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Edited by

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PREFACE

THIS is a companion volume to the first series of *Modern Trends in Endocrinology* published in 1958, and in a way both supplementary and complementary. It was visualized at the time. Everyone interested in these matters knows only too well that it is almost impossible to keep up to date with the present rate and volume of research in this subject. Several contributors to the first series were only too conscious that new trends were developing in their particular field as the volume was in the press, but it cannot be avoided in such a rapidly advancing subject, and, at any rate, it is not the object of this series.

In this second volume, as in the first, will be found a collection of endocrine topics covering a wide and integrated field. They have been chosen to highlight the fronts on which advances of special interest seemed to have been or are being made. The selection has of course been arbitrary but one hopes it will have followed the general trend of opinion. It has also been the objective to fill in some of the gaps which were unavoidable in the first series owing to shortage of space, and in addition to deal with similar topics or even the same topic from a different angle. In fact, the objective of the second series has been the same as that in the first; to provide as comprehensive a picture as possible of the trends and salients on an advancing front rather than to produce a comprehensive book on Endocrinology. There will still be gaps, as there are bound to be, because we are only dealing with modern trends.

In the first series we dealt fairly fully with the thyroid and adrenal hormones. In this series we supplement the thyroid chapters with one on 'Auto-immunity in Thyroid Disease', a rapidly developing subject, and 'Thyrotrophic Hormone'. With regard to the adrenals, we now have a chapter on 'Primary and Secondary Aldosteronism'. More attention is paid to the pituitary in the present volume—in the first the anterior pituitary hormones were largely discussed in separate chapters dealing with the target glands. Now the problem of the anterior pituitary hormones and their correlation, and the site of their localization and production, is reviewed, and there is a study of the function and action of growth hormone. There are chapters too on the pathological results of trauma to the pituitary and therapeutic ablation. There are several other surveys dealing with therapeutics—surgery in Cushing's disease, oral hypoglycaemic agents, oral contraceptives and the anabolic steroids. In fact the list of advances has been brought as much up to date as possible.

To get a picture of the whole in the two series one might recapitulate the topics in the first volume; thyroid hormones, antithyroid drugs, exophthalmos, tests of thyroid function, treatment of thyroid conditions with radio-active iodine, endocrine factors in diabetes mellitus, and in over- and under-nutrition, pituitary antidiuretic hormone, hormones of the sympathetic nervous system and adrenal medulla, the new adrenal cortical steroids, pituitary-adrenal factors in water and

electrolyte control, and in hypopituitary coma, adrenal function tests, cortisone and corticotrophin in rheumatic and allergic disorders, breast development and milk secretion, cancer of the breast and prostate, endocrine treatment of gynaecological disorders, infertility in the female, carcinoid tumours and serotonin, and the stress concept in clinical medicine. In the two volumes, therefore, many modern trends in endocrinology have been reviewed.

The authors of the present chapters are again all experts in their particular fields, and they have adopted the same approach as before—to present the position to date and in perspective, with their own personal views and a tie-up of loose ends. At the same time, a critical evaluation of the trend of events has been aimed at with pointers to the future where possible.

I would like to take this opportunity of expressing my cordial thanks to all who have contributed to the present volume, and especially to Professor Prunty who was so helpful in the selection of such a distinguished team. I would like to express too my thanks to Butterworths for taking such infinite trouble with the preparation of these volumes.

H. GARDINER-HILL

London
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CHAPTER 1

THE HORMONES OF THE ANTERIOR PITUITARY AND THEIR CORRELATION

T. SYMINGTON

INTRODUCTION

IN this chapter it is proposed to draw attention to the problems of extraction and assay of hormones and to assess the results as they reflect the hormone content of the pituitary gland, urine and blood in health and disease. Such problems will be discussed as they relate to the anterior pituitary hormones in the following order: gonadotrophin, adrenocorticotrophin, growth hormone, thyrotrophin and prolactin. Likewise, an attempt is made to review existing knowledge of the site of localization of these hormones in the cells of the anterior pituitary and the effect of recent experimental findings and histochemical techniques on our concepts of the site of hormone production in the cells.

