DIAGNOSIS AND TREATMENT OF PITUITARY TUMOURS: A STARTING-POINT FOR MANIPULATION OF CANCER WITH HYPOTHALAMIC HORMONES

DIAGNOSE EN BEHANDELING VAN HYPOPHYSETUMOREN:
EEN STARTPUNT VOOR
MANIPULATIE VAN KANKER MET HYPOTHALAME HORMONEN

J.G.M. Klijn

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DIAGNOSIS AND TREATMENT OF PITUITARY TUMOURS: A STARTING-POINT FOR MANIPULATION OF CANCER WITH HYPOTHALAMIC HORMONES

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Aan de nagedachtenis van mijn vader en moeder,
die voor een fijne jeugd en een goede opleiding hebben zorg gedragen.

Aan Maeriam,
als troost voor mijn frequente afwezigheid.

Aan mijn dochters Esmeralda en Marjolanda,
die mijn grootste rijkdom vormen.

Aan mijn patiënten,
in de hoop dat zij enige baat hebben gehad en zullen hebben van het beschreven onderzoek.

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PART II

PART I

DIAGNOSIS AND TREATMENT OF PITUITARY TUMOURS

CHAPTER I

INTRODUCTION
RECOGNITION AND THERAPEUTIC APPROACH OF THE PITUITARY TUMOUR;

Developments between 1500 - 1980

A. CLINICAL SYNDROMES

One of the earliest depictions of the symptoms of a pituitary tumour dates back to 1365 BC, in which the portrait head of the Pharaoh Akhenaten displays features suggestive for acromegaly (Belchetz 1984a). In 1524 the Italian anatomist Berengario da Carpi described for the first time the human pituitary gland (Gauger 1979) and in 1670 Richard Lower concluded from his own observations that substances passed from the brain via the infundibulum and pituitary stalk and thence were distilled in the blood. A few years later, in 1679, Theophile Bonet reported the occurrence of a pathological enlargement of the pituitary gland. Subsequently pituitary tumours causing visual loss were described by Vieussens in 1705 and hydrocephalus by Petit in 1718 (Belchetz 1984a). In 1759 de Haen referred to the presence of a pituitary tumour in an amenorrhoeic woman.

The term acromegaly was first used by Marie in 1886 to describe two patients with hypertrophy of the extremities, marked enlargement of the hands and feet, prognathism, macroglossia, thickened skin associated with headache, arthralgia and lethargy (Marie 1886). In a number of earlier case reports Marie recognized acromegaly retrospectively, beginning with Saucerottes's patient described in 1772. In 1882 already Minkowski reported the constant finding of pituitary enlargement in acromegaly and he suspected for the first time that these two findings were connected. It was in 1909 that Harvey

Cushing used the term "hyperpituitarism" in relation to acromegaly. Since in the last 20 years assays for serum growth hormone became available, a clear relationship was proven between hypersecretion of growth hormone and acromegaly (Daughaday 1968, Lamberts et al 1979b, Klijn et al 1980b, Lamberts 1986b).

Functional disturbances as amenorrhoea and galactorrhoea are classically described in three clinical syndromes (Boyd et al 1977, Archer 1977, Blackwell 1985); (1) the Chiari-Frommel syndrome (Chiari 1855, Frommel 1882) with persistence of postpartum amenorrhoea and galactorrhoea for more than one year after delivery and without evidence of a pituitary tumour; (2) the Argonz-Del Castillo syndrome (1953), the spontaneous onset of amenorrhoea and galactorrhoea without a pathological sella turcica and not related with pregnancy; (3) the Forbes-Albright syndrome, since Forbes et al in their original description (1954) speculated that inappropiate breast secretion was caused by abnormal and presumably excess secretion of prolactin from a pituitary tumour radiologically diagnosed by a pathological sella turcica. Prolactin was discovered in 1928 as a lactogenic substance present in extracts of the pituitary gland of the cow but only since 1970 definitely identified in the blood by a sensitive bioassay (Frantz and Kleinberg 1970, Frantz 1978). In 1971 Friesen et al developed a radioimmunoassay (Hwang et al 1971) and showed increased plasma prolactin concentrations and increased tumoural prolactin synthesis in a patient with a Forbes-Albright syndrome (Friesen et al 1972). Since that time a rapidly expanding literature has accumulated on the human physiology of pituitary prolactin secretion in normal and various pathologic conditions.

With the development of more sophisticated radiological techniques for visualization of the pituitary fossa it appeared that these three clinical syndromes are all part of the same condition and in the 1970s the terms "hyperprolactinaemic-anovulatory syndrome" (Bohnet et al 1976) or "galactorrhoea-amenorrhoea syndrome" (Boyd 1977) were used as more practical ones. The pituitary tumours were indicated as microprolactinomas (diameter less than 1 cm) and macroprolactinomas.

In 1932 Cushing described pituitary basophilism occurring in the pituitary-dependent form of hypercortisolism with bilateral adrenal

hyperplasia, which is eponymously named after him as Cushing's disease (Cushing 1932, Lamberts et al 1980a and 1982c).

All other types of pituitary tumours without clinical signs of hypersecretion often are indicated as "inert" or "non-functioning" chromophobe adenomas. In general, these tumours are very large at diagnosis and cause local problems as bone destruction and neurological disturbances with visual loss. Recently, however, it appeared that the majority of these tumours secrete other hormonal substances like inactive parts of hormones like for instance the alpha-subunits of glycoprotein hormones (Ridgway et al 1981, Borges et al 1984, Horvath and Kovacs 1984, Whitaker et al 1985).

B. DIAGNOSIS OF PITUITARY GLAND TUMOURS

In general, the diagnostic procedures in patients with (suspected) pituitary tumours consist of neuroradiological ophthalmological and endocrine investigations.

1. Radiological Evaluation

In the evaluation of the sella turcica plain skull films are the common mainstays of radiologic diagnosis (Laws and Houser 1979). The earliest published radiograph of a confirmed pituitary adenoma appeared in Harvey Cushing's 1909 article reporting a patient with acromegaly, who was operated upon successfully via a transsphenoidal approach. On plain skull films the most obvious pathologic change is gross enlargement. Furthermore "doubling" of the floor, erosion, clear bone destruction, blistering, slanting, or straightening of the dorsum sellae can be observed. Intrasellar calcification may occur normally or in association with craniopharyngiomas, pituitary adenomas or carotid aneurysms. However, detection of minimal changes in the form of the sella caused by microadenomas need more sophisticated techniques. The development of multidirectional polytomography constituted an important step forwards in the diagnosis of microadenomas

(Vezina and Sutton 1974, Laws and Houser 1979, Wolpert 1980 and 1986, Hall 1984b). Tomograms offer detailed evaluation of the bony sella and focal changes can more readily be recognized. However, there is also evidence against the diagnostic value of sellar tomography (Muhr et al 1980 and 1981, Hall et al 1984a), showing that it is not as accurate (compared to variations of the configuration of the normal sella) as many original workers made out.

The earlier introduction of ventriculography and pneumoencephalography (PEG) by Walter Dandy provided valuable tools for the assessment of suprasellar extension of pituitary tumours, while angiography is more important for detecting carotid artery aneurysms (Laws and Houser 1979). However, since the advent of computer tomography scanning (CT-scan) in the second half of the 1970's these invasive investigations lost their important role in radiographic diagnosis (Wolpert 1980, Kendall 1983). The CT-scan appeared not only useful in the detection of intrasellar and suprasellar tumour masses, but is also superior to carotid angiography and cavernous sinus venography with respect to detection of lateral tumour spread (Hoffman 1979). High-resolution CT-scan even demonstrate microadenomas within the sellar fossa (Bamberger Bozo 1980, Bernasconi 1982, Houser 1984, Buchfelder et al 1984, Nakagawa et al 1985, Schneider 1985). The PEG has the advantage to delineate an empty sella from a necrotic intrasellar tumour and to identify small suprasellar tumours not seen on CT (Wolpert 1980). However the indications for PEG have been reduced yet further by the introduction of CT metrizamide cisternography which may give additional information about the nature and extent of intrasellar and extrasellar tumour mass (Wolpert 1980 and 1986, Hall 1984c).

Recently investigations with nuclear magnetic resonance (NMR) have been reported to identify again more details of pituitary tumours (Wolpert 1986). Also, NMR has a greater potential value for tissue characterization (Kendall 1983). Metabolic studies can be performed with positron emission tomography (Muhr et al 1984).

2. Ophthalmological Evaluation

The relationship between pituitary tumours and visual impairment

was first noted in the late 1800s (Stefanis et al 1979). Since then therapeutic efforts have focused on operative removal of tumour to restore and preserve vision. Before the 1970s the reported incidence of visual disturbances in some large series were relatively high i.e. 42-80%, but today the incidence dropped to about 20% due to better radiological and biochemical techniques allowing earlier detection of pituitary disease (Gittinger 1980, Buchanan 1984). The characteristic visual field defect caused by a pituitary tumour is bitemporal hemianopsia (Stefanis 1979, Gittinger 1980, Buchanan 1984). In a typical case of progressive chiasmal compression from suprasellar tumour growth, the superior temporal quadrants are affected initially followed by the inferior temporal, the inferior nasal and ultimately the superior nasal quadrant (in that order) resulting in blindness. Besides optic nerve atrophy papilloedema can occur when obstruction of the third ventricle by a large tumour mass causes increased intracranial pressure. The combination of optic atrophy on one side and papilloedema on the other has been described by Foster Kennedy.

Furthermore lateral extension of pituitary tumours can cause partial or complete palsies of the oculomotor, the abducens or the trochlear nerves resulting in paralysis of the extraocular muscles with diplopia or panophthalmoplegia. A pituitary apoplexy can cause acute visual disturbances accompanied by headache, nausea and sometimes meningismus or altered mental status (Stefanis et al 1979, Gittinger 1980).

The most common ophthalmological evaluation (Stefanis 1979, Gittinger 1980, Buchanan 1984) consists of a) funduscopic examination b) Snellen's visual acuity testing, and c) perimetry with the Goldman perimeter. Visual field defects caused by glaucoma have to be excluded. Tangent screen testing for small deficits in red-color vision may detect early abnormalities. A more recent technique for early detection of chiasmal compression consists of electroretinography with visual evoked potentials (VEPs). Abnormalities of the VEP occur in various lesions of the visual pathway and in fact may be the only sign of these lesions (Gittinger 1980). This method is particularly useful in patients in whom perimetric data are unreliable.

3. Endocrine Evaluation

The endocrinology of the pituitary gland and its target organs started really in the 1920s (Belchetz 1984a). Pituitary extirpation experiments and the use of pituitary extracts established gradually the spectrum of pituitary hormones, namely: the early experiments of Evans and Long on the growth in rats (1921), the restoration of thyroid function by pituitary extract in 1926 by Foster and Smith, and the separation of the gonadotrophins into Prolan A and B by Ascheim and Zondek in 1928 (Belchetz 1984a).

The major achievements have been made since 1930, in which year the endocrinologist Philip Smith published his results of experiments in hypophysectomized rats (Sawyer 1982). These experiments showed clearly that the pituitary gland produces hormones stimulating the thyroid, adrenal cortex, gonads, and growth. In the early 1930s the steroid hormones of the gonads (oestradiol and testosterone) were identified and synthesized. Is his excellent review on neuroendocrinology Sawyer (1982) stated that the anterior pituitary was soon recognized as the "leader of the endocrine orchestra". In 1932 Meyer et al and Moore and Price showed that gonadal hormones had a reciprocal action on the anterior pituitary activity, later called negative feedback. In the same year Hohlweg and Junkman noted that "castration cells" failed to develop in the pituitary when separated from the brain by transplantation, and they proposed that a "hypothalamic sex-center" was involved in the feedback circuit. In 1933 the pituitary portal vascular system was discovered by Popa and Fielding and the idea concerning humoral mediators from the hypothalamus controlling pituitary functions was first proposed by Hinsey and Markee. The downward direction of blood flow was first observed by Houssay et al (1935) and Wislocki and King (1936). In the 1940s Geoffrey Harris and Green established the neurovascular regulation of the anterior pituitary by humoral factors synthesized in the hypothalamus and secreted into the hypophyseal portal system (Harris 1955). Also since 1940, the history of the isolation of pure pituitary hormones has been dominated by Chok Hao Li (see review Sawyer 1982).

Meanwhile progress was being made in research on the hypothalamus

(Sawyer 1982). In 1951 Bargmann and Scharrer published their classic concept of neurosecretion. In the next years Du Vigneaud elucidated the structure of the posterior pituitary octapeptides vasopressin and oxytocin. In the early 1960s McCann and Harvis independently demonstrated the existence of a specific gonadotrophin releasing factor (LHRH). However, the simpler tripeptide thyrotrophin releasing hormone (TRH) was the first hypophysiotrophic factor that was chemically identified simultaneously in 1969 by Guillemin and Schally. The structure of LHRH as a decapeptide was published two years later by Matsuo and Schally (1971). Again two years later Guillemin's laboratory reported the structure of somatostatin being a tetradecapeptide (Brazeau 1973).

After the discovery of prolactin as a lactogenic substance present in extracts of the pituitary gland of the cow, it was not until 1970 that Frantz and Kleinberg identified prolactin definitely as a distinct hormone in man separate from growth hormone by means of a sensitive bioassay (Frantz and Kleinberg 1970, Frantz 1978). In the following year Friesen et al developed a radioimmunoassay for the measurement of human prolactin in plasma (Hwang et al 1971). Since then an enormous number of papers about the regulation of prolactin secretion and its clinical significance have been published (Thorner 1977, Lamberts et al 1978, Macleod et al 1984, Macleod and Lamberts 1986). With the general availability of the prolactin radioimmunoassay, the more widespread use of electron microscopy and the immunohistochemical investigations, it has become clear that prolactin hypersecretion occurs in approximately 50-80% of all patients with pituitary tumours. Many of these had previously been considered to be nonfunctional.

With the introduction in the 1970s of radioimmunoassays for most pituitary hormones, bioassays became obsolete. Simultaneously, radioimmunoassays for the hormones produced in the target organs of pituitary hormones became available. In the same decade a great number of stimulatory and inhibitory pituitary function tests were introduced, for instance the TRH- LHRH- and metyrapone test, insulin tolerance test (I.T.T.) and glucose tolerance test (GTT) (Christy 1979, Jackson 1980b and 1982). In the investigations described in this

thesis most of these tests have been used extensively. The reader is referred to section 2.b. of chapter II. All these revolutionary developments in endocrinologic evaluation together with the advances in neuroradiological techniques and the expanding use of transsphenoidal microsurgery appeared to be of great value in improving diagnosis, treatment and follow-up of patients with pituitary tumours.

After the extensive research in the last 30-40 years on the influence of electrical stimuli and neurotransmitters on pituitary function, we have seen in the past few years an explosive expansion of the research on brain peptides. The structures of CRF and GRF have recently been unraveled. However, not only releasing and inhibitory hormones are found but also a wide spectrum of peptides determined both in and outside the central nervous system, for instance in the gut and various endocrine glands. It can be expected with confidence that other hypothalamic hormones will be identified and synthesized and that many as yet unknown peptides will be discovered. In addition, the production of potent analogues of some neuropeptides as LHRH and somatostatin has contributed to the development of new treatment modalities in cancer therapy also in our hands (Klijn and de Jong 1982, Klijn et al 1984b-e, Klijn et al 1985a and b, Klijn 1986a and b, Klijn et al 1987a-f, Blankenstein et al 1985, Debruyne et al 1985, Foekens et al 1986 and 1987, Schröder et al 1987a and b, de Jong et al 1987, Setyono-Han et al 1987).

C. TREATMENT OF PITUITARY ADENOMAS

In this century many approaches of therapy for patients with pituitary tumours have been developed and tried out (Table 1). However, there are only three main types of therapy: surgery, radiotherapy and medical treatment. In the past 15 years major advances in treatment were the development of transsphenoidal (micro)surgery and the discovery of bromocriptine. This dopamine agonist inhibits particularly the hormonal activity of prolactin secreting pituitary adenomas and decreases the size of the adenomas considerably in many of them. Furthermore, the recent development of

Table 1. Potential types of therapy for patients with pituitary tumours.

1 Surgical Treatment

A) Directed at the pituitary
1: transfrontal or intracranial extirpation of tumour.
2: transsphenoidal or extracranial operation with:
- microsurgery
- cryosurgery
- thermal ablation
- implantation of radioactive implants (see below)
3: stereotactic
a) cryosurgery
b) radiofrequency
c) ultrasound

B) Directed at end organ (adrenalectomy in Cushing's disease)

2 Radiation Treatment

1) Conventional external radiotherapy (supravoltage or 60 Co)
2) Heavy-particle radiation
a) proton beam with Bragg peak
b) alpha particles (helium ions)
3) Implants
a) yttrium - 90
b) gold - 198
c) radium

3 Medical Treatment
see Table 2

4 Combined Treatment

5 None

potent somatostatin analogues will probably become important for the treatment of acromegaly. Also the techniques used in radiation therapy have been improved

1. Surgery

1.a. Intracranial Surgery

In the first two decades after Marie's suggestion about a relationship between acromegaly and pituitary tumours most surgeons had the opinion that pituitary tumours were inoperable. However, in 1889 the first pituitary adenoma was removed by Victor Horsley (Fahlbusch and Marguth 1978, Post 1980a). This earliest surgical approach was by the intracranial route to decompress the optic chiasm from a pituitary tumour. By 1893, Caton and Paul reported an attempt at removal of a growth hormone producing tumor via craniotomy and middle fossa approach. In 1905 Krause c.s. suggested an approach through the anterior cranial fossa. The first small series of patients was reported by Horsley, who approached the tumours via the middle fossa using intrasellarly moved rhinoscopic mirrors (Horsley 1906). Two out of ten operated patients died. After the introduction of an extracranial approach (Schloffer 1907, Cushing 1909) Frazier performed both operations, transsphenoidal and transcranial, before 1913, with the view of using the transsphenoidal approach for small enclosed tumours and tumours growing downward, and the transcranial approach for tumours growing upward (Post 1980a). The mortality rates differed significantly: 3.4 % for the former and 30% for the latter. After a period of popularity a gradual reversion to the intracranial procedure occurred in the 1920's. After more than 200 transsphenoidal operations Cushing abandoned this approach in the late 1920's and became the champion of the transcranial approach which until recently was favoured by most neurosurgeons (see Laws et al 1979a). He developed the midline subfrontal approach which still remains a standard procedure. Cushing returned to the transcranial operation because of higher incidence of infection, cerebrospinal fluid rhinorrhoea (which would be overcome later) and higher rate of tumour recurrence after

transsphenoidal operation alone (67,2 % within five years versus 42,5 % after transfrontal surgery alone). Also the frequent occurrence of chiasmatic compression as an indication for surgery made the transcranial approach the more popular for the next several decades (Henderson 1939). The optic chiasm syndrome has always remained the most important indication for the subfrontal approach. In the absence of an optic chiasm syndrome or neurologic disorders, in general, most doctors were inclined to apply radiotherapy or to remain expectative because of the high mortality rates and the bad results of surgery in recurrent tumours. The findings of Jefferson (1940 and 1969) regarding the relationship between tumour size and operative risks have been confirmed by many subsequent workers (Bakay 1950, Horrax et al 1955, Svien et Colby et al 1969, Elkington and McKissock 1967, Wirth et al 1974). In patients with large tumours and massive suprasellar extension the mortality rate was higher (16.7-36 %) than in those with small tumours (2.5-6,4 %) with an overall mortality rate per series of 5.4 to 14.1 % (Post 1980c). For re-operations the mortality rate was very high: between 33 % (Wirth et al 1974) and 55 % (Elkington and McKissock 1967). Ray and Patterson (1971), reaching a very low overall primary mortality rate of 1,2 % found a mortality of 25 % with re-operations. The most frequent causes of death were intracranial or intratumoural bleeding, cerebral oedema or infarction, and brain-stem damage. The mortality rate decreased impressively (to 2-5 %) after the introduction of perioperative protection with high doses of corticosteroids preventing cerebral oedema and adrenal insufficiency (Ray and Patterson 1971; van der Zwan 1971; Taekman 1979; Post 1986). Nevertheless, the transsphenoidal operation is more and more favoured now even in the case of suprasellarly extending tumours (Laws et al 1977) because of good results and lower morbidity and mortality.

1.b. Extracranial Surgery

In 1907 Schloffer performed the first successful extracranial extirpation of a pituitary tumour via the transsphenoidal route in a man with symptoms of panhypopituitarism, severe headache and an optic chiasm syndrome. He gained access to the sella turcica by way of the

ethmoid cells and the sphenoid sinus by a modification of the operation of Giordano (1897), who used a route through the frontal sinus. In 1909 Cushing attempted to control acromegaly surgically by his first operation using the transsphenoidal approach. The symptoms and signs of acromegaly rapidly improved, and there ensued a large series of 247 pituitary adenoma patients operated transsphenoidally. Until then the operation involved considerable dissection of the frontal sinus, nose, ethmoids and sphenoids and was very disfiguring. In 1910 Halstead started with a transoronasal route with an incision through the mucosa above the upper teeth, utilizing the submucosal techniques of Kocher. Also in 1910, Oscar Hirsch performed the first of his 413 transsphenoidal operations (Hirsch 1910). The transsphenoidal route to the sella turcica was the operative method of choice until the 1920's (Halstead 1910, Hirsch 1910, Cushing 1912 and 1914, Frazier 1913).

After the return of Cushing to the subfrontal approach in spite of an overall mortality rate of only 5 % in a series of 231 transsphenoidally operated patients, Cushing's pupil Norman Dott (1925) and Hirsch remained faithful to the open transsphenoidal route. In 1959 the first report of lasting remissions of 20 to 30 years followed (Hirsch et al 1959a and b). Probably the longest reported survival is that of Mr. Owens, first operated transsphenoidally by Cushing and four years later reoperated by Hirsch utilizing the same approach for radium implantation to restore failing vision. He was in a good condition and introduced to the Cushing Society by Hirsch 48 years after the first operation (Hirsch 1959b, Hardy and Wigser 1965).

After 1940 Hamlin used transsphenoidal surgery successfully in a series of 104 patients (1962) with a mortality rate of 2 %, but the subfrontal approach was favoured by most surgeons especially because of the lower recurrence rate. However, the open transsphenoidal approach revived after the introduction of image-intensified fluoroscopy by Guiot (a student of Dott) in 1959 (Guiot and Thibaut 1959) and the further modernization of this procedure by the introduction of the operating microscope by Hardy (1962, 1965), who was trained by Guiot. The radiological control, the magnification and improved focal illumination has made the operation safer and

identification of tumour tissue easier. These technical advances made microsurgery possible. More than the technical improvements, however, Hardy's introduction of the concept of selective microsurgical removal of functioning pituitary adenomas has been responsible for the current intense neurosurgical interest. This has also been stimulated by the better detection of small hormonally active tumours due to the advances in endocrinology and radiology (radioimmunoassays for prolactin and growth hormone, polycyclic tomography of the sella). Later several surgeons have made their own modifications of this surgical technique (Wilson 1978 and 1984, Tindall et al 1978, Post 1980b). Some excellent reviews has been published by Post (1980a,b,c). In Europe the transsphenoidal operation became also very popular in the 1970's outside France (Giovanelli et al 1976, 1980 and 1984, Fahlbusch et al 1978, 1980, 1984 and 1985, Landolt 1984a and 1985b, Lüdecke et al 1983 and 1984). Some surgeons use the transsphenoidal route for cryosurgery, thermal ablation or implantation of radioactive isotopes (Post 1980b).

In 1969 de Lange started as the first surgeon in the Netherlands with the transsphenoidal technique using the operating microscope and radiological control in patients with pituitary adenomas. The results of the transsphenoidal operations performed in his department are reported in chapter II. In the beginning transsphenoidal microsurgery has been restricted to patients with prolactin and growth hormone secreting pituitary tumours, but later on it was applied also in patients with Cushing's disease and with hormonally inactive tumours.

Together with the advances in endocrinology transsphenoidal microsurgery, aimed at a selective removal of microadenomas, improved insight into the pathogenesis of pituitary tumours. So, it is clear now that there exist at least three types of pituitary dependent Cushing's disease: ACTH-producing microadenomas in the anterior pituitary, in the pars intermedia, and diffuse hyperplasia of corticotrophic cells in the anterior pituitary (Lamberts et al 1980a,c,d,e, 1981, 1982c, 1984a). However, other authors did not find evidence of intermediate lobe origin of corticotroph adenomas (McNicol et al 1986).

2. Radiation Treatment

Radiation therapy was first applied in the management of acromegaly. In 1907 successful treatment was carried out by Gramegma in a 45-year-old acromegalic female and by Bèclère in a 16-year-old girl with gigantism. Gramegma utilized an intraoral glass applicator and an early Crooks tube with voltage in the range of 80 KV, while Bèclère used 100 KV delivered via a five-field-treatment technique, a technique that is still being used in modified form by many radiotherapists (Emami 1980). Then in the 1910's local application of radium into the sphenoid sinus was tried by surgeons via the transnasal route (Quick 1920, Hirsch 1921), but soon abandoned because of high morbidity and mortality. A series of patients treated by external radiotherapy was first reported by Heinismann and Czerny (1926).

In the 1930's considerable controversy existed between neurosurgeons and radiotherapists as to which treatment modality was superior. Cushing did not favour radiotherapy above surgery. However, in a follow-up study of his series of 338 surgically treated patients, Henderson (1939) reported that the frequency of tumour recurrences within 5 years postoperatively decreased considerably by radiotherapy after surgery (from 58 to 26 %). The five year recurrence-free rate was 32.8 % with transsphenoidal operation alone and 57,5 % with subfrontal surgery alone, but increased to 65,3 % and 87,1 % respectively if the operation was followed by irradiation. This was a surprising result because radiation therapy consisted of only 500 rad given in two treatments. After the earlier recommendation of postoperative radiotherapy by Pfahler and Spachman (1935), Henderson's study can be considered the basis for combined surgical and radiotherapeutic approach to pituitary tumours. Subsequently, primary radiotherapy became also customary, especially in patients with tumours which did not compress the optic chiasm and/or the third ventricle.

In the 1940's, radiotherapy of pituitary tumours consisted of multiple small courses of low-dose exposure, separated by periods ranging from several weeks to years (Emami 1980). In 1949 Kerr as well

as Ellis advised a single intensive course, which in the next decades appeared superior over the prolonged low-dose technique. Currently conventional irradiation is given as a single course consisting of fractions of 180-200 rads, four or five per week, with a total dose of about 4.000 to 4.600 rads, calculated at the 95% isodose line. The total dose may be increased to 5.500 rads in the case of very large invasive tumours (Sheline 1979, 1982 and 1984). Detailed descriptions of the external radiation therapy techniques are given in the excellent articles of Emami (1981) and Sheline (1979, 1982 and 1984).

