androsterone is a type (Fig. 2, IV), closely related to testosterone, the hormone of the testis, have indeed a sex-hormonal virilizing action.

Conclusion

The outlines are beginning to appear of the most complicated pattern of the complementary actions of these hormones in relation to one another; of their balanced actions against those of other hormones—for example, of some of them against the actions of insulin; and of the control of their formation and output by the so-called adrenocorticotrophic hormone of the pituitary anterior lobe and apparently to some extent by the suprarenal medullary hormone adrenaline. I myself observed, as early as 1921, that a cat from which the suprarenals had been removed showed a greatly exaggerated sensitiveness to histamine, even before overt general signs of deprivation appeared. And, in more general terms, the part played by the cortical hormones in the whole scheme of the endocrine control of the body's functions and reactions, and especially, as Selye, of Montreal, has emphasized, in its adaptative reactions to almost all kinds of injury or stress, is undoubtedly one of central importance.

In further and most suggestive illustration of this function, in the case of at least one of the corticosteroids comes the recent news of therapeutic trials, at the Mayo Clinic and elsewhere, giving promise of a protective and curative effect of another member of the carbohydrate-controlling group, the 17-hydroxy-11-dehydrocorticosterone (Kendall's "Substance E," Fig. 2, III), against defective or perverted adaptative reactions responsible for rheumatoid arthritis. We shall wait eagerly for further evidence of advance in this new and surprising direction. And meanwhile I think you will agree that, if the anterior pituitary lobe occupies the conductor's rostrum in the endocrine orchestra, we must at least assign the leading violinist's desk to the suprarenal cortex. And surely no artificial monument to the memory of Thomas Addison could be so imposing as this great and now so rapidly growing tree of health-giving and life-saving knowledge, sprung from the seed which Addison dropped as he worked in this hospital with such patient devotion and with a clinical vision unsurpassed in its clarity and alertness.

The Report of the Food Investigation Board for the year 1939 has now been issued by His Majesty's Stationery Office, price 2s. 6d. Before publication could be effected when the report was originally prepared the type was destroyed by enemy action, and the document could not be issued until the present time because of restrictions on printing and paper. Some of the work described in the original paper has now been completed, and accounts have been published in various journals, to which references are given. Among the experiments described are tests on the control of microbial spoilage in meat by ultra-violet irradiation. Some retardation of bacterial growth was obtained, but the effect was not great. The storage of eggs was investigated, and experiments were carried out on freezing and storage of sprats before canning. Work was undertaken on the effect of acetylene and ethylene on the ripening of "Kelsey" South African plums. Treatment with either gas for three days accelerated ripening and improved the quality of the fruit, while untreated samples kept in air ripened slowly. Continued exposure to acetylene until the fruit ripened, however, made it too sweet. Research on blemishes on the rind of grapefruit stored at low temperatures, known as "low temperature spot," showed that wrapping in moisture-proof material instead of tissue-paper enabled lower temperature storage to be used, or the fruit to be stored longer. Other work included investigations of various methods of freezing strawberries, raspberries, and peas.

SOME PRINCIPLES UNDERLYING DETERMINATION OF SEX HORMONES*

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During the last two decades our knowledge of biological processes has been rapidly extending. Studies on sex hormones have had an outstanding influence on this advance. Research work and clinical experience have shown that specific principles formed in the pituitary, gonads, and adrenals profoundly affect the structure and function of the whole body. Therefore sex hormones, now available in highly purified preparations, have been introduced in many cases of gonadal and extragenital disorders either as substitution or as stimulation therapy. This use of hormones has enormously increased. However, it must always be remembered that no benefit can be expected from their promiscuous use. A critical attitude should be maintained in the management of endocrine disorders.

These disorders demand some understanding of sexual functions under various physiological and pathological conditions and a thorough knowledge of the hormone-producing organs and those involved in hormone metabolism. In cases of possible hormonal deficiency diagnosis and treatment are based both on the clinical picture and on the histological, biological, and chemical findings.

Any hormone determination is of value only in the light of a preceding clinical examination. Typical changes in weight, temperature, fatigue, appetite, growth and distribution of hair, and vascular, nervous, and other physical manifestations sometimes suggest the kind of sex-hormone imbalance present. The sexual sphere, as the most sensitive to hormonal influence, is the one most readily involved. Undisturbed sexual function may already exclude important hormonal disorders. Administration of sex-hormone preparations in such cases may be unwise and even harmful.

