REVIEW ARTICLE

THE HORMONES OF THE ANTERIOR LOBE OF THE PITUITARY GLAND

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Modern advances in our knowledge of the hormones of the anterior pituitary have been stimulated by the development of techniques for hypophysectomy by various people using various animals. In 1927, P. E. Smith[1] announced that rats could be conveniently hypophysectomised, and that this operation caused inhibition of growth and atrophy of the thyroid, adrenal cortex and gonads. All these effects could be reversed by the implantation of rat pituitaries. Much recent work has been based on this.

Great progress has been made in the fractionation of anterior pituitary extracts. Preparations of follicle stimulating hormone, luteinising hormone, prolactin, thyrotrophin, corticotrophin, and growth hormone have been almost completely freed of other forms of activity. It seems possible that all the effects of extracts of the anterior lobe may be due to these six substances, acting either independently of one another or together. Other hormones have been postulated with ketogenic, parathyrotropic, glycotropic, diabetogenic, pancreatrophic, and other effects, but it seems likely that the effects attributed to these hormones were really due to one or more of the above six substances.

The separation of the hormones has been achieved by fractional precipitation with organic solvents and inorganic salts, and by adjustment of the *p*H. A detailed account of this work has been given by Li and Evans[2]. Some of the main data are summarised in Table I. An extract prepared with 66 per cent. acid acetone contains prolactin and adrenocorticotrophic hormone (ACTH) and little of the other hormones. Prolactin is almost insoluble in salt-free water. Gonadotrophins from different species

TABLE I

Hormone	Follicle stimulating Hormone		Luteinising Hormone		Prolactin	Thyrotrophin	Adrenocorticotrophic Hormone	Growth
Animal	Sheep	Pig	Sheep	Pig	Sheep Ox	Ox	Sheep Pig	Ox
Molecular weight ...	70,000	—	40,000	100,000	26,500	10,000	20.000	44,250
Isoelectric *p*H	4·5	4·8	4·6	7·45	5·73	—	4·65	6·85
Per cent. saturation with $(NH_4)_2SO_4$ for precipitation	55-70	50-90	37-40	33-90	0	30-50	20	20-50
Solubility in 66 per cent. acid acetone	0	0	0	0	+	0	+	0
Sugar in molecule ...	+	+	+	+	0	+	0	0

appear to be different substances. In extracts of the pig's pituitary they can be separated by adjustment of the *p*H and in extracts of the sheep's pituitary they can be separated by precipitation with ammonium sulphate. Prolactin and adrenocorticotrophic hormone have seemed to have similar properties when obtained from different species, but the meaning of this fact is in doubt owing to recent evidence that the latter can be obtained in the form of quite small molecules.

GONADOTROPHINS

There are several different gonadotrophic substances, all of them probably glycoproteins, except luteotrophin which has been found to be identical with prolactin. It is important that these substances should not be confused with one another. Follicle stimulating hormone (FSH) usually denotes a substance obtained from the pituitary gland and should not be applied to the substance found in pregnant mare serum, although this also stimulates follicles. The two substances have quite different effects on male rats.

In the following list, follicle stimulating hormone, luteinising hormone (LH) and luteotrophin come from the pituitary, while human chorionic gonadotrophin (CG) and equine gonadotrophin (PMS) come from the placenta, and one or other of the last two is the main active constituent of most commercial gonadotrophins. The account of follicle stimulating hormone and luteinising hormone is based largely on that of Greep, van Dyke and Chow[3].

1. *The follicle stimulating hormone from the anterior pituitary* (*FSH, Thylakentrin*). In the female this stimulates the growth of follicles and increases the weight of the ovaries. If pure, it does not cause luteinisation directly. In intact animals luteinisation may occur eventually owing to the liberation of luteinising hormone from the animal's own pituitary. It used to be believed that follicle stimulating hormone caused the release of œstrogens from the ovaries, but there is evidence that pure preparations do not do this in hypophysectomised rats and that a small amount of luteinising hormone must be present for this effect to occur; pure follicle stimulating hormone thus has no effect on the uterus. In the male, it causes development of the seminiferous tubules, but not the release of androgens. The gonadotrophins in the urine in female castrates, or at the menopause or in male urine, are probably mainly follicle stimulating hormone mixed with a little luteinising hormone. There is no standard preparation. Highly purified preparations have been made from pig's pituitary by van Dyke and his colleagues[3] and from sheep's pituitary by Li, Evans and Simpson[4] in California.