External radiotherapy has meanwhile improved with the development of high-energy sources of röntgen irradiation and with the use of the cyclotron to produce high-energy particles proton beam: (Tobias et al 1952, McCombs 1957, Linfoot et al 1970, Linfoot 1979 and 1984, Lawrence 1957 and et al 1970, Kjellberg et al 1968 and 1980). After the development of stereotactic approaches to the pituitary in the 1950's, the use of intrasellarly placed radioactive isotopes also appeared successful in a considerable number of patients with pituitary tumours (Greenwood et al 1965, Hartog et al 1962 and 1965, Northfield 1949, Wright et al 1970, Joplin et al 1979 and 1984).

In conclusion, after 70 years of experience, there is no longer doubt that radiotherapy plays a major role in the management of pituitary adenomas. Almost all pituitary tumours treated by surgical excision (with the exception of the smaller ones) recurred within seven years, unless radiotherapy was added (Henderson 1939; Bakey 1950; Guiot et al 1967, Erlichman et al 1979). After the introduction of the GH and PRL assays, it appeared that after primary radiotherapy GH and PRL hypersecretion decreased gradually in nearly all patients with acromegaly or prolactinoma. However, in 50-75 % of the patients normal levels were not reached. Also in Cushing's disease external pituitary irradiation as the primary modality of therapy does not induce definite cure in the majority of the patients. On the other hand, there is in general no difference between radiotherapy alone and surgery in combination with radiotherapy with respect to the efficacy of tumour growth control (Emami 1980, Erlichman et al 1979). The average disease-free rate in 11 studies was 85 % five to ten years after treatment compared to 41% for surgery alone in patients with

pituitary tumours (Emami 1980). Erlichman et al (1979) found a median time to recurrence of 3.5 years. One patient showed a recurrence 17 years after initial therapy.

The frequency of failures after proton beam radiosurgical treatment is reported to be low (10 %). Kjellberg, who was an assistant of Hirsch and who became a supporter of focal radiosurgery with Leksell (in Stockholm), found an overall improvement in 90 % of patients with acromegaly and in 85 % of patients with Cushing's disease 24 months after radiosurgery with a remission rate of 60-65 % (Kjellberg and Kliman 1980).

Treatment of pituitary tumours by interstitial irradiation has also been proven to be an attractive type of radiotherapy (Joplin et al 1979 and 1984, Linfoot 1979 and 1984), especially in acromegaly and Cushing's disease. It causes a quick fall in GH and ACTH levels, i.e. of about 60 % within one year.

3. Medical Treatment

Before the extensive use of bromocriptine in medical practice for pituitary adenomas, various pharmacologic agents have been tried to control the secretion and/or the effects of pituitary hormones produced by hormonally active pituitary tumours (Table 2, Goldfine and Vigneri 1979, Soman 1979, Molitch 1980). The results of these types of treatments have generally been disappointing.

Oestrogens do not appear to decrease circulating GH levels. The moderate beneficial clinical effects in acromegaly are due to suppression of GH-mediated somatomedin generation in the liver (Wiedemann and Schwartz 1972, Wiedemann et al 1976). The amelioration of clinical symptoms is also apparent from reduction of urinary calcium and hydroxyproline excretion as well as a decrease of the serum phosphorus level (Schwartz et al 1969). There is no wide-spread use of this therapy because of the relatively short lived effect, the increased cardiovascular risks and the possible predisposition to apoplexy in pituitary tumours (Molitch 1980). It might be applied in

Table 2. Medical Treatment of pituitary tumours

Acromegaly	Prolactinoma	Cushing's disease
1) somatostatin analogues		
2) dopaminergic agents i.e.	1) dopaminergic agents	A)Directed at pituitary tumour.
- bromocriptine		1)dopaminergic agents
- lysuride		2)serotonine antagonists
- lergotrile mesylate		B)Directed at adrenals
		3)o'p' - DDD
3) antioestrogens	2) antioestrogens	4)aminoglutethimide
4) oestrogens	3) serotonine antagonists (probably via dopaminergic action)	5)metyrapone
		6)ketoconazole
5) medroxyprogesterone acetate	4) pyridoxine	C) Antiglucocorticoids
6) Alpha-adrenergic antagonists -phentolamine -fenfluramine		
7) Serotonine antagonist -cyproheptadine -metergoline-methysergide (probably via dopaminergic action)		
8) chlorpromazine		

acromegalic patients, who have not responded to other therapy. In patients with prolactinomas or mixed tumours oestrogens are contraindicated because of the stimulatory effects on prolactin secretion and on tumour growth; this is also applicable to patients with Cushing's disease because of retention of salt and water with cardiovascular risks.

Medroxyprogesterone acetate may decrease basal GH levels and the response of normal and tumourous GH-secretion to various stimuli as hypoglycemia and arginine (Simon et al 1967, Lawrence and Kirsteins 1970). Long-term follow-up showed improvement of soft-tissue swelling and headache in acromegalic patients (Lawrence and Hagen 1973). However, the success rate appeared to be low (10-20 %) with sometimes even a rise in circulating GH levels during this type of treatment (Soman 1979, Molitch 1980).

Alpha-Adrenergic antagonists as phentolamine and fenfluramine (Soman 1979), have been shown to lower plasma GH levels acutely in acromegalic patients (Nakagawa and Mashino 1973), probably by depletion of catecholamine concentration in the hypothalamus. There is search for new suitable drugs, but until now an agent effective in the majority of acromegalic patients is not available (Lamberts 1985a).

Chlorpromazine was initially reported by Kolodny et al (1971) to be effective in reducing GH levels and improving clinical features in a young man with acromegaly. However, the effectiveness has not been confirmed in later reports (Dimond et al 1973, Alford et al 1974). Chlorpromazine stimulates normal prolactin secretion. This stimulating effect is absent or decreased in patients with prolactinomas, but inhibition has been not observed. This drug is of no use in patients with prolactin secreting pituitary tumours.

Serotonin antagonists have been reported to inhibit both basal and stimulated plasma GH levels in normal people (Bivens et al 1973, Smythe and Lazarus 1974, Nakai et al 1974), and in acromegalics (Delitala et al 1976, Feldman et al 1976). However, Chiodini et al (1976) found no acute effect of cyproheptadine in four acromegalics.

Metergoline indeed inhibited GH release, but appeared to have dopaminergic effects because it can be blocked by the dopamine-receptor blocker pimozide.

Because serotonin is known to be stimulatory to PRL secretion several serotonin blocking agents have been used for treatment of patients with (micro)prolactinomas. Cyproheptadine and methysergide are relatively ineffective in lowering PRL levels, especially in long-term therapy and in patients with very high PRL levels (D'Agata et al 1977, Crosignani et al 1978, Ferrari et al 1976 and 1978, Wortsman et al 1979). However, metergoline, an antiserotoninergic drug but with dopaminergic properties (Chiodini et al 1976), appeared to be effective in restoring menses and PRL levels to normal in about one-third of the women treated (review Molitch 1980).

In Cushing's disease and Nelson's syndrome cyproheptadine has been reported to decrease ACTH secretion (Krieger et al 1975 and 1976, Cassar et al 1976). In ectopic ACTH secretion cyproheptadine may also be effective. Krieger reported successful responses in about 60 % of 80 patients with Cushing's disease (1979). Relapses occur in all patients when treatment is discontinued.

The drug o,p'-DDD (by selective destruction of the zona fasciculata and zona reticularis of the adrenal cortex (Lubitz et al 1973)) and aminoglutethimide or metyrapone (by blocking the production of cortisol) are effective in Cushing's disease (Child et al 1976, Molitch 1980). Recently, also treatment with high dose ketoconazole appeared effective (Loli et al 1986). However, they will not induce definitive cure in the patients.

Pyridoxine (vit B6), a cofactor in the biosynthesis of dopamine, was in 1976 reported to be effective in women with the galactorrhoea-amenorrhoea syndrome (McIntosch). However, Tolis et al (1977) found no effect of pyridoxine on normal PRL as well as GH secretion and an absence of therapeutic effects in the galactorrhoea-amenorrhoea syndrome.

Up to now dopaminergic agonists have turned out to be the most effective drugs in patients with pituitary tumours, especially in patients

with prolactinomas. In 1954 Shelesnyak noted that certain ergot alkaloids suppress prolactin secretion in rats (Thorner 1980b). Later on Zeilmaker and Carlsen (1962) showed that one of these drugs, ergocornine, acts directly at the pituitary. In the early 1960's a systematic pharmacological study of ergot alkaloids and ergot derivatives started with the goal to find an orally effective drug which could specifically inhibit prolactin secretion. These studies were carried out with the aid of bioassays of prolactin and measurement of clinical effects as galactorrhoea. The development of 2 - Br - Alpha - ergocryptine mesylate (CB-154, bromocriptine, Parlodel R) by Flückiger and Wagner in 1965 appeared in the 1970's to be a fundamental improvement of the medical treatment of pituitary tumours. In 1967 the drug was selected for further development as a therapeutic agent (Flückiger and Wagener 1968). At that time the mode of action was not known while interest in the role of dopamine in the regulation of prolactin secretion was growing rapidly (van Maanen and Smelik 1968; Fuxe et al 1970, Kordon 1971, Macleod and Lehmeyer 1973 and 1974, MacLeod et al 1984, Macleod and Lamberts 1986). After the identification (Frantz and Kleinberg 1970), isolation and purification (Guyda et al 1971) of prolactin and the development of a radioimmunoassay for this hormone (Hwang et al 1971), it appeared that the catecholamine dopamine is the most important physiological factor in the inhibition and regulation of prolactin release. There is abundant evidence that dopamine is identical or at least one of the previously proposed hypothalamic Prolactin Inhibiting Factors (PIF) (van Maanen and Smelik 1968; Schally et al 1976). Dopamine acts directly on the pituitary as proved in vitro (MacLeod and Lehmeyer 1973). In addition to this observation dopamine receptors have been found on lactotroph cells in the pituitary (Calabro and Macleod 1978, Caron et al 1978, Cronin et al 1978, 1979 and 1980).

Bromocriptine was shown to act by stimulating dopamine receptors (Corrodi et al 1973, Fux et al 1974). Dopamine receptors have been demonstrated in PRL, GH and ACTH secreting pituitary tumours (Cronin et al 1980). Following animal pharmacologic experiments (Flückiger and Wagner 1968; Billeter and Flückiger 1971) Lutterbeck et al (1971) showed that bromocriptine therapy can be used in the treatment of

galactorrhoea. The drug decreased plasma PRL concentrations in normal persons (Del Pozo et al 1972) and proved to be successful in restoring menstruation and fertility in hyperprolactinemic patients with an amenorrhoea-galactorrhoea syndrome (Besser et al 1972, Del Pozo et al 1974, Rolland et al 1974), which syndrome often appeared to be caused by a prolactin secreting (micro)adenoma (Vezina and Sutton 1974). Ovulation returned during bromocriptine therapy in about 85% of the patients within 10 months (Thorner et al 1980b), unless the reserve of the gonadotrophin secretion is disturbed (Lamberts et al 1978a and b). On the basis of 805 pregnancies documented by Sandoz (Thorner et al 1980b) and 1410 pregnancies reported by Turkalj et al (1982) there is no evidence that bromocriptine used until establishment of pregnancy is associated with an increased incidence of multiple births, fetal abnormalities, or increased abortion rates (Thorner et al 1980b, Griffith et al 1978, Rolland 1980, de Wit et al 1985, Weil 1986).

Confirming earlier animal work (Quadri et al 1972) Corenblum et al (1975) and other authors reported reduction in the size of prolactinomas (Corenblum 1978, McGregor et al 1979, Wass et al 1979, Thorner et al 1980a and b). This has been demonstrated by radiological and ophthalmological methods. This effect may become manifest within days to weeks after the start of bromocriptine treatment. Reviewing a great number of series Molitch (1980) reported that bromocriptine was successful in normalizing serum PRL levels in 80-90% of the patients with cessation of galactorrhoea in over 90% and restoration of normal menstrual pattern in 80-90%. In addition, I would like to refer to Chapter II, section 3.c.3.

In 1972 Liuzzi et al demonstrated that L-dopa which stimulates GH secretion in normal individuals has an paradoxical inhibitory effect on GH secretion in acromegaly. In 1974 he also reported an acute inhibitory effect of bromocriptine on GH levels in some acromegalics (1974a and b). This effect was more prolonged than that after L-dopa. Since then many investigators have reported fairly good results of long-term treatment of acromegaly with bromocriptine. Reviews of these studies (Goldfine and Vigneri 1979, Soman 1979, Molitch 1980, Vance et al 1984) show that bromocriptine causes a significant fall in GH

levels in about 70-75% of the acromegalic patients. However, a decrease to normal GH levels, less than 5 ng/ml, is achieved in only 20 % of the patients (Wass et al 1977, Vance et al 1984). Liuzzi et al (1974a) as well as our group (Lamberts et al 1979a, 1982a, 1983, Chapter VII) showed that bromocriptine was more effective in acromegalic patients with increased PRL levels and high responses of GH to TRH. In contrast to the report of Wass et al (1979) concerning reduction of tumour and sellar size by long-term treatment with bromocriptine in acromegalics, Lindholm et al (1981) reported no effect even on GH levels of bromocriptine in a double-blind study.

After the reports of the lowering of PRL and GH level in patients with prolactinomas and acromegaly by bromocriptine, Lamberts and Birkenhäger (1976; Lamberts et al 1980a, c and e) and Benker et al (1976) showed that bromocriptine also reduces ACTH and cortisol levels in some patients with Cushing's disease or Nelson's syndrome by a direct effect on the ACTH-secreting pituitary adenoma (Lamberts et al 1980a,c and e). Later, it became clear that bromocriptine reduced ACTH and cortisol levels, especially in patients with ACTH-secreting tumours originating from the intermediate pituitary lobe (Lamberts et al 1982c, Lamberts 1984a). However, especially in the practical management of Cushing's disease medical treatment should be regarded as a temporary adjunct to more definite treatment.

Anti-oestrogens as tamoxifen appear to enhance the sensitivity of prolactin secreting pituitary tumour cells to dopamine and bromocriptine (Lamberts et al 1980b). The use of the combined treatment with bromocriptine and tamoxifen may prove to be more effective than treatment with bromocriptine alone in some patients with PRL-secreting pituitary tumours (Lamberts et al 1982b, Volker et al 1982).

Somatostatin is a hypothalamic hormone acting at the level of the pituitary. It is capable of inhibiting the release of basal and stimulated GH secretion in healthy people and in patients with acromegaly (Brazeau et al 1973, Hall et al 1973, Besser et al 1974a and b, Yen et al 1974). This type of treatment with the natural hormone is not useful because of the short biological half-life, the

need of continuous parenteral administration, and the effect on the secretion of other hormones as insulin, glucagon, gastrin, renin, GIP and VIP and the postinfusion rebound hypersecretion (Guillemin and Gerich 1976; Reichlin 1983). After synthesis of the somatostatin analogues with a longer half-life (Sarantakis et al 1976, Grant et al 1976), it was not before 1983 that the first clinical trials have started.

Recently a long-acting somatostatin analogue (SMS 201-995, Sandoz)was shown to exert a profound inhibitory effect on GH release in most acromegalic patients without a rebound hypersecretion (Lamberts et al 1985b and c, Ch'ng et al 1985). Preliminary studies show that chronic treatment of acromegalic patients with SMS 201-995 results in (near) normalization of plasma GH and somatomedin-C levels, disappearance of the clinical symptoms and shrinkage of the pituitary tumours in many acromegalics (Lamberts et al 1985b and c and 1986a). For additional data see Chapter II, 3.b.3.b..

D. REFERENCES

The references of this chapter are cited together with those of chapter II.

CHAPTER II

OWN RESULTS AND RECENT LITERATURE DATA

A. AIMS OF THE STUDY

In order to make a well-founded choice between the therapeutic modalities presently available it is important to have a clear picture of the differences in the natural history of the various types of pituitary tumours. One of the most important questions regards possible differences in the tendency of the various tumours to grow extrasellarly. A related question concerns the relationship between the secretion rate of growth hormone, prolactin etc. and the size of the respective tumour. In other words: do high serum levels of these hormones in general indicate the presence of a large tumour and do moderately increased levels point to a smaller tumour?

A parallel and for the management sometimes crucial question is a possible relationship between tumour growth rate and the age of the patient. Furthermore, one can ask what - in a therapeutic sense - may be the significance of a combined secretion of growth hormone and prolactin by a pituitary tumour.

The therapeutic part of our study concerns the efficacy and the rapidity, with which the desired therapeutic endpoint may be reached by means of (transsphenoidal) surgery, irradiation or the combination of these treatments in the various types of pituitary tumours. Furthermore, we have considered the value of medical treatment as an additional or - possibly - as primary therapy.

B. PATIENTS, METHODS AND MATERIALS

1. Patients

During the time period of this study (1980 - 1985) the records of 357 patients, investigated and treated since 1950 in the University Hospital "Dijkzigt" and in the Dr. Daniel den Hoed Cancer Center/ Rotterdam Radio-Therapeutic Institute for intrasellar and suprasellar tumours were reviewed. The diagnostic and therapeutic results of specific studies in subgroups of patients have been reported in different publications. For the description of the patients the reader is referred to chapters III-VIII.

2. Methods and Materials

For radiological and ophthalmological examinations, and endocrine investigations we refer to chapter III. With a few exceptions, in all patients operated upon for an intrasellar tumour neurological and ophthalmological examination, plain sellar radiography, sellar tomography (sagittally and in part also frontally), pneumencephalography (PEG) and/or computertomography (CT), and/or bilateral carotid angiography have been performed preoperatively. When preoperatively a patient was examined in our own department a standard endocrine investigation was carried out, whenever possible, comprising estimation of basal plasma hormone levels (PRL,GH,TSH,LH,FSH,ACTH in Cushing disease, thyroid hormones, cortisol, testosterone or oestradiol-17betha) as well as stimulation tests (TRH-test, LHRH-test, metyrapone test). In acromegalic patients also a glucose tolerance test (G.T.T.) was performed. In some patients the response of GH and PRL to bromocriptin has been evaluated. In a few patients an insulin tolerance test and/or lysine-vasopressine test (later on replaced by the CRF-test) was carried out also. About 2-3 weeks postoperatively and with variable intervals after the subsequent external pituitary irradiation these endocrine investigations were repeated.

3. Measures and Procedures

3.a. Prae- and Postoperative Measures

A preoperative check-up was carried out by the ENT-specialist, who would perform the first part of the operation. This included nasal swab cultures. All patients received prophylactic ampicilline treatment. Peroperatively a corticosteroid cover was used in all patients. Patients operated upon subfrontally received over the day of operation 4 x 4 mgr. dexamethasone and patients operated upon transsphenoidally 4 x 100 mgr. hydrocortisone. In 7-10 days these corticosteroid dosages were diminished to a substitution level in the department of neurosurgery and terminated after transfer of the patients to the endocrine department. At least 4 days after terminating corticosteroid administration the stimulation tests were carried out on subsequent days in the following order: LHRH-, TRH-, metyrapone and glucose tolerance test.

3.b. Operative Procedure (transsphenoidal adenomectomy)

After an incision under the upper lip the nasal septum is uncovered and - after preparing off the mucosal membranes - largely removed and kept. A tunnel is made up to anterior wall of the sphenoid sinus. Here the neurosurgeon takes over, opens the sinus, removes the mucosal membrane and opens the sella with radiological monitoring by means of an image-intensifyer and using the operation microscope (magnification 10x). After coagulation the intrasellar dura is opened and the pathological content of the sella is removed. The sellar diaphragm is seen to descend. To prevent liquorrhoea, that may ensue when the diaphragma has been cut, the sella is filled with muscle tissue taken from the right thigh at the onset of the operation. Subsequently the sella is closed with muscle tissue that has been dipped in biological acryl-glue, that hardens after having been put in place. The ENT-specialist puts back the nasal septum and places tampons. Morbidity is usually small and the patient is mobilized within 24 hours.

3.c. Radiotherapeutic Procedure

The radiotherapeutic procedure is described in chapter VIII. During the period of irradiation cortisone substitution therapy was reinstituted until the results of the metyrapone test were known (after the end of the irradiation).

C. RESULTS AND DISCUSSION

1. Epidemiological and Clinical Features

1.a. Prolactinomas

The prolactinoma is probably the most common kind of pituitary tumour (Kovacs et al 1984, von Werder 1984, Landolt 1985a). The reported percentage of prolactinomas in groups of patients with pituitary tumours varies and is probably dependent on the referral patterns in the different centres. Epidemiologic studies have shown an increased incidence (more than twice) of pituitary adenomas in the last 10-15 years compared to prior decades, which increase may be partly due to a better detection of the adenomas and maybe partly due by the use of oral contraceptives (Peillon et al 1984, Molitch 1986). A population study performed by the Mayo Clinic found a mean annual incidence of diagnosis of pituitary tumours of 8.2 per 100.000 women aged 15 or greater (Molitch 1986). This figure is only about 0.1 % of the actual frequency of tumours known from autopsy studies. Subclinical microadenomas have been discovered in 1.5-26.7 % of 12 autopsy series, 40-50 % of them being prolactinomas (Burrow et al 1981, Peillon et al 1984, Molitch 1986).

Two types of prolactinomas with different symptomatology can be distinguished clinically: microprolactinomas and macroprolactinomas (von Werder 1984). They have different sex distributions, presenting symptoms and natural histories. Microprolactinomas are mainly detected in premenopausal woman and rarely in men (Fig. 1, Chapters III and IV,

Klijn et al 1980a and b, 1980, Nabarro 1982). Macroprolactinomas occur at all ages with a small preponderance in men namely about 60% (Klijn et al 1980, Nabarro 1982). In patients with microprolactinomas most symptoms are caused by hyperprolactinaemia per se (menstrual disorders, infertility, galactorrhoea, hypoestrogenism and sexual disfunction), while in patients with large tumours symptoms caused by local tumour extension (neurological and visual disturbances, hypopituitarism) are more predominant. With respect to the natural history microadenomas have on the average a slower growth rate while macroprolactinomas develop faster and are often invasive (von Werder 1984, Landolt 1984a). The frequency of capillaries in macroadenomas has been found to be decreased, but macroadenomas appear to be more sensitive than microadenomas to dopamine and bromocriptine both in vivo and in vitro (see review of Molitch 1986).

For detailed description of symptomatology I refer to articles of Biller et al (1980), Nabarro (1982) and Molitch (1986). Vetter et al (1974) found galactorrhoea in only 0,9% of nearly 5600 women examined, while in a study of Jones and Gentile (1975) concerning 800 women galactorrhoea occurred in 3,6% and 1% of women without and with ovulations, respectively. In other studies, however, galactorrhoea was observed in up to 46% of the examined women (Molitch 1986). Such variability might be caused by differences in breast examination technique, definition of galactorrhoea and selection of the examined population. The likelihood of detecting a pituitary adenoma in patients with galactorrhoea lies between 15-48% and rises to 34-59% when galactorrhoea is accompanied by amenorrhoea (Biller et al 1980). When amenorrhoea occurs alone, in the average 15% of the patients (range 2-26%) may have hyperprolactinaemia which occurs in even 30% of patients with menstrual dysfunction (Biller et al 1980, Molitch 1986). In patients with amenorrhoea for longer than 6 months detectable pituitary adenomas are found in 9-34% of the cases. When galactorrhoea occurs alone, hyperprolactinaemia has been found in 28% (range 0-60 %) of the patients (Molitch 1986). In combined series totaling 471 patients with both amenorrhoea and galactorrhoea, even 75 % were found to have hyperprolactinaemia (Molitch 1986). In most of these series, radiological evidence of tumour was apparent in about 50 % of the

patients. Absence of galactorrhoea does not exclude a prolactinoma. In patients with a pathological sella and hyperprolactinaemia galactorrhoea occurs in 28-56% of the patients with a higher incidence in young women with microprolactinomas (Chapter III, Klijn et al 1980a, Nabarro 1982) than in men or older women. In 70-90% of female patients with suspected prolactinomas menstrual disorders occur. The likelihood of the presence of a pituitary tumour increases with the duration of amenorrhoea and/or galactorroea (Biller et al 1980). In contrast to what is found in acromegaly (Chapter IV, Klijn et al 1980b) the age at diagnosis of a prolactinoma has no relationship to the duration of symptoms (Nabarro 1982). In our study (Chapter III) the mean duration of the galactorrhoe for the female patients was 4.25 years (for the male patients 6.5 years) and of the menstrual disorders 8 years. Headache is reported in (more than) half of patients with (micro)prolactinomas. In 469 women with secondary amenorrhoea and/or galactorrhoea Strebel et al (1986) found that headaches were four times more frequent in the presence of an adenoma than in its absence, suggesting that the space-occupying mass effect of a prolactinoma is responsible for headache. Weight increase (up to 40% of the patients) and hirsutism are observed in a minority of the patients.

In men with prolactinomas decreased libido and impotence are the most frequent symptoms besides local signs of tumour growth; galactorrhoea is found in 5-30 % (Chapter III, Klijn et al 1980a, Muhr et al 1985, Hulting et al 1985a, Weber et al 1985) in contrast to about 75% in female patients. Hyperprolactinaemia seems to be a minor cause of reproductive dysfunctions in men. The incidence of hyperprolactinaemia among 598 infertile men in our clinic was 2% (Weber et al 1985).

1.b. Acromegaly

Acromegaly is in nearly all cases caused by a GH secreting pituitary tumour. Only a few cases are reported with ectopic GRF secretion (Thorner et al 1982, Asa et al 1984, Spero and White 1985). The annual incidence is three cases per million in the Newcastle region in England and the prevalence of diagnosed cases up to 40 per

million (Alexander et al 1980). These numbers are comparable with the estimated incidence in our own region of the South-West part of the Netherlands. Davidoff (1926) found a family history in 4 of 100 cases (1 sister, 1 father, 1 uncle, 1 aunt). In our series of 117 patients acromegaly affecting a near relative occurred in 2 families (father and son; brother and sister).

The symptoms of acromegaly may be divided in those which result from local effects of the pituitary tumour itself (destruction and displacement of surrounding tissues) and those that results of excessive GH secretion. The clinical effects of excessive GH are numerous and are dominating the clinical picture. In fact, all systems and organs are more or less involved in the disease causing a broad spectrum of symptomatology as indicated in Table 1 of Chapter IV.

