating concerning the chemical constitution of these substances in some species. They are proteins, and thus rather complex. Fertilizin of sea urchins is a glycoprotein, similar to the human blood-group substances.

Fertilization is inhibited when excess dissolved fertilizin is present because the spermatozoa react with the dissolved fertilizin and can no longer bind to the fertilizin on the egg. The converse experiment with solutions of antifertilizin gives similar results. The dissolved fertilizin, present around the unfertilized eggs, can be "mopped up" by excess spermatozoa. This is part of the explanation for the large number of spermatozoa required in lower forms for successful fertilization, although only one normally enters the egg. In forms with internal fertilization the large minimum size of inseminates is due to the random nature of the movements of the spermatozoa, their ordinarily short life span, and the attrition that occurs in their passage up the female genital tract. Union of egg and spermatozoa is highly sensitive to changes in the surrounding medium such as can be produced endocrinologically and otherwise in the female tract.

While possible mechanisms, such as specific pinocytosis (phagocytosis) for engulfment of the sperm have been proposed from studies on lower animals, there is much that needs to be learned in this area for all animals and mammals in particular.

*Activation of egg.* Activation of eggs has been achieved artificially, but the physico-chemical basis for it is still mostly not understood. Many dramatic physico-chemical changes occur upon fertilization, including a change in electrical potential which disappears when the external potassium ion concentration is brought to a value near that of the inside, thus resembling muscle and nerve. Measurements with radioactive potassium show that this ion is largely "compartmentalized" before fertilization, while it exchanges freely with the outside after fertilization. Among other interesting properties may be cited the ability of unfertilized eggs to oxidize carbon monoxide. This falls off upon fertilization and development.

*Initiation of protein biosynthesis.* A primary part of the process of producing a new individual is the manufacture of specific proteins. This process is initiated at fertilization. Currently, great discoveries are being made concerning the manner in which the molecular structure of the proteins of the cell is determined by a "code" represented by the particular sequence of the units (nucleotides) comprising the deoxyribonucleic acid (DNA) that is the essential ingredient of the gene. DNA operates by transcribing its code on a particular kind of ribonucleic acid (RNA) termed "messenger RNA", which serves as the immediate template for protein synthesis. A synthetically produced polyribonucleotide can act as a messenger RNA so that the corresponding protein will be produced in a cell-free system consisting of certain ingredients (ribosomes, particular enzymes, amino acids, etc.) obtained from bacterial homogenates; this has also been done with sea urchins. Preparations from unfertilized eggs are as active as from fertilized eggs. The unfertilized egg evidently has a considerable number of unprogrammed ribosomes awaiting messenger RNA. Experiments with non-nucleated egg fragments, activated artificially, show that there may also be an inhibitor of the ribosomes accounting, along with lack of messenger RNA, for the inertness of the unfertilized egg.

## 5. GESTATION

### **Progestation**

After fertilization, the mammalian egg passes down the Fallopian tube, segmenting without increasing in size. After entering the uterus, it expands to form a hollow blastocyst, probably under the influence of some uterine factor at present unknown, and remains free in the uterus for a variable time. It becomes implanted by burrowing into the uterine mucosa,

by being engulfed in a crypt, or by expanding to fill the lumen of the uterus. At this stage, or possibly before, a signal is evidently sent to the hypothalamus indicating that there is an incipient pregnancy and initiating hypophysial changes that maintain the corpus luteum and throw the cycle out of gear. In human beings, the effect is to postpone the regression of the corpus luteum and the endometrial breakdown that would have brought on the next menstruation. In some species, including man, close timing is evidently required, but the nature of the signal sent from the uterus at the time of implantation is unknown and remains one of the outstanding problems of reproductive physiology.

The blastocyst can become implanted in the uterus only when the endometrium is in the receptive state, which normally occurs a few days after ovulation under the influence of ovaries with a functional corpus luteum. The exact nature of the endocrine conditions required has been the subject of a great deal of rather confused and often contradictory experimental work, based on removal of the ovaries or administration of hormones and other substances, or both, in studies on normal or delayed implantation and on the decidual cell reaction.

Delayed implantation in the post-partum rat and mouse may, according to various reports, be terminated by the administration of progesterone, by stopping its administration, or by the administration of oestrogen with or without progesterone. The most general conclusion would seem to be that progesterone plus oestrogen is needed. In the mated, non-lactating rat, delay in implantation induced by ovariectomy may be terminated by high doses of progesterone when ovariectomy is done on the second day after mating, but only by the simultaneous injection of oestrogen plus progesterone when ovariectomy is delayed to the fourth day. However, oestrogens are highly potent in preventing implantation in rodents, but only in doses 50-100 times that needed for initiating it. Anti-oestrogens also prevent implantation. Oestrogens suppress the decidual cell reaction and so does dimethylstilboestrol, a potent anti-oestrogen. The hypothesis that decidual formation in the rat follows histamine release in the uterus after oestrogen administration has received a great deal of attention, and it is known that intra-uterine administration of antihistamines suppresses decidual formation. There have, however, been criticisms of the histamine hypothesis.

Various techniques make it possible as a routine to bring about in mice the ectopic implantation of blastocysts removed from the uterus under the kidney capsule or in the spleen in either sex, or in the testis. No special hormonal conditions seem to be necessary for such implantations, and their effect on the ovarian cycle and mammary glands of the female host is not yet known. It seems, however, that the elaborate build-up of the uterus for implantation is necessary to overcome some otherwise inhibitory factor or to provide the conditions necessary for the transformation of the

segmented egg into the blastocyst. In any case, experimental ectopic implantation should prove most valuable in the analysis of the implantation feedback mechanism.

