BIOLOGICAL ACTIONS OF SEX HORMONES

BY

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SECOND EDITION

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PREFACE TO SECOND EDITION

The purpose and style of the first edition are here retained. Fresh material has been added, and in reply to the friendly comments of private correspondents and public reviewers some alterations have been made. Throughout the essay literary daintiness has been held of second importance to clarity of statement, and this principle has led to an amount of tautology which may trouble the refined reader. Little room has been spared for unconfirmed opinions, for the aim of the writer has been to collect facts and to co-ordinate them so that the subject of sex hormones may be regarded broadly without any obscurity derived from unproved ideas. The book is chiefly concerned with laboratory work and its outlook is almost confined to vertebrates, with mammals having the larger share. Quotations of recorded observation and experiment are numerous, though forming only a small proportion of the very large number which might have been usefully mentioned; however, they may be enough to help anyone who is beginning some particular study connected with sex hormones, and the papers to which they refer will certainly guide him to further sources of information. A good deal of space has been given to the primary discoveries from which later investigations have arisen; this respect for the past has perhaps been overdone, but our knowledge of the physiology of sex is a recent acquirement and its foundations are yet new enough to demand a careful inspection. Besides, things done are the seeds of things to be.

I am grateful to Professors G. A. R. Kon and C. W. Shoppee for useful advice, and those helpers who were named in the preface to the first edition are thanked once more. To the Royal Cancer Hospital (Free) and the Chester Beatty Research Institute I owe a special debt for their continued encouragement and support. I am also grateful to the Cambridge University Press for their unremitting attention to the many details required in first class publication, and to my friend Dr J. N. Goldsmith for his valuable help in arranging an excellent index.

H. B.

Marlborough, August 1948

PREFACE TO FIRST EDITION

In the last few years our comprehension of vital phenomena has been rapidly extending. The nature of the sex hormones, and the reactions of living tissues toward them, have been prominent in this advance, and it is now generally understood that compounds formed in the pituitary, gonads and adrenals radically affect the structure and functions of the body and the workings of the mind. To-day our knowledge of these matters is growing so fast that to keep abreast of it is not easy for those who are occupied with many other affairs. The author felt, therefore, that a co-ordinated summary of experimental inquiries in this field might be useful. In pursuing the idea attention has been confined almost entirely to biological work performed in the laboratory; the ultimate possibility of applying the experience so gained for the benefit of man has been the leading motive.

The essay can hardly be offered to the scientific world without an apology. Biological work is still largely confined to qualitative observation. Life is a changing process and in solving its problems we are often deprived of fixed and measurable data; moreover, the adaptability of living tissue to circumstance involves so many and such complex reactions that an exact prediction of the outcome of any extraneous influence cannot, as a rule, be stated in precise quantitative terms; nor can experimental results in this field be described adequately without specifying the conditions in which they were obtained. The presentation of the subject demanded by the latter drawback may, it is feared, be tedious to the reader, especially as the narrative contains many references to the literature. Sir James Paget complained of the difficulty of composing a readable scientific review, and the present writer is too modest to suppose that he has overcome the difficulty. It is hoped, however, that the matter contained in these pages may supply a trustworthy, though limited, foundation for further progress in both sex-hormone research and clinical practice.

The author would like to regard his book as a tribute to the pioneers of sex-hormone physiology, with special regard to John Hunter (1728–93), the first and greatest of them. More than a hundred years before the term hormone had been invented, Hunter showed that the accessory reproductive organs are largely dependent for their development and even for their existence on some influence derived from the gonads.

The writer regrets that much good work done in foreign lands has been given inadequate consideration, but it may be unnecessary or impossible to insert every detail into a picture; for general portrayal it is perhaps enough to draw the salient features as far as possible with fidelity of outline and correct emphasis.

My own experimental work on the sex hormones has been done, under the auspices of the British Empire Cancer Campaign, at the Chester Beatty Research Institute of the Royal Cancer Hospital (Free). To the authorities of these institutions, to Professor Kennaway the director, and to my other colleagues I should like to express gratitude for their interest and encouragement. I have

also to thank, most cordially, several donors for generous gifts of chemical material; among these donors are Dr Girard of Paris, Dr Laqueur of Amsterdam, Dr Macbeth of Organon, Limited, Mr Smart and Dr Miescher of the Ciba Company. To Dr Macbeth I am greatly indebted for extensive references to relevant literature. I should like also to acknowledge valuable advice from Dr F. H. A. Marshall. To Dr J. N. Goldsmith I am especially grateful; not only has he prepared an exceptionally helpful index but he has given great help with proof-reading and advice on various details. Lastly, I must mention the unremitting help which I have received from my wife, without whose aid this book could not have been written.

