

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. 303

**MECHANISM OF ACTION
OF SEX HORMONES
AND ANALOGOUS SUBSTANCES**

Report of a WHO Scientific Group

	Page
1. Introduction	3
2. Biosynthesis and metabolism of steroid hormones of the reproductive system	4
3. Physiological effects and mechanisms of action of the endogenous steroid hormones on the reproductive system	9
4. Exogenous sex-like steroids: their physiology, metabolism and ultimate disposition	12
5. Long-term effects of endogenous and exogenous steroids	17
6. Non-steroid suppressants and/or stimulants	21
7. Research needs	22
8. Recommendations	24

WORLD HEALTH ORGANIZATION

GENEVA

1965

WHO SCIENTIFIC GROUP ON MECHANISM OF ACTION OF
SEX HORMONES AND ANALOGOUS SUBSTANCES

Geneva, 8-14 December 1964

Members :

- Professeur E.-E. Baulieu, Laboratoire de chimie biologique, Faculté de Médecine de Paris, France (*Rapporteur*)
- Professor M. Carmack, Department of Chemistry, Indiana University, Bloomington, Ind., USA
- Professeur J. Ferin, Département de Gynécologie et d'Obstétrique, Université catholique de Louvain, Belgium
- Professor C. A. Gemzell, Department of Obstetrics and Gynaecology, University of Uppsala, Sweden (*Rapporteur*)
- Dr H. Gershberg, Director of the Diabetes and Endocrine Clinics, Department of Medicine, New York University School of Medicine, New York, USA
- Professor G. Hecht-Lucari, Department of Obstetrics and Gynaecology, University of Rome, Italy
- Professor F. H. Kemper, Pharmacological Institute, University of Münster, Westfalen, Germany (*Rapporteur*)
- Professor A. S. Parkes, Physiology Laboratory, University of Cambridge, England
- Professor G. Pincus, Director of Research, Worcester Foundation for Experimental Biology, Shrewsbury, Mass., USA (*Chairman*)
- Dr F. E. Szontágh, Professor of Obstetrics and Gynaecology, University Medical School, Szeged, Hungary (*Vice-Chairman*)
- Dr J. Zañartu, Jefe del Departamento de Fertilidad, Clínica Obstétrica Universitaria, Santiago, Chile

Secretariat :

- Dr R. T. Hill, Scientist, Maternal and Child Health, WHO (*Secretary*)
- Professor L. T. Samuels, Department of Biological Chemistry, University of Utah, Salt Lake City, Utah, USA (*Consultant*)

© World Health Organization 1965

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. Nevertheless governmental agencies or learned and professional societies may reproduce data or excerpts or illustrations from them without requesting an authorization from the World Health Organization.

For rights of reproduction or translation of WHO publications *in toto*, application should be made to the Division of Editorial and Reference Services, World Health Organization, Geneva, Switzerland. The World Health Organization welcomes such applications.

PRINTED IN FRANCE

MECHANISM OF ACTION OF SEX HORMONES AND ANALOGOUS SUBSTANCES

Report of a WHO Scientific Group

1. INTRODUCTION

1.1 The WHO Scientific Group on Mechanism of Action of Sex Hormones and Analogous Substances was convened in Geneva on 8-14 December 1964 to advise the Director-General of research needs in this field. Because of the widespread use of, and great interest in, such compounds, especially the orally active progestogens, for the control of the reproductive cycle, a thorough knowledge of their effects on the reproductive process, both immediate and following use over long periods, is vital.

1.2 To understand these effects it is essential to understand the basic mechanisms involved in normal reproduction; otherwise, the effects of exogenous agents on the reproductive process cannot be clearly distinguished from influences on unrelated systems. An assessment of the state of knowledge of endogenous processes, both normal and abnormal would seem, therefore, to be a base from which consideration of the effects of exogenous materials might be developed.

1.3 The exogenous materials which have thus far aroused interest in their effects on the reproductive process can be divided into three groups: (a) the natural hormones and steroids which resemble them but which have been chemically so modified that they are either not metabolized as readily or have different relative effects on various target tissues; (b) analogues of non-steroidal compounds having hormonal effects; (c) compounds not resembling substances with hormonal action but which affect the reproductive cycle. It is desirable to assess current knowledge of the mechanisms by which these various groups affect the reproductive process, whether through direct interaction with hormonal systems, by interference with some process essential to the initiation or completion of the hormonal reaction, or by action on the gamete. Knowledge of the effects of these compounds on processes other than reproduction, and on long-term changes in tissue response, also needs review. From such considerations the fields of research that seem to need greatest emphasis can be defined.

1.4 Before considering in more detail the present state of knowledge, it would be helpful to define the terminology used in this report. In referring to steroid compounds, commonly accepted trivial names have been used since they are familiar to biologists and clinicians as well as to chemists while the more precise but more cumbersome generic chemical names would often be confusing. The numbering of the carbon atoms and lettering of rings is illustrated in Figure 1. In the steroids, the steric position of groups attached to the nucleus is of vital importance; consequently those on the same side of the plane of four rings as the angular methyl groups are designated β and represented by a solid line attached to the nuclear rings, while those that are on the opposite or trans side are designated α and represented by dotted lines. When designating an isomer of a well-known compound where it is only a question of the α or β position of a substituent group, the prefix "epi" has been added to the trivial name.

1.5 To denote biological activities, the term androgen has been used to designate compounds that cause development of the male secondary sex organs. Oestrogens are those substances that cause changes in the uterus characteristic of the proliferative phase and/or cornification of the vagina when absorbed directly into the cells of these tissues, while the term pro-oestrogens is used to designate those substances that cause the uterine and vaginal changes only after passage through other organs. A substance that causes secretory changes in the oestrogen-primed uterus on direct application is designated a gestogen, while compounds that cause such changes only after passage through another tissue or where the direct action is unknown are called progestogens.

1.6 The gonadotrophins have been referred to on the basis of their action in the female. Follicle stimulating hormone (FSH) refers to a substance that causes development of the follicle and that stimulates spermatogenesis in the male. Luteinizing hormone (LH) causes further growth and ovulation in the FSH-developed follicle and formation of a corpus luteum, as well as stimulation of interstitial cells in the male. The gonadotrophic substance produced by the human placenta is named human chorionic gonadotrophin (HCG) on the basis of its origin.

