# PROLACTIN STATUS IN HEALTH AND DISEASE

by

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# TABLE OF CONTENTS

## VOLUME I

## ABBREVIATIONS

| | |
|---|---|
| ACTH | adrenocorticotrophin |
| BRCR | bromocriptine |
| BSA | bovine serum albumin |
| cf | compared to |
| cpm | counts per minute |
| CV | coefficient of variation |
| DA | dopamine |
| DHAS | dehydroepiandrosterone sulphate |
| $dH_2O$ | distilled water |
| DHT | dihydrotestosterone |
| eg | for example |
| F | females |
| FSH | follicle stimulating hormone |
| GH | growth hormone |
| GnRH | gonadotrophin releasing hormone |
| h | human |
| $^3H$ | tritium |
| hCG | human chorionic gonadotrophin |
| $H_2O_2$ | hydrogen peroxide |
| hPL | human placental lactogen |
| 5-HT | 5-hydroxytryptamine |
| kg | kilogram |
| l | litre |
| LH | luteinising hormone |
| LPO | lactoperoxidase |

| | |
|---|---|
| m | monkey |
| M | males |
| M | molar solution |
| mCi | milli curie |
| MCP | metoclopramide |
| ml | milli litre |
| mm | milli metre |
| mRNA | messenger ribonucleic acid |
| MSH | melanocyte stimulating hormone |
| mU | milli units |
| nmol | nano moles |
| o | ovine |
| $OE_2$ | oestradiol |
| p | porcine |
| PBS | phosphate buffered saline |
| PIF | prolactin inhibiting factor |
| pmol | picomoles |
| PRF | prolactin releasing factor |
| PRL | prolactin |
| PTH | parathormone |
| r | rat |
| rpm | revolutions per minute |
| RIA | radioimmunoassay |

| | |
|---|---|
| SA | specific activity |
| SD | standard deviation |
| SEM | standard error of mean |
| T | testosterone |
| TRH | thyrotrophin releasing hormone |
| TSH | thyroid stimulating hormone |
| U | units |
| µg | microgramme |
| µl | micro litre |
| < | less than |
| > | greater than |

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## DECLARATION

The work described in this thesis was performed in Glasgow Royal Infirmary, both in the wards and out-patient clinics of the University Department of Medicine and in the laboratories of the Radioimmunoassay Unit, Department of Biochemistry, from January 1977 to July 1979. The detailed planning of the work and its clinical and laboratory execution were performed entirely by the author unless specifically stated within the text or acknowledgements.

Some of the work described in this thesis has already been published:-

(1) Cowden, E.A., Ratcliffe, W.A., Ratcliffe, J.G., Dobbie, J.W. and Kennedy, A.C. (1978) Hyperprolactinaemia in renal disease. Clinical Endocrinology, 9: 241-248.

(2) Cowden, E.A., Ratcliffe, W.A., Beastall, G.H., Ratcliffe, J.G. (1979) The laboratory assessment of prolactin status. Annals of Clinical Biochemistry, 16: 113-121.

(3) Cowden, E.A., Ratcliffe, J.G., Thomson, J.A., Macpherson, P., Doyle, D., Teasdale, G.M. (1979) Tests of prolactin secretion in diagnosis of prolactinomas. Lancet, 1: 1155-1158.

Copies of these papers can be found inside the pocket in the back cover of the thesis (Volume 1).

The following papers based on work in this thesis have been presented personally by the author:-

(1) Society for Endocrinology (London) November 1977.
Hyperprolactinaemia in renal disease.

(2) The Renal Association (London) February 1978.
Association of hyperprolactinaemia and renal disease in man.

(3) The Institute of Neurological Sciences Research Workshop (Glasgow) March 1978.
Prolactin secreting tumours of the pituitary.

(4) Society for Endocrinology (London) May 1978.
Abnormal hypothalamic pituitary function in uraemia.

(5) VIIth International Congress of Nephrology (Montreal) June 1978.
Hypothalamic pituitary function in uraemia.

(6) Nephrology Division, University of California (San Diego) July 1978.
Prolactin and the kidney: a review.

(7) XIIth Acta Endocrinologica Congress (Munich) June 1979.
The role of dynamic tests of prolactin secretion in diagnosis of prolactinomas.

## PREFACE

The work presented in this thesis was performed from January 1977 to July 1979, during twelve months of which it was a full time commitment - a situation made possible by a generous grant from the National Kidney Research Fund. Moreover much of the initial motivation to develop an interest in prolactin was as a result of studies performed by Dr. J.W. Dobbie, Senior Lecturer in Medicine, within the University Department of Medicine in the Royal Infirmary.

In the course of an ultrastructural reconstruction of the fish glomerulus, Dr. Dobbie observed a phenomenon, in young rainbow trout and smolting salmon, which he termed "occlusive glomerular hyperplasia" (Dobbie, Moss and Kennedy, 1977). In these fish 40-50% of the glomeruli showed hypercellularity involving endothelial, mesangial and epithelial cells which resulted in occlusion of capillary and urinary spaces (Fig. 1). These changes were not apparent in fresh water fish (Fig. 2). In many species this process of smolting is controlled by prolactin (Lam, 1972) and Dr. Dobbie concluded that in these creatures smolting involved a state of reversible glomerular cellularity, the instigating hormone being prolactin.

Since proliferative glomerulonephritis is one of the most potent causes of irreversible renal failure in man, it was with interest that Dr. Dobbie noted the considerable similarity in morphological appearance of glomeruli in salt water fish and those of patients with severe proliferative glomerulonephritis (Fig. 3). It was tempting to speculate that prolactin might have some relevance in the control of cellular constituents in the human glomerulus.

Concurrent with these studies, an adolescent girl was admitted to the Renal Unit of the Royal Infirmary (Fig. 4) with rapidly progressive, crescentic glomerulonephritis. Renal biopsy revealed that 100% of glomeruli were severely affected, with crescent formation - diagnostic of "irreversible" renal damage. The child was initially treated by intermittent peritoneal dialysis but as expected her kidneys showed no sign of recovery; she was anuric and uraemic. Active therapy was discontinued but she was given chlorpromazine therapy as a sedative to ensure her comfort. However, this drug caused considerable enlargement and engorgement of her breasts and the girl began to lactate. Some days thereafter, the patient began to pass urine spontaneously, her blood urea fell and her general condition improved.

The fortuitous presentation of this patient at a time when the morphological aspects of smolting were first described suggested that it might be of practical importance to attempt to define prolactin status in human renal disease.

Thus, this thesis describes the setting up and validation of a radioimmunoassay for human prolactin and its application in the assessment of prolactin status in normal individuals and in patients with diverse pathological conditions. Particular experience and expertise has been accumulated in the diagnosis and treatment of prolactin secreting tumours of the pituitary gland and in the relationship between serum prolactin levels and renal disease in man.

## SUMMARY

Human prolactin was isolated in 1970 and thereafter sensitive, specific radioimmunoassays were developed for its measurement. This has enabled progress in understanding some of the factors which control serum prolactin concentrations in man; previously unrecognised prolactin secreting pituitary tumours may be diagnosed and the concept of a non-lactogenic role for human prolactin has emerged.

In this thesis, Chapter 1 presents a review of selected aspects of human prolactin pathophysiology. It is divided into sections and begins with an account of the evidence for the separate existence of human prolactin and a description of its isolation. The lactotroph is described and there follows a detailed consideration of the physiological control of prolactin secretion. Selected clinical aspects of prolactin secretion are discussed together with examples of the various investigative approaches used to identify a non-lactogenic role for human prolactin. The chapter ends with a statement of the aims of the thesis.

Chapter II commences with a discussion of the several techniques available for the measurement of prolactin concentration and includes an outline of the

principle of radioimmunoassay. A description of the prolactin radioimmunoassay used in the present studies follows, and includes a comparison of available reagents, details of the separation technique and method of calculation and analysis of results. Data on the specificity, accuracy of recovery and precision of the assay are provided together with a description of serum effects, parallelism studies and quality control data. The chapter concludes with a discussion of the advantages in the assay system adopted.

Chapter III describes a study to assess the importance of some factors which relate to interpretation of prolactin status in health and disease. Detailed reference ranges of basal prolactin levels were determined in 921 subjects in defined groups, including normal children, adults, pregnant females, patients on medication known to affect prolactin status, including the oral contraceptive and patients with a variety of pathological conditions. Assessment of the incidence of stress induced hyperprolactinaemia was made together with a measure of *intra* and *inter diem* variation in circulating prolactin levels. Prolactin response to defined stimulation and suppression tests was determined both in males and females and finally, based on this data, a

strategy to facilitate the laboratory assessment of prolactin status is presented.

A brief historical review of the "amenorrhoea-galactorrhoea" syndrome begins Chapter IV and there follows an account of 32 consecutive patients presenting with unexplained, symptomatic hyperprolactinaemia. Radiological abnormalities of the pituitary fossa suggested the presence of prolactinomas in 32% of these individuals but impaired prolactin response to stimulation and loss of the normal sleep peak of prolactin secretion were reliable indices of tumour, even when radiology was normal. Trans-sphenoidal microhypophysectomy proved effective, selective and safe therapy for patients with prolactinomas.

Twenty five per cent of subjects had neither biochemical nor radiological evidence of prolactin secreting tumour and these have been defined "functional" hyper-prolactinaemia. The clinical, biochemical and radiological features of this group are described together with their response to bromocriptine therapy, and possible underlying diagnoses in the group are considered. The chapter concludes with a discussion of the usefulness of dynamic tests of prolactin secretion in the diagnosis of radiologically occult microadenomas and in the provision

of an objective criterion for the comparison of patients treated in different ways. Finally, a strategy, based on this study, is described for the elucidation of unexplained hyperprolactinaemia.

Significantly elevated prolactin levels also occur in patients with renal disease and Chapter V deals with a survey of 357 patients with renal disease of variable severity and pathology. The prevalence of hyperprolactinaemia was determined and the relationship between hyperprolactinaemia and underlying pathology, creatinine concentration, duration of uraemia and drug therapy was examined. The arteriovenous concentration difference of prolactin across the normal kidney was measured. It was concluded that hyperprolactinaemia not attributable to drug therapy, occurred commonly in renal failure but not renal pathology _per se_. The relationship between prolactin and creatinine, reversion of prolactin levels towards normal after successful transplantation and arteriovenous concentration difference of prolactin across the normal kidney suggested that hyperprolactinaemia in uraemia may be accounted for, in part, by altered renal metabolism.

Deranged hypothalamic pituitary function in uraemia was not excluded in the survey of prolactin and renal disease. Chapter VI therefore deals with this aspect and

begins with a general review of endocrine status in uraemia. Thereafter, two studies are described (i) to determine overall basal hypothalamic pituitary status in 231 patients with progressive uraemia and after renal transplantation (ii) to define responses of several anterior pituitary hormones to stimulation and suppression in 19 maintenance Haemodialysis patients and in 6 patients after successful renal transplantation. Evidence is provided both of hypothalamic and pituitary dysfunction in uraemia, not ameliorated by dialysis but reversed by successful renal transplantation. It is concluded that endocrine dysfunction, and hyperprolactinaemia in particular, may be sensitive indices of non urea and creatinine related uraemic toxicity.

Circulating prolactin, like other peptide hormones, occurs naturally in several forms, which though immunologically indistinguishable are separable on the basis of size. Chapter VII describes a study to identify in uraemic sera, compared to pregnancy or prolactinoma sera or pituitary extracts, any changes in molecular heterogeneity as a result of altered renal metabolism of prolactin. Only one peak of immunoreactive prolactin,

similar to monomeric prolactin, was seen in uraemic sera with no evidence of aggregate or fragment formation. This unexpected finding provides additional data to suggest that hyperprolactinaemia in uraemia may be of biological significance.

The thesis concludes (Chapter VIII) with an assessment of the studies described and discussed in earlier chapters and potentially productive areas for future study are considered.

# CHAPTER 1

## INTRODUCTION AND HISTORICAL REVIEW

### 1 (1) Introduction

Although it had been established in the late 1920s that the anterior pituitary contained some agent which stimulated milk secretion in the developed mammary gland, Riddle, Bates and Dykshorn (1933) first used the term prolactin to describe the distinct anterior pituitary hormone which stimulated crop "milk" production when injected into pigeons or ring doves. Ovine prolactin was isolated and purified by 1937 (White, Catchpole and Long, 1937) and in species other than primates little difficulty was encountered in identifying distinct lactogenic hormones in pituitary extracts by the use of appropriate bioassays (Nicoll,1967, Frantz and Kleinberg, 1970).

Phylogenetically prolactin is probably the oldest of the pituitary polypeptide hormones and it serves a wide diversity of function in primitive vertebrates - over eighty physiological actions have been described (Bern and Nicoll, 1968, Nicoll, 1974). Mammotrophic

and lactogenic activity occur in many species including teleost fish and in these creatures, prolactin is also a major osmoregulatory hormone enabling survival in both salt and fresh water environments. In lower vertebrates prolactin is a growth and development factor; it may control steroidogenesis, fat deposition, erythropoiesis and in many birds it has important behavioural effects.

1 (2) Evidence for the separate existence of human prolactin (h PRL)

Until the early 1970s there was considerable doubt whether the human pituitary secreted a separate prolactin molecule (Sherwood, 1971). Supposed growth hormone or prolactin preparations from primate pituitary extracts invariably produced positive responses in both growth promoting and lactogenic bioassays. Although the ratios of growth hormone to prolactin activity varied considerably in different preparations, nevertheless the view emerged that in man growth and lactation were controlled by a single hormone (Bewley and Li, 1970).

However, considerable clinical and experimental data were available to suggest that this view was erroneous. (1) Few acromegalic patients with elevated growth hormone (GH) levels developed galactorrhoea and conversely in physiological and pathological lactation, it was rare to detect GH by available techniques.

(2) Individuals who had hereditary growth hormone deficiency could lactate quite normally (Rimoin et al., 1968). (3) Extracts of a pituitary tumour from a patient with galactorrhoea had considerable lactogenic activity but no growth promoting activity (Peake et al., 1969). (4) In post partum lactating females, lactogenic activity in plasma could not be neutralised with anti h GH serum whereas, lactogenic activity in plasma from acromegalic patients was neutralised (Frantz and Kleinberg, 1970). (5) In vitro studies with human pituitary tissue culture demonstrated that the secretion of GH decreased with time but secretion of PRL, as measured by bioassay, increased with time (Pasteels, 1972). These clinical and experimental observations were incompatible with the suggestions that h GH and h PRL were one and the same hormone.

1 (3) Isolation of human prolactin

In 1970 and 1971 respectively, human and monkey prolactin (m PRL) were isolated and clearly differentiated from growth hormone (Friesen, Guyda and Hardy, 1970, Guyda, Hwang and Friesen, 1971, Friesen and Guyda, 1971). This followed the recognition, initially in immunofluorescence studies (Herbert and Hayashida, 1970), that although h GH and h PRL are similar in physical, chemical and biological

properties, in its immunological properties h PRL is related to o PRL rather than to h GH. Hence although antisera to h GH or human placental lactogen (h PL) do not localise in prolactin secreting cells of the human pituitary, antiserum to o PRL does localise in these cells. The contribution of Friesen and his group in this field has been summarised (Friesen and Hwang, 1973).

Pituitary tissue fragments from both human and monkey sources were incubated in the presence of $^{3}H$-labelled leucine. Gel filtration of the proteins released into the incubation medium showed a single peak of radioactive material which cross reacted with antiserum to o PRL but not antiserum to h PL or h GH. Specific antibodies to h PL were then coupled to Sepharose and this immunoadsorbent was used to remove all GH from the pituitary fractions with retention of full PRL activity. Thus primate prolactin was separated from growth hormone. A method more suited to the larger scale purification of prolactin was later described (Hwang, Guyda and Friesen, 1972).

Also in 1971, using polyacrylamide gel electrophoresis at alkaline pH, a distinct band just behind GH was isolated from a homogenate of pituitary obtained from a female who died during pregnancy (Lewis et al., 1971).

This band, identified as PRL, proved identical to Friesen's material.

Immunisation of rabbits with these prolactin rich preparations yielded specific antisera and thus sensitive homologous radioimmunoassays for human prolactin were established (Hwang, Guyda and Friesen, 1971, Sinha et al., 1973).

In retrospect it is clear that two major factors contributed to the difficulty in isolating h PRL. (1) The considerable physical, chemical and biological similarity between h GH and h PRL - indeed h GH has 10-20% of the prolactin activity of the best o PRL standards. (2) h GH constitutes 5-10% of the dry weight of pituitary powder while the h PRL content is 0.05-0.1% i.e. GH is present in a one hundred fold greater amount.

Only in 1977 was the entire linear amino acid sequence of h PRL reported (Shome and Parlow, 1977; Fig. 5). The hormone, molecular weight 21,500 was composed of 198 amino acids with six half cystine residues. Extensive homology with p PRL (77%) o PRL (73%) r PRL (60%) was confirmed and contrasted with only 16% homology between h PRL and hGH, and 13% between h PRL and h PL.

1 (4) Source of human prolactin

Human prolactin is secreted by the anterior pituitary. It had been known for many years that the pituitary enlarged during normal pregnancy as a result of increase in the number and size of "pregnancy cells" (Erdheim and Stumme, 1909) and in 1961 it was postulated that these pregnancy cells secreted prolactin (Purves, 1961). This has been confirmed by immunofluorescent studies and it is now clear that prolactin secreting cells occur not only in pregnancy, when they constitute up to 50% of all acidophils (Goluboff and Ezrin, 1969), but also in non pregnant females and males. The cells are visible on light microscopy by special staining techniques (Herlant and Pasteels, 1967) and on electron microscopy their characteristic features include the presence of well developed endoplasmic reticulum and large pleomorphic secretory granules (Fawcett, Long and Jones, 1969).

The prolactin content of a normal pituitary gland has been variably reported as between 100-500 µg h PRL per gland (Friesen and McNeilly, 1977) and as such was initially the sole source of h PRL for purification. However, the observation that in early pregnancy prolactin concentrations in amniotic fluid were greatly in excess of those in either maternal or foetal serum (Tyson et al., 1972, Clements et al., 1977) caused speculation that its

origin might be foetal (Fang and Kim, 1975) maternal (Schenker, Ben-David and Polishuk, 1975) or placental (Friesen et al., 1972b). This speculation remains unresolved, although the observation has been unanimously confirmed and amniotic fluid now provides a potent source of h PRL which is chemically and immunologically indistinguishable from human pituitary prolactin (Friesen et al., 1972b). Indeed antisera raised to extracts of amniotic fluid have formed the basis of radioimmunoassays for h PRL (Cole and Boyns, 1973).

1 (5) Physiological control of prolactin secretion

Concepts of the physiological control of prolactin secretion in man are principally derived from experimental studies in animals (Tindal, 1974, Tindal, 1978).

Both in vivo animal studies and in vitro tissue culture data demonstrate that prolactin, unlike other anterior pituitary hormones, is under inhibitory control from the hypothalamus (Meites and Clemens, 1972, Pasteels, 1972). The cell membrane of the lactotroph is thought to be maintained in a state of hyperpolarisation by the specific interaction of a prolactin inhibiting factor (PIF) with its receptor on the pituicyte. The cell is thereby impermeable to Ca++ ions required for depolarisation and release of hormone granules. Thus

under normal circumstances the prolactin secreting cell is bathed in a constant, probably small supply of PIF the nature and origins of which are only recently apparent.

Although catecholamine and dopamine (DA) terminals abut directly on the portal capillary bed, suggesting that DA might be released directly into the portal circulation (Fuxe, 1963), initially, it was reported that injections of DA into portal capillaries failed to lower PRL levels (Kamberi, Mical and Porter, 1971). In a further study, however, half a million pig hypothalami were extracted and the fraction with greatest PIF activity was not only devoid of polypeptide but was rich in catecholamines, dopamine in particular. Further, when this preparation was dissolved in freshly made 5% glucose solution to protect it from oxidation, it did indeed inhibit PRL secretion when infused into pituitary portal vessels (Schally et al., 1974, Takahara, Arimura and Schally, 1974). Thus it was speculated that the early failure to demonstrate such an effect may have been due to oxidation and inactivation of dopamine.

Shaar and Clemens (1974) have confirmed the above results using hypothalamic extracts from the rat and in addition, by measuring PIF activity and catecholamine

content of both untreated hypothalamic extracts and "purified" fractions, they have concluded that the prolactin inhibiting activity of the hypothalamus can be totally accounted for by its catecholamine content. Thus there is now convincing evidence that PIF is dopamine itself; that it originates in granules in terminals of neurones which abut directly onto, and discharge into, portal blood vessels.

The control of prolactin secretion in birds is the opposite of that in mammals, that is the brain facilitates prolactin release (Meites et al., 1972). Hence it was of interest that in mammals thyrotrophin releasing hormone (TRH) released PRL as well as thyrotrophin (TSH) in vivo and in vitro (Bowers et al., 1971, Tashjian, Barowsky and Jensen, 1971). There has subsequently been speculation that TRH may be a physiological control mechanism for PRL release (Bowers, Friesen and Folkers, 1973). However, since post partum suckling, a potent stimulus for PRL release fails to release TSH (Gautvik et al., 1974) and there is no feedback effect of thyroid hormones on PRL release by TRH (L'Hermite et al., 1974), in man, the action of TRH on PRL secretion may be of pharmacological significance only. Nevertheless, there have been reports of a PRF distinct from TRH (Valverde-R, Chieffo and Reichlin, 1972, Dular et al., 1974) although the identity and physiological

significance of this factor remain speculative.

Since in the rat a minor stress such as ether anaesthesia causes a rapid but relatively small release of prolactin, while suckling results in a slightly delayed much larger release, two separate mechanisms may evoke PRL release (Terkel, Blake and Sawyer, 1972, Blake and Sawyer, 1972). A specific PRF may cause the relatively minor immediate release induced by stress, while the delayed but much larger release triggered by suckling may be due to a decrease of PIF activity.

The neurotransmitter serotonin has also been implicated in the physiological control of PRL secretion (Smythe, 1977). It facilitates release of PRL both in the rat (Lu and Meites, 1973) and in man (Kato et al., 1974) and in addition, blockade of 5-HT synthesis by parachlorophenylalanine blocks the release of prolactin in response to suckling, the effect being overcome by administration of 5-HT (Korden et al., 1973/1974). The physiological significance of this mechanism once again is not clear but theoretically at least serotonin may affect PRL secretion either directly, by induction of a PRF or by depletion of PIF at hypothalamic level, the latter alternative being most likely. Perhaps a serotonin secreting neurone may provide the "link" between those

cells directly involved in PRF or PIF synthesis and pathways leading to the higher centres involved in the control of prolactin secretion.

The central connections of those neurones involved in the control of PRL secretion are imperfectly understood and much available information is derived from electrophysiological studies and assessment of pituitary function following chemical implants, such as oestrogen, in defined areas of the hypothalamus (Tindal, 1978). Detailed anatomical studies using specific immunofluorescent stains for individual neurones, have expanded knowledge especially on the relationship between the arcuate and periventricular nuclei, median eminence and pituitary stalk (Fuxe,1963).

Neurones involved in the control of PRL secretion have their cell bodies in the arcuate and periventricular nuclei of the hypothalamus, the "hypophysiotrophic area", where they are in close physical contact with neurones involved in the control of other anterior pituitary hormones. Fairly short cytoplasmic extensions sweep down through the median eminence to abut onto the portal capillaries as previously described. The hypophysiotrophic area itself is connected to virtually all the other major brain centres. For example (1) the largest input direct from the cerebral cortex comes from the

hippocampus via the fornix system, and a smaller pathway derives from the orbito frontal cortex via the claustrum, external capsule and pre-optic area of the hypothalamus. (2) The stria terminalis and sublenticular systems bring fibres which originate in the olfactory and temporal lobe areas and relay in the amygdaloid body. Also from the basal ganglia comes the ansa lenticularis which carries fibres from the globus pallidus of the lentiform nucleus. (3) The large median forebrain bundle conducts signals from the reticular activating system in the mid and hind brain and the smaller dorsal longitudinal fasciculus brings input from the medial thalamic nuclei.

Some of these pathways terminate in the pre-optic area of the hypothalamus rather than directly into the arcuate and periventricular nuclei and, therefore, it has been postulated that "link" neurones may connect these two adjacent areas. One might speculate that these may be serotonin secreting neurones.

Finally, in rats having intact pituitary glands, and ectopic pituitaries transplanted under the renal capsule, PRL may exert a negative feedback, at hypothalamic level to inhibit further PRL release (Chen, Minaguchi and Meites, 1967).

In summary, the physiological control of prolactin secretion may be visualised at two levels (1) the central connections of those neurones which are directly involved in the control of PRL secretion. These derive from the cerebral cortex, basal ganglia, mid and hind brain (Fig. 6a), (2) stimuli propagated via any of these major central routes must effect their influence via a "final common pathway", in which the major control upon PRL secretion is inhibitory (PIF) although there is evidence for the presence of one or more prolactin releasing factors (PRF) and other neurotransmitters, for example, serotonin may be involved at some level. Prolactin may exert negative feedback control over its own secretion (Fig. 6b).

It is clear then that in vivo, the widely differing psychic, physical, chemical and pharmacological stimuli which have been observed to affect PRL status may do so by interaction at any of several sites.

It is also apparent that close physical proximity of key groups of specific hypophysiotrophic neurones within the arcuate nucleus and median eminence must facilitate chemical communication between them. Therein might lie a mechanism whereby events in one group of trophic neurones may initiate either synergistic or

reciprocal changes in cells directly controlling the secretion of different pituitary hormones. For example, such interaction between dopaminergic neurones and Gn RH secreting neurones may be responsible for the failure of LH and FSH response to the clinical and biochemical hypogonadism typical of hyperprolactinaemic states.

1 (6) Clinical aspects of prolactin secretion

With the development of sensitive radioimmunoassays for h PRL there has been progress in understanding of the normal physiology of prolactin secretion and of its relevance in clinical practice (Noel et al., 1972, Guyda and Friesen, 1973, Ehara et al., 1973, Noel, Suh and Frantz, 1974). This will be discussed in Chapter III. Furthermore, knowledge of the control mechanisms involved in prolactin secretion has clarified many alterations in circulating prolactin levels after such drugs as L-dopa (Kleinberg, Noel and Frantz, 1971) antipsychotic agents (Gold 1976, de Rivera et al., 1976) methyl dopa (Steiner et al., 1976) and bromocriptine (del Pozo et al, 1972).

Appreciation of pathological prolactin secretion has advanced considerably since the early recognition that not all patients who have elevated prolactin levels have galactorrhoea (Friesen et al., 1972b) and conversely, that patients with profuse galactorrhoea may have normal prolactin concentrations (Frantz, Kleinberg and Noel, 1972).

Deficiency of prolactin either alone or in combination with other hormones is rare (Turkington ,1972, Carlson, Brickman and Bottazzo, 1977) and in the adult, seems associated with few if any ill effects.

By contrast, pathological hyperprolactinaemia is a much more common abnormality and may have many causes (Table 1). Irrespective of its underlying aetiology, hyperprolactinaemia in the female is usually associated with amenorrhoea, infertility and in about 30% of patients, galactorrhoea (Jacobs et al. , 1976). Thus hyperprolactinaemia is particularly common in infertile patients (Franks et al., 1975) some of whom may have prolactin secreting tumours of the pituitary (Franks et al., 1977) a disease from which males are not exempt (Carter et al., 1978).

It is hardly surprising that initial interest and research has centred on those aspects of prolactin function and malfunction related to lactation and reproduction. It is, however, perhaps surprising that in 1979, there persists considerable debate on the subject of an appropriate strategy for the investigation of pathological hyperprolactinaemia; ignorance of the natural history of prolactin secreting tumours and great uncertainty in respect of the diagnosis and ideal treatment, if indeed any is required, for these commonest of all pituitary tumours (New England Journal of Medicine, 1979).

1 (7) Search for a non-lactogenic role for human prolactin

The recognition that circulating PRL levels are considerably higher than those of any other pituitary hormone (Friesen,1973) together with the observation that the adult male has PRL levels only slightly lower than those of the adult female (Guyda and Friesen, 1973) has evoked considerable interest in the identification of a non-lactogenic function for PRL in the human, particularly in view of the wide diversity of physiological function served by PRL in the animal kingdom.

1 (7)(a) Exogenous administration of prolactin

Determination of the effects of exogenously administered prolactin is a theoretically attractive approach for the elucidation of its physiological functions. However, practical problems associated with such studies abound, in the form of availability, purity, dosage and specificity of suitable preparations for administration. In the case of h PRL scarcity of suitable prolactin preparations is perhaps the greatest of these. Nevertheless, two interesting observations have been made following administration of ovine prolactin to human subjects.

In 1971 it was reported that intramuscular ovine prolactin when given to five healthy adult males resulted in decreased urinary excretion of sodium, potassium, and

water with consequent increase in serum osmolality (Horrobin et al., 1971). The authors thus concluded that prolactin may be an osmoregulatory hormone in man as it is in animals. Despite subsequent criticism of this work due to vasopressin contamination of ovine prolactin preparations (Carey, Johanson and Seif, 1977) the original investigators vigorously defend the validity of their observations and conclusions.

In quite different circumstances, Jepson and McGarry (1974) demonstrated a significant increase in red cell mass in response to ovine prolactin, in a small number of patients with chronic, but not acute, bone marrow failure thus confirming their earlier observation that prolactin has an erythropoietic effect in the mouse.

1 (7) (b) Metabolic disturbances in hyperprolactinaemia

Further evidence that prolactin may have a non-lactogenic function in the human may derive from detailed analysis of metabolic disturbance in individuals who are hyperprolactinaemic. Recently, interest has focused on two such disturbances - namely, deranged androgen metabolism and, more controversially, abnormalities in fluid and electrolyte balance.

Infertility due to hyperprolactinaemia is common and its cause remains unclear. In the male, there is some evidence that deranged metabolism of testosterone may be involved (Magrini et al., 1976). Six normal males

were made hyperprolactinaemic by the acute administration of sulpiride. Thereafter h CG stimulation tests provoked the same increment in circulating testosterone (T) levels as in controls but significantly lower levels of the more potent metabolite dihydrotestosterone (DHT) were seen in hyperprolactinaemic subjects. Thus in the male, acutely induced hyperprolactinaemia may decrease 5 $\alpha$reductase activity thereby decreasing conversion of T to its more active metabolite DHT. If persistent, this defect might effectively lower physiologically active androgen levels and contribute to hypogonadism.

In the hyperprolactinaemic female, deranged androgen metabolism also seems to occur but the relationship it bears to hypogonadism, if any, is unknown. A study of 35 hyperprolactinaemic females before and after bromocriptine therapy revealed significantly lower levels of the adrenal androgen dehydroepiandrosterone sulphate (DHAS) ($p < 0.0005$) after therapy, whilst levels of testosterone and androstenedione were unchanged (Carter et al., 1977). The authors concluded that prolactin stimulates adrenal androgen production in the female.

Similar results were obtained in smaller numbers of patients by Bassi et al., (1977), Kandeel et al., (1978), and Vermeulen and Ando (1978). The latter authors also noted that acutely induced hyperprolactinaemia, either by means of TRH or sulpiride, did not result in elevated

DHAS in normal individuals, suggesting that prolonged hyperprolactinaemia was required. They further observed that hyperprolactinaemic females on steroid replacement therapy did not show the anticipated increase in circulating DHAS levels and thus concluded that physiological ACTH secretion is a pre-requisite for the effect of prolactin on adrenal androgen secretion.

The relationship which prolactin bears to fluid and electrolyte balance in man is controversial and will be further discussed in Chapter V. Initial reports suggested that patients with prolactin secreting pituitary tumours exhibited significantly lower osmolar clearances in response to standard water loading than did controls (Buckman et al., 1976) but others failed to confirm this observation in acutely induced hyperprolactinaemia (Baumann and Loriaux, 1976).

1 (7) (c) Prolactin status in pathological conditions

Less direct evidence for a non-lactogenic fundtion for human prolactin may be obtained by the detailed assessment of prolactin status in a variety of pathological conditions. Such studies have proliferated with the wider availability of radioimmunoassays for h PRL and a selected summary of some of these studies is shown in Table 2.

One suspects that many of the reported anomalies in prolactin status may be the result rather than the cause of disease states. In addition, in some studies

conclusions are drawn from limited data obtained from small groups of patients whilst in others, no attempt is made to draw any meaningful conclusion from observed anomalies.

Nevertheless, it is quite conceivable that irrespective of its aetiology, in pathological situations hyperprolactinaemia may assume a significance not applicable in normal physiological circumstances. For example, it may be that stress induced hyperprolactinaemia may induce beneficial compensatory salt and water retention in the fluid and electrolyte depleted diabetic, whilst the same mechanisms may aggravate pre-existing hypertension in the stressed businessman. Furthermore, it may be no coincidence that there is excessive hyperprolactinaemia in molar pregnancy - a disease characterised by early and severe pre-eclampsia. Hyperprolactinaemia in renal failure may certainly be a significant factor in the development of hypogonadism but it might also be a compensatory erythropoietic factor which serves to ameliorate the profound anaemia in these patients. Moreover, it is of considerable interest that there was an increased incidence of elevated prolactin levels in individuals at "high risk" of developing breast cancer when compared to subjects who actually had the disease. This may mean that in certain susceptible individuals, prolonged exposure to elevated

prolactin levels may itself induce, or be a co-factor for the induction of, prolactin dependent carcinomata and promote their growth.

1(7)(d) Diseases in which prolactin may play a pathophysiological role

In general, our ignorance of the significance of physiological let alone pathological prolactin secretion in the human, lends itself to fanciful hypotheses on the possible role of prolactin in diverse conditions - a selection of which are noted in Table 3.

However, proposals such as these clearly arise because there are pathological conditions which we do not fully understand; abnormal prolactin status for which we can offer no satisfactory or significant explanation; animal studies which reveal hormone actions which may or may not be applicable and relevant to the human subject. The hypotheses, therefore, merit serious consideration and critical evaluation.

1(8) Aims of this thesis

The aims of this thesis may be summarised:

(i) As a general introduction, to provide a brief review of selected aspects of human prolactin.

(ii) To describe a radioimmunoassay which has been validated and used for the measurement of circulating prolactin concentrations in man.

(iii) To provide a strategy for the laboratory assessment of prolactin status, based on data obtained in diverse physiological and pathological conditions.

(iv) To demonstrate the role of dynamic tests of prolactin secretion in the diagnosis of small, radiologically occult prolactinomas by detailed clinical, biochemical and radiological assessment of a consecutive series of patients with unexplained symptomatic hyperprolactinaemia - the "Amenorrhoea-Galactorrhoea" syndrome.

(v) To define the incidence of hyperprolactinaemia in renal disease and provide evidence of altered renal metabolism of prolactin together with diffusely deranged hypothalamic pituitary function in uraemia.

(vi) To describe altered molecular heterogeneity of human prolactin in uraemic sera compared to other hyperprolactinaemic sera and pituitary extracts.

(vii) To provide an assessment of the studies described and suggest potentially productive areas of future study.

# CHAPTER II

## PROLACTIN ASSAY

### 2(1) Introduction

Prolactin may be measured by bioassay, radioimmunoassay and radioreceptorassay. The sensitivity and specificity vary greatly and, therefore, the method chosen must depend upon the primary purpose of the estimation.

### 2(1)(a) Bioassay

#### The pigeon crop sac assay

This is the original bioassay for prolactin and depends upon the ability of the hormone to stimulate proliferation and crop "milk" production by pigeon crop sac epithelium (Riddle *et al.*, 1933). Indeed this method remains the classic bioassay for prolactin and is still used for the comparison and standardisation of the biological potency of purified prolactin and lactogenic hormones. However, the technique is cumbersome, prone to non specific serum interference and even at its most sensitive (Nicoll, 1967) cannot detect normal circulating levels of prolactin. Moreover both h GH and h PL also

produce positive responses in the assay system (Forsyth, Folley and Chadwick, 1965, Forsyth and Edwards, 1972).

Stimulation of lobulo-alveolar mammary tissue in vitro

Four methods have been described which depend on the ability of prolactin to stimulate suitably prepared and maintained mammary gland in vitro. Two of these methods have a histological end point with an arbitrary assessment of the amount of secretion accumulating in the alveolar lumina and use either mid pregnant mouse mammary gland(Frantz and Kleinberg, 1970) or pseudo pregnant rabbit mammary gland (Forsyth and Myres, 1971). Two of the methods have biochemical end points and use mid pregnant mouse mammary glands. They depend on either the prolactin stimulated incorporation of $^{32}P$ into casein (Turkington, 1971) or the stimulation of N-acetyllactosamine synthetase activity (Lowenstein et al., 1971).

These assays are more sensitive than the pigeon crop sac assay but still do not measure normal basal prolactin levels; they both lack specificity, are imprecise, require specialised facilities, are time consuming and manpower intensive and have limited sample capacity.

2(1)(b) Radioimmunoassay

Radioimmunoassay (RIA) is a saturation analysis technique, the requirements for which are (i) a specific

antiserum for the antigen to be measured (ii) antigen which may be labelled with radioisotope ($^{125}I$) and retain immunological activity (iii) antigen for standardisation (iv) a technique for separation of antibody-bound and free fractions.

In principle, a trace amount of radioactively labelled antigen is incubated with a constant, limiting concentration of antiserum, binding about 50% of labelled antigen alone. Unlabelled antigen competes with, and progressively displaces antibody bound labelled antigen. Thus there is an inverse relationship between unlabelled antigen concentration and the amount of antibody bound labelled antigen. Quantitation depends upon efficient separation of antibody bound and free fractions.

The technique was first used to measure circulating levels of thyroxine (Ekins, 1960) and insulin (Yalow and Berson, 1960) and with the isolation of primate prolactin as discussed in Chapter 1, radioimmunoassays for human prolactin became feasible. Heterologous systems were described - that is RIAs in which the antiserum used is raised to prolactin of one species and tracer is prolactin from another species (Hwang et al., 1971, Jacobs, Mariz and Doughaday, 1972) but these were superseded by homologous RIAs in which prolactin of human origin was used for

immunisation, labelling and as standard (Friesen et al., 1972b, Sinha et al., 1973).

The major advantages of this technique are sensitivity (e.g. the ability to measure normal basal prolactin levels), specificity (e.g. no cross reaction with h GH or h PL), sample capacity and simplicity.

2(1)(c) Radioreceptor assay

The recurring criticism of radioimmunoassay is that it measures immunological activity which may be distinct from biological activity. Discrepancies between immuno-reactivity and bio-activity do occur. For example, a hormone precursor may be immunologically but not biologically active as may a partially degraded hormone fragment.

Thus in an effort to augment the established technique of radioimmunoassay, radioreceptor assays have been developed in which the binding agent is the physiological hormone receptor on the cell membrane of target tissues. Theoretically, these receptors bind only biologically active hormone and in the case of prolactin may be prepared from mammary glands of pregnant or pseudo-pregnant rabbits (Shiu and Friesen, 1976). The sensitivity and precision of such methods is usually much greater than conventional bioassays but the problem of lack of specificity recurs in that h GH and h PL cross react in the prolactin radioreceptor assay and non specific serum interference

is a practical problem (Friesen and McNeilly, 1977). Moreover, these assays measure only hormone binding and although this may be a pre-requisite for biological activity, may not be equated with it.

Nevertheless the development of radioreceptor assays represents an advance towards comparison of biological and immunological activity at relatively low hormone levels, a prospect not feasible with conventional bioassay techniques.

2(2) Radioimmunoassay of human prolactin

A homologous radioimmunoassay has been used to measure human prolactin concentrations in all of the studies presented in this thesis. Both the purified human prolactin and the antihuman prolactin serum were prepared in the laboratories of Professor H.G. Friesen and were kindly supplied by Dr. A. McNeilly.

Using these reagents, assay conditions were optimised to provide two systems; one for routine use, in which the mean sensitivity was 38 mU/l, range 27-54 (Table 4) and a more sensitive assay with mean sensitivity of 6 mU/l, range 2-15 (Table 5). Standard curves typical of each of these systems are shown in Fig. 7.

2(2)(a) Reagents

Tracer

Highly purified human pituitary prolactin (FR 75.7.10), 5 µg, was iodinated by the lactoperoxidase method (Thorell

and Johansson, 1971, Table 6). Lactoperoxidase (LPO) was supplied by Sigma London, bovine serum albumin (BSA) by Armour, hydrogen peroxide 30% w/v by BDH Chemicals and $^{125}I$ of specific activity 13-17 mCi/µg (IMS-30) was from the Radiochemical Centre, Amersham. The iodination reaction was terminated by simple dilution.

2 x 10 µl aliquots of the iodination mixture were removed, diluted with 90 µl 0.05M phosphate buffer pH 7.5 then applied to calibrated paper chromatographic strips (Whatman 3 mm) and electrophoresed for one hour (voltage 500v). $^{125}I$ incorporation and specific activity (SA) were calculated as described by Landon, Livanou and Greenwood (1967). Mean $^{125}I$ incorporation was 79%, range 71-84 and mean SA of labelled prolactin was 174 µCi/µg, range 162-194 in 14 iodinations.

The remainder of the iodination mixture was applied to a 40 x 1 cm column of G150 Sephadex which had been primed with 5% w/v BSA in phosphate buffered saline (pH 7.5, 0.05M phosphate, 0.9% w/v saline) (PBS). The column was eluted with 0.1% w/v BSA in PBS. Elution volumes of approximately 1 ml were collected using a Model 1200 Pup Golden Retriever automatic fraction collector (Instrumentation Specialties Co.). Measured

aliquots of each individual fraction were then counted in the gamma counter and an elution profile constructed (Fig. 8). The monomer peak was clearly separated from both "aggregated" prolactin and free iodine. It was possible, therefore, to select and pool appropriate fractions from the "monomer" peak and retain these at -20°C for use as assay tracer.

When maximal binding of this monomeric tracer was assessed in antibody excess (anti human prolactin serum FR AR 7-13, final dilution 1:5000), 94% of the tracer was bound and in assay binding of 50-60% was consistently obtained in the routine system.

A comparison of standard curves using tracers derived either from the "monomer" or "aggregate" peaks of the iodination profile is shown in Fig. 9. Moreover, the correlation between results obtained in 30 test sera using a 5 day old tracer compared with those using the same tracer 40 days old, was poor ($r = 0.71$), thus repurification was carried out on each occasion prior to addition of tracer to an assay. A disposable 10 ml pipette containing G150 Sephadex was used and the column eluted with 0.1% (w/v) BSA in PBS. Serial repurification profiles of the same tracer are shown in Fig. 10 and increasing aggregate and free iodine concentrations are

evident with time. However, significant amounts of "monomeric" tracer persisted for 4-6 weeks and thus provided a satisfactory life span for the tracer.

Alternative preparations of purified human prolactin were assessed for their suitability as source of assay tracer (Table 7). The most appropriate material was found to be FR 75.7.10 on the basis of high immunological activity, low non specific binding (NSB), increased assay sensitivity and prolonged working life span with repurification.

The more conventional iodination technique using the oxidising agent chloramine T (Hunter and Greenwood, 1962) was compared with lactoperoxidase iodination and, in agreement with Wood, Shahwan and Marks (1975), tracer produced by the lactoperoxidase method gave higher binding in assay, lower non specific binding and assays of greater sensitivity than those using tracer prepared with chloramine T (Table 8). The latter also had a shorter working life span.

Antibody

The rabbit anti human prolactin serum (FR AR 7-13) was used at a dilution of 1:28,000 to give 50-60% binding of tracer alone (Fig. 11).

VLS# 4 anti human prolactin serum (kindly supplied with VLS # 3 h PRL for iodination, by the National

Institute of Arthritis Metabolism and Digestive Diseases as part of their hormone distribution programme) was also tested at a final dilution of 1:30,000 and irrespective of whether this antiserum was used with VLS ≠ 3 tracer (NIH system) or with FR 75.7.10 tracer, it gave consistently lower binding in assay (by 10-20%), consistently higher NSB values (by 2-2.5x) with assays less sensitive than those using the routine Friesen system (FR AR 7-13 antiserum with FR 75.7.10 tracer).

For objective comparison, 30 test sera which had been assigned values in the routine Friesen system, were re-assayed in two other systems (1) the NIH system (2) a Mixed System using FR AR 7-13 antiserum with VLS ≠ 3 tracer. A closer correlation to the Friesen system was seen with the Mixed System ($r = 0.94$) than with the NIH system ($r = 0.88$) suggesting an antibody related discrepancy (Fig. 12).

Standard

Since March 1977, the first international reference preparation of human prolactin MRC 75/504 (650 milli international units/ampoule, from the National Institute for Biological Standards and Control, Holly Hill, Hampstead, London) has been used as a working standard, made up in assay diluent.

Prior to this working standards were prepared from Lowry Batch 3 purified human pituitary prolactin and were standardised using the MRC Research Prolactin Standard A 71/222 (10 milli units/ampoule) for absolute reference.

23 mU/l was equivalent to approximately 1 ng/ml of the VLS-NIH preparation.

2 (2)(b) Separation technique

Bound and free fractions were separated by a double antibody technique using a donkey anti rabbit serum (Wellcome Reagents Code RD 17) at a final dilution of 1:80 and carrier non-immune rabbit serum at a final dilution of 1:1000, which gave maximum precipitation of the bound fraction.

After an overnight incubation at 4°C, separation was by centrifugation at 4°C for 30 minutes at 2000 xg, after which the supernatant was aspirated and the precipitate counted in an automatic LKB Wallac 80000 gamma sample counter.

2 (2)(c) Calculation and analysis of results

Paper tape print-out from the automatic gamma counter was fed into a Hewlett Packard Model 10 programmable calculator via a Hewlett Packard Model 9863A paper tape reader. A plot of free fraction divided by bound

fraction against antigen concentration (the F/B plot) gave a standard curve which was close to linear. Little distortion was, therefore, involved if the standard curve was considered as a series of straight lines connecting adjacent points and this linear interpolation method was used to derive results automatically (Challand, Ratcliffe and Ratcliffe, 1975). Final results were printed using the Hewlett Packard calculator's alpha printer facility, along with parameters such as Δ values for all duplicates, standard curve mid point, assay mean, and a histogram of the Δ values obtained during the assay run.

2 (2)(d) Assay validation

Specificity

No significant cross reaction was demonstrated with either h GH or h PL over the range 0-5000 ng/ml and 0-100,000 ng/ml respectively (Fig. 13).

Accuracy of Recovery

Varying known concentrations of h PRL were added to aliquots of a horse serum which had been pre-assayed in the routine system and found to contain undetectable levels of h PRL. Six duplicate estimations of each of these (horse serum + h PRL) preparations were then

made in the same assay and within the range of prolactin levels tested, viz 49-300 mU/l, recovery of added h PRL was 97-108% (Table 9).

Serum effect

In the routine prolactin system described non specific serum effects were observed both with some horse sera (n = 4) and with "prolactin free" human sera from six patients being treated with the prolactin suppressing agent, bromocriptine (Fig. 14a). In the routine assay, the effect was small (<10% suppression of % tracer bound) but the effect was volume related (Fig. 14b).

In the assay conditions routinely used, the effect was judged sufficiently small to enable a diluent standard curve to be used.

Parallelism Studies

As a crude assessment of the comparability of the prolactin used as assay standard (MRC 75/504) and that measured in the clinical situation, sera from a series of patients who had elevated circulating prolactin levels of diverse aetiology were studied at serial dilution in assay diluent to ensure that they were parallel to the diluent standard curve. Typical examples of such parallelism studies are shown in Fig. 15.

Parallel serial dilutions were obtained in pregnant sera, pituitary tumours, drug induced hyperprolactinaemia, hypoglycaemic stress induced hyperprolactinaemia and in uraemic sera.

Precision within assay

Within assay precision was assessed by measurement of prolactin levels in twenty duplicate specimens of each of five sera which had mean prolactin levels 70, 140, 280, 360 and 560 mU/1. Coefficients of variation for each of these sera were 3%, 4.9%, 4%, 7.8% and 13% respectively.

Precision between assay

Between batch precision was assessed by the inclusion of four quality control sera at the beginning and end of every assay performed. The mean prolactin concentration in each serum was 70, 190, 360 and 560 mU/1 respectively. Over a period of 15 months during which 217 prolactin assays were performed, the between batch coefficient of variation in each of these sera was 8.4%, 5.4%, 7.8% and 9.2% respectively.

Quality control

In addition to the inclusion of internal quality control sera in all assays, the laboratory participated in the external National Quality Control Scheme for human prolactin (Dr. S. Jeffcoate, London).

For the 14 month period up to July 1978, results had a mean% difference from the all method mean of -34, SD 14. Two factors may have contributed to this discrepancy (i) the lack (at that time) of a widely available reference preparation for use as working standard (ii) the lack of uniformity of reagents and methods used by participating laboratories causing some groups to return consistently high results e.g. horse

serum reading 1200 mU/l. Clearly such high values cause skewing of the all method mean.

On a limited number of observations (n = 3) the mean inter assay C.V. of eight pools with prolactin levels in the range 40-1553 mU/l, was 8.1%.

The assay discriminated well between horse serum and a low human pool e.g. <42 mU/l (Routine system) compared to an all method mean of 192, and <31 (Routine system) compared to 176 (all method).

2(3) Statistics

Since the distribution of prolactin levels observed in healthy control subjects and in diverse pathological conditions was non-Gaussian, the Mann Whitney test was applied to assess statistical significance, by means of a Wang 600 programmable calculator (Wang Laboratories Inc.).

2(4) Discussion

For routine clinical use radioimmunoassay is the most suitable currently available technique for the measurement of prolactin concentrations. The system described has proved accurate, precise, sensitive and specific. Only small serum volumes were required and results of high diagnostic accuracy were available within one week. Although the method was entirely manual and hence labour intensive, one person was able to cope with a routine throughput of approximately 100 samples per week, each sample assayed neat and at dilution on the first occasion.

The method involved a well established technique for the separation of bound and free fractions and the method of calculation and analysis of results was one which was already validated and in routine use for other hormones within the radioimmunoassay unit.

Friesen's reagents proved the most suitable of those tested with high % binding in assay, low non specific binding and the tracer produced by lactoperoxidase iodination had a prolonged life span with repurification and hence was saving of iodination material and labour.

A clear advantage of the reagents used was the considerable flexibility which they conferred on the system. The routine system was robust, gave good precision over the normal range of prolactin concentrations and discriminated well between levels which were normal and those which were elevated. Moreover, minor modification of assay conditions such as the use of a disequilibrium time course, increase in standard/sample size and halving of working antibody titre produced a considerably sensitized system which was of particular value in the chromatographic studies of the molecular species of prolactin present in sera and pituitary extracts (Chapter VII).

Non specific serum effects may occur in radio-immunoassays and if present usually involve suppression

of the percentage of tracer bound at a given antigen concentration. The effect may be so marked as to necessitate preparation of standard solutions in appropriate antigen free serum rather than assay diluent, to avoid distortion as a result of comparison of serum samples to diluent standards.

The minor non specific serum effects observed in this assay enabled routine use of a diluent standard curve and hence avoided the necessity to identify and/or prepare a constant supply of prolactin free serum.

Finally, the parallelism which was demonstrated on serial dilution of various hyperprolactinaemic sera enabled confident, accurate measurement of prolactin levels, by dilution, in pathological hyperprolactinaemia.

# CHAPTER III

## THE LABORATORY ASSESSMENT OF PROLACTIN STATUS

### 3(1) Introduction

Measurement of serum prolactin concentration is now an essential part of the routine investigation of patients with suspected hypothalamic - pituitary dysfunction (Friesen et al., 1972a, Kleinberg, Noel and Frantz, 1977); indeed, hyperprolactinaemia is probably the most frequent hypothalamic - pituitary disorder seen in clinical practice, commonly presenting with amenorrhoea and infertility in females and impotence in males (Friesen and Hwang, 1973, Tyson et al, 1975). Gross abnormalities of prolactin secretion are readily detected by measuring basal prolactin levels and in general, the higher the level, the greater the chance that the patient has a prolactin secreting adenoma (Kleinberg et al., 1977). However, pituitary micro-adenomas are often associated with relatively modest degrees of hyperprolactinaemia and as such may be

difficult to distinguish from the many physiological and pathological conditions also associated with this biochemical finding (Table 1).

To assess the importance of some of the factors which are relevant to the interpretation of basal prolactin levels, detailed reference ranges in defined groups of patients were determined. In addition, serum prolactin responses to standardised dynamic tests were compared in normal subjects since these tests may be useful in the diagnosis of pituitary microadenomas in patients with modest hyperprolactinaemia (<1000 mU/l) and normal pituitary tomography. Moreover, the use of such tests may provide information about the pathophysiological factors involved in diverse hyperprolactinaemic states.

3 (2) Subjects studied and methods

Prolactin levels were studied in 921 subjects. None were receiving drugs known to affect prolactin secretion unless stated. Samples were taken between 09.00-12.00 unless otherwise indicated. Abnormal prolactin levels were confirmed on at least one further specimen. The following groups were studied.

3(2)(a) Normal subjects

Basal levels

Hospital Personnel: 100 subjects (50M, 50F mean age 31y, range 17-55).

Hospital in-patients: 50 subjects (30M, 20F mean age 48y, range 18-86) without evidence of renal, endocrine or malignant disease.

Hospital out-patients: 163 females (mean age 39y, range 17-71) attending a gynaecology out-patient clinic for the first time. Patients with amenorrhoea and infertility were excluded. Where prolactin levels were elevated, a second specimen was taken at the following visit to obtain an estimate of possible stress factors related to hospital attendance.

Females on oral contraceptive preparations as the sole drug: 104 subjects (mean age 28y, range 17-38). Samples were taken at routine clinic visits between 14.00-15.00.

Children with benign, non-endocrine disease: these comprised 11 normal babies (6M, 5F) aged less than 3 months and 30 children (13M, 17F) mean age 5y, range 3-13.

Serial levels

Within day variation was studied in six subjects (4M, 2F mean age 27y, range 24-30). Blood samples were taken via an intravenous cannula at hourly intervals from 08.00-20.00 during which time, subjects maintained normal activity.

Day to day variation was studied in ten subjects (6M, 4F mean age 28y, range 19-39) sampled daily for five days between 09.00-12.00.

Possible stress of venepuncture was studied in 12 males (6 hospital staff and 6 patients, mean age 27y, range 25-34). The patients were to undergo elective surgery for non-endocrine conditions the following day. Samples were taken from an intravenous cannula at 10 minute intervals for 90 minutes.

3(2)(b) Pathological Conditions

Hypothalamic-pituitary disease

Miscellaneous hypothalamic-pituitary disorders. This heterogenous group consisted of 27 patients (15M, 12F mean age 31y, range 12-64) with the following disorders:

| | |
|---|---|
| Kallman's Syndrome | n = 4 (M) |
| Nelson's Syndrome | n = 3 (1M, 2F) |
| GH deficiency | n = 5 (4M, 1F) |
| Craniopharyngioma | n = 5 (F, 2 patients post surgical treatment, 1 patient post radiation therapy) |
| Gonadotrophin (Gn) deficiency | n = 4 (M) |
| Panhypopituitarism | n = 6 (2M, 4F) |

Diagnosis in all cases was made on clinical, biochemical and radiological evidence and all patients were untreated at the time of sampling apart from the three patients noted above. Patients with "panhypopituitarism" had no clinical or biochemical evidence of any specific endocrinopathy.

Pituitary tumours

This group consisted of 42 patients (10M, 32F mean age 32y range 16-69) in the following categories:

| | |
|---|---|
| Prolactinoma | n = 16 (1M, 15F) |
| Acromegaly | n = 15 (6M, 9F) |
| Cushing's disease | n = 11 (3M, 8F) |

Diagnosis in all cases was established on clinical and biochemical grounds together with light and electron microscopy and immunoperoxidase studies on surgically removed tissue. In a number of cases samples were obtained both pre and post selective, curative surgical treatment.

Idiopathic hyperprolactinaemia

This group consisted of 10 females (mean age 26y, range 16-34) who presented with galactorrhoea and/or amenorrhoea and infertility and who had persistent

hyperprolactinaemia (i.e. elevated PRL levels on at least two separate occasions) with normal pituitary tomography and no other factor identified to account for their elevated prolactin levels.

Chronic Sick

Twenty subjects (8M, 12F mean age 42y, range 27-59) who had chronic progressive arthropathy for more than three years were studied. All patients were taking non steroidal anti inflammatory agents as the sole medication.

Renal failure

62 subjects (32M, 30F mean age 41y, range 17-45) with moderate chronic renal failure (creatinine <600μmol/1) were studied along with 13 patients (9M, 4F mean age 37y, range 18-49) who had severe chronic renal failure (creatinine >600 μmol/1). Thirty seven subjects on regular dialysis treatment (mean creatinine 1300 μμol/1 17M, 20F mean age 33y, range 16-52) were also studied.

None of these patients was on any drug therapy known to affect prolactin levels nor had they evidence of clinical endocrinopathy apart from five of the male dialysis patients who were impotent.

Primary Hypothyroidism

This group consisted of 50 female patients (mean age 44y, range 24-78) with gross primary hypothyroidism (mean T4 27 nmol/1, range <11-48; mean T3 0.6 nmol/1,

range <0.4-1.8; TSH all >50 mU/1). None of these patients had clinical evidence of any other endocrine abnormality.

Pituitary Ablation

This group comprised five patients (3M, 2F mean age 32y, range 27-49) who were insulin dependent diabetics and who had had attempted total hypophysectomy as a therapeutic manoeuvre for severe proliferative retinopathy. Macroscopically, hypophysectomy had been complete and samples were obtained from seven months to two years post surgery.

Drug Therapy

Samples were obtained from 68 patients (38M, 30F mean age 37y, range 16-72) who were on long term therapy with drugs which may affect basal prolactin levels viz., phenothiazines n = 21, αmethyl dopa n = 35, tricyclic antidepressants n = 8, metoclopramide n = 2, reserpine n = 2.

3(2)(c) Dynamic tests of prolactin secretion

Thyrotrophin Releasing Hormone (TRH-Roche) 200μg was given intravenously to 24 healthy subjects (14M, 10F mean age 29y, range 24-33) and blood was collected by serial venepuncture at 0', 20' and 60' after TRH administration.

Metoclopramide monohydrochloride (Maxolon-Beecham) 10 mg was given intravenously to 12 subjects (6M, 6F mean age 28y, range 25-33) and blood samples withdrawn at 0', 15', 30', 60' and 120' after metoclopramide.

L-dopa (Roche) 500 mg orally was taken by eight subjects (5M, 3F mean age 29y, range 26-33) and blood samples withdrawn at 0', 1h, 1.5h, 2h, 3h, 5h and 7h following ingestion of the drug.

Bromocriptine (Parlodel-Sandoz) 2.5 mg orally was taken by three subjects (2M, 1F mean age 28y, range 2[illegible] 30) following a meal and blood samples collected at 0', 1h, 1.5h, 2h, 3h, 5h and 7h thereafter.

Whereas in the TRH test blood was collected by repeated venepuncture, in all other tests, samples were taken via an indwelling intravenous cannula kept patent by heparinised saline. All subjects studied were fit and healthy on no drug therapy and they maintained normal activity during these tests. No side effects were noted apart from postural hypotension and vomiting following bromocriptine administration, thus restricting the number of subjects receiving this test.

3(2)(d) Radioimmunoassay of prolactin was by the routine system described in Chapter II. Prolactin levels in serum and plasma were not significantly different, nor

in specimens frozen immediately in a dry ice mixture or allowed to freeze at -20°C. Serum samples were routinely assayed, blood being separated within 12 hours of sampling and serum stored at -20°C until assay.