H. B.

London, 1944

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Chapter I. *The Nature and Functions of Gonadotrophins*

Introductory remarks. Inactivation, excretion, sources and distribution of gonadotrophin. The pituitary and placental gonadotrophins are not identical. The follicle ripening (FRH) and luteinizing (LH) hormones. The action of gonadotrophins on the ovary with special reference to hormonal balance. The action of gonadotrophins on the testis. Puberty and the awakening of sexual activity.

Introductory Remarks

THE reactions which take place between the anterior lobe of the pituitary and the endocrine glands are caused by hormones conveyed in the bloodstream. Though numerous nerve fibres connect the pituitary with the supraoptic nucleus and adjacent cells in the hypothalamus, they pass almost entirely to the posterior lobe; and the relatively sparse fibrils of the anterior lobe are derived mainly from the carotid plexus (Rasmussen, 1938, 1940; Hair, 1938; Ingram, 1940; Brooks & Gersh, 1941). The only notable influence these non-myelinated nerves exert over the anterior lobe of the pituitary is a regulation of its blood supply and its consequent ability to utilize any circulating hormones. Yohimbine, according to some observers though not to all, brings about dilatation of the pituitary blood vessels, and its reputed sexual effects have been explained largely or entirely by this vascular effect (D'Amour, 1934; Hechter, Lev & Soskin, 1940; Fugo & Gross, 1942; Sulman & Black, 1945). Oestrogens and androgens are both said to increase the general blood supply of the brain (Reiss & Golla, 1940), and the pituitary would, it is presumed, participate in this hyperaemia.

The vascular supply of the pituitary is very abundant, and includes a portal system. Arteries from the internal carotids supply a rich upper plexus in and about the pars tuberalis. From this plexus wide collecting veins descend to the anterior lobe of the pituitary where they drain into a secondary lower capillary plexus. The flow of blood in this system is from the upper to the lower plexus, and seems adapted for the transmission of hormones from the hypothalamus to the anterior lobe of the pituitary. A vascular system of this kind connecting the hypothalamus with the anterior lobe of the pituitary is present in frog, guinea-pig, rabbit, cat, dog, porpoise, monkey and man (Harris, 1948).

Several experimenters have confirmed the opinion of anatomists on the almost complete freedom of the anterior lobe of the pituitary from direct nervous control. Hinsey & Markee (1933) prevented impulses from reaching the pituitary through the carotid plexus, by dividing the sympathetic trunks in each side of the rabbit's neck below the cervical ganglion or by removing these ganglia, and the operations did not forbid subsequent mating and fertility. Severance of the pituitary stalk in

the rabbit has been found to permit coitus though it prevented the usual sequel of ovulation (Brooks, 1938, 1940; Brooks & Beadenkopf, 1940), and has even been followed by degeneration of the anterior lobe of the pituitary (Harris, 1937); but it seems possible that this difficult operation may have been accompanied by some coincident injury to the hypothalamus and other adjacent structures, for mating, ovulation, pregnancy, delivery and lactation will all occur in the rat after division of the pituitary stalk (Richter, 1933; Uotila, 1940; Dempsey & Searles, 1943).

So far as concerns afferent nervous impulses derived from various parts of the body and affecting the gonadotrophic functions of the pituitary, these impulses all appear to end in the region of the hypothalamus and the influence which they have upon the pituitary must be conveyed there by one or more hypothalamic hormones the nature of which is not yet known. Although electrical stimulation of the sympathetic trunks, or of the pituitary itself, does not cause the release of gonadotrophin in the rabbit, the same sort of stimulation (201 mv., 0·083 ma.) applied directly to the hypothalamus may do so (Markee, Sawyer & Hollinshead, 1946).

After this little anatomical survey, the gonadotrophins themselves may be considered. They receive their name because they govern the development and biological activities of the gonads.* Cushing & Goetsch (1915) showed that pituitary deficiency in man, whether naturally or artificially produced, is accompanied by atrophy of the gonads and adrenal glands; and Smith (1916, 1926*b*, 1927*a*), in a fine series of pioneer experiments, demonstrated that hypophysectomy in lower animals also is followed by atrophy of the gonads and adrenals, and he further showed that the normal condition of these glands may be largely restored by daily implants of pituitary tissue. He also discovered that in young healthy animals precocious puberty can be induced by similar pituitary implants (Smith & Smith, 1922; Smith, 1926*a*).