2. BIOSYNTHESIS AND METABOLISM OF STEROID HORMONES OF THE REPRODUCTIVE SYSTEM

2.1 All steroid hormones are probably formed from cholesterol. While all tissues forming steroid hormones have the ability to synthesize this compound, the proportion of plasma cholesterol that may be used in hormone synthesis may vary among different glands. The circulating

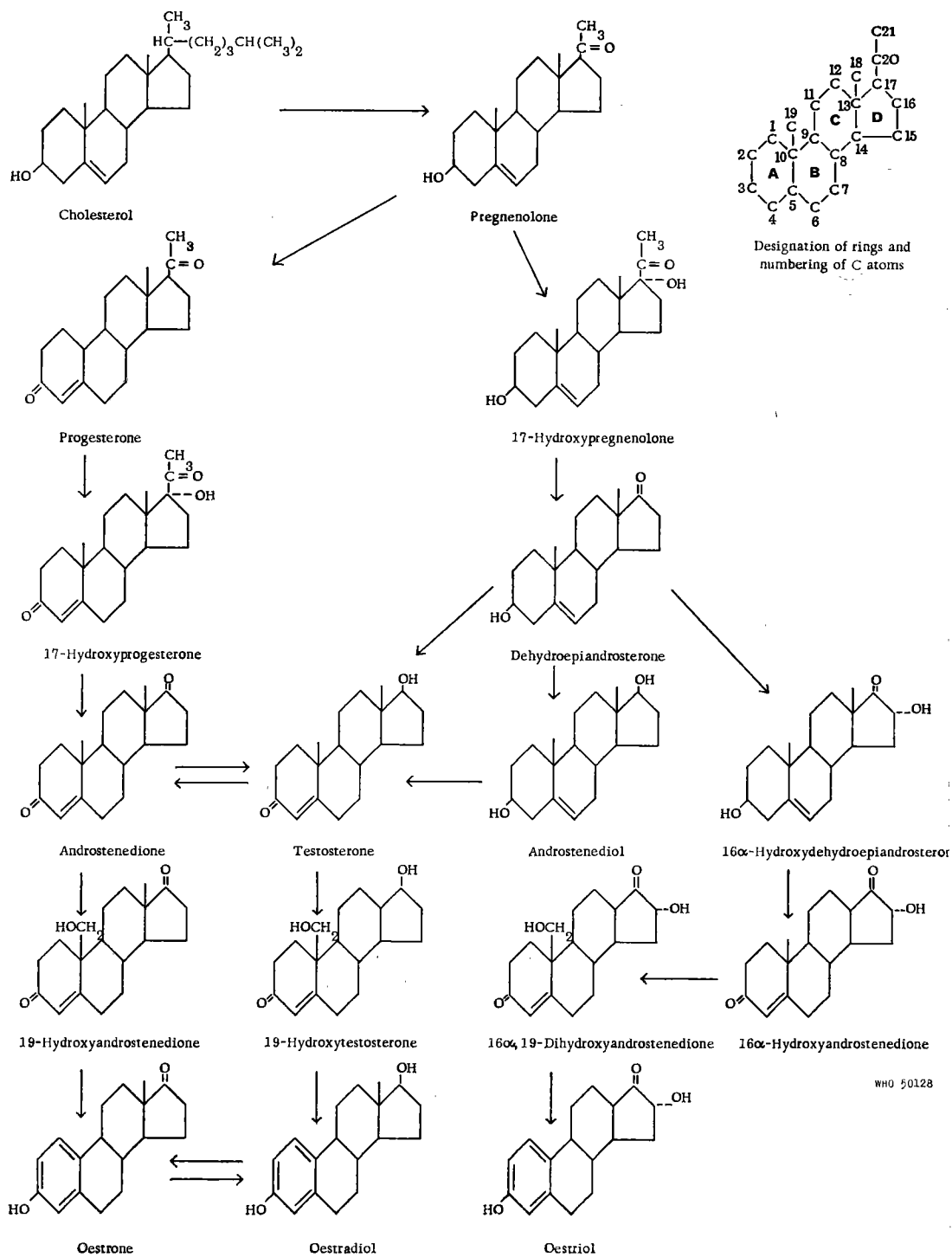
cholesterol has been shown to be a major precursor of adrenal and testicular hormones but its importance in the ovary is unknown.

2.2 The primary reaction in steroid hormone biosynthesis appears to be the cleavage of the six-carbon side-chain from cholesterol through successive hydroxylations to yield pregnenolone (3β -hydroxypregn-5-en-20-one) and isocaproic aldehyde. In the gonads, pregnenolone is metabolized by way either of progesterone or of 17-hydroxypregnenolone to the C_{19} and C_{18} steroids. The two general routes with their cross-overs are shown in Fig. 1. The same reactions are involved, the differences between the possible sequences being due to the different orders in which the hydroxylations, dehydrogenations and isomerizations take place. Whether the choice of routes is determined by the relative concentration and binding power of each intermediate for the same series of enzymes, or whether distinct enzymes preferring each substrate are involved in each reaction is still undetermined.

2.3 In the sequence of reactions where the shift of the double bond in ring A and the oxidation of the hydroxyl group on carbon 3 are the last steps, other changes can apparently occur while sulfuric acid is conjugated to the 3β -hydroxyl. Recent work has established that the human placenta is one organ in which the utilization of the Δ^5 - 3β -hydroxy sulfates is a major and essential factor in hormone biosynthesis. The trophoblastic cells that form the hormones apparently lack the side-chain-splitting enzyme. In the foetus, the adrenal gland has a large amount of this enzyme but is low in 3β -hydroxysteroid dehydrogenase. Large amounts of dehydroepiandrosterone sulfate and particularly of 16α -hydroxydehydroepiandrosterone sulfate flow back from the foetus to the placenta, where to a large extent they are converted into oestradiol, oestrone and oestriol. The recognition that the sulfates can be precursors of the hormones raises the question of the role of conjugates in the organism other than for excretion.

2.4 While the general pattern of biosynthesis appears to be the same in all steroid-forming tissues, the proportion of different enzyme activities determines the relative amounts of the various hormones secreted. The enzymes involved in aromatization are low in testicular interstitial cells and the major products are, therefore, androstenedione and testosterone. In the ovarian follicle cells, there are marked changes in hormonal output at the time of ovulation. Before ovulation the major secretory product is oestradiol, the activity of the aromatizing enzyme being relatively high. Afterwards, with the development of luteinization, progesterone and 20α -hydroxypregn-4-en-3-one become the major secretory products because of a decrease in 17α -hydroxylase and 17β -desmolase activities. At present there are two theories to explain this change. One holds that the enzyme systems within the same cells undergo alterations owing to the influence

FIG. 1. BIOSYNTHESIS OF STEROID HORMONES



WHO 50128

All 3β -hydroxy- Δ^5 - compounds can be in the 3-ester sulfate form.

of the luteinizing hormone; the other considers that the change is due to the activation of different cells, the entire enzyme sequence being present in the cells of the theca interna while the granulosa cells lack the two enzymes. In support of the latter view, it has been pointed out that during the preovulatory phase the granulosa appears inactive, having little blood supply and cells with little cytoplasm. Under the influence of luteinizing hormone the blood vessels invade the granulosa and the cells change to active luteal cells. The theca cells are quite active in the early phase and in the human being become luteinized where they remain active and account for the oestrogen secretion of the second half of the cycle. In the mare, where thecal luteinization does not appear to take place, no oestrogens are secreted during this period. Further elucidation of the two theories must await studies on isolated cell species.