Hormone assay

A hormone assay may be either chemical or biological and, though the former may be more precise, less laborious and less expensive, the latter is more specific and more sensitive. Most hormone assays originate as biological investigations but ultimately chemical procedures are developed. The catechol amine content of the adrenal medulla or the urinary catechol amines in phaeochromocytoma may be measured biologically but the fluorimetric and chromatographic methods now available are more satisfactory. Reliable chemical assays now replace the old bioassay methods for adrenal steroid hormones. Thus the ultimate aim in hormone investigations must be towards chemical assays but at present this is not possible with pituitary hormones that are assayed by biological means.

The essential requirements of a hormone assay are that the method should be reliable and practical. The ideal bioassay method should be highly specific for the hormone, extremely sensitive and with a high degree of precision and accuracy. Unfortunately very few assays fulfil these criteria. Many factors are encountered that determine whether or not a method is applicable to biological fluids such as blood or urine. Techniques may be satisfactory for the assay of the high concentrations of hormone in the pituitary, yet quite unsatisfactory for the much lower levels found in blood or urine. Strains of animals are important, for example, the urine of normal menstruating and postmenopausal women will cause hyperplasia of mammary tissue in one strain of weanling male mice and not in others. Different extraction procedures for ACTH in blood give different results even when the same assay method is used. Until recently, results were expressed in terms of animal units, but now attempts are being made to establish national and

possibly international standards so that the results of different laboratories can be more easily compared and assessed.

Perhaps the greatest stumbling-block to our understanding of the role of the anterior pituitary hormones in health and disease has been a lack of adequate techniques to measure the hormone content of the pituitary and of biological fluids, and the fact that each year new methods of extraction and assay have been described.

PITUITARY GONADOTROPHIN (HPG)

The term HPG is used to denote human pituitary gonadotrophins and it has been assumed by many workers that two gonadotrophins are formed by the human pituitary, one with follicle-stimulating activity (FSH) and the second causing luteinization (ICSH). This assumption is based largely on animal observations. ICSH (interstitial cell-stimulating hormone) has been isolated from the pituitary glands of sheep and pigs and in each case the hormone was shown to be a glycoprotein. Likewise, a highly purified preparation of FSH, also a glycoprotein, was isolated from these sources. So far, however, no such separation has been possible with gonadotrophins of human pituitaries.

Albert (1960), using his method of biological characterization referred to as a system of 'biological fingerprinting of gonadotrophins', suggested that HPG exists in the pituitary gland in the same form in men and women less than 50 years of age, is secreted into the blood in the same state but considerably modified in the urine. In addition the HPG prepared from the urine of men and women (young, old and castrate) is different from other well-known gonadotrophins, such as urinary human chorionic gonadotrophin (HCG), sheep follicle-stimulating hormone (FSH), luteinizing hormone (LH) and pregnant mare serum gonadotrophins (PMS) (Albert, Kelly and Kobi, 1958; Albert and Kelly, 1958).

Assay for pituitary gonadotrophin in urine

Assay measuring total gonadotrophic activity

No method is sufficiently sensitive for the blood assay of gonadotrophin and most of the available information has accrued from urinary investigations. Nevertheless, it is in this field that considerable confusion and differences in opinion exist. Some workers (Loraine, 1958; Albert, 1960) believe that for clinical purposes urinary gonadotrophins should be extracted from a 24- or 48-hour specimen of urine by the kaolin–acetone (KA) method and assayed by the mouse uterine weight method. This gives a measure of total urinary gonadotrophic activity and is generally believed to measure the combined effect of FSH and ICSH. It is a method that will measure total urinary gonadotrophic activity whether the hormone in urine is a single one with FSH and ICSH activity or more than one hormone with separate activities.

Early investigators in the field, using rats or mice for their assay, expressed the results as animal units, the unit being the quantity required to produce a given effect. The expression of biological assays in terms of animal units is unsatisfactory owing to variations in animal strains and the sensitivity of different strains. Again, it is difficult to compare results from different laboratories in different countries

when they are expressed in terms of animal units. The establishment in Great Britain of a standard urinary gonadotrophin preparation, HMG-20A, is a significant advance.