Further information on hormonal activity is obtained from histological and cytological examinations. In women, endometrial biopsy and vaginal smears reveal the uterine and vaginal response to ovarian stimulation. They are the simplest means of determining ovarian function. They show whether an ovarian deficiency exists and whether the disturbance involves suppression in the follicular or luteal phase, or both. They are used not only as diagnostic aids but also as a control to the efficacy of treatment. These clinical findings, however, vary with the responsiveness of the organ involved and are predominantly qualitative in character. They have to be completed by quantitative studies of sex hormones, data of which are provided by biological and chemical methods indicating hormone levels in blood and urine. These give evidence not only of the kind but of the amount of hormone produced. These determinations represent one of the most important diagnostic

*From a lecture delivered by Professor de Watteville to the Faculty of Ophthalmologists of London, at the Maternité of Geneva, on May 11, 1948.

and/or its metabolites, and (b) that no other substances present in the extract under consideration show the same properties or interfere with the reaction employed.

Unless these two facts have been established by biochemical research no chemical determinations are possible. That is why gonadotropins, for instance, as protein compounds of unknown chemical constitution, are tested exclusively by biological methods. (Compare, however, the work of Mello, 1943.) Bio-assay measurements are comparative and require the use of a reference standard, because differences in strain, living conditions, and age of test animals (mostly mice, rats, and rabbits) sometimes lead to divergent results. Therefore values obtained from biological tests by different research workers are comparable only if related to the international unit of a standard preparation.

Some clinicians prefer biological methods, arguing that biologically active principles can be measured only by the response of a living organism. But actually the respective proportions of active principles and the corresponding chemical fractions in human urine are fairly well known. Chemical and biological standard methods are therefore regarded as equally reliable and informative.

Gonadotropins

Aschheim and Zondek, and independently Smith, in 1926-7 observed that immature mice respond to implantations of pituitary or injections of human pregnancy urine with precocious ripening of their ovaries. This reaction is the effect of the gonadotropic hormones. *These are not sex-specific*, as they stimulate male as well as female gonads.

In the pituitary two fractions of gonadotropins are produced: the follicle-stimulating hormone stimulates primordial follicles of the ovary to develop into mature follicles, which in turn produce oestrogens; the luteinizing hormone transforms the ruptured follicles into corpora lutea, which in turn will produce progesterone.

Gonadotropins also have a stimulating effect on the size and functional activity of the testis.

A source of gonadotropins other than the pituitary is the placenta, which is the chief producer of the luteinizing factor. Tumours derived from chorionic tissues likewise produce gonadotropins, the amounts far exceeding those already high during pregnancy.

Generally, routine biological tests determine both fractions together. Separation of follicle-stimulating hormone and luteinizing hormone is done only in biological research work and is not used for clinical examinations.

Determinations of high gonadotropin levels in body fluids are of practical importance for the early diagnosis of pregnancy. A drop to subnormal values indicates foetal death. Excessive rise in gonadotropin titres as proved by a positive Aschheim-Zondek reaction with highly diluted samples of urine indicates pathological growth of hormone-producing chorionic tissues, such as hydatid mole, chorion-epithelioma, and some kinds of teratoma containing chorionic cells.

In conditions other than pregnancy only small amounts of gonadotropins are present. Therefore they must be concentrated before they can be measured by the biological tests available. During the menstrual cycle gonadotropin excretion rises and falls, with a peak about the time of ovulation. Several determinations have therefore to be made, the most important one being in mid-cycle.

In castrates of both sexes, in old men, in women at the menopause, and in conditions due to primary ovarian

failure a rise of gonadotropin levels has been demonstrated. This fact is said to be at least partly responsible for climacteric troubles.

The pituitary gland influences the whole endocrine system. Therefore control of gonadotropins is of interest in all cases in which endocrine dysfunction is suspected. High levels of gonadotropins are to be expected in pituitary basophilic hyperplasia or adenoma and in organic diseases of the hypothalamus. On the contrary, low values are found when the basophilic cells of the pituitary are destroyed by tumour growth—for example, in cases of chromophobic adenoma, eosinophilic adenoma, and cranio-pharyngioma or some other intracranial tumours—and in all cases of symptomatic or idiopathic pituitary atrophy, such as Simmonds's disease, Dercum's disease, and the syndromes of Fröhlich and Laurence-Moon-Biedl.