2.5 Since all the sex hormones are formed in the same sequence of reactions, a decrease in one enzyme activity, due either to inhibition or to decreased synthesis, may lead not only to a decrease in a hormone formed via this reaction but to increased secretion of another which is either a precursor or is formed from a precursor by an alternative series of reactions. An example of this has already been cited in the case of the secretion of progesterone in the corpus luteum due to an absence of 17 α -hydroxylase. An example in an abnormal condition is the increased output of androgens associated with a relative decrease in aromatizing activity in the polycystic ovary. The possibility that exogenous steroids may act as inhibitors of certain biosynthetic enzymes and thus produce changes in hormone balance must always be kept in mind.

2.6 The mechanisms by which the gonadotrophins regulate the output of the sex hormones are still not understood. One group has found evidence of stimulation of the conversion of cholesterol to pregnenolone by luteinizing hormone, while another has presented evidence of its action on cholesterol biosynthesis. The effect of luteinizing hormone on the synthesis of progesterone in the corpus luteum was blocked by puromycin, which inhibits protein synthesis. Some studies support an effect of human chorionic gonadotrophin (HCG) on 19-hydroxylation of androgens in the placenta. No direct effect of FSH on steroid biosynthesis has yet been demonstrated since a preparation free of LH has yet to be prepared. Since the actual sites of action in the biosynthetic sequence are not yet clear, nothing is known about the mechanisms by which the peptide hormones act on the enzyme systems.

2.7 The major hormone secreted in mammalian ovarian vein blood during the pre-ovulatory phase appears to be oestradiol. Oestrone is also secreted, as well as small amounts of progesterone, 17-hydroxyprogesterone, androstenedione and testosterone. After ovulation progesterone and 20 α -hydroxypregn-4-en-3-one become the major components,

although in the human being oestradiol is also present in large amounts. The major secretory products of the testis are testosterone and androstenedione. These steroids do not seem to be conjugated, but in the blood they form complexes with serum albumin and perhaps with other proteins that affect their diffusibility. In most tissues, concentrations are lower than in the blood plasma but there is now good evidence that oestradiol is concentrated in target tissues, such as the uterine endometrium and the hypothalamic nuclei controlling gonadotrophin production, while testosterone is concentrated in the prostate gland and seminal vesicles. These non-polar steroids also diffuse into the fat depots because of high lipid solubility.

2.8 The liver is the major site of further steroid hormone metabolism. The reactions are largely reductive, although some oxidative hydroxylations such as the formation of oestriol from oestradiol and oestrone occur. The 17-dehydrogenases are also active, so that testosterone from the blood will be in part excreted as androsterone and etiocholanolone. There are also very active sulfokinases and glucuronokinases present which readily conjugate the steroids as sulfates or glucuronosides. Far from being end-products, steroid sulfates, as already mentioned, can themselves be metabolized. Furthermore, the different types of conjugates appear to be distributed differently in the tissues and excreted by the kidney at different rates, the sulfates remaining longer in the circulation. Much remains to be done to elucidate the significance of these metabolic processes.

2.9 While the liver is the major organ of steroid inactivation, other tissues are not inert. The uterus reduces the ketone group on carbon-20 of progesterone to a hydroxyl radical. Many tissues seem to have small amounts of 17 β -dehydrogenase, 20-dehydrogenase and 6 β -hydroxylase activities, but their significance in endocrine physiology is unknown.

2.10 The relative roles of biliary and urinary excretion of steroids vary in different species and for different compounds. Studies have shown that the less polar steroids, and particularly the oestrogens, may be excreted into the intestinal tract, undergo conjugation or metabolism, and be re-absorbed. In man, some steroid sulfates are partly excreted in the bile and can cross the intestinal wall, reaching the blood without the splitting of the ester link. The effect of this hepato-enteric cycle has never been fully studied. In most species, e.g., rat, mouse, dog and bovine, the urine is a minor pathway, but it plays a more important part in the human being and the guinea-pig.

2.11 The reactions that the steroids undergo, both during biosynthesis and catabolism, involve other compounds that are essential for many other vital processes. During the early stages of cholesterol biosynthesis, adenosine triphosphate (ATP) is utilized; the various hydroxylations require reduced nicotinic adenine dinucleotide phosphate (NADPH),

while the dehydrogenases and reductases use nicotine adenine dinucleotide (NAD) and nicotine adenine dinucleotide phosphate (NADP). Uridine diphosphate glucuronic acid and phosphoadenosine phosphosulfate are used up during conjugation. Since all these substances are also required for other vital processes, particularly in the liver, their use in steroid metabolism may affect the rate of other reactions in the cell, and the reverse may also be true.

2.12 Analysis of urinary steroids can be very accurate, specific and relatively convenient. However, the numerous metabolic pathways as well as the individual variations in yield of metabolites preclude attaching too much significance to the results. Modern methods using radioactive-labelled material and gas chromatography allow determinations in the blood to be made with great sensitivity and specificity; the determination of the circulating sex hormones themselves can give much useful information and can serve to determine their production rate and their metabolic clearance rate. The application of these newer techniques will considerably increase knowledge of steroid metabolism, but much remains to be done.

3. PHYSIOLOGICAL EFFECTS AND MECHANISMS OF ACTION OF THE ENDOGENOUS STEROID HORMONES ON THE REPRODUCTIVE SYSTEM

3.1 Possible means of control of the reproductive function cannot be fully developed until the mechanisms by which the normal process is brought about are understood. Although the main features of the hormonal regulation of reproductive phenomena are known, there is much to learn about physiological mechanisms, and very little is known about the ultimate biochemical reactions affected by the hormones.