The clinical features and mode of presentations in our patients are generally in agreement with those in the literature. More detailed data of the clinical symptoms and pathology have been described in some reviews and more specific reports (Davidoff 1926, Cushing and Davidoff 1927, Atkinson 1932, Davis 1941, Kellgren et al 1952, Mastaglia et al 1970, Hirsch et al 1969, van der Zwan 1971, Daughaday 1974, Wass et al 1982, Quabbe 1984, Dinn and Dinn 1985); for epidemiology and mortality studies see the reports of Alexander et al (1980) and Wright et al (1970).

Our findings (Chapter IV) showed a few conspicuous differences with the early description by Davidoff (1926). In his series of 100 patients 62% of the patients had visual disturbances in contrast to 14% in our experience. Also headache was more frequent (87 vs 52%). This might be caused by an earlier detection at the present time or selection by differences in referral pattern to a surgical clinic on the one hand and to an endocrine clinic on the other hand. The mean delay between start of symptoms and time of diagnosis was 13 years in Davidoff's series and 6.5 years in our series. On the other hand the mean age at diagnosis appeared the same in both series (40 years). In addition, van der Zwan (1971) reported a mean delay of 8.4 years in a group of 89 acromegalic patients with roughly the same age distribution as in our series. The average age at diagnosis in Davidoff's series was lower in men (33 years) than in women (45 years)

in contrast to our findings. While in our material there is no selection in the direction of visual disturbance as a presenting symptom, the fact that the delay in diagnosis in men is twice as long as in women may be reconciled with Davidoff's data: in our material the men probably developed symptoms of acromegaly at an earlier age than the women.

Furthermore we observed arthropathy and back pain in 30 and 20% of the patients respectively while Davidoff and Cushing did not mention joint disease in their review (1926). A few years later Atkinson (1932) in his review of 1.319 cases reported the occurrence of joint pain in half of the patients. In our series a remarkable high frequency of inguinal hernia has been observed in the male patients (30%). This may be caused by chronic pulmonary disease and/or an inferior quality of soft tissue and/or a wide inguinal canal.

Most striking in our series is the negative relationship between tumour size and age on the one hand and a relatively short period intervening between onset of symptoms and the time of diagnosis on the other hand in the younger patients (Chapter IV). We concluded that in general the growth rate in the tumours which develop earlier in life is greater than in the others. Therefore these patients need to be treated rather agressively. Another reason for aiming at reaching normal GH levels is the relatively high contribution of cardiovascular, pulmonary, and malignant disease to the overall mortality, which is twice as high as expected in a normal population (Wright et al 1970; Chapter VIII).

2. Diagnosis of Pituitary Gland Tumours

2.a. Radiological and Ophthalmological Evaluation

The sella turcica can be pathological by abnormalities in form, size and bone density. Abnormal calcifications in and around the sella may indicate pathology, too. A diagnostic problem is the observation of a rather great variation in the configuration and size of the normal sella turcica (Di Chiro 1960). On the one hand small

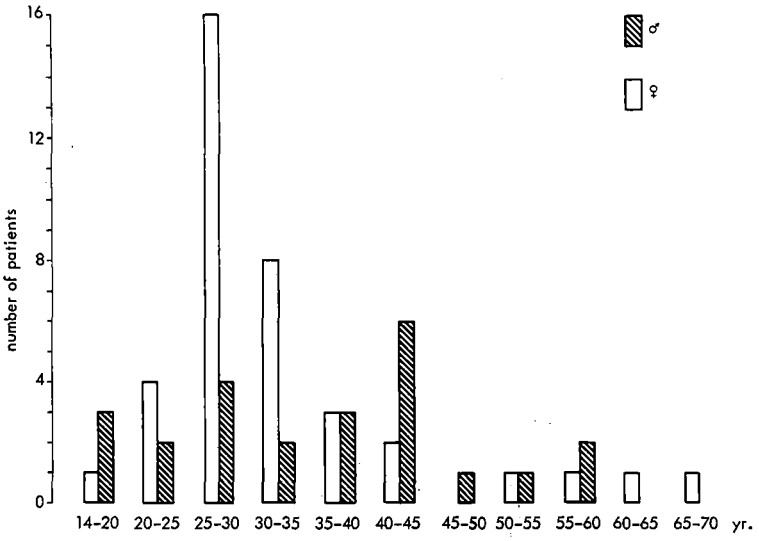

Fig. 1 Distribution of 62 patients with hyperprolactinaemia and a pituitary tumour according to age groups.

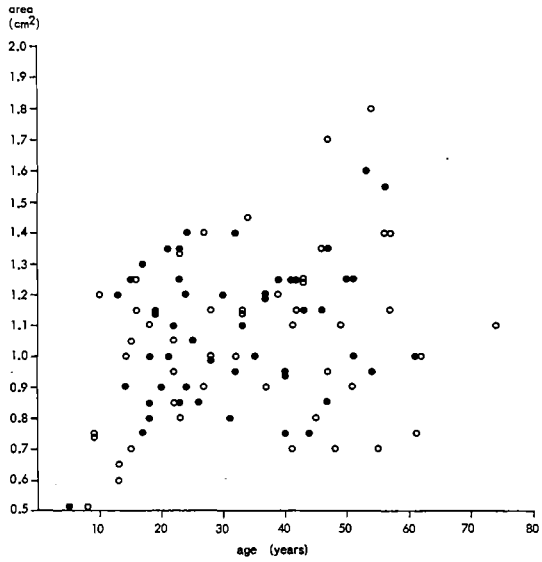

Fig. 2 The relationship of age and lateral area of the sella turcica in 100 consecutive neurological patients without evidence of pituitary pathology (n.s.; ● = ♀, o = ♂).

differences or alterations in relation to the "average" sella will sometimes not be recognized as pathological or wrongly labeled as pathological. On the other hand a microadenoma of less than 5 mm can be present in the pituitary gland without visible abnormalities of the sella turcica (Vezina and Sutton 1974). Although high-resolution computer tomography can detect such small tumours in some patients, the endocrine evaluation is of great importance in this situation. Microadenomas of 5-10 mm generally cause local alterations or asymmetry (of the front wall, the floor or dorsum) without enlargement of the sella. These local alterations frequently exist of thinning of the lamina dura and local bulging in the sphenoid sinus or deepening into the clivus. Adenomas larger than 1 cm (macroadenomas) always cause sellar enlargement. The form of the sella is dependent on the development of pre-and postsphenoid, planum sphenoidale, clivus and some ligaments.

During the growth the sella turcica increases gradually in size until the age of 18-20 years (Silverman 1957, Underwood et al 1976). Some authors have reported an increase in sellar size during the whole life period (Finby and Kraft 1972). This could be caused by a higher incidence of hyperplasia and "physiological" formation of adenomas at higher age. Costello (1936) and Mosca (1975) observed one or more microadenomas in respectively 22.5 and 20% of routine autopsies, especially above the age of 50 years. These observations are in contrast to other series with a lower incidence (see review Molitch 1986). On the average over 9.637 autopsies in 12 studies the incidence of microadenomas was 10.8 %.

In a study of our own (shortly referred to in chapter III) 100 consecutive neurological patients have been reviewed with respect to form and size of the sella turcica. We did not find a relationship between age and sellar size (Fig. 2). However, in older age groups the variation in size appeared to be greater than in the younger ones. In this control group of 100 patients without evidence of pituitary pathology length, height and width of the sella varied between 10-15 mm, 7-12 mm and 11-20 mm respectively. Camp (1923) found maximal values of 16 and 12 mm for length and height respectively. The 95% range of the normal lateral sellar area in our study was between 70

and 140 mm^2. Hare et al (1949) observed a maximal normal sellar area of 130 mm^2. The volume of the sella turcica can be calculated according to the formula (½ x length x height x width) of Di Chiro and Nelson (1962). In 17 out of our 100 "control" patients the sellar floor was not visible on plain frontal radiographs. In the other 83 individuals there was a good correlation ($r= +0.69$, $p < 0.0005$) between the results of our area measurement and those of the volume calculation (Fig. 3). We found a normal range of sellar volume of 454-1305 mm^3. The pituitary occupies about 70-80% of the sellar content. As Fischer and Di Chiro (1964) demonstrated in normal people a small lateral sellar area does not always indicate a small pituitary or sellar volume, but can be accompanied by a large width (and vice versa). This is in agreement with the absence of a correlation between sellar area and width ($n= 83$, $r= -0.179$) in our control group (Fig. 4). Seki (1965) even observed a tendency to a negative correlation between these 2 parameters in 500 normal adults. However, in 36 patients with an enlarged sella and hyperprolactinaemia (Fig. 5, $r= +0.63$, $p < 0.0005$: chapter III) and in 36 acromegalic patients ($r= +0.62$, $p < 0.0005$) we found a significant positive correlation between (log) area and (log) sellar width . Furthermore, we found a stronger correlation between (log) area and (log) volume in the patients with a pituitary tumour (Fig. 6, both groups with $r= +0.96$, $p < 0.0005$) than in the control group (Fig. 3). Therefore, the lateral area of the sella turcica, when necessary complemented with that of extrasellar tumour tissue (Table 3), is a good practical parameter of pituitary tumour size. A sella with a length larger than 15 mm and/or a height larger than 12 mm and/or a width larger than 20 mm, an area larger than 140 mm^2 or a volume larger than 1300 mm^3 has to be considered as pathological. In fact a sellar enlargement is a late symptom because in case of a hypersecreting pituitary tumour the clinical picture has already developed. Other causes of sellar enlargement than pituitary tumours could be extrasellar tumours (craniopharyngeomas, meningiomas, gliomas, neurinomas, chordomas, ependymomas, fibrous dysplasia, chondromas, metastases), granulomas, aneurysms, primary hypothyroidism, sphenoid mucocèle, increased intracranial pressure, etc.

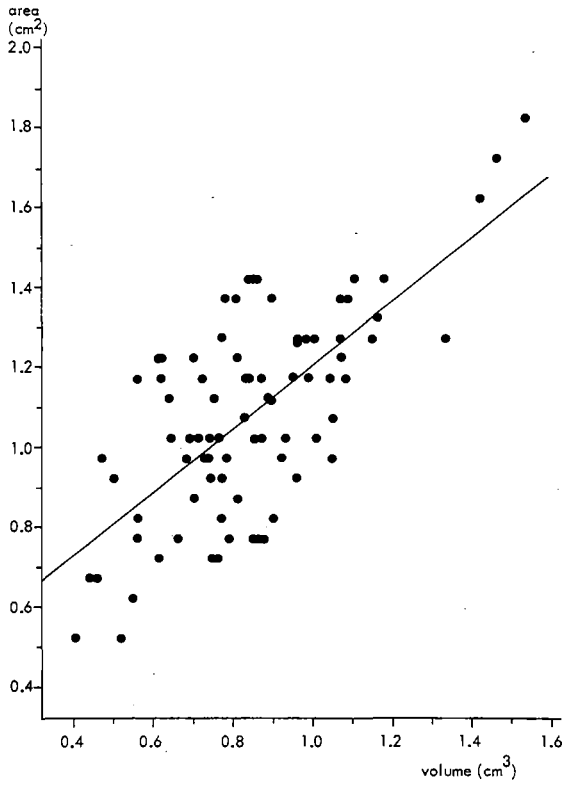

Fig. 3 The relationship between sellar area and volume in 83 neurological patients without evidence of pituitary pathology ($r = 0.69$, $p < 0.0005$).

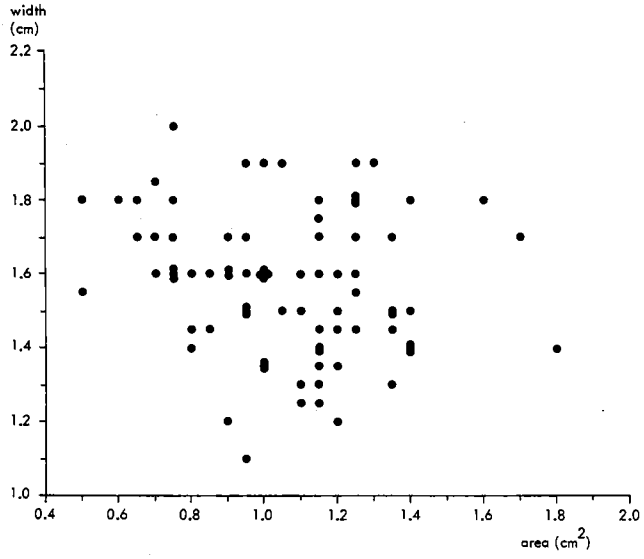

Fig. 4 The relationship between sellar area and sellar width in 83 neurological patients without evidence of pituitary pathology ($r = -0.1794$, n.s.).

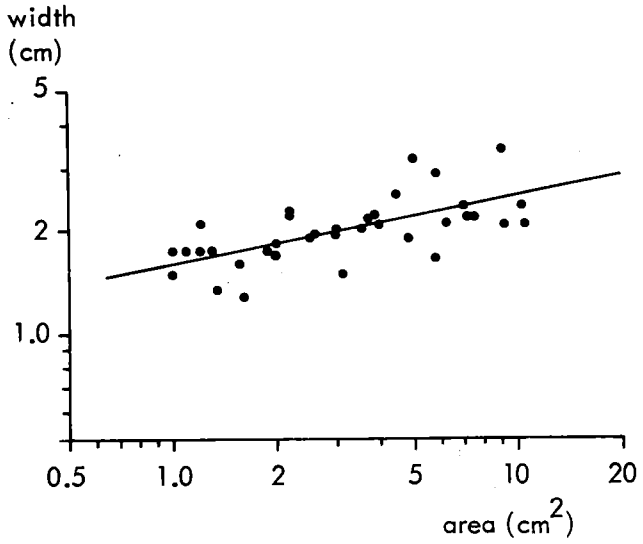

Fig. 5 The relationship between (log) sellar or tumour area and (log) sellar width in 36 patients with a prolactinoma ($r = 0.63$, $p < 0.0005$).

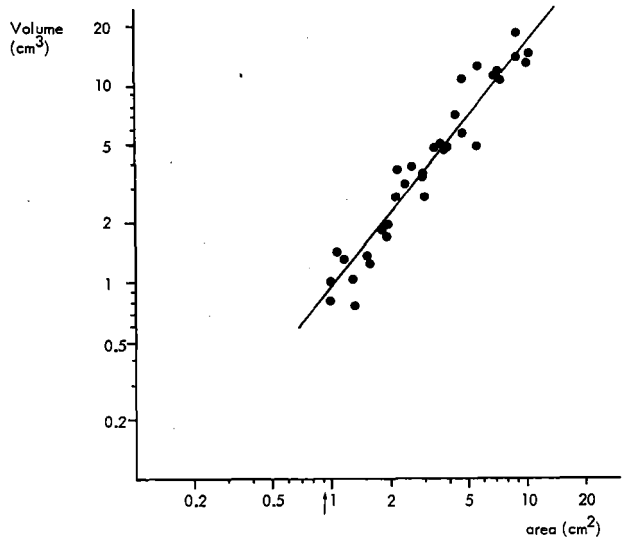

Fig. 6 The relationship between (log) sellar or tumour area and (log) volume in 36 patients with a prolactinoma ($r = 0.96$, $p < 0.0005$).

Table 3. Incidence of normal and pathological configuration of the sella turcica in relation to mean tumour size in 62 patients with hyperprolactinaemia and a pituitary tumour.

Radiological examination

	percentage	$\bar{x}$ tumor size
- Normal sella	6 %	1.1 cm^2
- Asymmetrical sella	19 %	1.2 cm^2
- Enlarged without extra-sellar extension	31 %	2.3 cm^2
- Extrasellar extension	44 %	7.3 cm^2
	100 %	

A sella turcica with a normal size may have an abnormal configuration. An asymmetrical sella frequently, but not always, indicates pathology. In that way Swanson and du Boulay (1975) found a thinning of the lamina dura in 16.5% and an asymmetrical sella in 31.7% of 85 normal indivuals. In our own study we observed an "asymmetrical" sella on the lateral X-ray in 19% of 100 controls, but after detailed investigation this appeared to be explained by overprojection of somewhat oblique X-ray or overprojection of lateral structures. Therefore plain frontal radiographs are necessary for judgment of possible asymmetry of the sellar floor. However, in 17% of our control group the floor was not visible.

2.b. Endocrine Diagnosis

After the introduction of radioimmunoassays for estimation of an increasing number of hormones the possibilities for endocrine evaluation of pituitary function are clearly ameloriated by means of measurement of basal plasma concentrations of different hormones and by the development of stimulation and inhibition tests. These endocrine function tests are focussed on hormonal hypersecretion and pituitary insufficiency.

2.b.1. Basal hormone concentrations and dynamic tests for hypersecretion.

In untreated patients with hyperprolactinaemia and a pituitary tumour we found a positive correlation between basal plasma PRL concentrations and both sellar or tumour area (Chapter III) and volume (Fig. 7A). In patients with acromegaly we also observed a positive correlation between basal plasma GH levels and both tumour area (Chapter IV) and volume (Fig. 7B). These results are confirmed by different other studies (Hardy 1983, Bertrand et al 1983, Fahlbusch et al 1984, Landolt 1984a). However there are only a few reports about the relationship between age, tumour size and basal plasma hormone concentrations (Landolt 1984a). We were the first to show a negative correlation between tumour size and age in patients with acromegaly

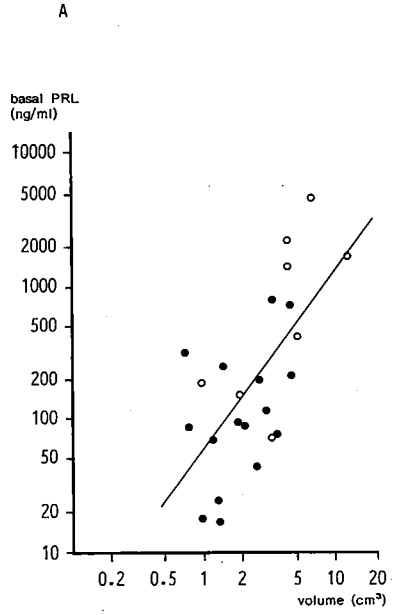

Fig. 7A The relationship between (log) sellar or tumour volume and (log) basal plasma prolactin concentrations in 24 untreated patients with a prolactinoma ($r = 0.66$, $p < 0.0005$; ● = ♀, o = ♂).

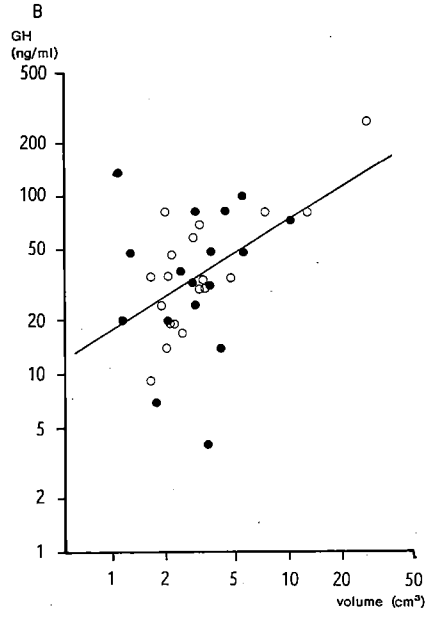

Fig. 7B The relationship between (log) sellar or tumour volume and (log) basal plasma growth hormone concentrations in 36 untreated acromegalic patients ($r = 0.48$, $p < 0.01$; ● = ♀, o = ♂).

(chapter IV). In patients with prolactinomas we did not observe such a relationship (Chapter IV). In patients with prolactinomas Landolt (1984a) found a comparable scatter diagram for age in relation to tumour size as in our study.

Dynamic tests for PRL and GH hypersecretion are described in various reviewing articles (Archer 1977, Christy 1979, Jackson 1980b, Guillemin 1983, Assies 1984, Davis and Hipkin 1984, Ho et al 1985, Faglia et al 1985, Holl et al 1985).

2.b.1.a. Hyperprolactinaemia and prolactinomas

Hyperprolactinaemiae can occur in different physiological and pathological conditions (Thorner 1977, Lamberts et al 1978, Ho et al 1985); for instance associated with prolactinomas, suprasellar tumours, hypothalamo-pituitary non-tumoural disease, various medications, thyroidal dysfunction, pregnancy and lactation, chronic renal failure etc. For differential diagnostic purposes the TRH-test is most commonly used wether or not in combination with other stimulation tests such as chlorpromazine and metoclopramide. In our patients with a prolactinoma, in whom a TRH-test was carried out, we found no relationship between basal PRL level and ΔPRL expressed in nanograms per milliliter, but there appeared a negative correlation between the basal PRL level and Δ PRL as a percentage (chapter V, Klijn et al 1981)). No correlation was found between ΔPRL and tumour size, ΔTSH, T4, T3 or age.

The clinical value of the TRH-test with respect to the possibility to distinguish prolactinomas from other causes of hyperprolactinaemia is still controversial (Chapter V, Klijn et al 1979 and 1981, Barbieri et al 1985). In a survey of the literature concerning 548 patiënts with a prolactinoma (reported in 35 studies) we found a blunted PRL response to TRH (increase < 100%) in 89% of the patients. Later on Barbieri et al found a somewhat higher incidence of an abnormal response to TRH (96%) in 167 patients with the clinical diagnosis prolactinoma, but no significant difference in the incidence in the patients with surgically documented prolactinomas (95% versus 91% in our report). The peak value and the range of the PRL response to TRH

in the control group in Barbieri's study was nearly the same as in our control group. The only difference found concerned the time of the peak value after the TRH injection: in Barbieri's study 49% of the individuals had a maximal PRL response at 10 minutes in contrast to only 7 (24%) of 29 control subjects in our study. The somewhat higher incidence of a blunted PRL response to TRH in prolactinoma patients in Barbieri's study may be explained by a) a difference in referral pattern (selectively to the neurosurgical department in Barbieri's centre in contrast to more varied disciplines in our study), b) lack of uniform guidelines for the diagnosis of prolactinoma, c) differences in sampling times. As we found no difference in effect between 200 and 400 /ug TRH (chapter V) the influence of the use of a different dose seems less important.

In agreement with our study Barbieri et al (1985) reported about 50% occurrence of a blunted PRL response to TRH in patients with hypothalamic or other pituitary disease. Furthermore, they observed blunted responses in half of the patients using dopamine antagonistic drugs and in a substantial proportion of patients with hyperthyroidism, while patients with hypothyroidism showed a normal or exaggerated response. On the basis of the two large studies of Barbieri et al. and of our group and of a number of other studies we conclude that the TRH-test may discriminate well between normal subjects and patients with prolactinoma, but in the differential diagnosis of hyperprolactinaemia it is of less value. Furthermore in patients with basal PRL levels of more than 500 /ug/l a TRH test is not strictly necessary because such high values are only observed in patients with prolactinomas and in a few patients with acromegaly.

Prolactinomas show a wide spectrum of insufficient responses to inhibition with dopamine. On the average twice as much dopamine appears to be necessary to decrease PRL secretion in hyperprolactinaemic states in comparison to control subjects (Bansal et al 1981, Assies 1984). The observed hyposensitivity may be the result of decreased dopamine binding to prolactinoma tissue and/or defective biological events after binding of dopamine to the cell membrane (Macleod et al 1984, Faglia et al 1985). Such biochemical defects in the pituitary may be a cause of prolactinoma development.

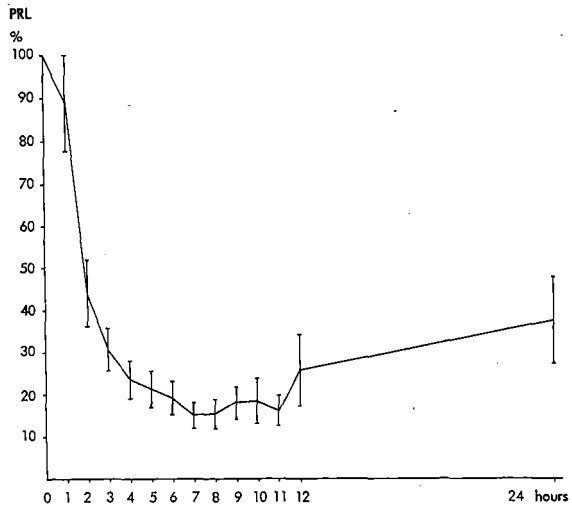

Fig. 8 The effect of the administration of one dose of 2.5 mg bromocriptine on percentual decrease of plasma prolactin concentrations in 14 patients with a prolactinoma (values in mean ± SEM).

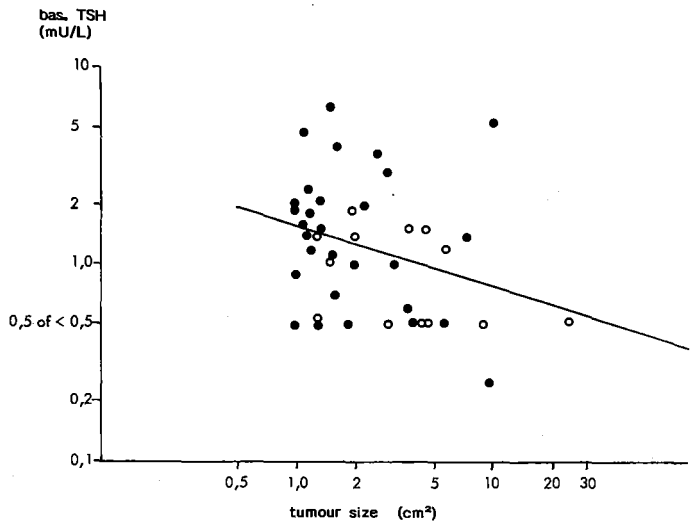

Fig. 9 The relationship between (log) tumour size (cm^2) and (log) basal plasma concentrations of TSH in 42 patients with hyperprolactinaemia and a pituitary tumour ($r = 0.32$, $p < 0.025$; ● = ♀, o = ♂).

In most of the applied dynamic tests drugs are used which interfere with dopamine synthesis, release, metabolism, reuptake or receptor binding (Assies 1984). For suppression tests various direct and indirect dopamine agonists are used. In our experience one dose of bromocriptine caused a mean maximal decrease to 15% of the pretreatment value 7 hours after administration to patients with a prolactinoma (Fig. 8), but a great variability in sensitivity of the lactotroph cell to bromocriptine and to endogenous dopamine has been described (Thorner et al 1980c, Ho et al 1985, Macleod and Lamberts 1986). More recently, the use of nomifensine, an agent which inhibits the re-uptake of dopamine in the central nervous system, appeared also not to be fully discriminative between physiological and pathological hyperprolactinaemic states (Ho et al 1985). Therefore the basal plasma PRL level appeared to be the best predictor of the presence of a prolactinoma, while in patients with only slight or moderate hyperprolactinaemia (<300 µg/l) a subnormal or absent response to either stimulatory or inhibitory agents is indicative for a pathological condition with the possibility of the presence of a microprolactinoma.