Assay methods for FSH and ICSH

Within recent years attempts have been made to develop urinary gonadotrophin methods that are more specific for FSH and ICSH and that estimate one or other of the two hormones. The assay for FSH used by Crooke and his co-workers employs the augmentation-principle developed by Steelman and Pohley (1953) for rats and by Brown (1955) for mice. Immature female mice injected with 40 i.u. of a preparation of HCG show a slight increase in ovarian weight. No additional effect is seen when doses of up to 1,000 i.u. of HCG are administered. When FSH or the unknown containing FSH is injected along with the HCG, a pronounced increase in ovarian weight results. Originally the urine was extracted by the kaolin adsorption method but more recently Butt, Crooke and Cunningham (1959) have found a more satisfactory extraction when benzoic acid–tungstic acid is used. The authors claimed that the new method can be assayed satisfactorily against standards prepared by the kaolin method since there is no departure from parallelism in the log-dosage response lines. This is contrary to the views of the group (Benz and his colleagues, 1959) who concluded that, 'for the assay of pituitary hormones in urine the method of preparation of extracts should be similar to that of the standard.'

The assay involving an increase in the ventral prostate in hypophysectomized immature rats (Loraine and Brown, 1954) is considered specific for ICSH and not affected by FSH. It is sufficiently sensitive to measure ICSH in normal and pathological conditions but is too laborious to be of value for routine clinical studies.

Problems associated with urinary gonadotrophins

It is obvious that there are many problems surrounding urinary gonadotrophins. Does the hormone exist in urine as a single substance with FSH and ICSH activity or are there separate hormones? If the hormone is a single substance, then the mouse uterine test, which is an indication of combined FSH and ICSH activity, must be considered as a satisfactory method for urinary gonadotrophin assay. In Great Britain the value of this test has been enhanced by the establishment of the national standard (HMG-20A) so that the results from different laboratories can be expressed in terms of this standard. Clearly the aim must be the establishment of an international standard and in this respect some progress is being made. In America a standard preparation (AMW) has been prepared by Albert (1956) and assayed against HMG-20A (Albert and his colleagues, 1958). Both standards have been prepared by the kaolin–acetone method, but tricalcium phosphate procedure has been excluded from the American standard. Again, whereas HMG-20A was prepared from postmenopausal women, AMW came from normal men. HMG-J was prepared on the Continent from postmenopausal urine, using a method depending on zeolite adsorption. A variety of bioassay methods having been used, the activity of HMG-20A has been compared on the one hand with the American preparation (AMW) and more recently with HMG-J, and though some progress has been made towards the establishment of a truly international standard, the conclusion reached was that in the assay of pituitary hormones in urine the method

of preparation of extracts should be similar to that of the standard. Much work is being done on methods of extraction of urinary gonadotrophin hormones and further advances can be expected in this respect. In summary, many workers assess urinary gonadotrophic activity in terms of both FSH and ICSH activity as assayed by the mouse uterine test, but Crooke and his colleagues have attempted to separate the gonadotrophic activity of urine and believe that whereas the mouse uterus test estimates FSH and ICSH activity, FSH only is estimated when the mouse ovarian test with HCG priming is used. Their work has been extended to include separation of FSH from HCG in the urine of pregnant women by using benzoic acid followed by kaolin extraction (Crooke and his colleagues, 1958).

In view of the difficulty of deciding whether urinary gonadotrophin is a single substance with FSH and ICSH activity or separate hormones that should be assayed separately, as well as the problems associated with the establishment of an international standard, it would be quite unwise to try to give normal and abnormal values for primary gonadotrophins.

The assay has an obvious place in the investigation of primary amenorrhoea in the young female. High urinary values would indicate primary ovarian dysfunction; low values would point to hypopituitarism. In the adult female the assay has a place in investigating the problems of the menopause and is of value in differentiating pituitary failure from anorexia nervosa. Likewise, estimation of urinary gonadotrophins is indicated in the cases of precocity and positive results are to be expected in the rare conditions of cerebral tumours causing isosexual precocity.

In conclusion, the mouse uterine test with kaolin–acetone extraction, which appears to measure combined FSH and ICSH activity, should be used for ordinary clinical investigation. Until established extraction methods for FSH and ICSH are found, assays for the separate hormones (FSH and ICSH) will remain a research problem, although offering most hope for our future understanding of the balance of these hormones in urine in pathological conditions.