3.2 Experimental evidence indicates that oestradiol is concentrated by the uterus and does not undergo metabolic oxidation-reduction at the C-17 level while it acts upon the target organ. However, much remains to be studied concerning the exact location of the hormone in the different parts of the uterus (myometrium, endometrium, connective and vascular tissue) and inside the cells. The histamine release from the mast cells seems involved in the water and sodium uptake of the oestrogenized uterus but the intimate mechanism of action is unknown. An early activation of the protein biosynthesis mechanisms has been demonstrated and measurements of ribonucleic acid, puromycin inhibition, etc. tend to give an important role to messenger ribonucleic acid activity. Increased entrance of amino acids and glucose into cells seems secondary to the above-cited reactions or similar ones. Neither the primary effect of

oestrogen, however, nor the connexion between histamine release and the protein responses is yet known. Oestriol acts upon the latter and not upon the former. The role of the placental oestrogen-dependent NADH-NADPH transhydrogenase and the potential importance of modifications of NADH-NADPH balance in placental function remain to be worked out. The biochemical mechanisms by which oestrogen specifically enters the target cells have still not been elucidated, nor has the mechanism of the recently demonstrated uptake of oestrogen by hypothalamic centres. Little is known concerning the uptake and mechanism of action of progesterone. *In vitro* systems are urgently needed to permit a more thorough study to be made of the mechanism of action of sex hormones.

3.3 Biochemical changes in the vagina due to oestrogens have not been clearly defined. In part, this is due to the general lack of detailed knowledge of the mechanism of keratinization in any tissue. At present there seems to be a close qualitative relationship between the activity of an oestrogenic compound on the uterus and on the vagina, but the quantitative relationship is not so close. Whether the action is *via* the same parts of the steroid molecule at the ultimate site of action in both tissues is unknown.

3.4 The concept of the ovarian cycle most widely accepted at present is that as the oestrogens rise they suppress the synthesis and release of FSH and cause an increase in LH through action on hypothalamic centres affecting the anterior pituitary gland. The simultaneous presence of the two hormones in proper proportion as FSH decreases and LH rises leads to ovulation. The subsequent increase in progesterone causes suppression of LH secretion. In women, the corpus luteum continues to secrete for a certain period, after which the progesterone and oestrogen levels fall and menstruation ensues. Whether the steroid chemical structure involved in pituitary suppression is exactly the same as that involved in the other effects of oestrogens and gestogens is not known. The question of the involvement of a luteotrophic hormone (LTH) in the maintenance of a functional corpus luteum in the human female is still not conclusively answered, but on the basis of present evidence this seems improbable.

3.5 Oestrogens have been shown to increase the speed of tubal transportation of ova in mice, rabbits, guinea-pigs, sheep and cows and seem to be the most prominent activators of normal transport. The mechanism of egg transportation through the fallopian tubes is not understood. It may take place without contractions and sometimes also without the help of the ciliary apparatus. An intrauterine device introduced in the monkey enhances tubal transportation. A number of compounds inhibiting the action of progesterone on the endometrium of the rabbit, most

of which have a degree of oestrogenic activity, appear to expedite the passage of ova from the fallopian tubes during early post-coital days and to expedite their discharge from the uterus. These ova do not appear to be damaged, since they may be transplanted to foster mothers and emerge as normal young.

3.6 The uterus appears to require specific endocrine stimulation for implantation of the blastocyst. In ectopic situations, as shown experimentally in the kidney or even the testis, the blastocyst may become implanted and for a time continue development under highly non-specific conditions. *In utero* the rate of development is primarily progesterone dependent. Implantation may also require the presence of histamine, although antihistamines administered to the general circulation have no inhibitory effect. In the rat, ergocornine, an antihistaminic agent, inhibits implantation directly, while in the human the effect seems to be due to inhibition of progesterone production by the corpus luteum.

3.7 Studies have shown that shortly after implantation the syncytial cells of the developing human embryo begin to produce HCG. It is assumed that this hormone maintains the corpus luteum of pregnancy until the placenta produces adequate amounts of progesterone, but how far HCG is also involved in stimulating the trophoblast to produce progesterone remains an unsolved question. The mechanism of ovulation inhibition during pregnancy and lactation is as yet undecided.

3.8 The oestrogens also have effects on other tissues than those associated with reproduction. Protein metabolism is affected. Oestrogens may increase the level of cortisol-binding globulin which in turn affects the pituitary-adrenal axis. Thyroxin-binding globulin also increases and influences thyroid function. Moreover calcium-phosphorus metabolism is affected by oestrogens, and it is known that these hormones lead to sodium and water retention in the organism. Fat deposition is also influenced. Glucose metabolism appears to be modified. Lactation, hair growth, erythropoiesis and lympholysis are all affected but, as in the case of all other effects of oestrogens on cells, the mechanisms are not understood.

3.9 As to extra-reproductive effects of progesterone, there is only limited evidence of metabolic influences; unlike oestrogens under many experimental conditions, progesterone has not been observed to cause any alterations of carbohydrate metabolism, etc. Effects on electrolyte metabolism have been established. Often progesterone and the oestrogens appear to be antagonistic in their gross effects. The same is true of androgens and oestrogens. The mechanisms of these antagonisms require study.

3.10 The effects of the sex hormones on the nervous system have been considered by the WHO Scientific Group on Neuroendocrinology and

Reproduction in the Human,¹ but certain influences might be mentioned. Not only are the oestrogens and androgens differentially concentrated in certain nuclei of the hypothalamus but amino acid incorporation into protein in these nuclei is increased at the same time. These are the nuclei which ablation studies indicate as the controlling regions of FSH inhibition and LH release. The thermogenic effect of progesterone is probably *via* a central mechanism. The relief of such conditions as severe premenstrual tension by progestogens indicates that the sex hormones have other influences on the central nervous system which are poorly understood.

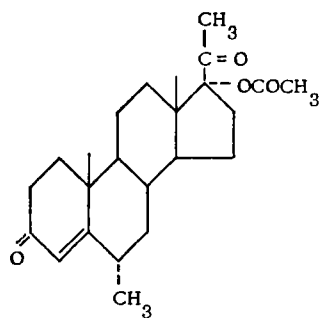
4. EXOGENOUS SEX-LIKE STEROIDS: THEIR PHYSIOLOGY, METABOLISM AND ULTIMATE DISPOSITION

4.1 The early work on administration of the naturally occurring sex hormones demonstrated that they were relatively inactive when given by mouth. The portion absorbed is largely removed from the portal blood as it passes through the liver. Efforts to modify the steroid molecule have led to the discovery of a number of synthetic steroids with oestrogen or gestogen function which are effective orally. Some highly effective non-steroidal oestrogens, such as diethylstilboestrol, have also been found. These are being widely used in human therapy today. A thorough understanding of their physiological effects and metabolism is therefore of great importance in medicine.