2.b.1.b. Acromegaly.

For the assessment of GH secretion a number of test are described (Ho et al 1985). Besides measurement of basal plasma GH levels some physiological (sleep, exercise) and pharmacological stimulation tests (L-dopa, arginine, insulin-induced hypoglycaemia, propranolol, clonidine) are applied, to which recently the GRF-test has been added. Furthermore, plasma GRF measurements may be helpful in diagnosing ectopic GRF producing tumours as a rare cause of acromegaly (Thorner et al 1984). Measurement of somatomedin C (SM-C) may be useful in patients with acromegaly, because of a possibly better correlation of the elevated SM-C levels with the clinical activity of acromegaly than the GH levels generally show (see reviews of Vance et al 1984 and Nortier and Croughs 1985c). However, increased SM-C concentrations are found in some physiological conditions (puberty, pregnancy) and in patients with acromegaloidism.

Since the observation in 1963 of Roth et al that glucose administration caused no or a subnormal suppression of plasma GH in acromegaly the oral glucose tolerance tests (OGTT) is commonly used as an inhibition test in patients with increased basal plasma GH levels. Even a paradoxical rise of plasma GH occurs in 20% of acromegalic patients (Ho et al 1985). However, an abnormal GH response to glucose cannot be taken as diagnostic because abnormal GH responses may occur in adolescents (Pieters et al 1980)and in some pathological conditions (anorexia nervosa, chronic renal failure, liver cirrhosis, Wilson's disease, acute intermittent porphyria, thyrotoxicosis). In acromegalic patients treated with dopamine agonists the interval between the last dose of the drug and the performance of an OGTT may be critical (Nortier and Croughs 1985c).

The most common cause of acromegaly is a GH secreting pituitary tumour. There is still considerable controversy as to whether these tumours arise by hypothalamic dysfunction or primary pituitary abnormalities (Ho et al 1985). The finding of an abnormal GH response to TRH and sometimes LHRH also suggests a pathological function of the tumour somatotrophic cells. However, there is recent in vivo and in vitro evidence that GRF may permit or facilitate GH responsiveness to TRH (Thorner et al 1983, Borges et al 1983). The TRH-test is commonly used in the diagnosis of acromegaly. However, like the GH response to oral glucose loading an abnormal GH response to TRH is not specific for acromegaly in the differential diagnosis of increased plasma GH levels. TRH-induced GH responsiveness may occur in patients with anorexia nervosa, diabetes mellitus, chronic renal failure and in children with tall stature. Therefore, the diagnosis of acromegaly is mainly made on clinical grounds although an abnormal GH response to glucose and TRH as well as elevated SM-C levels are helpfull to establish this diagnosis. On the other hand, in the differential diagnosis of acromegaly, syndromes with acromegaloid features in the presence of normal basal GH levels and function tests have been described (Mims 1978). In some studies increased levels of SM-C (Hoffenberg et al 1977) or other growth-promoting factors in the serum of the patients (Aschraft et al 1983) have been observed. We also described increased biological SM-C activity in four members of a

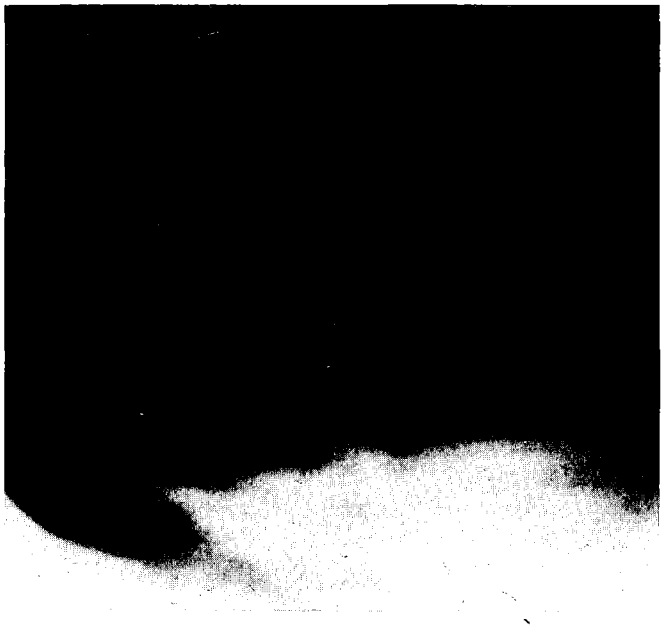

Plate 1 Ductus craniopharyngeus (arrow on cysternoplanigraphy) in a patient with acromegalic features and congenital abnormalities especially in the midline.

family with acromegaloid features, congenital abnormalities and a persistant ductus craniopharyngeus (Plate 1, Klijn et al 1981b).

Although dynamic tests can not specifically distinguish between the causes of elevated GH concentrations, it is our experience (Chapter VII Lamberts et al 1979a, 1982a and 1983) as well as of other groups (Liuzzi et al 1974a, Roelfsema et al 1979) that the GH response to TRH and/or to a single dose of bromocriptine are of prognostic value with respect to the effect of chronic bromocriptine therapy (see section on medical treatment 3.b.3.a.). Also the plasma PRL level and the simultaneous presence of PRL and GH in a GH-secreting pituitary tumour help to predict which patients with acromegaly are likely to respond to bromocriptine with an inhibition of GH secretion (Chapter VII, Lamberts 1979a, 1982a and 1983). Furthermore, the degree of GH suppression during somatostatin infusion may also have predictive value with respect to the results of chronic bromocriptine treatment, but this procedure appears to offer no additional information as compared to that obtained by the acute bromocriptine test (Nortier and Croughs 1985c).

2.b.2. Hormonal insufficiency

Both pituitary tumours with and without suprasellar extension as well as suprasellar tumours can cause insufficiency of the hypothalamo-pituitary-target organ axis. Endocrine test protocols vary among institutions, but they should be comprehensive enough to assess anterior and posterior pituitary lobe function. Measurement of the basal levels of pituitary hormones (LH, FSH, TSH, ACTH, PRL, GH) and of hormones derived from the target organs (testosterone, oestradiol, T4, T3, cortisol) offers often an insufficient reflection of the pituitary function. Therefore basal hormonal measurements are commonly combined with stimulation tests especially those directed at measurement of pituitary hormone reserve. The endocrine investigations and assays, used in our department, are described in chapter III. The most common tests for measurement of the dynamics of TSH, LH/FSH and ACTH are the TRH-, LHRH- and metyrapone-tests, respectively.

2.b.2.a. The pituitary-thyroidal axis.

In patients with hyperprolactinaemia and a pituitary tumour (Klijn et al 1980a) we found a negative correlation between pituitary tumour size and basal TSH (Fig. 9), the response of TSH to TRH (Δ TSH) and circulating T4 and T3 levels (Chapter III and VI, Table 4). These correlations were not present in the acromegalic patients (Klijn et al 1980c). The high incidence of an impaired TSH response (without hypothyroidism and independent of tumour size) is probably caused by suppression of TSH secretion rather than by destruction of thyrotrophic cells. This suppression of TSH secretion may be caused by an autonomous thyroid function in the presence of a more or less enlarged thyroid or by increased hypothalamic somatostatin secretion or both.

Patients with hypothyroidism due to hypothalamo-pituitary disease sometimes have slightly elevated TSH levels (Lamberton and Jackson 1983). In some patients with suprasellar tumour mass the TSH is immuno-reactive but not fully bioactive (Faglia et al 1979), while in others there may be an altered "set-point" of TSH secretion possibly resulting from deficient hypothalamic somatostatin secretion (Jackson 1980a). Increased plasma TSH concentration in the presence of hyperthyroidism can occur in patients with TSH (and TSH + GH) secreting pituitary tumours, which are very rare (Jackson 1980a, Meinders et al 1981).

Some authors (Faglia et al 1973) concluded that the TRH test could indeed be used in the differential diagnosis of secondary (subnormal TSH response) and tertiary hypothyroidism (exaggerated, delayed TSH rise). Other authors, however, observed a considerable overlap in the TSH response to TRH in patients with pituitary or hypothalamic hypothyroidism (Patel and Burger 1973, Snyder et al 1974, Jackson 1982, Lamberton and Jackson 1983). Furthermore, it has to be noted that TSH-secretion can be more or less influenced by spontaneous variation, sex, age, pregnancy or menstrual cycle, drugs, other endocrine diseases, systemic illnesses, anorexia nervosa, renal failure etc. These influences on TSH secretion are not always of clinical importance. In practice, the therapeutic decisions are made on the results of direct thyroid hormone measurements.

Table 4. Summary of radiological and endocrine data in 44 patients with acromegaly and 62 patients with a prolactinoma.

	Acromegaly	Prolactinoma
patients	21 ♀, 23 ♂	38 ♀, 24 ♂
mean age	♀ = 39 yr, ♂ = 40 yr	♀ = 32 yr, ♂ = 35 yr
mean tumor size	♀ = 3.50, ♂ = 3.76 cm^2	♀ = 2.88, ♂ = 6.08 cm^2
rad. extrasellar extension	32 %	44 %
rad. suprasellar extension	23 %	35 %
visual field defect	14 %	35 %
rad. extrasel. extension (tumorsize < 3 cm^2)	0 %	3 %
" " " (" > 3 cm^2)	64 %	92 %
" " " (" > 4 cm^2)	92 %	95 %
tumor size: basal GH or PRL level	$p < 0.0005$	$p < 0.0005$
tumor size: age	$p < 0.005$ (neg.)	n.s.
ΔTSH vs. control group (or < 5 mU/l)	56 % (48 %)	44 % (22 %)
hypothyroidism	2 %	25 %
log tumor size: log ΔTSH, T_4, T_3	n.s.	$p < 0.01$, $p < 0.005$, $p < 0.005$
log tumor size: log ΔFSH, log ΔLH	n.s.	$p < 0.005$ (both)
ΔFSH: ΔLH	n.s.	$p < 0.0005$
increased basal LH in men	14 % (n = 14)	7 % (n = 14)
" " FSH " "	64 % (n = 14)	7 % (n = 14)
mean testosterone level in men	0.32 µg %	0.34 µg %
impaired metyrapone test	11 %	17 %

2.b.2.b. The pituitary-gonadal axis

In patients with hyperprolactinaemia and a pituitary tumour we found a negative correlation between pituitary tumour size and gonadotrophin secretion under basal conditions (Fig. 10A and 10B for LH and FSH respectively) and after LHRH (Fig. 11 for LH, see Chapter III for Δ FSH). In female patients with microprolactinomas hyperresponses of FSH and LH to LHRH (in 50 and 60% of the patients, respectively) were frequently observed in contrast to the findings in patients with macroprolactinomas, in whom an insufficient response occurred in (more than) half of the patients (Klijn et al 1980a). Muhr et al (1985) found also elevated LH levels in 5 of 37 male patients with a prolactinoma and (sub)normal plasma testosterone concentration (4 men with microadenomas and one patient with a macroadenoma). Weber et al (1985) observed hyperresponses in a group of 13 men with microprolactinomas compared to normal controls.

In our acromegalic patients (Chapter IV) a decreased gonadotrophin secretion was present in 12 out of 42 (29%) investigated patients in contrast to about 50% of the prolactinoma patients with the same range of tumour size (Chapter III). All these 12 patients had macroadenomas with tumour sizes of more than 2.5 cm^2. On the other hand it was striking that a significant number of the acromegalics had increased basal FSH levels (Fig. 12B, Table 4 and 5) in the presence of normal LH and testosterone concentrations and normally sized (even sometimes large) testes. Lindholm et al (1977) have also observed a discrepancy between LH and FSH secretion. Elevated FSH with low or normal LH levels have also been described in patients with a gonadotrophin subunits producing tumour (Lamberton and Jackson 1983, Borges et al 1984). After exclusion of postmenopausal acromegalic women we did not observe a significant correlation between tumour size and the gonadotrophin response to LHRH (Fig. 12A).

Decreased plasma sex steroid concentrations in the presence of low gonadotrophin levels indicate hypothalamo-pituitary insufficiency. Failure to respond to a single dose of LHRH does not necessarily differentiate between hypothalamic and pituitary dysfunction since prolonged LHRH deficiency may impair pituitary responsiveness to

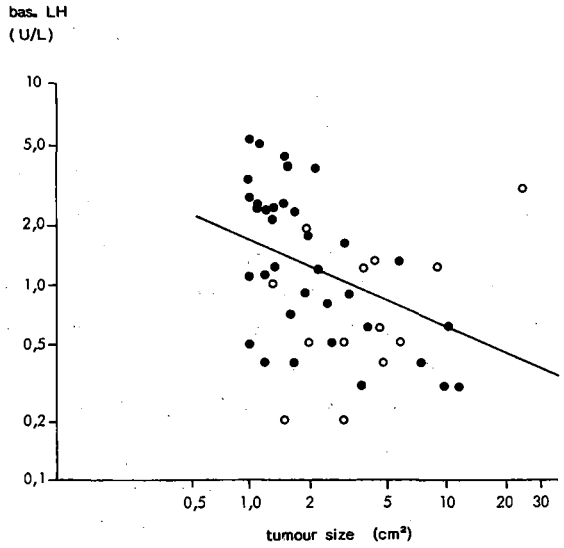

Fig. 10A The relationship between (log) tumour size (cm^2) and (log) basal plasma concentrations of LH in 48 patients with hyperprolactinaemia and a pituitary tumour (r = 0.38, $p < 0.005$; ● =♀ , o =♂).

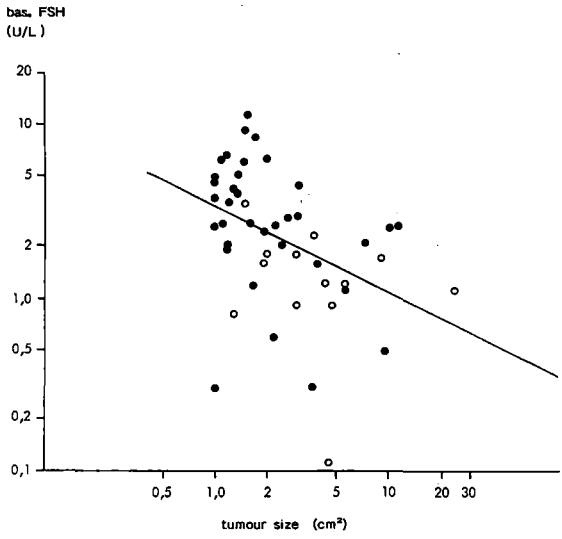

Fig. 10B The relationship between (log) tumour size (cm^2) and (log) basal plasma concentrations of FSH in 48 patients with hyperprolactinaemia and a pituitary tumour ((r = 0.41, $p <$ 0.005; ● = ♀, o = ♂).

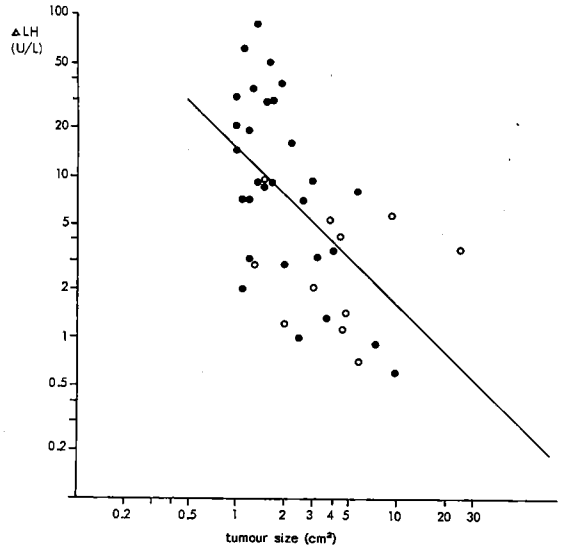

Fig. 11 The relationship between (log) tumour size (cm^2) and (log) the response of LH to LHRH (Δ LH) in 40 patients with hyperprolactinaemia and a pituitary tumour ($p < 0.0005$; ● = ♀ , o = ♂).

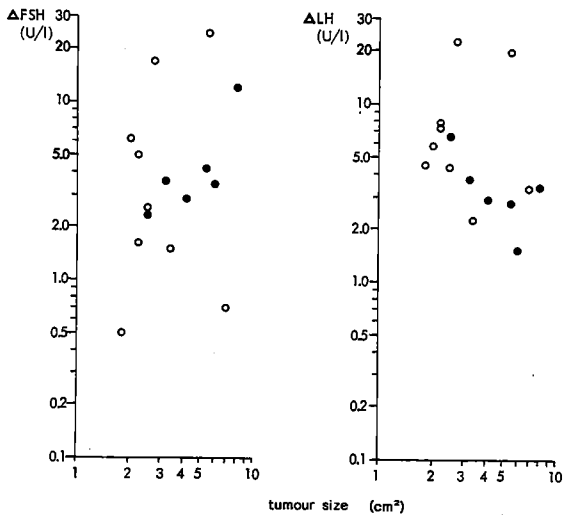

Fig. 12A The relationship between (log) tumour size (cm^2) and the LH and FSH response to LHRH in 6 acromegalic premenopausal women (●) and 9 acromegalic men (o) ($r = 0.26$ for Δ FSH and -0.35 for Δ LH, both n.s.).

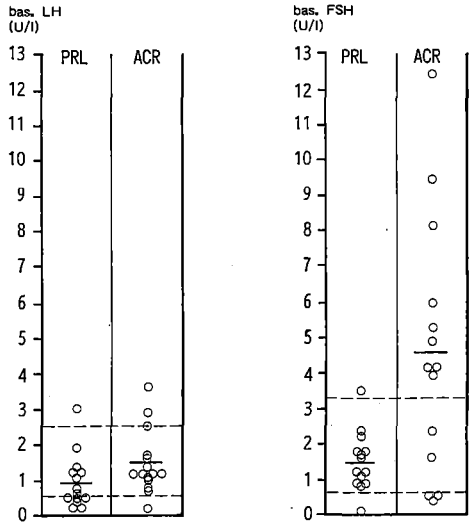

Fig. 12B Comparison of basal plasma LH and FSH levels in 14 male patients with prolactinomas (PRL) and 14-15 male patients with acromegaly (ACR). Increased basal FSH levels in 64% (9/14) of the acromegalic and in 7% (1/14) of the prolactinoma patients ($p < 0.01$).

Table 5. Effects of surgery and radiotherapy on various endocrine parameters in patients with acromegaly and in patients with prolactinomas or a non-functioning tumour.

SUMMARY OF RESULTS

Function	ACROMEGALY						PROLACTINOMA+NON-FUNCTIONING TUMOURS					
	before → after surgery			before → after p.o. rad.ther.			before → after surgery			before → after p.o. rad.ther.		
	n	mean value	p	n	X	p	n	X	p	n	X	p
basal TSH	30	1.8 → 1.8	n.s.	14		n.s.	21		n.s.	17		n.s.
ΔTSH (mU/l)	30	7.7 → 4.3	p<0.002	14	6.2 → 7.4	n.s.	21	10.4 → 6.1	p=0.02	17	8.1 → 12.4	p<0.02
T4 (µg%)	39	8.7 → 9.1	0.2>p>0.1	34			38	7.2 → 7.2	n.s.	29	8.2 → 6.8	p<0.01
basal LH	30	4.1 → 3.5	n.s.				23		n.s.	17		
basal FSH	29	10.6 → 9.8	n.s.				22		n.s.	17		
ΔLH (U/l)	29	10.6 → 7.6	p<0.01	9	6 ↓		23	6.0 → 3.4	p<0.01	17	3.1 → 3.1	n.s.
ΔFSH (U/l)	28	9.1 → 6.8	p<0.01	7	4 ↓		22	4.6 → 3.0	p<0.01	17	2.7 → 3.2	n.s.
"S" (µg%) after metyrapone	31	17 → 18	n.s.	18	20.8 → 12.6	p<0.001	23	16.0 → 16.1	n.s.	24	17.6 → 12.7	p=0.05

exogenous LHRH. Also the clomiphene test cannot differentiate between these two possibilities (Lamberton and Jackson 1983). Furthermore, it has to be noted that some drugs (antiphlogistic agents as indomethacin) and some systemic disorders (malnutrition, Cushing's syndrome, hypothyroidism, diabetes mellitus, renal failure, etc) may influence gonadotrophin secretion.

2.b.2.c. The pituitary-adrenal axis

A low plasma cortisol concentration (< 180 nmol/l) between 8-9 a.m. and a low 24-hour urinary 17-hydroxycorticosteroid and 17-ketosteroid excretion suggest adrenocortical failure (Lamberton and Jackson 1983). On the other hand, a normal basal a.m. cortisol value does not exclude ACTH deficiency. Furthermore, women taking oral contraceptives containing oestrogen may have spurious high plasma cortisol levels due to elevation of cortisol binding globulin (transcortin). To prove pituitary corticotrophic insufficiency stimulation tests are necessary. A rapid ACTH stimulation test is of restricted value in the diagnosis of pituitary-adrenal insufficiency because only prolonged ACTH deficiency is associated with adrenal atrophy. A metyrapone test is more commonly used and of greater value. In addition, pituitary ACTH reserve can directly be measured by determination of plasma ACTH or cortisol levels after an injection of lysine-vasopressin. However, at present the synthetic 41-peptide corticotrophin-releasing hormone (CRF) will doubtless become more widely used in the wake of TRH and LHRH administration (Hermus et al 1985). An insulin hypoglycaemia (I.T.T.) may be particularly sensitive in demonstrating ACTH deficiency when this deficiency is recent in onset (Lamberton and Jackson 1983), but on the whole discrepancies with the outcome of other tests are not uncommon as also in our experience (Table 6).

In nearly all our patients we have used the metyrapone test as the most practical and reliable one. As for pituitary thyrotrophic and gonadotrophic function we observed a negative correlation between pituitary tumour size and corticotrophic function (chapter III and IV, Fig. 13). Again the value of the index of tumour size (3 cm^2) proved to be critical, while the incidence of hormonal insufficiency appeared

Table 6. Comparison of four pituitary-adrenal function tests in 17 patients with a prolactinoma or a non-functioning tumour.

C.D.R. = cortisol dayrhithm; L.V.P.= lysin-vasopressin test.
+ = normal test; - = disturbed function or decreased cortisol response (<7 /ug%); n.d. = not done
( ) = insufficient hypoglycaemia

Patient	CDR	Metyrapone-t	I.T.T.	L.V.P.
1	±	-	-	-
2	+	-	n.d.	-
3	+	+	(-)	n.d.
4	+	-	+	+
5	n.d.	+	+	n.d.
6	n.d.	+	-	-
7	-	+	-	n.d.
8	+	+	+	n.d.
9	+	+	-	+
10	+	+	+	+
11	+	+	n.d.	n.d.
12	+	-	-	-
13	n.d.	+	+	n.d.
14	n.d.	+	+	-
15	n.d.	+	±	n.d.
16	n.d.	+	(-)	n.d.
17	n.d.	+	n.d.	+

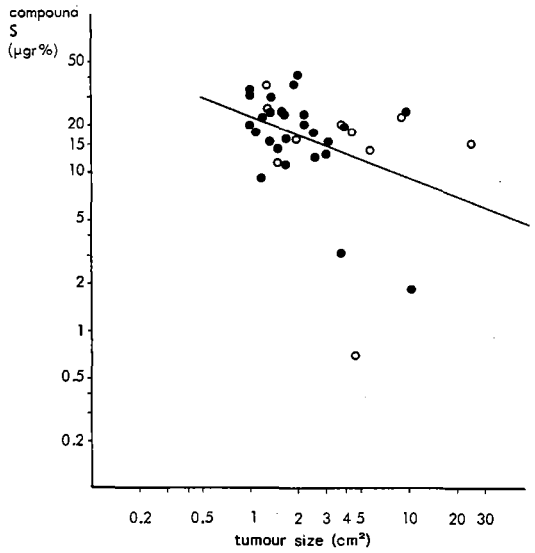

Fig. 13 The relationship between (log) tumour size and plasma desoxycortisol (compound S) concentrations after metyrapone in 36 patients with hyperprolactinaemia and a pituitary tumour ($p < 0.025$; ● = ♀, o = ♂).

to be much lower in acromegalic than in prolactinoma patients. A disturbed pituitary-adrenal axis occurred only in one patient with a prolactinoma and in one patient with acromegaly with a tumour size less than 3 cm^2 (Klijn et al 1980a and 1980b). In patients with macroadenomas ($\geq$ 1.5 cm^2) an impaired metyrapone test was found in 24% of the prolactinoma patients versus 11% of acromegalic patients, while the highest incidence (44%) occurred in patients with large macroprolactinomas ($\geq$ 3 cm^2).

2.b.2.d. Growth hormone deficiency

The tests commonly used to evaluate GH reserve are the insulin hypoglycaemia, L-dopa- and L-arginine test. Recently, a human pancreatic GH releasing factor (hpGHRF, a 40 aminoacid peptide isolated by Rivier et al 1982) appeared to be useful in the evaluation of pituitary GH reserve (Thorner et al 1983) in analogy with to TRH, LHRH and CRF. The I.T.T. is still considered the standard by which all others are judged, but may carry risk for patients who have low adrenal reserve, heart disease and epilepsy. Some investigators suggest pretreatment with oestrogen to enhance the response to the stimulus used, but this is no common practice.

In our patients with pituitary tumours we have not systematically looked for GH deficiency because of the lack of clinical relevance in adult patients. GH deficiency may predispose the patients to hypoglycaemia by increased sensitivity to insulin. Furthermore, it may also contribute to a loss of anabolic action as manifested by skin and muscle changes and to the development of osteoporosis in patients with hypopituitarism (Belchetz 1984b). Impaired GH secretion regularly occurs as an early phenomenon (Landon et al 1966) and is described as the most frequently disturbed pituitary anterior lobe function in patients with non GH-secreting pituitary tumours (Lamberton and Jackson 1983, Luger 1985).

2.b.2.e. Prolactin deficiency

Basal PRL levels of the deficient patient with a "non-functioning" pituitary tumour overlap with the lower range of normal necessitating

the use of stimulation tests to determine PRL reserve. TRH or a dopamine blocking agent, e.g. chlorpromazine, are used for this purpose. Low baseline levels of PRL with failure to respond to one or more stimulatory agents is good evidence for deficiency of PRL secretion by the lactotrophs of the anterior pituitary gland. In humans, however, the clinical relevance of PRL deficiency has not been proven. We did not investigate this aspect of pituitary disease.

2.b.2.f. Vasopressin deficiency

Diabetes insipidus is more common in patients with suprasellar tumours than in patients with intrasellar pituitary tumours (and after hypophysectomy). It is sometimes masked by adrenal insufficiency and became then manifest after cortison substitution.