ADRENOCORTICOTROPHIC HORMONE (CORTICOTROPHIN OR ACTH)

Since Li, Evans and Simpson (1943) and Sayers, White and Long (1943) extracted ACTH from sheep and ox pituitaries and believed them to be pure proteins, a tremendous amount of research has been done on this subject. ACTH obtained from concentrates of pituitary glands has several active components and the structure of one of these from pig pituitaries has been studied by Shepherd and his colleagues (1956). This is a polypeptide containing 39 amino acids. There is a slight difference in the amino acid arrangement in the sheep and ox hormone. The amino acid analysis of the human hormone appears to be identical to that of ox and sheep, although the sequence has not been completely determined (Lee, Lerner and Buettner-Janusch, 1959). This 39 amino acid polypeptide is the main active component of the present commerical preparations of corticotrophin prepared by the oxycellulose method. These contain other pituitary substances, however, but their function is not at present clear and the term 'corticotrophins' is used to denote adrenal-stimulating polypeptide preparations obtained by this method.

In 1950 the first international standard for ACTH was established by the World Health Organization when the material used for the standard was a relatively crude

preparation (LA-1-A). The international unit was defined as the activity contained in 1 mg of the international standard and was used until 1953 to assay crude ACTH preparations. Since the introduction of highly purified oxycellulose preparations no new international standard has been established and individual manufacturers have prepared their own oxycellulose purified standards, which have been assayed against the present international standard ACTH(2). It is hoped that a new international standard prepared from oxycellulose purified corticotrophin will soon be available and will be used by all manufacturing laboratories.

Numerous assay methods for corticotrophin have been devised. The original technique of Sayers, Sayers and Woodbury (1948) is based on the depletion of ascorbic acid from the adrenal gland of a hypophysectomized rat. This is a very reliable and practical method when carried out in a careful manner. It can measure 0·25 milliunits of ACTH. Modifications have been devised in which hydrocortisone pre-treatment is used instead of hypophysectomy. The strain of animal seems to be important in this test. Estimation of the ascorbic acid content of the adrenal effluent of the hypophysectomized rat after ACTH has been described (Munson and Toepel, 1958). This method is very sensitive but not so practical as the Sayers assay. Other assays depending on the production of corticosteroids have been used and are more direct. The 17-hydroxycorticosteroid content of the adrenal vein of a hypophysectomized dog (Nelson and Hume, 1955), the corticosterone output in the adrenal vein of the hypophysectomized rat (Lipscomb and Nelson, 1959) and the assay based on the corticosteroid production of excised rat adrenals (Saffran and Schally, 1955) are all satisfactory methods.

Corticotrophin levels in body fluids

Most investigations on gonadotrophins were concerned with the content of this hormone in urine, since assay methods for blood are not at present satisfactory. On the other hand, renal excretion is of little importance as a method of excretion of ACTH and it is doubtful if urinary ACTH analysis is of value. Accordingly, most biological investigations for ACTH have been concerned with the content of this hormone in blood. The results have varied greatly. When whole serum or plasma from a normal individual in doses of 10–30 ml is injected directly into the assay animal, no detectable ACTH activity is found using the ascorbic depletion method (Taylor, Albert and Sprague, 1949) or that involving the steroid content in the adrenal vein of the dog (Bethune, Nelson and Thorn, 1957; Nelson, 1960). When blood from a normal individual was collected in acetic acid, extracted with oxycellulose and the concentration equivalent to 40 ml of blood administered to a rat, no significant depletion of ascorbic acid was found (Paris and his colleagues, 1954). These workers, like Sydnor and his colleagues (1953), concluded that the concentration of ACTH in the blood of normal subjects was less than 0·5 milliunits per 100 ml blood. Davies, Currie and Symington (1960), however, starting with 1 l. of blood, which was collected directly into glacial acetic acid, extracted with oxycellulose and assayed by the adrenal ascorbic acid method, found the ACTH content of normal subjects to be 0·75 milliunits per 100 ml. When the injection material was prepared by acid–acetone extraction and assayed by ascorbic acid depletion (Bornstein and Trewhella, 1950; Montanari and his colleagues, 1951; Gray and Parrott, 1953), extremely high figures (60–197 milliunits per 100 ml) were recorded. The reason for this discrepancy is not obvious but points once

more to the very variable results that follow different methods of hormone extraction.