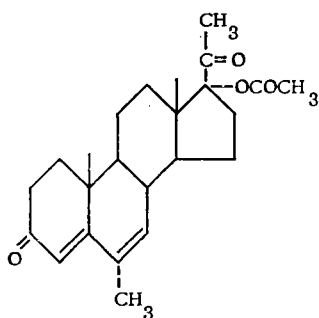
4.2 No adequate explanation has been obtained for the oral activity of these compounds; the fact that some derivatives are more active after oral administration than after injection or local application suggests that a hepatic metabolite could be involved. Only a few studies, however, have been made of the metabolism of these compounds. The metabolism of 19-nortestosterone compared to that of testosterone is characterized by the increased formation of 5 α reduced metabolites, more of which are sulfo-conjugated; they are therefore present in the organism longer than if they were conjugated to glucuronic acid. The metabolism of norethynodrel (VIII, Fig. 2) has been studied and the formation of 10 β -hydroxy-derivatives demonstrated. These appear to have some synergistic effect with oestrogens and possibly progestogens. A metabolic 21-hydroxylation of medroxyprogesterone (I) has been observed. The significance of the formation of this metabolite is unknown. It does not seem that the catabolic formation of known oestrogens accounts for the oestrogenic activity of the 19-norsteroids. Much more information is needed about the metabolism of these compounds before their mechanisms of action can be understood.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1965, 304.

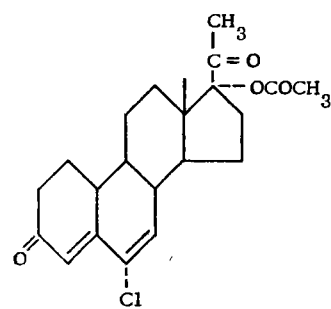
FIG. 2. SOME ORALLY ACTIVE SYNTHETIC GESTOGENS, PROGESTOGENS AND OESTROGENS



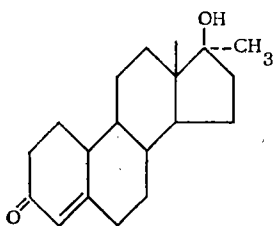
I. Medroxyprogesterone acetate
(methypregnone)



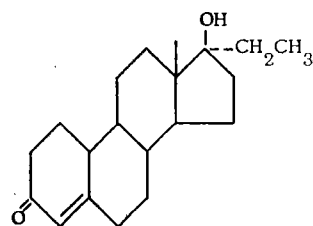
II. Megestrol acetate



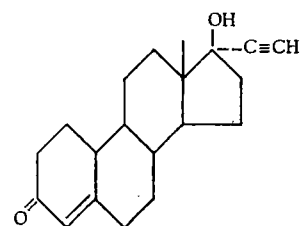
III. Chlormadinone



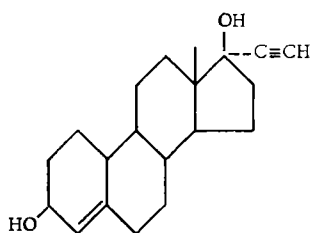
IV. Normethandrolone



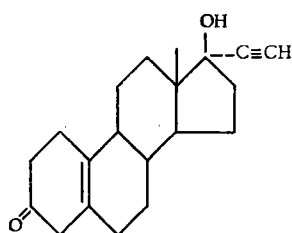
V. Norethandrolone



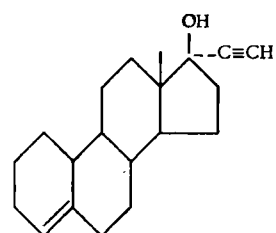
VI. Norethindrone



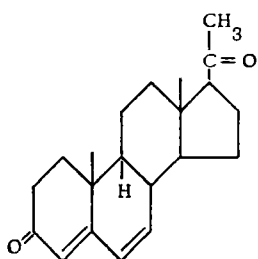
VII. Ethynodiol



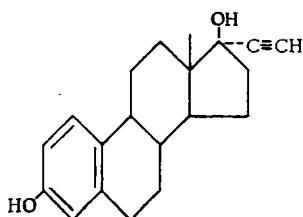
VIII. Norethynodrel



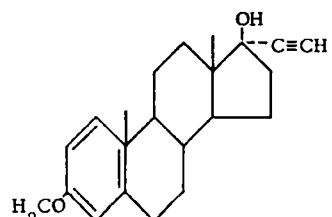
IX. Lynoestrenol



X. Dydroprogesterone



XI. Ethynylestradiol (EE)



XII. Mestrenol (EE3ME)

4.3 The influence of administered compounds on the secretion and metabolism of endogenous hormones has not been fully studied. Cortisol and dehydroepiandrosterone sulfate production rates are apparently not depressed. An increased binding of cortisol to plasma proteins has been demonstrated after norethynodrel plus mestrenol (XII) administration. Increased sulfo-conjugation of certain androgen metabolites and decreased formation of androstenediol from testosterone were also observed after lynoestrenol (IX) plus mestrenol administration. It seems certain that the catabolism of endogenous hormones can be modified by the anti-fertility compounds but systematic studies are needed.

4.4 The control of fertility by hormonal steroids may be due to their effects on (a) ovulation processes, (b) fertilization and the tubal transport of ova, (c) blastocyst development and implantation. All the major types of gonadal steroids (i.e., oestrogens, androgens and progestogens) have been shown to inhibit ovulation in the rabbit, but steroids having standard progestational potency generally tend to be more active than androgens and oestrogens in this species; moreover, among the various types of synthetic gestogens the 19-norsteroids and certain 6-substituted derivatives of 17-acetoxypregesterone tend to be the most active. Gonadotrophin inhibition (or depletion) of the rat hypophysis is obtained with prototypes of the major gonadal steroids but here the most active oestrogenic steroids tend to have high inhibiting potency. The androgens act primarily to deplete LH, while the gestogens and progestogens have a wide range of quantitative effects with certain 19-norsteroids being highly active as gonadotrophin suppressors.

4.5 The gestogens and progestogens most active as ovulation inhibitors and/or antigonadotrophins have in recent years been used as constituents of oral antifertility agents. When taken cyclically in adequate amounts by women in their fertile years, they lead to anovulatory artificial menstrual cycles which are best controlled in duration and normalcy of menstrual flow when a small amount of oestrogen is combined with the gestogen or progestogen. The constituents of these preparations are (a) certain 19-norsteroids — norethynodrel (VIII), norethindrone (VI), norethindrone-17-acetate, ethynodiol (VII) diacetate and lynoestrenol (IX) — and (b) 17-acetoxypregesterone derivatives — methypregnone (medroxyprogesterone acetate) (I), megestrol acetate (II) and chlormadinone (III) (see Fig. 2). The compositions of the oral antifertility preparations that have been most widely studied clinically are given in the accompanying table.