In conclusion pituitary function testing is important to confirm clinically suspected insufficiency and to detect early dysfunction without clinical symptoms. In patients with microadenomas pituitary hormone deficiencies are uncommon (Chapter III and IV; Valenta et al 1982), but pituitary function testing may be useful in such patients to provide baseline information for future care. In patients with macroadenomas GH and gonadotrophin secretion are most commonly affected, followed by TSH and ACTH secretion. Pituitary insufficiency occurs less frequently in acromegalic patients than in patients with prolactinomas or non-functioning tumours.

Investigators have to keep in mind that hypersecretion of hormones as PRL and GH can influence target organ function without destruction of trophic cells. For instance hyperprolactinaemia can lead to hypogonadism by partial interference with gonadotrophin actions on their gonadal receptors and by disturbance of pulsatile LH release (Bouchard et al 1985) while on the other hand macroprolactinomas can cause diminished gonadotrophin reserve. Another example is the described suppression of TSH secretion by GH excess mimicking destruction of thyrotrophic cells (Chapter VI).

3. Treatment of Pituitary Tumours

3.a. Introduction

Pituitary tumours may manifest themselves by 1) symptoms caused by extension of tumour growth locally and pressure upon the adjacent structures (mostly the optic chiasm) 2) symptoms caused by hypersecretion of various hormones (PRL, GH, ACTH) 3) symptoms caused by loss of normal pituitary functions.

Trying to combat these symptoms and complaints the treatment of pituitary tumours aims at 1)reducing pituitary tumour size 2) decreasing or normalizing hormonal hypersecretion 3) preserving normal pituitary functions 4) preventing tumour recurrence and 5) improving survival in the absence of serious side effects. Three main types of therapy are available: surgical, radiotherapeutical and medical treatment (Chapter I and VIII). We have studied retrospectively these therapeutical effects in (subgroups of) 336 patients with a pituitary tumour treated since 1950 in the University Hospital "Dijkzigt" and the Rotterdam Radio-Therapeutic Institute. For patients, methods and materials I would like to refer to section B.

3.b. Acromegaly

3.b.1. Surgical Treatment of Acromegaly

3.b.1.a. Transsphenoidal surgery

During the past two decades the transsphenoidal approach to the sella turcica has supplanted intracranial exploration for pituitary adenomas with only few exceptions in the case of brain invasion by the tumour. Even in the presence of supra- or parasellar extension of the pituitary adenoma with visual impairment, the transfrontal surgical approach has been generally replaced by the transsphenoidal one in view of the excellent results of decompression of the surrounding structures and the low morbidity of this operation.

3.b.1.a.1. Effects on clinical symptoms

Together with the effects of irradiation these will be discussed in section 3.b.2.a.

3.b.1.a.2. Effects on plasma basal and TRH stimulated growth hormone concentrations

The effect of transsphenoidal surgery on basal GH levels in our series is described in chapter VIII. The mean immediate decrease in plasma GH was found to be about 50%. The results of transsphenoidal surgery appeared to be dependent on preoperative GH levels. Patients with preoperative GH levels of higher than 31 ng/ml never reached "cure" by operation only. Some series showed better results than in our study with up to 60-90% of patients showing normalization of basal GH level within the first few months after surgery (Laws et al 1979a, Pearson et al 1981, Lüdecke et al 1984, Giovanelli et al 1984,Quabbe 1984, Serri et al 1985). This may be caused by the fact that these series includes more patients with microadenomas, in which treatment group the best results are achieved (Laws et al 1979, Serri et al 1985). However normal GH secretion is rare even after microsurgical normalization of plasma GH levels in acromegaly (Lamberts et al 1979e, Hulting et al 1982) and it does not guarantee complete removal of all tumour tissue. This can be shown by the persistence of a paradoxical increase of GH levels in response to TRH (Chapter VIII, Faglia et al 1978, Schaison et al 1983, Lüdecke et al 1984). In our experience none of the patients with a positive GH response to TRH before operation and a normal basal GH level after operation (< 5 ng/ml) showed absence of a response to TRH after operation. In general, the percentual GH response was similar before and immediately after operation (Fig. 14) Some authors report annihilation of the GH response to TRH after operation in a few patients, but a positive response may reoccur (Serri et al 1985). This may be explained by the observation that invasion of the dura mater was present in some 50 percent of the cases (Wrightson 1978, Ludecke 1984). On the basis of these findings it can be concluded that postoperative external pituitary irradiation (Chapter VIII) is probably indicated in most or all cases in order to prevent regrowth of the tumour.

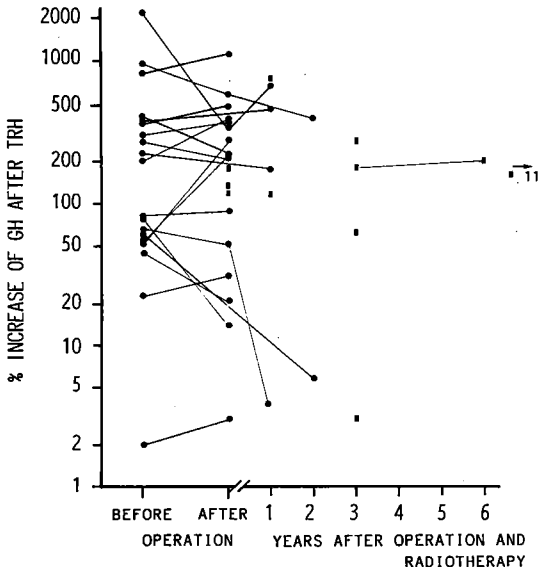

Fig. 14 The percentual growth hormone response to TRH before and immediately after operation. For a few patients only one measurement immediately after operation or later on was available (▮).

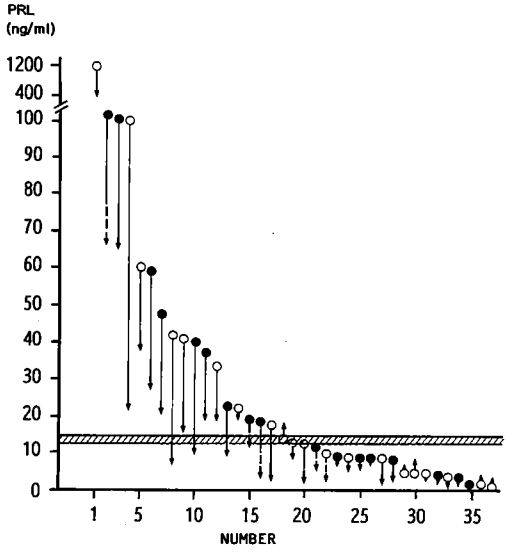

Fig. 15 The effect of transsphenoidal surgery on plasma prolactin concentrations in 37 acromegalic patients (——→; ● = ♀, o = ♂). In 6 patients plasma prolactin was measured 0.5-1 year after postoperative irradiation (----→). The shaded zone separates the upper limits for normal women and men respectively.

3.b.1.a.3. Effect on basal prolactin concentrations

In 37 out of 45 patients with acromegaly basal plasma PRL level was measured before transsphenoidal operation. In 20 of them (54%) an increased value was found (in women > 15 ng/ml, in men > 12 ng/ml). In 31 of these 37 patients plasma PRL levels were again measured in the postoperative phase, in 3 within 0.5 year after postoperative radiotherapy and in 3 other patients within one year after radiotherapy. In most patients we observed a significant decrease of the plasma PRL concentration after transsphenoidal surgery (Fig. 15). In 8 of the 20 patients (40%) with increased plasma PRL before surgery the PRL level became normal after operation. In 2 men, one with a slightly increased basal PRL of 22 ng/ml in the presence of a very large suprasellar tumor mass, no significant change in plasma PRL was found after transsphenoidal operation. In the 20 patients with increased preoperative PRL levels the mean percentual decrease by surgery was 53 $\pm$ 6.0% ($x \pm$ SEM). In 13 out of the 17 patients with normal preoperative plasma PRL levels also a "decrease" in basal PRL was found. However the mean decrease in the total group of 17 patients was only 20 $\pm$ 8.3% (i.e. to 80% of the preoperative value).

In general, there was no correlation between the percentual decrease of plasma PRL and of plasma GH by transsphenoidal surgery neither in the hyperprolactinaemic (Fig. 16A) nor in the normoprolactinaemic group (Fig. 16B) of acromegalic patients. However it was conspicuous that in 9 of the 10 patients with the highest basal PRL level ($\geq$ 40 ng/ml) the percentual decrease of plasma PRL by operation was higher than that of plasma GH (to 38 and to 70% of the preoperative value respectively). In contrast to that finding the mean percentual decrease of basal GH in the whole group of 20 hyperprolactinaemic patients was equal to that of the group of 17 normoprolactinaemic patients, namely to 54.7 $\pm$ 7.3% and to 51.3 $\pm$ 6.7% of the preoperative values respectively.

In 6 out of the 45 patients treated by transsphenoidal surgery plasma PRL was measured for the first time some years after postoperative irradiation. In only one patient plasma PRL (42.7 ng/ml) appeared to be elevated (4 years after irradiation). In the remaining two other patients plasma PRL has not been measured.

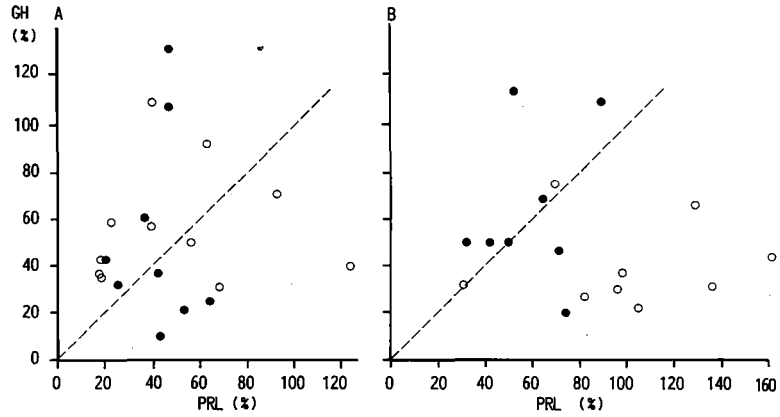

Fig. 16 The percentual decrease (100% = preoperative value) of plasma growth hormone and prolactin in 20 hyperprolactinaemic (A) and 17 normoprolactinaemic (B) acromegalic patients after transsphenoidal surgery. The 45° angle is indicated (● = ♀, o = ♂).

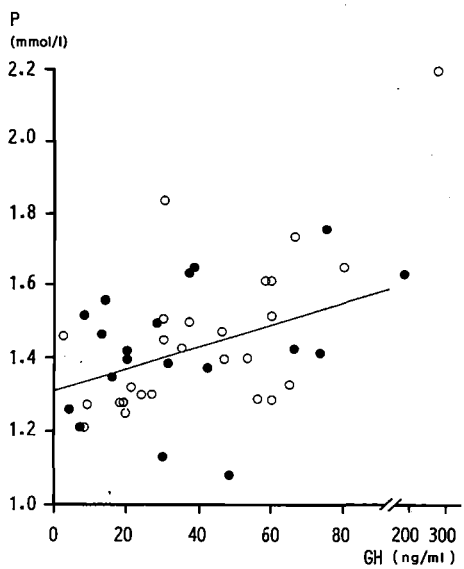

Fig. 17 Relationship between plasma growth hormone and phosphorus concentration in 45 acromegalic patients before transsphenoidal surgery ($r = 0.64$, $p < 0.0005$; ● = ♀, o = ♂).

3.b.1.a.4. Metabolic effects

Preoperatively there was a rough positive correlation between plasma GH and plasma phosphorus concentrations (Fig. 17). Immediately after operation there appeared to occur no significant decrease in plasma phosphorus levels. However, in the years after postoperative irradiation (Fig. 18A) or after irradiation only (Fig. 18B), plasma phosphorus clearly decreased in more than half of the patients. We have not systematically examined changes in the urinary excretion of calcium or hydroxyproline, which after successful therapy are known to decrease together with the GH levels (Roelfsema thesis 1972, Wass et al 1977, Wass 1984b). In addition, the carbohydrate tolerance can improve after therapy (Roelfsema et al 1979, Wass 1984b).

3.b.1.a.5. Effects on pituitary function

3.b.1.a.5.a. pituitary - thyroidal axis. There appeared to be no significant difference in basal plasma TSH before and after transsphenoidal surgery (n=30, n.s.). High normal levels before with low levels after operation and vice versa were found (Fig. 19). Mean plasma TSH level before and after operation were 1.80 mU/l and 1.84 mU/l, respectively (Table 5). In 7 of the 9 patients with a postoperative increase of the basal TSH level T4 appeared also slightly increased. However, pituitary TSH reserve, measured with TRH stimulation had significantly fallen after transsphenoidal surgery (Fig. 20): mean $\pm$ SEM: 7.65 $\pm$ 1.01 mU/l and 4.32 $\pm$ 0.63 mU/l, respectively ($p<0.002$). Twenty-two of 30 patients showed postoperatively a lower response to TRH. There was no significant decrease of the mean plasma T4 level (Fig. 21) after surgery, but rather a tendency to increase (8.67 /ug% and 9.06 /ug% respectively n=39, n.s.). Twenty-four of the 39 patients investigated showed a postoperative increase of T4. In 7 of the 10 patients with an increase above 10 ug% a TRH-test was carried out. Strikingly, in all of them a decrease of the TSH response to TRH was found.

In 14 patients plasma T3 was measured before as well as after operation. No significant difference between the averages was observed. In 8 patients there was an increase, in 6 a decrease of plasma T3.

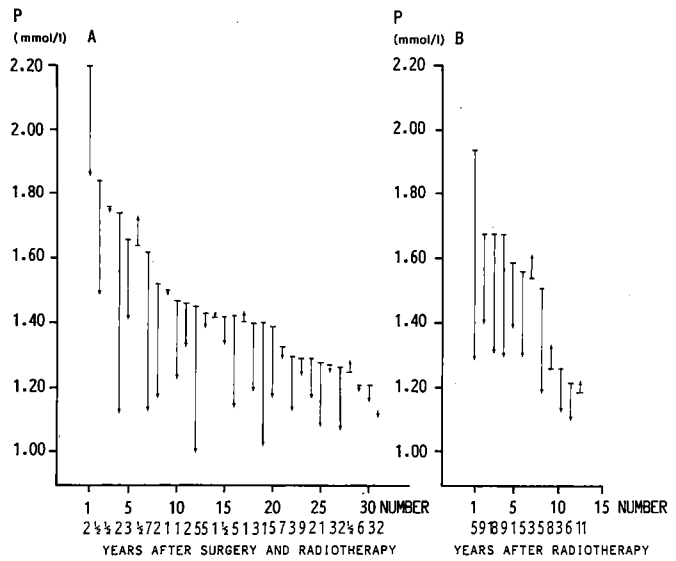

Fig. 18 Decrease in plasma phosphorus concentration after combined surgical and radiotherapeutical treatment (A) and after radiotherapy only (B) in 31 and 12 acromegalic patients respectively.

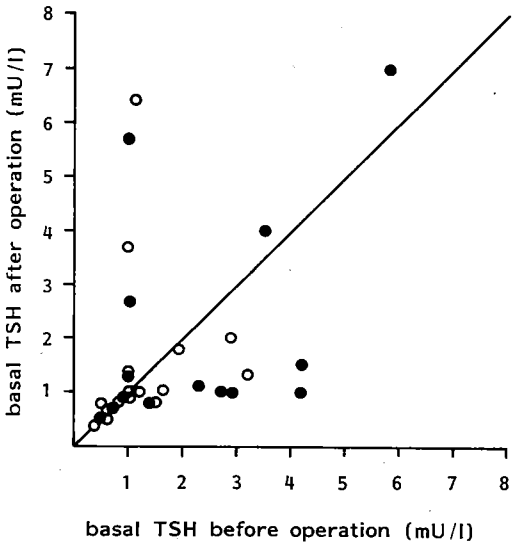

Fig. 19 Basal plasma TSH concentrations before and after transsphenoidal surgery in 30 acromegalic patients (n.s.). The 45° angle is indicated (● =♀ , o = ♂).

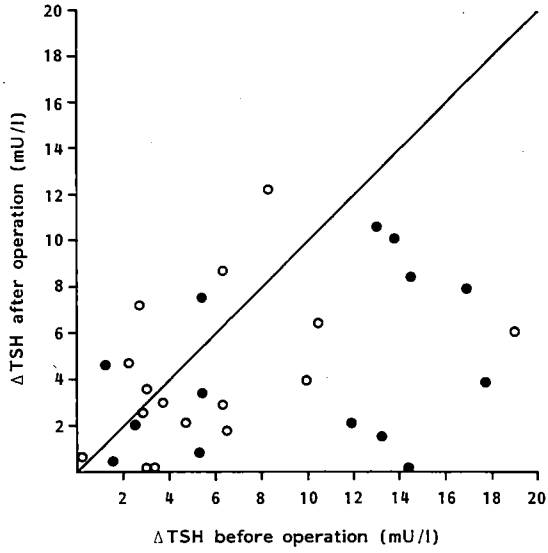

Fig. 20 The TSH response to TRH (Δ TSH) before and after transsphenoidal surgery in 30 acromegalic patients ($p < 0.002$; ● = ♀, ○ = ♂).

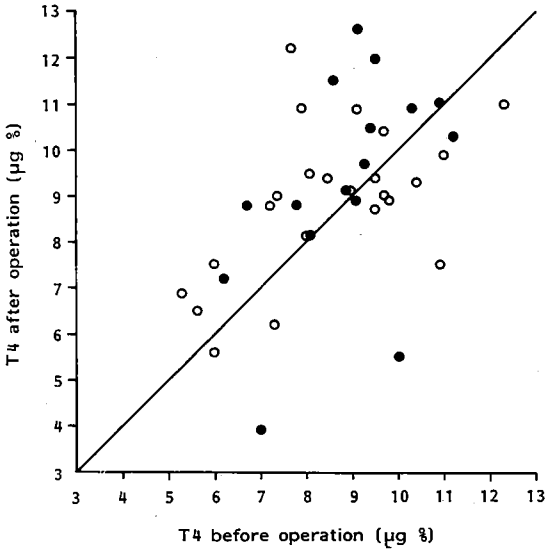

Fig. 21 Plasma T4 concentrations before and after transsphenoidal surgery in 39 acromegalic patients (n.s.; ● = ♀, ○ = ♂).

In 3 out of the 45 transsphenoidally operated acromegalic patients (6.7%) the thyroid function (measured by T4, FTI or PBI) was already subnormal before operation. This percentage increased to 11.1% (5 out of 45) postoperatively because 2 of the 42 euthyroid patients (4.8%) became hypothyroid after operation (see Chapter VIII).

3.b.1.a.5.b. pituitary - gonadal axis. No significant difference was present between prae-and postoperative basal LH (Fig. 22, n=30) and basal FSH levels (Fig.23, n=31). Postoperatively the mean basal level appeared only slighly decreased (Table 5) for LH (4.1 to 3.5 U/1) as well as for FSH (10.6 to 9.8 U/1). In contrast pituitary LH and FSH reserve (ΔLH and Δ FSH after LHRH) decreased significantly by transsphenoidal surgery (Fig. 24 and 25; both $p<0.01$, Wilcoxon rank test). Twenty two out of 30 patients and 21 out of 29 patients showed postoperatively a lower response to LHRH with regard to LH and FSH, respectively. Mean Δ LH decreased from 10.6 to 7.6 and mean Δ FSH from 9.12 to 6.79 U/1 (Table 5). Plasma testerosterone was measured in 13 men preoperatively as well as immediately postoperatively. There was no significant change after operation (average from 0.24 ug% to 0.21 ug%). In 7 women oestradiol-17B was measured pre- as well as postoperatively. The mean plasma level fell from 33 to 15 pg/ml with a marked decrease in 2 patients.

In 19 out of the 45 acromegalic patients transsphenoidally operated upon (42%) pituitary-gonadal function, measured by LHRH-test or urinary gonad stimulating hormone (GSH), was disturbed already before operation. The percentage of patients with a disturbed function increased to 24 out of 44 (54%) postoperatively as in 6 of the 26 patients (23%) the initially normal pituitary gonadotrophin reserve had decreased after surgery (Tables IIA and IIB in chapter VIII). In the immediate postoperative period none of the patients showed normalization of a decreased function already present before operation.

3.b.1.a.5.c. pituitary - adrenal axis. The pituitary-adrenal function was measured by determination of 11-desoxycortisol in plasma or increase of 17-OHCS in urine after metyrapone (see Chapter III).

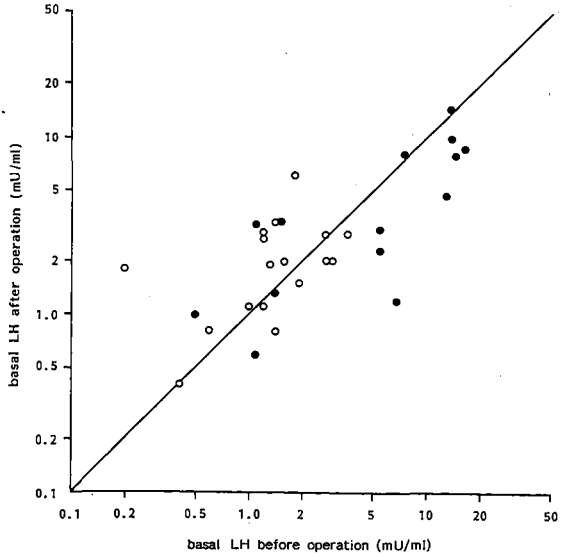

Fig. 22 Basal plasma LH concentrations before and after transsphenoidal surgery in 30 acromegalic patients (n.s.; ● = ♀, o = ♂).

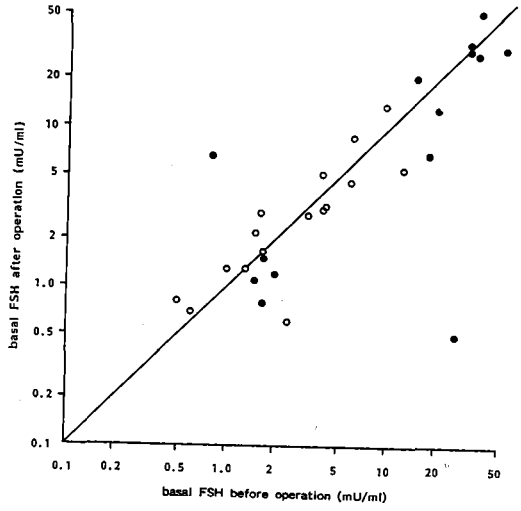

Fig. 23 Basal plasma FSH concentrations before and after transsphenoidal surgery in 30 acromegalic patients (n.s.; ● = ♀, o = ♂).

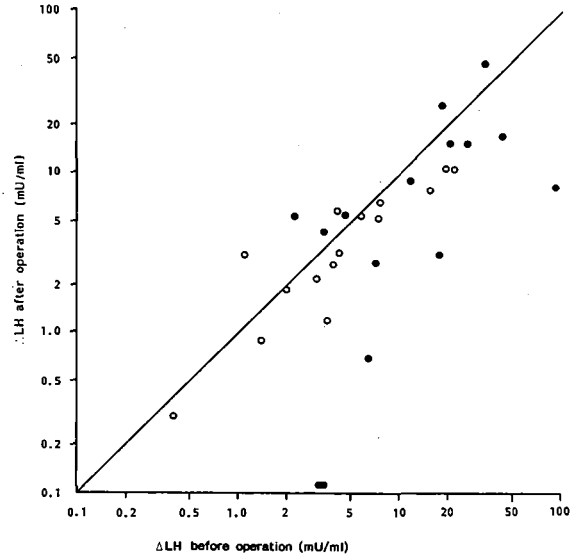

Fig. 24 The LH response to LHRH (Δ LH) before and after transsphenoidal surgery in 30 acromegalic patients ($p < 0.01$; ● = ♀, o = ♂).

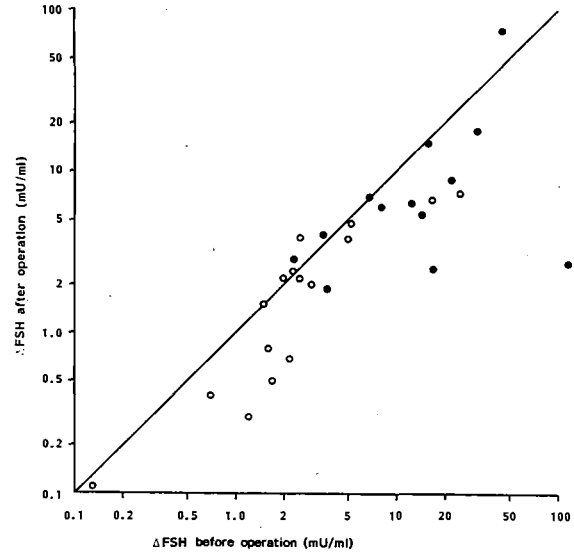

Fig. 25 The FSH response to LHRH (Δ FSH) before and after transsphenoidal surgery in 29 acromegalic patients ($p < 0.01$; ● = ♀, o = ♂).

Before operation the metyrapone-test appeared to be disturbed in 9 out of 45 patients (20%). Immediately after operation 3 patients received substitution with cortisone on clinical indication without available biochemical proof of pituitary-adrenal insufficiency. In 4 out of 33 patients with a normal test preoperatively (12%) pituitary-adrenal function became disturbed by transsphenoidal surgery, while in 5 patients a preoperatively subnormal function remained so. On the other hand in 4 out of 9 (44%) patients a preoperative insufficient function had become normal immediately after operation (Chapter VIII). So, in total a disturbed metyrapone-test was found in 9 out of 42 patients examined shortly after surgery (21%).

In 31 patients the metyrapone-test was carried out before as well as after operation with the determination of the 11-desoxycortisol concentration in plasma. In this group there was no significant decrease by operation (Chapter VIII). Mean 11-desoxycortisol level after metyrapone amounted to 18 $\pm$ 1.56 ug% postoperatively as compared to 17 $\pm$ 1.35 ug% before operation (Table 5).

3.b.1.b. Subfrontal surgery

Subfrontal surgery occurred rarely and this approach has been gradually replaced in the 1970s by transsphenoidal surgery even in the presence of clear suprasellar extension of tumour. By this approach it namely appeared possible to perform decompression and descent of the hypothalamus with minimal manipulation of the cerebrum causing no or less serious complications as sometimes observed after the subfrontal approach. Only 6 patients (3 women and 3 men) were treated by subfrontal surgery because of suprasellar extension. Amoung them was the young woman with the highest GH level. The patient with the largest tumour had undergone a subfrontal operation elsewhere without postoperative irradiation before his referral to our department. The effect of all forms of treatment on GH levels in these patients are summarized in Table 7. Two patients (A and E) appeared to be cured by subfrontal operation and postoperative radiotherapy. Three patients (B, C and G) were reoperated via the transsphenoidal route because of tumour growth or insufficient effect of the previous therapy on GH

Table 7 Effects of subfrontal surgery plus postoperative irradiation and subsequent forms of therapy on plasma growth hormone levels in 6 acromegalic patients (A-F). In another patient (G) operated elsewhere no postoperative irradiation was given.