### **Endocrinology of the foetus**

The hormones produced by the endocrine glands of the foetus have long been suspected of playing a role in foetal development and some definite information is now available.

No foetal gland so far has been found to be indispensable to the general body growth of the foetus, and the endocrine or metabolic conditions responsible for the development of "giant" foetuses still remain unknown. Various endocrine glands, however, are necessary for the production of a normal and properly differentiated foetus. The physiology of the foetal hypophysis has been a subject of numerous studies.

The role played by foetal testicular secretion in the differentiation of the genital system has been clearly demonstrated, but the nature of the substance or substances involved is obscure. Some observations suggest that two types of foetal hormone may be produced, one of an essentially inhibitory nature and the other resembling the adult hormone. The solution of this problem is of both theoretical and clinical interest. Some anomalies of the reproductive tract can be more easily interpreted on the assumption that they are caused by deficiency in one or the other of these hormones. It should be emphasized that the action of the foetal gonadal hormones leaves its mark on the organism throughout life and that disorders of these hormones cause practically irreparable disturbances. Moreover, the administration of certain synthetic progestogens to women in early pregnancy has produced virilism of the external genitalia of female foetuses.

The foetal thyroid secretes thyroxin in the rat. However, the way in which thyroxin acts on the foetal skeleton or, more importantly, on the maturation of the nervous system still remains almost completely unknown, despite the great clinical importance of the problem. One example of the action of hormones in the differentiation of a physiological function in the foetus is supplied by the control of foetal liver glycogen by means of a synergistic action between a pituitary hormone (probably somatotrophin) and an adrenocortical hormone. This has been demonstrated in the rabbit.

Recent studies suggest that the gonadal steroids exert an organizing action on the developing central nervous system of the foetus or new-born. It seems that, at least in the rat, the early foetal central nervous system is female in type, and that in the normal genetic male the nervous system differentiates under the influence of secretions from the foetal or neonatal testis. Sexual differentiation of the central nervous system is shown by the type of control it exerts over (a) the pattern of secretion of gonadotrophic

hormone from the anterior pituitary gland—that is, whether it is cyclic as in the female or acyclic as in the male; and (b) the pattern of sexual behaviour predominantly shown by an individual. Female rats that receive one injection of testosterone within 96 hours of birth fail to show sexual cycles when they become adult but show a steady level of ovarian function. Further, they do not exhibit any female pattern of sexual behaviour but may show marked male-type behaviour. Similar evidence is also available for female guinea-pigs. If the developing male rat is deprived of testicular hormone by castration at the time of birth, the central nervous system seems to remain female in type. Thus, an ovary transplanted into such an animal when adult shows that the nervous system is regulating gonadotrophic secretion in the rhythmic female pattern. These findings suggest that administration of certain androgenic or progestational compounds to pregnant women might give rise to disturbances of the central nervous system of the offspring. They may also perhaps throw light on certain types of aberrant sexual behaviour, and they are concordant with the fact that a female calf born twin to a bull after anastomosis of the foetal vessels is usually abnormal sexually.

The reaction discussed above may be analogous to that by which oestrogen administered to new-born male rats results in profound permanent testicular damage, with respect to both spermatogenesis and androgen biosynthesis, presumably by way of the hypothalamo-hypophysial system. Since progesterone will protect new-born female rats against the effects of administered testosterone, it is anticipated that progesterone will also protect the new-born male against the effect of oestrogen.

It is suggested that progesterone protects the sensitive hypothalamo-pituitary system from the damaging effects of excessive androgens or oestrogens. In humans, in the absence of the restraining influence of progesterone, it is possible that excessive oestrogens could cause hypogonadism in the male foetus and that excessive androgens in the female foetus could lead to abnormal ovaries, perhaps of the Stein-Leventhal type.

#### **Foetal and placental physiology**

The foetus and placenta are best considered as a whole, if only because they grow from a single cell and exhibit a reciprocating symbiotic partnership throughout intra-uterine life.

Intra-uterine life is conveniently regarded as falling into three periods. The first is that of differentiation and morphogenesis, during which the placenta is formed and the foetus develops indications of the organs it will need in later life. The second period is that of placental activity, in which the dominating factor is the growth of the placenta and foetal membranes and the production of the large volume of amniotic liquid (with allantoic in non-primates). The third period is that of foetal growth,

dating approximately from the age at which foetal weight exceeds the placental weight. In this period it is normal for the placenta to show senescent changes, particularly in human beings.

The first period is being studied by the experimental embryologist, especially in lower mammals. The genetic influence of the chromosomal pattern of the ovum stamps its lifelong imprint: specific differentiating enzymes are active, and viruses, drugs, and excess or deficiency of nutrients can cause teratological disaster.

The third period is being studied by the experimental physiologist, the agricultural scientist, the obstetrician, and the paediatrician, and derives its importance from the danger of premature delivery and precarious viability. The respiratory and circulatory systems and heat metabolism have been well evaluated in this period.

The second period has not been studied systematically. Sporadic interest in this or that phase of development has been shown by scientists working on problems of the adult. Evidence of such interest is the recent knowledge concerning acquired tolerance and immunology<sup>1</sup>. The second period has recently become important because certain experimental findings permit a reinterpretation of phenomena that may go some way towards increasing our understanding of prenatal development.