Noble (1938*a*) has described the progressive atrophy of the gonads and adrenals and of some of the accessory genital structures which follows hypophysectomy in male and female rats (Table 1).

TABLE 1. Progressive atrophy of reproductive organs after hypophysectomy in male and female rats (Noble, 1938*a*)

Males

Intervening time (weeks)	Number	Average weights of organs in mg.			
		Testes	Prostate	Seminal vesicles	Adrenals
1	12	1,204	120	52	12
2	12	854	109	56	12
4	8	366	87	42	8
6	5	273	54	34	5

Females

Weeks	Number	Average weights of organs in mg.		
		Ovaries	Uterus	Adrenals
1	4	24	142	28
2	6	22	129	23
4	4	17	117	14
6	3	15	82	8

* γονή = gonad, τροφή = nourishment.

The changes in the gonads and adrenal cortices after removal of the pituitary include shrinkage of nuclei and cytoplasm with arrest of secretion. These results can be prevented, or if already present can be reversed, by injecting extracts made from the anterior lobe of the pituitary of other animals into the muscles or subcutaneous tissues.

The chemical nature of the gonadotrophins has not been exactly determined. They are soluble in water, give the general reactions of proteins, and are precipitated without denaturation by ethyl alcohol. According to Askew & Parkes (1933) the ovulation-producing hormone of pregnancy urine is inactivated by heating to 100° C. in water, but loses none of its activity if kept at that temperature for 1 hour when dry; the results are unaffected by the exclusion of oxygen. From these and other observations it seems that the gonadotrophins are proteins or are so closely associated with proteins that their activity disappears when the latter are destroyed. The gonadotrophins also contain carbohydrate in the form of mannose and galactose. Among gonadotrophins from different sources Gurin (1942) has detected differences in the carbohydrate content.

The effects of proteolytic enzymes on gonadotrophins. Evans, Simpson & Austin (1933*b*) found that the gonadotrophin of pregnant mares' serum was but little affected by pepsin during 4 hours at 37° C. when the *p*H was between 4 and 5, though at a *p*H of between 1·8 and 2 its activity was destroyed. They note that other samples lost their potency when subjected to this *p*H in the absence of pepsin. Trypsin at a *p*H of 8·5 inactivated the hormone. Bates, Riddle & Lahr (1934) obtained FRH from beef pituitaries and digested it with trypsin at 37° C. and *p*H 8·0 for 2 hours. The material was then injected in four equal daily doses into immature ring doves whose testes were weighed 96 hours after the first dose, and compared with those of untreated birds of the same age. Comparison was also made with the testes of doves who had received injections of normal undigested FRH. The results show that the hormone had been destroyed by the tryptic digest (Table 2). (See also Riddle, Bates, Lahr & Moran, 1936.)

TABLE 2. The effect of trypsin on a gonadotrophic extract rich in FRH (Bates, Riddle & Lahr, 1934)

		Average weight of doves' testes 96 hours after the first injection (mg.)	
Material injected	Dose (mg.)	Uninjected control doves	Injected doves
Untreated FRH	4	8·7	49·2
	4	6·5	34·0
FRH after digestion with trypsin	4	6·5	6·8
	4	7·8	10·7
	8	6·3	6·6
	8	6·3	8·8

Van Dyke (1936) says that the gonadotrophin of pregnancy urine is rendered inert by boiling, ultra-violet light, hydrogen peroxide or trypsin, but not by pepsin, though according to Fevold (1937) it is destroyed by pepsin. Collip

(1937*b*) states that pituitary gonadotrophin is inactivated by prolonged boiling and is sensitive to alkali, losing its potency at a little beyond *p*H 8. Thyrotrophic hormone, he says, shows approximately the same reactions. Using extracts of sheep's pituitary McShan & Meyer (1938, 1939) found that LH* is largely if not entirely destroyed by trypsin when exposed to it for $3\frac{1}{2}$ hours at 37° C. and *p*H 8·0, and is relatively resistant to ptyalin, whereas FRH is resistant to trypsin and destroyed by ptyalin. Ch'en & Van Dyke (1939) found that tryptic digestion destroyed most of the luteinizing action of pituitary extracts whether obtained from sheep or horse but large doses of such digested extracts still caused some luteinization in the ovaries of hypophysectomized immature rats, showing that the destruction was not complete.

Chow, Greep & Van Dyke (1939) incubated extracts of fresh pig pituitaries at 37° C. with various proteolytic enzymes; some of their results are given in Table 3, and show that the gonadotrophin used was inactivated by trypsin, chymotrypsin and pepsin, but not by papain or carboxypeptidase.