Corticotrophin in blood in pathological conditions

A rise in the level of blood ACTH has been found in certain pathological conditions. In most cases, but not invariably, the level in Addison's disease is high (8–40 milliunits per 100 ml) and, though it is reasonable to expect values higher than normal in conditions of stress, this is not always so. Sydnor and his colleagues (1953) found raised levels in the adrenogenital syndrome due to congenital adrenal hyperplasia. Though this assay may be a useful diagnostic test in Addison's disease, there is no justification for removing the large volume of blood required for the test in conditions of stress or congenital adrenal hyperplasia. In Cushing's syndrome on the other hand, where the patient is hypertensive, the withdrawal of 1,000 ml of blood can be regarded as a therapeutic measure. Davies, Currie and Symington (1960) found the circulating level of ACTH in two patients with Cushing's syndrome to be in the region of 2 milliunits per 100 ml. Other workers (Taylor, Albert and Sprague, 1949; Paris and his colleagues, 1954) using the oxycellulose method, but with much smaller quantities of blood, did not detect ACTH in the blood in this condition. The results of Davies, Currie and Symington (1960), that the blood ACTH in Cushing's syndrome is approximately twice normal, would explain the histological finding of a broad zona reticularis in the adrenal gland; it would also explain the abnormal sensitivity of the Cushing's patient to the infusion of normal amounts of ACTH and the higher-than-normal content of 11β-hydroxylating enzyme (Symington and his colleagues, 1958). The finding of high plasma ACTH levels of 30–400 milliunits per 100 ml in two patients who had been adrenalectomized previously for Cushing's syndrome is interesting (Nelson, Meakin and Dealy, 1958; Rees and Bayliss, 1959). Chromophobe adenomas were present in two patients who had extreme pigmentation associated with the development of the pituitary tumours. The finding is interesting in view of Furth's experimental ACTH tumours and will be discussed later.

Corticotrophin in human pituitaries

Taylor, Loraine and Robertson (1953) found the ACTH concentration in lyophilized pituitary tissue from adults, infants and foetuses expressed in terms of dried weight to range from 49 to 588 i.u. per g. Hewett, Cruickshank and Currie (1954) analysed the ACTH content of 18 human pituitaries and found 205 i.u. per g of dried powder. Though we speak of ACTH as if it were a single substance, there is evidence that it exists in the pituitary in four or five forms, and Davies and Currie (1960), on the basis of their results with 2·5 per cent trichloracetic acid treatment of human pituitaries, believe that some is protein-bound and some present as a free polypeptide. It would be interesting to speculate that perhaps the polypeptide is available immediately on demand whereas the protein-bound represents the storage form. It is clear that much work remains to be done in this field, particularly on the effect of stress on the content and nature of ACTH in the human pituitary.

Corticotrophin and melanocyte-stimulating hormone (MSH)

The development of pigmentation of the skin in some cases of Addison's disease

and in patients treated with crude preparations of ACTH has stimulated a number of investigations into the relationship of the two hormones. Though skin and hair pigmentation sometimes follows administration of crude ACTH, this is rarely if ever seen with the new oxycellulose preparations. A study of the composition of ACTH compared with that of a α-MSH in pig is shown below.

	1 2	13 14 39
ACTH	Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.Gly.Lys.Pro.Val.Gly. . .	PheOH
α-MSH	CH_3.CO.Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.Gly.Lys.Pro.Val.NH_2	

The initial structure of the first 13 amino acids in ACTH and α-MSH is the same, but α-MSH has an acetyl group attached to serine, whereas ACTH has an additional 26 amino acids. Nevertheless, the MSH activity of ACTH is $\frac{1}{250}$ of that of α-MSH. Protein chemists have added a CH_3 CO grouping to the serine of ACTH and this has enhanced the MSH activity of ACTH only five times. It is obvious that the long chain of 26 amino acids plays some role in diminishing the MSH activity. Though MSH hormones may have little role in the body economy, observations on their structure may yet throw a great deal of light on the part played by the different amino acid groups or their structural configuration on the mechanism of action of ACTH.