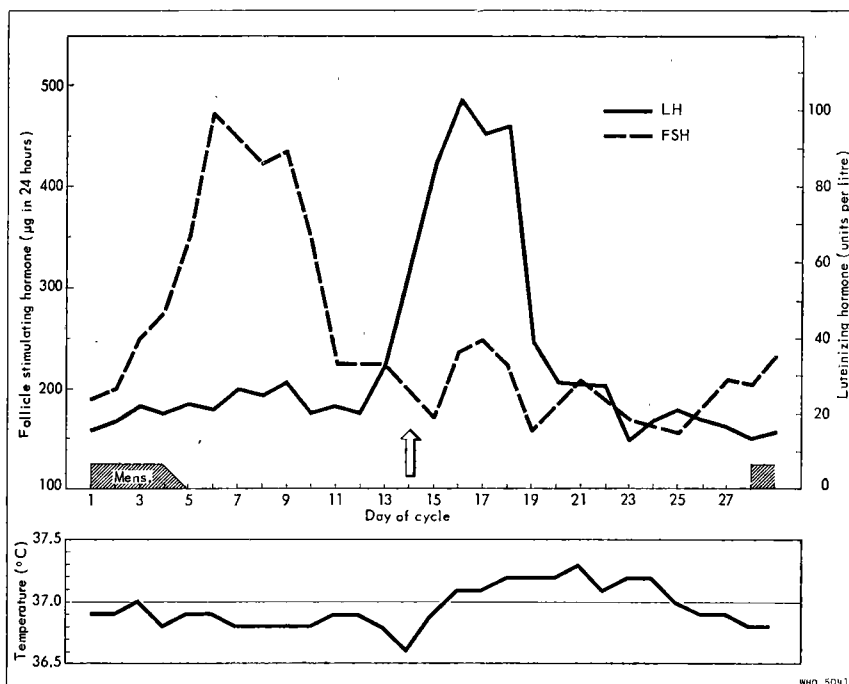
4.6 Although it is possible to make general statements about the effects of oestrogens, gestogens and progestogens on the release of pituitary gonadotrophins, the specific effects are difficult to evaluate. The widely used mouse-uterus test does not differentiate accurately between LH and

COMPOSITION OF SOME ORAL ANTIFERTILITY PREPARATIONS

Gestogen component		Gestogen content (mg/tablet)	Oestrogen content (mg/tablet)		
			Mestrenol	Ethynyl-oestradiol	
Progesterone derivatives	17-Acetoxyprogesterone derivatives	Dydroprogesterone	10	—	—
		Medroxyprogesterone acetate	2.5	—	—
			10	—	—
			10	—	50
			5	—	50
2	—	20			
	Megestrol acetate	4	—	50	
	Chlormadinone	2	80	—	
19-Norsteroid derivatives	Δ^4 -Oestrenolone derivatives	Norethindrone	5	—	—
			10	—	60
			5	—	150
			2	—	100
		Norethindrone acetate	5	—	—
			4	—	50
	Ethynodiol diacetate	2	100	—	
		1	100	—	
	Δ^5 (10)-Oestrano- lone	Norethynodrel	10	150	—
			5	75	—
Oestronol derivative	Lynoesstrenol	2.5	100	—	
		5	150	—	
		2.5	75	—	

FSH activity. Significant information about the effects of various steroid compounds on the release of FSH and LH has been obtained by the use of one assay specific for FSH and another for LH. During a normal menstrual cycle, FSH activity in urine, tested biologically by the ovarian augmentation test, is high during the menses and falls thereafter, reaching a low level at the time of ovulation. LH activity in urine, assayed biologically or immunologically, seems to be at a constant low level except for a marked rise during a four-day period at the time of ovulation (Fig. 3). Administration of oestrogens, or of oestrogens accompanied at the end

FIG. 3. VARIATION IN GONADOTROPHIN EXCRETION DURING THE NORMAL MENSTRUAL CYCLE



of the medication cycle by gestogens (sequential therapy), does not affect the LH peak, while the same doses of oestrogens and gestogens in combined therapy inhibit the peak. Preliminary results seem to show that in man FSH release is not affected.

4.7 The effect of exogenous oestrogens, gestogens and progestogens on the morphology and function of the ovaries is little known. An apparent, although not statistically significant, decrease in pregnanediol and oestrogen excretion observed by some laboratories in subjects receiving these compounds during the luteal phase only may point to an eventual inhibitory effect on an existing corpus luteum. Histological studies of ovaries under prolonged treatment with either combined therapy or gestogens alone showed no follicles in advanced development but the same proportion of atretic follicles. There is no evidence available of any genetic effect on the ovum or sperm. The administration of a single large intramuscular dose of gestogens produces prolonged ovarian arrest with amenorrhoea.

4.8. In animal studies, a number of artificial gestogens may act as substitutes for progesterone in sustaining normal blastocyst growth but others fail. It seems that derivatives of progesterone facilitate implantation whereas certain steroids of the 19-nor type (e.g., norethindrone, norethynodrel, lynoestrenol, ethynodiol diacetate) may inhibit it. A better understanding of the relative actions of the different compounds on the primary mechanisms in the different target cells would elucidate the reasons for these differences in over-all action.

4.9 The mode of application seems to modify the influence of certain steroids on the development of the foetus in the early stages. In some experiments in rats, it has been shown that certain gestogens and progestogens given orally have no effect but when given subcutaneously they produce some evidence of masculinization or feminization; others show such effects even when given by the oral route. Most of the compounds used in therapy have not been observed to affect the somatic development of the human foetus. One, however, norethisterone (or its acetate), has been reported to cause occasional manifestations of masculinization. Although these somatic effects on the foetus do not seem to be a serious hazard to clinical use of these compounds, further data are needed. The effects of the artificial gestogens and progestogens, when administered during the latter part of gestation, on the ultimate hypothalamo-hypophyseal cycle in the human being are also unknown. Since in rodents this cycle can be disturbed during the early post-natal period by the administration of androgens or oestrogens and protected by progesterone, the secretion of these compounds or their metabolites in the milk is a problem that requires urgent investigation.

5. LONG-TERM EFFECTS OF ENDOGENOUS AND EXOGENOUS STEROIDS

5.1 Both the steroid hormones and the synthetic analogues, when used during long periods, have effects on the reproductive tract that need evaluation. In the normal female, endogenous hormones are secreted cyclicly, involving the inter-related rise and fall of oestrogen and gestogen; this seems to be a protective mechanism of considerable significance. If there is continuous exposure to even low doses of oestrogens, either endogenous or exogenous, pathological effects are produced, the endometrium becoming hyperplastic. On the other hand, if progestogens and gestogens are given continuously at even low levels, amenorrhoea and sterility result, with regression of the endometrium to a thin layer having scant if any secretory activity. In the primate, break-through bleeding occurs

irregularly due to shedding of portions of an atypical thin mucosa. A similar effect has been observed in women under continuous gestogen treatment. These extreme changes are avoided when gestogens and progestogens are administered in a cyclic way, particularly if they are associated with small amounts of oestrogen, but during these artificially controlled cycles the uterine mucosa is abnormal if the ratio of oestrogens to gestogens is not carefully adjusted. Reports indicate, however, that the changes in the uterine mucosa are rapidly reversible, even after prolonged administration, and the endometrium becomes normal after treatment is discontinued. Thus far, there is no clear evidence of irreversible damage to the endometrium, fallopian tubes or the ovaries.