2. *The luteinising hormone, or interstitial cell stimulating hormone of the anterior pituitary* (*LH, ICSH, Metakentrin*). This stimulates the interstitial cells in the gonads of either sex of rat. In the male, this causes the release of androgens with secondary effects upon other organs such as the prostate and seminal vesicles. In the female, the effect depends very much on the presence of follicles in the ovary. In young hypophysectomised female rats there are no follicles and, though the effect on the interstitial cells can be detected histologically, there is little or no

increase in the weight of the ovary and no release of œstrogens. If such rats are first treated with follicle stimulating hormone to form follicles, the injection of luteinising hormone causes ovulation, luteinisation, and the release of œstrogens. This is sometimes referred to as a synergistic effect. Follicle stimulating hormone and luteinising hormone both may increase the weight of the ovaries, but a proper combination of them both has most effect. Under the action of the œstrogens released there is a striking increase in the weight of the uterus. There is no standard preparation of luteinising hormone. The hormone has been isolated as a single homogeneous protein from pigs' pituitaries by Chow *et al.*[7], and from sheep's pituitaries by Li, Simpson and Evans[6]. These two proteins were quite different from one another (*cf.* Table I).

3. *Luteotrophin (Prolactin).* Corpora lutea formed under the action of the two preceding hormones do not secrete progesterone until stimulated to do so by a third substance present in extracts of the anterior pituitary. This substance has been called luteotrophin, but it seems to be identical with prolactin, which is discussed in a separate section[7,8].

4. *Human chorionic gonadotrophin (CG).* This is formed in the placenta and is present in blood, urine and placental extracts during pregnancy, or in other conditions when chorionic tissue is present, as it is in chorionepitheliomata and hydatidiform moles. Its actions on rats are similar to those of luteinising hormone. It is not the same substance as luteinising hormone as obtained from the pituitary of sheep or pigs, but might possibly be identical with human luteinising hormone which has not been much studied. In monkeys (rhesus) its action on the ovary is depressant, and in man it has little stimulant action unless it is combined with follicle stimulating hormone or equine gonadotrophin (PMS)[9]. Such a combination is sold as "synapoidin" and has been shown to produce corpora lutea and hæmorrhagic follicles in women, but human chorionic gonadotrophin has no clear use in therapeutics yet. The international standard preparation contains 10 units per mg. A preparation containing 8,500 IU per mg. was made by Katzman *et al*[10]. Claesson *et al*[11] claim to have isolated the pure hormone.

5. *Equine gonadotrophin (PMS).* This is formed in the equine placenta and obtained from pregnant mare serum. It differs from all the other gonadotrophins in the fact that it is not excreted in the urine. Its main effect on the ovary is like that of follicle stimulating hormone (stimulation of follicles), but large doses cause luteinisation even in hypophysectomised rats. Its main effect on the testes is more like those of luteinising hormone and human chorionic gonadotrophin (stimulation of the interstitial cells with the liberation of androgens). The international standard contains 4 units per mg. A preparation containing 12,000 to 13,500 I.U. per mg. has been made[12].

Assays[13]. Estimates of gonadotrophins in blood, urine and tissue extracts are sometimes required in physiological and clinical research. Estimates of human chorionic gonadotrophin and equine gonadotrophin are also of commercial importance. These last two hormones are sometimes present during pregnancy in such overwhelming amounts that

blood or urine can be used directly without extraction, but more often it is necessary to make an extract in order to reduce the bulk and remove toxic substances and other hormones which would interfere with the test. The methods used include precipitation with alcohol or acetone, adsorption on various reagents, salting out with sulphates, and precipitation with tannic acid[14]. One simple method which has been found satisfactory for human chorionic gonadotrophin and for the gonadotrophins in urine after the menopause, involves adsorption on kaolin as recommended by Scott[15] followed by precipitation with acetone[16,17]. The adsorption leaves most of the salts behind and the acetone removes œstrogens. A litre of urine can thus be concentrated to about 400 mg. of solid material containing all the gonadotrophin, and quantities corresponding to 200 ml. of urine or more injected into each animal.

Standard preparations are available for equine gonadotrophin and human chorionic gonadotrophin, but not for follicle stimulating hormone and luteinising hormone. It is therefore difficult to design really satisfactory assays of these, and most workers use animal units. It might perhaps be justifiable to use the standards for equine gonadotrophin and human chorionic gonadotrophin for the assay of follicle stimulating hormone and luteinising hormone respectively, using female rats. This would not necessarily give consistent results, since the active principles in the standard and unknown preparations would be different, but it might easily be more reliable than the use of animal units, which are notoriously variable.