3 (2)(e) Statistical analysis was by the Mann Whitney test.

3(3) Results

3(3)(a) Normal subjects

Basal levels

The range of prolactin levels seen in hospital personnel and in-patients (the control range) was 60-375 mU/l (Table 10, Fig. 16). When compared to controls, significantly elevated prolactin levels ($p < 0.0005$) were noted even in the first trimester of pregnancy and concentrations rose until term, with a wide scatter. Oral contraceptive therapy and long term therapy with other defined drugs were associated with significant hyperprolactinaemia ($p < 0.005$).

Eighteen of the 163 patients (11%) attending a gynaecology clinic for the first time were found to have prolactin levels outwith the control range. On subsequent sampling 12 patients (7%) were found to have levels within the upper end of the control range whilst the remaining six patients had persistent hyperprolactinaemia. Four of these patients subsequently were found to be

taking drugs known to be associated with hyperprolactinaemia (oral contraceptive therapy n = 3, metoclopramide n = 1). Two patients (1000 and 1614 mU/l) had unexplained hyperprolactinaemia and detailed assessment subsequently revealed one of these to have a pituitary tumour.

No significant sex difference in basal prolactin levels was observed as may be seen in Fig. 17 which shows the relationship between prolactin level and age in both male and female adults as well as children. Significantly elevated prolactin levels ($p < 0.005$) were seen in infants under the age of three months, when compared both to older children and adults. Although in males, there was a trend towards lower basal prolactin levels with increasing age, this did not reach statistical significance. In females, however, the trend was more marked, and women >50y had significantly lower prolactin levels ($p < 0.005$) than younger women.

Serial levels

There was no significant trend in basal prolactin concentration in any of the 12 subjects who were venesected at 10 minute intervals after insertion of an indwelling intravenous cannula, the mean C.V. for the group being 11% (range 7-17%).

Throughout the day, however, a clear circadian pattern of prolactin secretion was observed (Fig. 18). Between 08.00-09.00h (mean PRL 236 mU/l, range 100-414) and between 17.00-20.00h (mean PRL 195 mU/l, range 86-391) prolactin levels were significantly higher ($p < 0.005$) than between 09.00-12.00h (mean PRL 133 mU/l, range 71-208).

In addition, from day to day, circulating prolactin varied in the same individual, the degree of variability being slightly, though not significantly, more marked in females (mean C.V. 28% range 25-32) than in males (mean C.V. 20% range 9-29) (Fig. 19).

3(3)(b) Pathological conditions

The range of prolactin levels seen in a defined selection of pathological conditions is shown in Table 11, Fig. 20.

Of patients with miscellaneous hypothalamic-pituitary disease, only three diagnostic categories were associated with abnormal prolactin levels viz:

(i) Craniopharyngioma - in which one untreated patient had an elevated prolactin level of 600 mU/l and a further patient, treated by external irradiation had a grossly elevated level of 2800 mU/l.

(ii) One out of four patients who had isolated gonadotrophin deficiency had a persistently modestly elevated basal prolactin level at 480 mU/l.

(iii) Three of the five patients who were otherwise panhypopituitary were found to have modestly elevated prolactin levels ( <750 mU/1) and this contrasted sharply with the uniformly undetectable levels seen in patients rendered panhypopituitary by elective surgical pituitary ablation.

Most patients who had prolactin secreting tumours of the pituitary gland, as expected, had grossly elevated circulating prolactin levels. However, five of these patients (31%) had moderately elevated levels ( <1200 mU/1) and there was overlap in basal prolactin levels with patients who had idiopathic hyperprolactinaemia.

Seven of the 15 (47%) untreated acromegalic patients were found to have hyperprolactinaemia and in all but two of the nine patients who were studied before and after selective, curative surgical treatment, prolactin levels were significantly reduced ($p < 0.005$).

Patients with active Cushing's disease, as a group, had prolactin levels significantly higher ($p < 0.05$) than those in control subjects although only three patients had frankly abnormal levels. As in acromegalic subjects, selective, curative microsurgery was associated with a fall in prolactin level.

The prevalence of hyperprolactinaemia in female patients who were hypothyroid, was 40% (20 patients), levels ranging from 360-2400 mU/1.

Hyperprolactinaemia occurred in 10 patients (16%) with moderate chronic renal failure but was more common both in severe chronic renal failure (77%) and dialysis patients (78%).

Patients with chronic progressive arthropathy had prolactin levels within the reference range.

3(3)(c) Dynamic tests of prolactin secretion

Administration of TRH (Fig. 21) and metoclopramide (Fig. 22) resulted in a prompt rise in circulating prolactin levels. The response evoked was greater in magnitude ($p < 0.005$) and more prolonged in duration following metoclopramide. In response to both stimulation tests, female subjects had significantly greater prolactin reserve than did males. This contrasts with the prolactin response to suppression tests in which the pattern of response was similar for both male and female subjects. L-dopa administration (Fig. 23) resulted in suppression of prolactin levels which was smaller in magnitude and shorter in duration than after bromocriptine administration (Fig. 24). The range of prolactin responses to stimulation and suppression tests is shown in Table 12.

3(4) Discussion

The basal prolactin levels seen in healthy neonates, children and adults in this study, are consistent with those previously described, in smaller groups of subjects (Friesen and Hwang, 1973, Guyda and Friesen, 1973).

Even in healthy individuals, however, several factors may affect prolactin status and appropriate interpretation of a given result demands adequate reference data in relation to factors such as sex, age, circadian rhythm, pulsatility of secretion, stress and drug ingestion (Jeffcoate,1978).

Mean basal prolactin levels are usually higher in females than in males (Ehara et al., 1973, Friesen and Hwang, 1973) though with a considerable overlap in their ranges (Frantz,1978) and in the present study, no statistically significant sex difference was observed, although no account was taken of the stage of the menstrual cycle in female subjects. Some authors have demonstrated significantly higher prolactin levels in the peri ovulatory and luteal phases of the cycle when compared to follicular phase levels (Franchimont et al., 1976) but other investigators have failed to demonstrate any consistent pattern in circulating prolactin during the menstrual cycle (Ehara et al., 1973, McNeilly and Hagen,1974). It is of interest, however, that in post menopausal females, a significant fall in prolactin levels was observed, which may well be oestrogen related, since no similar fall was seen in ageing males, and

Thorner and his colleagues (1977) have demonstrated a rise in serum prolactin levels which correlates with oestradiol levels in pubertal girls, but not boys. Thus, age, is also a factor which may affect basal prolactin status, at least in the female. The origin of the hyperprolactinaemia in neonates is unclear but is presumed to be of foetoplacental origin (Guyda and Friesen,1973) although it also may be oestrogen related.

It is established that considerably elevated prolactin levels occur during the hours of sleep (Sassin et al., 1973, Ehara et al., 1973) and it has been reported that in the early hours of the morning, prolactin levels may still be significantly elevated (Friesen and Hwang, 1973). The present data would confirm this, and, in addition, suggest a diurnal variation in prolactin secretion during waking hours, with higher prolactin levels in the early morning and a trend towards higher levels again in the evening. The ideal sampling time thus would be 09.00-12.00h when prolactin levels are at their nadir. The degree of individual variability in prolactin secretion from day to day is modest, but is more marked in females than in males - an observation which again may be related to the oestrogen status of the subject. This modest variation

in prolactin levels from day to day, together with the relative consistency in prolactin concentrations noted over a much shorter period of time (90 minutes), would suggest that for confirmation of hyperprolactinaemia, repeated sampling on several different days may be of more value than the usually advocated repeated sampling over a short period, via an indwelling cannula.

Prolactin release arising from the stress associated with major surgery or insulin induced hypoglycaemia is well established (Noel et al., 1972). The incidence of hyperprolactinaemia, attributable to the "psychic" stress of either hospital attendance or venepuncture is poorly documented, however, although a recent study suggests that 19% of women attending an infertility clinic have elevated prolactin levels, following clinical interview (Koninckx,1978). In the present study, there was no rise in prolactin level as a result of venepuncture per se, but 7% of females attending a general gynaecology clinic for the first time were noted to have elevated prolactin levels, which were normal on subsequent sampling, and thus may have been related to the stress of hospital attendance. It is clearly necessary, therefore, to confirm consistent elevation of prolactin levels before attributing any clinical abnormality to hyperprolactinaemia.

The relationship between prolactin and oestrogen levels in pregnancy is familiar and the stimulant effect of oestrogens on prolactin secretion has also been demonstrated experimentally (Carlson, Jacobs and Daughaday, 1973). Hence it is not surprising that patients taking oestrogen containing oral contraceptive preparations have a mean prolactin level higher than control subjects. Indeed 44% had frankly abnormal levels. Similarly, other drugs have been associated with hyperprolactinaemia e.g. αmethyl dopa (Steiner et al, 1976), phenothiazines (de Rivera et al., 1976) and metoclopramide (McCallum et al., 1976) and in the present study, long term therapy with αmethyl dopa, phenothiazines, tricyclic antidepressants, metoclopramide and reserpine were all associated with significantly elevated prolactin levels. The degree of hyperprolactinaemia, however, was modest, 95% of patients having levels <700 mU/l. This finding may account for the fact that none of these patients had any symptoms or signs which might be attributed to hyperprolactinaemia.

This study confirms that several pathological conditions may be associated with elevated prolactin levels although not chronic ill health, per se. The postulated aetiological mechanism involved in the production of

hyperprolactinaemia are diverse; e.g. disruption of the hypothalamic-pituitary stalk as may occur in pituitary tumours with supra-sellar extension; deranged hypothalamic control mechanisms to account for idiopathic hyperprolactinaemia; excessive stimulation by naturally occurring agents such as TRH in primary hypothyroidism and altered renal metabolism of prolactin with progressive uraemia. Autonomous production of prolactin by pituitary adenomas clearly results in the highest circulating levels of prolactin (Kleinberg et al., 1977, Frantz, 1978) but in this series 30% of confirmed tumours were associated with minor elevations in basal prolactin levels, such that they could not be distinguished from other causes of hyperprolactinaemia on this basis alone.

Since the early reports that TRH and L-dopa evoked, respectively, stimulation and suppression of prolactin levels in normal individuals (L'Hermite et al., 1972, Friesen et al., 1972a) it has been hoped that these tests of the autonomy of prolactin secretion would prove of value in the identification of tumour associated hyperprolactinaemia. Initial results were disappointing (Lamberts, Birkenhager and Kwa, 1976) but more recently, dynamic tests, particularly stimulation tests, have been found of value in the identification of prolactin secreting

tumours, particularly when the degree of hyperprolactinaemia is modest and pituitary radiology is entirely normal (Kleinberg et al., 1977, Chapter IV).

It is now established that metoclopramide is a potent stimulus to prolactin secretion (McCallum et al., 1976, Delitala et al., 1976, Judd, Lazarus and Smythe, 1976) and indeed not only is the magnitude of prolactin response greater than that seen following TRH, but it is also more prolonged in duration. In response to both TRH and metoclopramide stimulation, prolactin release is considerably greater in the female - a finding which may reflect greater oestrogen related prolactin reserve despite remarkably similar basal levels in both males and females. Acute administration of L-dopa and bromocriptine result in prompt suppression of prolactin levels in males and females alike; the extent and duration of suppression of prolactin levels, however, is much greater following bromocriptine. These different patterns of prolactin suppression in response to the acute administration of L-dopa and bromocriptine may well be related to the observed failure and success of these respective agents in the long term treatment of hyperprolactinaemic syndromes (Friesen et al., 1972a, Thorner et al., 1974).

## 3(5) Strategy for the laboratory assessment of Prolactin status

As a result of the studies described in this chapter, a strategy was drawn up for the laboratory assessment of prolactin status, in individuals with clinical presentations suggestive of hyperprolactinaemia:

1. Draw blood sample, under resting basal conditions, ideally between 09.00-12.00h though sampling between 09.00-17.00h is acceptable.
2. If an elevated prolactin level is demonstrated, when compared with appropriate age and sex related control subjects, exclude
   - (i) drug therapy (including oral contraceptive)
   - (ii) pregnancy
   - (iii) primary hypothyroidism
   - (iv) chronic renal failure
   - (v) hypothalamic-pituitary disease

   Relate level to appropriate reference ranges in groups (i) - (v).
3. Repeat sample:
   - (i) If both levels >700 mU/l, this is unlikely to be due to stress
   - (ii) If both levels >1000 mU/l, this is likely to be due to pituitary tumour.
4. If levels are modest and unexplained, and pituitary tomography is normal, undertake stimulation tests of prolactin secretion and compare responses with appropriate male or female control subjects.

# CHAPTER IV

## The Amenorrhoea - Galactorrhoea Syndrome

### 4(1) Introduction

In view of the current interest and controversy surrounding the amenorrhoea - galactorrhoea syndrome, it is somewhat chastening to learn that Hippocrates first described the clinical abnormalities over 2000 years ago, "If a woman is not with child, nor has brought forth, have milk, her menses are obstructed" (Bryner and Greenblatt, 1977).

By 1935 little progress had been made either in recognition of the syndrome or in study of its pathophysiology, since in that year, a historical review of "the syndrome embracing utero-ovarian atrophy with persistent lactation" cited only three such cases (Sharp, 1935). Two of these were described by Chiari, Braun and Spaeth in 1855 and the remaining case was reported by Frommel in 1881 - hence the "Chiari-Frommel" syndrome is used to describe onset of the characteristic features after parturition. Many years later it was recognised that the syndrome may arise de novo (Argonz and del Castillo, 1953) and in 1954, Forbes and her colleagues

demonstrated that the typical syndrome may occur in the presence of a pituitary tumour (Forbes et al., 1954). She further postulated that this tumour might be prolactin secreting - an astute speculation in view of contemporary doubt as to the existence of a distinct prolactin molecule in man.

Currently, the clinical syndrome of amenorrhoea, galactorrhoea and infertility, due to hyperprolactinaemia, is increasingly recognised (Thorner et al., 1974, Tyson et al., 1975, Jacobs et al., 1976) and it is further recognised that elevated prolactin levels occur in the male and may result in impotence (Franks et al., 1978). Moreover, hyperprolactinaemia and its associated clinical features may occur in a variety of conditions e.g. drug therapy, hypothyroidism, chronic renal failure ( as discussed in Chapter III)but nevertheless when these have been excluded, it is important to distinguish between patients who have discrete prolactin secreting tumours and those who have not.

In the past, radiological abnormality of the pituitary fossa has been judged the most useful diagnostic criterion of a pituitary tumour (Vezina and Sutton, 1974) but small changes revealed by radiology are difficult to interpret (Swanson and du Boulay, 1975). The diagnosis

of small prolactinomas is important, because treatment of large pituitary tumours with gross changes may be less effective (Werder et al., 1978, Tindall, McLanahan and Christy, 1978).

The aim of the present study of 32 hyperprolactinaemic patients was twofold (1) to investigate the value of dynamic tests of prolactin secretion in the diagnosis of pituitary adenomas (2) to assess the results of therapy in patients who had evidence of pituitary tumour and those who did not.

4 (2) Subjects studied and methods

4(2)(a) Subjects studied

Thirty two consecutive patients (1M, 31F mean age 28y, range 16-40) presented with amenorrhoea, infertility, galactorrhoea or visual disturbance. All had hyperprolactinaemia on at least two separate occasions (range basal PRL levels 480-18,000 mU/l; normal reference range 60-360). No other factor was identified to account for their clinical abnormalities and persistent hyperprolactinaemia. Clinical details of the patients are shown in Table 13.

4(2)(b) Methods

Serial linear tomography of the pituitary fossa

All patients were investigated by tomography of the pituitary fossa using the Mimer unit with a thickness of cut 0.27 cm. Films were independently reviewed without

knowledge of the clinical or operative findings and the appearance and volume of the pituitary fossa was determined (normal <1500 $mm^3$).

Anterior pituitary function

Anterior pituitary function was assessed in all patients by hormone measurements after an intravenous bolus of soluble insulin (0.1 U/kg) combined with thyrotrophin releasing hormone (TRH, 200 μg, Roche) and gonadotrophin releasing hormone (Gn RH, 100 μg, Ayerst). Normal minimal responses were defined as: cortisol increment >200 nmol/l and peak growth hormone levels > 16 mU/l in response to hypoglycaemia <2.2 mmol/l; TSH increment >3.6 mU/l at 20 min; LH increment > 3.0 U/l and FSH increment >1.0 U/l. The test was performed with the patient at rest, after an overnight fast and samples were obtained via an indwelling cannula at 0, 20, 45, 60, 90 and 120 minutes after administration of the drugs. Cannulae were *in situ* for at least 30 minutes before the test.

Dynamic tests of prolactin secretion

Circadian variation was assessed by blood sampling via an indwelling cannula during sleep and at mid morning, mid afternoon and mid evening during a 24 hour period.

Stimulation tests were TRH (Roche) 200 µg intravenously and metoclopramide (Beecham) 10 mg intravenously. Sampling details and reference data are shown in Table 12 and Figs 21 and 22.

Suppression tests were L-dopa (Roche) 500 mg orally and bromocriptine (Sandoz) 2.5 mg orally. Sampling details and reference data are provided in Table 12 and Figs. 23 and 24.

Patients were not fasted and maintained normal activity during these tests. No side effects were observed in any of the subjects studied.

Treatment

The treatment offered to any individual patient depended on the presumptive underlying diagnosis. If a prolactinoma was suspected, treatment was by trans-sphenoidal microhypophysectomy. If there was no radiological or biochemical evidence of pituitary tumour, then bromocriptine therapy was given to suppress prolactin levels into the control range.

Tumour histology

Histology was investigated by light and electron microscopy and immunoperoxidase staining for prolactin-secreting cells.

Post-operative assessment

Basal prolactin levels were measured 1-3 weeks post-operatively and anterior pituitary function was assessed as described above to define the selectivity of therapy. At least three months after therapy, prolactin response to TRH and metoclopramide was re-assessed. Objective clinical response was taken as the return of normal function i.e. regular menstruation, ovulation and/or restored fertility.

Hormone Assays

Serum prolactin was measured by the routine system described in Chapter II. All other hormones were measured by standard radioimmunoassays except serum cortisol which was measured by the method of Mattingly (1962).

4(3) Results

Using the investigative protocol described, two categories of patient were identified (1) those in whom there was biochemical and/or radiological evidence of pituitary tumour (Groups 1-3) and (2) those in whom there was neither biochemical nor radiological evidence of prolactinoma (Group 4 "Functional" hyperprolactinaemia).

4(3)(a) Prolactinoma patients

Twenty four patients (75%) had biochemical and/or radiological evidence of prolactin secreting tumour. Six of these patients (nos. 19-24, Group 3) are awaiting surgical identification and treatment of their tumours.

In all eighteen patients who have been treated, the presumed pre-operative diagnosis of prolactinoma was confirmed at operation (Table 14). Histologically nine tumours were chromophobe, six were amphophilic and three were eosinophilic (Fig. 25). Immunoperoxidase staining showed that all tumours were composed of prolactin secreting cells (Fig. 26) and electron microscopy confirmed the presence of plentiful, typical large pleomorphic secretory granules (Fig. 27).

Clinical details (Table 13)

Irrespective of the size of the tumour and the degree of hyperprolactinaemia, the commonest presenting complaints were amenorrhoea and infertility, galactorrhoea occurring in only 44%. The onset of symptoms was related either to childbirth or cessation of an oral contraceptive in 46% of patients.

Radiology (Table 14)

In ten of eighteen treated patients (56%), the pituitary fossa was radiologically normal (Group 1), and typical examples of normal pituitary radiology are shown in Figs. 28, 29 and 30 respectively. At operation, direct observation confirmed that nine of these ten patients had no bony abnormality, while a minor alteration in bone texture was detected in one.

The remaining patients had either general enlargement or localised abnormality of the pituitary fossa (Group 2). In one patient there was simple enlargement of the pituitary fossa, in one there was a minor loss of cortical definition, and in the remainder the floor of the fossa was asymmetrical, apart from the one male patient (18) whose pituitary fossa was virtually destroyed. Selected pituitary radiology of patients in Group 2 are shown in Figs 31-36.

In Group 2, basal prolactin levels were significantly higher, tumours were larger and the duration of symptoms was longer than in those patients with normal radiology.

Anterior pituitary function

Abnormalities of anterior pituitary function, other than hyperprolactinaemia, were identified in three Group 1 patients; two patients had a significantly impaired cortisol and growth hormone response to adequate hypoglycaemia and one had a delayed TSH response to TRH. In two of these patients, anterior pituitary function became normal after successful removal of their tumours. One patient in Group 2 had an impaired growth hormone reserve which was not affected by tumour resection.

Dynamic tests of prolactin secretion

A consistent abnormality in both groups was the complete absence of sleep associated increment in prolactin

levels (Fig 37). Both groups had grossly impaired prolactin responses to TRH (Fig 38) and metoclopramide stimulation (Fig 39), with minimal responses in patients with larger tumours.

Prolactin suppression patterns were less consistently abnormal although the mean prolactin response to L-dopa (Fig 40) and bromocriptine (Fig 41) was low normal in both groups. Only five patients failed to suppress normally after bromocriptine.

Treatment

Removal of the pituitary tumour was "curative" in fifteen of eighteen patients (mean basal prolactin 227 mU/1, range 88-340). In the other patients, one from Group 1 and two from Group 2 (1F, 1M) prolactin levels were significantly reduced (2500 mU/1 to 600; 9000 mU/1 to 1000; and 18,000 mU/1 to 1000).

Postoperative assessment of prolactin response to stimulation tests has been made in ten "cured" patients (Group A). Uniformly, they show reversion to normal prolactin response after TRH and metoclopramide stimulation (Table 15). In contrast, patients whose basal PRL levels were significantly reduced, but not normalised (Group B) retained their grossly subnormal responses to stimulation.

Treatment was "selective" in all patients i.e. no subject developed anterior pituitary dysfunction post-operatively.

No operative morbidity was observed apart from transient diabetes insipidus. Five patients are now pregnant (four from group 1), nine are ovulating (six from group 1) and galactorrhoea has been abolished.

4(3)(b) Patients with "functional" hyperprolactinaemia

Eight patients (25%) had neither radiological nor biochemical evidence of prolactin secreting tumours and thus were defined as having "functional" hyperprolactinaemia (Table 16).

Clinical details

On clinical grounds these patients (Table 13, Group 4) were indistinguishable from those patients with prolactin secreting tumours.

Radiology

In all patients, the pituitary fossa was radiologically normal both in its volume and contour.

Anterior pituitary function

No patient in this group demonstrated any abnormality of anterior pituitary function, other than persistent hyperprolactinaemia.

Dynamic tests of prolactin secretion

Subjects with "functional" hyperprolactinaemia had a normal diurnal variation in circulating prolactin levels. Although prolactin response to TRH and metoclopramide stimulation was less than in control subjects ($p < 0.05$) it was significantly greater than in patients with confirmed prolactinomas ($p < 0.005$). Indeed, a graded impairment of prolactin response to stimulation tests was seen from patients with "functional" hyperprolactinaemia to patients with small prolactinomas and subjects with larger tumours.

Like patients with prolactinomas, subjects with "functional" hyperprolactinaemia showed a normal suppression of prolactin levels following L-dopa and bromocriptine administration.

Treatment

All patients in this group were given bromocriptine, a dopamine agonist, in a final dose of 5 mg/day. At this dose, prolactin levels were normal in all subjects (mean basal PRL 74 mU/l, range 50-140), galactorrhoea was abolished and ovulation restored. No side effects of treatment were observed.

Three patients (25, 26 and 28) were given a six month course of the drug which was then stopped. In all three subjects, prolactin levels have remained within the normal range and there has been no recurrence of symptomatology (post bromocriptine follow-up ranges from 3-11 months).

The remaining patients continue on their course of therapy apart from one patient who is now 27 weeks pregnant and well.

4(4) Discussion

These results suggest that prolactin secreting tumours of the pituitary gland can be identified reliably by biochemical methods pre-operatively, even in patients in whom conventional tomography is normal. Impairment of the sleep associated increment in prolactin levels and prolactin responses to stimulation by TRH and metoclopramide provide an accurate means of diagnosing radiologically occult microadenomas.

Hitherto, the diagnosis of pituitary tumours has relied on radiological studies (El Gammal, 1977) which even experienced neuroradiologists find notoriously difficult to interpret because the anatomy of the fossa is variable and the characteristic radiological changes associated with microadenomas are subtle (Vezina and

Sutton, 1974). In addition, minor changes are frequently seen in skull radiographs of patients in whom there is no suspicion of endocrine abnormality (Swanson and du Boulay, 1975). Furthermore, small pituitary tumours may not result in surrounding bony abnormalities and so would elude the most sophisticated techniques (Jacobs et al., 1976, Thorner, 1977). This was so in nine patients in Group 1 in whom pituitary tomography was normal and in whom at operation the absence of a defect in bone structure around the tumour could be seen directly.

Gross abnormalities of prolactin secretion are readily detected by measuring basal prolactin levels and, in general, the higher the level, the greater the chance that the patient harbours a prolactin secreting adenoma (Kleinberg et al., 1977). However, 39% of patients with confirmed prolactinomas had modest hyperprolactinaemia (590-1600 mU/l) with levels characteristic of many associated physiological and pathological conditions (Chapter III). Although it was hoped that tests of autonomy of prolactin secretion might help in the recognition of prolactinomas (Friesen et al., 1972a)

recent reports have concluded that such tests have little diagnostic value (Jacobs et al., 1976, Tolis et al., 1974, Lamberts et al., 1976, Healy et al., 1977). However, several of these studies have assumed that normal radiology excludes a pituitary tumour. Thus the significance of altered prolactin responsiveness in hyperprolactinaemic patients with normal tomography, may have been overlooked.

The results in patients with radiologically occult prolactinomas contrast with those in patients with physiological hyperprolactinaemia e.g. post partum lactating women, in whom significant prolactin responses to stimulation (Barbarino et al., 1978) and suppression (Muller, Genazzani and Murru, 1978) have been demonstrated. It may be concluded, therefore, that dynamic tests such as TRH and metoclopramide stimulation are of considerable value in identifying hyperprolactinaemic patients with prolactin secreting adenomas, particularly those which are inapparent radiologically.

The ideal treatment of prolactinomas remains to be determined although several therapeutic approaches, of varying success, have been described (Thorner et al., 1974, Werder et al., 1978, Tindall et al., 1978, Hardy, 1973,

Kelly et al., 1978) Early recognition of such tumours should ensure optimal results from microsurgical techniques (Guiot, 1978) and in this series trans-sphenoidal micro hypophysectomy has proved effective, selective and safe. In particular, the observed restoration of normal prolactin responsiveness to stimulation tests, following removal of tumour, suggests that prolactinomas represent discrete pituitary lesions. Hence removal of such adenomas should result in lasting cure. In contrast, the persisting absence of normal prolactin responsiveness in patients whose basal prolactin levels, although substantially reduced, were not normalised, is highly suggestive of residual tumour.

The importance of early diagnosis of prolactinomas, when they are small, is emphasised by the presentation and outcome in the one male patient in the series (18) who represents a typical male prolactinoma. He presented late in the course of his disease, with visual field defect due to a large tumour with cystic suprasellar extension (Figs 31a, b) and he was panhypopituitary prior to any attempted surgery. Complete excision of his tumour was impossible, the patient required post-operative radiation therapy and is on permanent hormone replacement.

A more satisfactory outcome was noted in patient 3 whose amenorrhoea and infertility led to an early presentation, with biochemical diagnosis of a radiologically occult microadenoma (Fig 28) and selective trans-sphenoidal microsurgery effected clinical and biochemical cure with the patient now some 34 weeks advanced in pregnancy.

The dopamine agonist bromocriptine is also advocated for the treatment of the amenorrhoea-galactorrhoea syndrome (Thorner et al., 1975) but unfortunately its use has been associated occasionally, with rapid expansion of pre-existing tumours in subsequent pregnancies (Lamberts et al., 1977, Bergh, Nillius and Wide, 1978). In contrast, there have been recent reports that bromocriptine may effect reduction in size of large pituitary tumours in man (McGregor et al., 1979, Wass et al., 1979). However, as yet there are no definitive reports of cure of small prolactinomas by medical treatment. In any case, the identification of radiologically occult microadenomas by biochemical tests might allow selection of patients who require detailed supervision during and after bromocriptine induced pregnancy.

In general, a reliable practical method of identification of small prolactinomas, irrespective of radiological findings, will permit the choice between alternative methods of management to be made more rationally.

It will provide also an objective criterion for comparing groups of patients treated in different ways. The diagnostic strategy described in this study has been reliable and clinically useful in the identification of patients with prolactinomas. For routine purposes, the complete range of tests may not be necessary and assessment of the circadian prolactin profile and prolactin responsiveness to TRH and metoclopramide stimulation, together with tests of anterior pituitary reserve should enable accurate diagnosis of microadenomas which are radiologically occult.

Patients with "functional" hyperprolactinaemia (Group 4) are of particular interest. Biochemically, they are clearly distinguished from patients, with similar modestly elevated basal prolactin levels, who harbour radiologically inapparent micro-adenomas (Group 1). Since none of the patients with "functional" hyperprolactinaemia has undergone exploration of the pituitary, it is impossible to know that they do not have small microadenomas or indeed that they may develop these over the next few years. The pattern of increasingly impaired prolactin responsiveness to stimulation in Groups 4, 1 and 2 might be interpreted as a progressive phenomenon; patients in Group 4 may represent micro-adenomas at their earliest possible stage,

that is, when they are both radiologically and biochemically occult, Group 1 consisting of radiologically occult, biochemically evident micro-adenomas and Group 2 comprising tumours which are clearly apparent radiologically and biochemically.

However, the extent of the difference in response to stimulation between Group 4 and Group 1 patients is so great that it is barely conceivable that such a difference could be related to a smaller discrete pituitary lesion than the 2-3 mm micro-adenomas seen in Group 1 patients. Moreover, as previously mentioned, it has been demonstrated that patients with physiological hyperprolactinaemia in the puerperium stimulate and suppress basal prolactin levels, although not "normally", in a manner clearly different from patients with pituitary tumours. Further, three patients with gross hypothyroidism and modest hyperprolactinaemia (800, 1200 and 950 mU/l) have demonstrated a similar pattern of response to dynamic tests of prolactin secretion to that seen in subjects with "functional" hyperprolactinaemia; none of the hypothyroid patients had any evidence of pituitary enlargement and all have reverted to normal prolactin status following thyroxine therapy alone. Findings in

the puerperium and in hypothyroidism would thus suggest a true "functional", that is, hypothalamic "defect" in the control of prolactin secretion and I suggest this is the most likely explanation for "functional" hyperprolactinaemia.

The excellent clinical and biochemical response to bromocriptine therapy seen in hyperprolactinaemic patients is not new (Besser et al., 1972, Franks et al., 1977; Donald, Espiner and Livesey, 1978). Usually, however, in patients with tumours, withdrawal of the drug results in recurrence of symptomatology and biochemical abnormality. Further evidence that "functional" hyperprolactinaemia may be a true hypothalamic defect, is that three of the eight patients who have now completed a six month course of bromocriptine therapy have thereafter remained clinically, radiologically and biochemically normal, suggesting that normal physiological control of prolactin secretion has returned.

4 (5) Strategy for the elucidation of unexplained hyperprolactinaemia

Finally, a strategy for the differentiation of tumour associated and "functional" hyperprolactinaemia is given in Fig 42. This should enable a rational attempt at

differential therapy for hyperprolactinaemic states and clearly long term follow up both of patients with confirmed prolactinomas and those with presumed "functional" hypothalamic disorders should provide clarification and confirmation of preliminary diagnoses.

# CHAPTER V

## Prolactin and Renal Disease I

### 5(1) Introduction

Elevated serum prolactin concentrations occur commonly in patients with renal disease. Chirito, Gonda and Friesen (1972) reported that 20% of patients with chronic renal failure had hyperprolactinaemia, and elevated prolactin concentrations were described in patients on maintenance haemodialysis by Nagel et al., (1973) and Czernichow et al., (1976).

The significance of these observations was not clear, however, since patients with renal disease commonly take drugs, such as methyl dopa, which are known to alter serum prolactin and the role of the kidney in prolactin metabolism has not been defined.

In this study, the prevalence of hyperprolactinaemia in 357 patients with renal disease has been determined. In addition, the relationship between hyperprolactinaemia and underlying pathology, creatinine concentration, duration of uraemia and drug therapy has been assessed and arteriovenous concentration differences of prolactin

across the normal kidney have also been measured.

5(2) Patients studied and methods

5(2)(a) Patients studied

Blood samples were taken from 357 patients (187M, 170F, mean age 34.7y, range 14-75) with renal disease. The patients comprised two main groups:

Patients with impaired renal function

This group consisted of 210 patients in the following categories:

| | |
|---|---|
| Moderate chronic renal failure (CRF) (creatinine <600 μmol/l) | n = 87 |
| Severe chronic renal failure (creatinine >600 μmol/l) | n = 28 |
| Regular dialysis therapy (RDT) including home and hospital dialysed patients | n = 52 |
| Post renal transplantation | n = 43 |

Patients with normal renal function

These patients had normal renal function but had renal disease of the following pathologies:

| | |
|---|---|
| "Glomerulonephritis" (GN) | n = 56 |
| Chronic pyelonephritis (CPN) | n = 38 |
| Recurrent urinary tract infection (UTI) | n = 12 |
| Calculus disease | n = 14 |
| Polycystic kidneys | n = 12 |
| Systemic lupus erythematosus (SLE) | n = 15 |

The diagnoses were made by clinical, radiological and, where appropriate, histological criteria.

Patients undergoing elective cardiac catheterisation

Samples were also obtained from the renal artery and renal vein in seven patients undergoing elective left and right heart catheterisation for diagnostic purposes. None of these patients had any evidence of renal or endocrine disease, nor were they taking any medication known to alter serum prolactin concentration.

5(2)(b) Methods

All renal patients studied were hospital out-patients and samples were taken at routine clinic visits, the majority being taken between 09.00-12.00h under conditions of minimal stress. Dialysis patients were venesected immediately before haemodialysis. An elevated prolactin concentration was confirmed on at least one further sample.

Meticulous drug histories were taken from all patients, with particular reference to drugs known to affect prolactin concentrations. The most commonly prescribed drugs taken by the patients with renal disease were methyl dopa, prednisolone, and tricyclic anti-depressants. All dialysis patients were taking vitamin supplements (as orovite, fefol and ascorbic acid) and

aluminium hydroxide capsules. Four dialysis patients were taking 1 αhydroxy vitamin $D_3$ for renal osteodystrophy. All patients after renal transplantation received prednisolone and azathioprine.

The control group of patients comprised those described in Chapter III, including 20 "chronic sick" patients with chronic progressive arthropathy of at least three years' duration, requiring numerous hospital admissions.

Serum prolactin concentrations were measured in the routine system described in Chapter II. All renal artery and renal vein samples were assayed in the same batch.

The Mann Whitney test was used to assess statistical significance.

5(3) Results

5(3)(a) Control subjects

The reference prolactin range obtained as described in Chapter III, was 50-360 mU/l (mean 168). "Chronic sick" patients had prolactin levels within this reference range.

5(3)(b) Patients with renal disease

Elevated prolactin concentrations (>360 mU/l) were found in 113 (32%) of all renal patients. In 53 (47%)

of these patients, elevated concentrations (range 400-6454 mU/1, mean 1849) were possibly attributable to drug therapy (methyl dopa n = 39, prednisolone n = 8, tricyclic antidepressants n = 5). Not all patients on these drugs had elevated prolactin concentrations, however, and the prevalence of hyperprolactinaemia in patients receiving these drugs is shown in Table 17.

Excluding patients receiving one or more of these three drugs, prolactin concentrations were not significantly elevated in patients with renal disease and normal renal function (Table 18, Fig 43). The single exception was a 42 year old female with glomerulonephritis, who was post menopausal but had had primary infertility and who had persistently elevated prolactin concentrations (mean 1400 mU/1). Further investigation of this patient has revealed that although she has normal visual fields, there is marked asymmetry of the pituitary fossa on tomography with evidence of bone erosion, suggestive of the presence of a pituitary tumour.

In contrast, serum prolactin concentrations in patients with impaired renal function were significantly elevated ($p < 0.005$, Table 18), both in patients on drug therapy (Fig 44) and in those not taking any medication known to affect serum prolactin (Fig 45). There was a

significant correlation ($p < 0.005$) between prolactin and creatinine concentrations ($r = 0.45$) but no correlation was found between prolactin concentrations and age, sex, underlying diagnosis or duration of uraemia. Patients who had undergone successful renal transplantation had significantly lower prolactin concentrations than those patients undergoing regular dialysis treatment ($p < 0.001$).

5(3)(c) Arteriovenous concentration difference in PRL across the normal kidney

There was a significant decrease in prolactin concentration across the kidney in seven patients with non-renal, non-endocrine disease (Table 19, Fig 46).

5(4) Discussion

This study demonstrates that hyperprolactinaemia is commonly associated with renal disease, 32% of all renal patients studied having concentrations above the upper limit of the reference range ( >360 mU/l). Various commonly prescribed medications e.g. methyl dopa and neuroleptic drugs are known to affect prolactin concentrations (Steiner et al., 1976, de Rivera et al, 1976, Turkington, 1972) and in the present study drug therapy was a possible aetiological factor in 53 (47%) of all patients with hyperprolactinaemia. Methyl dopa

and tricyclic antidepressants specifically were associated with hyperprolactinaemia but in addition, many patients, notably those with SLE, who were taking prednisolone therapy, had elevated prolactin concentrations. This may represent a true association between prednisolone and hyperprolactinaemia, or alternatively, the relationship may be between hyperprolactinaemia and SLE itself. However, in the case of methyl dopa administration, the data indicate that 62% of patients with impaired renal function have elevated prolactin concentrations compared with 38% of those patients with normal renal function. This suggests that within the renal failure group, some patients had hyperprolactinaemia which was not solely due to drug therapy. Excluding patients taking these drugs, it was found that renal patients with normal kidney function had prolactin concentrations within the reference range, indicating that renal pathology, *per se*, was not associated with an elevated serum prolactin concentration.

In contrast, patients with impaired renal function had significantly elevated prolactin concentrations, confirming earlier reports (Chirito *et al.*, 1972, Nagel *et al.*, 1973). In addition there was a progressive rise in prolactin concentration as renal function deteriorated, in patients both on and off drug therapy known to affect

prolactin secretion. The correlation between creatinine and prolactin concentrations confirms that reported by Chirito et al., (1972) and the changes after renal transplantation support the view that restoration of renal function is associated with reversion of prolactin concentrations towards normal.

These findings, in conjunction with the observation that there is a consistent fall in prolactin concentration across the normal kidney, suggest that hyperprolactinaemia in renal failure, is attributable, in part, to altered renal metabolism. This mechanism may also explain the elevated concentrations of other hormones found in chronic renal impairment e.g. ßMSH-like immunoactivity (Gilkes et al., 1975, Smith et al., 1975). In man, there is evidence for the renal extraction of other peptides, e.g. insulin (Fine et al., 1976) and the isolated, perfused dog kidney has been shown to degrade parathyroid hormone with production of immunoactive fragments (Hruska et al., 1977). Moreover, using a fluorescein- labelled double antibody technique, it has been shown that in the rat, ovine prolactin gains access to the proximal tubular cells of the kidney by means of the glomerular filtrate (Donatsch & Richardson, 1975). It is therefore suggested that as the glomerular filtration rate falls with progressive

renal failure, plasma hormone clearance also declines, resulting in elevated circulating concentrations.

These data do not exclude the possibility of deranged hypothalamic-pituitary control mechanisms in chronic uraemia. Lim et al., (1977) have given a detailed account of thyroid dysfunction in chronic renal failure, which includes a subnormal pituitary TSH response to TRH; this abnormality has also been noted by Czernichow et al., (1976) who, in addition, observed an impaired prolactin response to TRH stimulation.

The significance of the hyperprolactinaemia of renal failure in man remains speculative. It is established that prolactin has a fundamental osmoregulatory role in many species of fish and amphibians (Lam, 1972) and Dobbie et al., (1977) have reported that the prolactin related "occlusive glomerular hyperplasia" seen in migrating fish shares many features with proliferative glomerulonephritis in man (Preface). It has also been demonstrated that prolactin concentration affects the severity of the chronic progressive nephropathy seen in some species of rat (Richardson & Luginbuhl, 1976). In man the possible role of prolactin as an osmoregulator remains controversial. Since the initial observation

(Horrobin et al., 1971) that intra muscular ovine prolactin reduced renal excretion of water, sodium and potassium in normal males, it has been reported that oral water loading of normal subjects produces a 50% suppression of serum prolactin from baseline concentrations and that this test is a useful discriminator between "functional" and tumour-associated hyperprolactinaemia (Buckman et al., 1973). These authors have also reported a decrease in osmolar clearance by the kidney in six subjects with small pituitary adenomas, associated with hyperprolactinaemia (Buckman et al., 1976). However, others have failed to confirm these results (Adler et al, 1975, Baumann & Loriaux, 1976, Baumann et al., 1977) and the latter authors conclude that prolactin is not an important osmoregulatory hormone in man.

# CHAPTER VI

## Prolactin and Renal Disease II

### 6(1) Introduction

Isolated clinical and biochemical endocrine abnormalities have been described in uraemia, in addition to hyperprolactinaemia (Klein and Kurokawa, 1978). However, the aetiological mechanisms involved in their production and the relationships between clinical and biochemical status have often remained obscure. It is difficult to define the relative contribution of factors such as primary hypersecretion of a hormone compared to secondary "feedback" stimulation or prolonged half life as a result of impaired renal excretion or metabolism. Moreover, there may be altered biological activity of endogenous hormone in the grossly deranged biochemical milieu of uraemia.

For example, abnormal carbohydrate metabolism is common in uraemia and is related to decreased sensitivity of peripheral tissues to insulin (de Fronzo et al., 1978); immunoreactive insulin levels are increased and there is

a delayed and diminished glucose response to exogenous insulin. Conversely, insulin response to sustained hyperglycaemia is biphasic with an early burst of insulin secretion followed by gradually increasing circulating insulin levels. However, elevated basal growth hormone levels and paradoxical growth hormone release during a glucose tolerance test also occur in uraemic patients and may contribute to their glucose intolerance (Orskov and Christensen, 1971). In addition, hyperglucagonaemia may be present (Bilbrey et al., 1974) as a significant contributing factor (Sherwin et al., 1976).

The clinical, radiological and biochemical features of secondary hyperparathyroidism are also common in chronic renal disease (Arnaud, 1973) as a result of impaired renal excretion of phosphate, lowered circulating Ca++, enhanced secretion of parathormone (PTH) and, in severe renal impairment, defective vitamin D synthesis. Indeed, this feedback mechanism forms the basis of Bricker's "trade off" hypothesis of uraemic toxicity (Bricker and Fine, 1978). However, in addition to hypersecretion of PTH in uraemia, impaired renal metabolism of the hormone results in accumulation of immunoreactive fragments of prolonged half life, both in man (Freitag et al., 1978) and in the dog (Hruska et al., 1977).

Similarly, factors influencing hypergastrinaemia in dialysis patients are not fully understood but, nevertheless, elevated gastrin levels may be of considerable clinical significance in relation to the increased gastric acid output and increased incidence of recurrent peptic ulceration occurring in these individuals (Doherty, 1978).

Overall assessment of hypothalamic-pituitary status in uraemia is poorly documented although isolated abnormalities of individual anterior pituitary hormones have been reported e.g. TSH (González-Barcena et al., 1973, Ramirez et al., 1976, Lim et al., 1977), GH (Orskov and Christensen, 1971, Czernichow et al., 1976), PRL (Nagel et al., 1973) and $\beta$MSH-like immunoreactivity (Gilkes et al., 1975, Smith et al., 1975) whilst ACTH levels are normal. In general, it has been assumed that impaired renal metabolism is the predominant underlying abnormality to account for elevated hormone levels.

Certainly impaired renal metabolism of prolactin may account, in part, for the elevated levels of this hormone in renal failure but this does not exclude the possibility of hypothalamic-pituitary dysfunction. The following study therefore was an attempt to define (i) basal hypothalamic pituitary status with progressive

uraemia (ii) responses of several anterior pituitary hormones to stimulation and suppression tests both in patients undergoing regular maintenance haemodialysis and following successful renal transplantation. Moreover, assessment of more than one pituitary hormone might enable patterns of abnormality to be identified.

6(2) Subjects studied and methods

6(2)(a) Basal hypothalamic pituitary status in uraemia

Patients studied

Blood samples were obtained from 231 patients (131M, 100F, mean age 32.4y, range 14-75) in the following categories:

| | |
|---|---|
| Moderate CRF (creatinine <600 μmol/1) | n = 89 |
| Severe CRF (creatinine >600 μmol/1) | n = 30 |
| RDT | n = 56 |
| Post renal transplantation | n = 46 |

Apart from prednisolone and azathioprine taken by patients following renal transplantation, the only drugs taken were aluminium hydroxide, oral iron and vitamins. No patient had clinical evidence of endocrine dysfunction other than five dialysis patients who were impotent.

Methods

All subjects studied were hospital out-patients and samples were taken at routine clinic visits, the

majority between 09.00-12.00h under conditions of minimal stress. Dialysis patients were venesected prior to haemodialysis. Any abnormal result was confirmed on at least one further specimen.

In each patient, the following were measured:
Total thyroxine ($T_4$), total tri-iodothyronine ($T_3$), TSH, Oestradiol ($OE_2$) in females, Testosterone (T) in males, sex hormone binding globulin (SHBG)
LH, FSH
Prolactin (PRL)
II hydroxycorticosteroids.

6(2)(b) Tests of anterior pituitary reserve

Patients studied

Anterior pituitary assessment was made in 19 patients (12M, 7F, mean age 33y, range 14-56) undergoing regular maintenance haemodialysis. Mean creatinine level was 1100 µmol/l, range 890-1400 and mean duration of dialysis was 14 months, range 2months-7years.

Six patients (3M, 3F, mean age 29Y, range 17-36) who had undergone successful renal transplantation at least six months previously, were also studied (mean creatinine 110 µmol/l, range 94-122).

Dialysis patients received aluminium hydroxide capsules and oral iron and vitamin supplements; after

renal transplantation, subjects received prednisolone and azathioprine.

Methods

All subjects underwent the following tests:

(i) TRH (Roche) 200 μg, was given, by intravenous bolus and blood collected at 0', 20' and 60' for measurement of basal $T_4$, $T_3$, TSH, PRL, GH and response of TSH, PRL and GH to stimulation.

(ii) Gn RH (Ayerst) 100 μg by intravenous bolus with blood samples at 0', 20' and 60' for measurement of basal $OE_2$ in females, T in males, FSH, LH and FSH/LH responses to stimulation. Ten of 19 dialysis patients and all post transplant patients received:

(iii) Metoclopramide (Beecham) 10 mg intravenously with blood samples at 0', 15', 30', 60' and 120' for PRL estimation.

(iv) L-dopa (Roche) 500 mg orally with blood samples at 0, 1, 1.5, 2, 3, 5 and 7h for PRL estimation.

(v) Bromocriptine (Sandoz) 2.5 mg orally with blood samples at 0, 1, 1.5, 2, 3, 5 and 7h for PRL estimation.

Tests were performed before dialysis in uraemic patients and blood was collected by an intravenous cannula which was *in situ* for at least 30' prior to the start of the test. No side effects of drug administration occurred.

All patients had serial linear tomography of the pituitary fossa performed as described in Chapter IV. Films were reviewed without knowledge of clinical or biochemical status.

6(2)(c) Control subjects

Eighty seven healthy volunteers (52M, 35F, mean age 31y, range 22-58), with normal renal function received TRH (n = 45), Gn RH (n = 15), metoclopramide (n = 16), L-dopa (n = 8) and bromocriptine (n = 3).

6(2)(d) Assays

Prolactin concentrations were measured by the routine system described in Chapter II. All other hormones were measured by standard radioimmunoassays with the exception of serum "cortisol" which was measured by the method of Mattingly (1962) and SHBG was assayed by a modification of the method of Rosner (1972).

6(3) Results

6(3)(a) Basal hypothalamic pituitary status in uraemia

Irrespective of category, all patients had normal basal levels of "cortisol" (mean concentration 392 nmol/l, range 286-540; reference range 270-690) and SHBG (mean concentration in males 32 nmol/l, range 8-48; reference range 5-45; mean concentration in females 54 nmol/l, range 30-68; reference range 25-65).

Elevated basal PRL concentrations, irrespective of drug therapy, occurred even in moderate chronic renal failure. Levels rose with progressive uraemia, were not normalised following institution of maintenance haemodialysis but did return towards normal after successful renal transplantation (Fig 45).

Basal gonadal status was also deranged in severe uraemia and biochemical abnormalities were reversed following restoration of normal renal function (Fig 47). When renal impairment was modest, basal levels of T, $OE_2$, FSH and LH were within the reference range in contrast to PRL. However, with progressive uraemia, concentrations of gonadal steroids fell significantly ($p < 0.0005$) and there was a concomitant rise in FSH and LH levels. Concentrations of the latter hormones, however, were less than might be expected for the degree of suppression of gonadal steroids. Moreover, there was a fall in FSH and LH levels in dialysis patients compared to undialysed patients with comparable uraemia, which did not reflect the progressive fall in gonadal steroids in these two groups.

In patients with moderate chronic renal failure, basal $T_4$, $T_3$ and TSH were normal (Fig 48). Concentrations

of $T_4$ and $T_3$ fell significantly ($p < 0.005$) as uraemia prof $T_4$ and $T_3$ fell significantly ($p < 0.005$) as uraemia ha progressed and continued to do so despite maintenance ur haemodialysis. Although TSH concentrations rose with we uraemia, they were within the reference range and hence th were inappropriate for the low $T_4$ and $T_3$ levels in returned to normal after successful renal transplantation.

6(3)(b) Tests of anterior pituitary reserve

TRH

Basal indices of thyroid function in the 19 dialysis patients given TRH are shown in Fig. 49. As expected, $T_4$ and $T_3$ concentrations were low compared to controls ($p < 0.005$) but whereas the mean TSH level was higher than in the control group, it was not significantly so.

In dialysis patients, TSH response to TRH (Fig 50) was significantly blunted at 20' but continued to rise until the end of the test.

Seven of 19 uraemic patients had resting basal GH levels >10 mU/l (Fig 51), with basal glucocorticoids uniformly unstressed, in the normal range. As a group, the dialysis patients demonstrated a heterogenous GH response to TRH, including those with a prompt and significant rise, those with delayed response and those with no response whatever.

Deranged hormone responses to TRH reverted to normal after successful renal transplantation (Table 20).

Gn RH

Basal parameters of gonadal status in 12M dialysis patients are shown in Fig 52. Testosterone levels were low and two patients (30y and 42y) had evidence of gonadal failure with LH and FSH values >50u/l. Of the remainder, LH levels were significantly greater than in controls whereas FSH levels, though tending to be higher, did not reach statistical significance. In 7F dialysis patients (Fig 53) the same pattern emerged viz. low basal $OE_2$, elevated LH, FSH similar to controls. One 56y old female had appropriate post menopausal levels of gonadotrophins.

Patients with gonadal failure excluded, LH response to Gn RH in uraemic males (Fig 54) was normal by 60' but there was a clearly subnormal FSH response. Similar results were seen in female dialysis patients (Fig 55).

Following transplantation, gonadotrophin responsiveness was normal (Table 20).

Tests of PRL secretion

The mean basal PRL level in 19 dialysis patients was 843 mU/l, range 196-4000.

In both male and female patients, a significantly blunted PRL response to TRH (Fig 56) and metoclopramide (Fig 57) was demonstrated, irrespective of basal PRL level. Moreover, there was no significant suppression of PRL levels either by L-dopa or bromocriptine (Fig 58) in uraemic patients compared to controls.

Patients with normal renal function after successful renal transplantation had basal PRL levels in the normal range and normal PRL responses to stimulation and suppression (Table 20).

6(3)(c) Pituitary radiology

Tomography of the pituitary fossa was normal both in dialysis patients and in those with functioning renal transplants.

6(4) Discussion

This study demonstrates that prolactin is but one of the abnormal pituitary hormones in uraemia. Indeed, virtually every hormone studied was deranged either basally or in response to dynamic tests but nevertheless, even when abnormalities were gross, restoration of normal renal function by successful renal transplantation resulted in return of physiological endocrine status.

It is now well established that in severe uraemia circulating levels of thyroxine and tri iodothyronine

are low despite normal levels of thyroid binding globulin and albumin (Ramirez et al., 1976, Lim et al., 1977). The present results are in agreement with this. It has been suggested that an intrathyroidal defect in thyroid hormone metabolism may account for low circulating thyroxine levels (Joasoo et al., 1974) and in addition there is deranged peripheral conversion of thyroxine to tri-iodothyronine (Lim et al., 1977). Previously, a high incidence of unexplained goitre has been reported in dialysis patients e.g. 58% compared to 8% in a comparable non uraemic population (Ramirez et al., 1973). None of the present patients had goitre or any clinical evidence of thyroid dysfunction.

The inappropriately low basal TSH levels in dialysis patients provides evidence, in addition, for a hypothalamic-pituitary defect in the control of TSH secretion in uraemia, as does the blunted and delayed TSH response to TRH stimulation. Others have reported that the TSH response to TRH stimulation in severe renal impairment, although delayed, is prolonged and have attributed this to prolonged half life of TRH and TSH in uraemia (Gonzalez - Barcena et al., 1973). Whereas this may well be the case, the more striking abnormality is the failure of

normal feedback stimulation of endogenous TSH in response to low circulating thyroid hormones and the relatively sluggish response to direct stimulation by exogenous TRH.

Deranged hypothalamic-pituitary-gonadal status in uraemia is perhaps more clearly related to overt clinical abnormality than hypothalamic-pituitary-thyroid dysfunction. Progression of renal failure is commonly associated with reduced libido, impotence in males, amenorrhoea in females and infertility in both sexes (Bailey, 1977). In males, testicular biopsy shows varying degrees of spermatogenic arrest resulting in oligospermia or azoospermia, whilst Leydig cells appear morphologically intact (Lim and Fang, 1975).

Previously, as in the present report, low levels of gonadal steroids with normal concentrations of sex hormone binding globulin, have been a consistent finding in uraemic subjects (Lim and Fang, 1975, Ølgaard, Hagen and McNeilly, 1975, Hagen et al., 1976).

Reports of gonadotrophin levels in uraemia, however, have been variable. Lim and Fang (1975), in addition to observing clinical and biochemical gonadal failure in several young uraemic males, found basal LH values to be modestly elevated whilst basal FSH levels were normal.

The present data is in close agreement with their results and moreover, a recent report suggests diminished renal clearance of LH occurs in uraemia (Holdsworth, Atkins and de Kretser, 1977).

Lim and Fang (1975) also comment on the inappropriately low basal levels of FSH and LH in uraemic males. Although they demonstrated a normal gonadotrophin response to clomiphene administration, they suggest that a defect in hypothalamic responsiveness to feedback control may be present in uraemia to account for the inappropriately low basal levels of LH and FSH. The responses of LH and FSH to direct pituitary stimulation by Gn RH, in the present study, would be consistent with this hypothesis, but also demonstrate diminished release of pituitary FSH to direct stimulation. Whilst the present data is consistent with that of Fang and Lim, others have reported significantly elevated FSH levels in uraemic females (Ølgaard et al., 1975) and in uraemic males (Hagen et al., 1976). These differences may reflect different populations under study and, in particular, differences in age and duration of dialysis.

Thus, in uraemia, there is evidence of (i) primary gonadal toxicity with clinical and biochemical gonadal failure (ii) hypothalamic-pituitary dysfunction with

inappropriate basal gonadotrophins and impaired FSH reserve to direct Gn RH stimulation.

Lack of PRL responsiveness to suppression and to stimulation, irrespective of basal prolactin level, is further evidence of diffuse hypothalamic-pituitary dysfunction in uraemia and cannot be accounted for solely by prolonged half life of the hormone. Impaired PRL response to stimulation by TRH has been reported in children (Czernichow et al., 1976) and the present results closely parallel those of Lim, Kathpalia and Frohman, (1979) who failed to show any suppression of PRL levels in uraemic patients during dopamine infusion. As in the present study, these authors noted reversal of prolactin abnormalities after renal transplantation. Thus, elevated basal prolactin levels in uraemia may result both from altered renal metabolism and from hypothalamic pituitary dysfunction. Hyperprolactinaemia is one of the most sensitive endocrine abnormalities in uraemia, occurring in patients with even moderate chronic renal failure and it seems likely that it will contribute to hypogonadism in uraemic patients no less than in those with prolactinomas (Chapter IV). The significance of elevated prolactin levels on renal function, blood pressure, and anaemia in dialysis patients remains speculative.

The present study confirms that elevated basal GH levels occur in dialysis patients and abnormal GH responsiveness to TRH stimulation again suggests deranged hypothalamic-pituitary control mechanisms. Similar abnormal GH release may occur in acromegalic patients (Gonzalez-Barcena et al., 1973) but in uraemia, GH abnormalities, like hyperprolactinaemia, are early manifestations of the syndrome occurring in patients with mild renal failure (Welssel et al., 1979).

Thus, in summary, overall assessment of hypothalamic-pituitary status in uraemia has shown that gross abnormalities of several pituitary hormones exist. There is evidence to suggest both hypothalamic defects e.g., inappropriate basal TSH, LH, FSH, GH and PRL levels and pituitary defects e.g. abnormal TSH response to TRH and impaired FSH response to Gn RH.

It is striking that, apart from a modest effect on gonadotrophin levels, institution of maintenance haemodialysis does not result in amelioration of endocrine dysfunction, while successful renal transplantation abolishes all endocrine abnormalities. Thus many hormonal abnormalities may arise primarily as a result of direct "uraemic toxicity" which is not urea or creatinine related.

Several substances have been termed "uraemic toxins" simply on the basis that they accumulate in the body fluids of patients with uraemia (British Medical Journal, 1977) but objective evidence that they are indeed "toxic" to humans or animals, is lacking . In view of the poor correlation between the toxic manifestations of uraemia and levels of creatinine and urea in serum, several theories emerged to account for this discrepancy (Bergstrom, 1975); the "small molecule" hypothesis incriminates substances such as methylguanidine as uraemic toxins; "middle molecules" with molecular weight 500-5000 will accumulate with conventional dialysis techniques and molecules in this range have been isolated from uraemic sera and are "toxic" in in vitro systems; and the "trade-off" hypothesis suggests that certain hormones may exert toxic effects as they accumulate due to homeostatic adaptations to the reduced glomerular filtration rate. It seems likely that clinical "uraemic toxicity" will result from a combination of these factors rather than one alone (Bergstrom and Furst, 1978).

Nevertheless, however mediated "uraemic toxicity", particularly at hypothalamic-pituitary level may contribute to the deranged hormone levels e.g. hyperprolactinaemia, which occur with impaired renal function.

# CHAPTER VII

## Size Heterogeneity of Human Prolactin

### 7(1) Introduction

Total immunoreactive hormone may not necessarily represent a homogenous population of molecular species. Rather, peptide hormones occur naturally in several forms, which though immunologically indistinguishable, are separable on the basis of size e.g. insulin (Roth, Gorden and Pastan, 1968),PTH (Berson and Yalow, 1968), ACTH (Yalow and Berson, 1971), GH (Goodman, Tanenbaum and Rabinowitz, 1972). Prolactin has also been shown to exist in more than one immunoreactive form in serum (Rogol and Rosen, 1974), amniotic fluid (Fang and Kim, 1975), pituitary extracts (Suh and Frantz, 1974) and cerebro spinal fluid (Kiefer and Malarkey, 1978).