5.2 When ovulation is inhibited over long periods of time, possibly by pituitary suppression, the ovaries still show developing follicles. The few investigations that have been made, however, indicate that there is no measurable accumulation of follicles over the number expected at the same age in a normal woman, and no fully matured follicles or signs of ovulation are present. While many more observations need to be made, there is no evidence at this time that the menopause will be significantly delayed.

5.3 The prolonged experimental administration of exogenous steroids has been shown to have effects on certain other endocrine functions. Studies of pituitary physiology have shown that continued suppression of trophic hormones by the secretory products of a target tissue may also affect the output of other trophic hormones. There is some evidence that when suppression of LH is maintained there is also some suppression of thyrotrophic hormone. Although observations on normal women under prolonged treatment have not so far indicated the production of significant thyroid abnormality, populations subject to thyroid disturbances should be studied carefully and adequate data need to be accumulated.

5.4 Any increased level of oestrogens leads to a higher concentration of corticosteroid-binding globulin in the plasma. This produces a stimulation of ACTH release to maintain normal levels of diffusible cortisol. At the same time, the ratio of urinary to plasma corticosteroid levels decreases. The changes are relatively small and thus far appear clinically insignificant, but whether the effect can be cumulative requires further clinical investigation.

5.5 Following long-term treatment with large doses of gestogens and progestogens, as in the treatment of endometriosis, etc., a low rate of adverse effects has been reported. The combined treatment has been used in many cases of functional sterility and experience seems to show that the treatment is especially valuable in women with polycystic ovaries.

5.6 An apparent increase in the pregnancy rate has been observed after cessation of the combined treatment, but the effect is difficult to evaluate as the normal pregnancy rate in different age groups is not known. Treatment of anovulatory sterility by this method is under intensive study.

5.7 Oestrogens administered over long periods produce decreased glucose tolerance in animals. This has also been observed in women during pregnancy as well as in women under chronic combined treatment for suppression of ovulation. This effect is greater than would be expected from the oestrogen alone. So far, no significant increase in insulin requirement in diabetics has been observed, and although there is no evidence of an increase in clinical diabetes due to treatment, the possibility of ultimately precipitating a clinical diabetes mellitus in subjects genetically conditioned or of increasing insulin requirements in an established clinical diabetes remains to be investigated.

5.8 An anabolic effect has been observed, particularly with the norsteroids having methyl or ethyl groups on carbon-17. Whether this is a direct effect of these steroids or is due to a metabolite is unknown.

5.9 Recently, consideration has been given to the occurrence of thromboembolic phenomena in women who have taken some of the gestogen-oestrogen or progestogen-oestrogen combinations. It has been shown, however, that the incidence is no higher than in a similar untreated population. Certain investigators have reported that in pregnancy as well as during chronic treatment with these compounds there is a small increase in the coagulability of the blood, but this is offset by increased fibrinolytic activity. Obviously, more controlled data are needed before reliable conclusions can be drawn regarding the influence of these compounds on the blood-coagulating mechanisms.

5.10 It is known that the use of certain steroids is related to liver disturbances (e.g., cholestatic jaundice), probably as the result of an anaphylactoid reaction. The prolonged use of the antifertility agents has not produced any permanent change in the usual liver function tests, but carefully controlled data should be obtained in patients with history or signs of liver disease who are under prolonged treatment.

5.11 Although oestrogens cause water and salt retention and oedema, the use of oestrogens in low cyclic dosage alone or combined with gestogens or progestogens does not seem to be contraindicated in congestive conditions resulting from chronic heart or kidney failure if adequate medical care is given in respect to salt restriction and judicious use of diuretics. The same appears to be true of subjects with hypertensive vascular disease.

5.12 One long-term effect of endogenous and exogenous oestrogens is the production of tumours in certain end-organs, either as primary

agents, as in the case of pituitary, ovarian, and testicular tumours in mice, or as facilitating agents, as in certain mammary tumours. Continuous exposure to the oestrogenic steroids for a considerable period of time seems to be important for primary incitation of tumour growth. If a cyclic regime is followed, with or without progestogens, this would not seem to be a probable hazard. In oestrogen-dependent tissues, the continuous administration of oestrogens and unopposed exposure to them, may precipitate tumour formation or stimulate tumour growth. Up to this time, however, present forms of cyclic treatment have not been related to tumour induction or stimulation in human beings. Further careful studies of the influence of these substances on tumour incidence and growth in man should be done.

5.13 Lactation in women is usually associated with amenorrhoea and anovulatory cycles. Resumption of ovulation is unpredictable. If the use of oestrogen-gestogen combinations is started soon after childbirth lactation may be interfered with. However, recent observations on the prolonged post-partum use of gestogens alone seem to show that lactation is maintained.

5.14 Little is known of the eventual passage of these compounds or their metabolites into the milk and their possible immediate or long-term effects on the child. As previously mentioned, this is a problem where research is urgently required, particularly as in many areas of the world prolonged breast-feeding is basic to child health.

5.15 Almost all sex hormones and their derivatives can exert a depressing effect on spermatogenesis, which is generally arrested at the spermatocyte stage. The recovery of testicular function seems to be much slower than the recovery of ovarian function. During the initial phase of testicular inhibition and during the recovery phase abnormal sperm may be produced. Morphologically abnormal sperm fail to enter the uterus.

5.16 Prolonged administration of oestrogens, gestogens and progestogens can reduce androgen production and thus influence male behaviour, depressing potency and libido. In the mouse, this effect is a result both of depression of the hypothalamic-hypophyseal system and of direct action on testicular interstitial cells, whereas in the rat only the first mechanism is involved. The suppression of androgen excretion and the influence on behaviour have been observed in man, but the degree to which the two mechanisms are involved has not been determined.

5.17 Since oestrogens and androgens in particular affect calcium and phosphorus metabolism, bone growth and epiphyseal union, the influence that continued use of exogenous steroids may have on epiphyseal union and body growth patterns needs careful investigation.