Oestrogens

The three naturally occurring oestrogens considered here are oestradiol, oestrone, and oestriol, the first being the most potent, the last-named the least potent. They are formed not only in the ovary and in the placenta but, in much smaller amounts, also in the adrenals and in the testes. Possibly only oestradiol is actually elaborated by the glands, the two other oestrogens being its metabolites. All three are excreted in urine, to a small extent as such, but mainly in conjugated form as sulphate or glucuronide. The only *inactive* metabolites known are the oestrane-diols, which, however, have no clinical importance.

The oestrogens stimulate proliferation of the myometrium, endometrium, and vagina, and furthermore they develop the mammary gland and other secondary sex characters.

The most widely employed biological test (Emmens, 1939; Pedersen-Bjergaard, 1939) is based on the ability of oestrogenic hormones to induce cornification of the vaginal epithelium in spayed mice and rats. Positive reaction is demonstrated by the presence of cornified cells in the vaginal smear. For the determination of low oestrogen concentrations the histological demonstration of vaginal mucification preceding cornification has been used (Fluhmann, 1930). These biological tests necessitate a previous chemical preparation of urine involving liberation of the active principles by hydrolysis of the inactive conjugated oestrogens, followed by extraction and concentration.

Numerous chemical procedures have been described in the literature using colour reactions of the oestrogenic phenols for their colorimetric determination. Unfortunately, they can replace bio-assay only in conditions such as pregnancy, when high levels are present. To the clinician it is much more important to determine the small amounts excreted during the menstrual cycle. This seems to be achieved by a fluorometric method (Jailer, 1947-8) the principle of which has long been in use in veterinary medicine as a qualitative pregnancy test in mares (Cuboni, 1934).

Chemical determination as well as bio-assays may be done after separating the three oestrogens in order to determine their respective amounts. This chemical separation (Pincus, 1945; Friedgood *et al.*, 1948) complicates the technique considerably, but nevertheless it may be worth while because of the additional information on oestrogen metabolism which can be obtained.

The rate of oestrogen excretion in urine varies during the stages of the menstrual cycle. The highest values are encountered at the end of the follicular phase, and considerable amounts are still present during the luteal phase.

Before the onset of the menstrual flow a sharp drop occurs. Thus several determinations are necessary to adjudge the oestrogenic activity of the ovary. This determination of the rate of oestrogen excretion may be of importance in the detection of slight pathological deviations of oestrogen levels, which may cause functional troubles even when no morphological changes or menstrual disorders are observed.

The same clinical symptoms may be produced by different oestrogen production, as, for example, in amenorrhoea, in which oestrogen values may be low or high, cyclic or acyclic.

As practically no oestrogens are detectable in menopausal women, positive tests suggest hormone-producing tumours of the ovary or of the adrenals. Also, in the male, tumours of the adrenals, and especially adrenal carcinoma, may produce considerable amounts of oestrogens.

Progesterone and Pregnanediol

Gonadotropins stimulate not only oestrogen but also progesterone production in the ovary. The latter hormone is elaborated in the corpus luteum and, from the third or fourth month of pregnancy onwards, in the placenta. Its principal actions are: (1) the secretory transformation of the uterine mucosa, and (2) the muscular relaxation of the whole uterus. These progestational changes allow of the implantation of the fertilized ovum and make possible the undisturbed growth of the embryo.

The biological assay of progesterone makes use of the effect of this hormone on the endometrium. The test animal is the immature (Clauberg, 1930) or castrated (Corner and Allen, 1929) rabbit, which must have received preparatory treatment with oestrogen in order to induce the proliferative phase of its endometrium. The test is positive if in the histological examinations signs of secretory transformation are observed. By intrauterine administration of the material to be tested (de Allende, 1940; Hooker and Forbes, 1947) even the small amounts of progesterone present in the blood of normal women during the second half of the menstrual cycle can be detected. The test, however, is not rigidly specific for progesterone, because two other hormones—namely, testosterone (from the testis) and desoxycorticosterone (from the adrenal cortex)—are also able to induce the transformation of the proliferated uterine mucosa into its secretory phase. Of course, the respective activities are not the same. The doses required, as compared with progesterone, are six times greater for desoxycorticosterone and twenty times greater for testosterone.