These experimental findings concern the placenta, the foetal fluids, and the foetus. The placenta transfers material to the foetus and metabolites to the mother by active consumption of energy rather than by passive diffusion under a pressure gradient. It secretes certain hormones to the mother, and it accelerates glucose transfer to the foetus (or *vice versa* according to the concentration gradient) by facilitating diffusion and also by exerting a one-way action, by various methods not yet completely understood but including the transforming or altering of molecules so that their return to the mother is impaired if not prevented. These products then exist in the foetal blood and extra-cellular fluid in concentrations normally exceeding those in the maternal blood. This group of products includes fructose in ungulates and cetaceans, placental glycogen in rodents and primates, potassium and calcium cations, phosphate ions as inorganic phosphates, phospholipids in rabbits and guinea-pigs, and the gross total plasma amino acid. It does not include water or sodium or chloride, which have certain peculiarities of their own, concerning osmotic pressure and active secretion, bound up with renal function in the foetus, where a hypotonic urine is excreted and goes into the amniotic fluid (and allantoic fluid when present).

Colloids are in higher concentrations in the maternal blood than in the foetal blood, so that colloidal osmotic pressure is greater in the maternal

---

<sup>1</sup> See page 26

blood plasma. Foetal colloids are synthesized within the foetus. Additional proteins do not pass the placenta in nutrient quantities but may do so in immunological quantities.

The foetus drinks its own amniotic fluid continuously, absorbing its contents; these circulate in the blood and are excreted in the urine to the amniotic fluids and reabsorbed again. Thus the extra-cellular fluid of the foetus is essentially in two parts, intra-foetal (which persists after birth), and extra-foetal, which is approximately 8 to 10 times greater in volume and content.

Lastly, the fluids decrease markedly in the third period and most of the dissolved material seems to be retained in the foetus. The use of labelled preparations has shown that fructose is laid down in the cell nuclei of foetal sheep, whereas glucose is distributed uniformly about the body. The specific properties of potassium, calcium, phosphate, and amino acids suggest that in the second period of pregnancy the placenta ensures in the total extra-cellular fluid a relatively small mass of special nutrients essential for key mechanisms during the rapid growth of the third period. The nutrients crossing the placenta in this period are less specific, are interchangeable, and supply bulk to the foetus. The critical growth of the placenta in the second period suggests that there should be some correlation between the weights of foetus and placenta at full term: that poor placentae might yield underweight foetuses and *vice versa*. Both correlations are well-known clinically.

#### **Uterine physiology in pregnancy and parturition**

The presence of marked species differences in the anatomy, physiology, and pharmacology of the myometrium does not permit the information obtained in laboratory animals to be extrapolated to man. This discussion, therefore, deals mainly with the human uterus.

The recording of amniotic pressure has shown that the pregnant human uterus has a low but detectable contractile activity during the first 30 weeks of pregnancy. This activity increases progressively during the last weeks of gestation and continues to augment during labour until delivery. A rapid decline in uterine motility occurs after parturition.

There is a great need for better knowledge of the mechanisms controlling the motility of the pregnant human uterus, since their activation determines the occurrence of labour, either at term or prematurely, whereas their failure leads to prolonged gestation.

Oxytocin apparently plays an important role in the control of human labour. During the last eight weeks of pregnancy the intravenous infusion of oxytocin in doses of about 8 mU/min/60 kg elicits uterine contractions identical to those recorded during normal spontaneous labour at term. Furthermore, several facts suggest that oxytocin is released during sponta-

neous labour in women. First, the recording of intramammary pressure demonstrates the occurrence of rhythmic contractions of the mammary myoepithelium, which is considered as a specific indication of oxytocin release. Second, a marked rise occurs in the oxytocin activity of human blood during labour; although the substance responsible for this rise has not yet been unequivocally identified, all the pharmacological or biochemical properties so far studied point to oxytocin. The neural control of oxytocin release has already been discussed<sup>1</sup>.

The sensitivity of the human uterus to oxytocin increases markedly from early pregnancy up to the 32nd-36th week and shows no significant changes during the last month of gestation or during labour. By contrast, in the rabbit, rat, sheep, and dog the uterus does not respond to oxytocin during the major part of pregnancy and becomes reactive only at full term.

*Oxytocinase.* An enzyme—oxytocinase—that inactivates oxytocin appears during early pregnancy in the blood plasma of women (but not in that of laboratory animals). The oxytocinase activity of human plasma increases with gestational age and reaches maximum values around the 36th week of pregnancy. No decline in oxytocinase activity occurs before the onset of labour or during parturition. The role of oxytocinase in human pregnancy and labour deserves further study.

*Oestrogens and progesterone.* The increase in the response of the human uterus to oxytocin roughly parallels throughout pregnancy the changes in myometrial weight and the concentration of actomyosin in the myometrium. There is general agreement that the anatomical as well as the biochemical "growth" of the pregnant uterus is promoted by the oestrogens produced in increasing amounts during gestation.