Studies of hormonal molecular heterogeneity under different conditions, along with pulse - chase experiments are often employed to investigate the synthesis, secretion and interaction between precursor, pro-hormone and hormone molecules. In the present study, the aim was somewhat the reverse viz. to identify in uraemic sera, compared to pregnancy or prolactinoma sera or pituitary extracts, any changes in molecular heterogeneity as a

result of altered renal metabolism of prolactin.

7(2) Materials and methods

7(2)(a) Serum and pituitary extracts

Blood samples were obtained from two patients with uncomplicated pregnancies, 13 and 28 weeks advanced respectively; two females with prolactinomas, S.C. and P.C.; two patients (1M, 1F) on regular maintenance haemodialysis for 11 months (creatinine level 940 and 1004 μmol/l respectively).

Serum was separated in a refrigerated centrifuge and stored at -20°C until used (not more than two weeks).

A normal pituitary gland was obtained at post mortem, <12 hours after death, along with portions of prolactinoma removed at selective transsphenoidal hypophysectomy from patients S.C. and P.C. whose blood samples had already been obtained.

Pituitary tissue was lyophilized to a constant weight and stored at -70°C until used (not more than 3 weeks).

7(2)(b) Gel filtration

Column

Sephadex G75 (fine) columns (90 x 1 cm) were prepared by packing slurries of previously swollen gel in degassed phosphate buffered saline (pH 7.5, 0.05M phosphate,

0.9% w/v saline) (PBS), containing 0.02% w/v sodium azide. Before use, gel columns were eluted for 48h with PBS containing 20 mg/ml human serum albumin. All filtrations were carried out at 4°C using PBS diluent containing 0.02% w/v sodium azide as eluant.

Constant volume fractions (1.5 ml) were collected in polystyrene tubes using an LKB 7000 Ultrorac fraction collector. Fractions were frozen at -70°C until assayed.

To correct for small differences in elution volume on different columns and in different filtrations on the same column, fractions were plotted as percentage of elution volume between blue dextran (void marker) and $^{125}I$ (salt marker).

The recovery of PRL immunoreactivity ranged from 81% to 98%.

Samples

One ml aliquots of serum samples were fortified with tracer amounts of blue dextran and $^{125}I$ before column application.

Lyophilized pituitary tissue was resuspended in 2 ml PBS. After mixing by rotation for 15 mins at 4°C and centrifuging at 2500 rpm for 10 mins at the same temperature, the supernatants were withdrawn and

1 ml aliquots fortified with tracer amounts of blue dextran and $^{125}I$ prior to column application.

7(2)(c) Prolactin radioimmunoassay

Prolactin concentration in serum, pituitary extracts and filtration fractions was measured by means of the routine or sensitive prolactin assay systems (described in Chapter II) as appropriate.

7(2)(d) Refiltration studies

Three fractions from peak 3 (monomer) h PRL, obtained from serum of prolactinoma patient S.C. and the same fractions from uraemic serum A, were separately pooled, freeze dried and resuspended in 1 ml horse serum. They were refiltered through similar columns and the PRL content in the refiltered fractions was assayed. Each elution pattern was compared to its original.

7(2)(e) Parallelism studies

Each fraction of peak 1 (void peak), 2 (20% elution volume peak) and 3 (monomer peak) from the 28w pregnancy serum, together with fractions of peaks 1 and 3 from prolactinoma patient S.C's serum and fractions from peak 3 of uraemic sera A, were assayed at several dilutions. Results were used to construct dose-displacement curves which were compared to the curve of h PRL standard 75/504.

7(2)(f) Standard

Six milli units of MRC reference h PRL preparation 75/504 were chromatographed as previously described.

7(3) Results

7(3)(a) Heterogeneity of h PRL after gel filtration

Immunoreactive h PRL appeared as three distinct peaks in MRC 75/504, normal pituitary extract and normal pregnancy sera (Fig 59). Three peaks with similar elution characteristics were observed in the pituitary extract and serum of prolactinoma patient S.C. (Fig 60) and patient P.C. (Fig 61).

Peak 1 eluted around the void volume, peak 2 had a mean % elution volume of 20 and peak 3, the major component in all samples, had a mean % elution volume of 33. The latter peak represented 87% of MRC 75/504 h PRL reference preparation and was assumed to be monomeric prolactin.

Immunoreactive h PRL in uraemic sera appeared as a sole, broad but symmetrical peak with elution volume similar to that of monomeric prolactin (Fig 62).

Quantitative data on the distribution of prolactin heterogeneity is given in Table 21. Prolactin of direct pituitary origin was similar in filtration profile irrespective of whether the pituitary was normal or

tumorous (peak 1 representing 7-16% of total immunoreactivity, peak 2, 6-20%). In sera from prolactinoma patients, there was a greater percentage of high molecular weight, peak 1 immunoreactivity (10-31%) compared to pituitary tissues, but peak 2 was comparable. Similarly, as pregnancy advanced, the relative contribution of peak 1 to total prolactin immunoreactivity was greater than in early pregnancy.

7(3)(b) Refiltration studies

Fractions of peak 3 from both prolactinoma and uraemic sera when refiltered, retained their original positions with elution volumes 32.8 and 33.9% respectively.

7(3)(c) Parallelism studies

Fractions from peaks 1, 2 and 3 all gave parallel dose-response curves compared to the standard reference preparation MRC 75/504.

7(4) Discussion

Although the heterogeneity in molecular size of human prolactin in blood and pituitary is well established, the nature, source and inter-relationships between the various molecular species is poorly understood.

While an early report of serum filtration (Rogol and Rosen, 1974) identified only two immunoreactive

prolactin peaks (similar to peaks 2 and 3 in the present study), subsequent authors have repeatedly shown the presence of three major immunoreactive components of circulating prolactin, as in the present study. Peak 1 in this study has previously been termed aggregate (Suh and Frantz, 1974) void fraction (Gala, Van de Walle and Hoffman, 1977), big-big prolactin (Garnier et al., 1978); peak 2 in the current study has previously been termed big prolactin (Suh and Frantz, 1974, Guyda,1975, Garnier et al., 1978) and medium prolactin (Fang and Refetoff, 1978) and peak 3 in this report has almost unanimously been termed little or small prolactin.

The formal identity of these three immunoreactive peaks is yet to be established but their respective molecular weights have been determined chromatographically to be 170,000 daltons (peak 1); 48,000 daltons (peak 2) and 23,000 daltons (peak 3) (Fang and Refetoff,1978). They have been found in the circulation (Suh and Frantz, 1974, Guyda,1975, Garnier et al., 1978), in amniotic fluid (Fang and Kim, 1975), pituitary extracts (Suh and Frantz, 1974) and cerebro spinal fluid (Kiefer and Malarkey, 1978). Garnier et al., (1978) in a study of normal sera after TRH stimulation found the percentage distribution of prolactin immunoreactivity between peaks 1, 2 and 3 to be 5.1 $\pm$ 1.7, 9.1 $\pm$ 0.9 and 85.8 $\pm$ 2.3

respectively. These values agree well with the distribution found in MRC 75/504 reference preparation and in normal pituitary extract in the present study.

The factors which may influence the pattern of prolactin heterogeneity and possible interconversion between one component and another, remain speculative.

Peak 1 is generally presumed to be aggregated material (Suh and Frantz, 1974) and indeed it may simply be an artefact of freezing or storage (Garnier et al., 1978). Certainly, it has been shown to occur in increased amounts after freezing and thawing of serum (Suh and Frantz, 1974, Kiefer and Malarkey, 1978) and in addition, the latter authors noted increased peak 1 immunoreactivity in the same serum filtered at $4^{o}C$ rather than $23^{o}C$. They also describe considerably increased peak 1 component in cerebro spinal fluid, when compared either to serum or pituitary tissue and Gala et al., (1977) similarly found increased void immunoreactivity in serum after glucose tolerance testing. No satisfactory explanation has been offered for these findings.

Fang and Refetoff (1978) in observing the distribution of $^{125}I$ labelled prolactin in human serum have also claimed that peak 1 may be an artefactual

product caused by binding of prolactin to serum proteins. They suggest that the void peaks obtained in human tissue may result from blood contamination and the present observation of increased peak 1 immunoreactivity in sera, compared to normal or tumorous pituitary in the same patient, would support this view.

Peak 2 prolactin immunoreactivity has been variously identified as (i) a prolactin dimer, produced naturally or as an artefact of handling (Rosen and Rogol, 1974) (ii) monomeric prolactin loosely associated with another component of as yet undetermined nature (Suh and Frantz, 1974) (iii) a prolactin prohormone (Guyda, 1975).

Certainly in the present study the immunoreactivity of all three components was stable and their immunochemical properties were similar. This is consistent with previous reports (Rosen and Rogol, 1974, Suh and Frantz, 1974 and Guyda, 1975). Less consistent, however, are reports of interconversion of the various components. Suh and Frantz (1974) reported that peak 2 might redistribute between peaks 1 and 3 after repeated freezing and thawing and this observation was confirmed by Garnier et al., (1978). However, others have demonstrated no interconversion on refiltration of prolactin from tumour or sera (Guyda, 1975, Kiefer and Malarkey, 1978, Fang and Refetoff, 1978).

Radioreceptor assays have been used to assess the relative binding activity of the various filtration peaks and again results are conflicting. Similar immunological and receptor activity in all three components was found by Guyda (1975) and by Fang and Refetoff (1978). Garnier et al., (1978) however, reported that peak 2 immunoreactivity was much less active than monomeric prolactin in radio-receptor assay.

The latter authors conclude that peak 2 prolactin immunoreactivity represent a native prolactin dimer linked by intermolecular disulfide bonds, arising in the lactotroph as a post-synthetic product or derivative. They believe it does not represent a true precursor prohormone. This view is strongly supported by the work of Evans, Hucko and Rosenfeld (1977) who demonstrated that mRNA isolated from a line of functional rat pituitary tumour cells ($GH_3$) added to a cell free protein synthesizing system derived from wheat embryo, caused biosynthesis of only one protein immunoprecipitated by prolactin antiserum. The protein "pre-prolactin" represented the initial product of translation of mRNA and was only 2000-3000 daltons greater in mass than prolactin. The authors also demonstrated that the mRNA was not large enough to code for the larger forms of immunoreactive prolactin demonstrated by gel filtration.

The finding of only one peak of immunoreactive prolactin in uraemic sera was unexpected. In parallel with pregnancy and prolactinoma sera in which circulating levels of prolactin were similar, and because of the presumed prolonged half life of prolactin in uraemia, one might have expected an increase in peak 1 immunoreactivity. None was detectable nor was any peak 2 reactivity demonstrable.

A possible explanation for this observation may be that circulating uraemic toxins result in disaggregation rather than accummulation of void volume components and in addition, cause cleavage of the putative disulphide bridges linking constituent molecules of peak 2.

Moreover, although the monomer peaks in uraemic sera were broad and may consist of several components, the mean elution volume of the peak was similar to that of monomeric prolactin in hyperprolactinaemic sera and pituitary extracts; dose-response curves of this peak were parallel to that of MRC 75/504 reference preparation and there was no evidence of low molecular weight immunoreactive fragments (analagous to PTH fragments) as a result of altered renal metabolism of prolactin.

Whereas this finding was unexpected, it provides additional data to suggest that the elevated levels of prolactin in uraemic sera, however mediated, may well be of biological significance and hence of clinical relevance.

# CHAPTER VIII

## CONCLUDING REMARKS

It has been suggested that prolactin may be "the" ancestral pituitary hormone, derived from a primordial peptide by gene duplication and evolving into placental lactogen and growth hormone (Schwartz, 1973). Its apparent antiquity, versatility of action and physiological evolution during the course of vertebrate phylogeny have made prolactin the focus of much attention from comparative endocrinologists, neuroendocrinologists and zoolgists. However, only recently has it been possible for the clinician to study human prolactin status in health and disease and thus gain some appreciation of its relevance in clinical practice.

Clearly a pre-requisite for such studies is the availability of sensitive and specific methods for prolactin assay and the development of homologous radioimmunoassays as discussed in Chapters I and II, has provided this. However, suitable assay reagents remain scarce and thus currently in the United Kingdom there is no standardisation either of reagents or methods.

Consequently results from different laboratories may not be comparable and thus each centre should accumulate adequate control data using their own system (Chapter III) before embarking on routine diagnostic or special research studies. Only then may accurate interpretation of results be possible with knowledge of the precision of the method, the distribution of levels in healthy populations and appreciation of the endogenous physiological, pathological and pharmacological factors which may influence the result.

Presently, there is a national quality control scheme for prolactin, organised by the Chelsea Hospital for Women, London (Dr. S. Jeffcoate) whose aim is to make participating laboratories aware of their errors and to improve comparability of results through adoption of common reagents and procedures e.g. use of the new international reference preparation MRC 75/504 is now strongly recommended. In the future, hopefully, a plentiful source of compatible antiserum and tracer, such as those used in the present studies, will be identified and adopted, along with a common assay protocol.

In clinical practice, the diagnosis and treatment of prolactin secreting tumours of the pituitary, remain problematic for a variety of reasons. The natural

history of prolactinomas is not known; certainly, some tumours do progress with time (e.g. patient 16 in the present series) but others may not, and currently, the clinician cannot predict which course is likely in any individual patient. Moreover, there is little knowledge to enable even evaluation of the relative risk of progression of an untreated tumour. In the past, there have been diagnostic difficulties since it was suspected that normal pituitary radiology did not exclude the presence of a small prolactinoma and therapeutic problems have arisen with the availability of alternative forms of therapy viz. surgery and medical management, neither of which are without risk.

The present studies clearly demonstrate that small prolactinomas may exist even when pituitary radiology is normal and moreover a pattern of prolactin responsiveness, reliably indicating prolactinoma, has been identified. The possibility that some patients may harbour micro-adenomas that are both radiologically and biochemically occult (false negatives) has not been excluded and it is also possible that with further experience, patients may be encountered with absent responses to sleep, TRH and

metoclopramide who do not have prolactinomas (false positives). Evaluation of these possibilities requires further data on the spectrum of biochemical responses and natural history of prolactinomas. Nevertheless, the diagnostic strategy described has been clinically useful and provides a significant improvement in sensitivity of detection of small prolactinomas over conventional tomography.

In the future, general adoption of a combined biochemical and radiological classification of hyperprolactinaemic subjects should enable comparison of groups of patients treated by different methods and hence form the basis for a more rational approach to therapy. Moreover, patients with small prolactinomas, identified biochemically, may be followed prospectively and thus provide objective data on the natural history of prolactin secreting tumours.

One might anticipate that dynamic tests of prolactin secretion other than those discussed in this thesis may prove equally, if not more effective in the identification of radiologically occult prolactinomas. For example, the advantages of a single diagnostic test are obvious and recently it has been suggested that nomifensine, an antidepressant drug, fails to suppress prolactin levels

in patients with prolactinomas whilst doing so in patients with puerperal hyperprolactinaemia (Muller et al., 1978). The report however requires confirmation and the present strategy provides the basis for an objective comparison of the diagnostic accuracy of nomifensine and any future diagnostic agents.

The clinical relevance of hyperprolactinaemia in uraemia remains speculative. The data presented in Chapters V, VI and VII define prolactin abnormalities in human renal disease and provide evidence of at least two aetiological mechanisms to account for hyperprolactinaemia in chronic renal failure. They do not, however, contribute to understanding the significance of hyperprolactinaemia other than indirectly, by the demonstration of large quantities of apparently intact monomeric prolactin in uraemic sera. Assessment of the biological activity of this material, or perhaps more realistically, the binding activity in radioreceptor assay, would further strengthen the conviction that elevated prolactin levels in uraemia may indeed be of biological significance.

Elevated prolactin levels may affect the uraemic patient in two ways (1) by contributing to hypogonadism as in prolactinoma patients (2) by affecting renal

morphology and function. The first of these alternatives is likely and since symptomatic hypogonadism is both common and distressing in young uraemic males, there is ample justification for a suitably controlled, therapeutic trial of bromocriptine to improve clinical and biochemical gonadal function. Since acute administration of a small dose of bromocriptine fails to lower circulating levels of prolactin in uraemic subjects, high doses of the drug over prolonged periods may be required for effect.

The second alternative, that hyperprolactinaemia may affect renal structure and function in uraemic subjects, is more difficult to test in man. A multiplicity of factors relating to the patient's general condition and non-feasibility of serial renal tissue for examination, make it improbable that such a study would provide results which were easily interpreted or conclusive.

Under these circumstances, animal studies may be an alternative approach. Standard kidney micropuncture techniques would provide an ideal opportunity to document the effect of prolactin infusion on glomerular and tubular function in the isolated nephron of the rat.

Both healthy and diseased animals might be studied under the influence of physiological and supra physiological levels of prolactin. In addition, experimentally induced acute renal failure in the rat would enable sequential study of endocrine status with progressive uraemia and further, the effect of prolactin or bromocriptine pre-treatment, on the development of nephropathy, might be observed. Such studies might provide evidence to justify a therapeutic trial of prolactin stimulating or suppression agents in humans with declining renal function.

Finally, in man, it is also possible that endocrine dysfunction and hyperprolactinaemia in particular, may be a sensitive index of non urea and creatinine related uraemic toxicity and thus may provide a useful "marker" for the assessment of new dialysis schedules and techniques.

Thus, a knowledge of prolactin status in health and disease is now essential to a wide spectrum of clinicians from neurosurgeons to nephrologists. Hyper-prolactinaemia is common, has many causes, and although

currently knowledge of its consequences is restricted to effects on reproductive function, this may not be the case for much longer as the search for a non lactogenic function for human prolactin continues. The present studies have been concentrated on limited and specific aspects of prolactin status in health and disease but the potential for fascinating, clinically relevant research into prolactin pathophysiology in diverse situations, is enormous.

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The references are listed according to the General Regulations for Higher Degrees, University of Glasgow and the Harvard system is used. In listing references alphabetically according to the initial letter of the surname of the first author, prefixes (e.g. del) are not used (e.g. papers by del Pozo *et al.*, are listed under P). Papers by authors with surnames beginning with Mc or Mac are listed after those beginning with M and if more than one article by the same author in the same year is referred to, then they are given postscripts after the date e.g. Friesen *et al.*, (1972a) and Friesen *et al.*, (1972b). The convention of initial capitals for the first word only in the title of a paper, but throughout the title of a journal or book is adhered to.

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# HYPERPROLACTINAEMIA IN RENAL DISEASE

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SUMMARY

Basal prolactin concentrations in 357 patients with renal disease of defined pathology have been compared with those in 210 control subjects. Elevated prolactin concentrations were found in 113 renal patients (32%) including 53 patients in whom elevated concentrations were possibly attributable to drug therapy. In the remaining 60 patients who had hyperprolactinaemia not attributable to drugs, elevated concentrations ($P < 0.005$) were found exclusively in patients with impaired renal function. A significant correlation was observed between prolactin and creatinine concentrations in these patients ($r = 0.45$ $P < 0.005$) and prolactin reverted towards normal after successful renal transplantation. A significant arteriovenous prolactin concentration difference across the kidney (mean 16% range 8-29% $P < 0.02$) was found in seven patients with non-renal non-endocrine disease.

It is concluded that the hyperprolactinaemia found commonly in patients with impaired renal function is only partly attributable to drug therapy. The positive correlation between prolactin and creatinine reversion of prolactin towards normal after successful transplantation and arteriovenous hormone concentration differences across the normal kidney suggests that the kidney has a important role in prolactin metabolism. Abnormal regulation of prolactin secretion in renal failure may also be involved.

Elevated serum prolactin concentrations occur commonly in patients with renal disease. Chirito *et al.* (1972) reported that 20% of patients with chronic renal failure had hyperprolactinaemia, and elevated prolactin concentrations were described in patients on maintenance haemodialysis by Nagel *et al.* (1973) and Czernichow *et al.* (1976). The significance of these observations is not clear, however, since renal patients commonly take drugs, such as methyldopa, which are known to alter plasma prolactin and the role of the kidney in prolactin metabolism has not been defined.

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We have determined the prevalence of hyperprolactinaemia in 357 patients with renal disease and assessed the relationship of hyperprolactinaemia with the underlying pathology, creatinine concentration, duration of uraemia and drugs administered. Arteriovenous concentration differences of prolactin across the normal kidney have also been measured.

## METHODS

### *Patients studied*

Blood samples were taken from 357 patients (187 M, 170 F, mean age 34.7y, range 14-75y) with renal disease. The patients comprised two main groups: (1) *Patients with impaired renal function* (n = 210): These included moderate chronic renal failure (CRF) (creatinine < 600 μmol/l) (n = 87), severe C.R.F. (creatinine > 600 μmol/l) (n = 28), regular dialysis therapy (R.D.T.) (n = 52), including both home and hospital dialysed patients, and patients after successful renal transplantation (n = 43). (2) *Patients with normal renal function*, but renal disease of the following pathologies: 'Glomerulonephritis' (G.N.) (n = 56), Chronic pyelonephritis (CPN) (n = 38), recurrent urinary tract infection (UTI) (n = 12), calculus disease (n = 14), polycystic kidneys (n = 12) and systemic lupus erythematosus (SLE) (n = 15).

The diagnoses were made by clinical, radiological and, where appropriate, histological criteria.

All renal patients studied were hospital outpatients and samples were taken at routine clinic visits, the majority being taken between 9.00 am and 12 mid-day under conditions of minimal stress. Dialysis patients were venesected immediately before haemodialysis. An elevated prolactin concentration was confirmed on at least one further sample. Meticulous drug histories were taken from all patients, with particular reference to drugs known to affect prolactin concentrations. The most commonly prescribed drugs taken by the renal patients were methyldopa, prednisolone and tricyclic antidepressants. All dialysis patients were taking vitamin supplements (as orovite, fefol and ascorbic acid) and aluminium hydroxide capsules. Four dialysis patients were taking 1α hydroxy vitamin $D_3$ for renal osteodystrophy. All patients after renal transplantation received prednisolone and azathioprine. Samples were also obtained from the renal artery and renal vein in seven patients undergoing elective left and right heart catheterization for diagnostic purposes. None of these patients had any evidence of renal or endocrine disease, nor were they taking any medication known to alter plasma prolactin concentration. All renal artery and renal vein samples were assayed in the same batch.

Resting blood samples were also taken from 190 control subjects, comprising hospital staff and patients without endocrine disease or not taking drugs known to affect prolactin secretion. Additional samples were taken from 20 'chronic sick' patients who had chronic progressive arthropathy for at least 3 years, requiring numerous hospital admissions, and who were taking only non-steroidal anti-inflammatory drugs.

### *Assay*

Serum prolactin was measured by a specific radioimmunoassay using MRC prolactin preparation 75/504 for standardisation. $^{125}$I-labelled prolactin was prepared by lactoperoxidase iodination of purified human prolactin (Dr H. Friesen, h PRL 75.7.10) and repurified by Sephadex G150 (15 cm × 0.6 cm) column chromatography immediately before each assay. FR AR7-13 antiserum (Dr H. Friesen) was employed at a final dilution of 1:28,000.

Labelled prolactin (50 pg) was added after one day pre-incubation of antiserum with test sample and the incubation continued for a further 1 day. Bound and free fractions were separated by double antibody using a donkey anti rabbit serum (Wellcome Reagents Code RD17). The detection limit of the assay was 20 mU/L which corresponds to 1 ng/ml in the VLS-NIH preparation. The mean within assay coefficient of variation was 5% and between assay variation 10% over the clinically relevant range.

*Statistics*

Since the distribution of prolactin concentrations in control subjects and renal patients was non gaussian the Mann Whitney test has been used to assess statistical significance.

## RESULTS

*Control Subjects*

The reference prolactin range obtained in hospital staff and patients and in the 'chronic sick' patients was 50–360 mU/L (mean 172). There was no significant difference between these groups nor was there a significant sex difference.

Table 1. Incidence of hyperprolactinaemia in renal patients receiving one or more of three commonly prescribed medications

| Group | Methyldopa | Prednisolone | Tricyclic anti-depressants |
|---|---|---|---|
| Renal failure (n = 63) | 31/50 (62%) | 2/8 (25%) | 5/5 (100%) |
| Renal disease (n = 33) | 8/21 (38%) | 8/12 (67%) | – |

Table 2. Prolactin concentrations in patients with renal disease with or without impaired renal function

| Group | N | Mean creatinine μmol/l | Mean prolactin mU/L | Absolute range prolactin mU/L |
|---|---|---|---|---|
| *Normal renal function* | | | | |
| GN | 45 | 105 | 178 | 88–1400 |
| CPN | 30 | 96 | 175 | 68–312 |
| UTI | 9 | 111 | 162 | 70–366 |
| Calculus | 13 | 84 | 173 | 68–324 |
| Polycystic kidneys | 11 | 82 | 188 | 71–360 |
| SLE | 7 | 99 | 197 | 117–310 |
| *Impaired renal function** | | | | |
| C.R.F. creatinine < 600 μmol/l | 62 | 292 | 270 | 88–1670 |
| C.R.F. creatinine > 600 μmol/l | 13 | 1140 | 562 | 123–1300 |
| RDT | 37 | 1280 | 880 | 253–4500 |
| Post renal transplantation† | 43 | 159 | 382 | 100–2000 |

*Many patients in this group on oral iron, vitamins, aluminium hydroxide.
†All patients in this group on prednisolone and azathioprine.

*Renal Patients*

Elevated prolactin concentrations (> 360 mU/L) were found in 113 (32%) of all renal patients. In 53 (47%) of these patients, elevated concentrations (range 400-6454 mU/L, mean 1849) were possibly attributable to drug therapy (methyldopa n = 39, prednisolone n = 8, tricyclic antidepressants n = 5). Not all patients on these drugs had elevated prolactin concentrations however, and the prevalence of hyperprolactinaemia in patients receiving these drugs is shown in Table 1.

Excluding patients receiving one or more of these three drugs, the prolactin concentrations were not significantly elevated in patients with renal disease and normal renal function (Table 2). The single exception was a 42-year-old female with glomerulonephritis, who was post-menopausal with primary infertility and who had persistently elevated prolactin concentrations (mean 1400 mU/L). Further investigation of this patient has revealed that

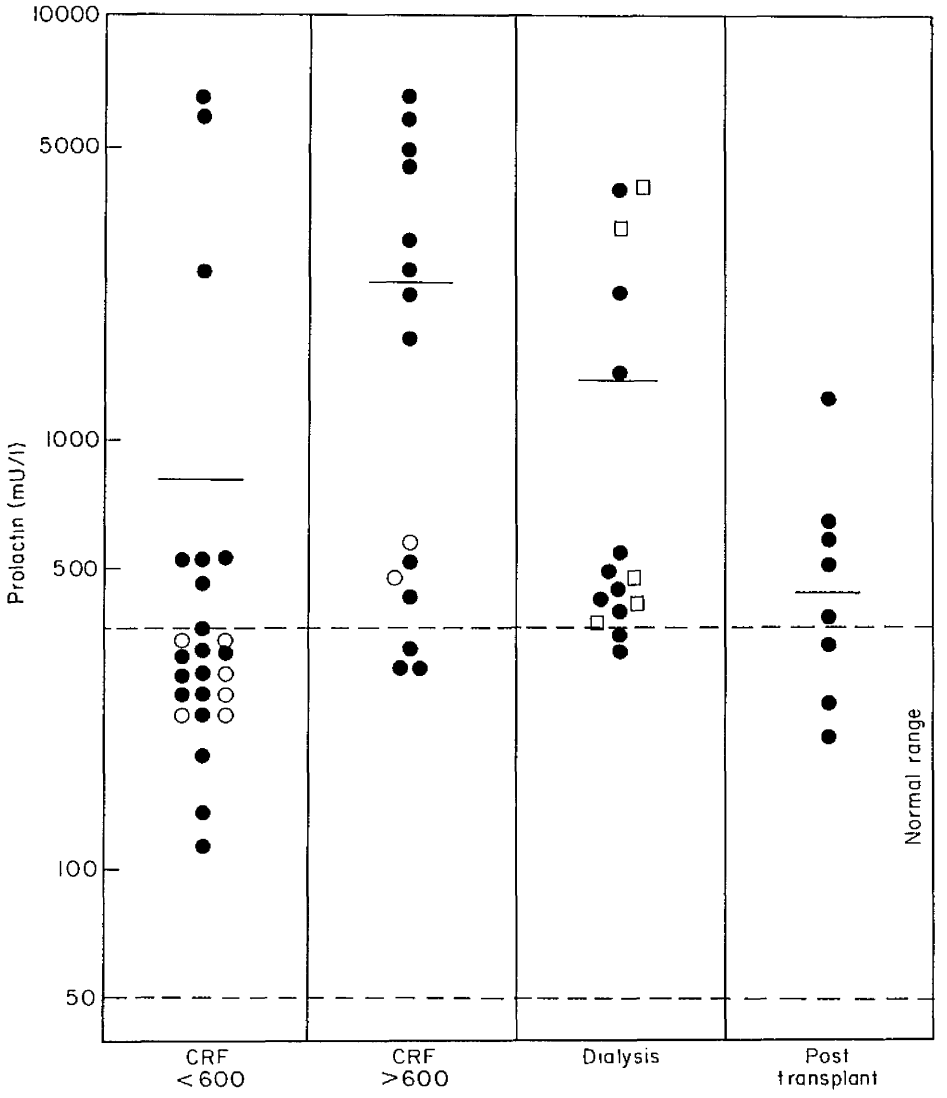


Fig. 1. Prolactin concentration in patients with impaired renal function, who were receiving one or more drugs known to affect prolactin level. • Methyldopa; ○ Prednisolone; □ Tricyclic antidepressants. (Dotted lines indicate the absolute reference range; horizontal bars indicate the mean prolactin concentration in each group studied.)

although she has normal visual fields, there is marked asymmetry of the pituitary fossa on tomography with evidence of bone erosion, suggestive of the presence of a pituitary tumour.

In contrast, plasma prolactin concentrations in patients with impaired renal function were significantly elevated ($P < 0.005$) (Table 2), both in patients on drug therapy (Fig. 1) and in those not taking any medication known to affect plasma prolactin (Fig. 2). There was a significant correlation ($P < 0.005$) between prolactin and creatinine concentrations ($r = 0.45$) but no correlation was found between prolactin concentrations, and age, sex, underlying diagnosis or duration of uraemia. Patients who had undergone successful renal transplantation had significantly lower prolactin concentrations than those patients undergoing regular dialysis treatment ($P < 0.001$).

There was a significant decrease in prolactin concentration across the kidney in seven patients with non-renal, non-endocrine disease (Table 3) ($P < 0.02$, mean fall 16%, range 8-29%).

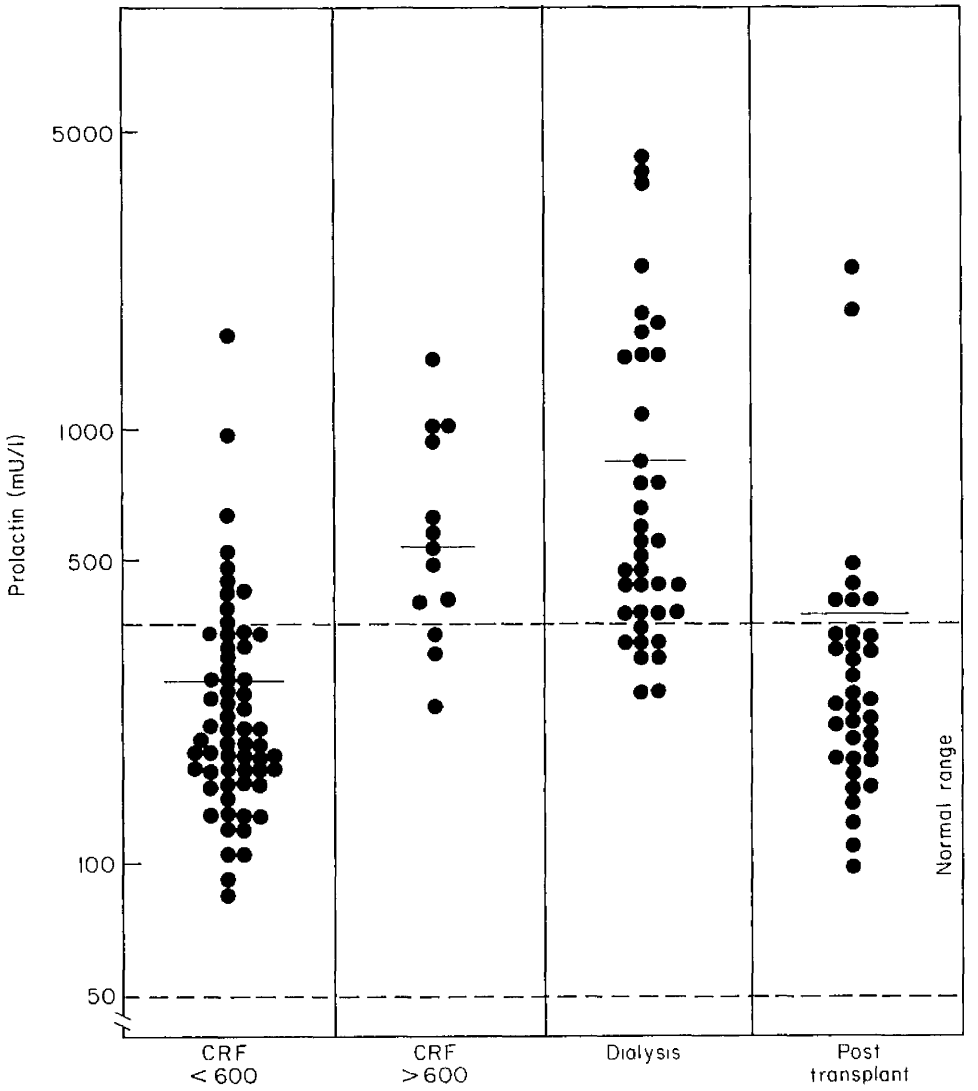


Fig. 2. Prolactin concentration in patients with impaired renal function who were on no medication known to alter prolactin secretion. (Dotted lines indicate the absolute reference range; horizontal bars indicate the mean prolactin concentration in each group studied.)

Table 3. Arteriovenous concentration difference of prolactin across the normal kidney

| Patient | Prolactin mU/L | | % fall in PRL |
|---|---|---|---|
| | Renal artery | Renal vein | |
| 1 | 360 | 330 | 8 |
| 2 | 205 | 150 | 27 |
| 3 | 242 | 215 | 11 |
| 4 | 163 | 148 | 9 |
| 5 | 146 | 103 | 29 |
| 6 | 370 | 313 | 15 |
| 7 | 342 | 287 | 16 |
| Mean | 261 | 219 | 16 |

## DISCUSSION

We have demonstrated that hyperprolactinaemia is commonly associated with renal disease, 32% of all renal patients studied having concentrations above the upper limit of the reference range (> 360 mU/L). Various commonly prescribed medications. e.g. methyldopa and neuroleptic drugs are known to affect prolactin concentrations (Steiner *et al.*, 1976, de Rivera *et al.*, 1976, Turkington, 1972) and in the present study drug therapy was a possible aetiological factor in 53 (47%) of all patients with hyperprolactinaemia. Methyldopa and tricyclic antidepressants specifically were associated with hyperprolactinaemia but in addition, many patients, notably those with SLE, who were taking prednisolone therapy, had elevated prolactin concentrations. This may represent a true association between prednisolone and hyperprolactinaemia, or alternatively, the relationship may be between hyperprolactinaemia and SLE itself. However, in the case of methyldopa administration our data indicate that 62% of patients with impaired renal function have elevated prolactin concentrations compared with 38% of those patients with normal renal function. This suggests that within the renal failure group, some patients had hyperprolactinaemia which was not solely due to drug therapy. Excluding patients taking these drugs, it was found that renal patients with normal kidney function had prolactin concentrations within the reference range, indicating the renal pathology, *per se*, was not associated with an elevated plasma prolactin concentration.

In contrast, patients with impaired renal function had significantly elevated prolactin concentrations, confirming earlier reports (Chirito *et al.*, 1972, Nagel *et al.*, 1973). In addition we have demonstrated a progressive rise in prolactin concentrations as renal function deteriorates, in patients both on and off drug therapy known to affect prolactin secretion. The correlation between creatinine and prolactin concentrations confirms that reported by Chirito *et al.* (1972) and the changes after renal transplantation support the view that restoration of renal function is associated with reversion of prolactin concentrations towards normal.

These findings, in conjunction with the observation that there is a consistent fall in prolactin concentration across the normal kidney, suggest that the hyperprolactinaemia of renal failure, is attributable, in part, to altered renal metabolism. This mechanism may also explain the elevated concentrations of other hormones found in chronic renal impairment e.g. $\beta$ MSH-like immunoactivity (Gilkes *et al.*, 1975, Smith *et al.*, 1975). In man,

there is evidence for the renal extraction of other peptides, e.g. insulin (Fine *et al.*, 1976) and the isolated, perfused dog kidney has been shown to degrade parathyroid hormone with production of immunoactive fragments (Hruska *et al.*, 1977). Moreover, using a fluorescein-labelled double antibody technique, it has been shown that in the rat, ovine prolactin gains access to the proximal tubular cells of the kidney by means of the glomerular filtrate (Donatsch & Richardson, 1975). It is therefore suggested that as the glomerular filtration rate falls with progressive renal failure, plasma hormone clearance also declines, resulting in elevated circulating concentrations.

These data do not exclude the possibility of deranged hypothalamic-pituitary control mechanisms in chronic uraemia. Lim *et al.* (1977) have given a detailed account of thyroid dysfunction in chronic renal failure, which includes a subnormal pituitary TSH response to TRH; this abnormality has also been noted by Czernichow *et al.* (1976) who, in addition, observed an impaired prolactin response to TRH stimulation.

The significance of the hyperprolactinaemia of renal failure in man remains speculative. It is established that prolactin has a fundamental osmoregulatory role in many species of fish and amphibians (Lam, 1972) and Dobbie *et al.* (1977) have reported that the prolactin related 'occlusive glomerular hyperplasia' seen in migrating fish shares many features with proliferative glomerulonephritis in man. It has also been demonstrated that the prolactin concentration affects the severity of the chronic progressive nephropathy seen in some species of rat (Richardson & Luginbuhl, 1976). In man the possible role of prolactin as an osmoregulator remains controversial. Since the initial observation (Horrobin *et al.*, 1971) that i.m. ovine prolactin reduced renal excretion of water, sodium and potassium in normal males, it has been reported that oral water loading of normal subjects produces a 50% suppression of serum prolactin from baseline concentrations and that this test is a useful discriminator between functional and tumour-associated hyperprolactinaemia (Buckman *et al.*, 1973). These authors have also reported a decrease in osmolar clearance by the kidney in six subjects with small pituitary adenomas, associated with hyperprolactinaemia (Buckman *et al.*, 1976). However, others have failed to confirm these results (Adler *et al.*, 1975, Baumann & Loriaux, 1976, Baumann *et al.*, 1977). The latter authors conclude that prolactin is not an important osmoregulatory hormone in man. Further investigation of the possible inter-relationship of prolactin and plasma osmolality in normal and hyperprolactinaemic individuals is clearly required to clarify the situation.

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# Laboratory assessment of prolactin status

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SUMMARY The laboratory assessment of prolactin status was evaluated by detailed study of 921 subjects (587 normal subjects and 334 patients with pathological conditions). The effect on serum prolactin levels of age, sex, circadian rhythm, pulsatility of secretion, stress, drug ingestion, and pregnancy was defined in normal subjects. The normal prolactin responses to stimulation (TRH, metoclopramide) and suppression (L-dopa, bromocriptine) were also determined. Basal prolactin levels were measured in patients with defined pathological conditions including prolactinoma, idiopathic hyperprolactinaemia, acromegaly, Cushing's disease, chronic renal failure, primary hypothyroidism, pituitary ablation, Kallman's syndrome, Nelson's syndrome, growth hormone deficiency, gonadotrophin deficiency, craniopharyngioma, panhypopituitarism, and chronic progressive arthropathy. Based on these data, a strategy for the routine laboratory assessment of prolactin status is outlined.

Measurement of serum prolactin concentration is now an essential part of the routine investigation of patients with suspected hypothalamic-pituitary dysfunction (Friesen *et al.*, 1972; Kleinberg *et al.*, 1977); indeed, hyperprolactinaemia is probably the most frequent hypothalamic-pituitary disorder seen in clinical practice, commonly presenting with amenorrhoea and infertility in women and impotence in men (Friesen and Hwang, 1973; Tyson *et al.*, 1975). Gross abnormalities of prolactin secretion are readily detected by the measurement of basal prolactin levels, and, in general, the higher the level, the greater the chance that the patient has a prolactin-secreting adenoma (Kleinberg *et al.*, 1977). However, pituitary microadenomas are often associated with relatively modest degrees of hyperprolactinaemia and as such may be difficult to distinguish from the many physiological and pathological conditions also associated with this biochemical finding (Table 1).

We have assessed the importance of some of the factors that are relevant to the interpretation of basal prolactin levels by determining detailed reference ranges in defined groups of patients. We have also compared serum prolactin responses to standardised dynamic tests in normal subjects since these tests may be useful in the diagnosis of pituitary microadenomas in patients with modest hyperprolactinaemia (up to 1000 mU/l) and normal pituitary tomography.

Table 1 *Hyperprolactinaemia*

| *Physiological*, eg | *Pathological*, eg |
|---|---|
| i Newborn | i Hypothalamic disorders |
| ii Pregnancy | ii Pituitary disorders |
| ii Nursing mothers | iii Hypothalamic–pituitary stalk 'irritation', eg, head injury |
| iv Stress | iv Primary hypothyroidism |
| v Sleep-related nocturnal rhythm | v Renal disease |
| vi Drugs (*a*) dopamine receptor blockers, eg, phenothiazines, metoclopramide | vi Idiopathic |
| (*b*) dopamine depleting agents, eg, α methyl dopa, reserpine | |
| (c) others, eg, oestrogens, TRH | |

## Subjects studied and methods

Prolactin levels were studied in 921 subjects. None was receiving drugs known to affect prolactin secretion unless stated. Samples were taken between 0900 and 1200 unless otherwise indicated. Abnormal prolactin levels were confirmed on at least one further specimen. The following groups were studied:

### NORMAL SUBJECTS

(*a*) *Basal levels*

(i) *Hospital personnel* 100 subjects (50 men and 50 women, mean age 31, range 17-55 years).

(ii) *Hospital inpatients* 50 subjects (30 men and 20 women, mean age 48, range 18-86 years) without evidence of renal, endocrine, or malignant disease.

(iii) *Hospital outpatients* 163 women (mean age 39, range 17-71 years) attending a gynaecology outpatient clinic for the first time. Patients with amenorrhoea or infertility were excluded. If prolactin levels were elevated, a second specimen was taken at the next visit to obtain an estimate of possible stress factors related to hospital attendance.

(iv) *Women on oral contraceptive preparations as the sole drug* 54 women (mean age 32, range 22-41 years). These patients were sampled between 1800 and 2000.

(v) *Women at various stages of uncomplicated pregnancies* 104 women (mean age 28, range 17-38 years). Samples were taken at routine clinic visits between 1400 and 1500.

(vi) *Children with benign, non-endocrine disease* 11 normal babies (6M, 5F) aged less than 3 months and 30 children (13M, 17F) mean age 5, range 3-13, years.

*(b) Serial levels*

(i) *Within-day variation* was studied in six subjects (4M, 2F, mean age 27, range 24-30 years). Blood samples were taken via an intravenous cannula at hourly intervals from 0800 to 2000 during which time subjects maintained normal activity.

(ii) *Day-to-day variation* was studied in 10 subjects (6M, 4F, mean age 28, range 19-39 years) sampled daily for five days between 0900 and 1200.

(iii) *Possible stress of venepuncture* was studied in 12 men (6 hospital staff and 6 patients, mean age 27, range 25-34 years). The patients were to undergo elective surgery for non-endocrine conditions the next day. Samples were taken from an intravenous cannula at 10-minute intervals for 90 minutes.

## PATHOLOGICAL CONDITIONS

*Hypothalamic-pituitary disease*

(i) *Miscellaneous hypothalamic-pituitary disorders* This heterogenous group consisted of 27 patients (15M, 12F, mean age 31, range 12-64 years) with the following disorders: Kallman's syndrome, n = 4 (4M); Nelson's syndrome, n = 3 (1M, 2F); growth hormone (GH) deficiency, n = 5 (4M, 1F); craniopharyngioma, n = 5 (5F) (2 patients post-surgical treatment, 1 patient post radiation therapy); gonadotrophin deficiency, n = 4 (4M), panhypopituitarism, n = 6 (2M, 4F). Diagnosis in all cases was made on clinical, biochemical, and radiological evidence, and all patients were untreated at the time of sampling apart from the three patients noted. Patients with 'panhypopituitarism' had no clinical or biochemical evidence of any specific endocrinopathy.

(ii) *Pituitary tumours* This group consisted of 42 patients (10M, 32F, mean age 32, range 16-69 years) in the following categories: prolactinomas, n = 16 (1M, 15F); acromegaly, n = 15 (6M, 9F); Cushing's disease, n = 11 (3M, 8F). Diagnosis in all cases was established on clinical and biochemical grounds together with light and electron microscopy and immunoperoxidase studies on surgically removed tissue. In a number of cases, samples were obtained both before and after selective, curative surgical treatment.

*Idiopathic hyperprolactinaemia*

This group consisted of 10 women (mean age 26, range 16-34 years) who presented with galactorrhoea and/or amenorrhoea and infertility and who had persistent hyperprolactinaemia (ie, elevated PRL levels on at least two separate occasions) with normal pituitary tomography, and no other factor identified to account for their elevated prolactin levels.

*Chronic sick*

Twenty subjects (8M, 12F, mean age 42, range 27-59 years) who had had chronic progressive arthropathy for more than three years were studied. All patients were taking non-steroidal anti-inflammatory agents as the sole medication.

*Renal failure*

(i) Moderate chronic renal failure (creatinine $< 600$ $\mu$mol/l) 62 subjects (32M, 30F, mean age 41, range 14-75 years); (ii) severe chronic renal failure (creatinine $> 600$ $\mu$mol/l) 13 subjects (9M, 4F, mean age 37, range 18-49 years); (iii) regular dialysis treatment (mean creatinine 1300 $\mu$mol/l) 37 subjects (17M, 20F, mean age 33, range 16-52 years). None of these patients was on any drug therapy known to affect prolactin levels, nor had they evidence of clinical endocrinopathy apart from five of the male dialysis patients who were impotent.

*Primary hypothyroidism*

This group consisted of 50 women (mean age 44, range 24-78 years) with gross primary hypothyroidism (mean T4 = 27 nmol/l, range $<$11-48; mean T3 = 0·6 nmol/l, range $<$0·4-1·8; TSH all $>$50 mU/l). None of these patients had clinical evidence of any other endocrine abnormality.

*Pituitary ablation*

This group comprised five patients (3M, 2F, mean age 32, range 27-49 years) who were insulin-dependent diabetics and who had had total hypophysectomy as a therapeutic manoeuvre for severe

proliferative retinopathy. Macroscopically, hypophysectomy had been complete, and samples were obtained from seven months to two years postoperatively.

*Drug therapy*

Samples were obtained from 68 patients (38M, 30F, mean age 37, range 16-72 years) who were on long-term therapy with drugs which may affect basal prolactin levels, *viz*, phenothiazines, n = 21; α-methyl dopa, n = 35; tricyclic antidepressants, n = 8; metoclopramide, n = 2; reserpine, n = 2).

DYNAMIC TESTS OF PROLACTIN SECRETION

(i) Thyrotrophin releasing hormone (TRH—Roche), 200 μg, was given intravenously to 24 healthy subjects (14M, 10F, meanage 29, range 24-33 years) and blood was collected by serial venepuncture at 0, 20, and 60 minutes.

(ii) Maxolon (metoclopramide monohydrochloride—Beecham), 10 mg, was given intravenously to 12 subjects (6M, 6F, mean age 28, range 25-33 years), and blood samples were withdrawn at 0 15, 30, 60, and 120 minutes.

(iii) L-dopa (Roche), 500 mg orally, was taken by eight subjects (5M, 3F, mean age 29, range 26-33 years), and blood samples were withdrawn at 0, 1, 1·5, 2, 3, 5, and 7 hours after ingestion of the drug.

(iv) Parlodel (Bromocriptine—Sandoz), 2·5 mg orally, was taken by three subjects (2M, 1F, mean age 28, range 26-30 years) after a meal, and blood samples were collected at 0, 1, 1·5, 2, 3, 5, and 7 hours thereafter.

Whereas in the TRH tests blood was collected by repeated venepuncture, in all other tests samples were taken via an indwelling, intravenous cannula kept patent by heparinised saline. All subjects studied were fit and healthy and on no drug therapy; they maintained normal activity during these tests. No side effects were noted apart from postural hypotension and vomiting after bromocriptine administration, thus restricting the number of subjects receiving this test.

RADIOIMMUNOASSAY OF PROLACTIN

Serum prolactin levels were measured by a specific radioimmunoassay using MRC prolactin preparation 75/504 (650 mU/ampoule) as standard. Purified human prolactin (Dr. H. Friesen 75.7.10) was iodinated by the lactoperoxidase method and purified by Sephadex G150 chromatography; the monomer peak was retained for use as tracer. Before its addition to an assay, the tracer was further repurified by Sephadex G150 chromatography in a 10 ml disposable pipette. 50 μl aliquots of standard or sample were incubated with 50 μl rabbit anti-prolactin serum (FR AR 7-13, Dr. H. Friesen) at a final dilution of 1:28 000. After overnight incubation at 4°, repurified $^{125}$I-labelled prolactin (50 μl) was added, and the mixture was incubated for 24 hours at 4°. Bound and free fractions were separated after a further overnight incubation with precipitating donkey anti-rabbit serum.

The detection limit of the assay was 30 mU/l, and mean precision was 5% CV (within batch) and 10% CV (between batch).

Prolactin levels in serum and plasma were not significantly different, nor in specimens frozen immediately in a dry ice mixture or allowed to freeze at −20°. Serum samples were routinely assayed, blood being separated within 12 hours of sampling, and serum was stored at −20° until assay.

STATISTICAL METHODS

Since the distribution of prolactin levels within the groups studied was non-Gaussian, the Mann-Whitney test was used to assess statistical significance.

## Results

NORMAL SUBJECTS—BASAL SAMPLING

The range of prolactin levels seen in hospital personnel and inpatients (the control range) was 60-375 mU/l (Table 2, Fig. 1). Compared with controls, significantly elevated prolactin levels

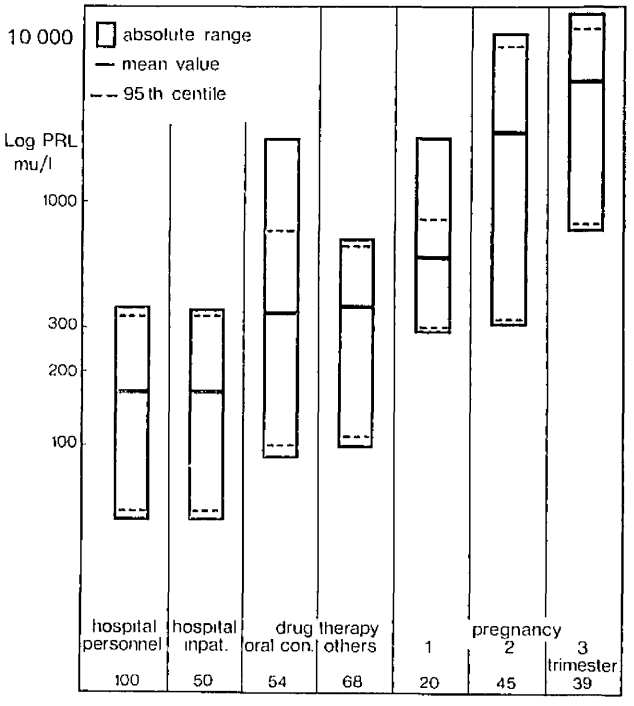


Fig. 1 *Basal prolactin levels in 'normal' subjects.*

Table 2 *Basal prolactin level (mU/l)*

| *Subjects group** | *n* | *Mean* | *Absolute range* | *Upper 95th centile* |
|---|---|---|---|---|
| Hospital personnel | 100 | 168 | 50-375 | 340 |
| Hospital inpatients | 50 | 169 | 50-360 | 320 |
| Children < 3mth | 11 | 4133 | 543-13 000 | — |
| 3mth-12yr | 30 | 128 | 85-299 | 250 |
| Normal pregnancy | | | | |
| Trimester 1 | 20 | 590 | 290-1750 | 830 |
| 2 | 45 | 1962 | 304-4760 | 4200 |
| 3 | 39 | 3166 | 770-5700 | 5000 |
| Oral contraceptive | 54 | 356 | 90-1720 | 750 |
| Drug therapy | 68 | 370 | 100-710 | 680 |
| *Pathological conditions* | | | | |
| Chronic sick | 20 | 185 | 70-370 | 310 |
| Miscellaneous hypothalamic–pituitary disorders | 27 | 368 | 115-2800 | 700 |
| Pituitary tumours | | | | |
| Prolactinomas | 16 | 8140 | 590-18 000 | 10 000 |
| Acromegaly | 15 | 490 | 62-2400 | 684 |
| Cushing's syndrome | 11 | 320 | 68-560 | 450 |
| Idiopathic hyperprolactinaemia | 10 | 826 | 440-1240 | 1189 |
| Chronic renal failure | | | | |
| Moderate | 62 | 270 | 88-1670 | 700 |
| Severe | 13 | 562 | 123-1700 | 1100 |
| Dialysis | 37 | 880 | 253-4500 | 4000 |
| Primary hypothyroidism | 50 | 491 | 115-2400 | 1400 |
| Pituitary ablation | 5 | < 50 | < 50 | — |

*As defined in text

($P < 0{\cdot}0005$) are noted even in the first trimester of pregnancy, and concentrations rise until term with a wide scatter. Oral contraceptive therapy and long-term therapy with other defined drugs are associated with significant hyperprolactinaemia ($P < 0{\cdot}005$).

Eighteen of 163 patients (11%) attending a gynaecology clinic for the first time were found to have prolactin levels outwith the control range. On subsequent sampling 12 of these patients (7%) were found to have levels within the upper end of the control range while the remaining six patients had persistent hyperprolactinaemia. Four of these patients were found subsequently to be taking drugs known to be associated with hyperprolactinaemia (oral contraceptive therapy n = 3, metoclopramide n = 1). Two patients (1000 and 1614 mU/l) had unexplained hyperprolactinaemia and are now undergoing detailed assessment of their hypothalamic-pituitary function.

No significant sex difference in basal prolactin levels was observed, as may be seen in Fig. 2, which shows the relationship between prolactin level and age in both men and women as well as in children. Significantly elevated prolactin levels ($P < 0{\cdot}005$) are seen in infants under the age of 3 months when compared both with older children and with adults. Although, in male subjects, there is a trend towards lower basal prolactin levels with increasing age, this does not reach statistical significance. In female subjects, however, the trend is more marked, and women of over 50 years have significantly lower prolactin levels ($P < 0{\cdot}005$) than younger women.

NORMAL SUBJECTS—SERIAL SAMPLING

There was no significant trend in basal prolactin concentration in any of the 12 subjects in whom venesection was carried out at 10-minute intervals after insertion of an indwelling intravenous cannula,

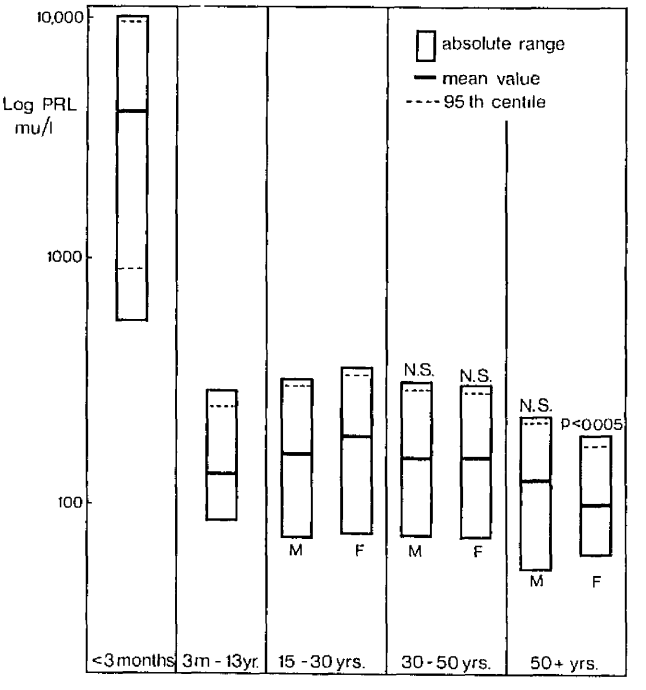


Fig. 2 *Age and sex related prolactin levels.*

the mean CV for the group being 11% (range 7-17%).

Throughout the day, however, a clear circadian pattern of prolactin secretion was observed (Fig. 3). Between 0800 and 0900 (mean PRL 236m U/l, range 100-414) and between 1700 and 2000 (mean PRL 195 mU/l, range 86-291) prolactin levels were significantly higher ($P < 0.005$) than between 0900 and 1200 (mean PRL 133 mU/l, range 71-208).

In addition, circulating prolactin varies in the same individual from day to day, the degree of variability being slightly, though not significantly, more marked in female (mean CV 28%, range 25-32%) than in male subjects (mean CV 20%, range 9-29%).

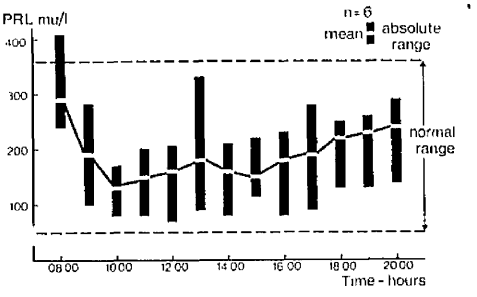


Fig. 3 *Within-day variation in prolactin levels.*

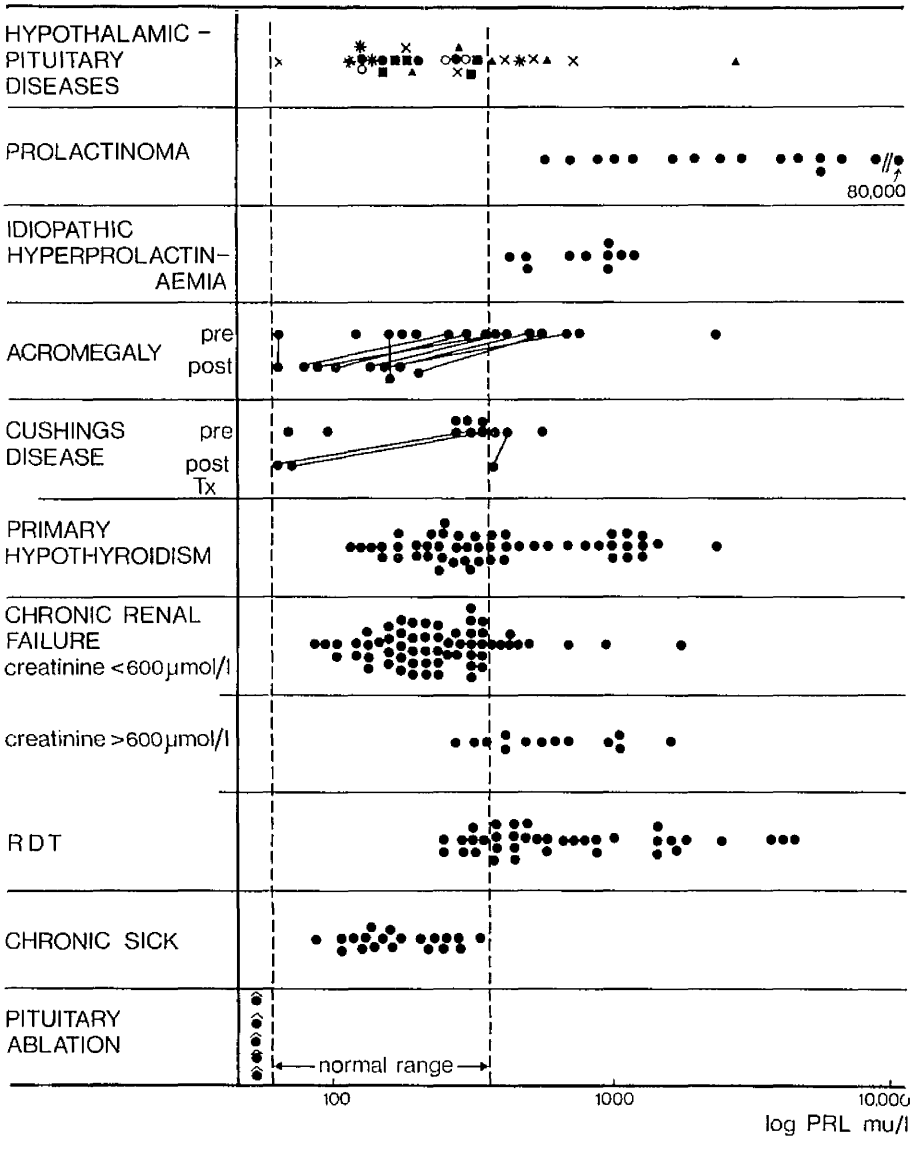


Fig. 4 *Basal prolactin levels in pathological conditions.* ●*Kallman's;* ○*Nelson's;* ■ *GH deficiency;* ▲ *craniopharyngioma;* ✳ *Gn deficiency;* × *panhypopituitarism.*

PATHOLOGICAL CONDITIONS

The range of prolactin levels seen in a defined selection of pathological conditions is shown in Table 2 and Figure 4.