The results of experiments on normal animals are liable to be complicated by the release of gonadotrophins from the animal's own pituitary. Results with hypophysectomised animals are easier to interpret, but even then the assay of mixtures of gonadotrophins is difficult because they often increase and may decrease one another's actions.

Assay of follicle stimulating hormone and luteinising hormone. According to Greep *et al.*[3] the ventral lobe of the prostate of young hypophysectomised male rats provides a test for luteinising hormone which is unaffected by the presence of follicle stimulating hormone in the solutions used. The hormone is injected for 4 days and the glands are then weighed. The specificity of this test appears to be unique. No other case is known in which the weight of an organ is increased by one of these hormones and not by the other. Greep *et al.*[3] use similar rats for the assay of follicle stimulating hormone, but assess the results by weighing the testes. This test is not quite specific, since luteinising hormone also increases the weight of the testes. Female rats give comparable results. Follicle stimulating hormone alone has a large effect on the weight of the ovaries and no effect on the weight of the uterus. Luteinising hormone has no effect on the weight of either ovary or uterus unless the animal has first been exposed to follicle stimulating hormone, but if follicles are first formed by the action of follicle stimulating hormone the effect of luteinising hormone is best shown by its enormous effect on the weight of the uterus.

The most popular way of estimating the gonadotrophins present in

uterine weight or on vaginal smears the result depends on the release of œstrogens from the animal's own ovary and is useless unless œstrogens have been completely excluded by suitable methods of extraction. The weight of the prostate of young rats provides a convenient and accurate method which is not affected by œstrogens and does not involve extraction[17]. Using the prostatic weight method a study of the chorionic gonadotrophin levels in blood and urine has been made in normal pregnancy, diabetic pregnancy and cases of pre-eclamptic toxæmia. The curve for the excretion in normal pregnancy has two distinct phases. In the first trimester very high figures, e.g., 20,000 to 40,000 I.U. per 24 hours are obtained, and there is great variation between individual patients. Toward the end of the first trimester the excretion falls and in the second and third trimesters the excretion remains in the range 4,000 to 11,000 I.U. per 24 hours ($P = 0{\cdot}99$). The mean value for this period of pregnancy was found to be 7,400 I.U. per 24 hours and the variation between individual patients was much less marked than in the first trimester[17].

In the second and third trimesters of pregnancy an excretion consistently above 11,000 I.U. per 24 hours must be regarded as pathological. This was found in a proportion of pregnant diabetics[23,24]. In cases of pre-eclamptic toxæmia, urinary and serum levels in mild and moderate cases were within normal limits but in severe and fulminating cases serum and urinary levels were often abnormally high. Cases of essential hypertension in pregnancy showed no abnormality in the levels either in blood or urine. Throughout these studies the concentration in the serum was shown to be quantitatively similar to the concentration in the urine. The mean renal clearance in normal pregnancy was found to be 0·95 ml./min. $\pm$·04 (standard error of the mean). Severe cases of pre-eclampsia and pregnant diabetic women had a significantly lower renal clearance than was found in normal women[19].

The administration of stilbœstrol to normal and diabetic women depresses the urinary excretion of chorionic gonadotrophin[24]. This depression, however, is evanescent and with continued therapy an "escape" phenomenon occurs, the excretion returning approximately to its original level. A similar effect with stilbœstrol has been noted on the serum concentration of chorionic gonadotrophin.

Assay of equine gonadotrophin. The most popular method of assaying this hormone is by its effect upon ovarian weight. Other methods depend upon vaginal smears, corpus luteum formation, the weight of the uterus or seminal vesicles, and ovulation[25,26].

PROLACTIN

It was first shown by Stricker and Grueter[27] that the anterior pituitary had some influence upon the mammary gland. These workers injected anterior lobe extracts into pseudopregnant rabbits, either normal or oophorectomised, on the 10th day of their pseudopregnancy and observed enlargement and development of the mammae followed by a profuse secretion of milk. They were unable to produce lactation in immature rabbits by the administration of pituitary extracts, and concluded that

morphological changes must occur in the gland before lactation will ensue. Since this pioneer work these observations have been extended to numerous other species[28,29], and it has also been shown that hypophysectomy performed during pregnancy will prevent lactation[30]. It has now been definitely confirmed that the anterior pituitary elaborates a substance known as prolactin, which is the essential factor in the initiation of lactation and is probably also important in its maintenance[31]. It exercises a direct effect on the mammary tissue as its actions are still produced in hypophysectomised, oophorectomised or adrenalectomised animals. Other names for prolactin are galactin or mammotrophin.