Patient	Sex	Age	GH value (ng/ml) preop.	GH value (ng/ml) postop.	↓	years of follow-up ¼	´	1	1´	2	2´	3	3´	4	4´	5	5´	6	6´	7	7´	8	9	10	11
A	♂	14	>80	38	↓	17		11						1		1							1		1
B	♂	22	>80	33	↓			25	60	↓ 18	10	7		4		2									
C	♀	25	720	840	↓		320	130		81	250	CB x=160(45-283)				190	↓ 260	410	185	195	CB 225	236			
D	♀	56	↓83	61			67	25			31	25				31	CB 17	22	23	CB + TAM 16	15				
E	♀	20	49	24	↓	129	9	CB 14	13	CB 11	9	8	5												
F	♀	32	7	9		4	11	5	↓12	6	8														
G	♂	25	37	10				18				40	285	↓ 201 330	↓ 410	CB 160	203	180							

↓ = radiotherapy

↓ = transsphenoidal surgery

⊢–⊣ = period of medical treatment (CB = bromocriptine; TAM = tamoxifen)

levels. One patient (F) had preoperatively a relatively low GH level (7 ng/ml) for the size of the tumor (3.6 cm^2). There was no significant decrease in GH level during a follow-up of 2½ years. In another patient (D) normal GH levels have not been obtained after 8 years in spite of treatment with irradiation, subfrontal surgery, transsphenoidal surgery and bromocriptine plus tamoxifen. The estimated mean decrease in GH level by subfrontal operation in all patients is about 30% (i.e. to 70% of the preoperative GH level).

3.b.2. Radiation Treatment of Acromegaly

Sixtyfour of the 67 acromegalic patients received radiotherapy in the course of their disease: 20 as the first form of therapy, 40 as additional treatment after surgery and 4 as the third or fourth form of therapy. One patient refused radiotherapy and two patients with only slightly increased GH levels were treated with bromocriptine only. Four patients were treated without success with bromocriptine only before irradiation. So 24 patients were irradiated before or without surgery: 22 with conventional radiotherapy, one with yttrium and one with proton beam irradiation. Another patient was treated after transsphenoidal surgery and conventional radiotherapy with proton beam irradiation.

3.b.2.a. Effect of surgery and irradiation on clinical symptoms

Twentyfour of the 45 patients operated upon transsphenoidally had complaints about headache of varying intensity before operation. In 9 of these 24 (37%) patients headache disappeared or became less after operation. In one patient headache developed and in another patient the complaint worsened after operation because of sinusitis. In 4 patients headache increased after postoperative radiotherapy. Seven out of 23 patients, who had been irradiated primarily, started to complain or showed an increase of headache after radiotherapy (one of them after proton beam therapy).

In 22 of 45 patients hyperhydrosis disappeared or lessened markedly after surgery and radiotherapy: in 12 of them immediately after

operation and before postoperative irradiation. Seven of the patients primarily irradiated showed a decrease of hyperhydrosis later on.

Acroparaesthesias had disappeared or had become less prominent in 16 patients after operation and radiotherapy; in 8 this was already apparent after operation only. In 17 patients soft tissue swelling decreased during the years of follow-up after transsphenoidal surgery and irradiation as observed by the patient and/or his medical doctor.

In general, it can be stated that we observed progressive loss of complaints concerning hyperhydrosis, acroparaesthesias and soft tissue swelling over the years after surgery and irradiation or after primary radiotherapy.

With respect to cardiovascular disease it has to be pointed out that a study of Baldwin et al (1985) suggests that a slow reduction in serum GH levels does not delay the development of cardiovascular complications.

3.b.2.b. Effects on growth hormone secretion

3.b.2.b.1. Effects on basal plasma growth hormone concentration

In 18 out of 22 patients, treated primarily with conventional radiotherapy, evaluation of the effect on GH levels was possible. One other patient received radiotherapy because of a recurrence of tumour growth two years after transsphenoidal surgery (without postoperative irradiation because of serious postoperative complications). The individual results of conventional radiotherapy in these 19 patients are shown in Chapter VIII. In one patient no plasma GH value before irradiation was known. In general a satisfying decrease of GH levels was obtained during the years of follow-up after irradiation.

Seven out of the 19 patients reached a GH level of (less then) 5 ng/ml and an other two less than 10 ng/ml. Subsequently 5 patients have undergone transsphenoidal surgery and 4 were treated with bromocriptine because of an insufficient decrease of plasma GH. Some patients had very high levels before irradiation. Before treatment the mean GH level in this group of primarily irradiated acromegalic patients was much higher than that in the group of patients that

underwent transsphenoidal surgery (Fig 26). This can be explained by the fact that irradiation was the first choice of therapy for patients without suprasellar extension of pituitary tumour in the time period before transsphenoidal surgery was introduced in our academic hospital and by the fact that some patients had already been irradiated elsewhere before referral to our department. The mean GH level fell in 7 years from 112 ng/ml to 19 ng/ml and subsequently remained at about that level (Fig. 26). In contrast, the mean GH level in patients treated with transsphenoidal surgery followed by irradiation fell in 4 years to below 5 ng/ml (Fig. 26). Comparing the results as a percentage of the GH level before treatment, radiotherapy as single treatment caused a decrease in plasma GH of 40% in the first year (Chapter VIII). After 1½- 2 years the same effect as immediately by transsphenoidal surgery was reached, i.e. a decrease of about 50% of the preoperative value. After 3 years of treatment there was no significant difference between the percental decrease in GH level after irradiation only or after transsphenoidal surgery followed by irradiation. However, when we divide the group of surgically treated patients in subgroups with a relatively successful or unsuccessfull surgical intervention, the "successfull" group showed over seven years a significantly larger percental decrease of GH level than the group of patients treated with radiotherapy only (Chapter VIII). The group of patients in whom immediately after surgery no or only a decrease of the GH level of less than 30% was found, showed the same curve of the mean GH level after postoperative irradiation as the group of patients treated with primary radiotherapy only (Chapter VIII). Comparing the results of combined surgical and radiotherapeutical treatment with those of radiotherapy only it has to be pointed out that his study was non-randomized and retrospective.

3.b.2.b.2. Effect on growth hormone response to TRH

In only 4 patients a TRH-test with estimation of GH could be carried out before treatment with irradiation because in most patients radiotherapy had already been applied before the TRH-test came into use or because of referral after radiotherapy. The percentual increase of GH in response to TRH in these 4 patients was 914, 685, 31 and 2%,

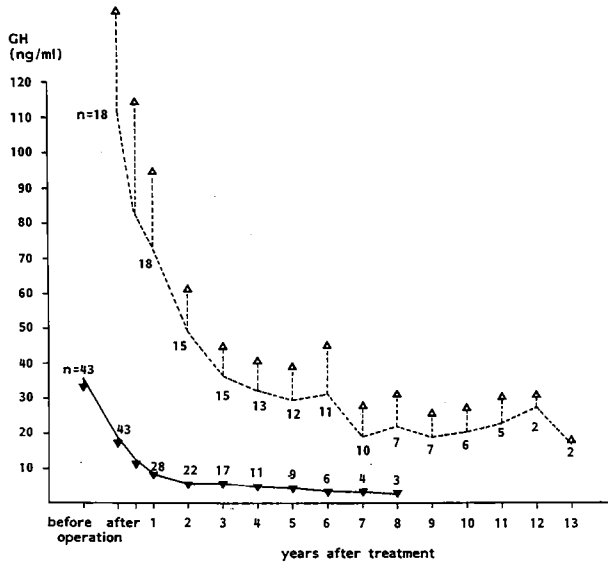

Fig. 26 Absolute plasma growth hormone levels (mean ± SEM) before and after combined surgical and radiotherapeutical treatment (n = 43) and before and after single treatment with radiotherapy (n = 18) in 61 acromegalic patients.

respectively. In the first patient the GH response to TRH decreased from 914 to 437% 6 months and to 32% one year after radiotherapy. In another 11 patients a TRH-test was performed after an average of 8 years (after radiotherapy only). In 5 patients the GH response to TRH appeared more than 100%, in 3 patients 50-100% and in 3 less than 50%.

3.b.2.c. Effect on basal prolactin level

In 4 patients basal plasma PRL levels were measured before radiotherapy. In 2 of them the prolactin concentration was increased (96 and 22 ng/ml). In the patient with the highest value plasma PRL decreased to about 30 ng/ml in one year after radiotherapy and remained at that level for the following 3 years of follow-up. In the patient with 22 ng/ml before radiotherapy plasma PRL level did not decrease significantly in the first 2 years after radiotherapy (18 ng/ml), but appeared to be 3 ng/ml 5 years after radiotherpy and after cessation of treatment with bromocriptine for 2 years.

In 14 patients basal PRL level was measured for the first time after radiotherapy (after an average of five years). Plasma PRL was increased in 6 patients. In three of these six patients plasma PRL was followed for several years without other therapy. In the first patient plasma PRL decreased from 77 to 37 ng/ml during a follow-up of 5 years, in the second PRL fell from 45 to 27 ng/ml during 2 years follow-up and in the third from 142 to 71 ng/ml during one year follow-up. In general, in the hyperprolactinaemic acromegalic patients, in so far evaluable, a decrease of the total plasma PRL concentration with at least 50% after single treatment with conventional irradiation was noted.

3.b.2.d. Metabolic effects

Plasma phosphorus decreased in 9 of the 12 evaluable patients treated by radiotherapy only (Fig. 18B). The reader is further referred to section 3.b.1.a.4. of this chapter.

3.b.2.e. Effect on pituitary function

3.b.2.e.1. Pituitary-thyroidal axis

In 5 patients treated by radiotherapy only, the response of TSH to TRH (Δ TSH) was measured before as well as in the years after radiotherapy. In 3 there was a decrease, in 2 an increase of Δ TSH. Also in 14 patients transsphenoidally operated upon, who were without substitution therapy, a TRH-test was carried out before giving postoperative radiotherapy and again after at least half a year following radiotherapy (mean: 2 year). In 9 there appeared an increase of Δ TSH; in 5 patients a decrease was found. In the whole group of 14 patients ΔTSH was 6.2 $\pm$ 0.9 (S.E.M.) mU/l before postoperative irradiation (Table 5) versus 7.4 $\pm$ 1.4 after (not significant).

In only one of 23 patients treated with radiotherapy only, hypothyroidism existed already before treatment. In the 22 patients who were euthyroid before treatment (one subsequently treated with proton beam irradiation) no hypothyroidism developed after radiotherapy (Chapter VIII).

As mentioned before, in 5 out of 45 patients (11%) treated by transsphenoidal surgery hypothyroidism was found immediately after operation. In two of them this was caused by the operation itself (see section 3.b.1.a.5.a.). In 34 patients thyroid function was determined in the follow-up after postoperative irradiation. In the other patients the follow-up was too short or thyroid function has not been checked. In 5 of these 34 patients investigated in follow-up hypothyroidism was already present before irradiation. In 5 out of the 29 euthyroid patients (17%) hypothyroidism developed in the years after radiotherapy (range 1-4 year; mean: 2 years). Overall, therefore, after the combination of transsphenoidal surgery and postoperative radiotherapy hypothyroidism was found in 10 out of 34 patients (29%) (see Tables IIA and B in Chapter VIII).

3.b.2.e.2. Pituitary-gonadal axis

In the majority of the patients treated with radiotherapy only, no LHRH test was carried out.

In only 9 of the 45 patients treated surgically by transsphenoidal

operation puitary LH and FSH reserve was measured without substitution before postoperative irradiation as well as in the years after irradiation (range 1-3 year, mean 1.5 years). In the other patients no evaluable data are available because of measurement of urinary GSH after operation instead of carrying out a LHRH test, the prescription of substitution therapy after surgery or no LHRH test being carried out in the (short) period of follow-up. In these 9 evaluable patients ΔLH decreased in 6 patients, and ΔFSH in 4 of 7 patients (Table 5). In 14 of the 22 patients, treated with radiotherapy only, pituitary gonadal function was measured before therapy by LHRH-test or measurement of urinary GSH. In 3 of them (21%) this function was disturbed. After radiotherapy pituitary-gonadal function was investigated in 18 patients, and appeared to be disturbed in five of them (28%). In 12 patients the same type of investigation before and after radiotherapy revealed the development of an impairment in one (8%) and recovery of pituitary-gonadal function in another patient (8%).

After transsphenoidal surgery (see section 3.b.1.a.5.b.) a decreased pituitary-gonadal function was observed in 24 out of 44 patients (54%). After postoperative radiotherapy a disturbed pituitary-gonadal function appeared in 20 of 33 (61%) patients examined (Table IIA and B in Chapter VIII). In one (8%) of the 12 remaining patients with a normal function after operation this function became impaired after radiotherapy. In contrast, in 1 of 20 patients (5%) with an impaired function after surgery recovery of the pituitary-gonadal function was observed after additional radiotherapy, while another patient (not examined after operation) after both surgery and radiotherapy showed normalization of a preoperatively decreased reserve.

3.b.2.e.3. Pituitary - adrenal axis

In the group of acromegalic patients, primarily treated with conventional radiotherapy, two out of 22 examined patients (9%) showed the development of a disturbed metyrapone test before treatment. In another patient this test was not repeated after radiotherapy. In 4 of the 19 patients (21%) with a normal pituitary-adrenal function before

primary irradiation an insufficient reserve was detected in the years after irradiation (range 2-13 years).

As mentioned before (see section 3.b.1.a.5.c. of this chapter), a subnormal result of the metyrapone test was found in 21% of the patients after transsphenoidal surgery as compared to 20% preoperatively. This percentage turned out to be increased to 59% after radiotherapy, namely in 19 out the 32 who could be examined in that period (Chapter VIII). In 10 out of 23 patients (43%) with a normal pituitary-adrenal function after operation this function became subnormal in the years after postoperative irradiation (Tables IIA and B in Chapter VIII) with a median duration of 1 year (mean of 2 years; range ½ - 6 years). No recovery of an impaired function was observed after additional radiotherapy. In 18 patients the metyrapone test was carried out with determination of plasma desoxycortisol both before as well as after postoperative irradiation (median: 1 year, mean 1.7 year, range 0.5-6 years) There was a significant decrease of the response of 11-desoxycortisol after metyrapone in the years after radiotherapy (Fig. 9 of Chapter VIII) with a mean value of 20.8 ug/% before and 12.6 ug/% after postoperative irradiation ($p < 0.001$; Table 5).

3.b.3. Medical Treatment of Acromegaly

3.b.3.a. Treatment with bromocriptine

Thirty-one acromegalic patients have been treated with bromocriptine. For 10 of them this was the primary treatment, for 8 the second therapy, for 12 the third and for 1 the fourth form of therapy.

3.b.3.a.1. Effects on clinical symptoms

Seven of 10 patients with more or less severe complaints of headache had relief during treatment with bromocriptine. In 6 out of 17 patients with hyperhydrosis and 3 of 13 patients with paraesthesias these complaints diminished during bromocriptine treatment. Of all 31 patients 8 patients found the soft tissue swelling unchanged or had

doubt about it. We have not exactly measured the degree of soft tissue swelling. Side effects are reported in section 3.d.3.

3.b.3.a.2. Effect on basal growth hormone during chronic treatment

For the plasma GH concentration before and under bromocriptine treatment the mean of two or more samples was taken. Nine of the 10 patients, untreated before institution of bromocriptine therapy, could be evaluated with regard to change in plasma GH levels. The mean decrease in plasma GH concentration appeared to be 45%. Six patients had a decrease of more than 30% (Fig. 27A).

In the group of 20 evaluable patients, previously treated with surgery and/or radiotherapy, the mean decrease in GH was 21%. Ten of them had a decrease of more than 30% (Fig. 27B). In some of them this decrease may be regarded as a (late) effect of radiotherapy, but part of the patients had stable plasma GH concentrations before bromocriptine treatment. In a few patients (3 or 4) a clearcut increase of plasma GH was seen under treatment with bromocriptine. Four patients showed an escape of plasma GH during chronic bromocriptine treatment after an initial good response as previously reported in detail with respect to one of these 4 patients (Lamberts et al 1979a).

3.b.3.a.3. Relationship between plasma prolactin, the magnitude of the increment of growth hormone to TRH, and the acute and chronic effects of bromocriptine

The relationship between plasma PRL, the magnitude of the increment of GH in response to TRH and the decrease of plasma GH after one single dose of 2.5 mg bromocriptine have been reported by our group before (Lamberts et al 1979a, 1982a, 1983, Chapter VII). The degree of suppression of plasma GH in response to 2.5 mg bromocriptine appeared positively correlated with the plasma PRL level and with the increase of GH after TRH. A homogeneity in the responsiveness of GH to TRH and to bromocriptine was confirmed in 74% of all 72 patients and in 92% of clearly hyperprolactinaemic acromegalic patients (Lamberts et al 1982a).

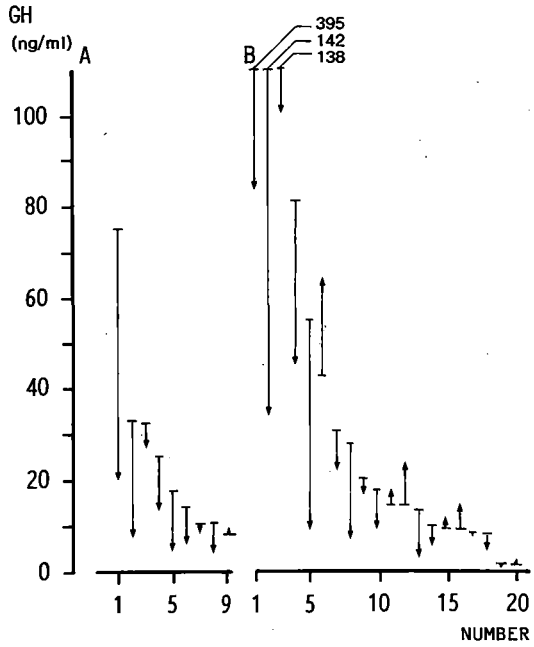

Fig. 27 Absolute decrease of plasma growth hormone concentrations during chronic bromocriptine treatment in 9 otherwise untreated patients (A) and in 20 acromegalic patients pretreated by surgery and/or irradiation (B).

In a collaborative study we found hyperprolactinaemia in 33 (42%) of 79 untreated acromegalic patients (Lamberts et al 1982a). Inhibition of GH secretion by more than 50% after administration of one dose (2.5. mg) bromocriptine occurred in 22% of normoprolactinaemic, in 53% of the mild hyperprolactinaemic and in 88% of the clearly hyperprolactinaemic patients. An increase of GH secretion by more than 100% of the basal value in response to TRH was observed in 44% of the normoprolactinaemic, in 59% of the mildly hyperprolactinaemic and in 75% of the clearly hyperprolactinaemic patients. In a later study (Lamberts et al 1983, Chapter VII) we found that the immunohistochemical presence or absence of PRL in pituitary tumour tissue of 35 transsphenoidally operated acromegalic patients was correlated with the in vivo sensitivity of GH secretion to one test dose of bromocriptine and to TRH. Plasma GH levels from 2 till 10 hour after the administration of one dose of 2.5 mg bromocriptine measured before operation were significantly more suppressed in patients with mixed GH/PRL containing adenomas than in those with pure GH-containing pituitary adenomas, being 38 $\pm$ 4% and 65 $\pm$ 4% (S.E.M.) of basal values, respectively ($p < 0.01$).

In this paragraph we describe the results of a study of the GH lowering effect of chronic bromocriptine treatment in relation to plasma PRL levels, the response of GH to TRH and response to bromocriptine in the acute test measured before start of chronic bromocriptine treatment.

- Significance of the basal plasma PRL level for the effect of treatment with bromocriptine.

A positive relationship between the plasma PRL level and the percentual decrease in GH during chronic treatment with bromocriptine was found (Fig.28). Untreated and pretreated patients with high plasma PRL levels (>40 ng/ml) showed a pronounced decrease of GH during chronic treatment: in 6 of 7 cases this amounted to more than 50%. The untreated and pretreated patients with normal or only slightly increased plasma PRL levels showed a highly variable response of GH to chronic bromocriptine treatment: in 4 of 21 the level was reduced by more than 50%, while in most cases there was no response and in a few

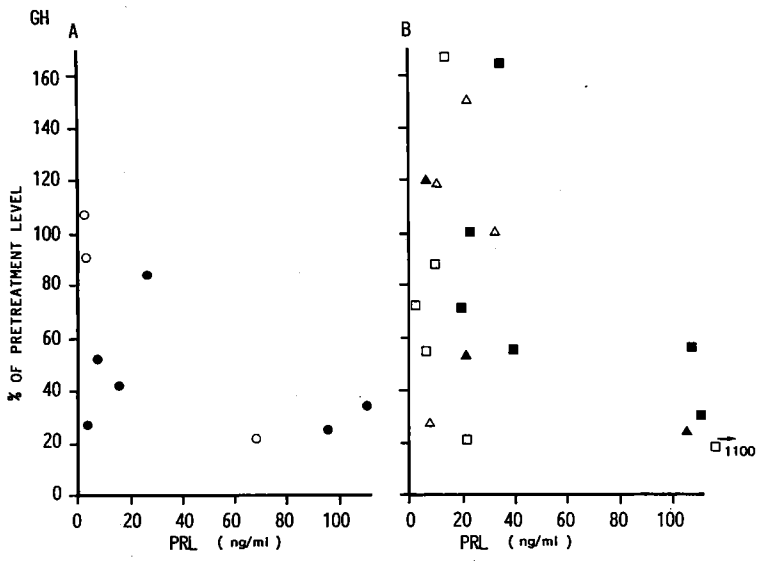

Fig. 28 Growth hormone levels during chronic bromocriptine treatment as percentage of pretreatment values in relation to plasma prolactin concentrations before bromocriptine treatment in 9 otherwise untreated (A) and 19 acromegalic patients pretreated by surgery and/or irradiation (B). "Pretreatment level" means the level before bromocriptine treatment. Closed symbols = ♀ , open symbols= ♂.
□ = Surgery + irradiation.
△ = Radiotherapy only.

even an increase in GH. An increase of GH in a few patients during chronic treatment with bromocriptine has been also reported by Roelfsema et al (1979) and Belchetz (1984c).

- Significance of the GH response to TRH for the effect of treatment with bromocriptine.

Only 1 out of 10 untreated and pretreated patients with a response of GH to TRH of less than two times the basal value showed a decrease of GH by more than 50% during chronic treatment with bromocriptine (Fig. 29). Furthermore, only three out of 18 untreated and pretreated patients with an increment of GH after TRH up to four times the basal value exhibited a good response of plasma GH i.e. a decrease of the mean level by more than 50% during treatment. However, in all 6 patients with an increment of more than four times the basal value of GH after TRH chronic treatment with bromocriptine was successful (>50% decrease).

- Significance of the acute bromocriptine test for the effect of chronic treatment.

A highly significant positive correlation between the decrease of GH after one test dose of 2.5 mg and the decrease during chronic treatment was observed (Fig. 30; in previously untreated patients $p<0.005$, in treated patients $p<0.01$). Five of the patients, treated before with surgery and/or radiotherapy (Figure 30B) showed a stronger effect during chronic treatment than in the acute test in contrast to the patients not treated before (Figure 30A). Possibly there was an additional effect of the previous radiotherapy in these patients. On the other hand, 10 of the 15 previously treated patients showed a fall of more than 50% of the GH level after one dose of bromocriptine. Five out of these 10 patients with a good decrease of GH during the acute test did not show a decrease of more than 50% during chronic treatment.

Overall, we observed a mean decrease of plasma GH concentration by 45% during chronic bromocriptine treatment. This is comparable with the results reported by Cozzi et al (1986) who found a reduction of 53% of

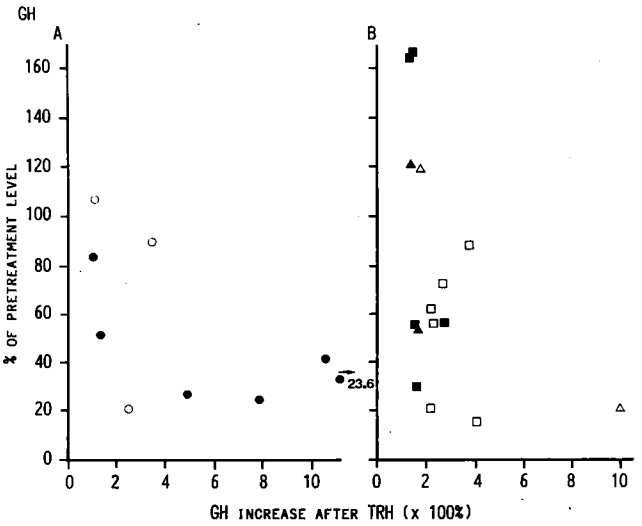

Fig. 29 Growth hormone levels during chronic bromocriptine treatment as percentage of pretreatment values in relation to the growth hormone response to TRH before bromocriptine treatment in 9 otherwise untreated (A) and 15 acromegalic patients pretreated by surgery and/or irradiation (B).
Closed symbols = ♀ , open symbols= ♂.
□ = Surgery + irradiation.
△ = Radiotherapy only.

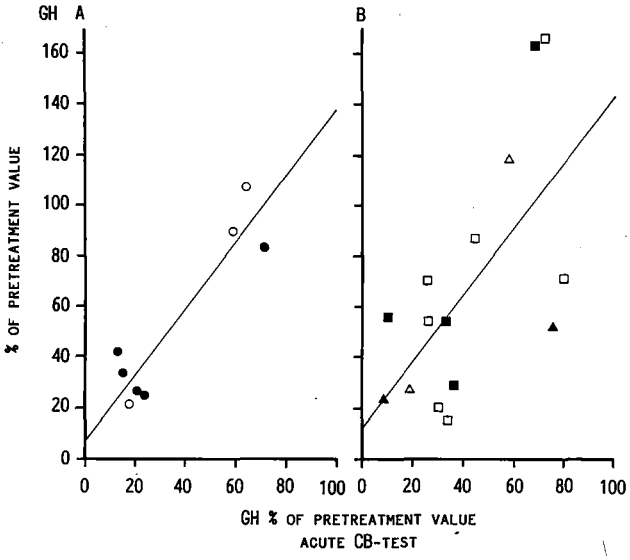

Fig. 30 Growth hormone levels during chronic bromocriptine treatment as percentage of pretreatment values in relation to the decrease of growth hormone after one dose of bromocriptine in 8 otherwise untreated (A) ($r = 0.92$, $p < 0.005$) and 15 acromegalic patients pretreated by surgery and/or irradiation (B) ($r = 0.64$, $p < 0.01$).
Closed symbols = ♀ , open symbols= ♂.
□ = Surgery + irradiation.
△ = Radiotherapy only.

the mean GH level (from 34 to 16 ng/ml) in 24 active acromegalic patients, and with the results of Bell et al (1986), who reported a 58% reduction during 2 treatment periods in 10 patients (decrease from 49.6 to 20.5 and 20.8 ng/ml respectively). Roelfsema et al (1979) reported a mean decrease of 62% in 10 responding and no change in 5 non-responding patients after one year of treatment.