6. NON-STEROID SUPPRESSANTS AND/OR STIMULANTS

6.1 Much information has accumulated in recent years concerning non-steroid substances that affect some aspects of reproductive endocrine functions. Most of these compounds have been investigated in animal experiments; of these, some have been found to be effective in man while others were inactive.

6.2 There are some psychopharmacological drugs that act on both the female and male reproductive systems of animals. In addition, monoamine oxidase and histamine inhibitors, as well as autonomic blocking agents, can interfere with the reproductive function. In the therapeutic dose-range, nearly all these substances show no such effects in man. On the other hand, radiomimetic alkylating chemicals, cytostatically active agents, antimetabolites, and certain antibiotics and antimitotics that influence the reproductive system in animals also have a similar action in man when given therapeutically. More research is needed to understand the mechanism of these effects in man.

6.3 Various non-steroid compounds found in plants have been reputed in folk medicine to be antifertility agents, but most have proved to be ineffective when studied under controlled conditions. A few appear to have activity on the reproductive system with few adverse effects on other functions. In some plants, substances have been found which seem to owe their suppressive effect on reproduction to their oestrogenic action. Most of these compounds appear to be pro-oestrogens; the active metabolites are in most cases unknown. A more complete knowledge of these compounds and their metabolites might lead to the synthesis of usable agents for control of reproductive processes.

6.4 Among plants not having oestrogenic effects, several varieties of lithosperm have had reputations as antifertility agents among primitive people in widely separated parts of the world. Considerable work has been done recently on this group of plants and the effects of their active principles. As a result of these investigations, there is now evidence that some substances contained in lithosperm may inactivate certain pituitary hormones and also affect the pituitary cells of origin. The exact nature of the interactions remains to be established. Chemical studies have indicated that catechols able to assume an *o*-benzoquinone structure may be the active constituents. Recent investigations indicate that substances of similar nature may be found in other plants.

6.5 Other plant ingredients, e.g., "rottlerin" from *Mallotus philippinensis* or the active compound of *Caladium seguinum*, also seem to deserve consideration. Further investigations of the reproductive effects of

compounds found in plants are urgently needed. Moreover, the elucidation of the mechanism of the chemical interaction between the plant constituents and the peptide hormones may give clues to the synthesis of additional active compounds.

7. RESEARCH NEEDS

After evaluating the present status of knowledge, and in view of the world-wide interest in all aspects of fertility, the Scientific Group considers that strong support should be given to the active investigation of the problems listed below. No attempt has been made to place these problems in order of priority. The references in parentheses indicate the relevant passages in the body of the report.

(1) Sex hormone production and metabolism during the entire course of foetal and post-natal development in mammals, particularly in the human (2.1);

(2) The isolation and purification of enzymes involved in steroid metabolism and their mode of action; the investigation of inhibitors of these enzymes and their mechanisms of action may have practical consequences (2.2);

(3) The biosynthetic functions of the different cellular elements in steroidogenic tissues (2.4);

(4) The role of conjugated steroids and steroid complexes in hormone biosynthesis, in the entrance of hormones into target cells, and in their ultimate mechanism of action (2.3; 2.8; 2.10);

(5) The effects of steroid analogues on endogenous steroid biosynthesis and metabolism and the mechanisms of these effects (4.3; 5.16);

(6) The basic biochemical mechanisms of action of endogenous steroids in different target organs, including the hypothalamus (2.9; 3.2; 3.3);

(7) The primary sites involved and the mechanisms by which general metabolic changes, including those affecting the central nervous system, are brought about by the steroid hormones (2.10; 3.8; 3.10);

(8) The regulation, metabolism, sites and mechanisms of action of the gonadotrophins, particularly FSH and HCG; to achieve this, further work on the purification of these hormones is required (2.6; 3.4; 3.7);

(9) The distribution, metabolism, ultimate disposal and mechanism of action of steroid analogues and their metabolites (4.2; 4.8);

(10) The influence of steroid analogues on protein metabolism and growth; studies of carbohydrate and lipid metabolism are also indicated (5.7; 5.8; 5.10; 5.17);

(11) The response of the primate ovarian tissues to exogenous steroids using advanced microscopic and histochemical techniques (4.7; 5.2);

(12) The action of the hormone analogues on the hypothalamo-hypophyseal system by specific assay methods for individual trophic hormones (4.6; 5.16);

(13) The effects of steroid analogues on the trophoblast and the foetus, in both the early and the late stages of pregnancy (4.9);

(14) The extent to which the artificial steroids and their metabolites are excreted in milk, the biological activity of the excreted compounds, and their influence on the quantity and composition of milk (4.9; 5.14);

(15) The actions of these compounds on spermatogenesis, and on sperm metabolism in various regions of the male and female genital tract (5.15);

(16) The effects of compounds and mixtures used for control of reproductive functions on blood coagulation, both in animals and in man; these effects should be studied by scientists versed in the chemistry of blood coagulation (5.9);

(17) Long-term effects of steroid treatments on endocrine, metabolic, circulatory and tumorigenic phenomena in man (5.1; 5.3; 5.4; 5.6; 5.10; 5.11; 5.12);

(18) The mechanisms of action of compounds that are not hormonometric but affect fertility in either the male or female (6.2; 6.4; 6.5).

In addition to the above specific research needs, the Scientific Group wishes to make note of the following generalizations:

(1) A search should be made, both among synthetic and natural products, for substances having no hormonal action but with the following biological properties (6.5):

(a) reversible inhibition of spermatogenesis without harm to other tissues, particularly testicular interstitial cells;

(b) inactivation of specific gonadotrophins *in vivo*, or blocking of the action of specific gonadotrophins on the target tissues; an FSH inactivator would be particularly interesting.

(2) More studies should be carried out in lower primates because there are marked species differences in steroid metabolism and in the response of target tissues, and in these functions primates most resemble man.

(3) There is an urgent need for the immediate organization of well-controlled statistical studies which would provide, in addition to data on reproductive effects, adequate information for ultimate evaluation of the significance of the possible effects mentioned under research needs 13, 16 and 17 listed above.

8. RECOMMENDATIONS

The Scientific Group recommends that WHO:

(1) make available to under-developed countries the services of competent and experienced scientists, to teach and train students and staff of educational and research institutions in the chemistry and physiology of steroids and steroid-like substances;

(2) strengthen, in so far as possible, the use of training grants to personnel from less developed countries for their training in centres which emphasize research in problems of reproduction;

(3) consider the convening of a group of specialists for the purpose of recommending dose-ranges for the clinical use of active materials in problems of reproduction and fertility;

(4) lend its support towards the furtherance of physiological research on steroids and steroid-like substances with special emphasis on the use of primates;

(5) encourage the search for natural and synthetic substances which affect the reproductive process without serious influence on other functions.