Actions of Prolactin. Normal lactation is affected by the pituitary, gonads, adrenal cortex and thyroid. The first step is the development of the mammary tissue during pregnancy, under the action of gradually increasing quantities of œstrogens and progesterone coming at first from the ovaries and later from the placenta. Although there is some species variation, it is now generally agreed that œstrogen acts mainly on the duct system and progesterone mainly on the lobulo-alveolar system. Œstrogens and progesterone together produce complete differentiation of the mammary tissue, but the actual flow of milk in the post-partum period is due to the liberation of prolactin from the anterior lobe of the pituitary. Prolactin itself cannot induce milk secretion unless the breast has been previously primed by œstrogens and progesterone. Nelson[32] believes that the suppression of lactation during pregnancy results from the high œstrogen titre present in body fluids, and stresses the inhibitory action of œstrogens on prolactin secretion. Meites and Turner[33], however, have recently questioned this view and suggest that œstrogens may actually stimulate prolactin secretion, and that during pregnancy progesterone inhibits this stimulant action of œstrogens.

Prolactin stimulates the crop glands of pigeons and doves, and this action is employed as a means of assaying the hormone[31]. In these birds rapid proliferation of the epithelial lining of the crop glands occurs; the epithelium becomes heaped up and shows numerous mitotic figures. In addition, prolactin increases the production of "crop milk," which is a caseous fluid consisting mainly of desquamated epithelial cells.

Prolactin undoubtedly has a stimulant action on mammary secretion, but there has been some controversy as to whether it causes actual growth of mammary tissue. Corner[28] produced both mammary growth and lactation in the rat by the administration of pituitary extract rich in prolactin. Using the delicate technique of intraduct injection Lyons[34] obtained evidence of hyperplastic changes in alveoli of oophorectomised virgin rabbits pretreated with œstrogen and progesterone. He concludes that in this species prolactin can actually cause tissue growth.

Prolactin has been used by Folley and Young[35] to increase the milk yields of lactating cows. These investigators believe that adrenocorticotrophic hormone helps to maintain milk production in combination with prolactin. Prolactin also stimulates maternal instincts in rats[31] and broodiness in hens. In pigeons it causes growth of the body as a whole, the most prominent effect being splanchnomegaly.

Evidence has recently accumulated that the gonadotrophic hormone, luteotrophin, is identical with prolactin. Luteotrophin stimulates and maintains the activity of corpora lutea and causes the secretion of progesterone. It inhibits the effect of œstrogens in producing cornification of the vagina in hypophysectomised rats and causes mucification of the vaginal mucosa instead. This has been made the basis of a method of assay[7]. Similar effects are produced by prolactin, and Cutuly[36,37] has shown that prolactin will maintain pregnancy in pregnant rats hypophysectomised on the 9th day of gestation. This effect is presumably due to its action on corpora lutea.

Assay. The most popular method of assay of lactogenic preparations depends on the increase in combined *weight of the crop glands* in pigeons[31]. Numerous investigators have called attention to the necessity for rigid standardisation of technique when this procedure is employed. The following factors have been found to influence the response—season of the year, body weight of the birds, strain and race of pigeon, environmental temperature, route of injection and volume of solution injected. In addition, all assays should be expressed in terms of the international standard of prolactin. Variants of this test are the *minimum stimulation method*[38] in which the crop gland is dissected out and merely examined against the light for a positive reaction, and the sensitive *local intradermal* or *micro method* introduced by Lyons and Page[39] in which the solutions under test are injected immediately over the crop gland. Prolactin may also be assayed by the induction of lactation in oophorectomised guinea-pigs[40] or rabbits, but these methods are less quantitative, more variable and less reliable than those employing pigeons.

This hormone may be assayed by the mucification reaction of the vaginal mucosa[7] or by the production of traumatic placentomata in the uteri of rats[8]. Prolactin is the only pituitary hormone for which there is an international standard preparation. By definition 1 unit = 0·1 mg. of this preparation.

Chemistry. Purified preparations of prolactin were first obtained by Lyons[41] and the hormone was isolated in pure form by Li *et al.*[42] Electrophoretic and solubility studies have shown that prolactin behaved like a pure protein. The molecular weight is estimated as 26,500 and the *iso*-electric *p*H is 5·5. The activity is 30 international units per mg.