In general, approximately 50-80% of patients treated with bromocriptine (10-60 mg per day) have a significant (>50%) reduction in serum GH levels (Lamberts et al 1979e, Vance et al 1984, Nortier and Croughs 1985c), with a fall to the normal range (<5 ng/ml) in only 15-20% of the patients. In a review of 10 studies Vance et al (1984) reported suppression of GH levels in 210 (73%) out of 288 patients and normalization of GH levels in 35 (20%) out of 172 patients. Clinical improvement with regard to sweating, ring size, urinary hydroxyproline excretion, and glucose tolerance occurred in the majority (70-92%) of the patients. Some authors (Clemmons et al 1979, Wass et al 1982) found that the clinical activity of the disease correlated better with plasma concentrations of somatomedin-C, through which the biological effects of GH are considered to be mediated. However in 3 other studies (Moses et al 1981, Stonesifer et al 1981, Nortier et al 1985a), the contrary was found. Roelfsema et al (1987) observed that somatomedin-C levels were increased without exception in 24 patients with active acromegaly and normal in 45 inactive treated patients. Somatomedin-C levels were significantly correlated with GH levels. Bromocriptine treatment induced preferentially a reduction of little GH (Nortier et al 1985b); a very good correlation between the decrease of little GH and total GH was found, and both were significantly correlated with the clinical response.

Five predictive tests for GH suppression during chronic bromocriptine treatment have been reported. We showed that in general GH suppression during chronic treatment was more pronounced 1) in patients with hyperprolactinaemia, 2) in patients with a high GH response to TRH, and 3) especially in patients with a pronounced decrease of GH after one test dose of bromocriptine. Other authors did also observe a good correlation between the plasma GH response to a single dose of bromocriptine and that to chronic bromocriptine

treatment (Belforte et al 1977, Roelfsema et al 1979, Nortier et al 1984a). The value of hyperprolactinaemia is controversial (Nieuwenhuijzen Kruseman et al 1983, Nortier and Croughs 1985c, Cozzi et al 1986). The degree of GH suppression during somatostatin infusion (fourth test) may also have predictive value but Nortier and Croughs (1985c) concluded that the somatostatin infusion did not offer additional information to that of the acute bromocriptine and TRH test in normoprolactinaemic acromegalics. Recently, Cozzi et al (1986) reported the predictive value of the GHRH-test (fifth test) with respect to response to chronic bromocriptine therapy. It appeared that GH release after GHRH was lower in patients responsive to bromocriptine than in bromocriptine nonresponders. In addition, the initial response of GH to GHRH was unaffected by bromocriptine treatment in contrast to the response to TRH, which, statistically significant initially only in bromocriptine responders, was reduced by bromocriptine treatment. These authors suggested that cells sensitive to bromocriptine and TRH but not to GHRH (lactotroph-like) and cells sensitive to GHRH but not to bromocriptine (pure somatotrophs) may coexist in GH-secreting adenomas.

3.b.3.b. Treatment with somatostatin analogues

For the effects of somatostatin analogue treatment I refer to our paper on: The sensitivity of growth hormone and prolactin secretion to the somatostatin analogue SMS 201-995 in patients with prolactinomas and acromegaly (Lamberts et al 1986a) and previous reports (Lamberts et al 1984c, 1985b and c).

The somatostatin analogue SMS 201-995 (Sandostatin) has recently been shown to be effective in suppressing GH secretion in most acromegalic patients. In the present study it was investigated whether PRL release in prolactinoma and acromegalic patients might also be sensitive to SMS 201-995 and whether co-secretion of PRL in acromegaly plays a role in determining the sensitivity of GH secretion to SMS 201-995. The subcutaneous administration of 50 /ug SMS 201-995 did not affect high plasma PRL levels in four microprolactinoma patients. Therapy of one of these patients for 3 days with 50 /ug three times a

day also did not affect PRl levels. The single administration of 50 /ug SMS 201-995 in 22 acromegalic patients lowered plasma GH levels for 2-6 hours to less than 5 /ug/l in 14 patients and to less than 50% of control values in 16 patients. In 18 of these 22 patients the immunohistochemical picture of the pituitary tumour was known. Eleven patients had pure GH-containing tumours and in seven patients there were mixed GH/PRL-containing tumours. In two of these latter patients there was evidence for GH and PRL being secreted by the same tumour cells. The sensitivity of GH secretion to SMS 201-995 did not differ between the patients with pure GH or mixed GH/PRL-containing adenomas. Plasma PRL levels were not affected by SMS 201-995 in the patients with pure GH-secreting tumours, but were significantly suppressed in four of the seven patients with mixed GH/PRL-containing tumours. Chronic treatment for 16 weeks of one patient with a mixed GH/PRL-containing tumour with SMS 201-995 (100 /ug three times a day) resulted in normalization of both the increased GH and PRL levels. It is concluded that SMS 201-995 does not affect tumorous PRL secretion in patients with pure prolactinomas. In acromegalic patients with mixed GH/PRL-containing tumours PRL secretion in some patients is sensitive to SMS 201-995, making these patients good candidates for chronic treatment with the analogue. The simultaneous presence of PRL in the GH-secreting pituitary tumour or the presence of hyperprolactinaemia in acromegalics does not play a role in the sensitivity of GH secretion to the somatostatin analogue.

3.c. Prolactinomas and "Non-Functioning" Tumours

Patients with "non-functioning" pituitary tumours mainly have macroadenomas. Microadenomas are especially found in the group of premenopausal patients with prolactinomas because hyperprolactinaemia early caused symptoms like menstrual disorders and infertility. The world over there is no uniform approach as to the treatment of microadenomas. In the United States and some large neurosurgical centres in Europe there is a preference for microsurgical adenomectomy while in other centres medical treatment with dopamine agonists is commonly used. Some centres combine these two lines of treatment. In

Rotterdam we have usually treated patients with microprolactinomas with dopamine agonists and patients with large tumours with both surgical and medical treatment. Primary radiotherapy is not suitable for obtaining immediate effects with respect to recovery of fertility or debulking of a large tumour mass, but secondary radiotherapy is effective in preventing tumour recurrences.

3.c.1. Surgical Treatment (transsphenoidal and subfrontal) of Prolactinomas and Non-Functioning Tumours

The results of surgical treatment are dependent on tumour size, preoperative basal PRL level, age and duration of amenorrhoea, which parameters are more or less related to each other (Wilson 1984, Hardy 1983, Fahlbusch 1980, Fahlbusch et al 1984, Fahlbusch and Buchfelder 1985, Landolt 1984a, Landolt and Froesch 1985b, Derome et al 1980 and 1984, Lüdecke et al 1983, Teasdale 1983, Randall et al 1985, Post 1980a,b,c, and 1986, Maira et al 1985). In several large series the best results of transsphenoidal surgery were shown in patients with microprolactinomas (< 10 mm) and patients with preoperative PRL-levels less than 200 ng/ml (Fahlbusch 1980, Fahlbusch et al 1984, Fahlbusch and Buchfelder 1985, Hardy 1983, Derome et al 1980 and 1984, Bertrand et al 1983, Lüdecke et al 1983, Post 1986). Normalization of plasma PRL concentrations was obtained in about 60-85% of patients with microprolactinomas. The best results were seen in patients with microprolactinomas less than 5 mm and plasma PRL levels less than 100-200 ng/ml (80-93% normalization).

Restoration of menses varied in the reported series with a range of about 40-70% with approximately a 50 percent success rate in achieving pregnancy in those who chose to become pregnant (Post 1986). Normal menses and fertility may be restored even when postoperative prolactin values remain slightly or moderately elevated.

Transsphenoidal microsurgical treatment appeared successful both in female and male patients with prolactinomas (Serri et al 1980, Hardy 1983, Bertrand et al 1983, Muhr et al 1985), but the results in women are better because in the average men have larger tumours.

Recurrence of hyperprolactinaemia is reported in 6 - 50% of the

patients with normalization of plasma PRL immediately after surgery (Bertrand et al 1983, Faglia et al 1983, Serri et al 1983, Fahlbusch et al 1984, Rodman et al 1984, Parl et al 1986, Buchfelder et al 1985, Schlechte et al 1986). In none of these series postsurgical irradiation was applied. In his review concerning 8 studies Parl noted recurrence of hyperprolactinaemia after surgical removal of microprolactinomas in 47 out of 238 (20%) patients during long-term follow-up of 0.5 - 10 years. Zervas calculated a recurrence rate at 5 years of 5% in 222 patients with preoperative plasma prolactin levels less than 200 ng/ml and of 7% in 132 patients with preoperative plasma levels greater than 200 ng/ml (Post 1986). Serri et al (1983) reported recurrence of hyperprolactinaemia after selective transsphenoidal adenomectomy in 50% of 24 patients after a mean follow up of more than 6 year (range 5-10 year). There was no radiological evidence of tumour recurrence in any patient. Clinical and biological features before surgery had no predictive value with regard to the long-term outcome. However, the immediate postoperative level of plasma PRL was significantly lower in patients in whom normoprolactinaemia was maintained than in those who relapsed. Post (1986) reported that a postoperative prolactin level of more than 10 ng/ml seems predictive of patients who might relapse. Maybe the best results with respect to both short and long-term effects are reached in the centres where aethanol irrigation of the removal cavity is applied after adenomectomy (Wilson and Dempsey 1978, Nicola et al 1980, Bertrand et al 1983).

In patients with macroadenomas the incidence of surgical failure is very high (70-100%) increasing with tumour size from 72% failures in patients with small intrasellar macroadenomas to 95-100% failures in patients with a large suprasellar extension (Fahlbusch 1980, Fahlbusch et al 1984, Fahlbusch and Buchfelder 1985). Normalization of a preoperative PRL level of more than 500-600 ng/ml appeared to be difficult i.e. only in about 15% (Hardy 1983, Fahlbusch and Buchfelder 1985).

In 16 consecutive transsphenoidally operated patients with a macroprolactinoma and known pre- and postoperative PRL levels we observed an immediate decrement of the mean plasma PRL concentration

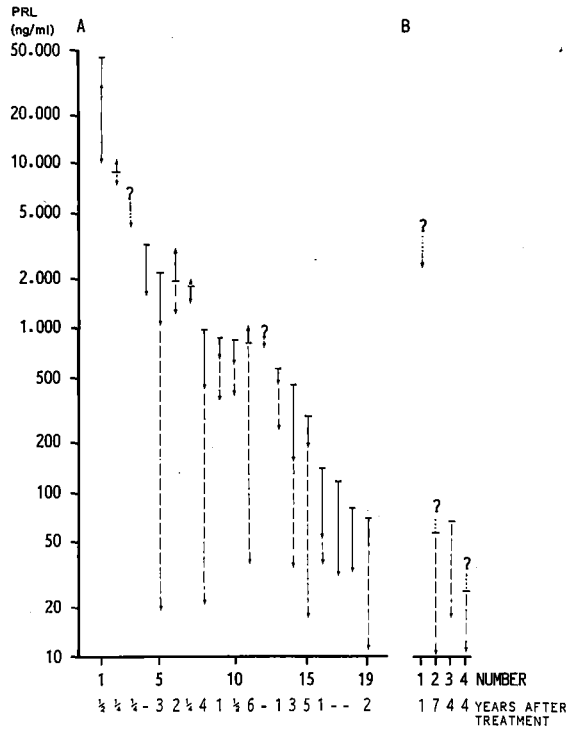

Fig. 31 Decrease of plasma prolactin concentrations by transsphenoidal surgery (⟶) and (postoperative) irradiation (....>) in 19 prolactinoma patients treated with combination therapy (A) and in 4 patients treated with radiotherapy only (B).
? = unknown pretreatment value.

by surgery to 67 ± 9% (mean ± SEM) of the preoperative value (Fig. 31; chapter VIII) without normalization in any of the patients. Subfrontal extirpation of tumour is mainly meant for decompression of surrounding tissues and causes on the average less decrease in plasma PRL levels (Fig. 32).

In 65 patients with a prolactinoma or "non-functioning" tumour transsphenoidal or subfrontal surgical treatment did not cause a significant decrease of basal TSH, LH and FSH or the reaction of desoxycortisol to metyrapone (Fig. 33, left). In contrast a significant decrease of the pituitary reserve of TSH (Fig. 34), LH (Fig. 35) and FSH (Fig. 36) occurred (cf. Chapter VIII). While the mean TSH response to TRH decreased from 10.4 to 6.1 mU/l (Table 5) the plasma thyroxine level remained unchanged at 7.2 µg% (Fig. 37, Table 5). Mean ΔLH and ΔFSH (LHRH-test) decreased from 6.0 to 3.4 and from 4.6 to 3.0 U/l, respectively. In general these results are in agreement with data in the literature, which show that the incidence of hormonal insufficiency after surgery is very low in patients with microadenomas but not uncommon in patients with macroadenomas. We did not observe a significant improvement of preoperative impaired pituitary reserve after (partial) removal of a macroadenoma as reported by some authors (McLanahan et al 1979, Baha'Uddin et al 1982, Teasdale 1983, Ohta et al 1985).

For the effects of surgery, recurrence-rate and survival I refer to Chapter VIII. In our study it appeared for the first time that the combined therapy of surgery and postoperative radiotherapy was superior to radiotherapy alone with respect to long-term survival. Both subfrontal and transsphenoidal decompression can result in recovery of visual acuity (Teasdale 1983). Indeed, sometimes a rapid improvement is noticed immediately after operation. Marked improvement is more likely when the history is short and when the loss of vision before surgery is not extensive. Fahlbusch (1980) and Post (1986) reported that visual improvement can be expected in more than 80% of the patients (see also 3.e.).

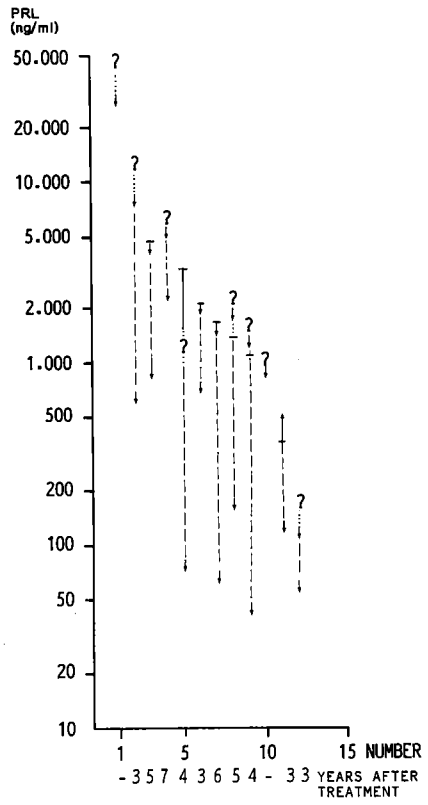

Fig. 32 Decrease of plasma PRL concentrations by subfrontal surgery (——→) and by postoperative irradiation (....>) in 12 patients with a prolactinoma.
?. = unknown pretreatment value.

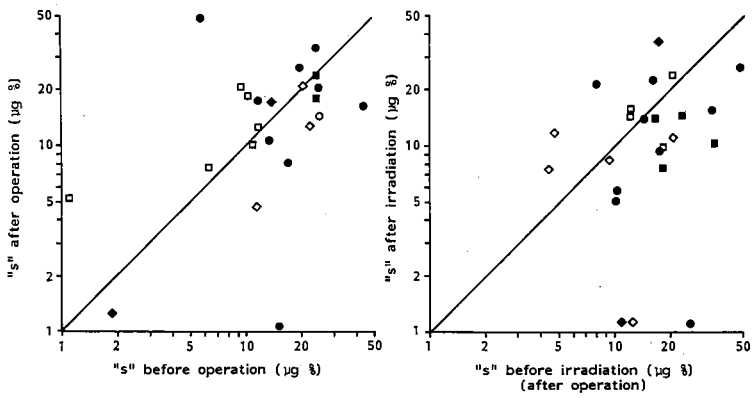

Fig. 33 Plasma desoxycortisol (compound S) concentrations after metyrapone measured before and after transsphenoidal or subfrontal surgery (n = 23, n.s.) and before and after postoperative irradiation (n = 24, p = 0.05) in patients with a prolactinoma or a non-functioning tumour. The 45° angle is indicated.
Closed symbols = ♀ , open symbols = ♂.
○ = Prolactinoma - transsphenoidal operation.
□ = Non-functioning tumour - transsphenoidal operation.
◇ = Prolactinoma - subfrontal operation.

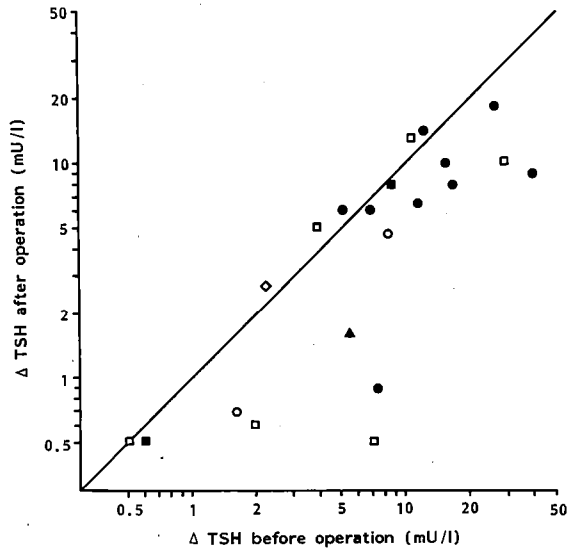

Fig. 34 The TSH response to TRH (Δ TSH) before and after transsphenoidal or subfrontal surgery in 21 patients with a prolactinoma or a non-functioning tumour ($p = 0.02$). The 45^{o} is indicated.
Closed symbols = ♀ , open symbols = ♂.
○= Prolactinoma - transsphenoidal operation.
□= Non-functioning tumour - transsphenoidal operation.
◇= Prolactinoma - subfrontal operation.
△= Non-functioning tumour - subfrontal operation.

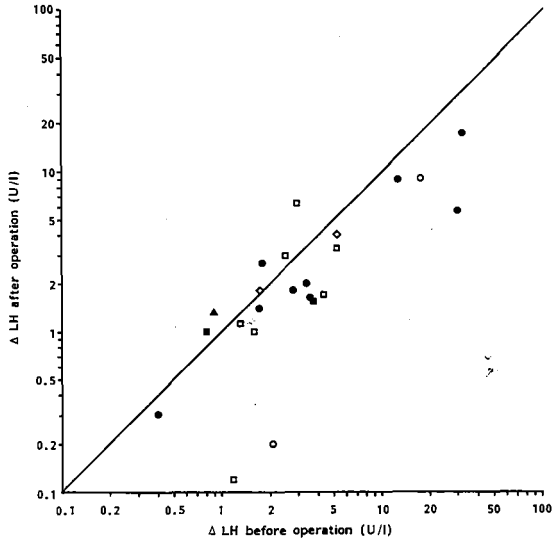

Fig. 35 The LH response to LHRH (Δ LH) before and after transsphenoidal or subfrontal surgery in 23 patients with a prolactinoma or a non-functioning tumour ($p < 0.01$). For symbols: see legends of figure 34.

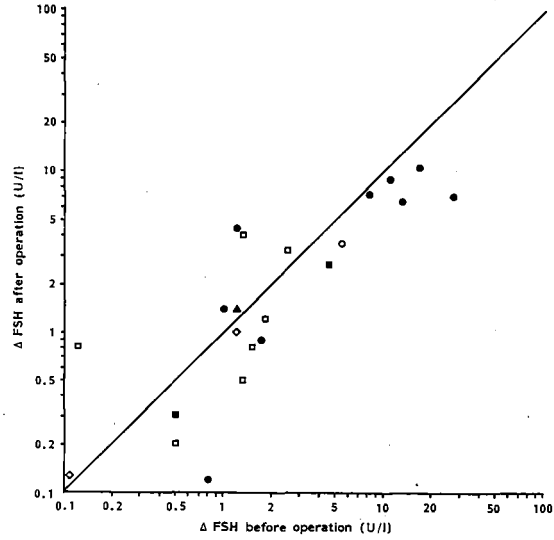

Fig. 36 The FSH response to LHRH (Δ FSH) before and after transsphenoidal or subfrontal surgery in 22 patients with a prolactinoma or a non-functioning tumour ($p < 0.01$). For symbols: see legends of figure 34.

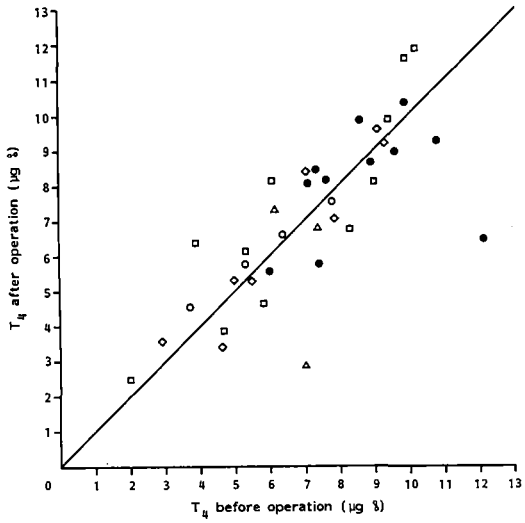

Fig. 37 Plasma T4 concentration before and after transsphenoidal or subfrontal surgery in 38 patients with a polactinoma or a non-functioning tumour (n.s.).
For symbols: see legends of figure 34.

3.c.2. Radiation Treatment of Prolactinomas and Non-Functional Tumours

Radiotherapy is not commonly used as primary treatment of patients with prolactinomas because of the observation that irradiation appears rarely to be curative on a short-term basis. Only a few small series have been published (Gomez et al 1977, Samaan et al 1977, Antunes et al 1977, Sheline 1982, von Werder 1982, Nabarro 1982, Sheline and Tyrell 1984, Coulter 1984b). Radiation therapy has mostly been applied in patients who continued to have elevated PRL levels after surgery or who had large adenomas. Normalization of serum PRL is related to pretreatment PRL level and tumour size (Sheline 1982, Nabarro 1982). In patients with large tumours and very high PRL levels, irradiation has produced a progressive decrease in PRL levels but only occasionally to normal values (Sheline 1982). When the PRL levels are below 6-9 times the upper limit of the normal range reduction to normal values may occur within 2-3 year (Sheline 1982, Nabarro 1982). Nevertheless both in patients with micro- as wel as macroprolactinomas there is a wide variation in response.

The decrease in mean PRL concentration by irradiation has been reported to vary from 62 to 93% (Antunes et al 1977, Gomez et al 1977, Kleinberg et al 1977, Sheline 1982, von Werder 1982) 2-6 years after external irradiation. Kleinberg et al (1977) showed in 15 patients treated with surgical resection and in 8 treated with radiotherapy that transsphenoidal surgery and radiotherapy were equally effective with a similar reduction in mean PRL level. Menses occasionally resumed. After the suggestion of Landolt et al (1979) that postoperative radiotherapy might have a synergistic effect together with bromocriptin treatment, Eversmann et al (1979) showed no difference in PRL levels in patients with macroprolactinomas treated with radiotherapy and bromocriptine or with bromocriptine alone.

For our results I refer to chapter VIII and Table 5. Sixteen patients, who had been operated upon transsphenoidally for a macroprolactinoma showed up to 6 years ($x = 3$ years) after surgery and postoperative irradiation a maximal decrease of PRL levels of 90% (Fig. 31A). Only four patients were treated with radiotherapy alone

(Fig. 31B). Two patients reached normal PRL levels 7 and 4 years after the radiotherapy while menses returned in the first patient.

In our patients with prolactinomas and non-functioning tumours gonadotropin secretion appeared to fall not significantly in the years after postoperative radiotherapy (Figs. 38 and 39). However, mean plasma T_4 decreased significantly from 8.2 to 6.8 /ug% (n=29, $p<0.01$, Fig. 40, Table 5) in the presence of an increase of the TSH response to TRH from 8.1 to 12.4 mU/l (n=17, $p<0.02$, Fig. 41, Table 5) indicating hypothalamic insufficiency as reported to occur also in patients irradiated for non-pituitary disease (Shalet 1983, Ahmed and Shalet 1984, Lam et al 1986). In our group of patients the mean desoxycortisol concentration after metyrapone administration decreased also: from 17.6 to 12.7 /ug% (n=24, p=0.05, Fig. 33 right, Table 5). Three patients developed severe adrenal insufficiency.

External radiotherapy has been advocated as prophylactic treatment before induction of ovulation in patients with large prolactinomas who wish to become pregnant (Thorner et al 1979, Albrecht and Betz 1986). Although prophylactic radiotherapy might reduce this number of complications during pregnancy, complications cannot be totally prevented (von Werder 1982, Albrecht and Betz 1986) as described before in 3 of our patients (Lamberts et al 1977 and 1979c).

The majority of large pituitary adenomas with hyperprolactinaemia or without known endocrine function can not be resected completely. Therefore postoperative irradiation is useful for growth control rather than for curation. Patients treated with radiotherapy alone and those treated by partial resection plus postoperative irradiation experienced approximately equal recurrence-free rates i.e. 90% after 5 years of follow-up in contrast to 25% of patients treated by surgery alone (Erlichman et al 1979, Sheline 1982, Chapter VIII). Although radiotherapy alone is effective, particularly in patients with smaller tumours, it is generally preferred to surgically "debulk" the tumour mass before the application of irradiation because of 1) the possibilities of confirmation of diagnosis, 2) rapid decompression of the optic system in case of suprasellar extension, and 3) improvement of long-term survival (Chapter VIII).