The biological assay of progesterone gives positive results only with certain tissue extracts and with blood, but in urine practically no progesterone can be detected. This is explained by the rapid inactivation of this hormone within the body, leading partly to the formation of pregnanediol. This progesterone metabolite is eliminated in urine in conjugated form as sodium pregnanediol glucuronide. Its chemical constitution differs only slightly from that of the parent compound, progesterone, but nevertheless it has no biological activity at all and can be determined only by chemical methods.

We use a method of our own (de Watteville *et al.*, 1948) which is relatively simple, reliable, and rapid. It is based on hydrolysis of the glucuronide and simultaneous extraction of the freed pregnanediol, followed finally by chromatographic isolation of the pure compound by means of an aluminium oxide column. The crystalline pregnanediol is weighed, and its purity can be controlled by determination of the melting-point.

Clinical experience has shown that, broadly, urinary pregnanediol levels parallel progesterone action observed in the organism. We are entitled, therefore, to use the pregnanediol level in urine as a reliable indicator of progesterone production by the corpus luteum or the placenta. The minimal amount of pregnanediol derived from normal adrenal steroids is too small to interfere with determinations during the menstrual cycle or in pregnancy. It acquires, however, some value as a diagnostic aid when considerably increased by certain adrenal tumours or adrenal hyperplasia. Still, the chief importance of pregnanediol determination lies in gynaecological and obstetrical diagnosis.

Together with the assay of gonadotropins, pregnanediol is employed as a test for normal or disturbed pregnancy. In threatened abortion normal or decreased pregnanediol levels are most informative regarding whether the further evolution will be good or bad. In cases of threatened or doubtful missed abortion the decision whether to interrupt pregnancy or not can almost exclusively be based on pregnanediol values.

Pregnanediol determinations are also very useful during the second half of the menstrual cycle. Normal values between the sixteenth and twenty-sixth days of the cycle are conclusive evidence that a normal corpus luteum is present. On the other hand, zero values are found only in anovulatory cycles when follicle rupture does not occur and no corpora lutea are formed. Finally, if pregnanediol values are too low, or if the period of excretion is too short, then progestational deficiency is strongly suggested.

It is well known that the ovary is not autonomous. Corpus luteum function, as well as follicle ripening, depends upon pituitary gonadotropins, and its disorders may be caused either by a primary ovarian deficiency or by disturbances of the pituitary. The latter, however, may be responsible for quite a lot of other more general disorders—for instance, post-partum obesity, which shows luteal deficiency of pituitary origin and can be successfully treated with pituitary luteinizing hormone. The meaning of all this is that normal pregnanediol values, if considered together with the clinical picture, enable us to exclude either ovarian failure or disorders of the gonadotropic function of the pituitary.

Androgens and 17-Ketosteroids

Testosterone is the characteristic endogenous hormone of the testicle. In the human body it is converted partly to androsterone and other 17-ketosteroids of less or no biological activity. These conversion products are excreted by the kidneys, but no testosterone is found in urine. Androgenic activity as well as 17-ketosteroids have been demonstrated in the urine of children and adults of both sexes. This is explained by the fact that androgens and 17-ketosteroids are not only of gonadal but also of adrenal origin. The normal output of 17-ketosteroids varies with age and sex; an extensive study of this subject (including standard curves) has been published by Hamburger (1948). In women, adrenals are by far the most prolific source of androgenic substances and 17-ketosteroids, the concentration of these being almost the same in normal and ovariectomized women, but less than in urine of normal men. Nevertheless, the ovaries also seem to be capable of androgen formation, as is strongly suggested by the fact that virilism may be caused by arrhenoblastoma. In men, both adrenals and testes are concerned with androgen and 17-ketosteroid elaboration: it is supposed that the testes account for 20 to 40% of the urinary 17-ketosteroids.

Determination values, therefore, are not a conclusive index of spermatogenic function, but in case of deficiency they may provide a clue to the incretory status of the testes and indicate a deficiency of the testicular hormone. Eunuchoids, for instance, excrete appreciably less androgens than normal men. In cases with destructive processes of the adrenals, such as Addison's disease, the values found are also low.