Clinical Applications. Prolactin is at present of more value in veterinary than in human medicine. Most attempts to demonstrate it in the urine of lactating women have met with little success but Lyons and Page[39] using acetone precipitation to extract the urine claimed to have demonstrated prolactin in 8 cases using their intradermal method of assay. Using a similar assay method, Meites and Turner[43] extracted post-partum urine by alcohol precipitation and by dialysis followed by evaporation. Prolactin was demonstrated in all the cases studied. It was concluded that a relationship probably exists between the quantity of lactogenic hormone in the urine and the level of milk secretion. In the therapeutic field the evidence is conflicting. Winson[44] and Kenney and King[45] obtained encouraging results when prolactin was administered to puer-

peral cases. In the majority of women a significant increase in the milk yield was obtained. Stewart and Pratt[46], however, observed no effect on milk secretion even when large doses of prolactin were administered to lactating women.

THYROTROPHIN

In 1888, Rogowitsch[47] described enlargement of the pituitary after thyroidectomy. This was the first hint of a relation between the two glands. In 1914, Adler[48] found that the destruction of the hypophysis delayed the development of tadpoles and at about the same time Gudernatsch[49] found that the administration of thyroid accelerated their development. Allen[50] showed that the thyroids of the hypophysectomised tadpoles showed signs of inactivity and the Smiths[51] showed that these thyroids could be reactivated by the administration of anterior pituitary. This evidence of a stimulant action of the pituitary on the thyroid was soon extended to other species.

The first solutions of thyrotrophin were made by Loeb and Bassett[52]. The hormone can be extracted with water from fresh gland or acetone-dried gland and purified by fractionation with acetone or salts, or by adsorption. It is not precipitated by 8 per cent. trichloracetic acid and may have quite a low proportion of protein-like material. Since preparations have been made with very little effect in tests for any of the other five hormones discussed here, there is no doubt that thyrotrophin can be separated from these other substances. The preparation made by White and Ciereszko[53] was a white powder easily soluble in water, and homogeneous in the Tiselius apparatus and the ultracentrifuge with an apparent molecular weight of about 10,000. 1 μg. of this powder produced a histological response on the thyroid of a 3-day-old chick when given once per day for 5 days.

Under the action of thyrotrophin the thyroid discharges its colloid so that the iodine content of the gland falls, but the cells increase in height and multiply so that the weight of the gland increases. There is a great increase in mitotic figures[54] and in the oxygen consumption of the tissue[55,56]. This active tissue has great avidity for circulating iodine, as can be shown by the fact that, if radioactive iodine is injected, it accumulates in the thyroid which has been stimulated by thyrotrophin even more rapidly than in the normal thyroid. The hormone increases the turnover of iodine in the gland, which makes thyroxine more rapidly and liberates it into the circulation. This causes various secondary effects such as a rise in the metabolic rate and heart rate, increased sensitivity to oxygen, creatinuria and increased calcium excretion. When continued injections of thyrotrophin are given they lose their effect owing to the development of antihormones[57,58,59]. Thyrotrophin also causes exophthalmos, especially in guinea-pigs. This effect is not produced by thyroxine and cannot in any case be secondary to an action on the thyroid, since it occurs in animals which have been deprived of this gland[60,61].

The rate of liberation of thyrotrophin from the pituitary depends on the concentration of thyroxine in the blood. If this is reduced by drugs, such as thiouracil, which inhibit the formation of thyroxine, the rate of

liberation of thyrotrophin increases and the thyroid increases in size. This effect can be prevented by the injection of thyroxine, which inhibits the release of thyrotrophin[62].

Assay. Methods of assay of thyrotrophin are numerous and four main experimental animals have been used. These are the chick, the guinea-pig, the rat and the tadpole.

The thyroid of the 1-day-old chick is one of the most sensitive methods of assay available. Smelser[63] states that it is 10 times more sensitive than the thyroid of the guinea-pig. The end-point may depend on the increase in weight of the gland[64] or on the histological changes[65]. Rawson *et al.*[66] calculate the mean acinar cell heights expressing the results by a process analogous to the Price-Jones curve for red blood cells. Dorfman[67] uses the iodine content of the chick thyroid. This has the advantage of technical simplicity if radio-active iodine is employed.

The guinea-pig thyroid has also been used frequently for the assay of thyrotrophin. Loeb[68] based his observations on the "mitotic index," i.e., the increase in mitosis produced by the administration of thyrotrophin. Most other workers have used either histological signs of stimulation[69,70] or the resulting increase in weight[71,64]. Recently, De Robertis and Del Conte[72] have introduced a new and extremely sensitive method depending on the determination of the number of colloid droplets in the cells of the guinea-pig thyroid, the gland being prepared by a freez-drying procedure. This method is claimed to be several hundred times more sensitive than any other. De Robertis[73] has applied this method to the assay of thyrotrophic hormone in human blood.