Of patients with miscellaneous hypothalamic-pituitary disease, only three diagnostic categories were associated with abnormal prolactin levels, viz:

(i) Craniopharyngioma—in which one untreated patient had an elevated prolactin level of 600 mU/l, and a further patient, treated by external irradiation, had a grossly elevated level of 2800 mU/l.

(ii) One out of four patients with isolated gonadotrophin deficiency had a persistently modestly elevated basal prolactin level at 480 mU/l.

(iii) Three of the five patients who otherwise showed panhypopituitarism were found to have modestly elevated prolactin levels (up to 750 mU/l), in contrast to the uniformly undetectable levels seen in patients with panhypopituitarism after elective surgical pituitary ablation.

Most patients who had prolactin-secreting tumours of the pituitary gland, as expected, had grossly elevated circulating prolactin levels. However, five of these patients (31%) had only moderately elevated levels (up to 1200 mU/l), and there was overlap in basal prolactin levels with patients who had idiopathic hyperprolactinaemia.

Seven of 15 (47%) untreated acromegalic patients were found to have hyperprolactinaemia; in all but two of the nine patients who underwent selective curative surgical treatment, prolactin levels were significantly ($P < 0{\cdot}005$) reduced by the treatment. A group of patients with active Cushing's disease had a mean prolactin level significantly higher ($P < 0{\cdot}05$) than that of a group of control subjects, although only three of the patients had frankly abnormal levels. As in acromegalic subjects, selective, curative microsurgery was associated with a fall in prolactin level.

The prevalence of hyperprolactinaemia in female patients who were hypothyroid was 40% (20 patients), the levels ranging from 360 to 2400 mU/l.

Hyperprolactinaemia occurred in 10 patients (16%) with moderate chronic renal failure but was more common both in severe chronic renal failure (77%) and in dialysis patients (78%).

Patients with chronic progressive arthropathy had prolactin levels within the reference range.

DYNAMIC TESTS OF PROLACTIN SECRETION

Administration of TRH (Fig. 5a) and metoclopramide (Fig. 5b) resulted in a prompt rise in circulating prolactin levels. The response evoked was greater in magnitude ($P < 0{\cdot}005$) and more prolonged in duration after metoclopramide administration. In response to both stimulation tests, female subjects had significantly greater prolactin reserve than did male subjects. This finding is in contrast to the prolactin response to suppression tests which was similar for both male and female subjects. Bromocriptine administration resulted in a more profound and prolonged suppression of prolactin levels than did L-dopa (Table 3).

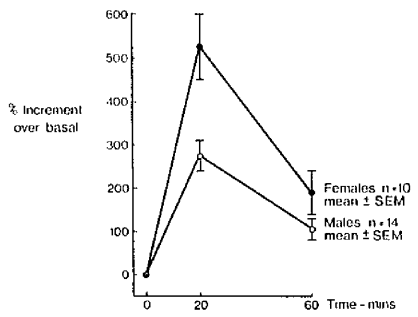


Fig. 5a *Prolactin response to TRH stimulation in 'normal' subjects.*

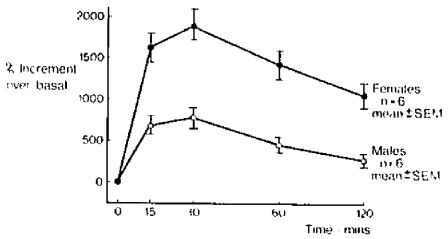


Fig. 5b *Prolactin response to metoclopramide stimulation in 'normal' subjects.*

Table 3 *Prolactin responses to stimulation and suppression test: normal subjects*

| | Sex | n | Mean PRL concentration difference % | Range |
|---|---|---|---|---|
| *Stimulation tests** | | | | |
| TRH | M | 14 | 278 | 64-474 |
| | F | 10 | 528 | 272-955 |
| Metoclopramide | M | 6 | 721 | 428-1039 |
| | F | 6 | 1940 | 1267-2946 |
| *Suppression tests*** | | | | |
| L-Dopa | M + F | 6 | 63 | 44-72 |
| Bromocriptine | M + F | 3 | 86 | 73-95 |

*Response calculated as: $\frac{\text{Peak PRL after stimulation}}{\text{Basal PRL}} \times 100$

**Response calculated as:

$$100 - \left(\frac{\text{Trough PRL after suppression}}{\text{Basal PRL}} \times 100\right)$$

## Discussion

The basal prolactin levels seen in healthy neonates, children, and adults in this study are consistent with those previously described in smaller groups of subjects (Friesen and Hwang, 1973; Guyda and Friesen, 1973). Even in healthy individuals, however, several factors may affect prolactin status, and appropriate interpretation of a given result demands adequate reference data in relation to factors such as sex, age, circadian rhythm, pulsatility of secretion, stress, and drug ingestion (Jeffcoate, 1978).

Mean basal prolactin levels are usually higher in female than in male subjects (Ehara *et al.*, 1973; Friesen and Hwang, 1973) though with a considerable overlap in their ranges (Frantz, 1978). In our study, no statistically significant sex difference was observed, although no account was taken of the stage of the menstrual cycle in women. Some authors have demonstrated significantly higher prolactin levels in the peri-ovulatory and luteal phases of the cycle when compared with follicular phase levels (Franchimont *et al.*, 1976) but other investigators have failed to demonstrate any consistent pattern in circulating prolactin during the menstrual cycle (Ehara *et al.*, 1973; McNeilly and Hagen, 1974). It is of interest, however, that in postmenopausal women we have observed a significant fall in prolactin levels, which may well be oestrogen-related, since no similar fall is seen in ageing males; Thorner and his colleagues have demonstrated a rise in serum prolactin levels, which correlates with oestradiol levels in pubertal girls, but not boys (Thorner *et al.*, 1977). Thus, age is also a factor that may affect basal prolactin status, at least in female subjects. The origin of the hyperprolactinaemia in neonates is not clear but is presumed to be of fetoplacental origin (Guyda and Friesen, 1973) although it may also be oestrogen-related.

It is established that considerably elevated prolactin levels occur during the hours of sleep (Ehara *et al.*, 1973; Sassin *et al.*, 1973), and it has been reported that, in the early hours of the morning, prolactin levels may still be significantly elevated (Friesen and Hwang, 1973). Our data confirm this and, in addition, suggest a diurnal variation in prolactin secretion during waking hours, with higher prolactin levels in the early morning and a trend towards higher levels again in the evening. The ideal sampling time thus would be between 0900 and 1200, when prolactin levels are at their nadir. The degree of individual variability in prolactin secretion from day to day is modest but is more marked in female than in male subjects, an observation which again may be related to the oestrogen status of the subject. This modest variation in prolactin levels from day to day, together with the relative consistency in prolactin concentrations noted over a much shorter period of time (90 minutes), suggests that for confirmation of hyperprolactinaemia repeated sampling on several different days may be of more value than the usually advocated repeated sampling over a short period via an indwelling cannula.

Prolactin release arising from the stress associated with major surgery or insulin-induced hypoglycaemia is well established (Noel *et al.*, 1972). The incidence of hyperprolactinaemia, attributable to the 'psychic' stress of either hospital attendance or venepuncture is poorly documented however, although a recent study suggests that 19% of women attending an infertility clinic have elevated prolactin levels after clinical interview (Koninckx, 1978). In the present study we have failed to demonstrate any rise in prolactin levels as a result of venepuncture *per se*, but 7% of women attending a general gynaecology clinic for the first time were noted to have elevated prolactin levels, which were normal on subsequent sampling and which may have been related to the stress of hospital attendance. It is clearly necessary, therefore, to confirm consistent elevation of prolactin levels before attributing any clinical abnormality to hyperprolactinaemia.

The relation between prolactin and oestrogen levels in pregnancy is familiar, and the stimulant effect of oestrogens on prolactin secretion has also been demonstrated experimentally (Carlson *et al.*, 1973). Hence, it is not surprising that patients taking oestrogen-containing, oral contraceptive preparations have a mean prolactin level higher than that of control subjects. Indeed, 44% had frankly abnormal levels. Similarly, other drugs have been associated with hyperprolactinaemia, for example, α-methyl dopa (Steiner *et al.*, 1976), phenothiazines (de Rivera *et al.*, 1976), and metoclopramide (McCallum *et al.*, 1976), and in the present study, long-term therapy with α-methyl dopa phenothiazines, tricyclic antidepressants, metoclopramide, and reserpine were all associated with significantly elevated prolactin levels. The degree of hyperprolactinaemia, however, was modest, 95% of patients having levels less than 700 mU/l. This finding may account for the fact that none of these patients had any symptoms or signs that might be attributed to hyperprolactinaemia.

We have confirmed that several pathological conditions may be associated with elevated prolactin levels although not chronic ill health *per se*. The postulated aetiological mechanisms involved in the production of hyperprolactinaemia are diverse: for example, disruption of the hypothalamic-pituitary stalk as may occur in pituitary tumours

with suprasellar extension; deranged hypothalamic control mechanisms to account for idiopathic hyperprolactinaemia; excessive stimulation by naturally occurring agents such as TRH in primary hypothyroidism; and altered renal metabolism of prolactin with progressive uraemia. Autonomous production of prolactin by pituitary adenomas clearly results in the highest circulating levels of prolactin (Kleinberg *et al.*, 1977; Franz, 1978), but, in our series, 30% of confirmed tumours were associated with minor elevations in basal prolactin levels, such that they could not be distinguished from other causes of hyperprolactinaemia on this basis alone.

Since the early reports that TRH and L-dopa evoked, respectively, stimulation and suppression of prolactin levels in normal individuals (Friesen *et al.*, 1972; L'Hermite *et al.*, 1972), it has been hoped that tests of the autonomy of prolactin secretion would prove of value in the identification of tumour-associated hyperprolactinaemia. Initial results were disappointing (Lamberts *et al.*, 1976) but recently we, and others, have found dynamic tests, particularly stimulation tests, to be of value in the identification of prolactin-secreting tumours, particularly when the degree of hyperprolactinaemia is modest and pituitary radiology is entirely normal (Healy *et al.*, 1977; Kleinberg *et al.*, 1977; Cowden *et al.*, 1979).

It is now established that metoclopramide is a potent stimulus to prolactin secretion (Delitala *et al.*, 1976; Judd *et al.*, 1976; McCallum *et al.*, 1976), and indeed not only is the magnitude of prolactin response greater than that seen after TRH, but it is also more prolonged in duration. In response to both TRH and metoclopramide stimulation, prolactin release is considerably greater in women, a finding that may reflect greater oestrogen-related prolactin reserve despite remarkably similar basal levels in men and women. Acute administration of L-dopa and bromocriptine results in prompt suppression of prolactin levels; the extent and duration of suppression of prolactin levels, however, are much greater after bromocriptine. These different patterns of prolactin suppression in response to the acute administration of L-dopa and bromocriptine may well be related to the respective failure and success of these agents observed in the long-term treatment of hyperprolactinaemic syndromes (Friesen *et al.*, 1972; Thorner *et al.*, 1977).

In conclusion, we suggest the following strategy for the laboratory assessment of prolactin status:

1 Draw blood sample, under resting basal conditions, ideally between 0900 and 1200, though sampling between 0900 and 1700 is acceptable.

2 If an elevated prolactin level is demonstrated, when compared with appropriate age and sex related control subjects, exclude:

(i) drug therapy (including oral contraceptive)
(ii) pregnancy
(iii) primary hypothyroidism
(iv) chronic renal failure
(v) hypothalamic-pituitary disease

Relate level to appropriate reference ranges in groups (i) to (v).

3 Repeat sample:

(i) If both levels > 700 mU/l, this is unlikely to be due to stress
(ii) If both levels > 1000 mU/l, this is likely to be due to pituitary tumour.

4 If levels are modest and unexplained, and pituitary tomography is normal, undertake stimulation tests of prolactin secretion and compare responses with appropriate male or female control subjects.

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# TESTS OF PROLACTIN SECRETION IN DIAGNOSIS OF PROLACTINOMAS

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***Summary*** Prolactin-secreting tumours of the pituitary were identified and treated by transsphenoidal microsurgery in fourteen infertile females with hyperprolactinæmia. Resting prolactin levels were 590–9000 mU/l (mean 3400). In seven patients, tomography of the pituitary fossa was normal and resting prolactin levels were 590–6000 mU/l (mean 3400). In these patients the pre-operative diagnosis of prolactinoma in these patients was made by demonstrating loss of the normal circardian prolactin profile and impaired prolactin response to intravenous thyrotrophin-releasing hormone (T.R.H.) and metoclopramide stimulation. Prolactin response to the acute oral administration of L-dopa and bromocriptine was of less diagnostic value. Preoperative assessment of anterior pituitary function identified abnormalities other than hyperprolactinæmia in four patients (28%). Post-operative assessment indicated that microsurgery was curative in twelve patients (86%), selective in all, and without significant side-effect. It is concluded that dynamic tests such as T.R.H. and metoclopramide stimulation have considerabe value in identifying hyperprolactinæmic patients with prolactin-secreting adenomas, particularly those which are radiologically occult.

## Introduction

THE clinical syndrome of amenorrhœa, galactorrhœa and infertility, due to hyperprolactinæmia, is increasingly recognised.[1–3] Hyperprolactinæmia and its associated clinical features may arise in a variety of conditions —e.g., drug therapy, hypothyroidism, chronic renal failure—but when these have been excluded it is important to distinguish between patients who have discrete prolactin-secreting tumours and those who have not. Radiological abnormality of the pituitary fossa has been judged the most useful diagnostic criterion of a pituitary tumour[4] but small changes revealed by radiology are difficult to interpret.[5] The diagnosis of small prolactinomas is important, because treatment of large pituitary tumours with gross changes may be less effective.[6,7] We have studied fourteen infertile hyperprolactinæmic females and investigated the value of dynamic tests of prolactin secretion in the diagnosis of pituitary adenomas.

## Patients

Clinical details of the fourteen patients (mean age 29, range 16–40), are shown in table I. All had hyperprolactinæmia on at least two separate tests (mean basal prolactin 3400mU/l, range 590–9000; normal reference range 60–360). No factor

TABLE I—CLINICAL DETAILS

| Patient | Age at diagnosis (yr) | Duration of symptoms (yr) | History† | Amenorrhœa | Galact-orrhœa | Infertility | Other |
|---|---|---|---|---|---|---|---|
| *Group 1 (normal radiology)* | | | | | | | |
| 1 | 21 | 1 | + | + | – | + | Secondary infertility |
| 2 | 23 | 1 | + | + | – | + | Primary infertility |
| 3 | 27 | 2 | + | + | – | + | Primary infertility |
| 4 | 39 | 11 | + | + | + | – | .. |
| 5 | 35 | 18 | – | + | + | – | .. |
| 6 | 24 | 3 | – | + | – | + | Primary infertility |
| 7 | 36 | 11 | – | – | – | + | Anovulatory bleeding |
| Mean (range) | 29 (21–39) | 6·8 (1–18) | | | | | |
| *Group 2 (abnormal radiology)* | | | | | | | |
| 1 | 16 | 5 | – | + | – | – | .. |
| 2 | 34 | 14 | – | + | + | – | .. |
| 3 | 32 | 6 | + | + | – | + | Secondary infertility |
| 4 | 28 | 4 | + | + | – | + | Primary infertility |
| 5 | 30 | 14 | – | – | – | + | Anovulatory bleeding |
| 6 | 31 | 10 | – | + | – | – | .. |
| 7 | 40 | 16 | + | + | + | – | .. |
| Mean (range) | 30 (16–40) | 9·8* (4–16) | | | | | |

*$p<0.05$. †Postpartum or after oral contraceptive use.

TABLE II—PROLACTIN-SECRETING TUMOURS OF THE PITUITARY

| — | Basal prolactin (mU/l) | Radiology | Fossa volume ($mm^3$) | % change from basal prolactin level | | | | | Anterior pituitary function | Tumour size (mm) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Sleep peak | T.R.H. | Meto-clopramide | L-dopa | Bromo-criptine | | |
| *Group 1* | | | | | | | | | | |
| 1 | 900 | Normal | 1000 | 16 | 19 | 47 | 50 | 77 | Impaired G.H. response<br>Impaired cortisol response | 3·5 |
| 2 | 590 | Normal | 900 | 46 | 89 | 65 | 64 | 86 | Impaired G.H. response<br>Impaired cortisol response | 6·0 |
| 3 | 1000 | Normal | 1080 | Nil | Nil | 31 | 34 | 86 | Normal | 4·0 |
| 4 | 2500 | Normal | 1020 | Nil | 37 | 12 | 25 | 81 | Normal | 3·0 |
| 5 | 2990 | Normal | 940 | Nil | Nil | Nil | 57 | 66 | Borderline G.H.<br>Delayed T.S.H. response | 8·0 |
| 6 | 6000 | Normal | 1200 | Nil | Nil | Nil | 67 | 66 | Normal | 5·0 |
| 7 | 1600 | Normal | 1050 | Nil | 68 | 85 | 65 | 85 | Normal | 5·5 |
| Mean | 2200 | | 1028 | | 34 | 34 | 52 | 78 | | 5·0 |
| *Group 2* | | | | | | | | | | |
| 1 | 1200 | Loss of definition in cortex | 860 | Nil | 15 | 37 | 63 | 86 | Normal | 8·0 |
| 2 | 5800 | Asymmetry | 1700 | Nil | Nil | 13 | 46 | 78 | Impaired G.H. response | 8·0 |
| 3 | 4900 | Double floor | 1500 | Nil | Nil | 7 | 54 | 67 | Normal | 6·0 |
| 4 | 4200 | Double floor | 1300 | Nil | Nil | Nil | 44 | 78 | Normal | 8·5 |
| 5 | 1690 | Asymmetry | 1300 | Nil | Nil | Nil | 43 | 66 | Normal | 6·0 |
| 6 | 9000 | Enlarged fossa | 1780 | Nil | Nil | Nil | 63 | 78 | Normal | 10·0 |
| 7 | 5500 | Asymmetry | 1600 | Nil | 80 | 67 | 84 | 93 | Normal | 10·5 |
| Mean | 4600* | | 1434 † | | 13 | 18 | 57 | 78 | | 8·0† |
| Reference mean (range) | (60–360) | (<1500) | (<1500) | 112 (92–257) | 528 (272–955) | 1920 (1267–2946) | 63 (44–72) | 86 (73–95) | | |

* $P<0.005$; † $P<0.05$; (group 1 *vs.* group 2).

other than pituitary tumour was identified to account for their clinical abnormalities and persistent hyperprolactinæmia. These patients were identified within a larger, consecutive group of women presenting with hyperprolactinæmia and its associated clinical syndrome; the rest had no radiological or biochemical evidence of pituitary tumour.

## Methods

### *Serial Linear Tomography of Pituitary Fossa*

All patients were investigated by tomography with the Mimer unit (thickness of cut 0·27 cm). Films were independently reviewed without knowledge of the clinical or operative findings and the appearance and volume of the pituitary fossa were determined (normal <1500 $mm^3$).

### *Anterior Pituitary Function*

This was assessed by hormone measurements after an intravenous bolus of soluble insulin (0·1 U/kg) combined with thyrotrophin-releasing hormone (T.R.H.) (200 μg, Roche) and gonadotrophin-releasing hormone (100 μg, Ayerst). Normal minimal responses were defined as: cortisol increment >200 nmol/l and peak growth hormone levels >16 mU/l in response to hypoglycæmia <2·2 mmol/l; T.S.H. increment >3·6 mU/l at 20 min; luteinising hormone increment >3·0 U/l and follicle-stimulating hormone increment >1·0 U/l. The test was done with the patient at rest, after an overnight fast. Samples were obtained via an indwelling cannula at 0, 20, 45, 60, 90, and 120 min after administration of the drugs. Cannulæ were in situ for at least 30 min before the test.

### *Dynamic Tests of Prolactin Secretion*

*Circadian variation* was assessed by blood sampling via an indwelling cannula during sleep and at mid-morning, mid-afternoon, and mid-evening during a 24-h period.

*Stimulation tests* (see fig. 1 for sampling details and table II for reference data) were T.R.H. (Roche) 200 μg intravenously and metoclopramide (Beecham) 10 mg intravenously.

*Suppression tests* (see table II for reference data) were L-dopa (Roche) 500 mg orally and bromocriptine (Sandoz) 2·5 mg orally. Samples were taken 0, 1, 1·5, 2, 3, 5, and 7 h after ingestion of both drugs. Subjects had not fasted and maintained normal activity during these tests. No side-effects were observed.

### *Treatment*

Treatment was by transsphenoidal microhypophysectomy.

### *Tumour Histology*

Histology was investigated by light and electron microscopy and immunoperoxidase staining for prolactin-secreting cells.

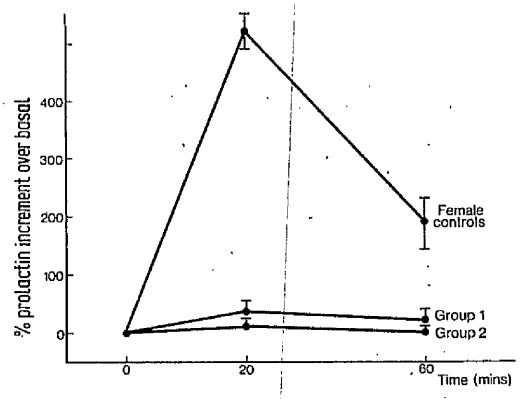


**Fig. 1—Prolactin response to T.R.H. stimulation.**

Mean (and S.E.M.).

*Postoperative Assessment*

Basal prolactin levels were measured 1–3 weeks post-operatively and anterior pituitary function was assessed as described above to define selectivity of therapy. Objective clinical response was taken as the return of normal function—i.e., regular ovulation and/or restored fertility.

*Hormone Assays*

Serum-prolactin was measured by a specific radioimmunoassay standardised with MRC preparation 75/504 (650 mU/ampoule).[8] The assay detection limit was 30 mU/l with mean precision coefficients of variation of 5% within batch and 10% between batch. All other hormones were measured by standard radioimmunoassays except serum-cortisol which was measured by the method of Mattingly.[9]

## Results

In all fourteen patients, a presumed pre-operative diagnosis of prolactin-secreting tumour was confirmed at operation (table II). Histologically, seven tumours were chromophobe, five were amphophilic, and two were eosinophilic. Immunoperoxidase staining showed that all tumours were composed of prolactin-secreting cells.

*Clinical Details (table I)*

Irrespective of the size of the tumour and the degree of hyperprolactinæmia, the commonest presenting complaints were amenorrhœa and infertility, galactorrhœa occurring in only 29%. The onset of symptoms was related either to childbirth or cessation of an oral contraceptive in half the patients.

*Radiology (table II)*

In seven of the fourteen patients the pituitary fossa was normal on radiology (group 1). At operation, direct observation confirmed that six of these seven patients had no bony abnormality, while a minor alteration in bone texture was detected in one.

The remaining patients had either general enlargement or localised abnormality of the pituitary fossa (group 2). In one patient there was simple enlargement of the pituitary fossa, in one there was a minor loss of corticol definition, and in five the floor of the fossa was asymmetrical. In group 2, basal prolactin levels were significantly higher, tumours were larger, and the duration of symptoms was longer than in those patients with normal radiology.

*Anterior Pituitary Function*

Abnormalities of anterior pituitary function, other than hyperprolactinæmia, were identified in three group 1 patients; two patients had a significantly impaired cortisol and growth-hormone response to adequate hypoglycæmia and one had a delayed T.S.H. response to T.R.H. In two of these patients, anterior pituitary function became normal after successful removal of the tumours. One patient in group 2 had an impaired growth-hormone reserve which was not affected by tumour resection.

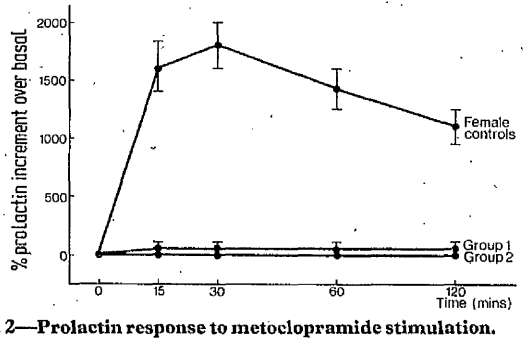


**Fig. 2—Prolactin response to metoclopramide stimulation.**
Mean (and S.E.M.).

*Dynamic Test of Prolactin Secretion*

A consistent abnormality in both groups was the complete absence of sleep-associated increment in prolactin levels. Both groups had grossly impaired prolactin responses to T.R.H. (fig. 1) and metoclopramide stimulation (fig. 2) with minimal responses in patients with larger tumours.

Prolactin suppression patterns were less consistently abnormal although the mean prolactin response to L-dopa was low normal in both groups. Only four patients failed to suppress normally with bromocriptine.

*Treatment*

Removal of the pituitary tumour was "curative" in twelve of fourteen patients (mean basal prolactin 227 mU/l, range 88–340). In the other patients, one from each group, prolactin levels were significantly reduced (2500 mU/l to 600; 9000 mU/l to 1000).

Treatment was "selective"—i.e., anterior pituitary dysfunction did not develop in any patient postoperatively. No operative morbidity was observed apart from transient diabetes insipidus. Five patients are now pregnant (four from group 1), six are ovulating (three from group 1), and galactorrhœa has been abolished in all who had it.

## Discussion

Our results suggest that prolactin-secreting tumours of the pituitary gland can be identified reliably by biochemical methods pre-operatively even in patients in whom conventional tomography is normal. Impairment of the sleep-associated increment in prolactin levels and prolactin responses to stimulation by intravenous T.R.H. and metoclopramide provide an accurate means of diagnosing radiologically occult microadenomas. Hitherto, the diagnosis of pituitary tumours has relied on radiological studies[10] which even experienced neuroradiologists find notoriously difficult to interpret because the anatomy of the fossa varies and the characteristic radiological changes associated with micro-adenomas are subtle.[4] In addition, minor changes are frequently seen in skull radiographs of patients in whom there is no suspicion of endocrine abnormality.[5] Furthermore, small pituitary tumours may not result in surrounding bony abnormalities and so would elude the most sophisticated techniques.[3,11] This was so in six of our patients in group 1 in whom pituitary tomography was normal and in whom at operation the absence of a defect in bone structure around the tumour could be seen directly.

Gross abnormalities of prolactin secretion are readily detected by measuring basal prolactin levels and, in general, the higher the level the greater the chance that the patient harbours a prolactin-secreting adenoma.[12] However, 35% of our patients with confirmed prolactinomas had modest hyperprolactinæmia (590–1200 mU/l) with levels characteristic of many associated physiological and abnormal conditions.[13] Although it was

hoped that tests of autonomy of prolactin secretion might help in the recognition of prolactinomas[14] recent reports have concluded that such tests have little diagnostic value.[3,15–17] However, several of these studies have assumed that normal radiology excludes a pituitary tumour. Thus the significance of altered prolactin responsiveness in hyperprolactinæmic patients with normal tomography may have been overlooked. The results in patients with radiologically occult prolactinomas contrast with those in patients with physiological hyperprolactinæmia—e.g., post-partum lactating women, in whom significant prolactin responses to stimulation[18] and suppression[19] have been demonstrated. We conclude, therefore, that dynamic tests such as T.R.H. and metoclopramide stimulation are of considerable value in identifying hyperprolactinæmic patients with prolactin-secreting microadenomas, particularly those which are inapparent radiologically.

The ideal treatment for prolactinomas remains to be determined although several therapeutic approaches, of varying success, have been described.[1,6,7,20,21] Early recognition of such tumours should ensure optimal results from microsurgical techniques[22] and in our series transsphenoidal microhypophysectomy has proved effective, selective, and safe. The dopamine agonist bromocriptine is also advocated for the treatment of the amenorrhœa-galactorrhœa syndrome but unfortunately its use has been associated occasionally with rapid expansion of pre-existing tumours in subsequent pregnancies.[23,24] The identification of radiologically occult microadenomas by biochemical tests might allow selection of patients who require detailed supervision during and after bromocriptine-induced pregnancy.

In general, a reliable practical method of identification of small prolactinomas, irrespective of radiological findings, will permit the choice between alternative methods of management to be made more rationally. It will provide also an objective criterion for comparing groups of patients treated in different ways. The diagnostic strategy described in this study has been reliable and clinically useful. For routine purposes, the complete range of tests may not be necessary and we now rely on assessment of the circadian prolactin profile and prolactin responsiveness to T.R.H. and metoclopramide stimulation, together with tests of anterior pituitary reserve.

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# SPECIFIC ALLERGIC SENSITISATION TO FILARIAL ANTIGENS IN TROPICAL EOSINOPHILIA SYNDROME

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***Summary*** Reaginic antibodies to antigens from the human filarial parasites *Wuchereria bancrofti* and *Brugia malayi* and the animal parasite *Dirofilaria immitis* were studied by histamine release from basophils in 7 patients with tropical eosinophilia (T.E.) and 18 patients with other manifestations of filarial infection (lymphatic changes or symptomless microfilaræmia). All the patients had antibodies to all three filariæ but T.E. patients were more highly sensitised. T.E. patients responded more to antigens from microfilariæ than did patients with non-T.E. filariasis and responded more to microfilarial antigens from the human parasites than to those from the animal parasite. These findings support the view that T.E. is a form of occult filariasis which results from host hypersensitivity to the microfilarial stage of parasites which, in other individuals, cause the more common lymphatic manifestations of filarial disease.

## Introduction

TROPICAL eosinophila (T.E.) is a chronic lung disease

DR COWDEN AND OTHERS: REFERENCES—*continued*

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# PROLACTIN STATUS
# IN HEALTH AND DISEASE

by

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# TABLE OF CONTENTS

## VOLUME I

## LIST OF TABLES

## LIST OF FIGURES.

## TABLE 1

## HYPERPROLACTINAEMIA: A CLASSIFICATION

| PHYSIOLOGICAL e.g. | PATHOLOGICAL e.g. |
|---|---|
| 1. Newborn | 1. Hypothalamic disorders |
| 2. Pregnancy | 2. Pituitary disorders |
| 3. Nursing mothers | 3. Hypothalamic - pituitary stalk "irritation" e.g. head injury |
| 4. Stress | 4. Primary hypothyroidism |
| 5. Sleep related nocturnal rhythm | 5. Renal disease |
| 6. Drugs | 6. Idiopathic |
| (a) dopamine receptor blockers e.g. phenothiazines metoclopramide | |
| (b) dopamine depleting agents e.g. αmethyl dopa reserpine | |
| (c) others e.g. oestrogens TRH | |

TABLE 2

SELECTED REVIEW OF LITERATURE ON PROLACTIN STATUS IN PATHOLOGICAL CONDITIONS

| Pathological Condition | Patients studied | Results | Conclusions | Author | Year |
|---|---|---|---|---|---|
| Affective Illness | n = 20 with bipolar affective disorders | PRL suppression to L-dopa >(p <0.001) in bipolar disease cf unipolar disease or controls | Neuroendocrine control differs in patients with bipolar and unipolar affective disorders | Gold et al | 1976 |
| | n = 7 with Huntington's Chorea | (1) Basal PRL > in choreic patients cf controls (p <0.02) (2) PRL suppression to bromocriptine <(p <0.005) of controls | There is abnormal central dopaminergic neurotransmission in Huntington's Chorea | Caraceni et al | 1977 |
| | n = 8 with Huntington's Chorea n = 23 first degree relatives | (1) Basal PRL < in choreic patients cf controls (p <0.004)) (2) PRL response to TRH <(p< 0.004) and chlor-promazine <(p <0.001) cf controls (3) 5/23 relatives had poorly sustained PRL response to TRH | (1) There is dopaminergic predominance in Huntington's Chorea (2) Abnormal tests may be early markers of disease | Hayden et al | 1977 |

TABLE 2 (continued)

| Pathological Condition | Patients studied | Results | Conclusions | Author | Year |
|---|---|---|---|---|---|
| Hypertension | n = 19 males with essential hypertension | (1) Elevated basal PRL ($p < 0.001$) in hypertension (2) Bromocriptine lowered blood pressure | Decreased dopaminergic activity in essential hypertension | Stumpe et al | 1977 |
| | n = 49 (16M) with essential hypertension | Basal PRL significantly ($p < .025$) lower in hypertensive patients | Apparent decrease in levels may reflect older age of predominantly female group of hypertensives | Holland and Sanchez | 1977 |
| Liver disease | n = 3 with liver failure, ascites, oliguria | Elevated basal PRL (50-100 ng/ml, normal <25 ng/ml) | The observation requires further elucidation | Horrobin, Manku and Nassar | 1974 |
| | n = 7 males with cirrhosis of liver | (1) Mean basal PRL normal (2) No circadian variation in PRL secretion | The observed dischronia may account for clinical features e.g. gynaecomastia, hypogonadism, fluid imbalance | Tarquini et al | 1977 |
| | n = 10 (9M) with severe liver disease | (1) Mean basal PRL normal (2) PRL response to TRH >controls ($p < 0.02$) | Observations may be related to excess circulating oestrogen ± deranged neuroendocrine control | Panerai et al | 1977 |

TABLE 2 (continued)

| Pathological Condition | Patients studied | Results | Conclusions | Author | Year |
|---|---|---|---|---|---|
| Breast Cancer | n = 115 with Breast Cancer n = 64 with "high risk" of Breast Cancer | (1) Basal PRL in breast cancer the same as controls (2) Elevated PRL ($p < 0.004$) in "high risk" group | None related to significance of PRL abnormalities in "high risk" group. | Kwa et al | 1974 |
| Diabetes Mellitus | n = 6 "labile" insulin dependent diabetics | (1) Basal PRL normal (2) No nocturnal peak in PRL secretion in 5/6 | None related to PRL abnormality | Drejer et al | 1977 |
| | n = 8 with hyperglycaemic keto-acidosis | Log serum PRL and serum sodium had negative correlation ($r = -0.61$ $p < 0.01$) | PRL elevated in keto-acidosis and returns to normal with therapy | Hanssen and Torjesen | 1977 |
| | n = 10 diabetics 5 with, 5 without, severe deteriorating retinopathy | PRL higher in patients without retinopathy | PRL unlikely to be involved in pathogenesis of diabetic retinopathy | Hunter et al | 1974 |
| | n = 55 diabetics of whom n = 26 with severe retinopathy | (1) Basal PRL normal in all (2) PRL "significantly" higher in non retinopathic patients | Suggest therapy to stimulate PRL secretion in diabetics with retinopathy | Harter et al | 1976 |

TABLE 2 (continued)

| Pathological Condition | Patients studied | Results | Conclusions | Author | Year |
|---|---|---|---|---|---|
| Molar Pregnancy | n = 20 females with molar pregnancy | (1) Basal PRL > in molar cf normal pregnancy ($p < 0.001$) (2) PRL response to arginine > in molar cf normal pregnancy ($p < 0.001$) | h PL inhibits PRL and oestrogen stimulates. In molar pregnancy, oestrogen > h PL | Mochizuki et al | 1976 |
| Nutritional Disorders | n = 8F with anorexia nervosa n = 9F with refractory obesity | Basal PRL > 5/8 anorexic patients and 2/8 with obesity | None related to PRL status | Harrower et al | 1977 |
| | n = 7M with obesity | Basal PRL and response to TRH were normal before and during prolonged fast | Abnormalities restricted to hypothalamic - pituitary - thyroid axis | Carlson et al | 1977 |
| Renal Disease | n = 77 (38M 39F) with renal failure | (1) 20% patients had elevated PRL (2) No PRL response to L-dopa was noted | There may be a deficiency of PIF secretion in renal failure | Chirito, Gonda and Friesen | 1972 |
| | n = 10 male patients on regular dialysis therapy | (1) Basal PRL increased ($p < 0.01$) (2) Failure of suppression after L-dopa (3) Failure of stimulation after TRH | Hypothalamic - pituitary dysfunction exists in chronic renal failure | Ramirez et al | 1977 |

TABLE 3

SELECTED CONDITIONS IN WHICH PROLACTIN MAY PLAY A PATHOPHYSIOLOGICAL ROLE

| | Condition | Author | Year |
|---|---|---|---|
| 1. | Post myocardial infarction arrythmias | Horrobin *et al* | 1973 |
| 2. | States of altered immune response e.g. pregnancy, sarcoidosis, liver failure, renal failure, cancer | Karmali, Lauder and Horrobin | 1974 |
| 3. | Migraine | Nader *et al* | 1974 |
| 4. | Pre eclamptic toxaemia | Horrobin | 1975 |
| 5. | Epileptiform seizures | Horrobin | 1975 |

TABLE 4

Radioimmunoassay of Human Prolactin: Routine System

**Reagents**

| | |
|---|---|
| 1. Diluent buffer | 0.05M phosphate buffer pH 7.5<br>0.25% w/v BSA<br>0.02% w/v sodium azide |
| 2. Standard prolactin | MRC 75/504 (650 mU/ampoule) was used to give working assay standards $P_0$-$P_8$ in the range 0-1000 mU/l, stored at -20°. |
| 3. Antiserum | FR AR 7-13 rabbit anti human prolactin serum used at final dilution 1:28,000 |
| 4. Tracer | FR 75.7.10 $^{125}$I h PRL, approximately 50 pg/tube |
| 5. Separation | Donkey anti rabbit serum 1:80 final dilution<br>Normal rabbit serum 1:1000 final dilution |

**Procedure**

| | | |
|---|---|---|
| Day 1 | Diluent | 250 µl |
| | Standard or sample | 50 µl |
| | Antiserum | 50 µl |
| | Incubate at 4°C | |
| Day 2 | Tracer | 50 µl |
| | Incubate at 4°C | |
| Day 3 | Normal rabbit serum | 50 µl |
| | Donkey anti rabbit serum | 50 µl |
| | Incubate at 4°C | |
| Day 4 | Centrifuge, separate and count | |
| | Calculate results | |

TABLE 5

Radioimmunoassay of Human Prolactin: Sensitive System

Reagents

| | | |
|---|---|---|
| 1. | Diluent buffer | 0.05M phosphate buffer pH 7.5<br>0.25% w/v BSA<br>0.02% w/v sodium azide |
| 2. | Standard prolactin | MRC 75/504 (650 mU/ampoule) was used to give working assay standards $P_0$-$P_8$ in the range 0-500 mU/l, stored at -20°. |
| 3. | Antiserum | FR AR 7-13 rabbit anti human prolactin serum used at final dilution 1:56,000 |
| 4. | Tracer | FR 75.7.10 $^{125}$I h PRL, approximately 50 pg/tube |
| 5. | Separation | Donkey anti rabbit serum 1:80 final dilution<br>Normal rabbit serum 1:1000 final dilution |

Procedure

| | | |
|---|---|---|
| Day 1 | Diluent | 200 µl |
| | Standard or sample | 100 µl |
| | Antiserum | 50 µl |
| | Incubate at 4°C | |
| Day 4 | Tracer | 50 µl |
| | Incubate at 4°C | |
| Day 5 | Normal rabbit serum | 50 µl |
| | Donkey anti rabbit serum | 50 µl |
| | Incubate at 4°C | |
| Day 6 | Centrifuge, separate and count | |
| | Calculate results | |

TABLE 6

## Lactoperoxidase Iodination of Human Prolactin

METHOD

| REAGENT | VOLUME |
|---|---|
| 5 µg h PRL | 10 µl |
| 0.5M PBS, azide free | 10 µl |
| $^{125}I$ 1 mCi | 10 µl |
| 5 µg LPO | 5 µl |
| $H_2O_2$ (50 µl 30% + 750 ml $dH_2O$, freshly made just prior to addition) | 5 µl |
| MIX FOR 3 MINS. | |
| $H_2O_2$ (as above) | 5 µl |
| MIX FOR 3 MINS. | |
| 0.1% BSA (w/v) in 0.05M PBS | 1 ml |
| MIX THOROUGHLY | |

2 x 10 µl aliquots for assessment of $^{125}I$ incorporation and specific activity

G150 Sephadex column chromatography for isolation of assay tracer

TABLE 7

Comparison of various purified human prolactin preparations as source of assay tracer

| Material | % Incorporation | % Binding in assay | Non Specific Binding (NSB) | Sensitivity in Assay (mU/l) | Life Span of Tracer |
|---|---|---|---|---|---|
| FR 75.7.10 | 79 | 58 | 3 | 32 | 4-6 weeks |
| * VLS ≠ 3 | 90 | 43 | 11 | 64 | 2-4 weeks |
| ** Lowry batch 2 | 48 | 34 | 2 | 100 | NS |
| batch 3 | 43 | 27 | 3 | 108 | NS |
| batch 4 | 74 | 25 | 12 | 90 | NS |

All tracers assessed with FR AR 7-13 antiserum

* Kindly supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases as part of their hormone distribution programme.

** Kindly supplied by Dr. P. Lowry, London

NS not studied

TABLE 8

Comparison of Tracers formed by Chloramine T and Lactoperoxidase iodination techniques

| Method | % Incorporation | % Binding in Assay | Non Specific Binding (NSB) | Sensitivity in Assay (mU/l) | Life Span of Tracer |
|---|---|---|---|---|---|
| LPO | 74 | 59 | 2 | 27 | 6 weeks |
| Chl T | 94 | 30 | 9 | 80 | 2 weeks |

LPO = Lactoperoxidase

Chl T = Chloramine T

TABLE 9

Radioimmunoassay of Human Prolactin:

Recovery Experiment

| h PRL added mU/l | Mean h PRL measured mU/l | Mean % Recovery | S.D. | C.V. |
|---|---|---|---|---|
| 49 | 47 | 97 | 9.3 | 11 |
| 98 | 100 | 102 | 4.3 | 4.6 |
| 195 | 211 | 108 | 7.4 | 7.9 |
| 300 | 309 | 103 | 6.0 | 5.8 |

S.D. Standard deviation

C.V. Coefficient of variation

n = 6 Duplicate estimations

TABLE 10

BASAL PROLACTIN LEVEL (mU/1): NORMAL SUBJECTS

| Group * | n | Mean | Absolute Range | Upper 95th centile |
|---|---|---|---|---|
| Hospital personnel | 100 | 168 | 50-375 | 340 |
| Hospital in-patients | 50 | 169 | 50-360 | 320 |
| Children - Age <3 months | 11 | 4133 | 543-13,000 | - |
| 3m-12y | 30 | 128 | 85-299 | 250 |
| Normal Pregnancy | | | | |
| Trimester 1 | 20 | 590 | 290-1750 | 830 |
| 2 | 45 | 1962 | 304-4760 | 4200 |
| 3 | 39 | 3166 | 770-5700 | 5000 |
| Oral Contraceptive | 54 | 356 | 90-1720 | 750 |
| Drug Therapy | 68 | 370 | 100-710 | 680 |

* as defined in text.

TABLE 11

BASAL PROLACTIN LEVEL (mU/l): PATHOLOGICAL CONDITIONS

| Group * | n | Mean | Absolute Range | Upper 95th centile |
|---|---|---|---|---|
| Chronic Sick | 20 | 185 | 70-370 | 310 |
| Miscellaneous Hypothalamic - pituitary disorders | 27 | 368 | 115-2800 | 700 |
| Pituitary Tumours: | | | | |
| Prolactinomas | 16 | 8140 | 590-18000 | 10000 |
| Acromegaly | 15 | 490 | 62-2400 | 684 |
| Cushings | 11 | 320 | 68-560 | 450 |
| Idiopathic hyperprolactinaemia | 10 | 826 | 440-1240 | 1189 |
| Chronic Renal Failure: | | | | |
| Moderate | 62 | 270 | 88-1670 | 700 |
| Severe | 13 | 562 | 123-1700 | 1100 |
| Dialysis | 37 | 880 | 253-4500 | 4000 |
| Primary Hypothyroidism | 50 | 491 | 115-2400 | 1400 |
| Pituitary Ablation | 5 | <50 | <50 | - |

* as defined in text.

TABLE 12

PROLACTIN RESPONSES TO STIMULATION AND SUPPRESSION TESTS:

NORMAL SUBJECTS

| Stimulation Tests * | | n | Mean PRL concentration difference % | Range |
|---|---|---|---|---|
| TRH | M | 14 | 278 | 64-474 |
| | F | 10 | 528 | 272-955 |
| Metoclopramide | M | 6 | 721 | 428-1039 |
| | F | 6 | 1940 | 1267-2946 |
| **Suppression Tests** ** | | | | |
| L-dopa | M + F | 6 | 63 | 44-72 |
| Bromocriptine | M + F | 3 | 86 | 73-95 |

* Response calculated as $\frac{\text{Peak PRL after stimulation}}{\text{Basal PRL}} \times 100$

** Response calculated as $100 - \frac{\text{Trough PRL after suppression}}{\text{Basal PRL}} \times 100$

TABLE 13

"Amenorrhoea - Galactorrhoea Syndrome": Subjects Studied: Clinical Details

| Diagnostic Category | Patient | Sex | Age at Diagnosis (y) | Duration of symptoms (y) | * History | Amenorrhoea | Anovulatory Bleeding | Galactorrhoea | Infertility | Othe |
|---|---|---|---|---|---|---|---|---|---|---|
| GROUP 1 | 1 | F | 21 | 1 | + | + | - | - | + | - |
| | 2 | F | 23 | 1 | + | + | - | - | + | - |
| Confirmed | 3 | F | 27 | 2 | + | + | - | - | + | - |
| Prolactinoma: | 4 | F | 39 | 11 | + | + | - | + | - | - |
| Radiologically | 5 | F | 35 | 18 | - | + | - | + | - | - |
| Occult | 6 | F | 24 | 3 | - | + | - | - | + | - |
| | 7 | F | 36 | 11 | - | - | + | - | + | - |
| | 8 | F | 27 | 3 | + | - | + | + | + | - |
| | 9 | F | 28 | 1 | + | + | - | - | + | - |
| | 10 | F | 27 | 2 | - | + | - | + | + | - |
| GROUP 2 | 11 | F | 16 | 5 | - | + | - | - | - | - |
| | 12 | F | 34 | 14 | - | + | - | + | - | - |
| Confirmed | 13 | F | 32 | 6 | + | + | - | - | + | - |
| Prolactinoma: | 14 | F | 28 | 4 | + | + | - | - | + | - |
| Radiologically | 15 | F | 30 | 14 | - | - | + | - | + | - |
| Evident | 16 | F | 31 | 10 | - | + | - | - | - | - |
| | 17 | F | 40 | 16 | + | + | - | + | - | - |
| | 18 | M | 36 | 12 | - | - | - | - | + | V- |

TABLE 13 (Continued)

| Diagnostic Category | Patient | Sex | Age at Diagnosis (y) | Duration of symptoms (y) | * History | Amenorrhoea | Anovulatory Bleeding | Galactorrhoea | Infertility | Oth |
|---|---|---|---|---|---|---|---|---|---|---|
| GROUP 3 | 19 | F | 16 | 2 | - | + | - | + | - | - |
| | 20 | F | 26 | 2 | + | + | - | + | + | - |
| Prolactinoma: | 21 | F | 24 | 1 | + | - | + | - | + | - |
| Awaiting | 22 | F | 26 | 3 | - | + | - | - | + | - |
| Confirmation | 23 | F | 36 | 10 | - | + | - | - | + | V. |
| | 24 | F | 18 | 6 | - | + | - | - | - | - |
| GROUP 4 | 25 | F | 24 | 3 | - | + | - | + | - | - |
| | 26 | F | 28 | 4 | - | - | + | - | + | - |
| "Functional" | 27 | F | 33 | 3 | - | + | - | - | + | - |
| Hyper- | 28 | F | 23 | 4 | + | - | + | - | + | - |
| prolactinaemia | 29 | F | 24 | 1 | - | + | - | + | + | - |
| | 30 | F | 29 | 4 | + | - | + | - | + | - |
| | 31 | F | 28 | 3 | - | + | - | + | + | - |
| | 32 | F | 32 | 13 | + | + | - | + | - | - |

*post partum or post oral contraceptive

V.S. visual symptoms

TABLE 14

Confirmed prolactinomas: Results of investigations

| | Basal PRL mU/l | Radiology | Fossa Vol. $mm^3$ | % Change from basal PRL level | | | | | Anterior Pituitary Function | Tumour Size mm |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Sleep Peak | TRH | MCP | L-dopa | BRCR | | |
| GROUP 1 | | | | | | | | | | |
| 1 | 900 | Normal | 1000 | 16 | 19 | 47 | 50 | 77 | Impaired GH and cortisol responses | 3.5 |
| 2 | 590 | Normal | 900 | 46 | 89 | 65 | 64 | 86 | Impaired GH and cortisol responses | 6.0 |
| 3 | 1000 | Normal | 1080 | Nil | Nil | 31 | 34 | 86 | Normal | 4.0 |
| 4 | 2500 | Normal | 1020 | Nil | 37 | 12 | 25 | 81 | Normal | 3.0 |
| 5 | 2990 | Normal | 940 | Nil | Nil | Nil | 57 | 66 | Borderline GH Delayed TSH response | 8.0 |
| 6 | 6000 | Normal | 1200 | Nil | Nil | Nil | 67 | 66 | Normal | 5.0 |
| 7 | 1600 | Normal | 1050 | Nil | 68 | 85 | 65 | 85 | Normal | 5.5 |
| 8 | 1730 | Normal | 950 | Nil | 54 | 66 | 59 | 78 | Normal | 4.5 |
| 9 | 2000 | Normal | 1000 | Nil | 23 | 27 | 32 | 70 | Normal | 7.0 |
| 10 | 1400 | Normal | 900 | Nil | 59 | 72 | 68 | 82 | Normal | 2.0 |
| Mean | 2071 | | 1004 | | 35 | 41 | 52 | 78 | | 4.9 |

TABLE 14 (continued)

| | Basal PRL mU/l | Radiology | Fossa Vol. $mm^3$ | % Change from basal PRL level: Sleep Peak | TRH | MCP | L-dopa | BRCR | Anterior Pituitary Function | Tumour Size mm |
|---|---|---|---|---|---|---|---|---|---|---|
| GROUP 2 | | | | | | | | | | |
| 11 | 1200 | Loss definition in cortex | 860 | Nil | 15 | 37 | 63 | 86 | Normal | 8.0 |
| 12 | 5800 | Asymmetry | 1700 | Nil | Nil | 13 | 46 | 78 | Impaired GH response | 8.0 |
| 13 | 4900 | Double floor | 1500 | Nil | Nil | 7 | 54 | 67 | Normal | 6.0 |
| 14 | 4200 | Double floor | 1300 | Nil | Nil | Nil | 44 | 78 | Normal | 8.5 |
| 15 | 1690 | Asymmetry | 1300 | Nil | Nil | Nil | 43 | 66 | Normal | 6.0 |
| 16 | 9000 | Enlarged fossa | 1780 | Nil | Nil | Nil | 63 | 78 | Normal | 10.0 |
| 17 | 5500 | Asymmetry | 1600 | Nil | 80 | 67 | 84 | 93 | Normal | 10.5 |
| 18 | 18000 | Destroyed | >2000 | Nil | Nil | Nil | 57 | 78 | Panhypopituitary | 15.0 |
| Mean | 6286* | | 1505* | | 12 | 16 | 57 | 78 | | 9.0** |
| Reference Mean | 168 | | | 112 | 528 | 1920 | 63 | 86 | | |
| Range | (60-360) | | (<1500) | (92-257) | (272-955) | (1267-2946) | (44-72) | (73-95) | | |

* p <0.005

** p <0.05 (group = cf group 2)

TABLE 15

Confirmed prolactinomas: Post operative results of dynamic tests of prolactin secretion

| | Basal PRL mU/l | % Increment after TRH | % Increment after MCP |
|---|---|---|---|
| GROUP A "CURES" | | | |
| n = 10 | | | |
| Pre-operative mean | 2800 | 25 | 37 |
| range | (590-5500) | (0-89) | (0-67) |
| Post-operative mean | 184 | 487 | 1849 |
| range | (88-290) | (198-640) | (1160-2450) |
| GROUP B "FAILURES" | | | |
| n = 3 | | | |
| Pre-operative mean | 9833 | 12 | 4 |
| range | (2500-18,000) | (0-37) | (0-12) |
| Post-operative mean | 867 | 0 | 0 |
| range | (600-1,000) | (0) | (0) |
| Reference range | (60-360) | (272-955) | (1267-2946) |

## TABLE 16

## "Functional" Hyperprolactinaemia: Results of Investigations

| Diagnostic Category | Basal PRL mU/l | Radiology | Fossa Vol. $mm^3$ | % Change from basal PRL level: Sleep Peak | TRH | MCP | L-dopa | BRCR | Anterior Pituitary Function |
|---|---|---|---|---|---|---|---|---|---|
| **GROUP 4** | | | | | | | | | |
| 25 | 1273 | Normal | 1040 | 268 | 454 | 1368 | 69 | 88 | Normal |
| 26 | 456 | " | 880 | 90 | 467 | 961 | 74 | 92 | " |
| 27 | 800 | " | 980 | 104 | 291 | 430 | 53 | 90 | " |
| 28 | 499 | " | 1200 | 87 | 455 | 1292 | 57 | 91 | " |
| 29 | 800 | " | 990 | 190 | 210 | 68 | 41 | 79 | " |
| 30 | 1040 | " | 1340 | 100 | 300 | 414 | 46 | 74 | " |
| 31 | 540 | " | 800 | 188 | 279 | 200 | 70 | 78 | " |
| 32 | 480 | " | 850 | 195 | 189 | 341 | 68 | 82 | " |
| Mean | 736 | | 1000 | 153 | 331 | 635 | 60 | 84 | |
| **PROLACTINOMA GROUP 1** | | | | | | | | | |
| Mean | 2071 | | 1004 | 6 | 35 | 41 | 52 | 78 | |
| Range | (590-5000) | | (900-1200) | (0-46) | (0-89) | (0-85) | (25-68) | (66-86) | |
| **PROLACTINOMA GROUP 2** | | | | | | | | | |
| Mean | 6286 | | 1505 | Nil | 12 | 16 | 57 | 76 | |
| Range | (1200-18,000) | | (360 ->2000) | Nil | (0-80) | (0-67) | (43-84) | (66-93) | |
| **REFERENCE** | | | | | | | | | |
| Mean | 168 | | | 112 | 520 | 1920 | 65 | 86 | |
| Range | (60-360) | | <1500 | (92-257) | (272-955) | (1267-2946) | (44-72) | (73-95) | |

TABLE 17

Incidence of hyperprolactinaemia in renal patients receiving one or more of three commonly prescribed medications

| Group (as defined in text) | Methyldopa | Prednisolone | Tri-cyclic anti-depressants |
|---|---|---|---|
| Renal failure (n = 63) | 31/50 (62%) | 2/8 (25%) | 5/5 (100%) |
| Renal disease (n = 33) | 8/21 (38%) | 8/12(67%) | - |

TABLE 18

**Prolactin concentrations in patients with renal disease, not receiving methyl dopa, prednisolone or tricyclic antidepressants**

| Group (as defined in text) | n | Mean creatinine μmol/l | Mean prolactin mU/l | Absolute range prolactin mU/l |
|---|---|---|---|---|
| **Normal renal function** | | | | |
| GN | 45 | 105 | 178 | 38-1400 |
| CPN | 30 | 96 | 175 | 68-312 |
| UTI | 9 | 111 | 162 | 70-366 |
| Calculus | 13 | 84 | 173 | 68-324 |
| Polycystic kidneys | 11 | 82 | 188 | 71-310 |
| SLE | 7 | 99 | 197 | 117-360 |
| **Impaired renal function*** | | | | |
| C.R.F. creatinine <600 μmol/l | 62 | 292 | 270 | 88-1670 |
| >600 μmol/l | 13 | 1140 | 562 | 123-1300 |
| RDT | 37 | 1280 | 880 | 253-4500 |
| ≠ Post renal transplantation | 43 | 159 | 382 | 100-2000 |

*Many patients in this group on oral iron, vitamins, aluminium hydroxide

≠All patients in this group on prednisolone and azathioprine

TABLE 19

Arteriovenous concentration difference of prolactin across the normal kidney

| Patient | Prolactin mU/l | | % fall in PRL |
|---|---|---|---|
| | Renal artery | Renal vein | |
| 1 | 360 | 330 | 8 |
| 2 | 205 | 150 | 27 |
| 3 | 242 | 215 | 11 |
| 4 | 163 | 148 | 9 |
| 5 | 146 | 103 | 29 |
| 6 | 370 | 313 | 15 |
| 7 | 342 | 287 | 16 |
| Mean | 261 | 219 | 16 |

TABLE 20

## Endocrine Status in Dialysis and Successfully Transplanted Patients, compared to Controls

| | | Control Group | | Dialysis Patients | | Post Transplant Patients | |
|---|---|---|---|---|---|---|---|
| | | mean | range | mean | range | mean | range |
| Basal T4 | nmol/l | 105 | 78-148 | 72 | 20-120 | 108 | 84-130 |
| T3 | nmol/l | 1.8 | 1.0-2.8 | 1.3 | 0.5-2.2 | 2.0 | 0.9-2.6 |
| TSH | mU/l | 2.4 | 1.8-6.8 | 4.4 | <1.8-8.0 | 3.4 | <1.6-8.2 |
| Maximal TSH increment (mU/l) to TRH | | 9.5 at 20' | 6.5-11.4 | 5.6 at 60' | 5.4-6.5 | 8.7 at 20' | 6.0-10.4 |
| Basal GH | mU/l | 2.8 | <0.8-5.4 | 9.4 | <0.8-40 | 3.9 | <0.8-7.4 |
| GH response to TRH | | Nil | | Variable | | Nil | |
| Basal T (males) | nmol/l | 34 | 17-66 | 12 | 9-17 | 27 | 16-39 |
| $OE_2$ (females) | pmol/l | 570 | 180-700 | 190 | 160-240 | 320 | 220-400 |
| FSH | U/l | 2.8 | 0.5-6.5 | 6.9 | 1.4->50 | 4.4 | 1.9-6.4 |
| LH | U/l | 4.5 | 1.0-10.0 | 13.1 | 1.0->50 | 6.4 | 2.4-8.0 |
| Maximal FSH increment (U/l) to Gn RH | | 3.4 | 2.9-4.8 | 1.1 | 0-2.5 | 3.5 | 2.8-5.1 |
| Maximal LH increment (U/l) to Gn RH | | 34 | 28-49 | 30 | 25-32 | 30 | 22-37 |
| Basal PRL | mU/l | 154 | 62-300 | 843 | 196-4000 | 210 | 84-340 |
| Percentage change from basal after TRH M | | 278 | 64-474 | 20 | 0-54 | 204 | 98-259 |
| F | | 528 | 272-955 | 38 | 12-60 | 410 | 210-740 |
| MCP M | | 721 | 428-1039 | 52 | 0-74 | 800 | 540-900 |
| F | | 1940 | 1267-2946 | 68 | 0-82 | 1340 | 980-2000 |
| L-dopa | | 63 | 44-72 | 10 | 0-18 | 64 | 40-78 |
| Bromocriptine | | 86 | 73-95 | 13 | 0-17 | 88 | 76-98 |

TABLE 21

Heterogeneity of h PRL after gel filtration

| Sample | Mass of tissue extracted mg | h PRL (extract or serum) mU/l | % Composition Peak 1 | Peak 2 | Peak 3 |
|---|---|---|---|---|---|
| MRC 75/504 | - | - | 7.0 | 5.9 | 87.1 |
| Normal Pituitary | 15 | 2400 | 9.2 | 11.6 | 79.2 |
| Pregnancy 13w | - | 840 | 10.4 | 15.4 | 74.2 |
| 28w | - | 1840 | 23.8 | 23.4 | 52.8 |
| Prolactinoma: SC | | | | | |
| Pituitary | 2.7 | 64000 | 15.7 | 15.4 | 68.9 |
| Serum | - | 8000 | 31.4 | 12.9 | 55.7 |
| Prolactinoma: PC | | | | | |
| Pituitary | 2.1 | 11800 | 13.2 | 20.4 | 66.4 |
| Serum | - | 1000 | 23.3 | 18.3 | 58.4 |
| Uraemic Serum A | - | 4000 | Nil | Nil | 100 |
| Uraemic Serum B | - | 1200 | 3 | Nil | 97.0 |

FIGURE 1

"Occlusive glomerular hyperplasia"
in a salt water fish.
An electron micrograph showing
crescent formation.