In some castrated and menopausal women and in those with arrhenoblastoma 17-ketosteroids tend to be increased. Urines from cases with adrenal hyperplasia or tumour yield enormous quantities of certain 17-ketosteroids. Therefore a rise in 17-ketosteroid output may be regarded as an aid in the differential diagnosis between Cushing's disease due to adrenal cortical tumour and that of pituitary origin where the level is apparently within normal limits.

The biological assay (Gallagher *et al.*, 1937) is based on the ability of androgenic material to promote comb-growth in the capon. To replace the biological test Zimmermann's (1935, 1946) colorimetric *m*-dinitrobenzene reaction, as modified by Callow *et al.* (1938) and many other workers, is now widely used for the determination of urinary non-phenolic 17-ketosteroids. To increase the specificity of this method it is strongly recommended to eliminate unspecific chromogens from the extract, using chromatography (Talbot *et al.*, 1941), charcoal (Consolazio and Talbott, 1940), or Girard's reagent T (Talbot *et al.*, 1940). Even then the 17-ketosteroid values obtained are too high if the 20-ketosteroids of pregnancy urine are present to contribute to the colour intensity. Pincus's (1943) antimony trichloride reagent seems to be more specific, but it does not react with dehydro-isandrosterone, an important 17-ketosteroid in the β -fraction. The neutral steroid fraction of human urines can be separated into an α - and a β -fraction of ketosteroids (Pincus, 1945). The determination of these two fractions may aid in the differential diagnosis between adrenal cortical hyperplasia and adrenal carcinoma, the β -fraction being markedly increased in the latter.

Much useful information could be gained from fractionating the extracts still further in order to trace individual steroids instead of groups. Unfortunately, the chemical procedure involved (Dingemans *et al.*, 1946) is too laborious to permit its use in daily clinical routine work.

The value of the determination of androgens and 17-ketosteroids in the diagnosis of gonadal and adrenal disorders is limited, because of great individual variations in both normal and abnormal subjects. Repeated determinations in the same subject, however, may yield some information about the evolution of an endocrine disease and about the therapeutic success. With regard to single determinations, significance can be attributed only to the very high figures occurring in cases of adrenal tumour.

Conclusions

Clinical endocrinology, although complicated, is of increasing importance. In order to stress this importance we may give some final conclusions: (1) Hormone determinations are of value only when considered simultaneously with, and in close relation to, the clinical picture of a case. (2) The usefulness of routine hormone determinations is threefold—theoretical, diagnostic, and therapeutic. (3) Their *theoretical* importance lies in basic research work on hormone metabolism, when we are trying to learn everything about the fate of hormone substances within the body, how they are produced, transformed in the target organ, inactivated, and excreted. (4) In *diagnosis* they provide valuable information on glandular functions, indicating whether

these are normal, increased, or deficient. (5) In *therapy*, at last, they help us to judge the effect of hormone treatment on internal secretion—whether we have succeeded in stimulating, substituting, or regulating the functions we meant to influence.

Hormone research and its clinical applications are in full progress, but much work has yet to be done. We can be sure that the result of future research will extend still further the assistance which the physician already receives from the laboratory.

Readers not familiar with the steroid nomenclature mentioned in this paper are referred to the instructive survey by Mason (1948).

We are indebted to Harris and Thimann (1943-7), Burrows (1945), Hamblen (1945), and Pincus (1947-8), whose books and reviews may profitably be consulted for further and more detailed information.

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The Ministry of National Insurance reports that seven million new claims to sickness benefit have been made in the first year of the scheme that started on July 5, 1948—an average of 140,000 a week, about one half of which included claims for dependants. New claims rose to a peak of 220,000 during the week ending Feb. 22; the present rate is about 100,000 a week. About three-quarters of a million claims have been made for Industrial Injuries benefits of all kinds during the first year. Some 16,000 claims are made each week for injury benefit, and claims to disablement benefit are being received at the rate of nearly 6,000 a month. Claimants are being examined by 103 medical boarding centres to assess the degree of disablement, and 13 medical appeal tribunals hear appeals against assessments. Just under 200 claims a month have been received for the death benefit paid to the widow or to certain other dependants of a man who has died as a result of an industrial accident or disease.