Evans[74] employs histological changes in the thyroid of hypophysectomised female rats as an indication of the degree of thyroid stimulation. A similar method was used by Hertz and Oastler[75] in a study of thyrotrophin in clinical states. The original method of Collip and Anderson[59] depending on the increase in metabolic rate produced by thyrotrophic extracts in hypophysectomised rats is not now widely used. Dvoskin[76] found that the administration of extracts containing thyrotrophic activity caused the formation of intracellular colloid droplets in the thyroid epithelium of hypophysectomised rats but unfortunately, the specificity of this test was doubtful.

D'Angelo, Gordan and Charipper[77] have used the starved tadpole for the assay of thyrotrophin. The administration of graded doses of thyrotrophic extract induced metamorphosis characterised by progressive loss of body weight, increase in hind limb length and activation of the thyroid gland.

Clinical. Injections of thyrotrophin produce a condition which resembles Graves' disease not only in the secondary effects due to thyroxine, but also in the appearance of the thyroid gland itself, and perhaps also in the exophthalmos[61]. In Graves' disease the gland also shows increased avidity for iodine and this fact has been made the basis of various tests of thyroid function.

Several attempts have been made to demonstrate increased amounts of thyrotrophin in the blood or urine of cases of Graves' disease, but these attempts have generally been unsuccessful[65,75]. De Robertis[73], however,

used the very sensitive method of assay depending on the presence of colloid droplets in the cells of the guinea-pig thyroid and found evidence of thyrotrophin in the blood of some cases of Graves' disease and some cases of myxœdema. He interprets the results by assuming that in some cases the disease is primarily in the pituitary and in others primarily in the thyroid. These interesting results require further confirmation.

Adrenocorticotrophic Hormone

The hormone in pituitary extracts which stimulates the adrenal cortex is called adrenocorticotrophic hormone (ACTH) or corticotrophin. Proteins with high activity of this kind were prepared in 1943 by salt fractionation of sheep pituitary[78] and by *iso*-electric precipitation from hog pituitary[79]. Another method combining both techniques gave a higher yield[80]. These protein preparations had a molecular weight of about 20,000 and were similar to one another, but more active preparations with lower molecular weights have been made[81,82,83] and it seems probable that the protein acts as a carrier for an active molecule which is thought to be a polypeptide, containing 7 aminoacids or less. The commercial preparations available contain protein and are generally contaminated with posterior lobe hormones. Doses are commonly given in terms of mg. of Armour's standard preparation.

Actions. It acts primarily on the adrenal cortex causing a loss of ascorbic acid and cholesterol, an increase of the weight of the gland and histological changes, mainly in the zona fasciculata. The zona glomerulosa is said to be affected in the rat by changes in mineral metabolism but not by this hormone[84,85].

It also causes the release of steroids from the adrenals, and various compounds can be detected either chemically or biologically in the urine. These substances may be divided into three classes according to their effects.

(1) Substances such as desoxycorticosterone which have no oxygen in position 11 cause the retention of sodium and chlorine with initial loss of potassium and have little or no other known effects. Death following adrenalectomy is mainly due to loss of sodium, and it is therefore clear that this action is essential to life. The typical changes in the urinary excretion of minerals have been produced by preparations containing this hormone, and this fact suggests that it releases these substances of this type, but there is no direct evidence of this; the interpretation of the results is complicated by the presence of small amounts of posterior lobe hormones in the preparations used.

(2) Substances with oxygen in position 11 (" 11-oxysteroids ") have much less effect on mineral metabolism, but some of them have other effects. The presence of a hydroxyl group in position 17 seems to increase these effects, which are particularly marked in cortisone (Kendall's compound E). They cause a loss of protein and an increase of the carbohydrates in the body. There is a negative nitrogen balance and an increased loss of uric acid in the urine. The excretion of creatinine is not

changed and the effect can conveniently be followed by estimating the ratio of uric acid to creatinine in the urine. Protein is lost from lymphatic tissue which decreases in weight. Lymphocytes and eosinophils disappear from the circulating blood and are presumably destroyed; on the other hand, the neutrophil leucocytes increase in the blood[86]. The blood sugar rises, sugar tolerance falls and glycosuria may occur. These effects resemble diabetes, and these hormones are sometimes said to cause an insulin-resistant diabetes. On the other hand, they increase the storage of glycogen, and in this they are opposed to pancreatic diabetes. These effects are also produced by adrenocorticotrophic hormone which, therefore, releases steroids of this type. According to Conn *et al*[87,88], the amount of sugar in the urine after adrenocorticotrophic hormone may be so large that it is necessary to assume that the utilisation of sugar in the body is decreased; the amount of extra sugar that could be formed from protein would not be enough to account for the results. These same workers found that adrenocorticotrophic hormone caused a fall in the blood glutathione, and that the injection of glutathione diminished the glycosuria[89]. There is, at present, no evidence that the doses of adrenocorticotrophic hormone used clinically can cause permanent diabetes.