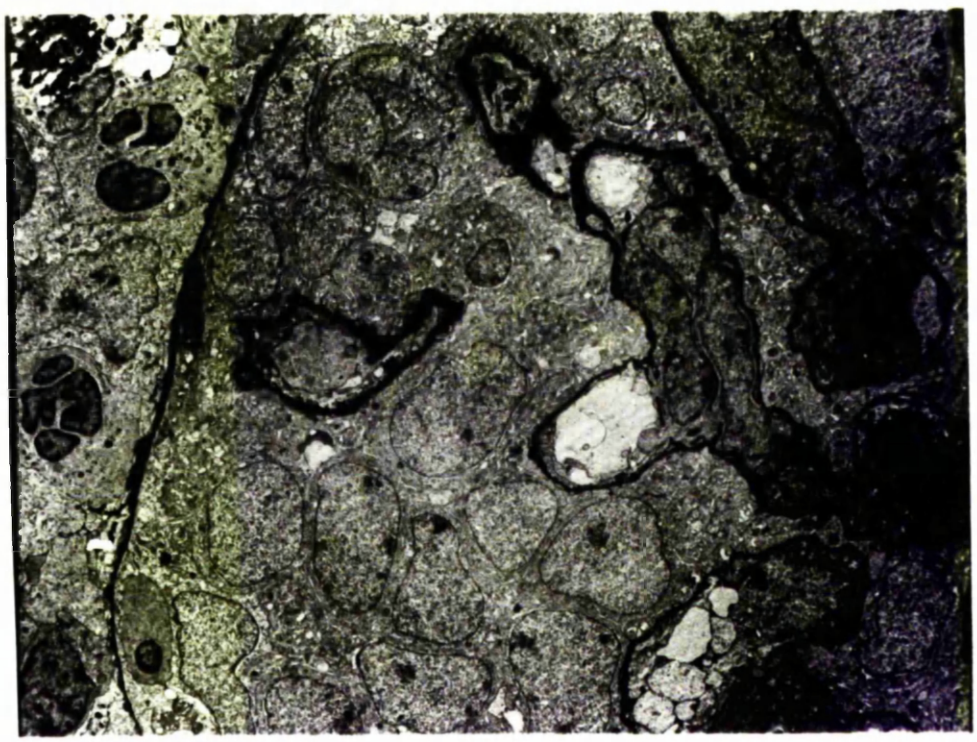


FIGURE 2

Glomerulus of a fresh water fish.
An electron micrograph showing open
glomerular structure with no
evidence of crescent formation.

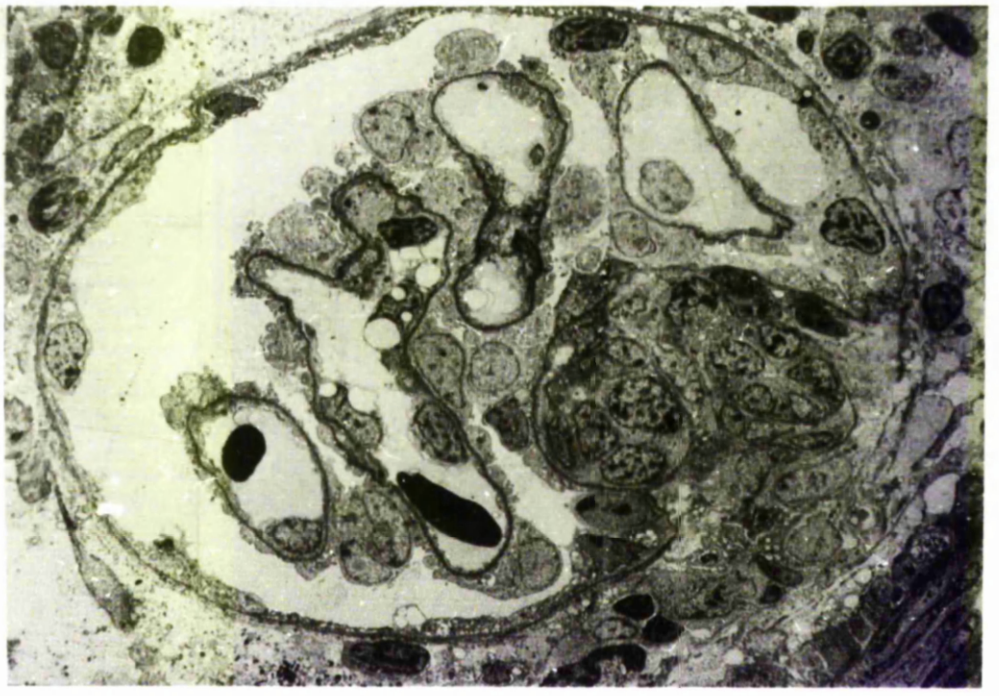


FIGURE 3

Schematic comparison between glomerular structure in fresh and salt water fish and in human proliferative glomerulonephritis.

MCGN: mesangio-capillary glomerulonephritis.

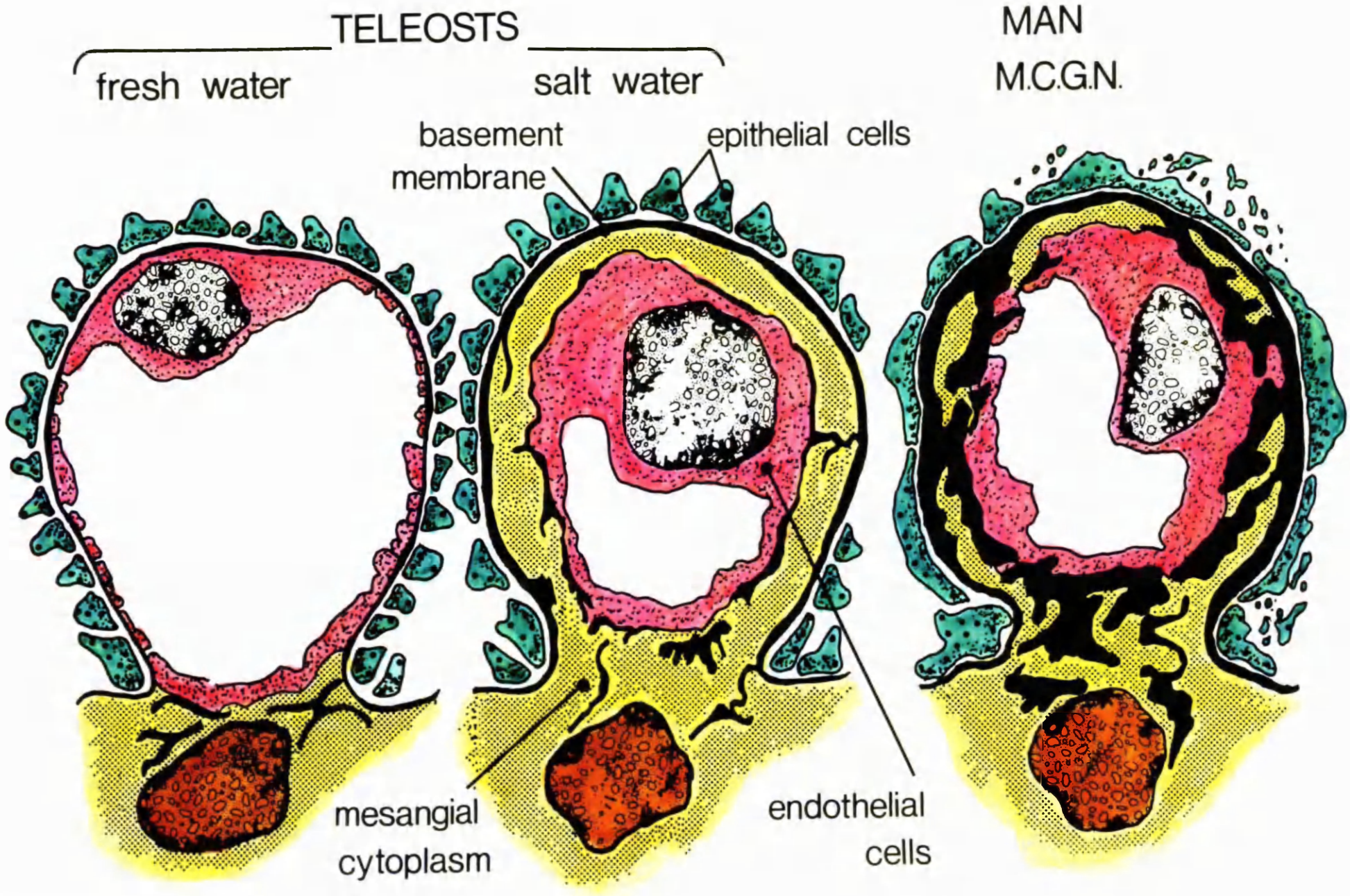

TELEOSTS
fresh water
salt water
MAN
M.C.G.N.
basement
membrane
epithelial cells
mesangial
cytoplasm
endothelial
cells

FIGURE 4

Clinical course of patient A.C.

P.D: peritoneal dialysis.

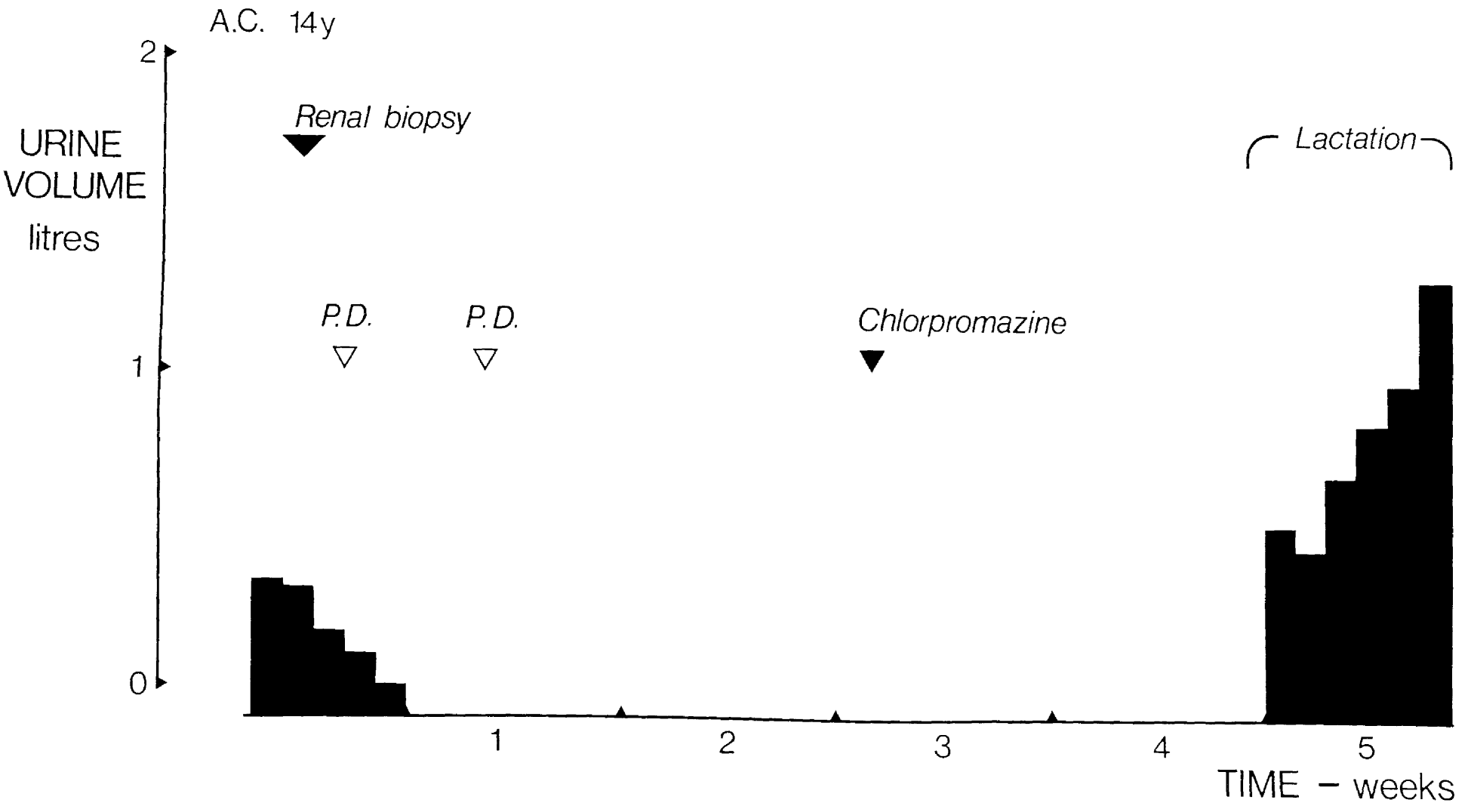

A.C. 14y
2
1
0
URINE VOLUME litres
Renal biopsy
Lactation
P.D.
P.D.
Chlorpromazine
1
2
3
4
5
TIME – weeks


FIGURE 5

The linear amino acid sequence of human prolactin

FIGURE 6

A concept of the physiological control of prolactin secretion.

(a) Central connections

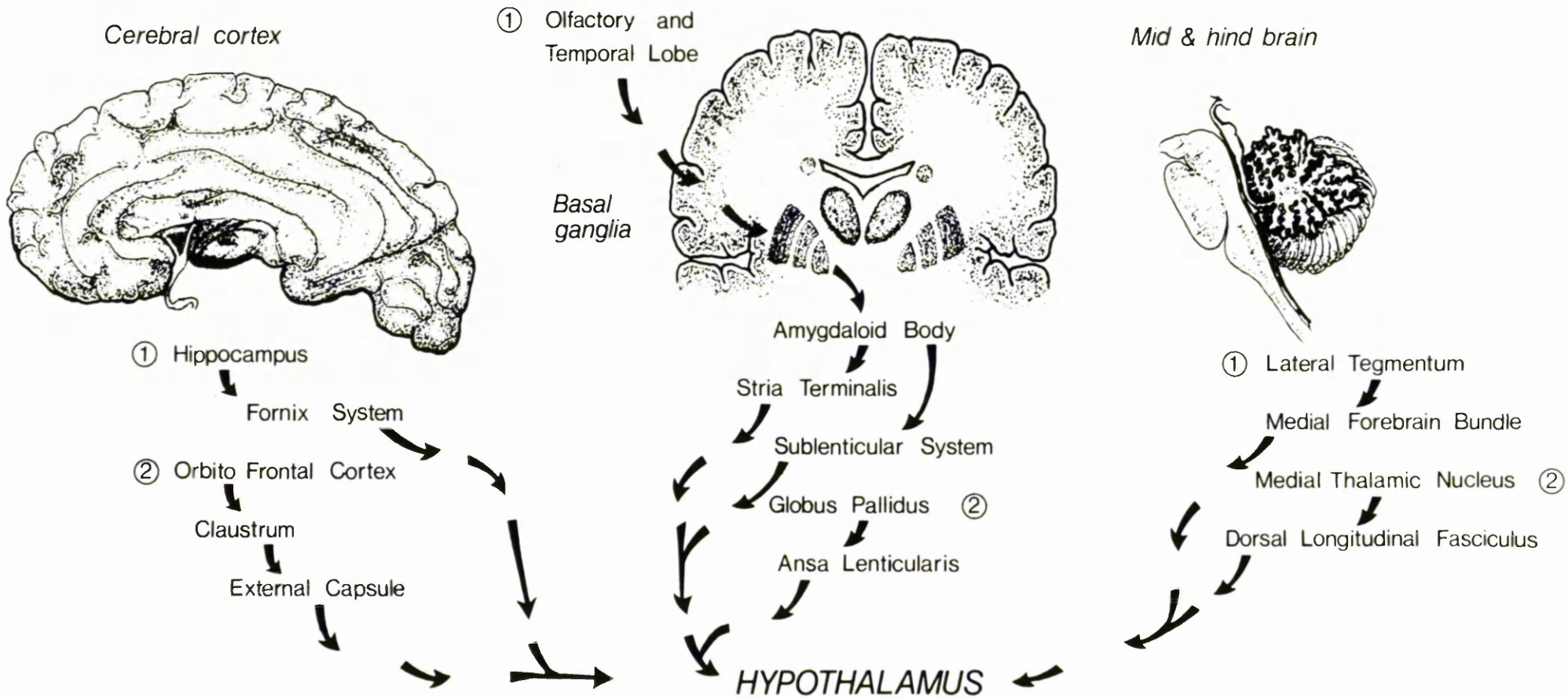

Cerebral cortex
① Olfactory and Temporal Lobe
Mid & hind brain
Basal ganglia
① Hippocampus
Fornix System
② Orbito Frontal Cortex
Claustrum
External Capsule
Amygdaloid Body
Stria Terminalis
Sublenticular System
Globus Pallidus ②
Ansa Lenticularis
① Lateral Tegmentum
Medial Forebrain Bundle
Medial Thalamic Nucleus ②
Dorsal Longitudinal Fasciculus
HYPOTHALAMUS

FIGURE 6

A concept of the physiological control of prolactin secretion.

(b) Final common pathway

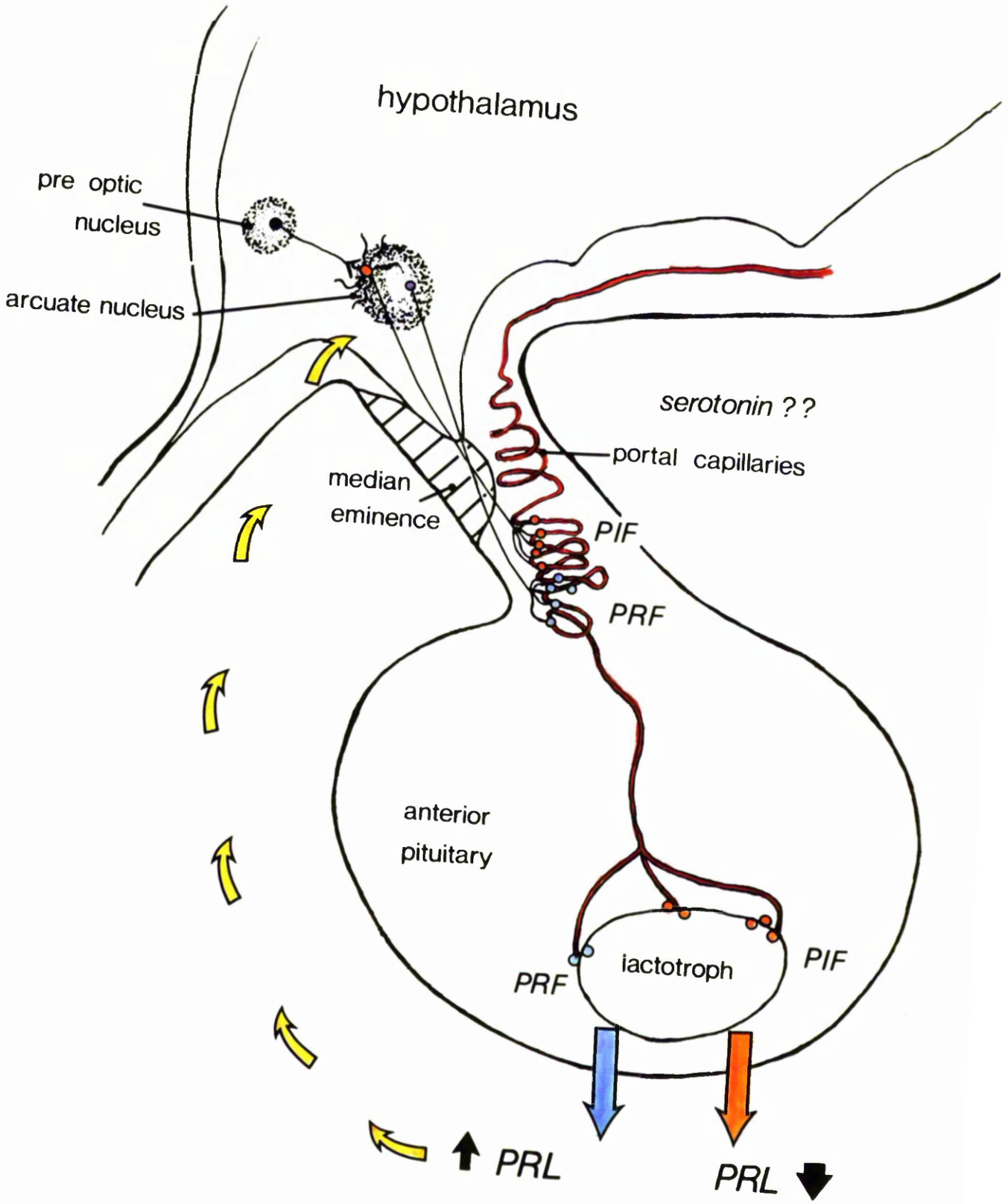

hypothalamus
pre optic
nucleus
arcuate nucleus
serotonin ??
portal capillaries
median
eminence
PIF
PRF
anterior
pituitary
lactotroph
PRF
PIF
PRL
PRL

FIGURE 7

Radioimmunoassay of human prolactin: Standard Curves. Assay conditions as described in text.

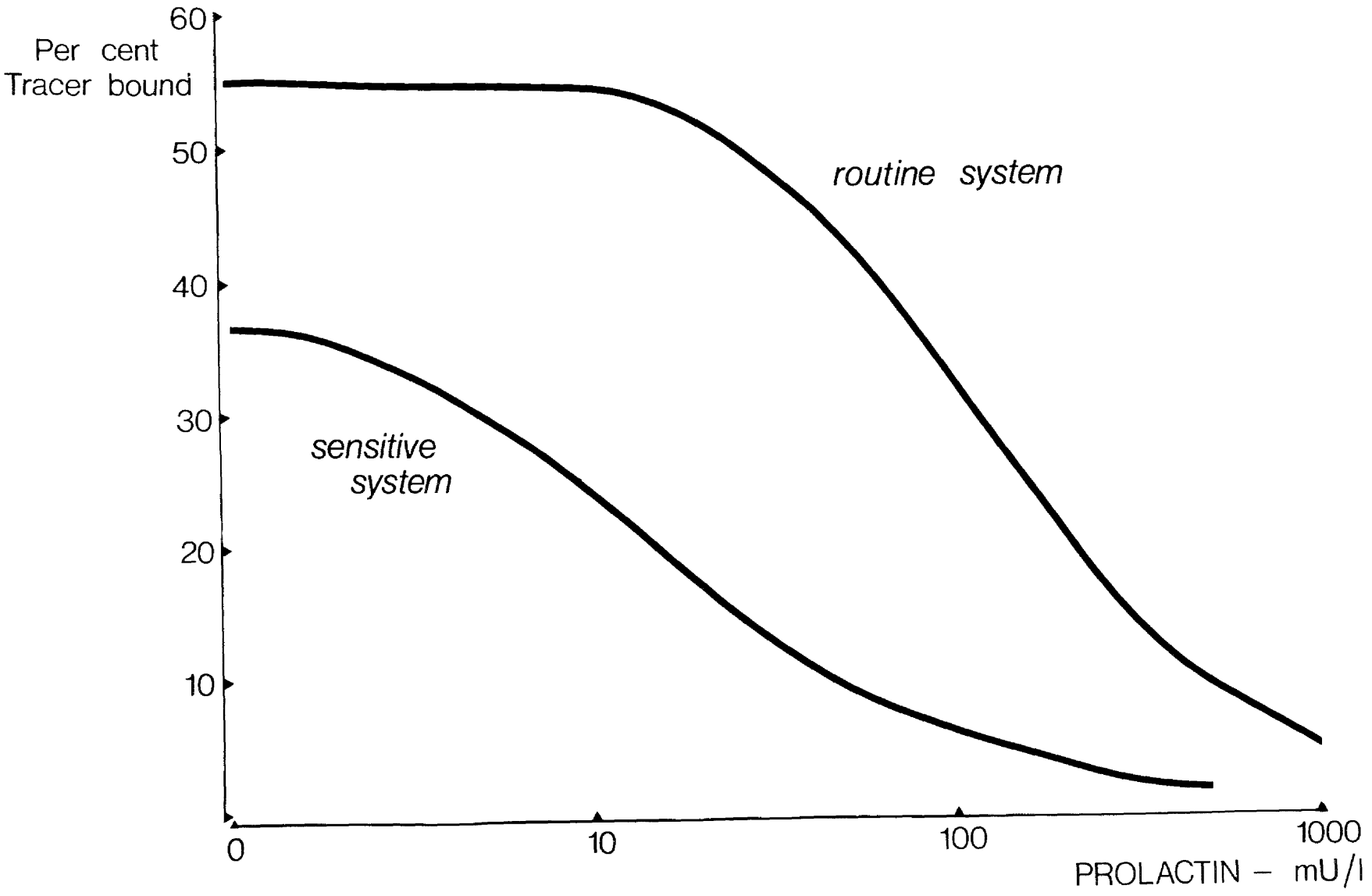

60
Per cent
Tracer bound
50
40
30
20
10
routine system
sensitive
system
0
10
100
1000
PROLACTIN – mU/l

FIGURE 8

Human prolactin FR 75.7.10: Iodination Profile.

Method as described in text.

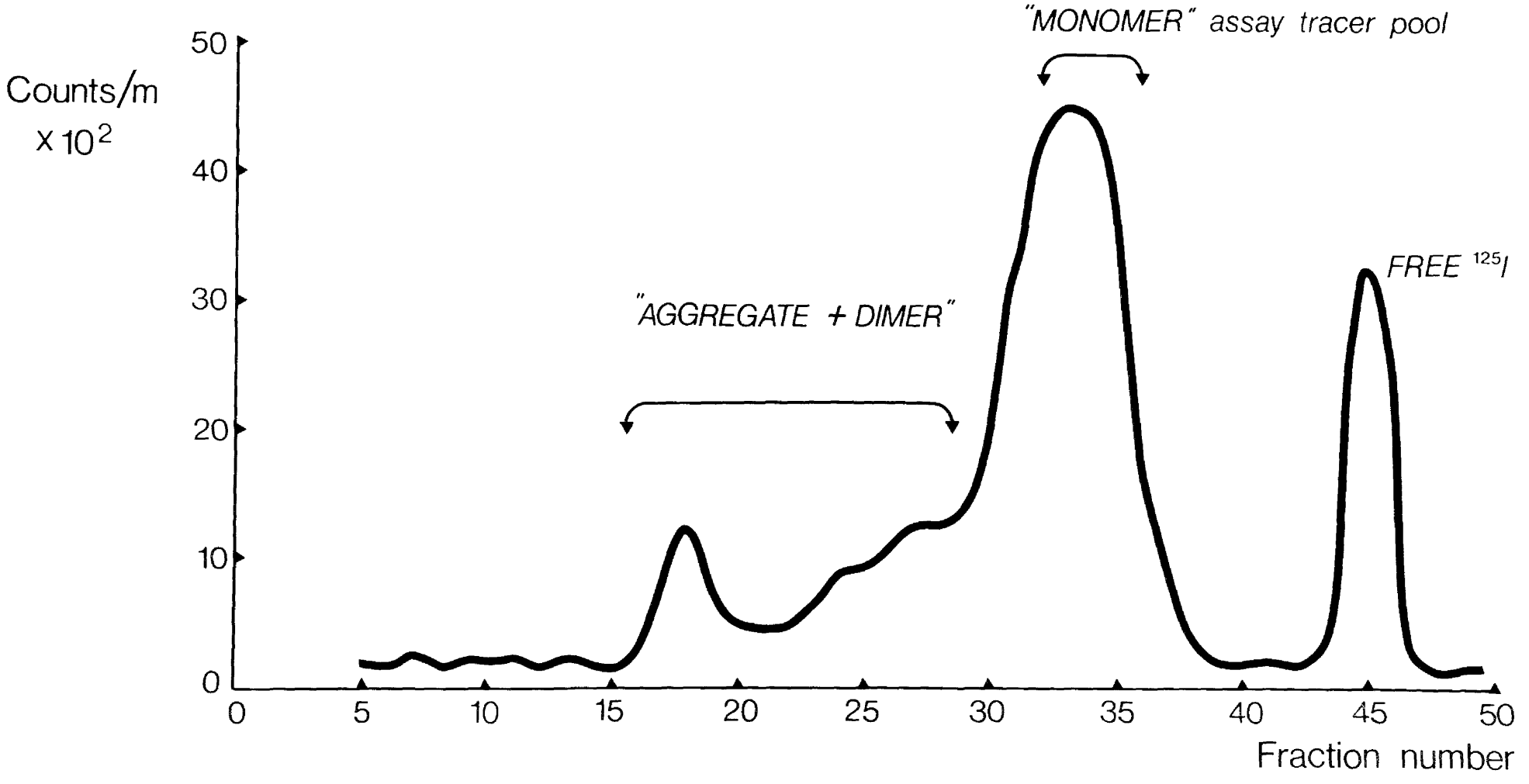

"MONOMER" assay tracer pool
FREE 125I
"AGGREGATE + DIMER"
Counts/m
x 10²
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
Fraction number

FIGURE 9

Radioimmunoassay of human prolactin: Comparison of the binding of "monomer" and "aggregate" tracer material in standard curve.

NSB: non specific binding.

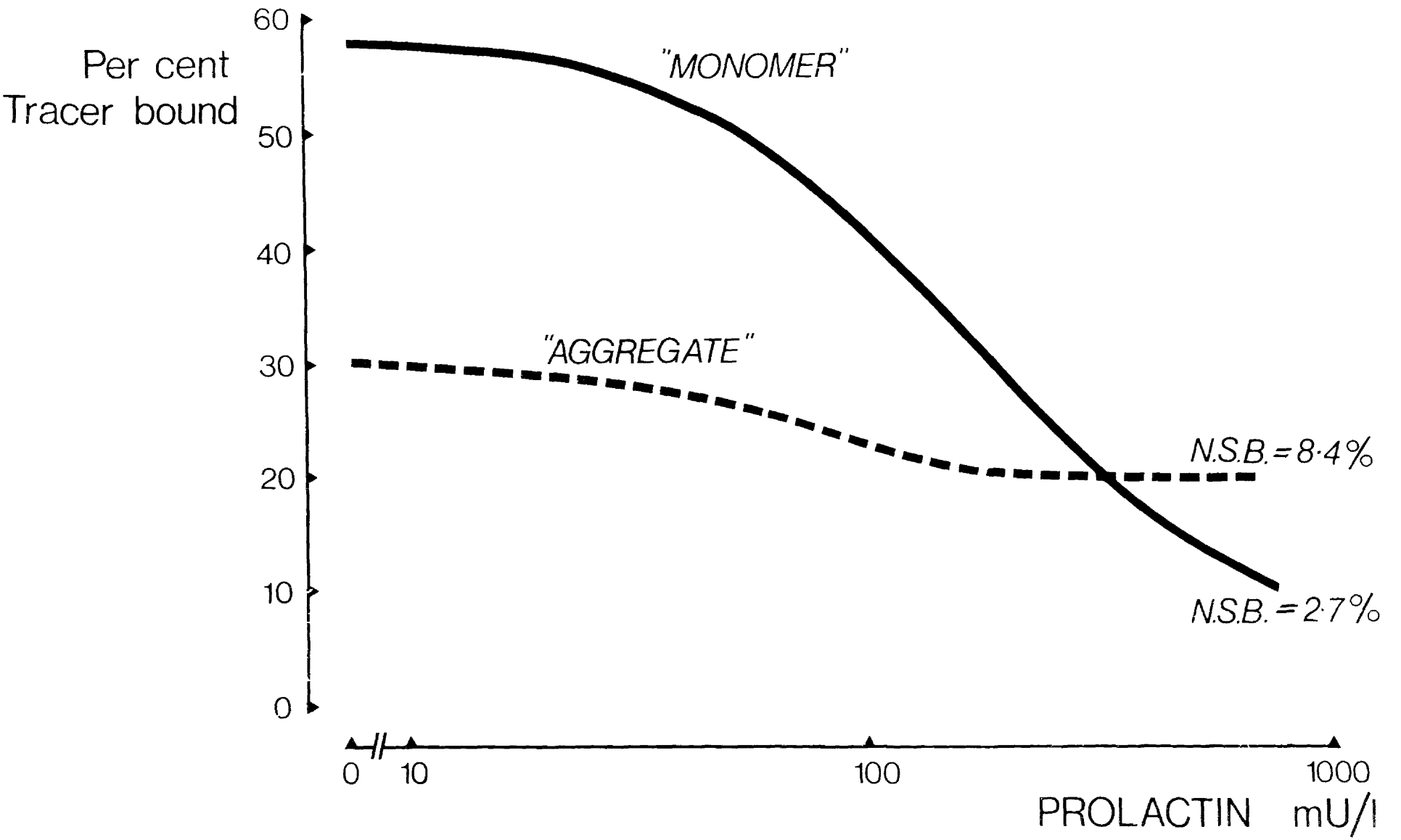

Per cent
Tracer bound
60
50
40
30
20
10
0
"MONOMER"
"AGGREGATE"
N.S.B. = 8·4%
N.S.B. = 2·7%
0
10
100
1000
PROLACTIN mU/l

FIGURE 10

$^{125}$I human prolactin 75.7.10: Serial repurification profiles.

Label prepared on 5.1.77 and repurified, as described in text, from 5-40 days after preparation.

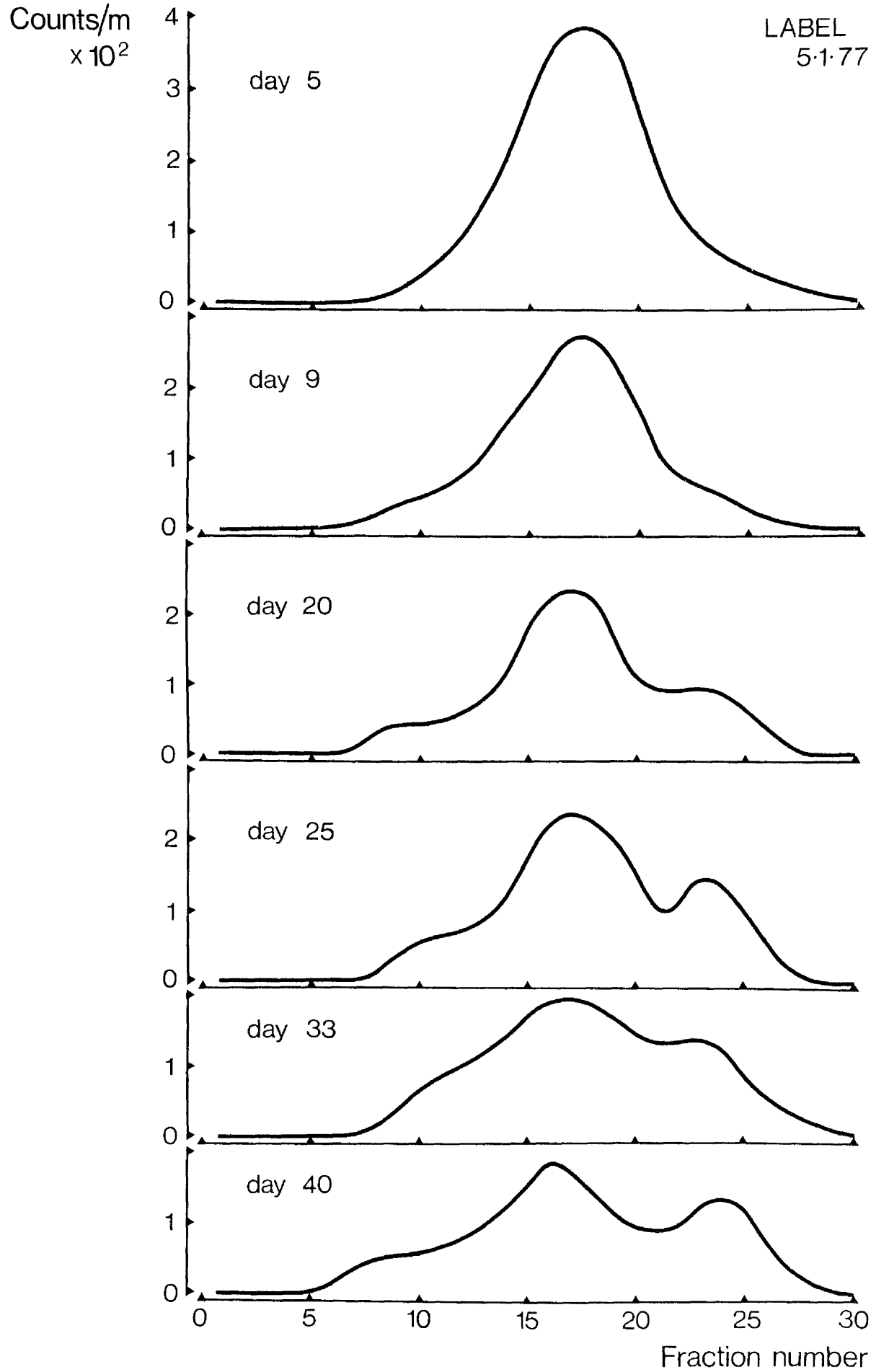

Counts/m
x 10²
LABEL
5·1·77
day 5
day 9
day 20
day 25
day 33
day 40
0
1
2
3
4
5
10
15
20
25
30
Fraction number

FIGURE 11

Antibody dilution curve for FR AR7-13 rabbit anti human prolactin serum.

Routine working antibody dilution 1:28,000.

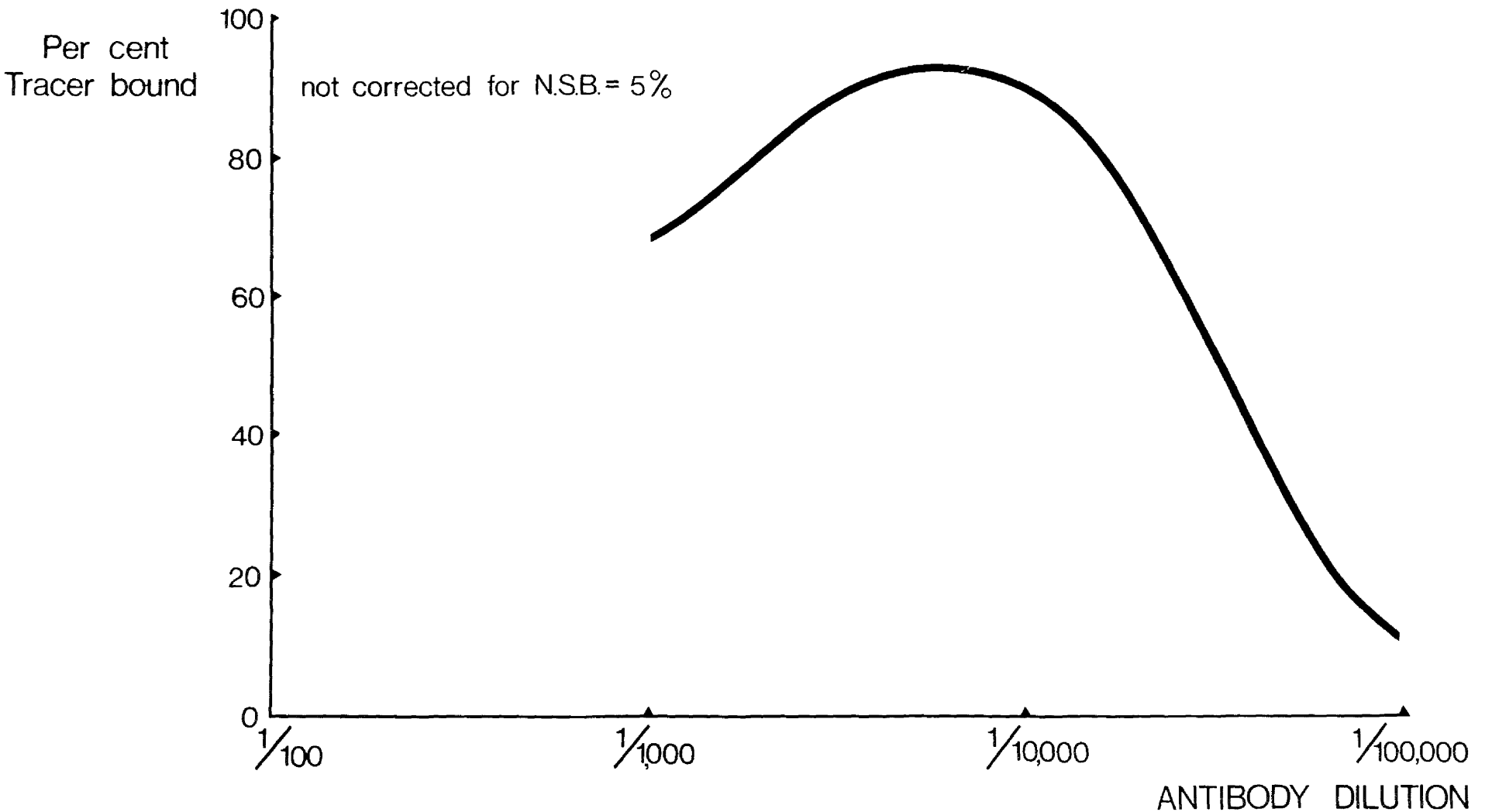

Per cent
Tracer bound
100
80
60
40
20
0
not corrected for N.S.B. = 5%
1/100
1/1,000
1/10,000
1/100,000
ANTIBODY DILUTION

FIGURE 12

Linear regression curves comparing serum prolactin levels measured in three systems (i) Friesen (Routine) system (ii) NIH system (iii) Mixed system.

Assay systems as described in text.

Values in Friesen system plotted on the X axis.

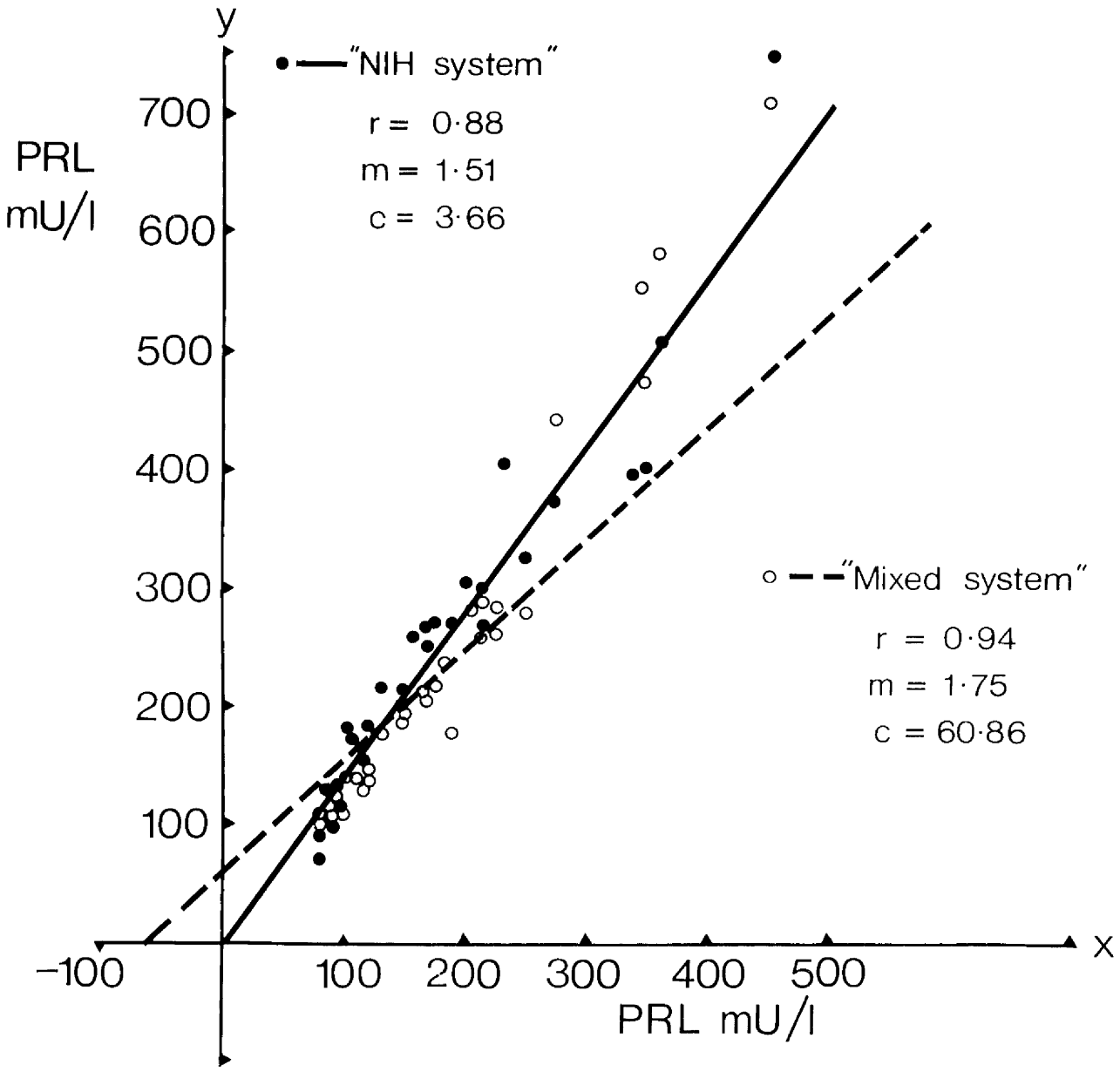

y
"NIH system"
r = 0·88
m = 1·51
c = 3·66
PRL
mU/l
700
600
500
400
300
200
100
"Mixed system"
r = 0·94
m = 1·75
c = 60·86
x
−100
100
200
300
400
500
PRL mU/l

FIGURE 13

Radioimmunoassay of human prolactin: Specificity of FR AR 7-13 antiserum.

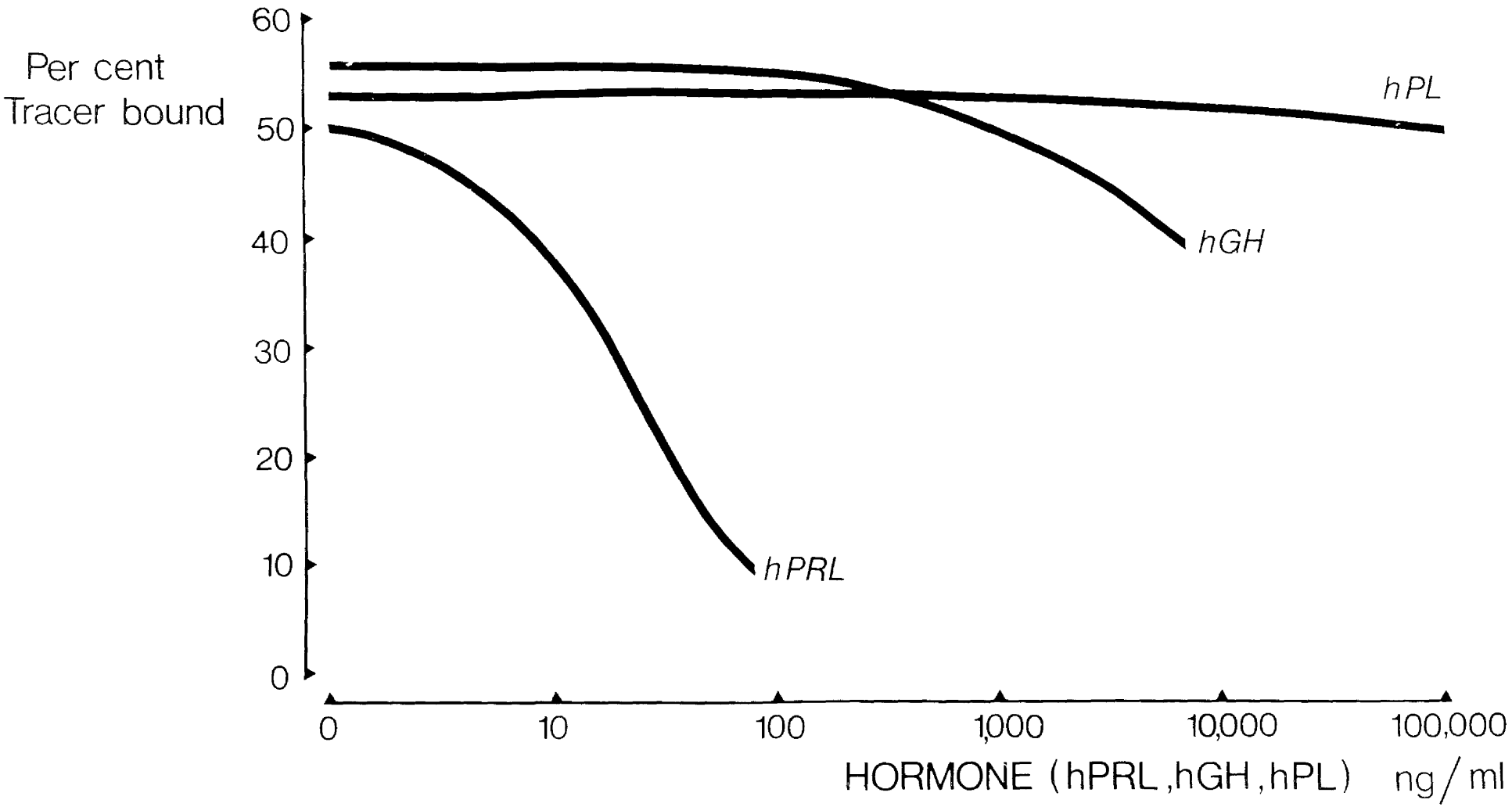

Per cent
Tracer bound
60
50
40
30
20
10
0
hPL
hGH
hPRL
0
10
100
1,000
10,000
100,000
HORMONE (hPRL, hGH, hPL) ng/ml

FIGURE 14

(a) Radioimmunoassay of human prolactin: Serum Effects 1. Coded lines represent the mean suppression of diluent standard curve by 50 µl horse sera (n = 4) and 50 µl human "prolactin free" sera (n = 6).

(b) Radioimmunoassay of human prolactin: Serum Effects 2. Coded lines represent the mean suppression of diluent standard curve by varying volumes of horse sera (n = 4).

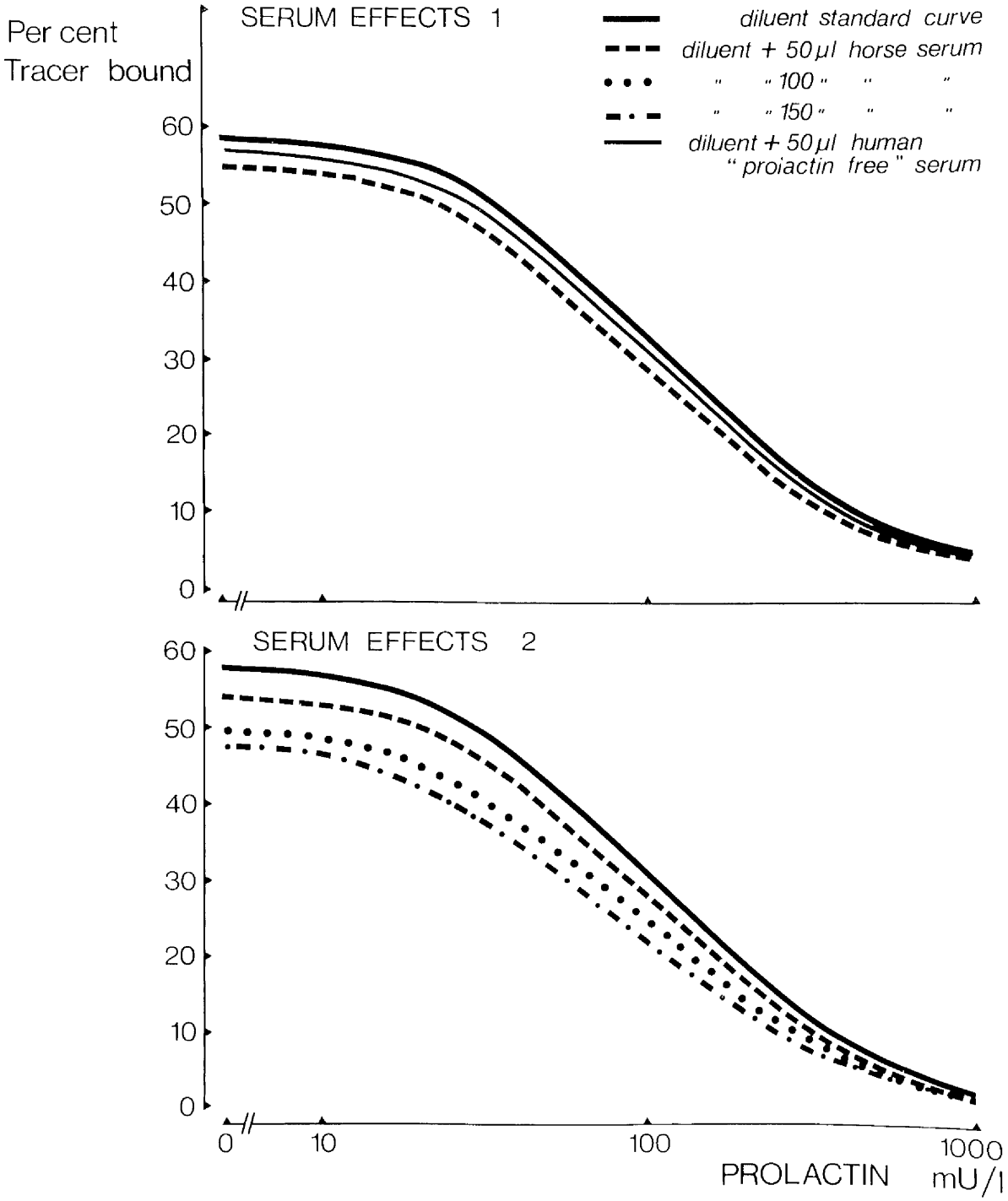

SERUM EFFECTS 1
Per cent
Tracer bound
diluent standard curve
diluent + 50µl horse serum
" " 100 " " "
" " 150 " " "
diluent + 50µl human
" proiactin free " serum
60
50
40
30
20
10
0
SERUM EFFECTS 2
60
50
40
30
20
10
0
0
10
100
1000
PROLACTIN mU/l

FIGURE 15

Radioimmunoassay of human prolactin: Parallelism studies. Serial dilutions of sera from patients with uraemia and the amenorrhoea-galactorrhoea syndrome, inhibit binding of $^{125}I$ human prolactin tracer to antibody in parallel with MRC 75/504 reference preparation.

*PROLACTIN R.I.A. STANDARD CURVE & PARALLELISM STUDIES.*

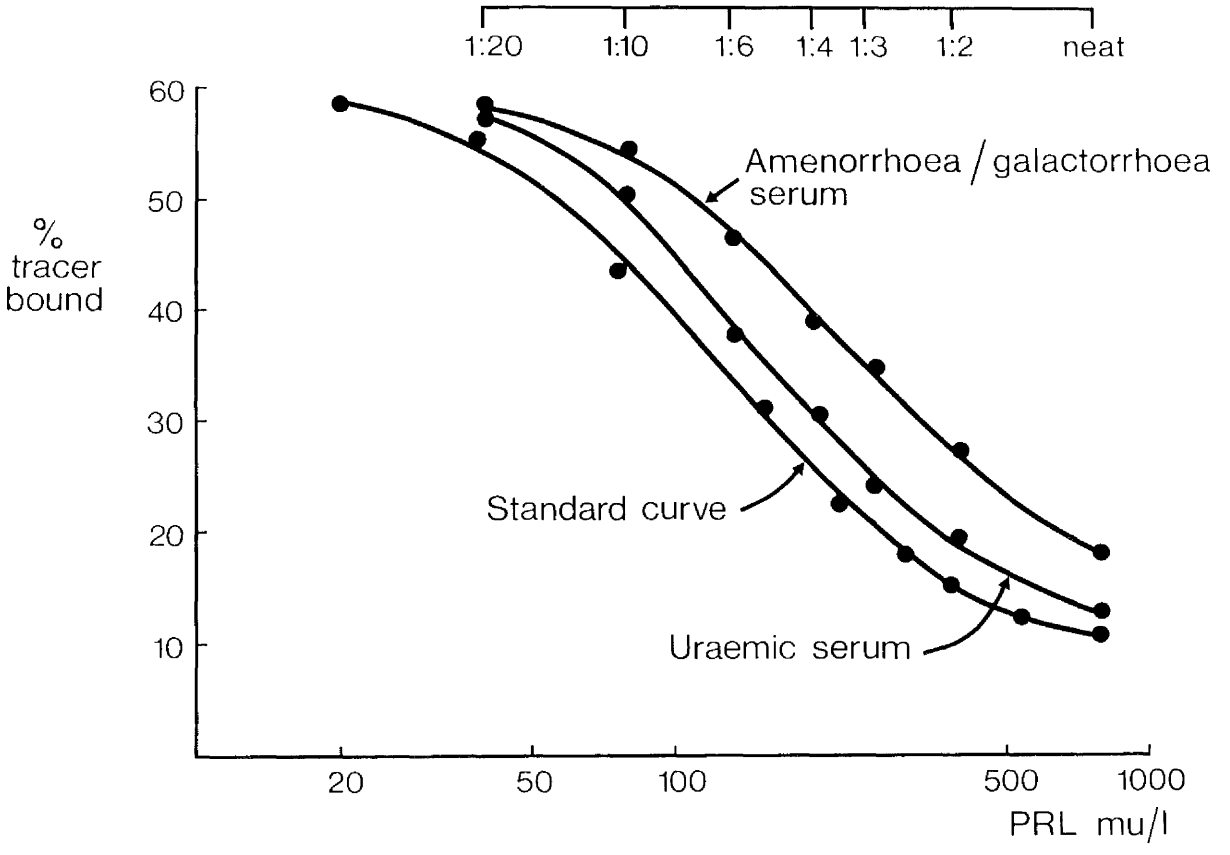


FIGURE 16

Basal prolactin levels in normal subjects.