TABLE 3. The effect of proteolytic enzymes on pituitary gonadotrophin (Chow, Greep & Van Dyke, 1939)

Enzyme	*p*H of digest	FRH	LH	Thyrotrophin
Pepsin	4·57	+		+
Papain	4·57	−	−	−
Trypsin	8·69	+ −	+	+
Chymotrypsin	8·69	+ −	+	+
Carboxypeptidase	8·69	−	−	−

NOTE. + = inactivated; − = not inactivated; + − = partially inactivated.

Abramowitz & Hisaw (1939) have also investigated the action of proteolytic enzymes on three different gonadotrophins, namely purified FRH, LH extracted from the pituitaries of sheep, and a placental gonadotrophin derived from the urine of pregnant women. Their findings indicate some differences in the proteolytic reactions of the extracts which were tested (Table 4).

TABLE 4. Proteolysis of pituitary and placental gonadotrophins (Abramowitz & Hisaw, 1939)

		Pituitary gonadotrophin		Placental
Enzyme	*p*H of digest	FRH	LH	gonadotrophin
Papain	7·1	+ −	−	+
Trypsin	7·1	+	+	+
Chymotrypsin	7·6	+ −	+ −	+
Crude ptyalin	7·1	+	−	+

NOTE. + = inactivated; − = not inactivated; + − = partially inactivated.

Li (1940) ground and treated the pituitaries of gonadectomized rats with trypsin at *p*H 9·6 and incubated the material for 2 hours at 38° C., after which it was assayed on 21-day-old female rats, eight doses being given in 4 days, and the findings were compared with those obtained by pituitaries which had not been

* For brevity and ease of discussion follicle ripening and luteinizing hormones will be referred to as FRH and LH respectively.

treated with trypsin (Table 5). His results show that the extract used which was rich in FRH was to a large extent inactivated by trypsin; the high degree of alkalinity of the digest will be noted.

TABLE 5. The effect of trypsin on the gonadotrophic potency of the rat's pituitary (Li, 1940)

Sex of pituitary donor	Total dose (mg.)	Pituitary treated by trypsin	Mean weight of ovaries in test rats (mg.)	Mean weight of uterus in test rats (mg.)
Female	5	−	69·76	82·10
Female	10	+	23·73	63·77
Male	5	−	66·65	82·89
Male	10	+	22·81	40·35

The results of proteolysis which have just been mentioned, though they are not all in complete agreement, suggest that the gonadotrophins, if not themselves proteins, are dependent for their activity on a close association with protein. Spielman & Meyer (1937), having examined the electrophoretic properties of placental gonadotrophin, believe that it probably consists of a specific principle combined with a non-specific carrier. They arrived at this conclusion by observing that the hormone may be still active biologically in spite of a change of its isoelectric point.

The physico-chemical nature of human placental gonadotrophin was investigated by Gurin, Bachman & Wilson (1940), who found by electrophoresis that their preparation was a protein, nearly electro-chemically homogeneous, with a mobility of $4{\cdot}8 \times 10^{-5}$ sq.cm. sec.$^{-1}$ volt^{-1} at a *p*H of 7. Analysis indicated it to be a glycoprotein. In the ultracentrifuge it sedimented as a single component, the calculated sedimentation constant being approximately 5×10^{-13} cm. sec.$^{-1}$ dyne^{-1}, which suggests that the minimum molecular weight lies between 60,000 and 80,000. The isoelectric point was between *p*H 3·2 and 3·3. The gonadotrophins undergo bacterial decomposition, and cannot be given effectively by the mouth (Evans & Long, 1921), though some response has been thought to follow oral administration in man (Goetsch & Cushing, 1913; Goetsch, 1916).

As a rule gonadotrophins are not stored appreciably in the body; they are inactivated in the bloodstream and in some animals are in part excreted with the urine.

Inactivation, Excretion, Sources and Distribution of Gonadotrophin

Inactivation in the living body. Geist & Spielman (1934) collected blood from the two ends of the severed umbilical cord of a baby and identified gonadotrophin in blood from the placental end in a concentration of 165 r.u. per litre, while none was recognizable in the blood obtained from the foetal end (see p. 13).

It was shown by Lipschutz & Vivaldi (1934) that human placental gonadotrophin when given intravenously to a rabbit disappears rather rapidly from the blood. Six to 8 hours after the intravenous injection of 100 r.u. only 20 per cent could be recovered from the blood, and 10 hours after injection only 10 per cent could be recovered.