Though much has been learned about corticotrophin, many problems still remain. Although the ascorbic depletion and different steroid assays mentioned do not reflect the same adrenal activity, it can be said that both indicate steroidogenic activity of the hormone. Since a number of active fractions exist in the pituitary, the question is whether they are all steroidogenic, or whether some are adrenal weight factors (Stack-Dunne and Young, 1951). It may be that the different biological activities can be explained on the different rates of absorption of ACTH from the site of injection (Astwood, Raben and Payne, 1952). There is no doubt that different adrenal effects result from single intravenous injections of ACTH compared with prolonged administration of the hormone. If 75 units oxycellulose purified ACTH are given as a continuous intravenous transfusion to a patient during the operation for adrenalectomy and the adrenal vein cannulated, the cortisol–corticosterone ratio remains at 1–2:1 and there is no alteration in the activity of the dehydrogenase enzymes (glucose-6-phosphate, 6 phosphogluconic acid). If, however, the patient has been given intramuscular injections of oxycellulose ACTH gel, 75 units daily for three days before the operation, and a similar dose of soluble ACTH is infused intravenously during the operation, the cortisol–corticosterone ratio in the adrenal effluent rises to 3–10:1, the ribonucleic acid content of the adrenal cortex increases markedly and there is a significant increase in the dehydrogenase enzymes mentioned above (Studzinski, 1960). Since similar changes occur in the adrenal cortex in conditions of stress, it is possible that the initial changes described above are due to a sudden release of ACTH and later changes the result of a slower, more continuous release of hormone from the pituitary. On the other hand, the changes may be the result of different constituents of the hormone having different biological properties. Investigation of the effect of crude and purified fractions of ACTH are being undertaken at present to clarify this point.

It is well established (Symington and his colleagues, 1958) that the adrenal gland in Cushing's syndrome may be 'normal' in size or markedly hyperplastic. If

this difference is due to the presence of an adrenal growth factor in the pituitary glands of Cushing patients with hyperplastic glands and its absence from those pituitaries where the adrenals are 'normal', then the assays based on ascorbic depletion or steroidogenesis will not detect the difference. In view of these remarks it becomes obvious now that any observations made on the balance of ACTH may have to be revised in the light of further work.

GROWTH HORMONE (SOMATOTROPHIN OR SOMATOTROPHIC HORMONE (STH))

Growth hormone has been prepared from ox pituitary glands and is protein in nature. A large number of assay methods has been devised, such as the increase in weight or increase in tail-length of hypophysectomized rats. Until recently the most sensitive method was the increase in width of the proximal epiphyseal cartilage of tibia in hypophysectomized rats. The high degree of specificity claimed for this method has been questioned, since it was noted that thyroid hormones, TSH and ACTH all interfere with the response. Accordingly, a true test animal should be hypophysectomized, adrenalectomized and thyroidectomized. Such methods have not detected growth-promoting activity in urine in normal or pathological conditions, but, by using the tibial epiphysis test, such activity has been detected in the plasma in cases of gigantism.

Read and Stone (1958) have described an immunological assay for human growth hormone in serum; it is a haemagglutinin method based on the tanned red-cell technique. Antibodies to human growth hormone have been prepared with relatively small total doses of growth hormone and since a titre of about $\frac{1}{1400}$ has been obtained, this considerably lessens the amount of serum required for the assay. The standard hormone preparation used was Raben's (Homo No. 6) growth hormone preparation (Read, 1960). The reliability of an immunological assay for any type of pituitary hormone will depend on the specificity of the anti-serum and so far no non-specific reactions have been recorded for growth hormone. This is surprising in view of the observations by Cruickshank and Currie (1958) that anti-serum to ACTH would react with TSH and HMG when the gel-diffusion technique was used.

The results obtained by Read and his co-workers are, however, very encouraging and, although Read stated that the data are inadequate to establish normal values for either adults or children, significant differences occur in normal adults, in children, in hypopituitarism and in hyperpituitarism (Read, 1960). If the reaction is found to be as specific as it appears, a wide field of clinical investigation will be opened. The relationship of growth hormone to diabetogenic hormone is intriguing. Does the diabetogenic hormone really exist as such or is the effect mediated by growth hormone or ACTH or a mixture of both (Young, 1953)? What is the level of growth hormone in diabetes mellitus, and in pregnancy complicated by diabetes? These are only some of the many problems that can be tackled if this immunological assay proves to be specific, but care must be taken to ensure that this is so and that the anti-serum to growth hormone will not react with the other pituitary hormones.