Groups as defined in text.

Numbers in each group indicated at bottom of each column.

BASAL PROLACTIN LEVELS IN "NORMAL" SUBJECTS.

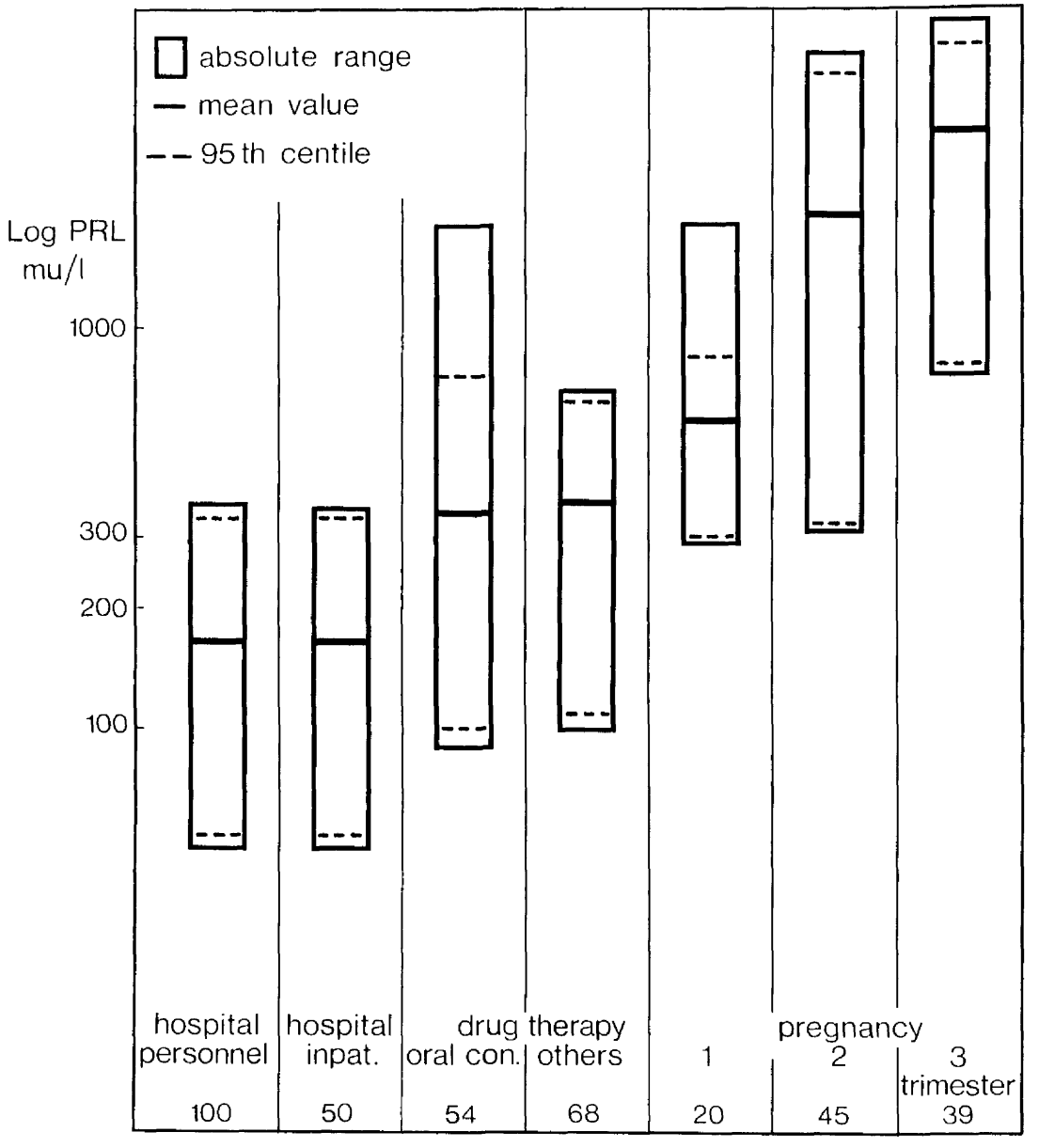


FIGURE 17

Age and sex related prolactin levels

M males F females

N.S. not significant

$p < 0.005$, females aged 15-30 years versus females aged 50+ years.

AGE AND SEX RELATED PROLACTIN LEVELS.

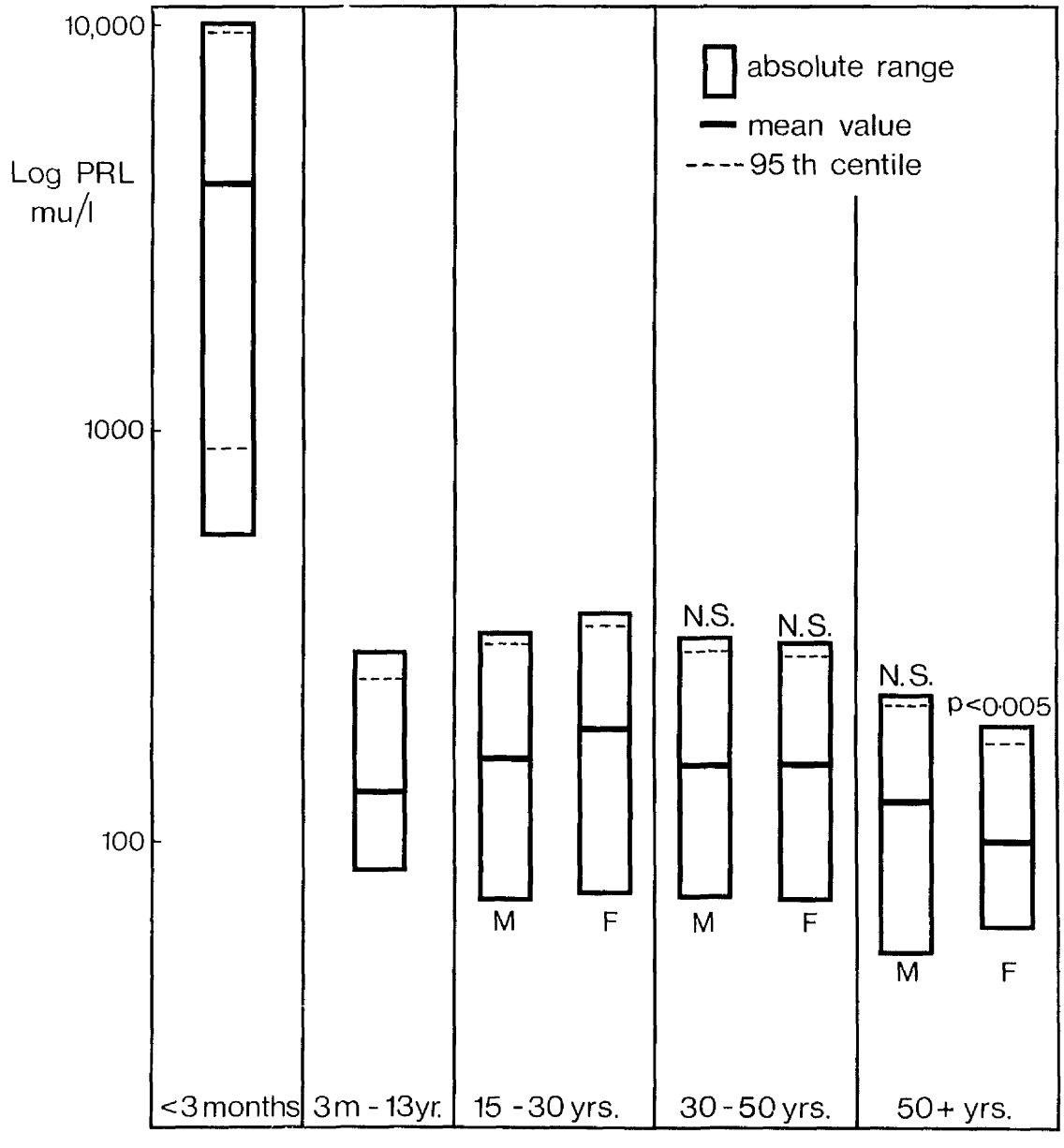


FIGURE 18

Within day variations in prolactin levels.

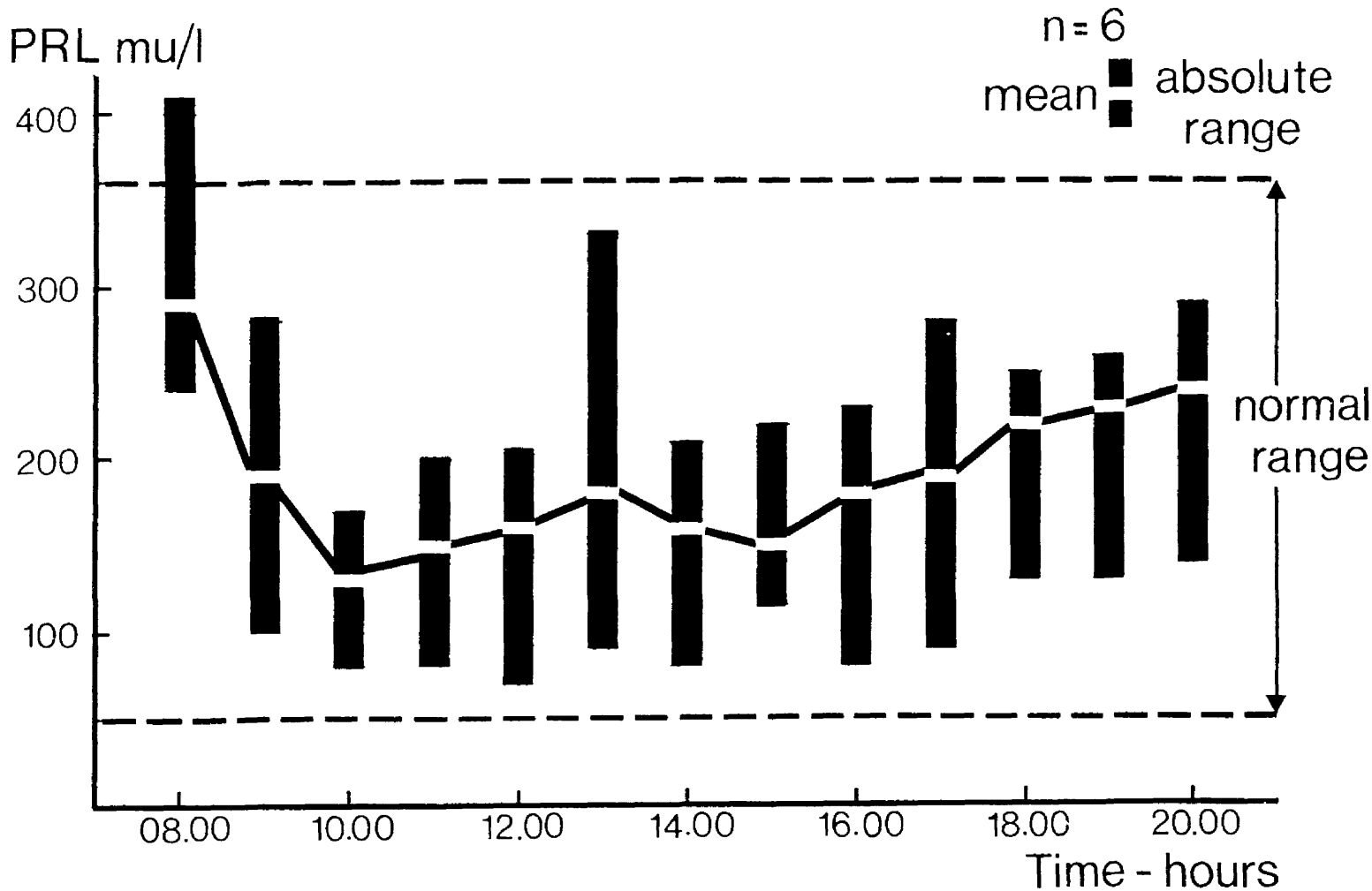

PRL mu/l
n = 6
mean
absolute range
400
300
200
100
normal range
08.00
10.00
12.00
14.00
16.00
18.00
20.00
Time - hours

FIGURE 19

Day to day variations in prolactin levels

Day to day variations in Prolactin levels.

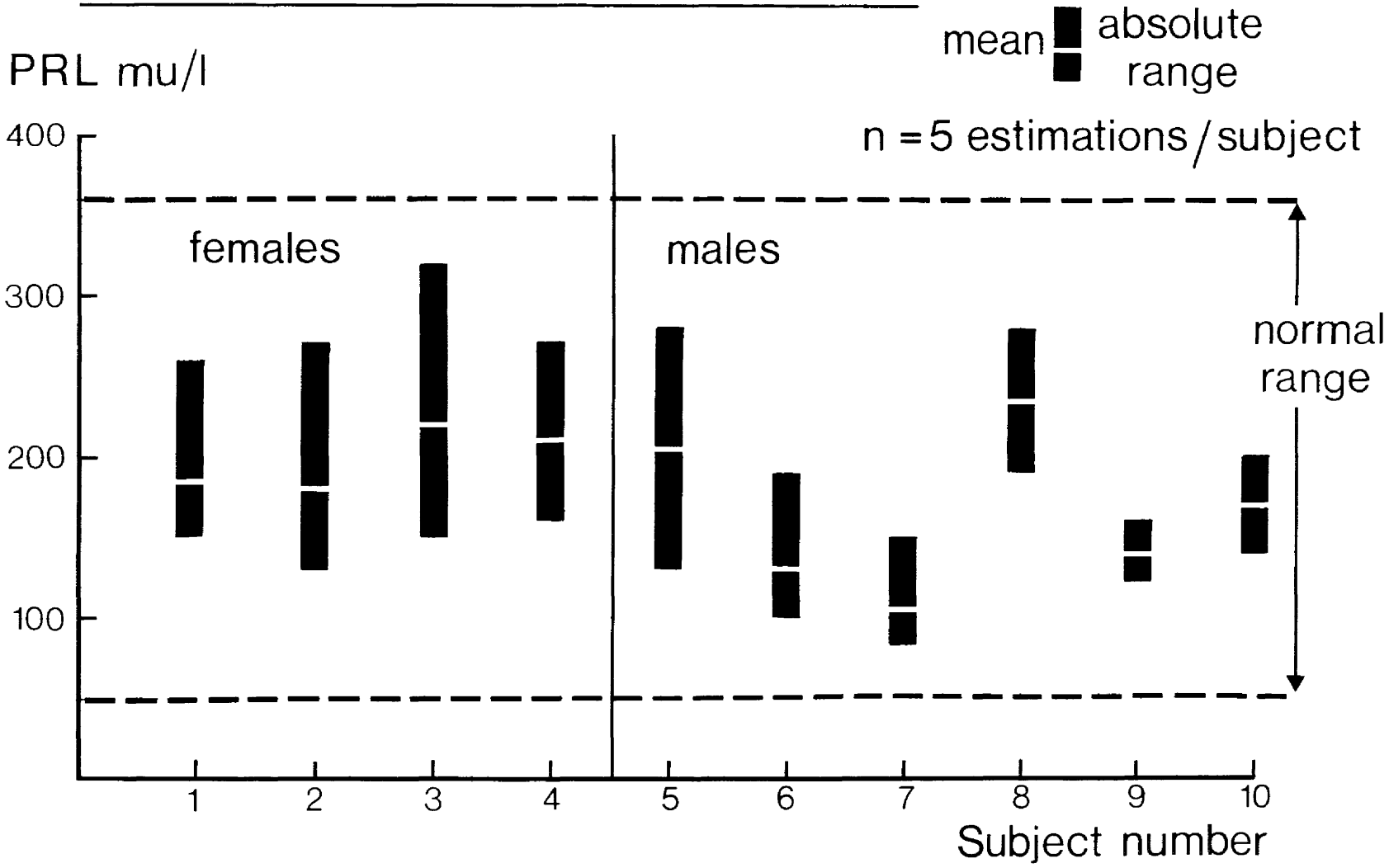


FIGURE 20

Basal prolactin levels in pathological conditions

Groups as defined in text

Tx: treatment

RDT: regular dialysis treatment

Gn: gonadotrophin

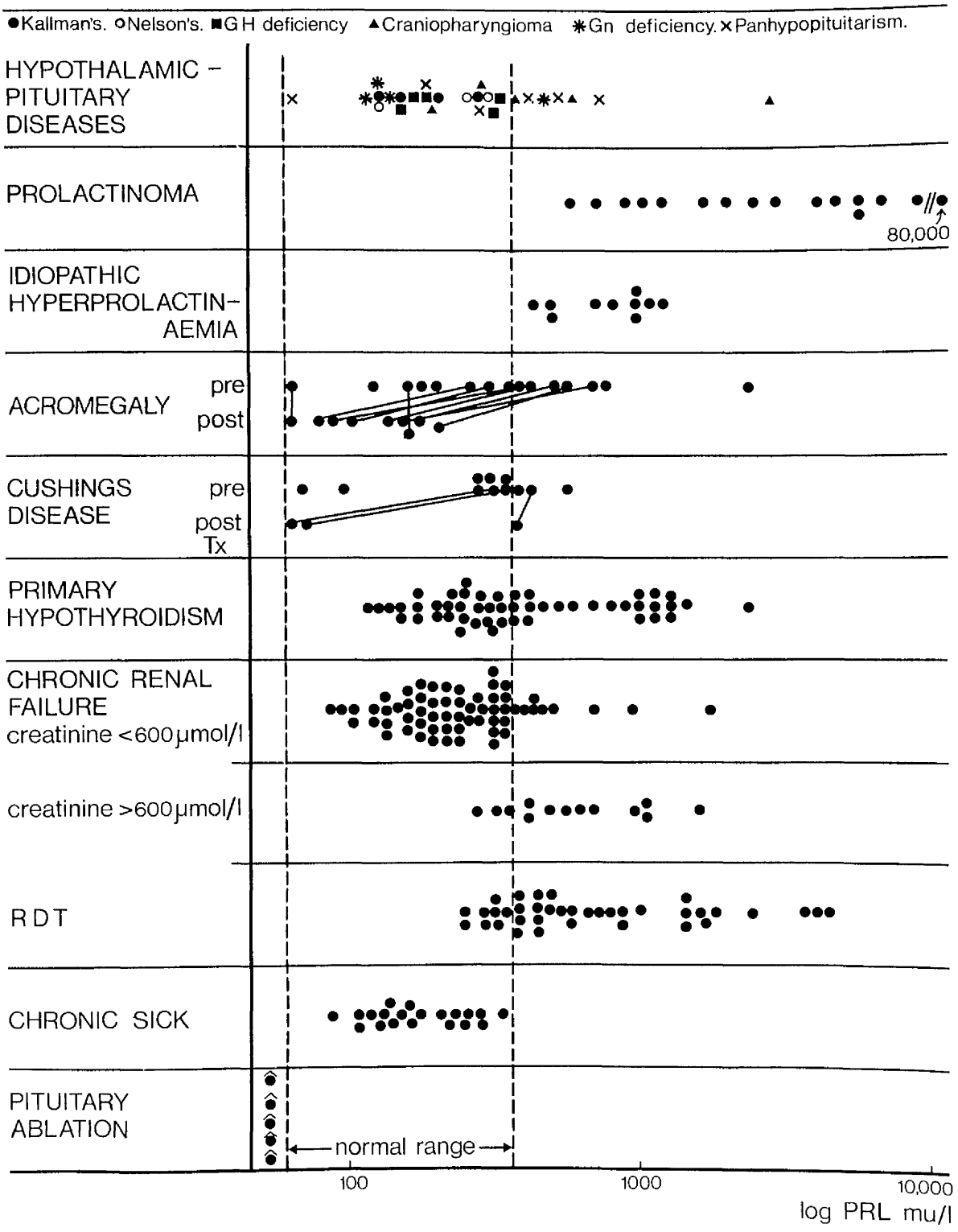

●Kallman's. ○Nelson's. ■G H deficiency ▲Craniopharyngioma ✱Gn deficiency. ×Panhypopituitarism.
HYPOTHALAMIC – PITUITARY DISEASES
PROLACTINOMA
80,000
IDIOPATHIC HYPERPROLACTIN-AEMIA
ACROMEGALY
pre
post
CUSHINGS DISEASE
pre
post Tx
PRIMARY HYPOTHYROIDISM
CHRONIC RENAL FAILURE
creatinine <600µmol/l
creatinine >600µmol/l
RDT
CHRONIC SICK
PITUITARY ABLATION
normal range
100
1000
10,000
log PRL mu/l

FIGURE 21

Prolactin response to TRH stimulation: Normal subjects.

SEM: standard error of mean

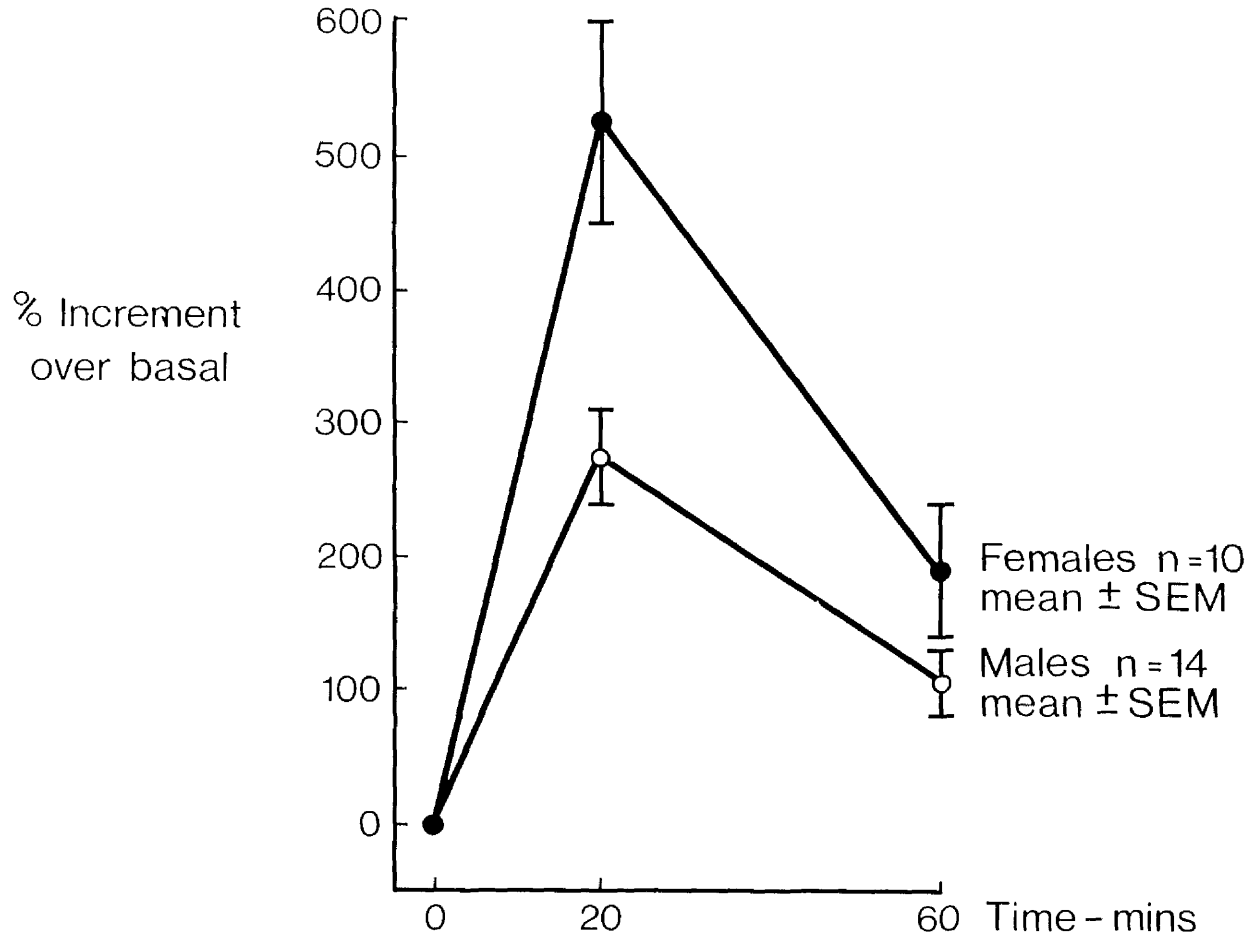

% Increment over basal
600
500
400
300
200
100
0
0
20
60
Time - mins
Females n = 10
mean ± SEM
Males n = 14
mean ± SEM

FIGURE 22

Prolactin response to metoclopramide stimulation:

Normal subjects.

SEM: standard error of mean.

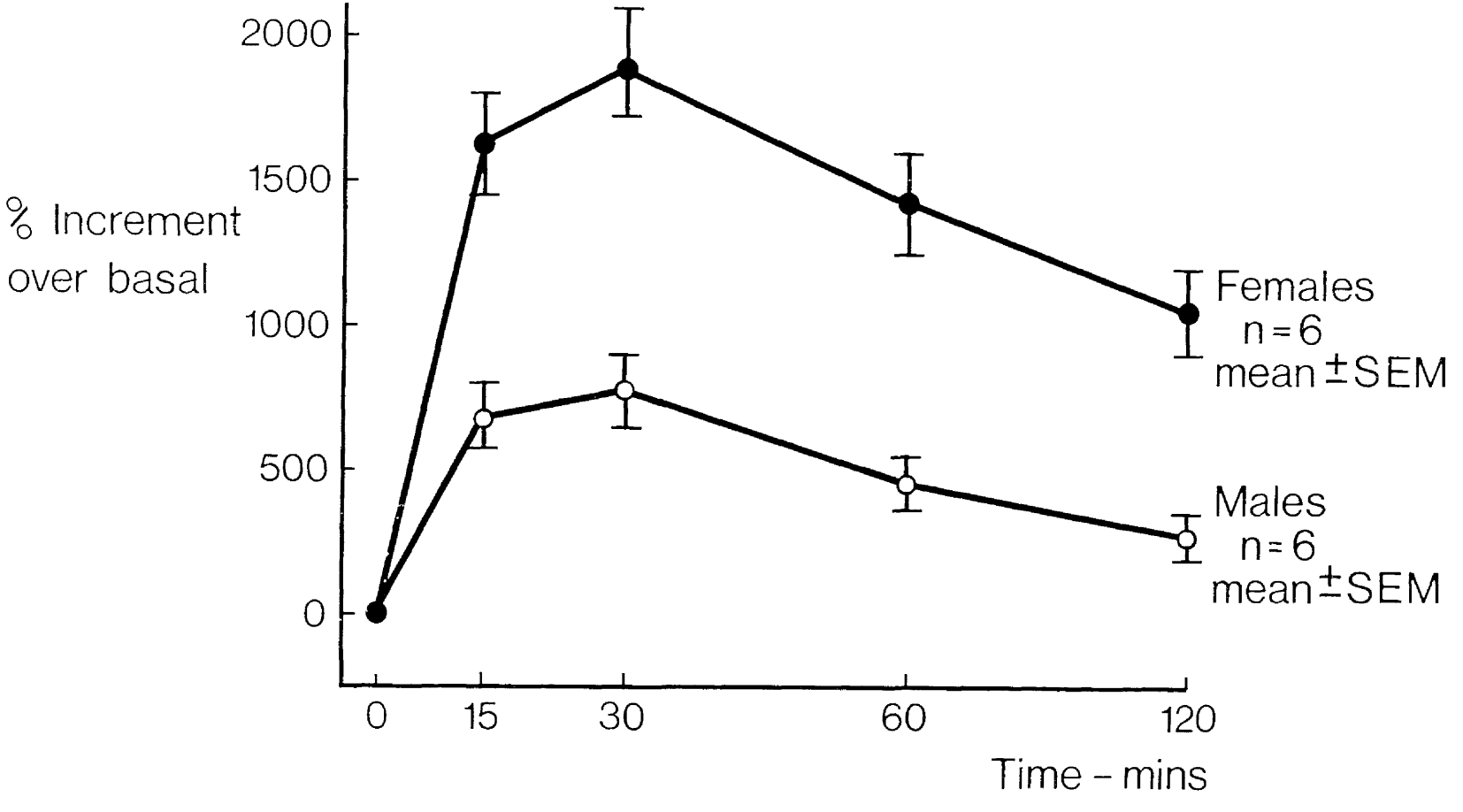

2000
1500
1000
500
0
% Increment over basal
0
15
30
60
120
Time - mins
Females
n = 6
mean ± SEM
Males
n = 6
mean ± SEM

FIGURE 23

Prolactin suppression by L-dopa: Normal subjects. Controls as defined in text.

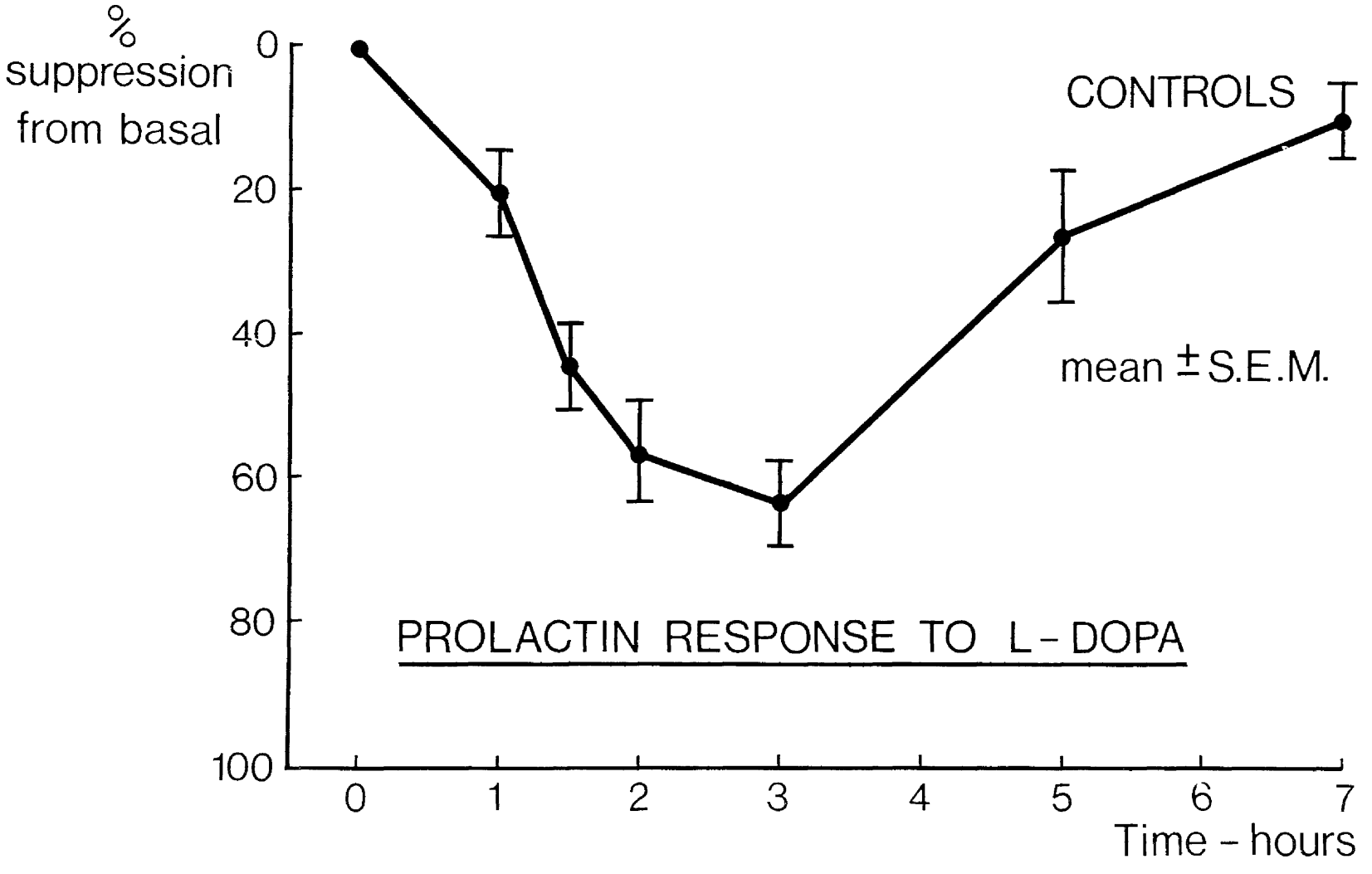

%
suppression
from basal
0
20
40
60
80
100
CONTROLS
mean ± S.E.M.
PROLACTIN RESPONSE TO L-DOPA
0
1
2
3
4
5
6
7
Time - hours

FIGURE 24

Prolactin suppression by Bromocriptine: Normal subjects. Controls as defined in text.

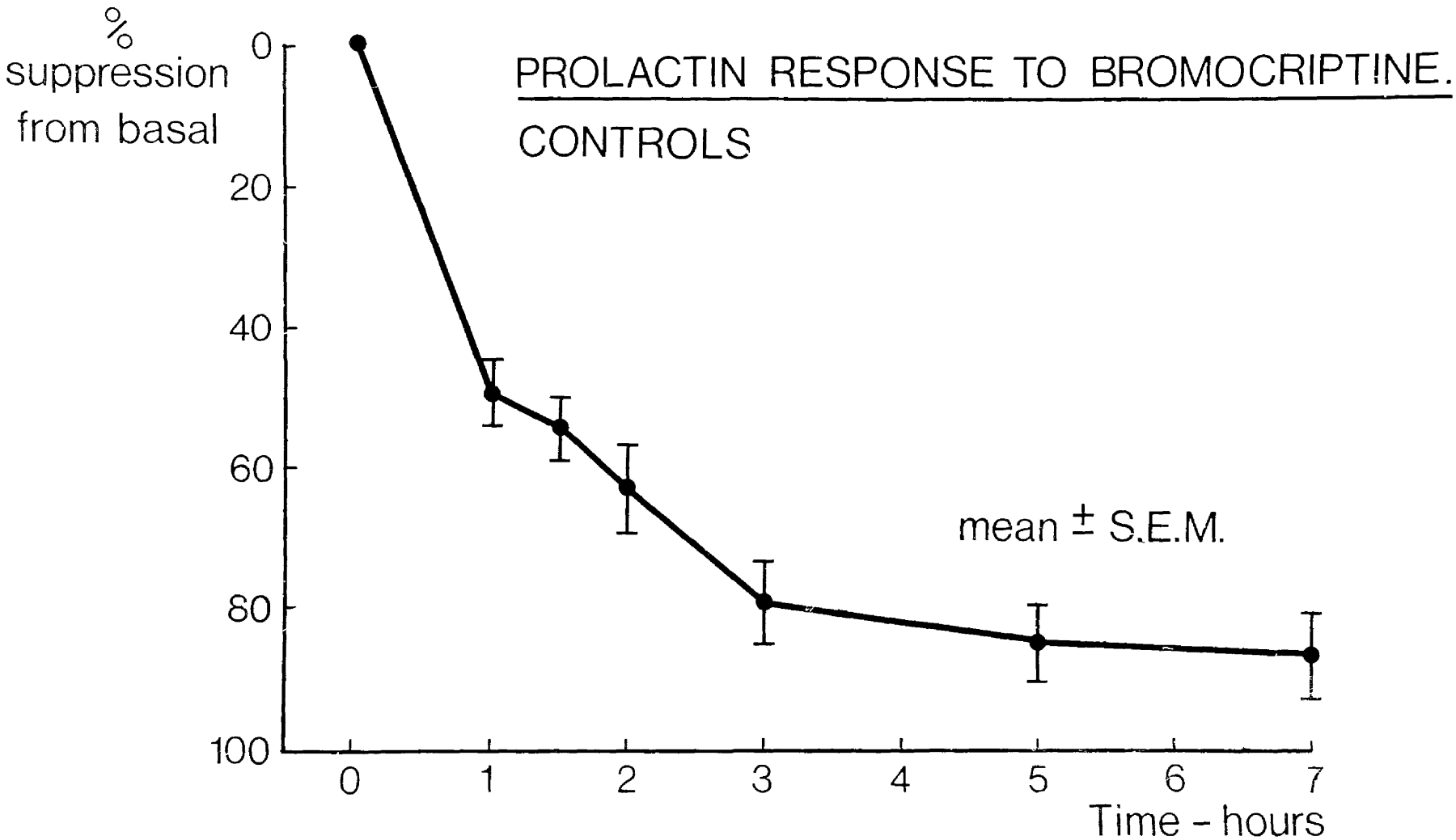

%
suppression
from basal
0
20
40
60
80
100
PROLACTIN RESPONSE TO BROMOCRIPTINE.
CONTROLS
mean ± S.E.M.
0
1
2
3
4
5
6
7
Time - hours

FIGURE 25

Prolactin secreting tumours of the pituitary:

Light microscopy.

A: Chromophobe adenoma

B: Eosinophilic adenoma

C: Amphophilic adenoma

Magnification x 250.

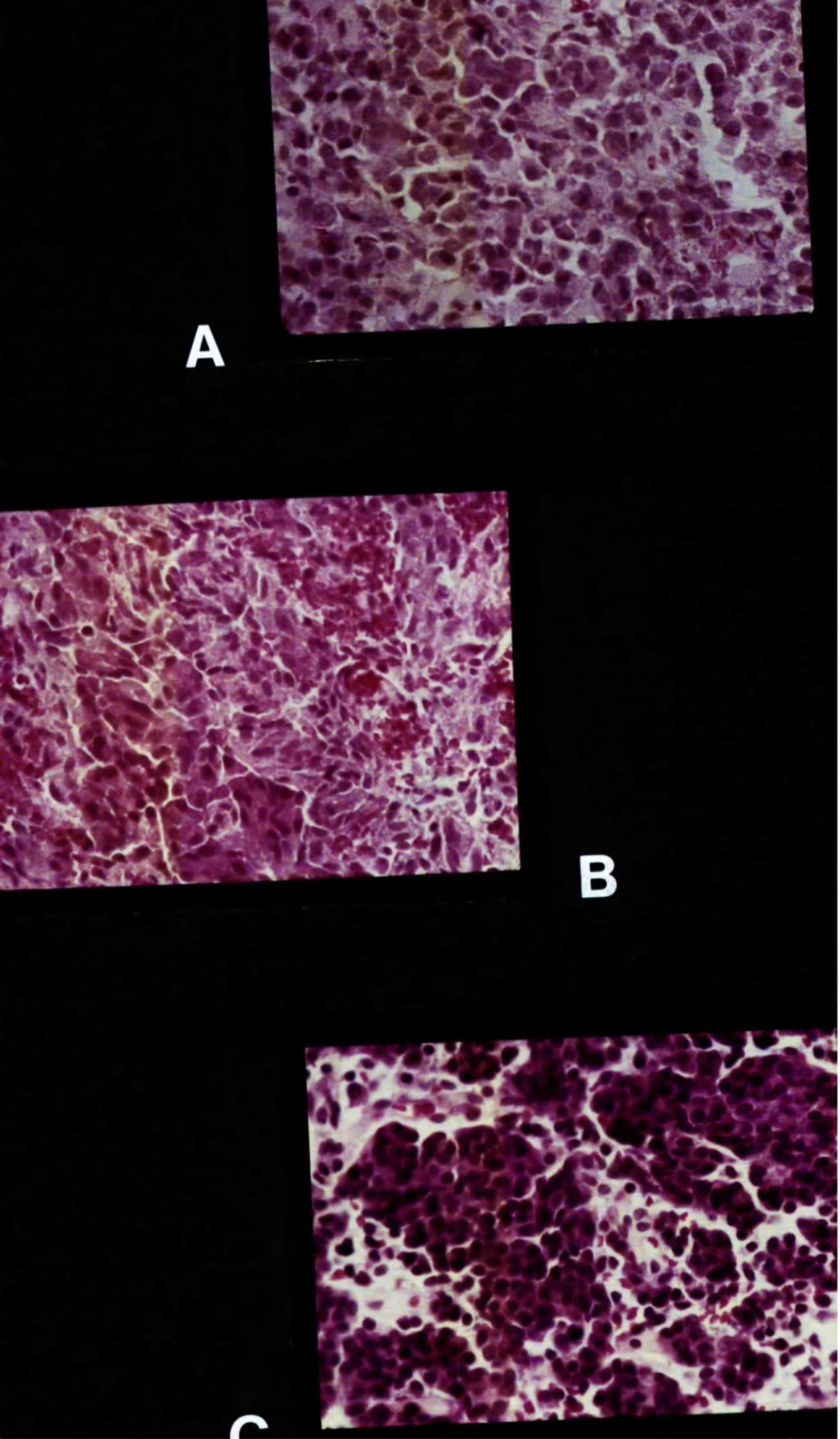

A
B
C

FIGURE 26

Prolactin secreting tumours of the pituitary: Immunoperoxidase staining.

(i) Normal, scattered distribution of prolactin cells in 27y old male who died in a road traffic accident.

(ii) Prolactin cells of increased size, number and activity in 29y old female who died sixteen weeks advanced in pregnancy.

(iii) Profuse, variably active prolactin secreting tumour cells with loss of normal pituitary architecture.

Magnification x 565.

i
ii
iii

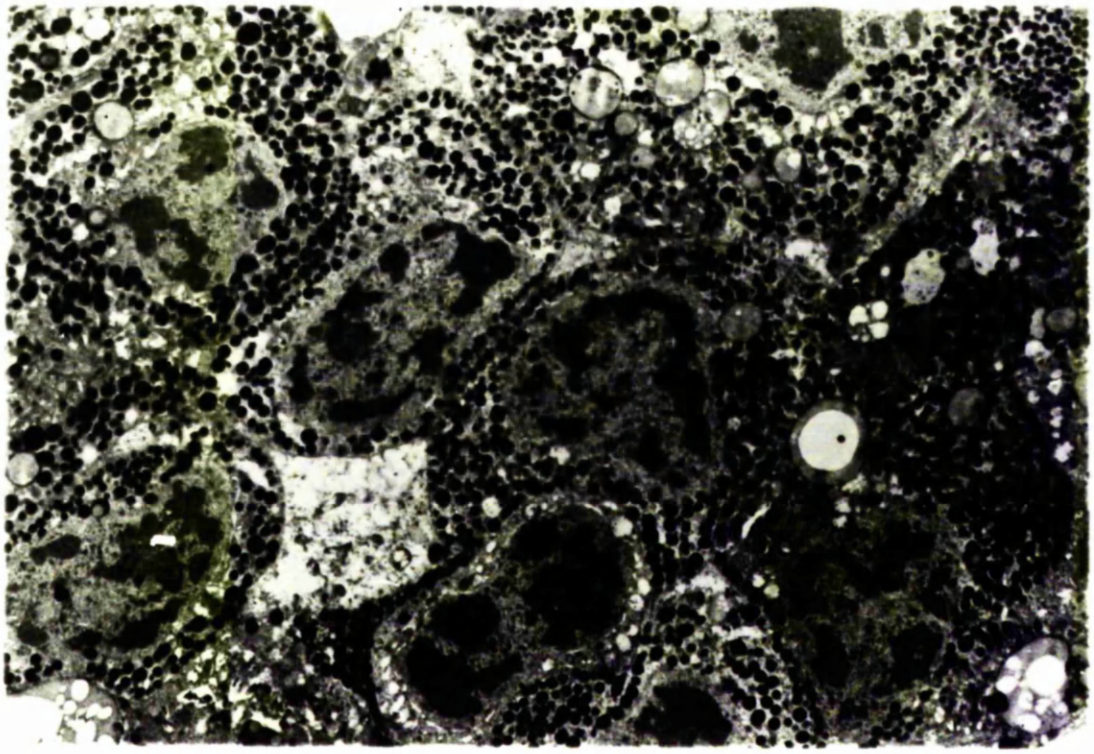
FIGURE 27

Prolactin secreting tumours of the pituitary:

Electron microscopy.

Plentiful large pleomorphic secretory granules.

Magnification x 9000.



Radiographs of the pituitary fossa are arranged as follows, unless otherwise stated:

| | |
|---|---|
| View A | Lateral Xray of skull |
| View B | Lateral tomogram |
| View C | Antero-posterior tomogram. |

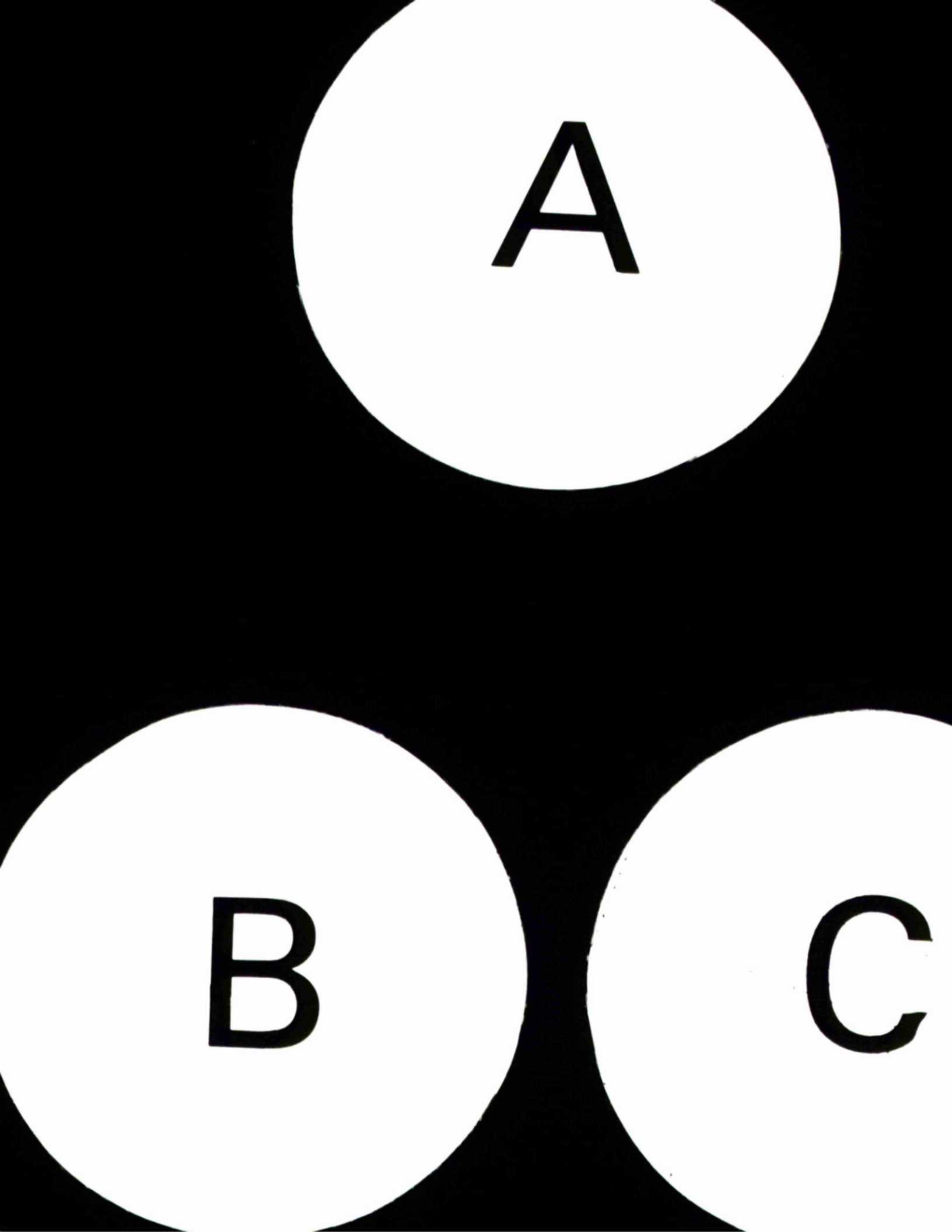

A
B
C

FIGURE 28

Normal pituitary tomography: Patient 3

1 pituitary fossa

2 dorsum sellae

3 sphenoid sinus

4 floor of the pituitary fossa


1
2
3

FIGURE 29

Normal pituitary tomography: Patient 4.

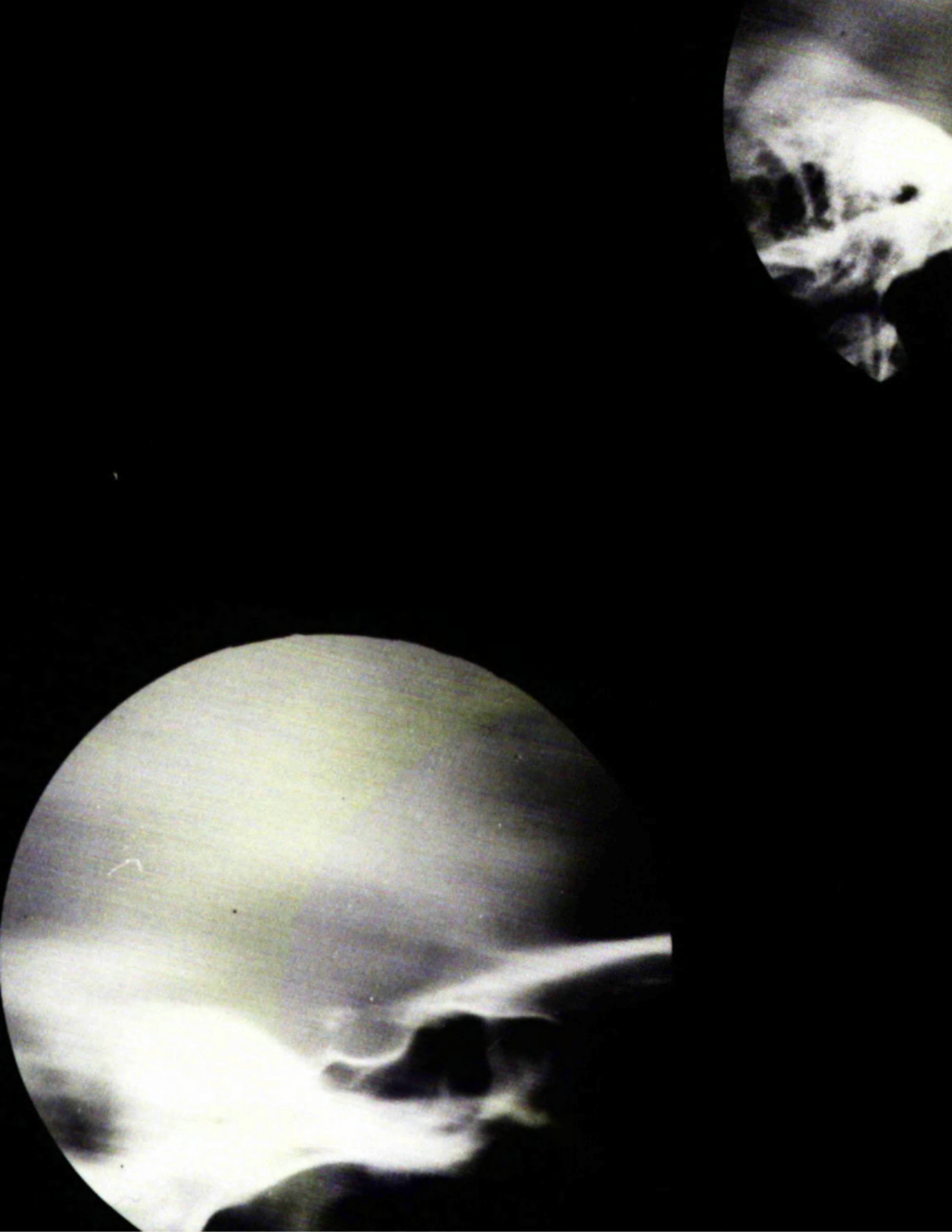


FIGURE 30

Normal pituitary tomography: Patient 6.

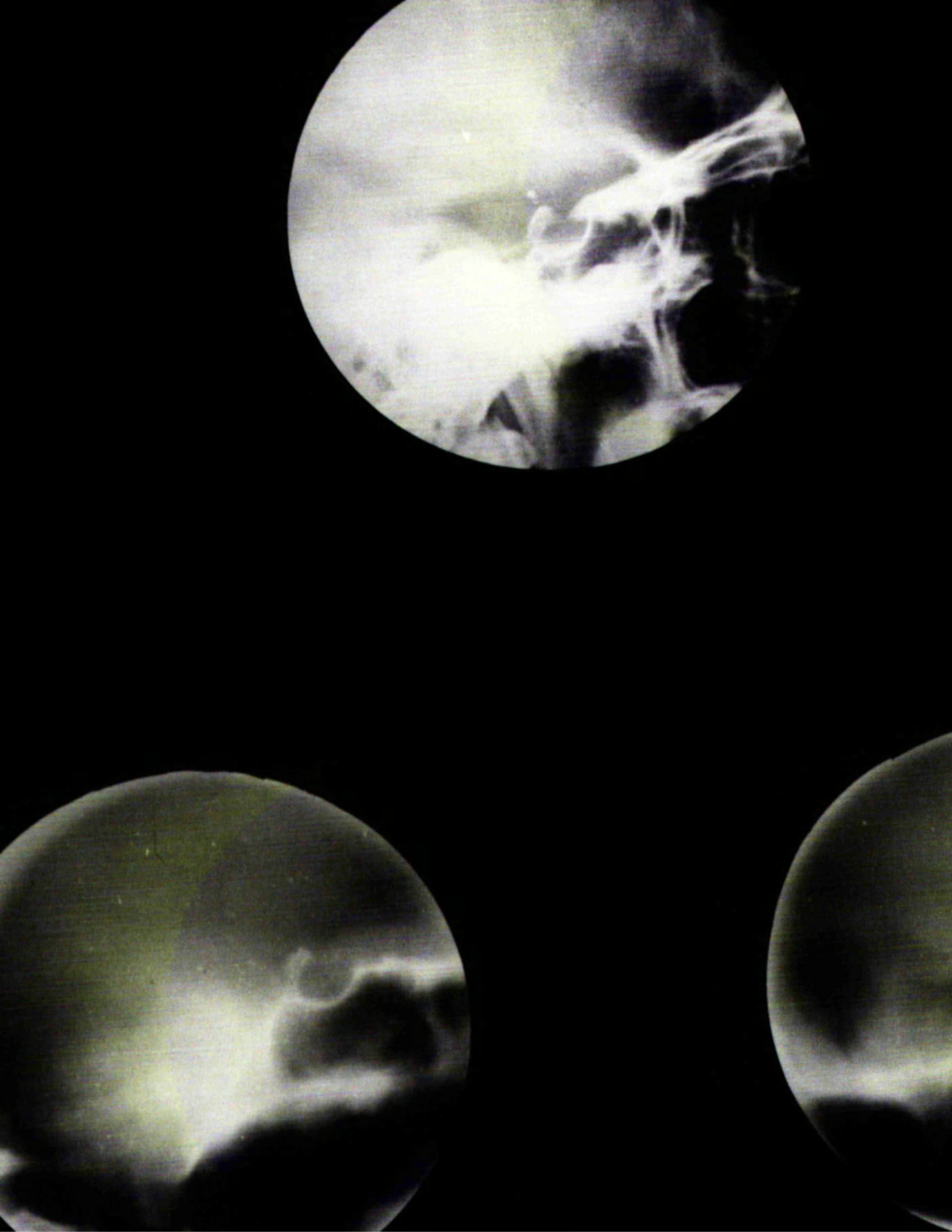


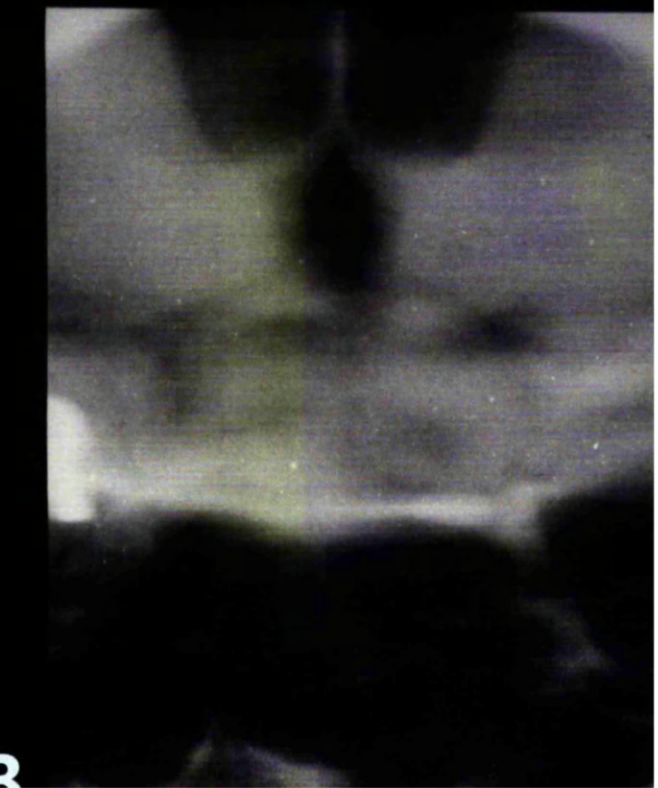
FIGURE 31 (a)

Pituitary radiology: Patient 18

View A Lateral tomogram showing ballooning of the pituitary fossa and erosion of bony margin.

View B Antero-posterior tomogram during air encephalography showing bulging of the fossa floor and large supra sellar extension of tumour.

A



B

FIGURE 31 (b)

EMI scan: Patient 18

A fluid level is clearly demonstrated in the supra sellar extension of the pituitary tumour.