(3) Various androgens are found in cortical extracts. Some of the symptoms of Cushing's disease have been attributed to the release of these androgens, but there is no direct evidence that this occurs. Adrenocorticotrophic hormone has been found to cause a rise[91], and cortisone a fall[95,98], in urinary 17-ketosteroids, which probably consist largely of androgens. These facts support the theory that adrenocorticotrophic hormone causes the release of a 17-ketosteroid from the adrenals, or of some other substance (not cortisone) which is converted into a 17-ketosteroid in the body.

Assay. Most methods depend on observations of the rats' adrenals after hypophysectomy. This operation causes lipoids to disappear from the cortex. One test depends on the repair of this change. Another test depends on the maintenance of the weight of the rats' adrenals after hypophysectomy[2]. The most popular method depends on the estimation of ascorbic acid in the adrenals[90]. This test can estimate about 0·5 μg. of the standard preparation and detect adrenocorticotrophic hormone in the blood following an injection[91] or during Addison's disease[92].

Release. There is evidence that adrenaline stimulates the adrenal cortex[93] probably by causing the release of adrenocorticotrophic hormone from the pituitary. It is also released in various conditions of stress such as hæmorrhage, scalding, exposure to cold, etc.[94].

Clinical use. Various workers have described the actions on man[91,95,96,97]. A wide variety of diseases are being treated experimentally with it, but it is still too soon to assess its range of usefulness. The discovery of the dramatic effect of cortisone in producing complete, though temporary, relief of the symptoms of rheumatoid arthritis led to the trial of adrenocorticotrophic hormone in this condition[98], and there is no doubt that it is very effective when injected in a dose of 10 mg. equivalents of

standard hormone every 6 hours[97]. The effect is a dramatic one. Pain and stiffness disappear from the joints in a few hours, appetite improves and weight increases; there is definite evidence of healing in the synovial membranes, and a rapid fall in the sedimentation rate and a rise in the concentration of hæmoglobin in the blood. The daily dosage is less than that of cortisone, and there is some evidence that the factor of safety is larger. Similar results have been obtained in rheumatic fever[99]. Adrenocorticotrophic hormone is also used to test the functional state of the adrenal cortex. It causes various measurable effects due to the release of corticoids; if these effects do not occur the cortex must be defective and a diagnosis of Addison's disease may be made. In Thorn's test[96] a count of the eosinophils is made by a special technique. If this does not fall the cortex is abnormal.

Growth Hormone

From a study of growth abnormalities in man it has been known for many years that the anterior lobe of the pituitary exerts a profound influence on skeletal growth. In 1885, Pierre Marie[100] described the clinical condition of acromegaly. This disease is believed to result from the excessive elaboration of growth hormone during adult life and occurs usually in association with an eosinophil adenoma of the anterior lobe. A similar pituitary lesion occurring in pre-adult life before ossification is complete produces gigantism. Conversely, pituitary failure during childhood or adolescence results in various forms of dwarfism. In 1910, Crowe, Cushing and Homans[101] hypophysectomised dogs and found that the growth of these animals was retarded. In 1921 Evans and Long[102] produced experimental gigantism in rats after prolonged treatment with extracts of ox pituitary. Putnam, Benedict and Teel[103] observed gigantism, acromegaly and splanchomegaly in bulldogs after the administration of pituitary extracts and Smith[104] showed that hypophysectomised rats ceased to grow and that a resumption of growth could be obtained by giving anterior lobe extracts.

Action. The action of this hormone in skeletal growth is independent of other endrocrine glands in that it causes growth in the absence of the adrenals, thyroid or gonads[105,106]. It does not depend on the improvement of appetite[107]. It increases the weight of most organs, including the liver, kidneys and ovaries, but it has a particularly large effect in increasing the weight of the thymus[108,109]. These effects depend upon an increase in the amount of protein and water in the organs; the amount of fat is generally diminished. The formation of new protein thus seems to play a fundamental part in the response. As might have been expected from these facts, there is a decrease in the non-protein nitrogen in the blood and in the excretion of urea. Nitrogen is retained in the body and used for the synthesis of protein[108,110,111].