The role of progesterone in the control of the growth and contractility of the human myometrium is a controversial matter. According to the classic view, progesterone inhibits uterine contractility, reduces myometrial response to oxytocin, and prevents both spontaneous and oxytocin-induced labour. The evidence supporting this view has been obtained mainly in rodents, particularly the rabbit, and has then been extrapolated to man. However, it has not yet been demonstrated that progesterone inhibits the contraction of the human myometrium, particularly during the second half of pregnancy. There are many facts that would be difficult to interpret if such an inhibitory effect existed. First, the amount of progesterone in the human placenta as well as its concentration in the blood reaches maximum values in late pregnancy and shows no decline before the onset of labour or during the process of parturition; only after the placenta is delivered is there a fall in plasma progesterone and urinary

---

<sup>1</sup> See page 6.

pregnanediol. Second, the administration of large doses of progesterone or certain synthetic progestogens has failed to prevent the spontaneous onset of premature or full-term labour and has had no effect on the myometrial response to oxytocin.

There is some indication, however, that during the first half of human pregnancy progesterone may inhibit uterine motility. It has also been suggested that placental progesterone exerts a local inhibitory effect on the neighbouring myometrium. Differences in functional properties have been found between the placental and non-placental areas of the uterus, but it has not yet been demonstrated that this effect is mediated by progesterone.

#### **Effects of labour on the human foetus**

Several factors may interfere with foeto-maternal exchanges during labour, causing foetal hypoxia, hypercapnia, acidosis, and related metabolic changes. Among these factors uterine contractions deserve special attention because they constitute an indispensable component of labour.

By compressing the intramyometrial region around the blood vessels supplying maternal blood to the intervillous space, normal strong uterine contractions cause transient episodes of asphyxia in the foetus, which in turn produce parallel transient falls in the foetal heart rate and in the partial pressure of oxygen in the foetal tissues. The amplitude of these falls in the foetal heart rate and partial pressure of oxygen is a function of the intensity of the corresponding uterine contractions. Abnormal decrease in maternal arterial pressure potentiates the foetal effects of uterine contractions, whereas the administration of oxygen has the opposite results. The influence of uterine vasoconstriction, maternal anaemia, maternal hypoxaemia (due to depression of the respiratory centre by sedatives), and placental insufficiency deserves much attention, since all these factors may greatly enhance the foetal asphyxia caused by uterine contraction.

Atropine greatly reduces the amplitude of the falls in the foetal heart rate, showing that the foetal vagus plays an important role in their production. Atropine might prove to be a useful drug in foetal asphyxia since it increases the heart rate and cardiac output, thus improving the blood supply to foetal tissues, particularly the brain.

The stimulation of uterine contractions beyond physiological limits by means of oxytocic drugs has deleterious effects on the foetus. Abnormally frequent and strong uterine contractions cause, as the initial effect, a marked and persistent fall in the foetal heart rate and partial pressure of oxygen. If uterine hypercontractility persists the foetus may die in 15 to 30 minutes. If the foetus survives, the heart rate may return gradually to what is currently considered as the "normal range", in spite of the persistence of uterine hyperactivity and a low partial pressure of oxygen—at

least in peripheral foetal tissues. In this stage of apparent "adaptation", it is very difficult to diagnose foetal distress clinically.

It remains to be determined whether the short periods of hypoxia caused by normal uterine contractions or the more prolonged and marked drops in partial pressure of oxygen provoked by uterine hyperactivity cause long-term effects in the foetus. Preliminary results obtained in the rhesus monkey indicate that permanent damage to the brain leading to severe neurological disturbances is produced by uterine hyperactivity.

## 6. BIOCHEMISTRY OF THE SEX STEROIDS

### Biosynthetic pathways

Apart from the gonadotrophins, the sex hormones are steroidal compounds, produced primarily from cholesterol in the ovaries, testes, adrenal cortex, and placenta. The basic common pathway to the three classes of sex hormones, progestational hormone, androgens, and oestrogens, involves the sequence: cholesterol  $\rightarrow$  pregnenolone  $\rightarrow$  progesterone  $\rightarrow$  testosterone  $\rightarrow$  oestradiol-17 $\beta$ .

The biosynthetic steps from cholesterol to pregnenolone involves the 20 $\alpha$ , 22-dihydroxycholesterol intermediate which is converted directly to pregnenolone and isocaproic aldehyde by a desmolase reaction. This biosynthetic step is regulated by adrenocorticotrophic hormone (ACTH) in the adrenal gland and apparently by FSH and LH in the corpus luteum. The regulation of this step in other portions of the ovary and in testicular and placental tissue has yet to be studied.

The progestational hormone, progesterone, is formed by a simple enzymatic oxidative step from pregnenolone. Progesterone and pregnenolone are important substrates for androgen biosynthesis.

Androgen biosynthesis occurs in all four steroid-producing tissues, with adult testes producing a preponderance of the most active androgen, testosterone. Androgens are produced by six interrelated pathways, but the quantitatively most important route appears to be: progesterone  $\rightarrow$  17-hydroxyprogesterone  $\rightarrow$   $\Delta^4$ -androstene-3,17-dione  $\rightarrow$  testosterone. The latter pathway produces a maximum of  $\Delta^4$ -androstene-3,17-dione and a minimum of testosterone. A second pathway of progesterone  $\rightarrow$  testosterone acetate  $\rightarrow$  testosterone  $\rightarrow$   $\Delta^4$ -androstene-3,17-dione appears to be of particular importance in the adult testis and produces a maximum of the highly active testosterone and a minimum of the less active  $\Delta^4$ -androstene-3,17-dione.