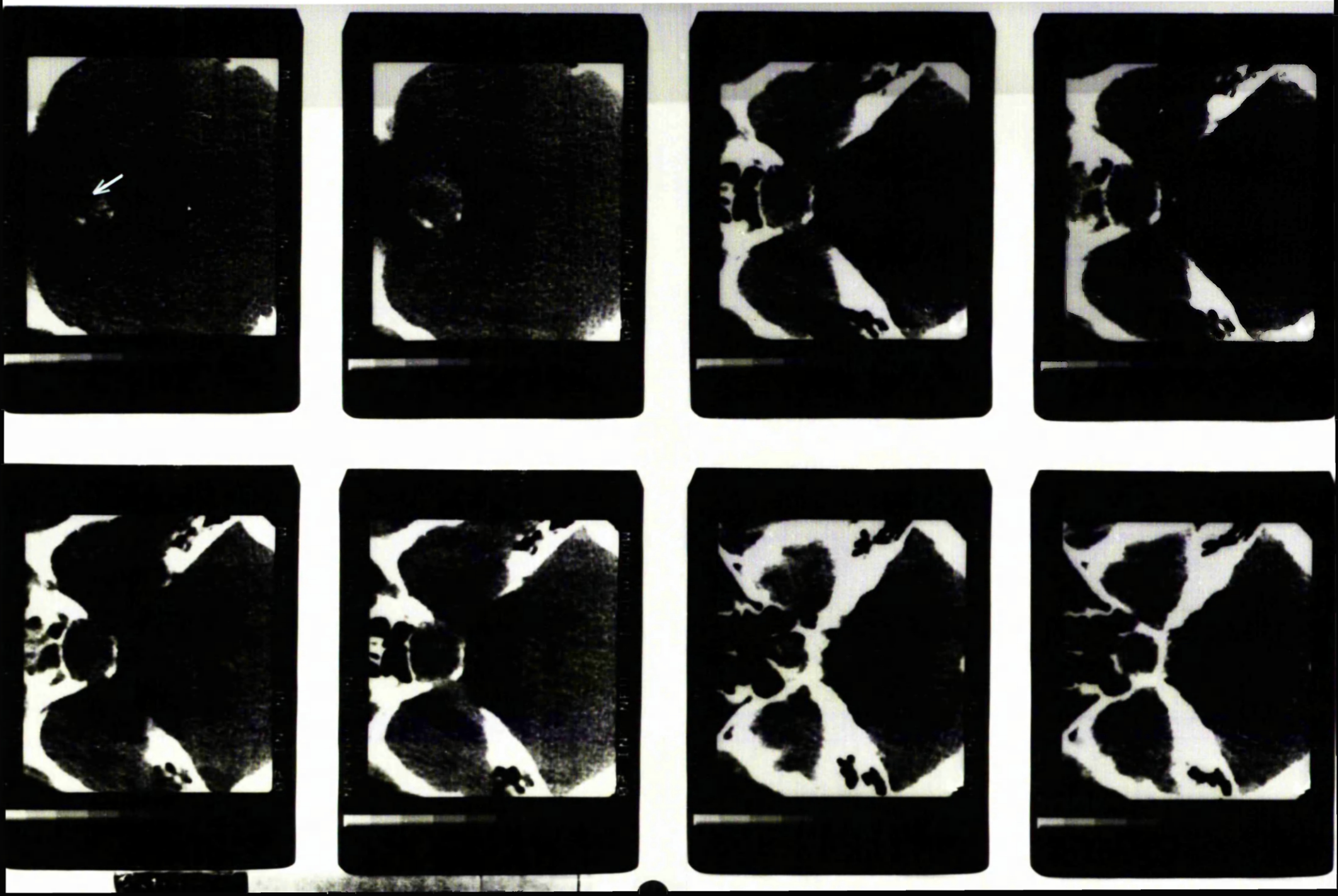


FIGURE 32 (a)

Pituitary radiology: Patient 16

An enlarged pituitary fossa with an inferior bulge seen on lateral tomogram.

FIGURE 32 (b)

Pituitary radiology: Patient 16.

A comparison of 1974 and 1978 films.

View A Lateral Xray of skull 1974

View B Lateral Xray of skull 1978,
showing extension of inferior bulge.

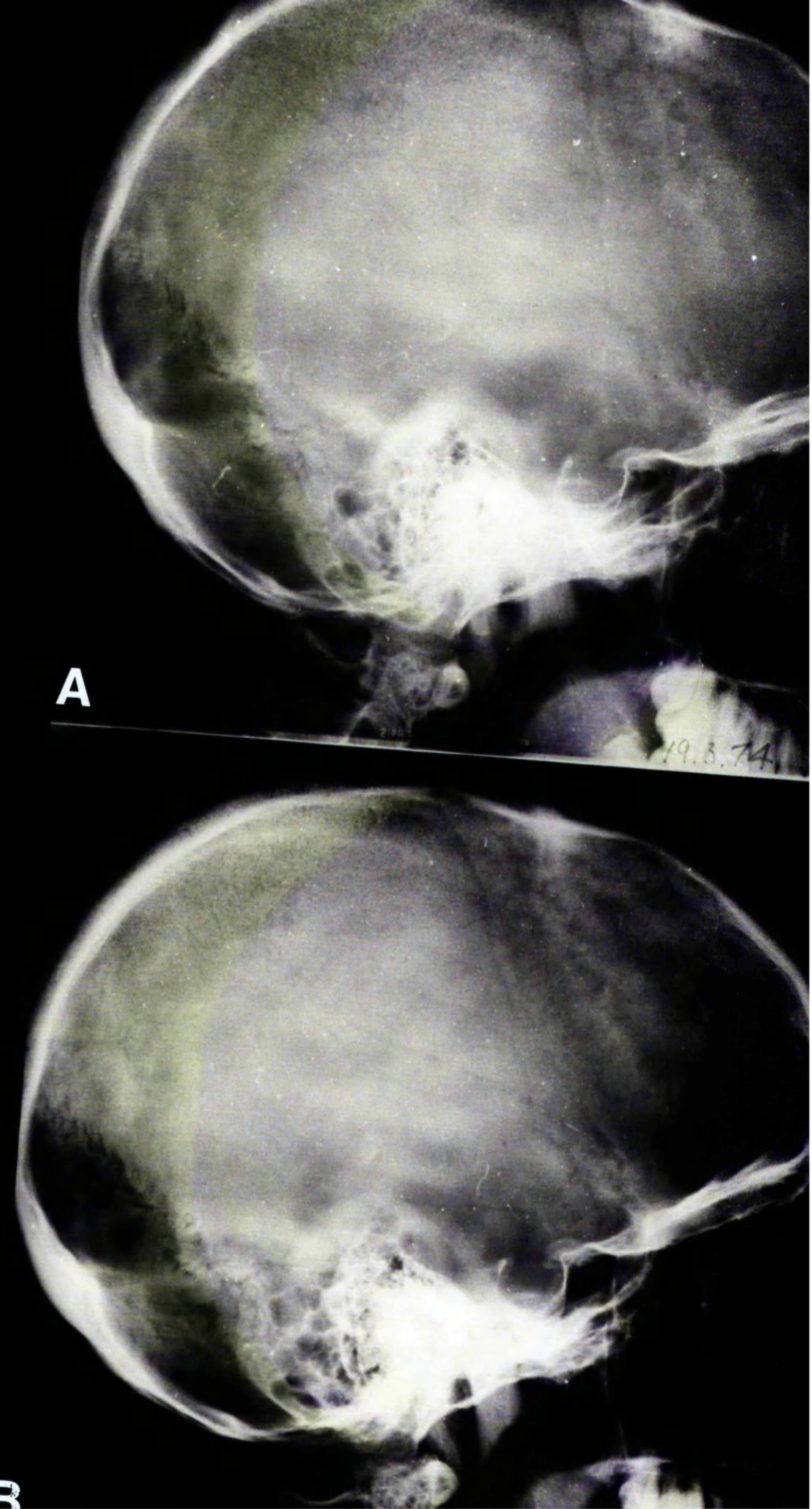

A
19.8.74
B

FIGURE 33

Pituitary radiology: Patient 12
Lateral tomogram showing erosion
of bony margins and bulge into
sphenoid sinus.

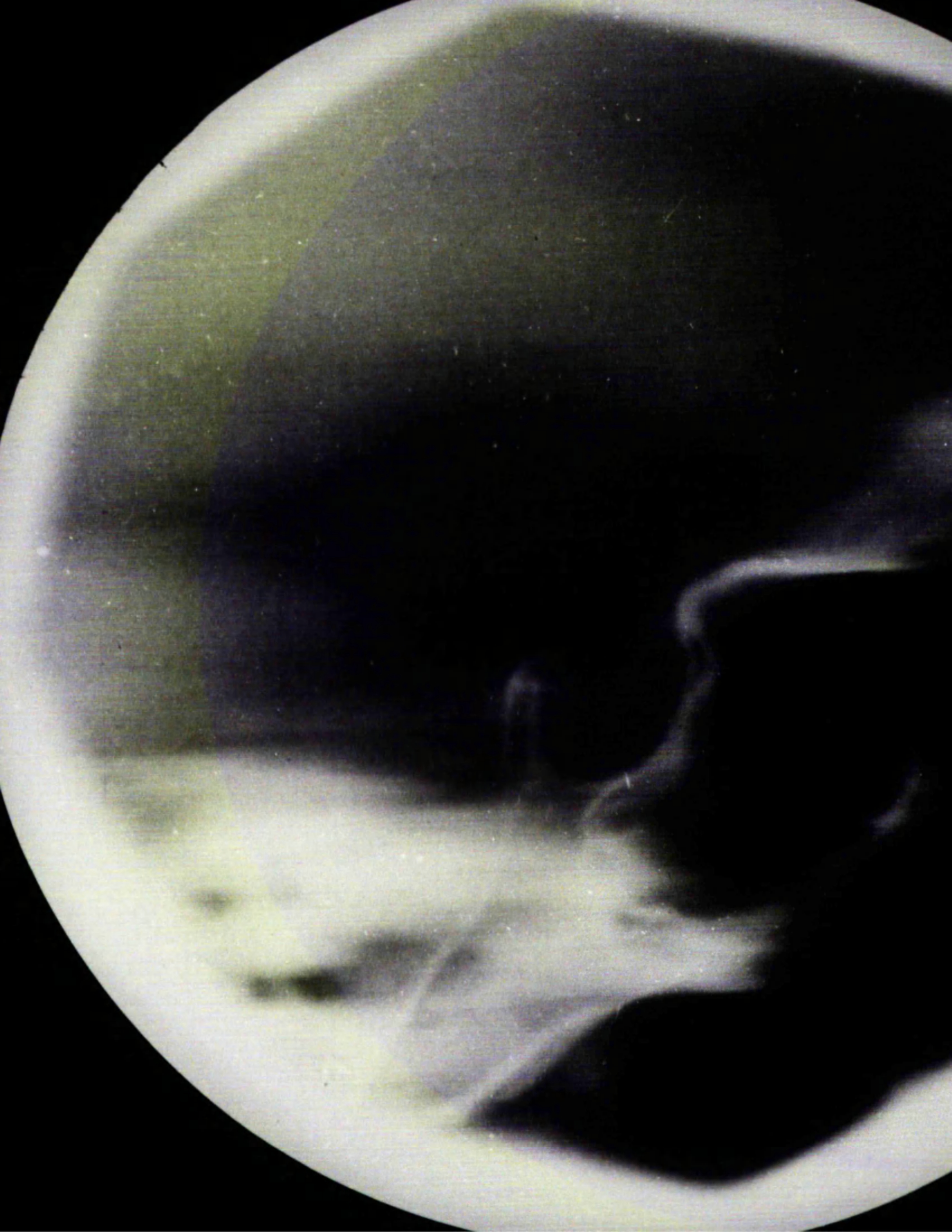


FIGURE 34 (a)

Pituitary radiology: Patient 13

The floor of the fossa is double in contour both in the lateral Xray of skull and lateral tomogram which also shows a thin dorsum sellae. Antero posterior tomography confirms asymmetry of the fossa floor.

FIGURE 34 (b)

Pituitary radiology: Patient 13.

A comparison of pre and post operative films.

View A Pre operative lateral Xray of skull

View B Post operative lateral Xray of skull showing diaphragma sellae marker in situ and remodelling of dorsum sellae. Film taken six months post operatively.

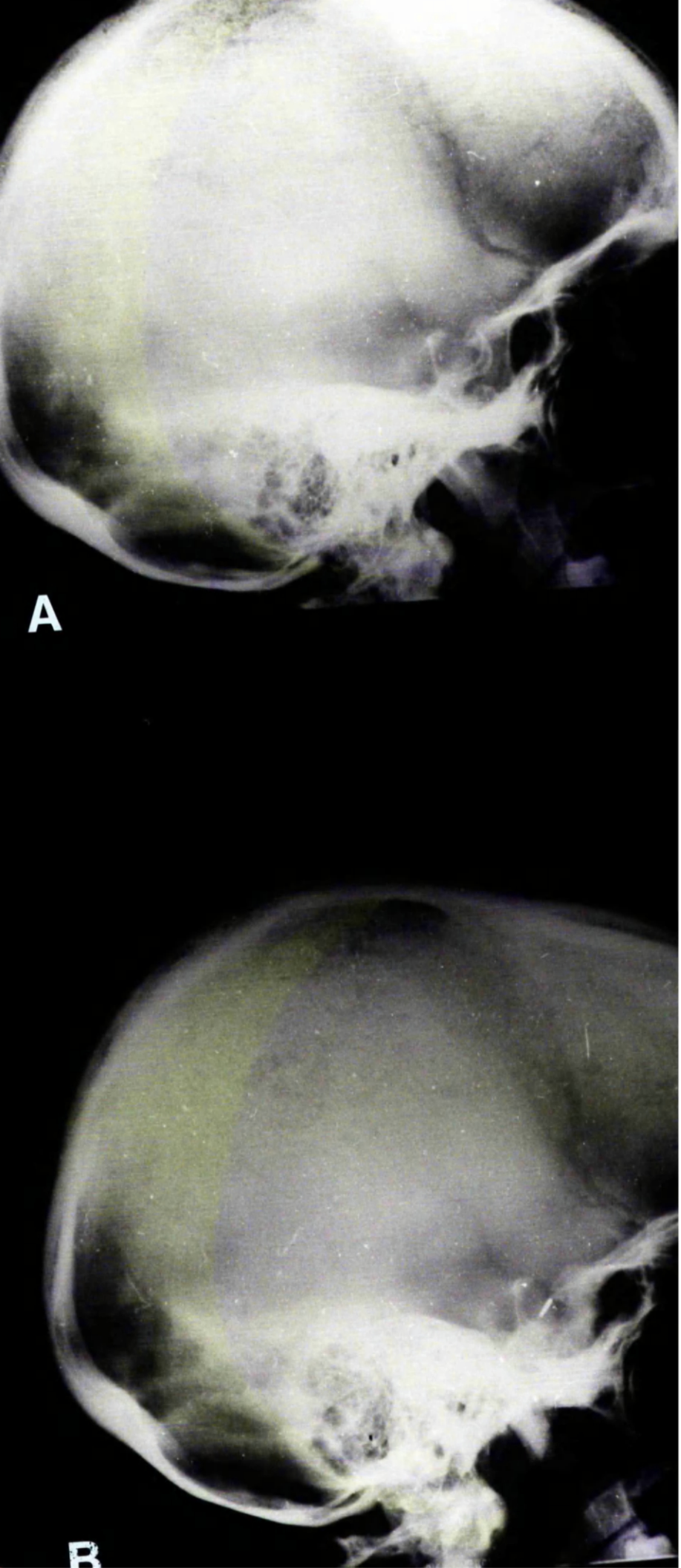

A
B

FIGURE 35

Pituitary radiology: Patient 14

A pituitary fossa of normal size but with a floor of double contour.

FIGURE 36

Pituitary radiology: Patient 11

Pituitary fossa of normal size with a suggestion of loss of cortical definition inferiorly, on lateral Xray of skull. This is confirmed on lateral tomogram.

Note also failure of pneumatisation of the sphenoid sinus and soft tissue swelling of adenoids.

FIGURE 37

Prolactinomas: Absence of diurnal variation in prolactin levels.

Groups as defined in text.

# DIURNAL VARIATION IN PROLACTIN LEVELS.

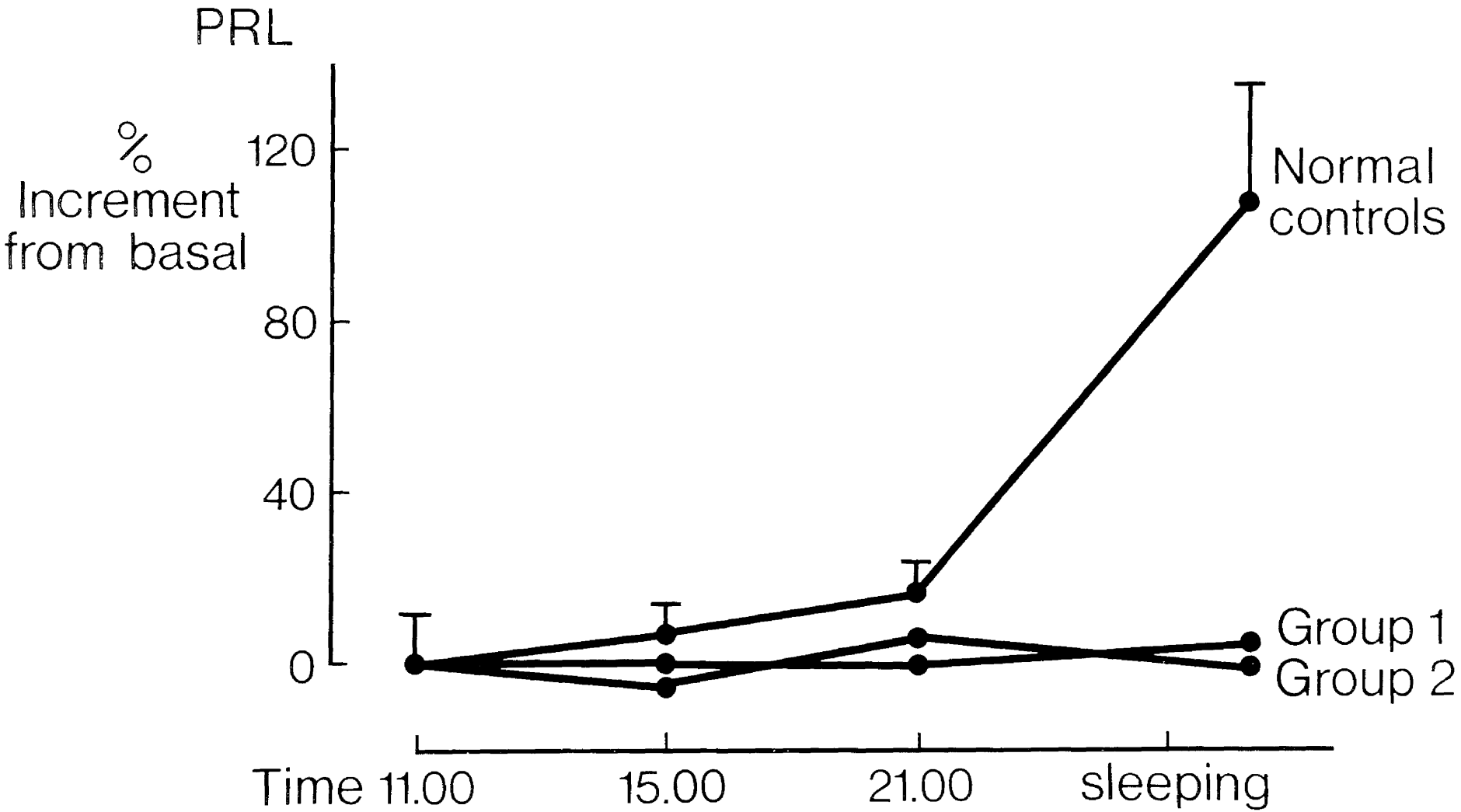


FIGURE 38

Prolactinomas: Prolactin response to TRH stimulation. Groups as defined in text.

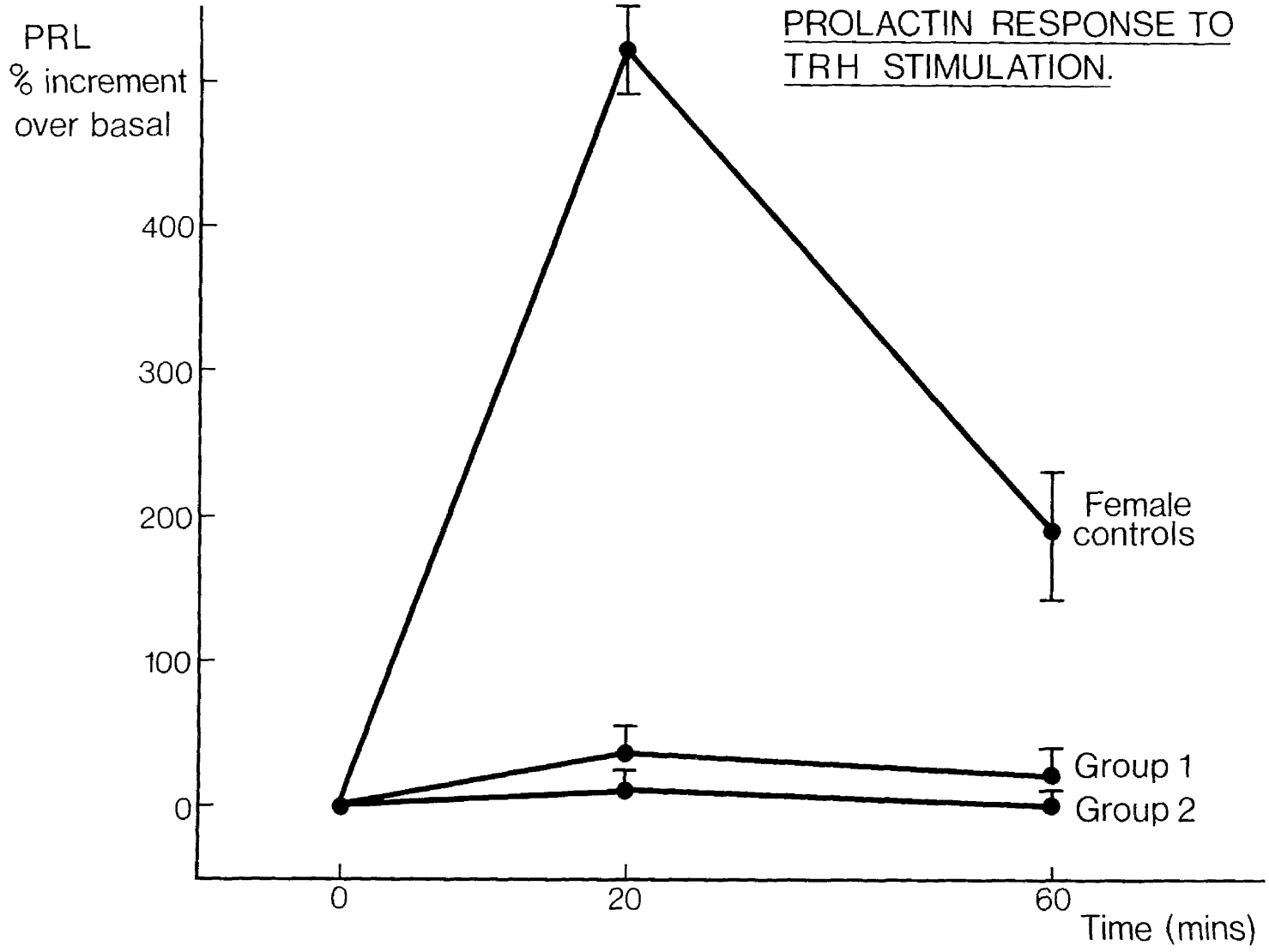

PROLACTIN RESPONSE TO TRH STIMULATION.
PRL
% increment over basal
400
300
200
100
0
0
20
60
Time (mins)
Female controls
Group 1
Group 2

FIGURE 39

Prolactinomas: Prolactin response to Metoclopramide stimulation.

Groups as defined in text.

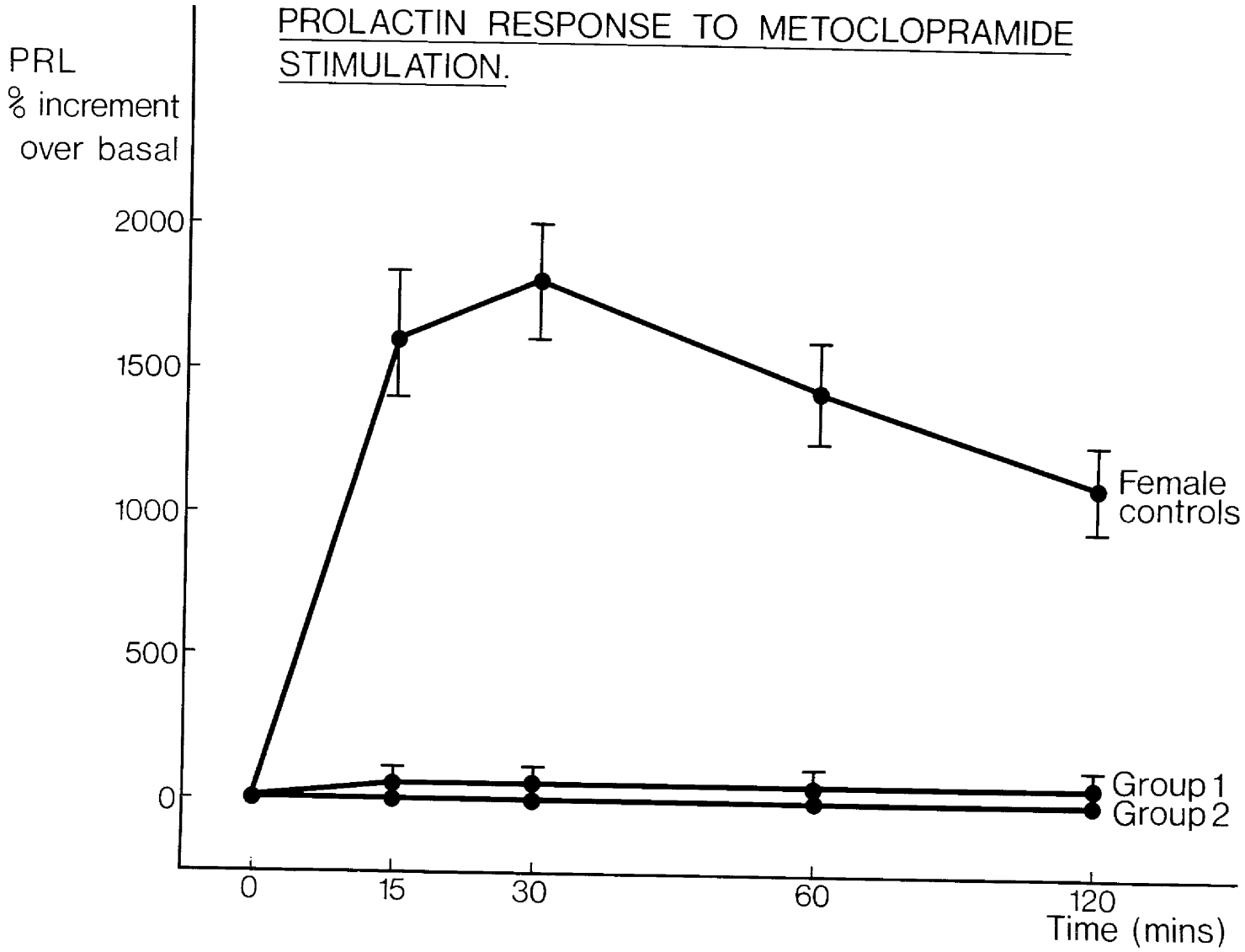

PROLACTIN RESPONSE TO METOCLOPRAMIDE STIMULATION.
PRL
% increment over basal
2000
1500
1000
500
0
0
15
30
60
120
Time (mins)
Female controls
Group 1
Group 2

FIGURE 40

Prolactinomas: Prolactin response to L-dopa.

Groups as defined in text.

SEM: standard error of mean.

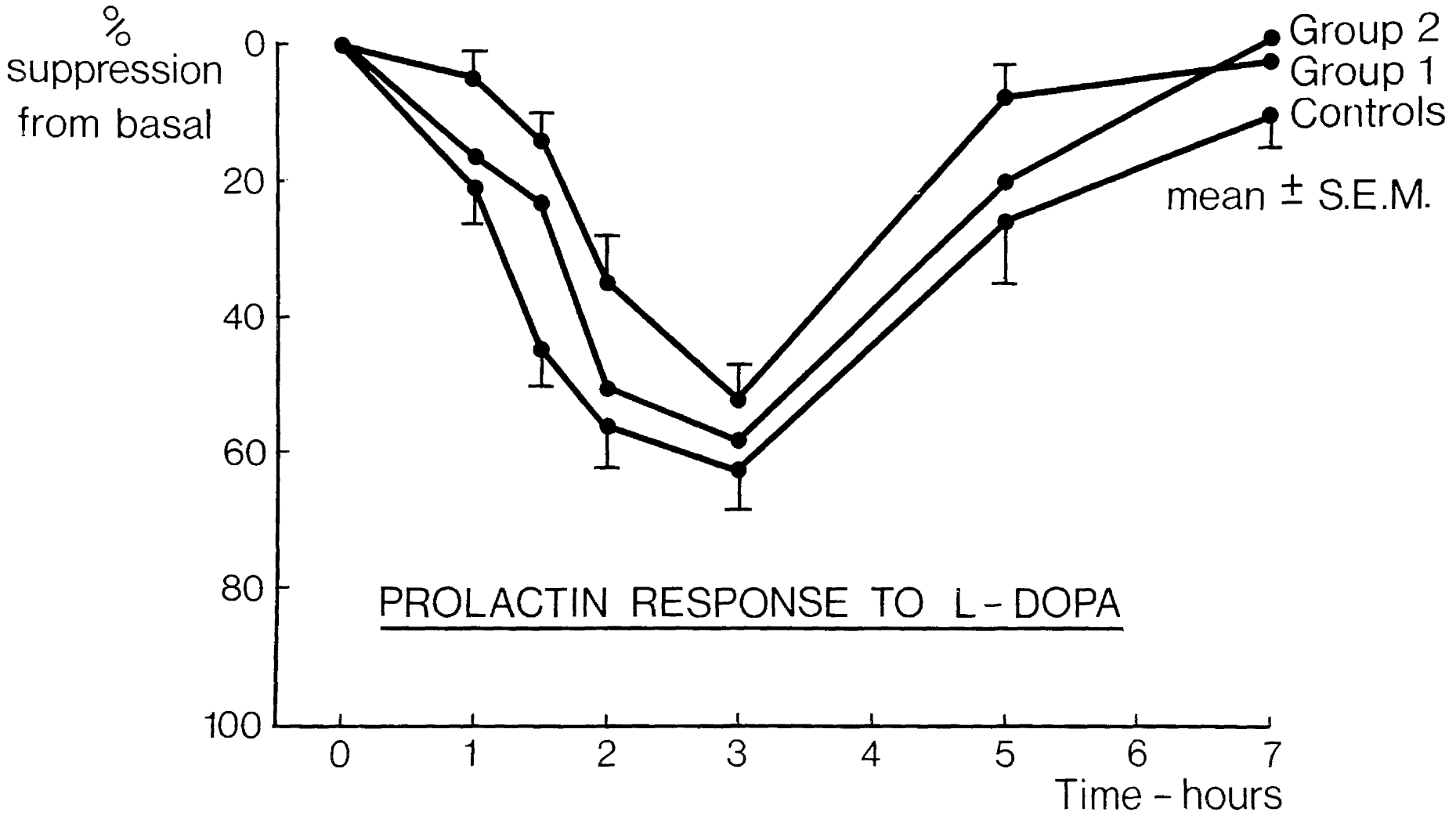

%
suppression
from basal
0
20
40
60
80
100
0
1
2
3
4
5
6
7
Time - hours
Group 2
Group 1
Controls
mean ± S.E.M.
PROLACTIN RESPONSE TO L-DOPA

FIGURE 41

Prolactinomas: Prolactin response to Bromocriptine.

Groups as defined in text.

SEM: standard error of mean.

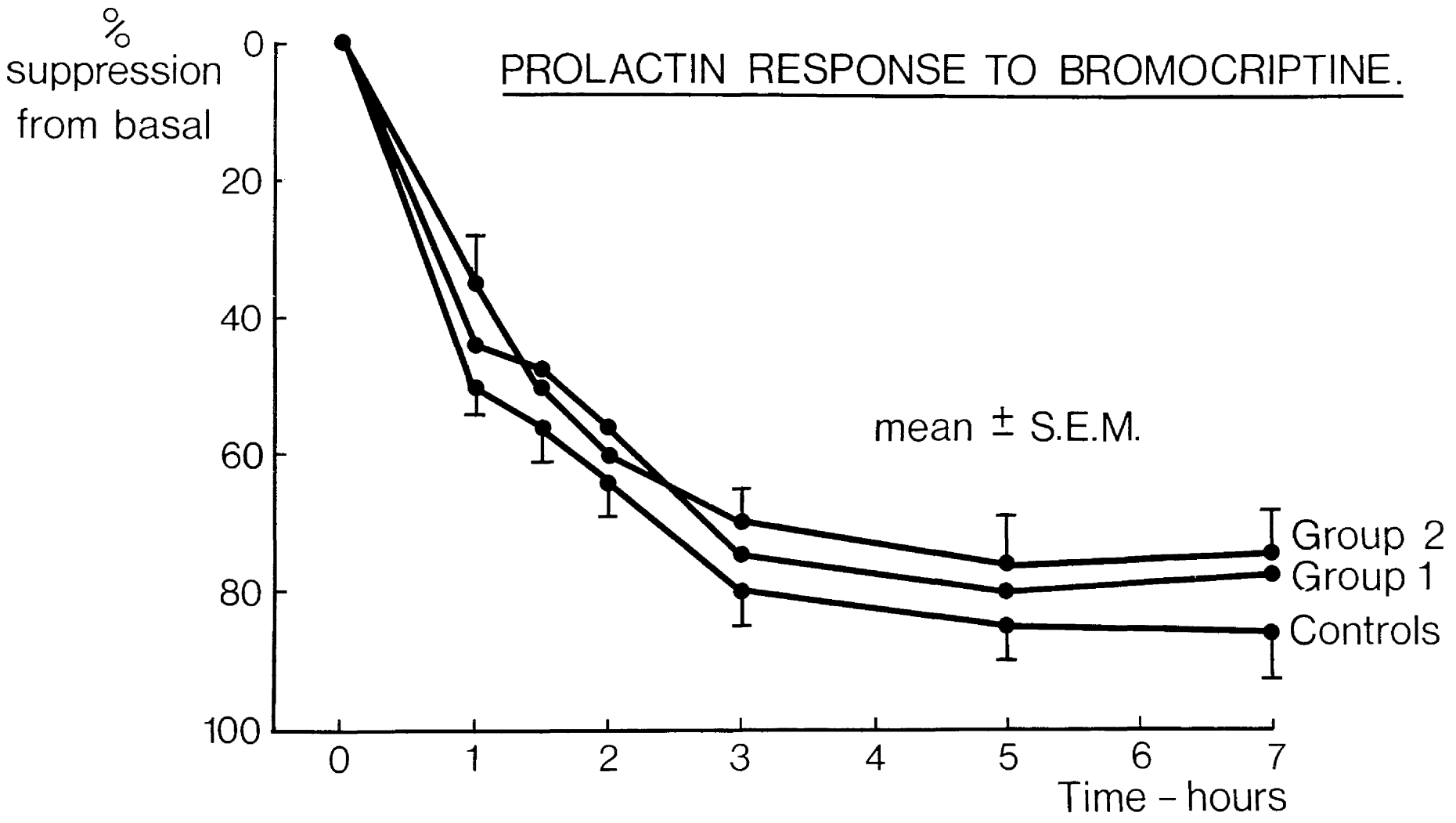

% suppression from basal
PROLACTIN RESPONSE TO BROMOCRIPTINE.
mean ± S.E.M.
Group 2
Group 1
Controls
0
20
40
60
80
100
0
1
2
3
4
5
6
7
Time - hours

FIGURE 42

Strategy for the elucidation of unexplained hyperprolactinaemia.

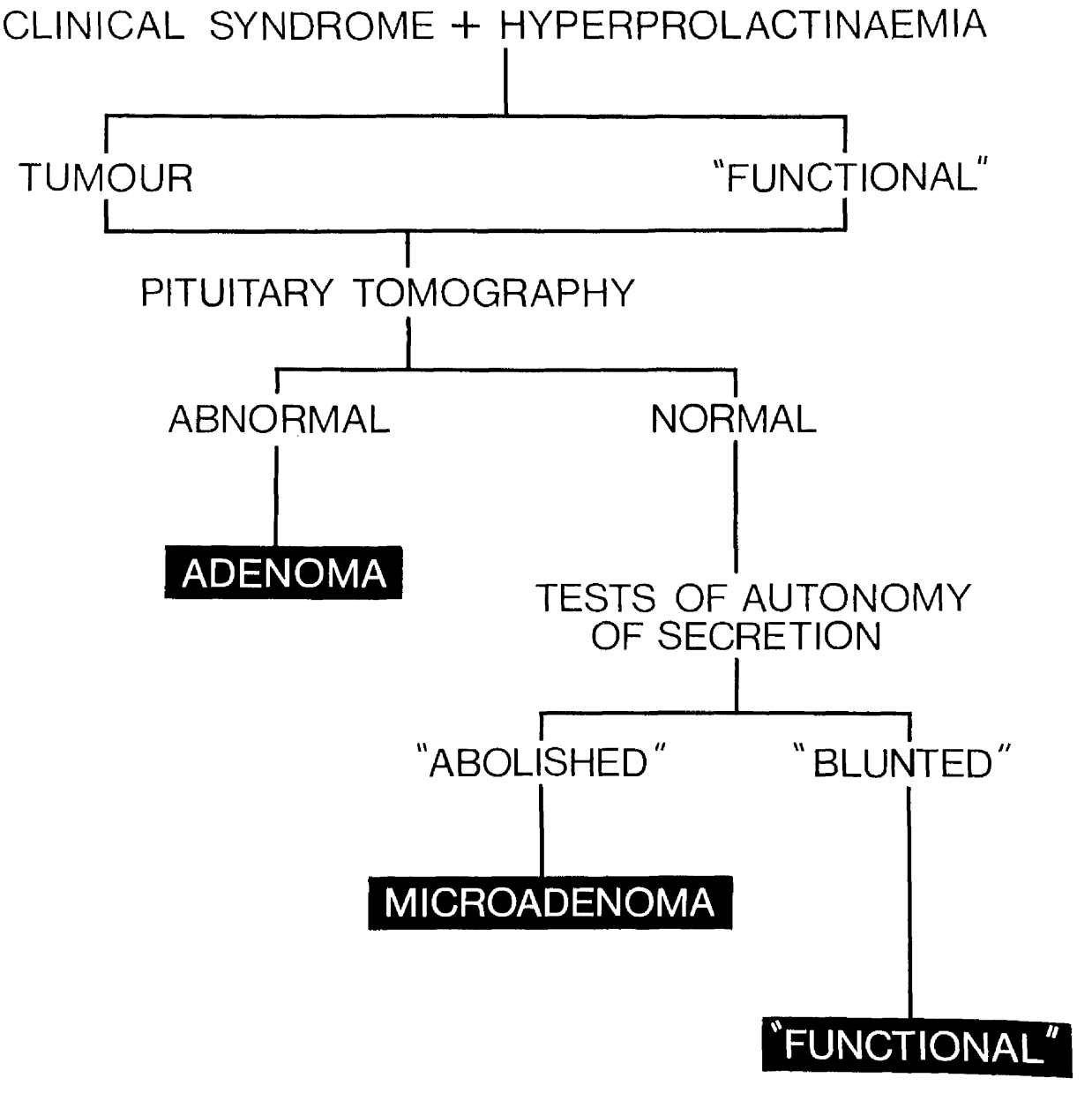

CLINICAL SYNDROME + HYPERPROLACTINAEMIA
TUMOUR
"FUNCTIONAL"
PITUITARY TOMOGRAPHY
ABNORMAL
NORMAL
ADENOMA
TESTS OF AUTONOMY OF SECRETION
"ABOLISHED"
"BLUNTED"
MICROADENOMA
"FUNCTIONAL"

FIGURE 43

Serum prolactin concentrations in patients with renal disease but normal renal function.

Groups as defined in text.

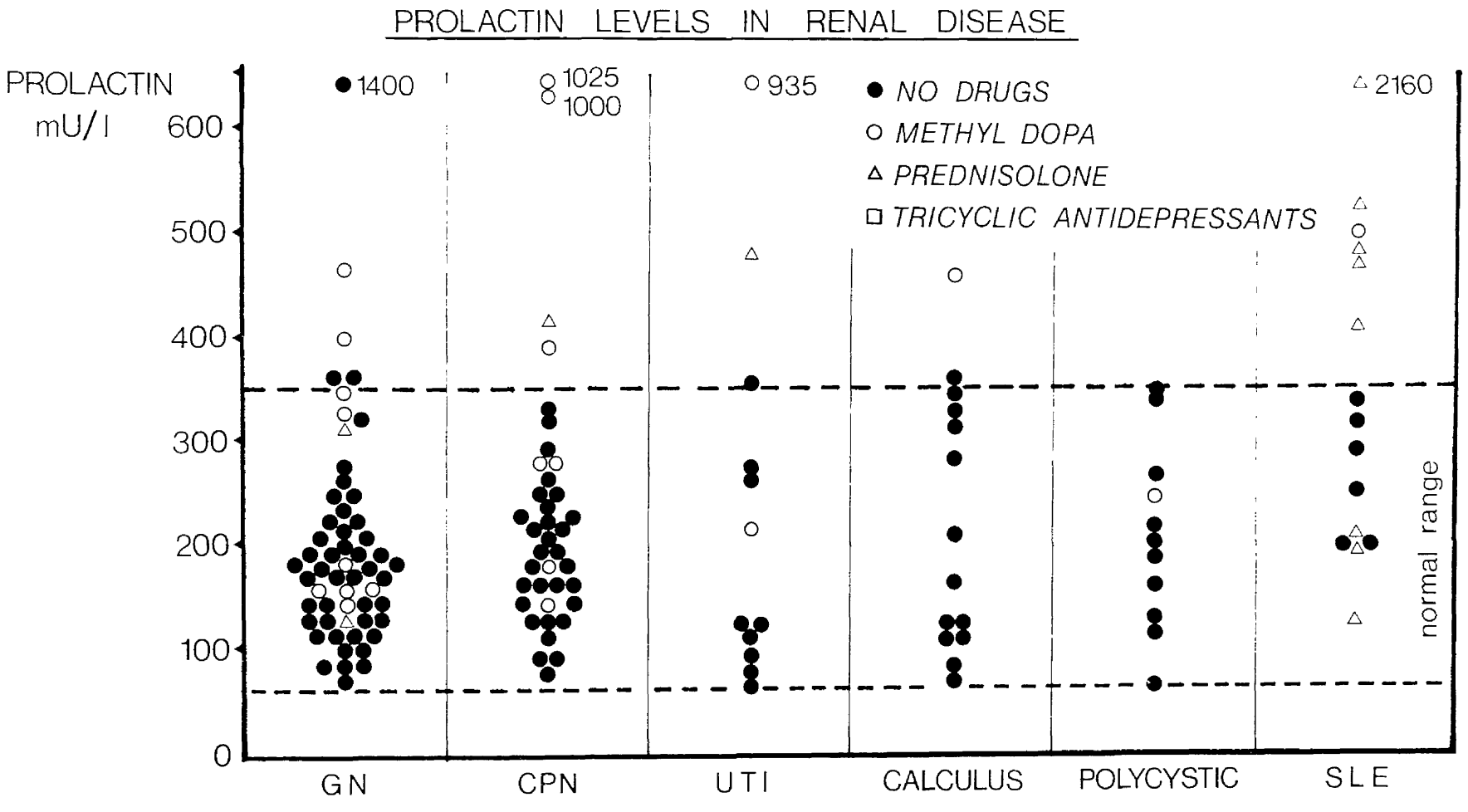

PROLACTIN LEVELS IN RENAL DISEASE
PROLACTIN
mU/l
600
500
400
300
200
100
0
1400
1025
1000
935
2160
NO DRUGS
METHYL DOPA
PREDNISOLONE
TRICYCLIC ANTIDEPRESSANTS
normal range
GN
CPN
UTI
CALCULUS
POLYCYSTIC
SLE

FIGURE 44

Serum prolactin concentrations in patients with impaired renal function, who were receiving one or more drugs known to affect prolactin level.

(i) Groups as defined in text.

(ii) Horizontal bars indicate the mean prolactin concentration in each group.

*PROLACTIN LEVELS IN PATIENTS WITH IMPAIRED RENAL FUNCTION ON DRUG THERAPY*

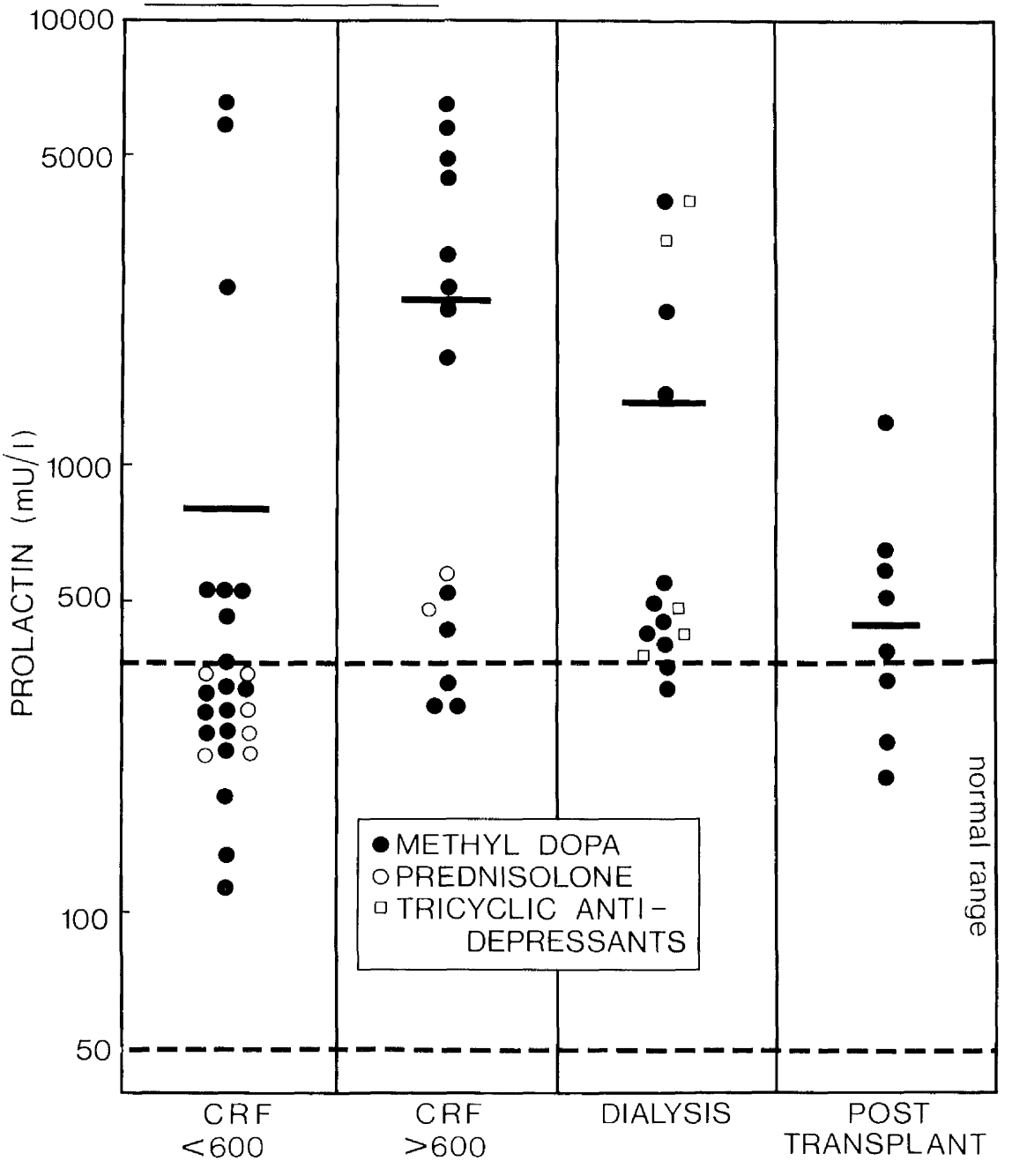


FIGURE 45

Serum prolactin concentrations in patients with impaired renal function, who were on no therapy known to alter prolactin secretion.

(i) Groups as defined in text.

(ii) Horizontal bars indicate the mean prolactin concentration in each group.

*PROLACTIN LEVELS IN PATIENTS WITH IMPAIRED RENAL FUNCTION*

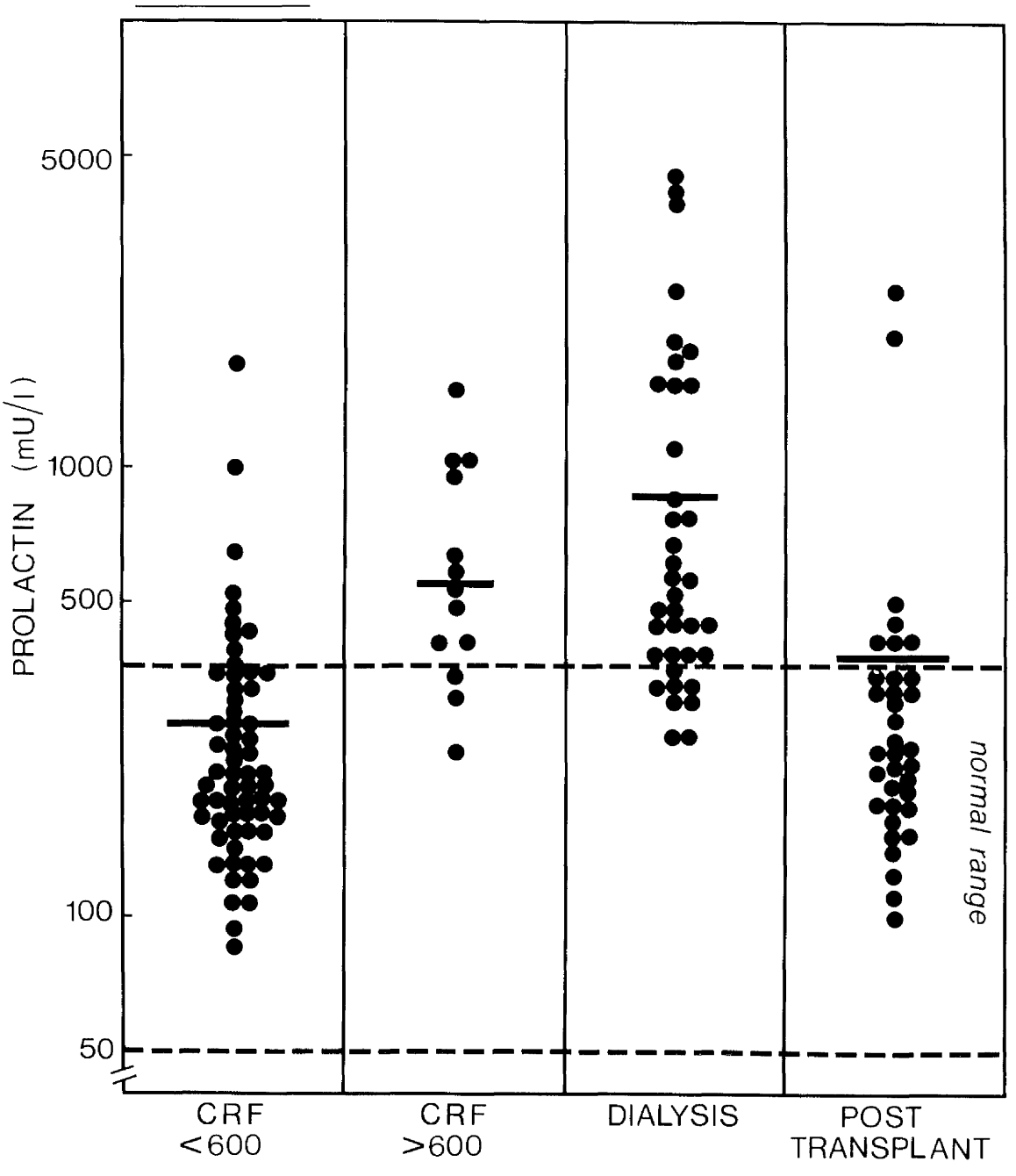


FIGURE 46

Arteriovenous concentration difference of prolactin across the normal kidney.

$\dashv\vdash$ mean $\pm$ SEM

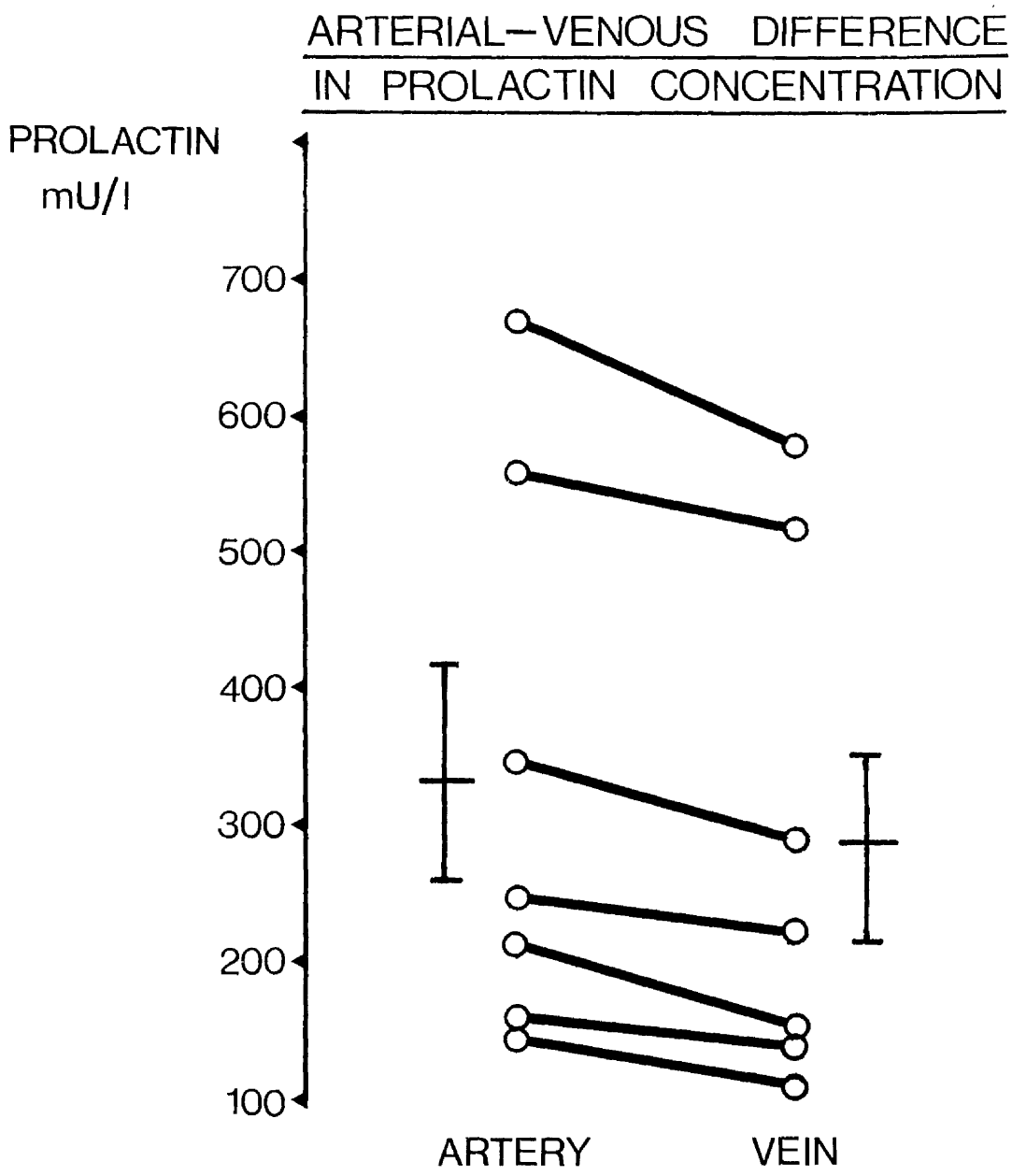

ARTERIAL–VENOUS DIFFERENCE
IN PROLACTIN CONCENTRATION
PROLACTIN
mU/l
700
600
500
400
300
200
100
ARTERY
VEIN

FIGURE 47

Basal gonadal status with progressive uraemia and after successful renal transplantation.

(i) Groups as defined in text.

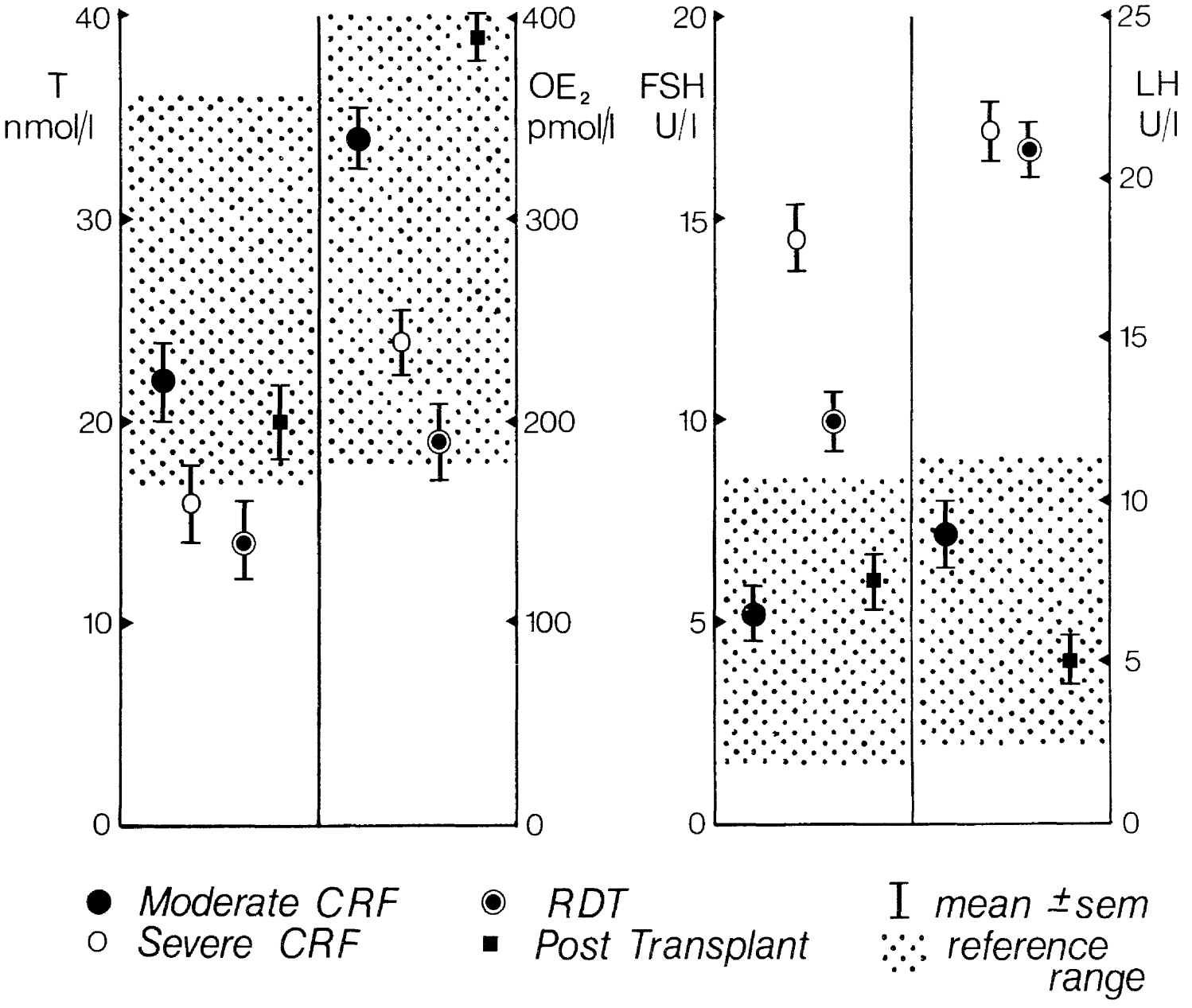

40
T
nmol/l
30
20
10
0
400
OE2
pmol/l
300
200
100
0
20
FSH
U/l
15
10
5
0
25
LH
U/l
20
15
10
5
0
Moderate CRF
Severe CRF
RDT
Post Transplant
mean ±sem
reference range

FIGURE 48

Basal thyroid status with progressive uraemia and after successful renal transplantation.
Groups as defined in text.