Growth hormone also increases the weight of the bones. This effect is accompanied by an increase in the concentration of inorganic phosphate in the plasma of men, dogs or rats[112]. There is a rise in alkaline phosphatase content of the bones[110] and of the plasma[113], but no change was

detected in the D-amino acid oxidase in liver or kidney, or in the succinic acid dehydrogenase in muscle[114]. These effects are best seen in young animals, but older animals can also respond, as is shown when acromegaly develops after growth has ceased. Becks *et al.*[115] found that the administration of purified growth hormone to hypophysectomised rats reawakened osteogenic and chondrogenic processes in the epiphyseal cartilage of the tibia even after a post-operative interval of a year or more. The changes produced were similar to those in the young normal growing rat.

Evidence is accumulating that the diabetogenic hormone of the anterior pituitary does not exist as a separate entity and that this effect is probably mediated by the growth hormone and the adrenocorticotrophic hormone[116]. Pure growth hormone, prepared by the method of Li *et al.*[117], has been found to be actively diabetogenic in adult intact cats. Young[109,118] believes that a close relation exists between the growth stimulating and diabetogenic actions of anterior lobe extracts. Prolonged administration of actively diabetogenic extracts to puppies initially causes acceleration of growth uncomplicated by diabetes mellitus. If, however, the treatment is continued for several months the animals cease to grow and a diabetic condition supervenes. Milman and Russell[119] administered pure growth hormone to fasting partially depancreatised or alloxan diabetic rats and observed pronounced hyperglycæmia. This effect was not evident in normal rats, which do not develop diabetes so easily as cats or dogs.

Chemistry. Li *et al.*[117] isolated the growth hormone in pure form from alkaline extracts of ox anterior pituitary. The hormone was found to be a protein of molecular weight 44,250. It was relatively insoluble in water. The *iso*electric *p*H was 6·85 and the hormone behaved as a homogeneous substance as shown by the results of experiments involving electrophoresis, diffusion and solubility. The hormonal activity was destroyed by treatment with pepsin and trypsin. Amino and tyrosine groups appeared necessary for the action of the growth hormone. The method used by Wilhelmi *et al.*[120], involved the use of alcohol precipitation at low temperatures, the method being similar to that used by Cohn in fractionating plasma proteins. The chief advantages of this technique are its relative ease and the very high yields of the hormone obtained. It was found, however, that the crystals so prepared exhibited two components on electrophoretic examination and therefore the preparation is less pure than that of Li *et al.*[117].

Assay. Three main methods of assay are available for the growth hormone[2].

1. *Body growth of normal rats.* Six-month-old female rats which have almost stopped growth are used. These animals can be made to grow by the administration of growth hormone. The chief disadvantage of this test is its relative insensitivity, and a long period of injections (up to 20 days) is required[121]. It seems however to be an accurate method[122].

2. *Body growth of hypophysectomised rats.* This test is more sensitive than the preceding. Young female rats 28 to 30 days at operation are used and injections are commenced 10 to 14 days after hypophysectomy.

Injections are continued for 10 days. When the weight gain is plotted against the logarithm of the dose a straight line is obtained.[123].

3. *Tibia of hypophysectomised rats.* In 1943, Evans, Simpson, Marx and Kilbrick[124] described a new method of assay of growth hormone depending on the increase in width of the proximal epiphyseal cartilage of the tibia in hypophysectomised rats. This test is claimed to be 3 times as sensitive as that depending on the body growth in hypophysectomised animals. In addition, injections are given for only 3 days. The technique is similar to that employed in the " line test " for vitamin D and if the width of the uncalcified cartilage is plotted against the logarithm of the dose a straight line is obtained. Kinsell *et al.*[125] detected growth hormone in acromegalic blood by this method.

SUMMARY

Six active substances are found in extracts of the anterior pituitary. All of them have been isolated as pure, or nearly pure, proteins. Much progress has been made recently in the development of sensitive methods of assay, and if all these methods are as good as they claim to be, it is now possible to estimate all these hormones in the blood or urine of patients when their concentrations are abnormally high. On the other hand, there are no standard preparations of these substances. Since the gonadotrophic proteins isolated from sheep are not the same as the gonadotrophic proteins isolated from pigs, there should perhaps be a set of standards for each species, but there is no evidence for this at present.

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