Recent detailed studies on the biosynthetic capabilities of the Stein-Leventhal ovary provide a possible explanation for the hirsutism and virilism, or both, observed in some of these patients. These polycystic

and enlarged ovaries produce three to five times more androgens per unit weight of tissue than normal ovaries. More detailed analysis involving radioactive substrates indicates that these ovaries, from the point of view of biosynthesis, appear to resemble the adult human testis both in the amounts of androgens produced and the pathways involved.

Biological conversion of androgens to oestrogens—thus a route for oestrogen biosynthesis—had been suggested for many years, but it remained for radiochemical techniques to establish this pathway beyond a reasonable doubt. Most studies have been carried out with human full-term placenta, but the three interrelated pathways appear to occur in all steroid-producing tissues. The principal pathway involves testosterone  $\rightarrow$  19-hydroxytestosterone  $\rightarrow$  oestradiol-17 $\beta$ , and the last step of this reaction may be rate-determined by gonadotrophic hormones.

The elucidation of the basic biosynthetic pathways solves the enigma that faced investigators for many years. Particularly confusing was the fact that all three types of sex steroid hormones are always present in all ovarian, placental, testicular, and adrenal cortical tissues. Now, it is clearly apparent that there is a carefully integrated scheme interrelating the three hormonal activities in the sequence progesterone  $\rightarrow$  androgen  $\rightarrow$  oestrogen. Glandular specificity is a quantitative rather than a qualitative matter, and sexuality represents a balance of endocrine factors rather than an absolute condition. The intimate regulation of sex hormone biosynthesis, the enzymes involved, and biosynthetic modifications leading to grossly abnormal states are yet to be examined in depth and in particular relation to the many basic problems of human reproduction.

#### **Determination of androgens and oestrogens**

The problem of evaluation of the effective concentration of androgens and oestrogens has been complicated by the fact that each class of substances is represented by many compounds in body fluids. These compounds, of varied structures and biological activities, are present in blood in only trace quantities. Rather than attempt to assess the precise concentration of each of these substances, it appears to be more profitable to consider methods for the determination of the "available strong androgen and oestrogens", which in man and at least some other species are testosterone, oestradiol-17 $\beta$ , and oestrone. Accordingly, micro methods have been developed for the assay of testosterone, oestradiol-17 $\beta$ , and oestrone in human plasma.

An oestrogen method sufficiently sensitive and reproducible for studies on human plasmas is the following. Plasma, after addition of insignificant quantities of oestrone-6,7- $^3\text{H}$  and oestradiol-17 $\beta$ -6,7- $^3\text{H}$ , is extracted with a mixture of chloroform and ether. The residue is purified by solvent partition, separation of the phenolic material, and paper chromatography.

The oestrogens are finally determined by phosphoric acid fluorescence, and recovery corrections are made on the basis of the  $^3\text{H}$  determination.

This method can detect 0.004  $\mu\text{g}$  of oestrogen per 100 ml plasma. The plasma of normal women contains about 0.058  $\mu\text{g}$  of oestrone at midcycle and about 0.023  $\mu\text{g}$  of oestrone at the end of the menstrual flow. Corresponding values for oestradiol-17 $\beta$  are 0.026  $\mu\text{g}$  and 0.010  $\mu\text{g}$  respectively. The plasma of normal men contains 0.023  $\mu\text{g}$  and 0.003  $\mu\text{g}$  of oestrone and oestradiol-17 $\beta$  per 100 ml respectively. A patient with a granulosa cell tumour of the ovary had plasma values of 0.35  $\mu\text{g}$  and 0.10  $\mu\text{g}$  per 100 ml respectively for oestrone and oestradiol-17 $\beta$ .

Testosterone in human plasma was determined by the following procedure. Plasma was extracted with a mixture of chloroform and ether after an insignificant weight of testosterone-7 $\alpha$ - $^3\text{H}$  had been added to control recovery. The residue was solvent-partitioned and paper-chromatogrammed. The testosterone was then converted to oestrogens by means of a human placental enzyme preparation and the oestrogens determined as described under the oestrogen method. By this method, nine normal men (23-74 years) had a mean value of 0.56  $\mu\text{g}$  per 100 ml plasma while 10 normal women (22-35 years) had a value of 0.12  $\mu\text{g}$ . Hirsute women (Stein-Leventhal syndrome) showing essentially normal urinary 17-ketosteroids had a mean value of 0.33  $\mu\text{g}$  while five virilized women (Stein-Leventhal syndrome), also with normal urinary 17-ketosteroids, had a mean value of 0.49  $\mu\text{g}$ . A highly virilized woman with an ovarian hilus-cell tumour (aged 68 years) whose 17-ketosteroid value was 3 mg per day had the enormous value of 2  $\mu\text{g}/100$  ml plasma before surgery and 0.04  $\mu\text{g}$  after removal of the tumour.

#### **Cellular effects of steroid hormones**

Studies on the mechanism of sex hormone action are being directed mainly towards control of some phase of protein synthesis and towards cell permeability processes. The former studies have indicated the importance of both androgen and oestrogen effects on microsomal protein synthesis in prostatic and uterine tissue respectively. In other studies, especially in the mouse, similar effects of androgen stimulation have been observed on protein synthesis in the kidney.