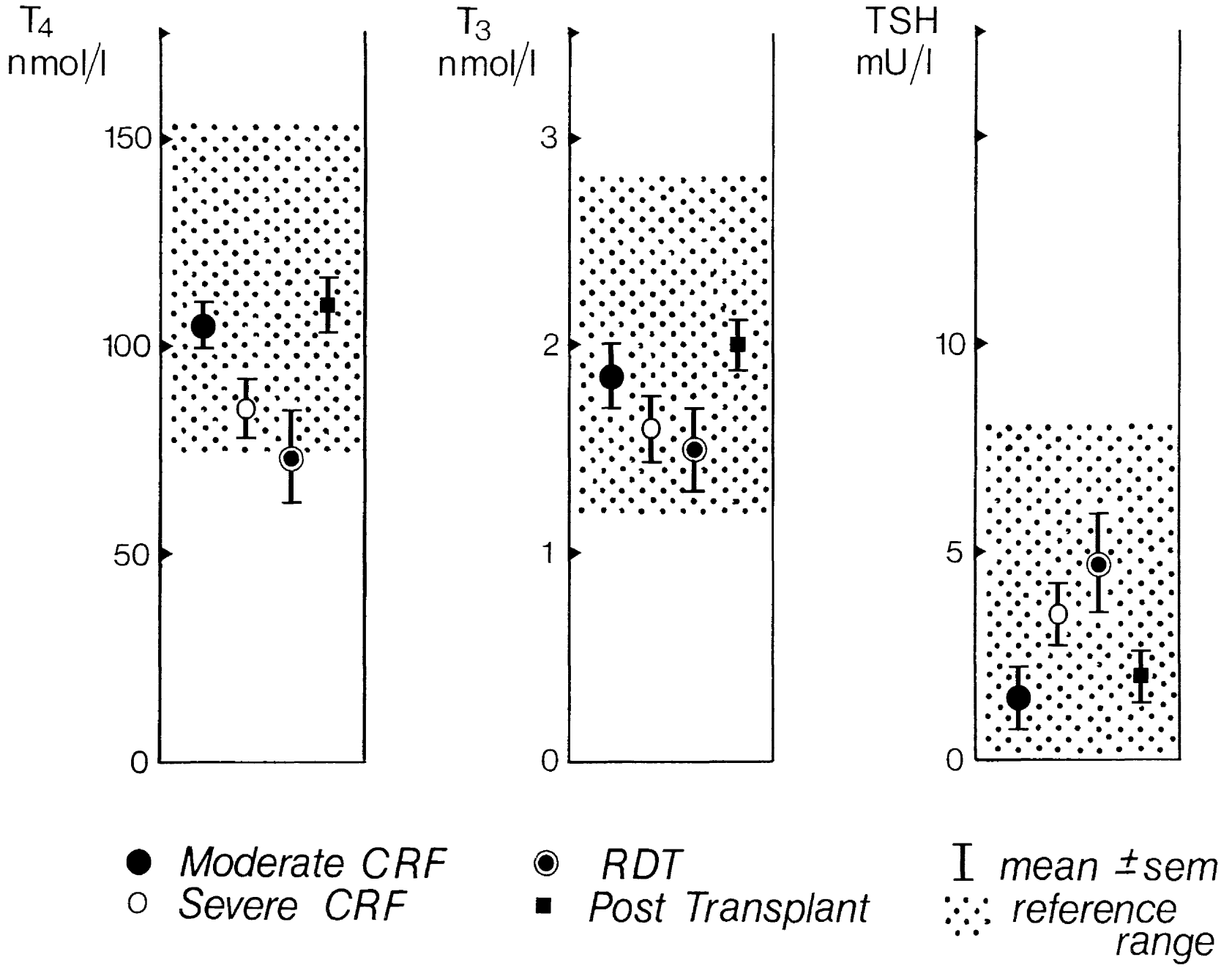

T4
nmol/l
150
100
50
0
T3
nmol/l
3
2
1
0
TSH
mU/l
10
5
0
Moderate CRF
Severe CRF
RDT
Post Transplant
mean ±sem
reference range

FIGURE 49

Basal thyroid status in dialysis patients.
Dotted lines indicate the absolute range
of values in control subjects.
Horizontal bars indicate the mean
concentration in each group.

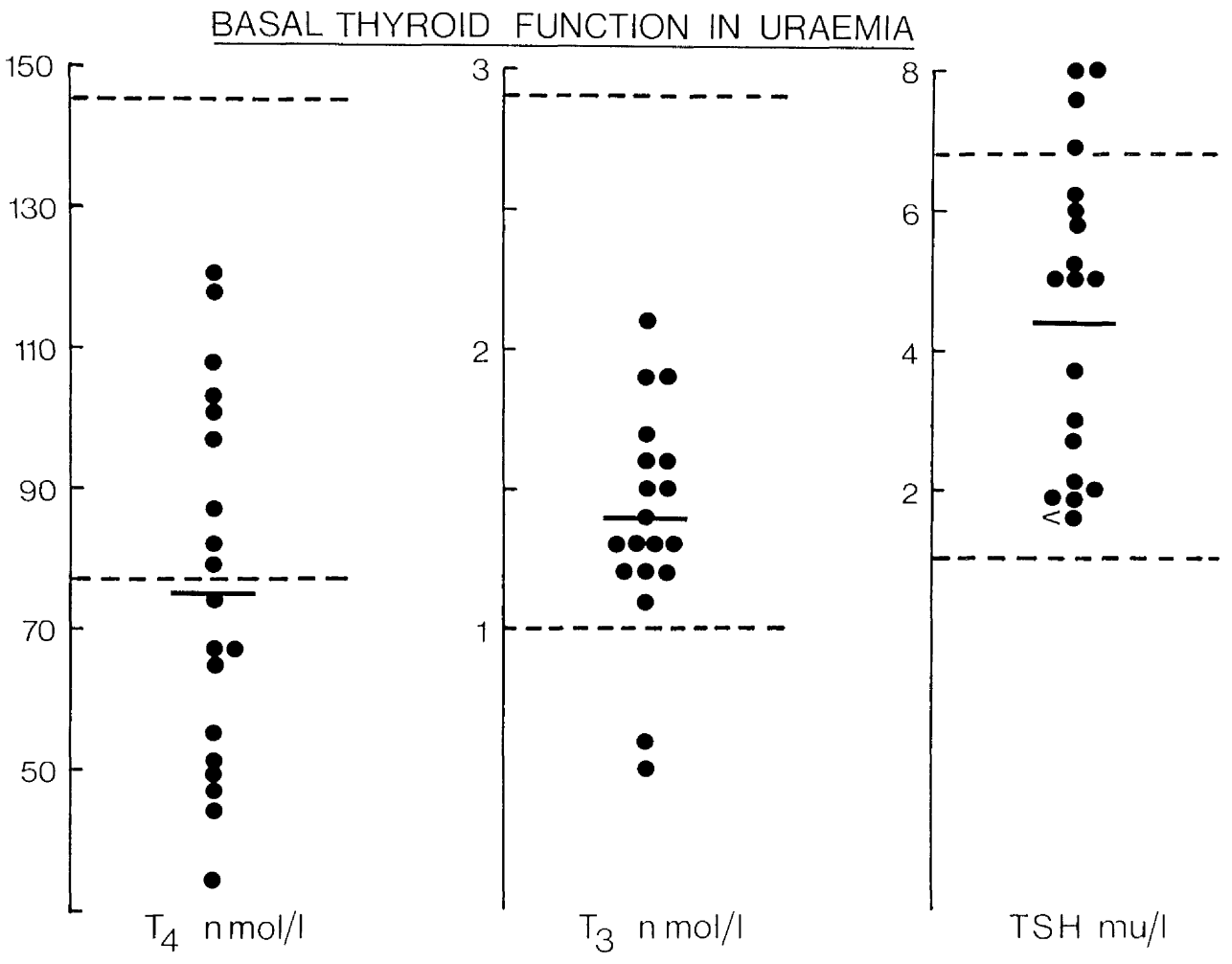

BASAL THYROID FUNCTION IN URAEMIA
150
130
110
90
70
50
$T_4$ n mol/l
3
2
1
$T_3$ n mol/l
8
6
4
2
<
TSH mu/l

FIGURE 50

TSH response to TRH: Uraemic subjects and controls mean ± standard error of mean plotted.

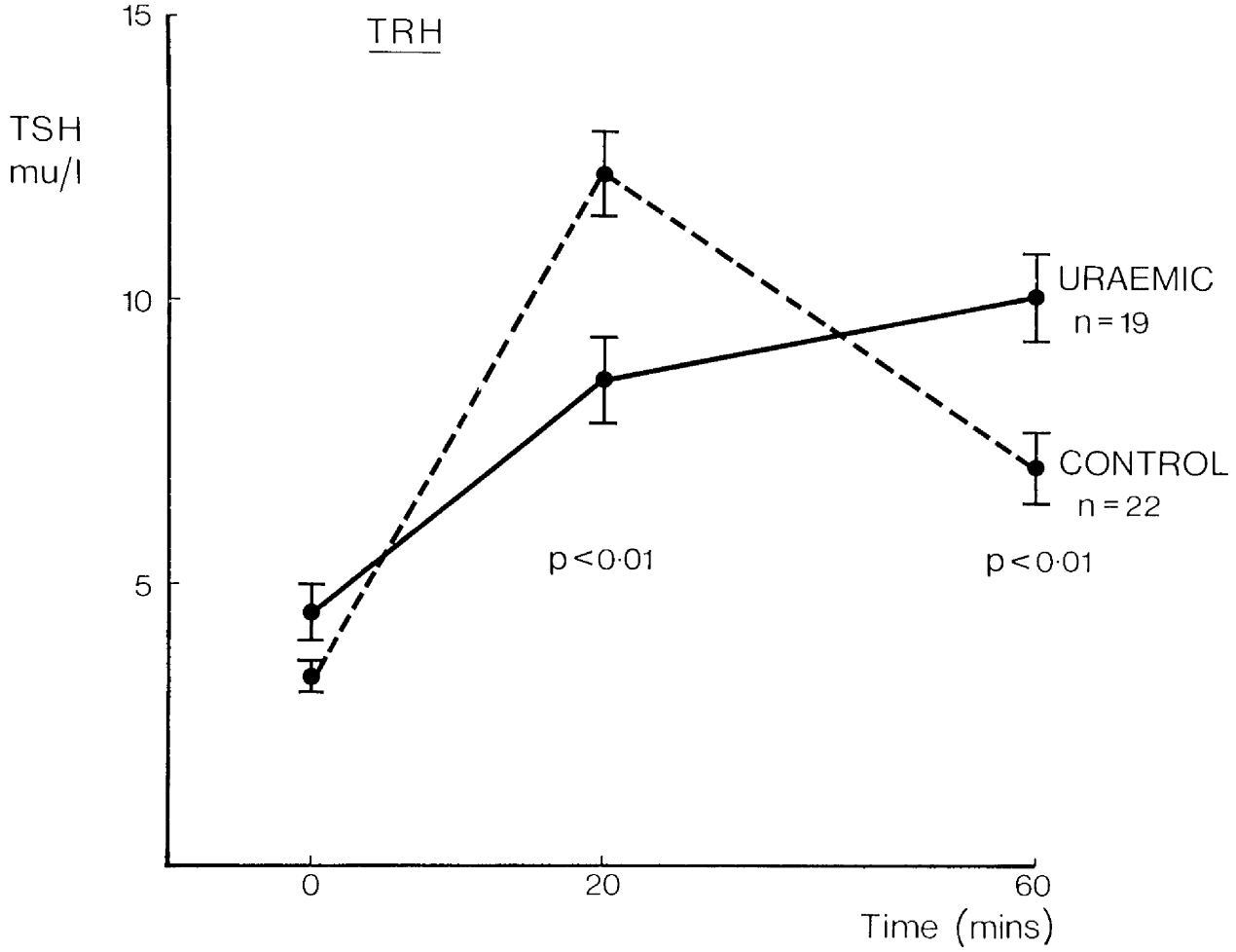

TRH
TSH
mu/l
15
10
5
0
20
60
Time (mins)
URAEMIC
n = 19
CONTROL
n = 22
p < 0·01
p < 0·01

FIGURE 51

GH response to TRH: Uraemic subjects and controls.

Individual responses plotted for each uraemic subject.

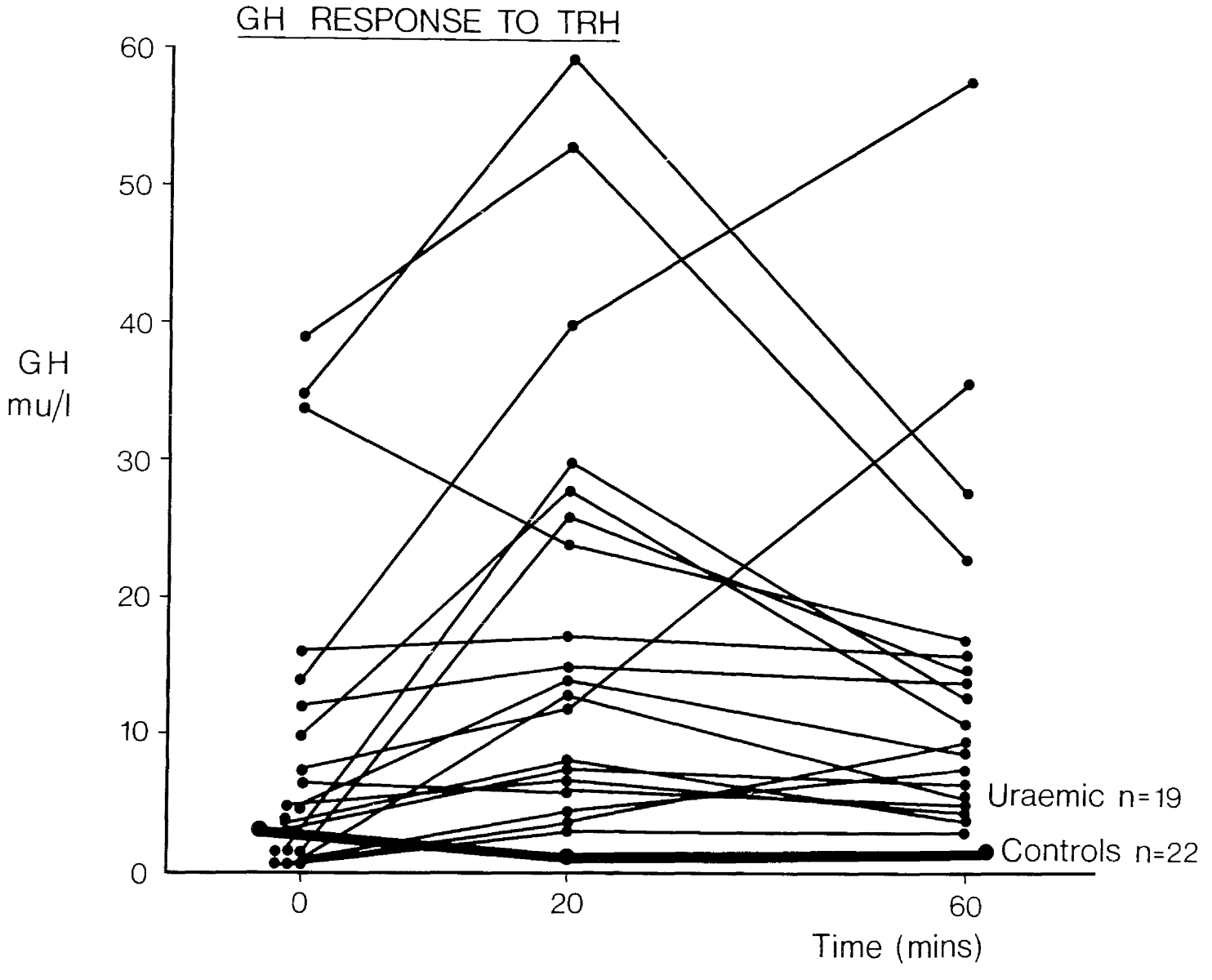

GH RESPONSE TO TRH
60
50
40
30
20
10
0
GH
mu/l
0
20
60
Time (mins)
Uraemic n=19
Controls n=22

FIGURE 52

Basal gonadal status in male dialysis patients. Dotted lines indicate the absolute range of values in control subjects.

## BASAL GONADAL FUNCTION : MALES

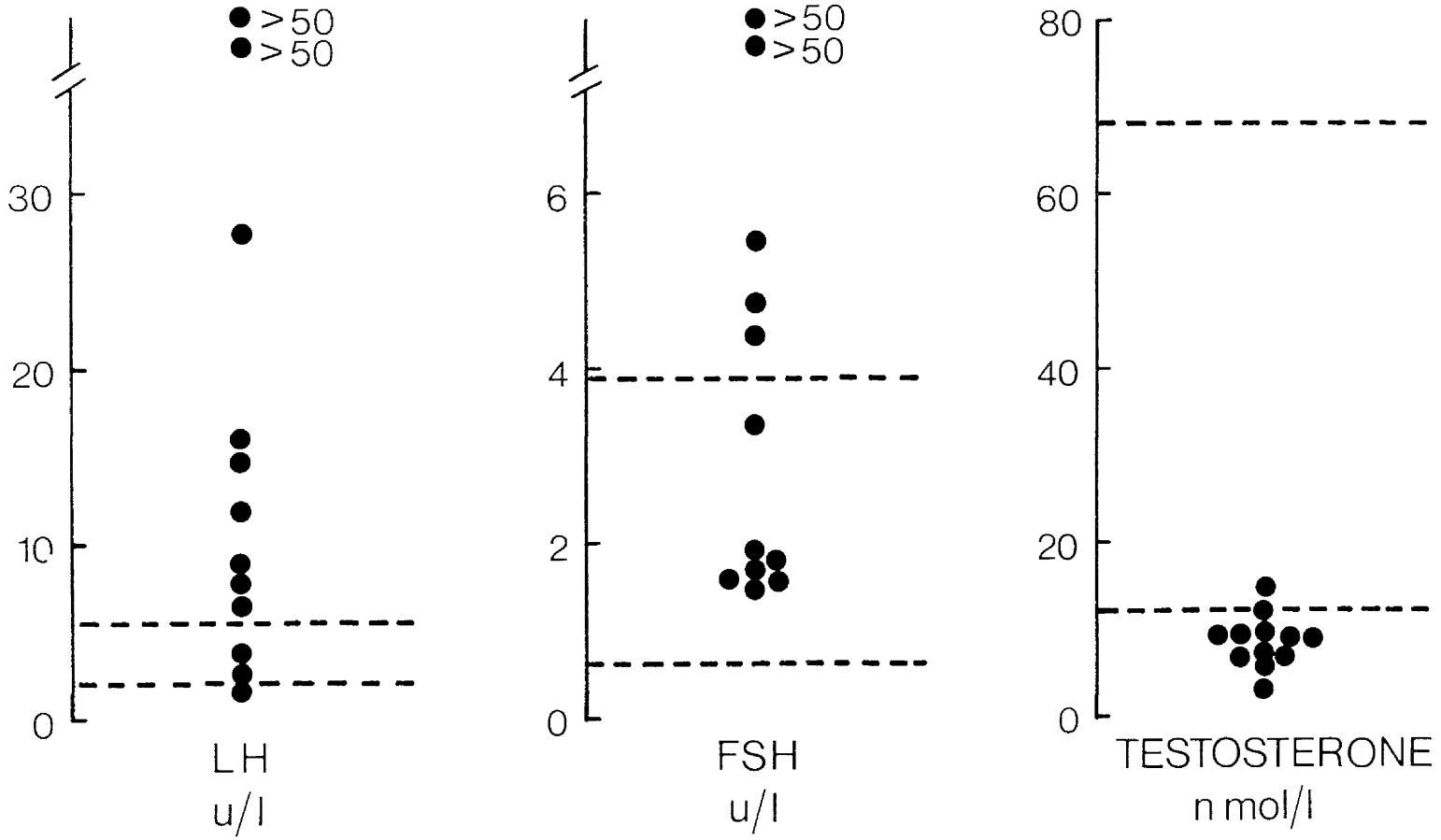


FIGURE 53

Basal gonadal status in female dialysis patients. Dotted lines indicate the absolute range of values in control subjects.

## BASAL GONADAL FUNCTION: FEMALES

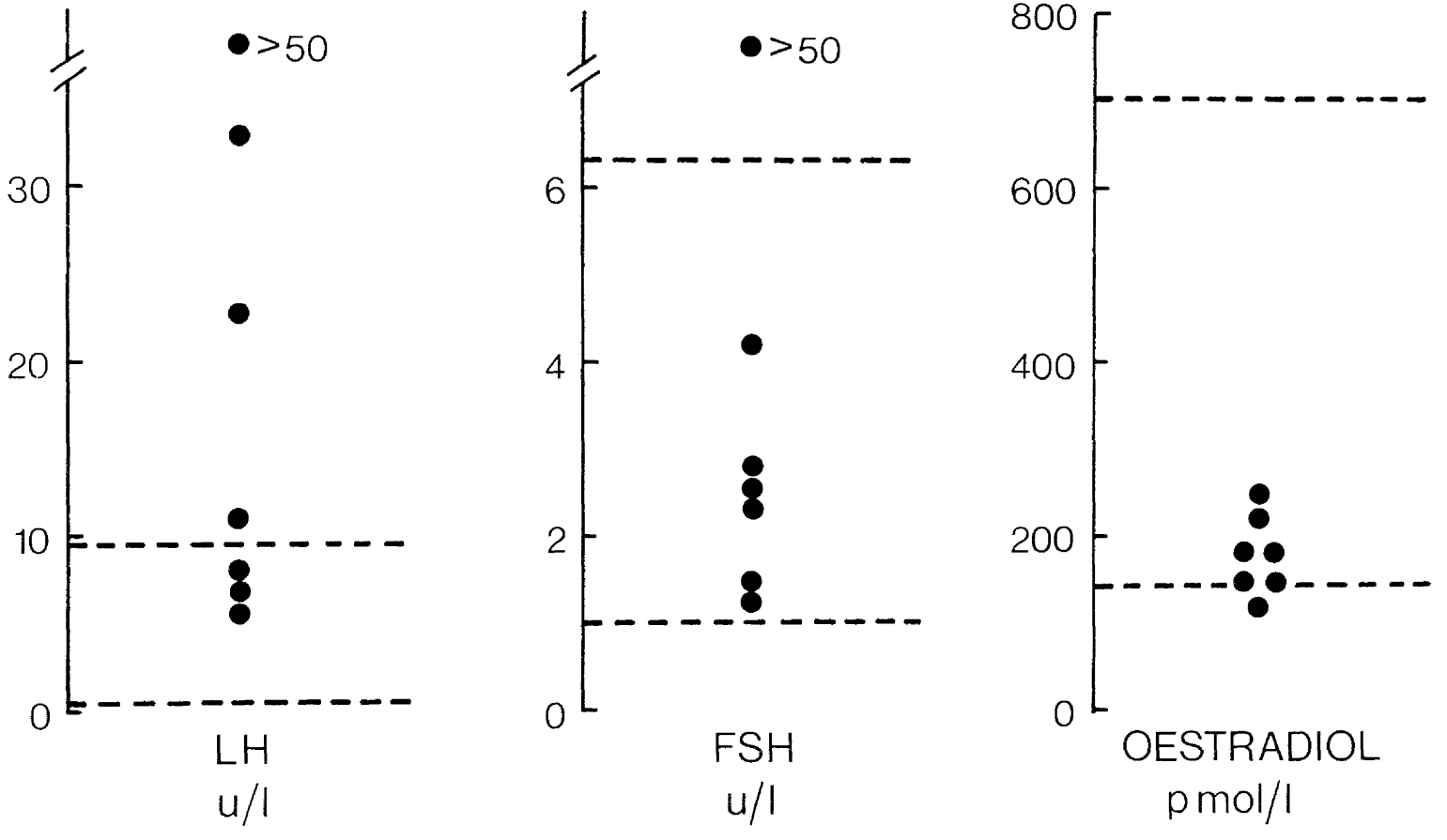


FIGURE 54

LH and FSH response to Gn RH: Uraemic males and controls.

Mean ± standard error of mean plotted.

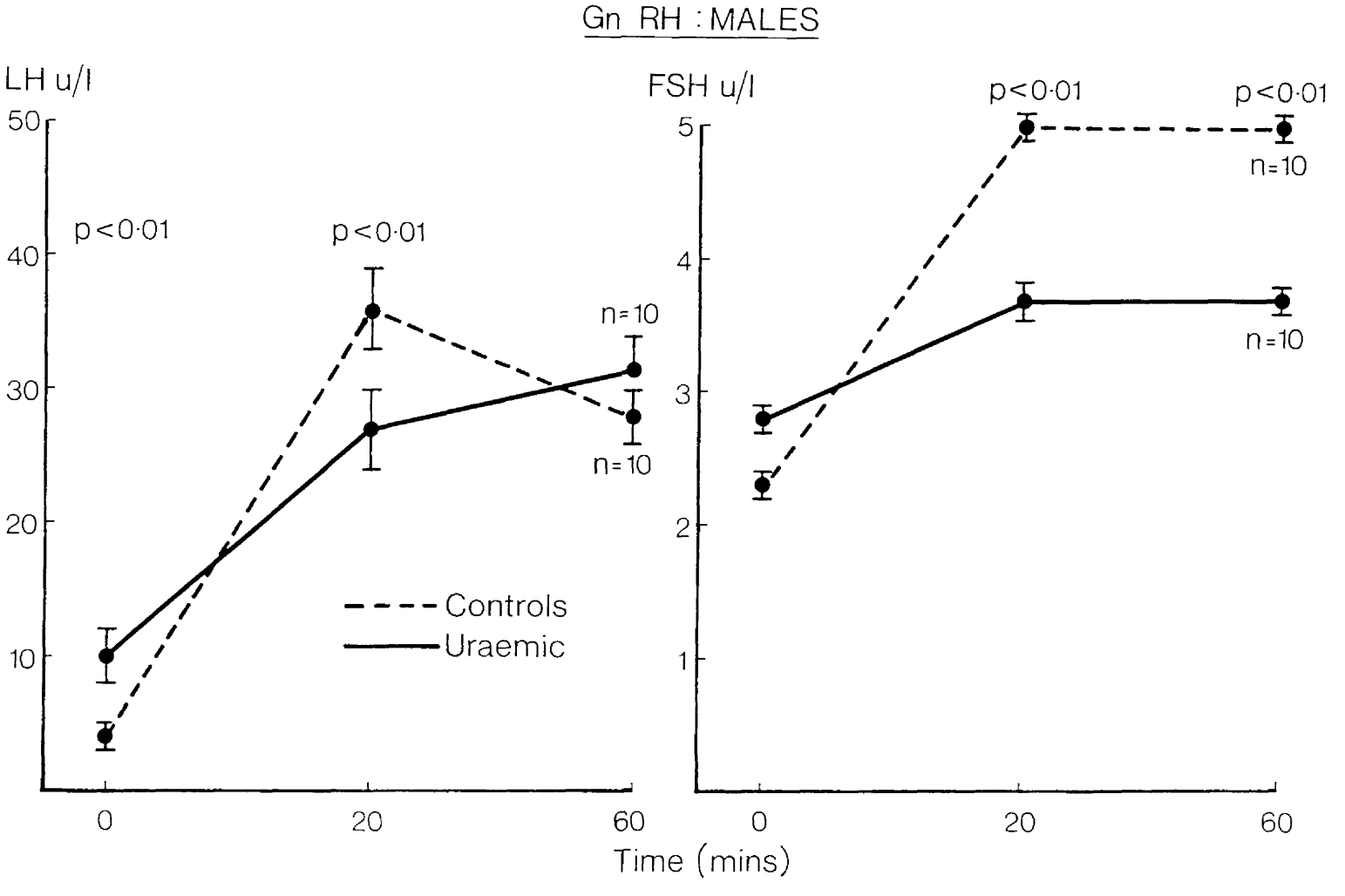

Gn RH : MALES
LH u/l
50
40
30
20
10
p<0·01
p<0·01
n=10
n=10
Controls
Uraemic
FSH u/l
5
4
3
2
1
p<0·01
p<0·01
n=10
n=10
0
20
60
0
20
60
Time (mins)

FIGURE 55

LH and FSH response to Gn RH: Uraemic females and controls.

Mean ± standard error of mean plotted.

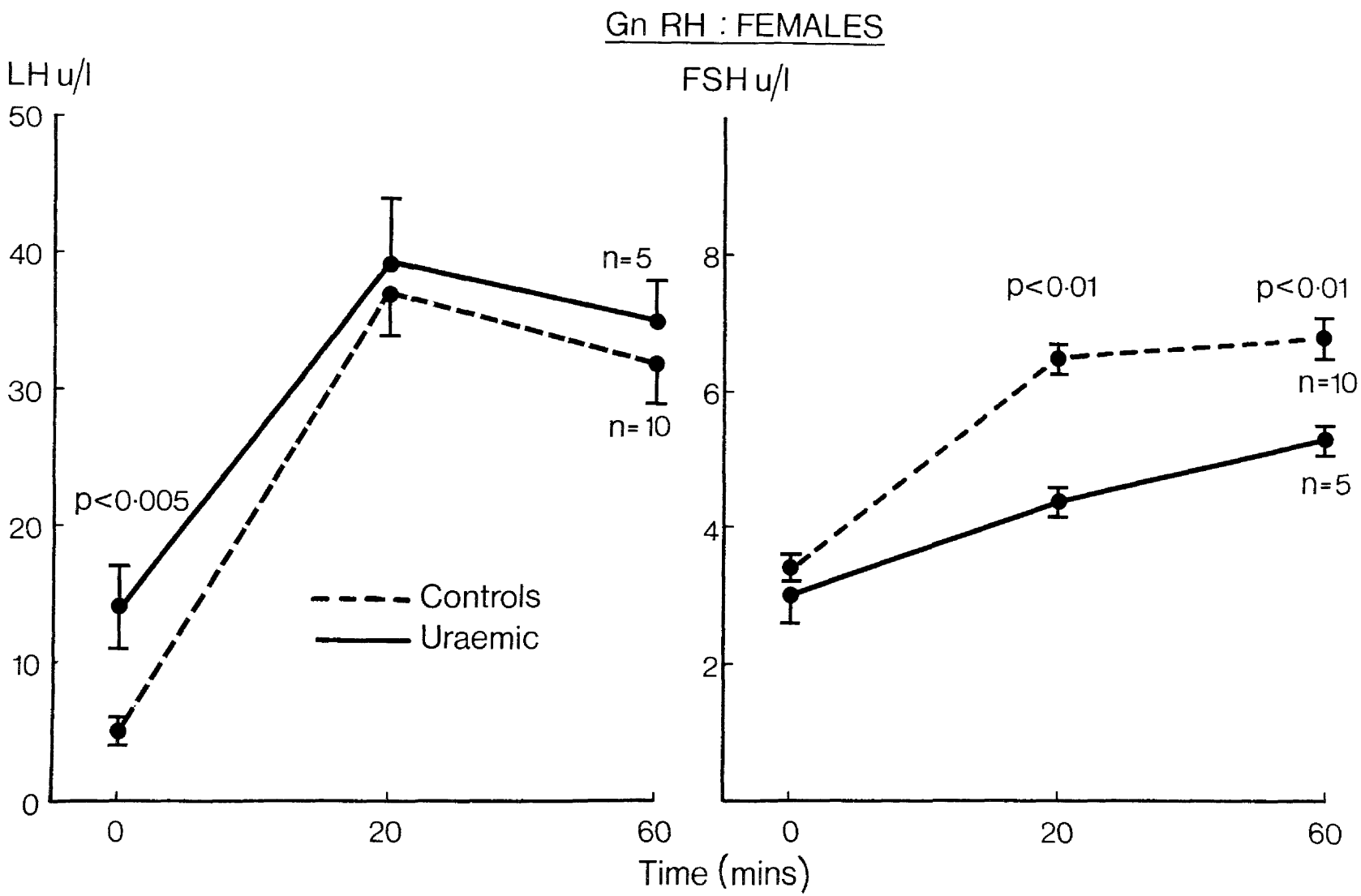

Gn RH : FEMALES
LH u/l
FSH u/l
50
40
30
20
10
0
8
6
4
2
n=5
n=10
p<0·005
p<0·01
p<0·01
n=10
n=5
Controls
Uraemic
0
20
60
0
20
60
Time (mins)

FIGURE 56

Prolactin response to TRH: Uraemic subjects and controls. Mean ± standard error of mean plotted.

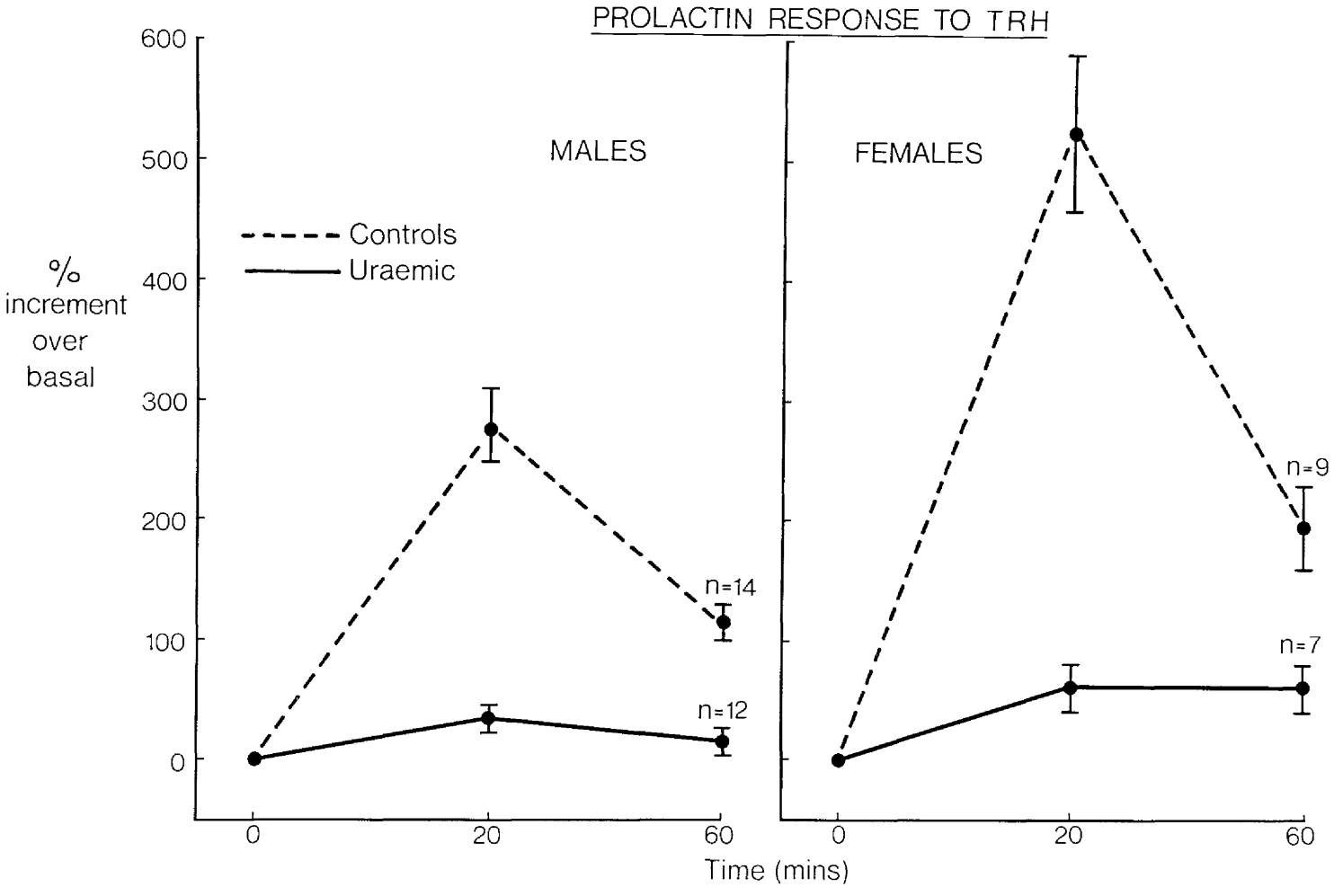

PROLACTIN RESPONSE TO TRH
MALES
FEMALES
Controls
Uraemic
% increment over basal
600
500
400
300
200
100
0
n=14
n=12
n=9
n=7
0
20
60
Time (mins)

FIGURE 57

Prolactin response to Metoclopramide: Uraemic subjects and controls.

Mean ± standard error of mean plotted.

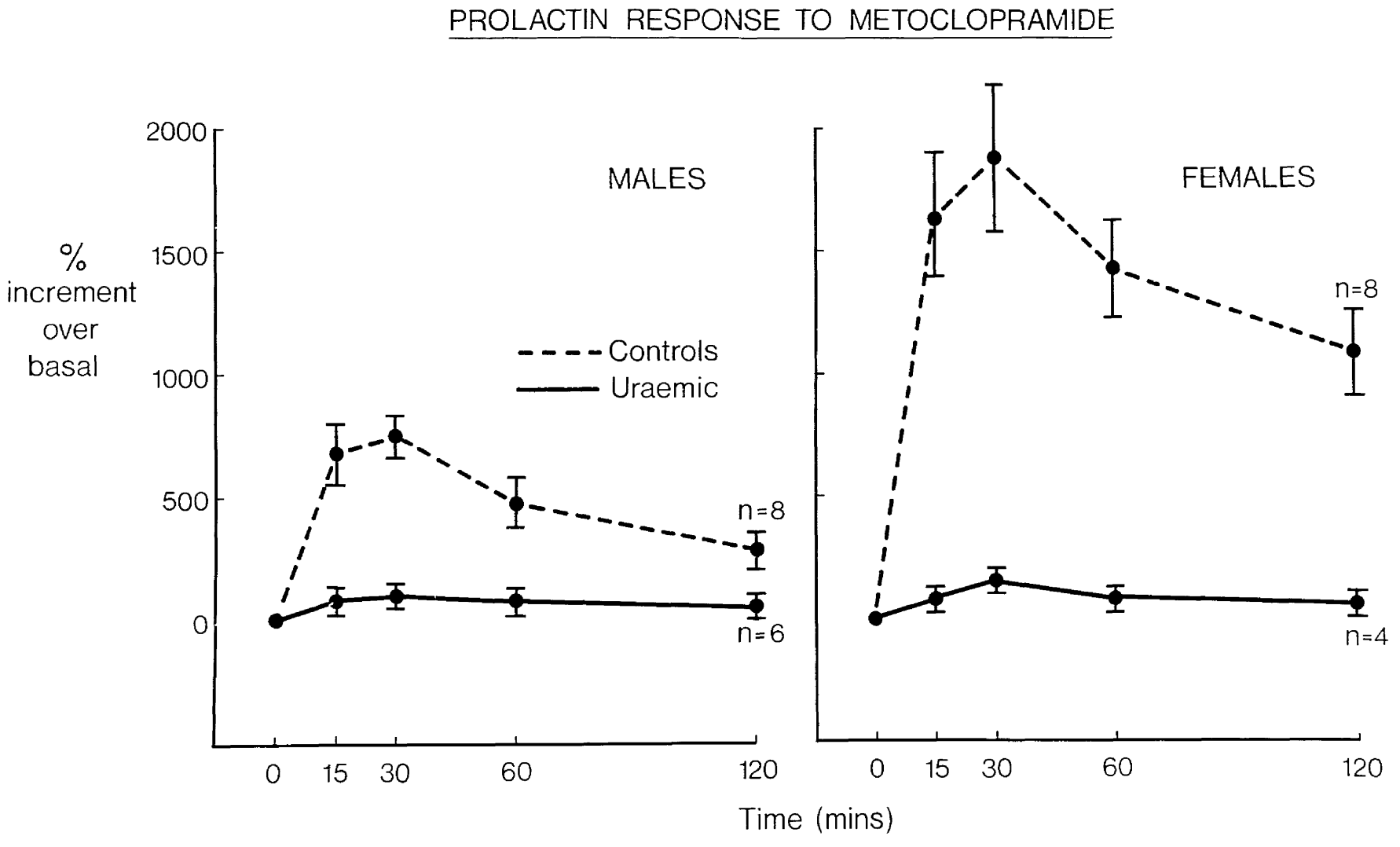
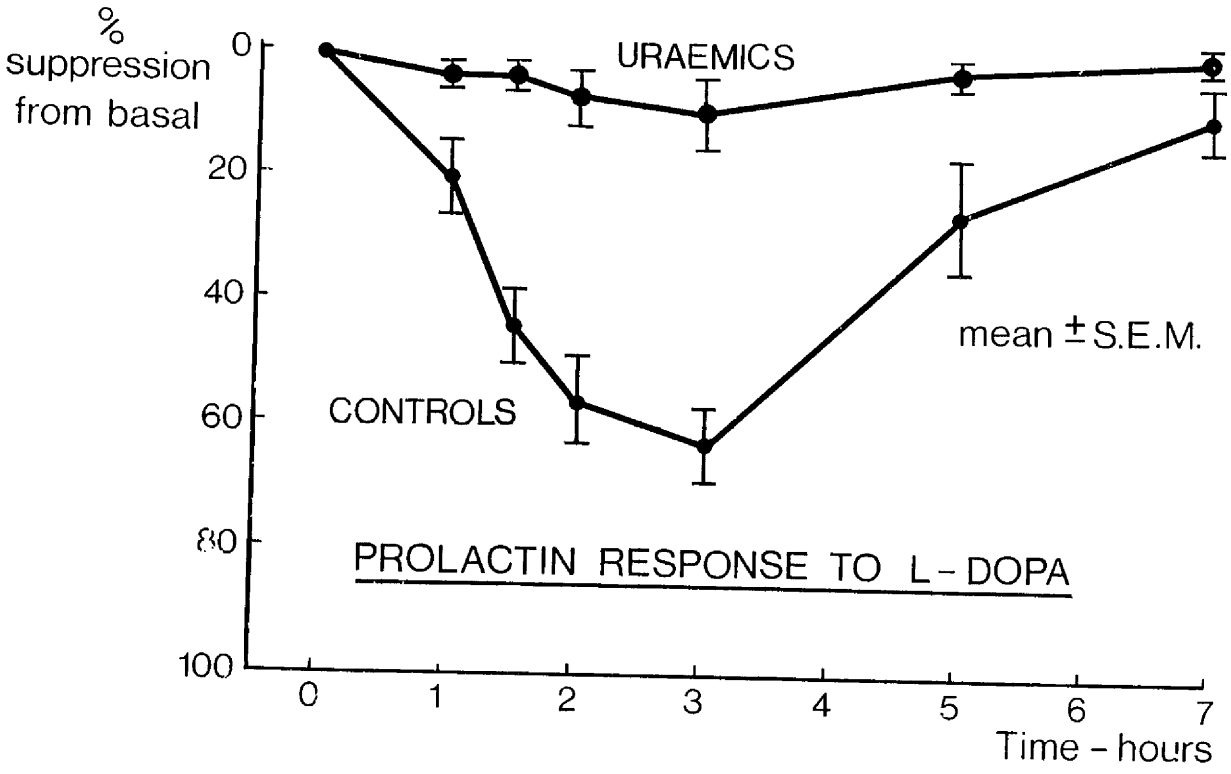

PROLACTIN RESPONSE TO METOCLOPRAMIDE
% increment over basal
2000
1500
1000
500
0
MALES
FEMALES
Controls
Uraemic
n=8
n=6
n=8
n=4
0
15
30
60
120
Time (mins)

FIGURE 58

Prolactin response to L-dopa and Bromocriptine: Uraemic subjects and controls.


% suppression from basal
0
20
40
60
80
100
URAEMICS
mean ± S.E.M.
CONTROLS
PROLACTIN RESPONSE TO L-DOPA
0
1
2
3
4
5
6
7
Time - hours

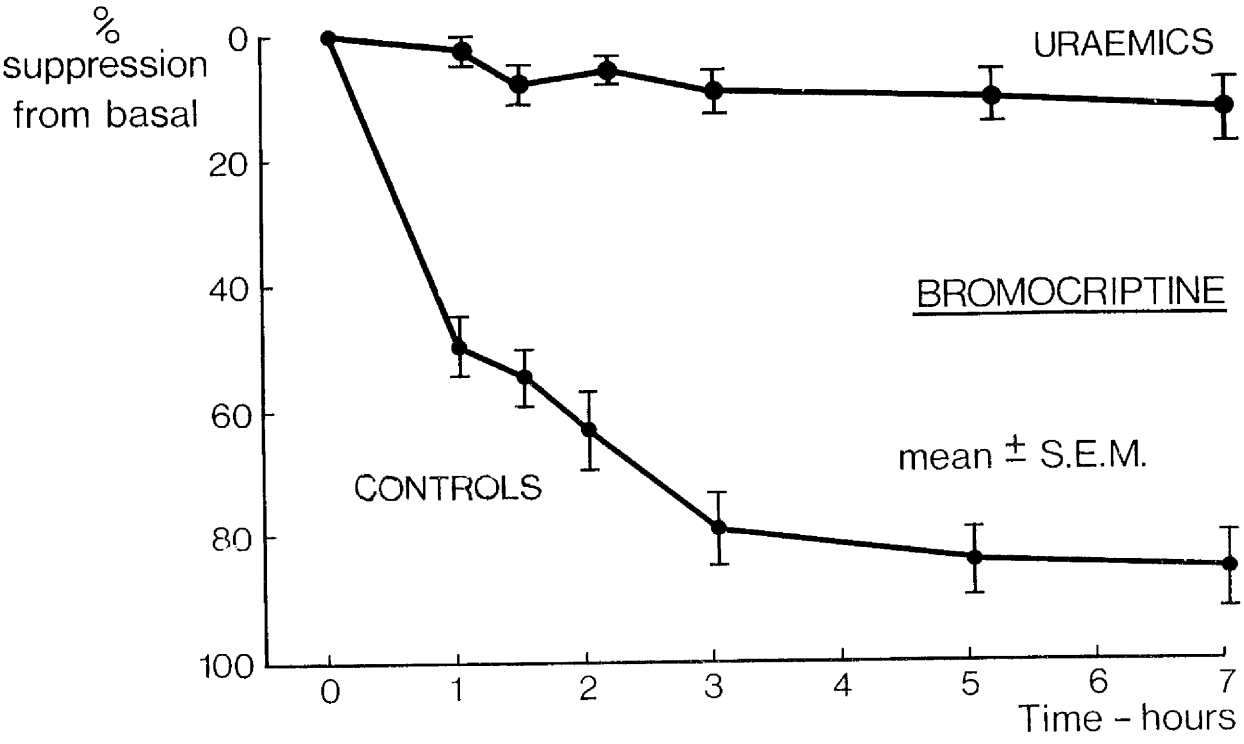

% suppression from basal
0
20
40
60
80
100
URAEMICS
BROMOCRIPTINE
mean ± S.E.M.
CONTROLS
0
1
2
3
4
5
6
7
Time - hours

FIGURE 59

Human prolactin heterogeneity after gel filtration: 1. MRC 75/504 h PRL reference preparation, normal pituitary extract and pregnancy sera.

Methods as described in text.

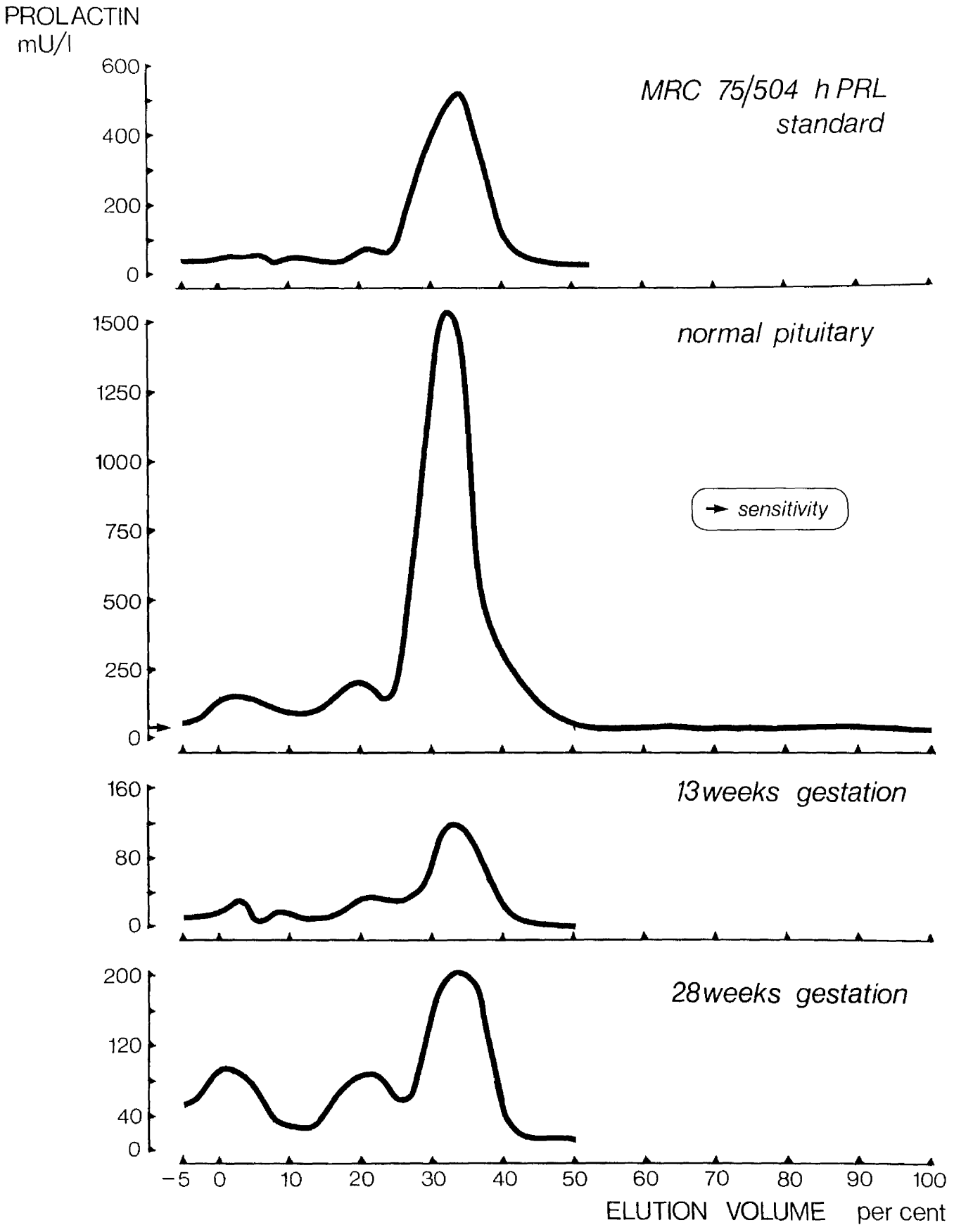

PROLACTIN
mU/l
600
400
200
0
MRC 75/504 h PRL
standard
1500
1250
1000
750
500
250
0
normal pituitary
sensitivity
160
80
0
13 weeks gestation
200
120
40
0
28 weeks gestation
−5
0
10
20
30
40
50
60
70
80
90
100
ELUTION VOLUME per cent

FIGURE 60

Human prolactin heterogeneity after gel filtration: 2. Prolactinoma patient S.C: Pituitary extract and serum. Methods as described in text.

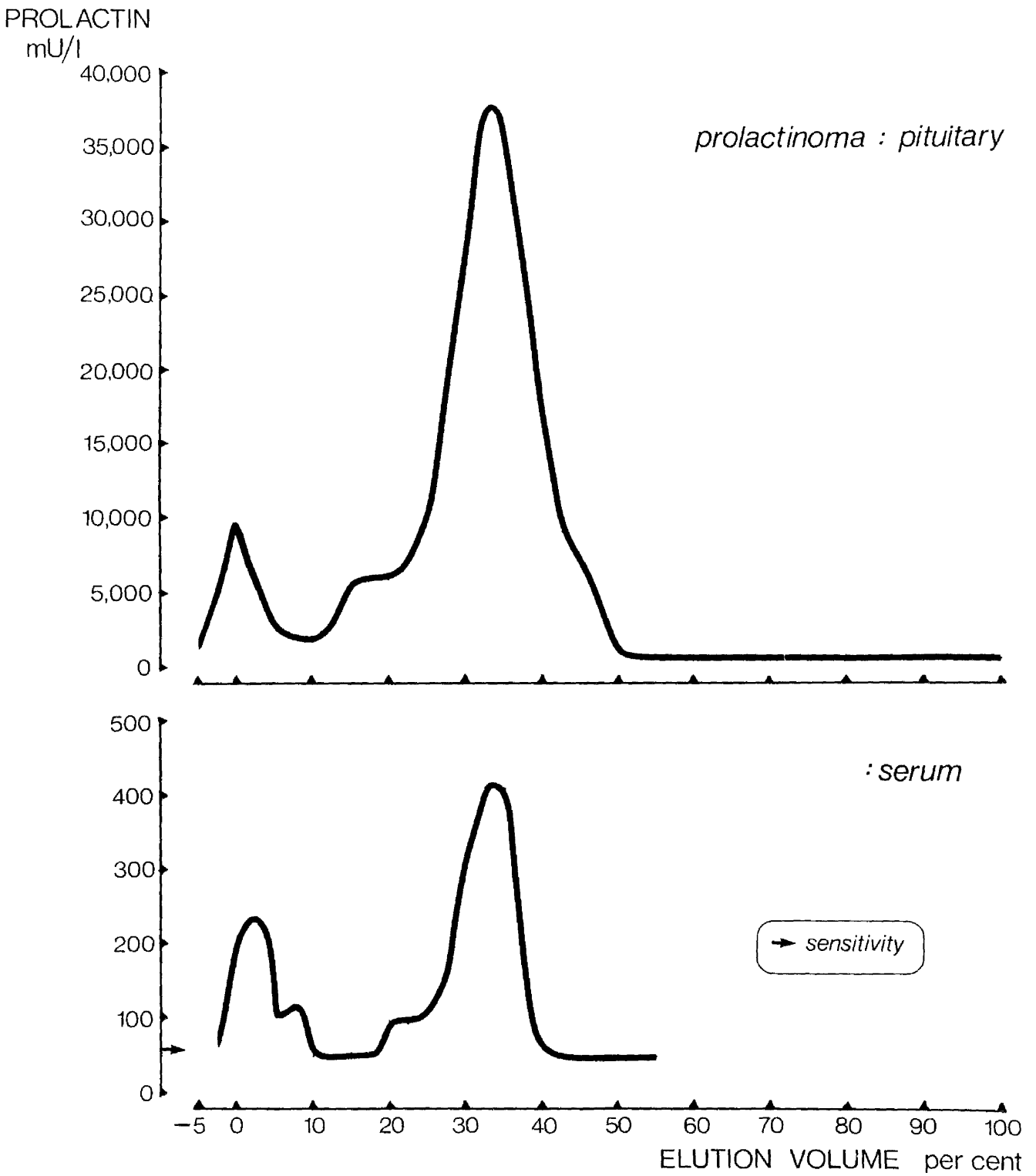

PROLACTIN
mU/l
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
prolactinoma : pituitary
500
400
300
200
100
0
: serum
sensitivity
−5
0
10
20
30
40
50
60
70
80
90
100
ELUTION VOLUME per cent

FIGURE 61

Human prolactin heterogeneity after gel filtration: 3. Prolactinoma patient P.C: Pituitary extract and serum. Methods as described in text.

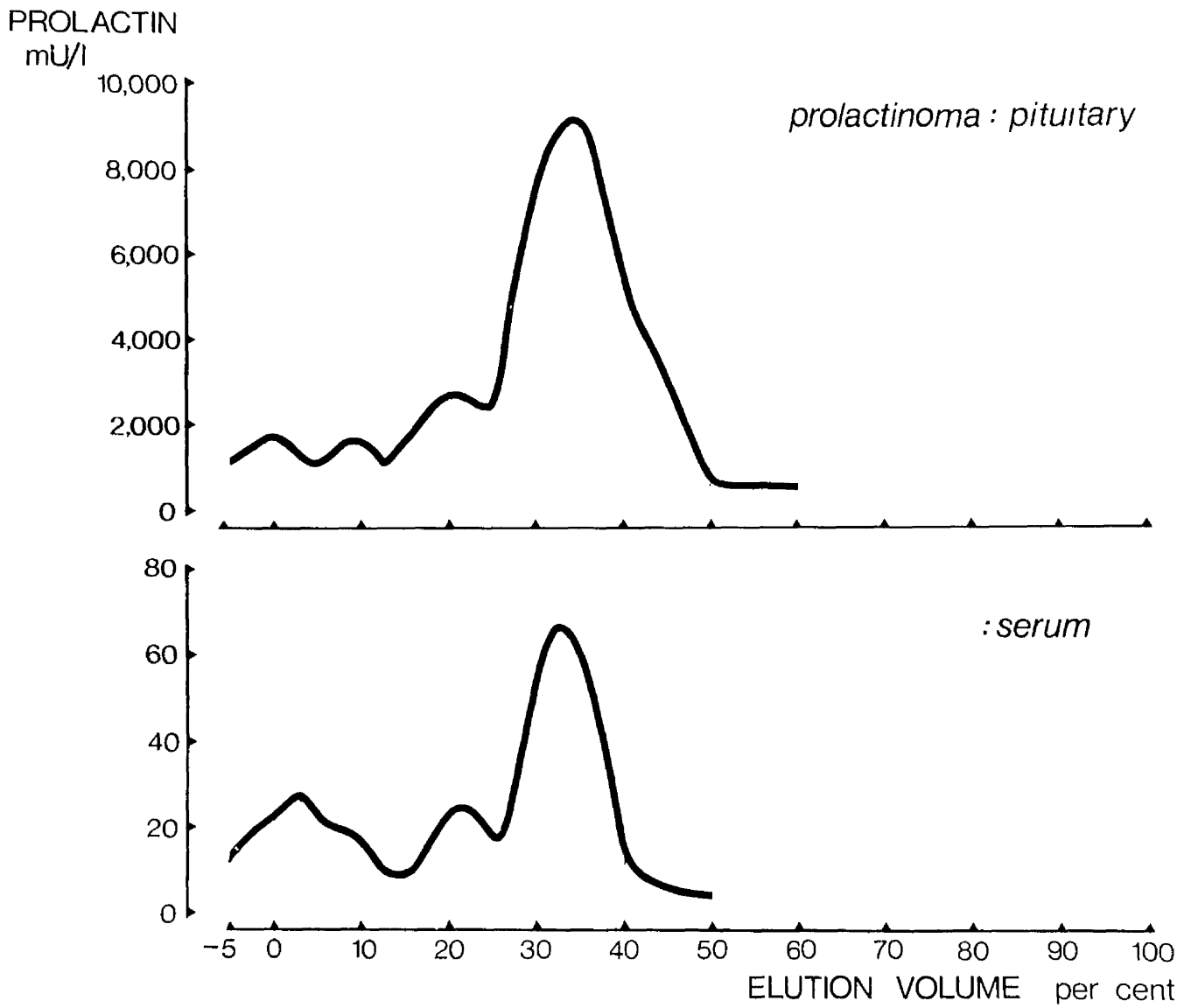

PROLACTIN
mU/l
10,000
8,000
6,000
4,000
2,000
0
prolactinoma : pituitary
80
60
40
20
0
: serum
−5
0
10
20
30
40
50
60
70
80
90
100
ELUTION VOLUME per cent

FIGURE 62

Human prolactin heterogeneity after gel filtration: 4. Uraemic sera A and B.

Methods as described in text.