Detailed studies of the sequence of anabolic events following oestradiol-17 $\beta$  stimulation of the rat uterus have revealed that only the most active oestrogen, oestradiol-17 $\beta$ , can be detected in this target tissue, and that lipid metabolism is stimulated initially, then followed by protein synthesis. An *in vitro* cell-free system to study the action of androgens, oestrogens, and progesterone is still lacking.

## 7. IMMUNOLOGICAL ASPECTS OF REPRODUCTION

Actual accomplishments in immuno-reproduction are not very great, but the general increased understanding of antigen-antibody reactions, of the structure of antibodies, and of the mechanism of antibody formation encourages optimism. The achievement of controlled "splitting of the antibody" leads to the expectation of early chemical characterization of the active sites of the molecule. Current advances in protein biosynthesis also lead to the expectation that the cell-free production of antibody is close at hand.

*Immunization with seminal materials.* A recent fairly comprehensive tabulation of reports since the beginning of this century lists some 225 sets of experiments on the production of antibodies against sperms. Of these, 54 included antifertility tests, mostly reported as positive. An analysis of the evidence does not, however, permit the simple conclusion that antifertility effects are readily obtained in lower mammals or in humans. Consistent effects in experiments with guinea-pigs apparently depend upon addition of an adjuvant of the Freund type (killed mycobacteria, mineral oil, and an emulsifying agent), which is noxious to the recipients. Passive immunization has not given clear effects, but *in vitro* experiments amply demonstrate that specific antibodies, even when made non-agglutinating, can destroy the fertilizing ability of homologous sperm without visible damage to these cells.

Immunization of males with homologous (or autologous) spermatozoa or testicular extract in Freund's adjuvant results in an apparently reversible suppression of the germinal epithelium of the testis. The accessory organs are essentially unimpaired, showing that the androgenic activity of the interstitial cells is not affected. Aspermatogenesis of this kind has been achieved in the guinea-pig, the rat, and possibly the monkey, but not in the rabbit, fowl, or opossum. Passive transfer has been effected by lymph node cells but not by serum.

*Immunization with placental embryonic and foetal materials.* In human beings, habitual aborters may have antibodies against placental proteins. In laboratory animals, abortion can be induced by anti-placental antibodies, which also cross-react with and damage the kidney.

The foetal placenta possesses transplantation antigens and also immunologically competent cells. The latter could explain the rapid growth of the placenta as a response to foreign antigens from the mother; and could be a basis for the final detachment of the placenta at parturition as a graft-versus-host reaction.

Immunization as a result of pregnancy occurs in Rhesus (RH) factor haemolytic disease of the new-born. The antifertility effect of ABO incom-

patibility is small but significant, and it may operate to protect against sensitization to foetal RH antigens. This area warrants further exploration with respect to overcoming sterility and eliminating possible deleterious effects on development.

The blocking of cell division and of development by antibodies is readily demonstrated in certain invertebrates. A purified constituent (fertilizin) of the plasma membrane of the egg of the sea-urchin is an effective antigen, but the internal constituents of the egg are not very effective.

*Antihormones and anti-enzymes.* Hetero-immune antisera neutralize homologous gonadotrophic hormones *in vitro* and may inactivate both exogenous and endogenous gonadotrophins *in vivo*. The antibodies are directed not against the hormonal but rather the species-specific chemical features. Anti-gonadotrophins are being used for diagnosing pregnancy and may prove useful for detecting ovulation. Antibodies against the steroid sex hormones may also have diagnostic uses.

Neutralization of hyaluronidase by antibodies may destroy the fertilizing capacity of the spermatozoa. The specificity problems are like those for antihormones.

*Natural antibodies.* Research is required into the occurrence of ABO antigens on spermatozoa and into the action on spermatozoa of lectins and of the various natural hetero-agglutinins that commonly occur in animal sera. A report that the "blood-group" of the mature spermatozoon is determined by its haploid genetic composition still lacks confirmation, but it has encouraged the speculation that selective fertilization might be accomplished by use of specific antibodies.

*Acquired tolerance.* The administration of foreign antigens to foetal or new-born animals can result in suppression of the ability of the animal to respond immunologically to the same antigen for a considerable period or even permanently in later life. This is a highly active field which undoubtedly will contribute greatly to knowledge of how the capacity for immunological response develops and, at the same time, provide important new ways for exploring other developmental processes.

## 8. PHARMACOLOGICAL ASPECTS OF REPRODUCTION

### Antibiotics and antimetabolites

The use of antibiotics in pregnancy must be viewed with caution because of their possible unfavourable effects on the maternal and foetal organisms. There is no doubt that some disturbances appear in various organs after treatment with antibiotics and that the placenta is crossed by many of these substances.

Administration of tetracycline to pregnant animals may induce fatty degeneration of the liver parenchymal cells and of the epithelium of the renal tubules and hyperplasia and degeneration of the beta cells of the pancreatic islets in both mother and offspring. Pathohistological changes in the pancreas after administration of a toxic dose of tetracycline correspond to those occurring in pancreatic diabetes. There is some evidence that tetracyclines can produce congenital deformities of the limbs of the developing chick embryo. A remarkable report describes one case in which a mother received a tetracycline during early pregnancy and gave birth to a child with deformities of both hands. Bone marrow depression and aplastic anaemia caused by chloramphenicol and jaundice of the new-born caused by novobiocine have been reported. The strong affinity of tetracyclines for calcifying tissues may explain the discoloration of the teeth of children treated with this antibiotic. It is necessary to draw attention to the potentially unfavourable effects of antibiotics on the pregnant woman and the foetus.

Reports have now been published showing that thiouracil induces leukopenia and agranulocytosis. Among different antimetabolites used as drugs, the derivatives of purine and pyrimidine bases and their nucleotides (fluoruracil, bromuracil, thiouracil, the barbiturates, mercaptopurine, azaguanine, etc.) require careful study. Interference with nucleic acid metabolism by these substances, or their incorporation into the natural nucleic acid, may cause a flow of incorrect genetic information, leading to the synthesis of "pathological" protein molecules and the occurrence of disease due to the change in the genetic code. This may exert an essential effect on the functional activities of the testes and ovaries and also of other endocrine glands, thus creating unfavourable conditions for processes that play an important role in human reproduction.

#### **Other inhibitory compounds**

Recently, new assays for inhibitory compounds have been developed and important new compounds, steroid and non-steroid, have been synthesized. Considerable research will be needed before the full potential of these compounds is realized. It is expected, however, that these inhibitory substances will be of importance for studies on the mechanism of action of sex hormones as well as practically, for therapy in animals and man.

Anti-androgenic compounds are substances that inhibit the action of androgens on target organs. In a typical assay, testosterone is administered to stimulate the secondary sexual characters or the glands and the test material simultaneously administered at a second site. Progesterone is an effective anti-androgen by the usual tests, and under certain conditions may have this function in pregnancy. Other anti-androgens include 19-norprogesterone, 6-dehydroretrotestosterone, 17,17-dimethyl-19-norandrosta-4, 13-

dien-3-one, 6-chloro-  $\Delta^6$ -dehydro-17-acetoxypregesterone and certain non-steroid tricyclic compounds.

Anti-oestrogens are defined as substances that inhibit the action of oestrogens on target tissues. In a typical assay, the uterus of the immature mouse is stimulated with oestrone and the test material is administered simultaneously at a second site. Progesterone and testosterone are effective anti-oestrogens, but considerably less active than certain  $17\alpha$ -alkyl-19-nortestosterone derivatives and 6 and/or 16 substituted progesterone derivatives.

Anti-ovulatory compounds can prevent ovulation in the mated rabbit, and their relative potencies can be quantitated by the reduction in the number of eggs ovulated. Active compounds include steroids of varied structures, particularly, but not necessarily, progestational agents. The inhibitory activity is not correlated with any of the more traditional hormonal activities. The mechanism of the inhibition is not established.

Recent studies have demonstrated that androgenicity in rats as measured by seminal vesicle and prostate responses is not necessarily correlated with the ability of steroids to inhibit particularly the production and release of pituitary FSH. Certain non-naturally-occurring steroids with novel substituents at positions 6 and 16 possess high anti-pituitary activity. Non-steroidal compounds e.g., ICI Compound 33828, have been developed which possess this anti-pituitary activity without possessing any other hormone-like activity.

## 9. RECOMMENDATIONS

The Group, having made a very wide survey of the biology of human reproduction, and having considered areas in which knowledge, facilities, or support are at present inadequate, recommends :

(a) that WHO assist in the development of fundamental knowledge of the biology of human reproduction and of other fields on which that knowledge is based ;

(b) that WHO convene meetings of appropriate specialist groups to consider practical methods of implementing the following proposals, which are *not* arranged in any particular order.

### 1. *Organization of surveys of :*

environmental and ethnic variation in human reproductive function ;  
human fertility in relation to blood groups and other immunological factors.

2. *Provision of services :*

the organization of a world-wide collection of human pituitary glands ;  
the establishment of a centre for the provision of labelled steroids,  
polypeptides, nucleotides, and special amino acids ;  
the establishment of an information centre on steroids and polypeptides ;  
the establishment of an information centre on human cell lines ;  
the establishment of an information and supply centre for new and  
existing laboratory animals .

3. *Promotion of research on :*

the effects of labour on the human foetus ;  
neuroendocrinology, including the effects of psychological factors,  
normal and abnormal, on human reproduction ;  
protein biosynthesis in prenatal development ;  
the intermediate metabolism of the foetus, especially in the control and  
development of function ;  
the physiology of the gametes, especially *in vitro* ;  
the mechanism of action of sex hormones and analogous substances,  
especially that of orally active progestogens ;  
the physiology of lactation, including galactopoiesis ;  
the biochemistry and microbiology of the female genital tract, with  
special reference to implantation and feedback ;  
the biochemical aspects of spermatogenesis.

---

**WORLD HEALTH ORGANIZATION  
TECHNICAL REPORT SERIES**

<i>Recent reports :</i>		Price		
No.		s.d.	\$	Sw. fr.
237	<b>(1962) Requirements for Biological Substances : 7. Requirements for Poliomyelitis Vaccine (Oral)</b> Report of a Study Group (29 pages) . . . . .	1/9	0.30	1.—
238	<b>(1962) The Teaching of Genetics in the Undergraduate Medical Curriculum and in Postgraduate Training</b> First report of the Expert Committee on Human Genetics (19 pages) . . . . .	1/9	0.30	1.—
239	<b>(1962) Internationally Acceptable Minimum Standards of Medical Education</b> Report of a Study Group (59 pages) . . . . .	3/6	0.60	2.—
240	<b>(1962) Principles Governing Consumer Safety in Relation to Pesticide Residues</b> Report of a meeting of a WHO Expert Committee on Pesticide Residues held jointly with the FAO Panel of Experts on the Use of Pesticides in Agriculture (18 pages) . . . . .	1/9	0.30	1.—
241	<b>(1962) Joint FAO/WHO Expert Committee on Meat Hygiene</b> Second report (87 pages) . . . . .	5/-	1.00	3.—
242	<b>(1962) Standardization of Reporting of Dental Diseases and Conditions</b> Report of an Expert Committee on Dental Health (23 pages)	1/9	0.30	1.—
243	<b>(1962) Expert Committee on Malaria</b> Ninth report (43 pages) . . . . .	3/6	0.60	2.—
244	<b>(1962) Dental Education</b> Report of an Expert Committee on Dental Health (32 pages)	1/9	0.30	1.—
245	<b>(1962) Joint FAO/WHO Expert Committee on Nutrition</b> Sixth report (65 pages) . . . . .	5/-	1.00	3.—
246	<b>(1962) Occupational Health Problems in Agriculture</b> Fourth report of the Joint ILO/WHO Committee on Occupa- tional Health (61 pages) . . . . .	3/6	0.60	2.—
247	<b>(1962) Expert Committee on Trypanosomiasis</b> First report (57 pages) . . . . .	3/6	0.60	2.—
248	<b>(1962) Radiation Hazards in Perspective</b> Third report of the Expert Committee on Radiation (37 pages)	3/6	0.60	2.—
249	<b>(1962) The Quality Control of Pharmaceutical Preparations</b> Report on a European Technical Meeting (35 pages) . . . . .	3/6	0.60	2.—
250	<b>(1963) Urban Health Services</b> Fifth report of the Expert Committee on Public Health Administration (35 pages) . . . . .	3/6	0.60	2.—
251	<b>(1963) Cancer Control</b> First report of an Expert Committee (27 pages) . . . . .	1/9	0.30	1.—
252	<b>(1963) Training of Psychiatrists</b> Twelfth report of the Expert Committee on Mental Health (39 pages) . . . . .	3/6	0.60	2.—
253	<b>(1963) Conference on Medicine and Public Health in the Arc- tic and Antarctic</b> Report (29 pages) . . . . .	1/9	0.30	1.—
254	<b>(1963) Public Health Responsibilities in Radiation Protection</b> Fourth report of the Expert Committee on Radiation (23 pages) . . . . .	1/9	0.30	1.—

No.		Price		
		s.d.	\$	Sw. fr.
255	(1963) CCTA/WHO African Conference on Ancylostomiasis Report (30 pages) . . . . .	1/9	0.30	1.—
256	(1963) The Care of Well Children in Day-Care Centres and Institutions Report of a Joint UN/WHO Expert Committee convened with the participation of FAO, ILO and UNICEF (34 pages)	3/6	0.60	2.—
257	(1963) Training of the Physician for Family Practice Eleventh Report of the Expert Committee on Professional and Technical Education of Medical and Auxiliary Personnel (39 pages) . . . . .	3/6	0.60	2.—
258	(1963) Expert Committee on Medical Assessment of Nutri- tional Status Report (67 pages) . . . . .	5/-	1.00	3.—
259	(1963) Expert Committee on Biological Standardization Fifteenth report (60 pages) . . . . .	3/6	0.60	2.—
260	(1963) The Public Health Aspects of the Use of Antibiotics in Food and Feedstuffs Report of an Expert Committee (30 pages) . . . . .	1/9	0.30	1.—
261	(1963) Expert Committee on Health Statistics Eighth report (34 pages) . . . . .	3/6	0.60	2.—
262	(1963) Expert Committee on Gonococcal Infections First report (70 pages) . . . . .	5/-	1.00	3.—
263	(1963) Measles Vaccines Report of a WHO Scientific Group (37 pages) . . . . .	3/6	0.60	2.—
264	(1963) Second Joint FAO/WHO Conference on Food Additives Report (12 pages) . . . . .	2/6	0.50	1.50
265	(1963) Insecticide Resistance and Vector Control Thirteenth report of the WHO Expert Committee on Insec- ticides (227 pages) . . . . .	12/-	2.25	7.—
266	(1963) Social Aspects in the Teaching of Obstetrics and Gynaecology Fourth report of the WHO Expert Committee on Maternal and Child Health (22 pages) . . . . .	1/9	0.30	1.—
267	(1964) General Practice Report of a WHO Expert Committee (24 pages) . . . . .	1/9	0.30	1.—
268	(1964) Genetics of Vectors and Insecticide Resistance Report of a WHO Scientific Group (39 pages) . . . . .	3/6	0.60	2.—
269	(1964) Promotion of Medical Practitioners' Interest in Preventive Medicine Twelfth report of the WHO Expert Committee on Profes- sional and Technical Education of Medical and Auxiliary Personnel (22 pages) . . . . .	1/9	0.30	1.—
270	(1964) Rehabilitation of Patients with Cardiovascular Diseases Report of a WHO Expert Committee (46 pages) . . . . .	3/6	0.60	2.—
271	(1964) Atmospheric Pollutants Report of a WHO Expert Committee (18 pages) . . . . .	1/9	0.30	1.—
272	(1964) WHO Expert Committee on Malaria Tenth report (52 pages) . . . . .	3/6	0.60	2.—
273	(1964) WHO Expert Committee on Addiction-Producing Drugs Thirteenth report (20 pages) . . . . .	1/9	0.30	1.—