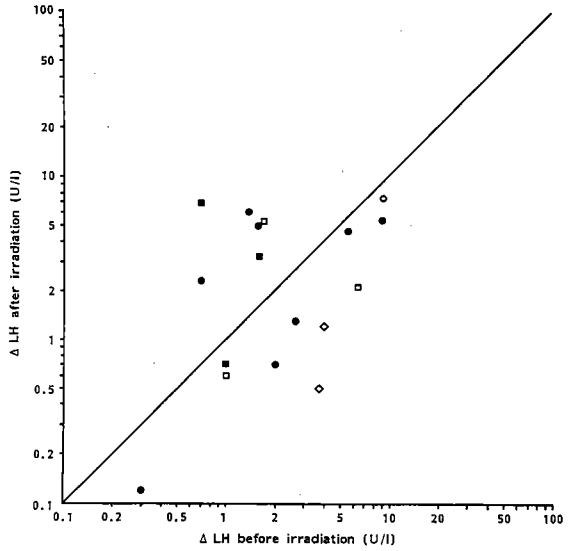

Fig. 38 The LH response to LHRH (ΔLH) before and after postoperative irradiation in 17 patients with a prolactinoma or a non-functioning tumour (n.s.).
For symbols: see legends of figure 34.

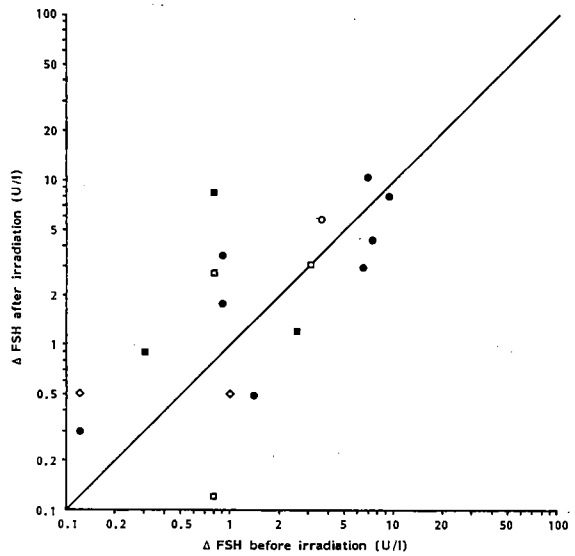

Fig. 39 The FSH response to LHRH (Δ FSH) before and after postoperative irradiation in 17 patients with a prolactinoma or a non-functioning tumour (n.s.).
For symbols: see legends of figure 34.

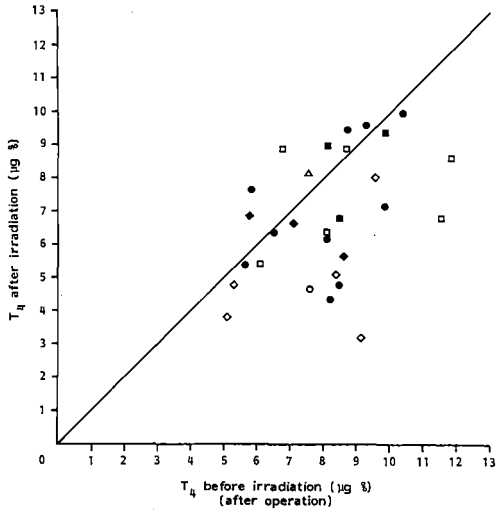

Fig. 40 Plasma T4 concentrations before and after postoperative irradiation in 29 patients with a prolactinoma or a non-functioning tumour ($p < 0.01$).
For symbols: see legends of figure 34.

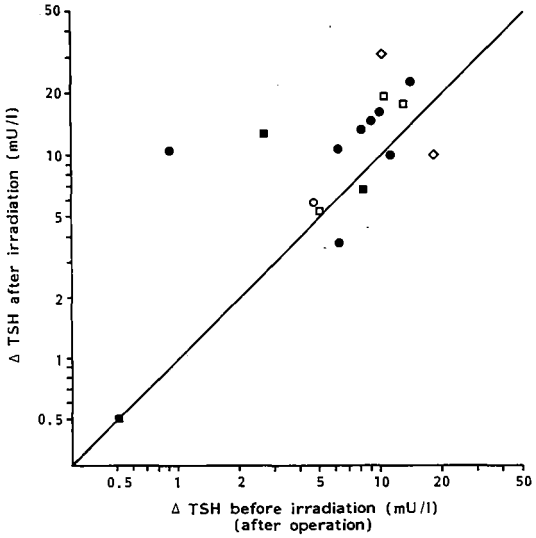

Fig. 41 The TSH response to TRH (Δ TSH) before and after postoperative irradiation in 17 patients with a prolactinoma or a non-functioning tumour ($p < 0.02$).
For symbols: see legends of figure 34.

3.c.3. Medical Treatment of Prolactinomas and Non-Functioning Tumours

3.c.3.a. Background

The medical management of pituitary tumours has advanced rapidly over the past 15 years. Especially in the treatment of prolactinomas medical treatment plays an important role. An increasing number of PRL lowering drugs has been investigated and are available. Nearly all of them are dopamine receptor agonists. Dopamine inhibits the PRL secretion (van Maanen and Smelik 1968, Macleod and Lehmeyer 1974). Most experience has been obtained with bromocriptine (Parlodel R , Sandoz) which drug was developed by Flückiger (Flückiger and Wagner 1968, Thorner et al 1980b, Flückiger 1985). This ergot derivative with long-acting dopamine agonistic properties (Corrodi et al 1973, Fuxe et al 1974, Calabro and Macleod 1978) suppresses PRL secretion by a direct effect at the level of the lactotrophs via the dopamine receptor (Pasteels et al 1971, Tashjian and Hoyt 1972, Mashiter et al 1977). Prolactin secreting adenomas contain a high affinity dopamine receptor which interacts with both agonists and antagonists in a similar fashion as reported for the anterior pituitary gland of several species (Cronin et al 1978, 1979 and 1980). In the early seventies a favourable effect in patients with galactorrhoea and/or amenorrhoea and hyperprolactinaemia has been described with a fall in PRL levels and restoration of gonadal function (Varga et al 1972, Besser et al 1972, Del Pozo et al 1974, Thorner et al 1974, Rolland et al 1974). Later on bromocriptine appeared to cause tumour size reduction in animal models (Macleod and Lehmeyer 1973, Lloyd et al 1975) and in patients with prolactinomas (Corenblum et al 1975, 1978 and 1983, Vaidya et al 1978, Ezrin et al 1978, McGregor et al 1979, Landolt et al 1979, Wass et al 1979, von Werder 1982).

3.c.3.b. Pharmacokinetics

The pharmacokinetics of bromocriptine (Moult 1984b) were studied by Schran et al (1980) and Thorner et al (1980b and c). After oral

administration resulting in 28% absorption from the gastrointestinal tract blood peak levels are achieved 1-1.5 hour later. A linear relationship was found between dose and serum level. The half-life appeared to be 3 hours and no bromocriptine was detectable after 11-14 hours. The fall of plasma PRL concentration starts within one hour with a nadir at 7 hours to about 15% of the pretreatment value (Fig. 8; Thorner 1980b and c). Two major metabolites of bromocriptine have been identified. A daily dose of 5-7.5 mg bromocriptine is commonly used, but in patients with plasma PRL concentrations of more than 1000 ng/ml these levels do not always return to normal even with a dose of 40 mg per day (von Werder 1984). Bromocriptine is almost exclusively excreted in the feces (98%), and there is a 21 hour lag time representing intestinal passage of the drug. Total urinary excretion is 2% of the dose (Vance et al 1984).

3.c.3.c. Effects on plasma prolactin levels

The aims of medical treatment are 1) suppression of hyperprolactinaemia and galactorrhoea 2) restoration of gonadal function and 3) reduction of tumour size. Bromocriptine appears effective in all forms of hyperprolactinaemia, physiological (Rolland and Schellekens 1973) or pathological (Moult 1984b). In patients with prolactinomas there is a broad spectrum of responsiveness. A significant decrease of plasma PRL levels occurs in 90-95% of the patients (del Pozo et al 1983, Landolt 1985a, and Landolt and Froesch 1985b). Normalization of plasma PRL, however, has been reported in 65-95% of women with prolactinomas (Fossati et al 1976, del Pozo et al 1983, Hall et al 1984a, Winkelmann et al 1984, Vance et al 1984, Landolt 1985a, von Werder 1984, Kuhn et al 1985, Robbins 1986), and in 54% of men with hyperprolactinaemia persisting after surgery (Grisoli et al 1980). Reviewing the literature, Vance et al (1984) reported normalization of PRL in 229 (82%) of 280 hyperprolactinaemic women and in 66 (72%) of 92 patients with macroprolactinomas. There is a trend towards higher PRL normalization rates in patients with lower than in patients with higher (> 160 ng/ml) plasma PRL concentrations (del Pozo et al 1983).

3.c.3.d. Effects on clinical symptoms

As a consequence of (near-)normalization of plasma PRL concentration galactorrhoea ceases in 95% of the patients (del Pozo et al 1983) and gonadotrophin pulsatility returns (Bohnet et al 1976, Moult 1984b) followed by restoration of LH positive feedback (Aono et al 1979) and rise in circulating oestradiol levels. Vaginal lubrication and libido improve during treatment and the risk of premature osteoporosis will be diminished. Within 3 months after start of treatment menstruation recurs in 70% of the patients but delays up to 10 months have been reported (Thorner et al 1978 and 1980, Moult 1984b). Ultimately in about 90% of the patients regular menstruation is seen within one year (Thorner and Besser 1978, Thorner et al 1980b, Franks and Horrocks 1984). The delay in the recurrence of menstruation was greater in patients with macroprolactinomas but unrelated to the duration of amenorrhoea. In the series of 31 patients of Thorner and Besser 26 (84%) conceived on bromocriptine. In 54 infertile women Bergh and Nillius (1982) reported a pregnancy rate of 91% and an ovulation rate of 94% by bromocriptine treatment. Rolland (1980) observed pregnancies in 80% of 57 infertile women mostly with microprolactinomas within 10 months. In a review of the literature Weil (1986) reported an average pregnancy rate of 81% in 614 hyperprolactinaemic infertile women in 26 studies.

3.c.3.e. Effects in men with prolactinomas

In men with prolactinomas (mostly macroadenomas) plasma testosterone may increase during treatment (Prescott et al 1982, Mancini et al 1984, Franks and Horrocks 1984, Vance et al 1984, Muhr et al 1985) but recovery of potency occurs mainly after normalization of plasma PRL levels. Thorner and Besser (1978) and del Pozo et al (1983) reported normalization of libido in about 80% of the subjects. In patients with persisting gonadotrophin deficiency bromocriptine treatment has been combined with testosterone replacement (Nagulesparen et al 1978, Moult 1984b). Sperma counts may increase during treatment (Mancini et al 1984, Murray et al 1984).

3.c.3.f. Effects on tumour size

Reduction in tumour size occurs in 30 to 100% of patients with prolactinomas. In five reviews of series of patients the incidence of tumour regression has been reported to be 90% of 49 (Bergh and Nillius 1982a, Nillius and Bergh 1984), 73% of 105 (Frantz 1982), 58% of 71 (del Pozo et al 1983), 62% (Landolt 1985a), and 73% of 147 patients (Robbins 1986). Improvement or normalization of campimetric lesions during therapy was recorded in 26 (59%) of 44 subjects (del Pozo et al 1983). Muhr et al (1985) reported reduction of tumour size measured by CT-scan in 8 of 10 males with prolactinomas and normalization or improvement in 13 of 15 men with visual field defects.

The extent of reduction in tumour size varies between 30-99% (Boyd 1982, Wolleson et al 1982, Liuzzi et al 1985, Landolt et al 1984b and 1985c, Molitch et al 1985) with an average of about 50% (Landolt 1985). In about 5% of the patients an increase in tumour size has been observed during bromocriptine treatment, sometimes despite a significant fall in serum prolactin (Franks and Horrocks 1984). These patients may have had non-hormone-secreting tumours causing secondary hyperprolactinaemia by compression of the pituitary stalk. An escape after remission of prolactinomas and during continuing treatment with bromocriptine has been shown in a few patients (Breidall et al 1983, von Werder 1984, Wass 1984c). In case of morphological increase in tumour size during bromocryptine therapy an intratumoural haemorrhage has to be considered (Hall et al 1984a). Until now tumour response can not be predicted.

Tumour shrinkage can occur rapidly within 3 days (Thorner et al 1980a) or more delayed. Shrinkage of tumour occurs nearly almost in the first 6-12 months of treatment (Thorner et al 1981, Johnston et al 1983, Warfield et al 1984, Landolt et al 1985c, Robbins 1986). Improvement of disturbed visual fields and endocrine functions may occur in up to 50% of patients with macroprolactinomas. Molitch et al (1985) even reported an improvement of visual fields in even 9 of 10 patients in whom they were abnormal; Sieck et al (1986) observed improvement of visual abnormalities in 7 of 12 patients within one week. However, in some patients, first neuroradiological signs of

tumour shrinkage can be observed only after prolonged medical treatment up to 2 years (Liuzzi et al 1985). If the tumour shrinks during the first year, further tumour reduction after the first year appeared to occur in only 2 of a series of 38 patients (Liuzzi et al 1985).

A reduction of plasma PRL levels can occur without reduction in tumour size and vice versa (von Werder 1984, von Werder et al 1985). On the long-term sella-volume reduction and/or recalcification of the bony structures of the sella has been observed (Liuzzi et al 1985). Tumour size reduction seems to occur more frequently in patients with microprolactinomas than in those with macroadenomas as reported for the former in 44 of 46 patients (96%) by Cecotto et al (1985). Addition of tamoxifen may improve responsiveness to bromocriptine (de Quijada et al 1980, Lamberts et al 1980b and 1982b, Volker et al 1982). Recently Koizumi and Aono (1986) reported two patients who could not tolerate a large dose of bromocriptine but became pregnant after combined treatment with bromocriptine and tamoxifen.

3.c.3.g. Histological effects

Bromocriptine induces inhibition of prolactin gene transcription from DNA to messenger RNA (Maurer 1980 and 1981). The cells present the morphological pattern of reduced protein synthesis. Histological and cytological features of prolactinoma cells during treatment are extensively described in some reports (Nissim et al 1982, Tindall et al 1982, Landolt 1984a, 1985a and c, Derome et al 1984). Shrinkage of the nucleolus, the rough endoplasmatic reticulum and the golgi system has been shown. The cells shrink by an average of 25% in comparison with cells in untreated samples (Landolt 1984a, 1985a and c). Cell death is rare. In a study of Gen et al (1984 and 1985) microscopic examination of biopsy specimens from only one bromocriptine treated prolactinoma showed signs of tumour cell necrosis.

3.c.3.h. Praeoperative treatment with bromocriptine

Landolt et al (1982 and 1985a) reported increased perivascular fibrosis when patients had been treated for periods of 3 months or

longer giving the adenoma a rubber-like consistency and rendering surgical removal more difficult. After pretreatment with bromocriptine for one year or more normalization of PRL levels could not be achieved surgically. These findings are not completely confirmed by other studies (Faglia et al 1983). Derome et al (1984) found a slightly but significantly lower surgical cure rate of 65% in bromocriptine-treated patients as compared with 78% in non-pretreated cases; there was no such difference between pretreated and non-pretreated acromegalic patients. On the other hand Ludecke et al (1983) found that normalization of PRL levels by transsphenoidal surgery was slightly more common in patients who had been pretreated with bromocriptine (58%) than it was in untreated patients (46%) with macroprolactinomas. Also our surgeons did not meet specific problems in bromocriptin-pretreated patients.

3.c.3.i. Effects after withdrawal of bromocriptine

A complete (morphological) remission of prolactinomas obtained by medical treatment is rare. Very few patients with large prolactinomas enjoy continuing benefit after stopping bromocriptine treatment (Thomas and Hall 1983, Liuzzi et al 1985). Reexpansion may occur within a few days after dopamine agonist withdrawal with shrinkage again after reinstitution of the treatment (Thorner et al 1981). This can not be explained by a cytotoxic effect of dopamine agonists. However, persisting (partial) suppression of PRL secretion after terminating long-term treatment has been observed in some patients with macro-and microadenomas (Eversmann et al 1979, Hall et al 1984a, Moriondo et al 1983 and 1985, Robbins 1986). For 6 to 25 months after withdrawal of bromocriptine treatment Winkelman et al (1984) found persisting bromocriptine induced normoprolactinaemia in 7 of 38 patients (18%) with micro-and macroprolactinomas. The remaining patients showed an increase of serum PRL after bromocriptine withdrawal, the extent of which was dependent on the duration of the preceding normoprolactinaemia. In the patients with persisting partial PRL suppression they found a significant correlation between the degree of persisting PRL suppression and the duration of bromocriptine

therapy, such in agreement with the findings of Eversmann et al (1979). However, in contrast to the findings of Eversmann the suppression of serum PRL did not correlate to the value of PRL levels before treatement. In patients with microprolactinomas Moriondo et al (1983 and 1985) observed persisting normoprolactinaemia in 5 out of 30 patients (17%) after stopping bromocriptine treatment. On the other hand Bergh and Nillius (1982) reported a return of PRL concentrations to pretreatment values in 42 of 49 patients (86%) within 2 months of stopping bromocriptine and in 5 other patients PRL levels rose steadily over the next 1-2 years. However the return of hyperprolactinaemia does not necessarily signify reexpansion of the tumour (Franks and Horrocks 1984).

3.c.3.j. Effects in patients with non-functioning pituitary tumours

Although isolated case reports on the occurrence of tumour size reduction have been published, dopamine agonists seems to be of limited value in patients with "non-functioning" pituitary adenomas (Liuzzi et al 1985). Bromocriptine was effective in reducing tumour size in only 1 out of 20 patients, but did not prevent tumour growth in 4 other patients as was suggested by the worsening of visual fields (Verde et al 1985). Grossman et al (1985b) found no significant tumour shrinkage in 15 patients with large "non-functioning" pituitary tumour. In a small comparative study Pullan et al (1985) observed that macroprolactinomas frequently shrink rapidly when treated with bromocriptine, whereas non-functioning tumours seldom show such a dramatic response. Bevan and Burke (1986) found that non-functioning tumours possess high affinity membrane-bound dopaminergic binding sites similar to those in normal pituitary and macroprolactinomas, but apparently fewer in number than in the latter resulting in absence of tumour regression.

3.c.3.k. Bromocriptine induced pregnancy

After institution of bromocriptine treatment in infertile women with (micro)prolactinomas about 80-90% will become pregnant within one

year (Rolland 1980, Bergh and Nillius 1982). Weil (1986) found an average success rate of 81% (range 56-100%) in 26 studies concerning totally 614 patients. Bromocriptine crosses the placenta but no teratogenic effects of bromocriptine have been observed (Thorner et al 1980b, Weil 1986). Data collected from 1410 pregnancies in which bromocriptine had been given during early pregnancy showed a normal incidence of congenital malformations (Turkalj et al 1982). No adverse effects upon placental function or increased abortion rate has been found. Plasma oestradiol and progesterone levels appeared not significantly different from those in patients with spontaneous pregnancies (Rolland 1980). Multiple pregnancy rate (1.77%) was reported not to be increased. The body weight of the children was found to be normal (Rolland 1980). Bromocriptine treatment during pregnancy appeared not harmful to the fetus (de Wit et al 1985, Weil 1986), but is not necessary because the risk of tumour complications in carefull supervised patients is low (Nillius and Bergh 1984). Visual field defects were observed in 4 and radiologic progression in 17 out of 300 pregnancies. Later on Nillius et al (1985) reported an incidence of 2% of 3.7% of these complications respectively during 488 pregnancies in 430 hyperprolactinaemic women. Other authors reported a higher incidence (5-35%) especially in untreated patients with macroprolactinomas (see reviews of von Werder et al 1985, Nillius et al 1985, Albrecht and Betz 1986, Weil 1986). Radiotherapy before pregnancy induction is not always effective (Lamberts et al 1977 and 1979c). If necessary, bromocriptine treatment may be reinstituted or a transsphenoidal operation may be performed (Nillius and Bergh 1984, Weil 1986).

Although some authors have discouraged patients with prolactinomas to breast-feed their babies in order to prevent stimulation of the pituitary lactotrophs Nillius and Berg (1984) find no reason to withhold their patients from the advantage of breast-feeding because they did not observe any untoward effects. After pregnancy plasma PRL levels are in some patients reported to be lower than before it, sometimes followed by spontaneous recovery of the menstrual cycle (see review Franks and Jacobs 1983a). In 31 patients with micro-prolactinomas the mean PRL level decreased significantly from 110 to

76 ng/ml after bromocriptine-induced pregnancy (Nillius and Bergh 1984). Franks and Jacobs (1983a) and Franks and Horrocks (1984) observed "cure" in about half of 28 patients with microprolactinomas after bromocriptine induced pregnancies, but the PRL response to TRH remained abnormal. Rolland (1980) observed a spontaneous second pregnancy without reinstitution of bromocriptine induction treatment in 4 out of 19 patients.

3.d. Side effects

3.d.1. Surgery

Since the use of high dose corticosteroid treatment peroperatively (Kendall 1949) the mortality rate of subfrontal hypophysectomy has been decreased significantly (Post 1986). Van der Zwan (1971) reported a mortality rate of 5% in 80 patients operated in Leiden and Rotterdam between 1959 and 1970. After the introduction of microscopical transsphenoidal surgery the risk of cerebral bleeding, cerebral oedema and death decreased further in comparison with the results of the subfrontal approach of pituitary tumours. The operative mortality of transsphenoidal surgery in earlier large series ranged between 0 and 2% and is at present about 0.25% (range 0-1%) (Wilson 1984, Post 1986, Laws and Kern 1979c, Lüdecke et al 1984).

The complications of transsphenoidal surgery in the first one hundred of our patients since 1969 are summarized in Table 8. Sixty patients had no complication at all, while 8 patients needed pitressine for less than one day. Sixteen patients developed diabetes insipidus for more than one day, but in only one patient permanent substitution with a synthetic analogon of antidiuretic hormone (ADH) was necessary. In 2 of the 11 patients with CSF rhinorrhoea a reoperation was necessary to stop the leakage of liquor. Six out of these 11 patients with CSF rhinorrhoea developed meningitis. Severe sinusitis occurred in 2 patients. Six patients had nasal complications. Three patients showed transient neurological deficits: one with diplopia, two with hemiparesis. A more serious complication has been observed in an acromegalic patient with carotid-cavernosus

Table 8. Incidence of various kinds of complications in 100 transsphenoidally operated patients.

	In total	Acromegaly	Prolactinomas and non-functioning tumours	Suprasellar tumours
Patients	100	48	47	5
Mortality	0	0	0	0
No complications	60	25	32	3
Diabetes insipidus (< 1 day)	8	6	1	1
Transient diabetes insipidus (> 1 day)	15	6	9	0
Permanent diabetes insipidus	1	0	1	0
CSF rhinorrhoea without meningitis	5	5	0	0
CSF rhinorrhoea with meningitis	6	2	3	1
Sinusitis	2	2	0	0
Epistaxis	4	3	1	0
Anosmia	1	1	0	0
Nasal septum perforation	1	1	0	0
(Transient) neurological disturbances (pareses)	3	3	0	0
Carotid-cavernosus shunting	1	1	0	0

shunting. In one patient the sella turcica was not reached by the surgeon. In conclusion, no significant complications occurred in about 70% of the patients, while permanent complications have been encountered in only a few percent (<5%) of them.

The incidence of complications was higher (7 out of 8) in the first years (1969-1972) after introduction of this surgical technique in comparison to later on. It has to be noted that in the first four patients the sella turcica was approached via the sinus frontalis and ethmoidalis, and not transnasally. The overall incidence of these complications in our series are more or less comparable with those reported in the literature. For a more detailed description of specific complications of transsphenoidal surgery I would like to refer to some reviewing articles (Laws and Kern 1979c, Post 1980b and 1986).

Nicola et al (1980) reported occurrence of diabetes insipidus in 11% of 289 patients and Fahlbusch et al (1980) in 17% of 366 patients. However, in the latter study only 3% of the patients needed treatment for diabetes insipidus for more than 3 months. In other studies permanent diabetes insipidus has been found in 4-5% of the patients (Post 1980b and 1986, Lüdecke et al 1984). Derome et al (1980) observed a secondary rhinorrhoea in 1.6% of 1200 transsphenoidal operation for pituitary adenomas; a part of these patients was cured by mere lumbar punctures (or a lumbar drainage); only 1% required a second transsphenoidal surgical procedure while multiple reoperations (2 and 3) were necessary for 2 patients. This author suggested a relationship with radiotherapy (shrinkage of a residual tumoural plug) in patients with occurrence of secondary rhinorhoea later on, i.e. a few weeks or months after surgery. Nicola et al (1980) described this complication in 2.9% of 289 patients and Lüdecke et al (1984) in 4.5% of 224 patients. Furthermore Nicola reported occurrence of neurological disturbances in 3% and death in 1% of his patients. In total, transient complications were observed in 17.6% of his series (20% in patients with macroadenomas) while permanent complications occurred in 6% of his patients, especially in patients with firm tumours.

Nicola et al (1980) reported 4 cases with postoperative visual

deterioration and 3 out of 294 cases with oculomotor palsy. The frequency of meningitis was low (less than 1%). In his review Post (1980b and 1986) reported an incidence of 1% for intracranial hematomas, 1-4% for visual complications, and 1-4% for meningitis. Dobozi and Landolt (1980) reported nasal septum perforation in 30% of their 199 patients, but only 2% of the patients had clinical symptoms. Five percent of their patients judged the nasal function worsened (dry, crust formation), but 17% better because of correction of a nasal septum deviation. Surgical treatment of pituitary tumours often results in a further reduction of the pituitary functions making substitution therapy with hormones necessary, but an amelioration can be obtained in some patients (Happ et al 1980).

The value of cauterizing fluids in the tumour bed is uncertain. Nicola et al (1980) reported a higher frequency of diabetes insipidus after rinsing with cauterizing fluids such as alcohol, but the percentage of patients cured by surgery for microadenomas increased from 61 to 77% by the use of alcohol, but not with Zenker solution (64% cure). Bertrand et al (1983) found no correlation between the appearance of transient diabetes insipidus and the use of 94% ethanol irrigation of the removal cavity. Giovanelli et al (1980) stated that preventive swabbing of the residual cavity with chemical agents does not seem to be effective in reducing the failure rate; because of this point of view they have never used chemicals. Our surgeons have used Zenker solution in the first few patients, but abandoned this additional treatment because of a serious complication in one patient (paresis of the nervus abducens postoperatively) and because of their impression that the solution was not effective.

3.d.2. External radiotherapy

Conventional external radiotherapy is usually considered to involve a low risk for the patients. Early radiation effects of the cranium and soft tissues occurred in up to 40% of patients (Baglan and Marks 1981). Indeed serious complications are rare, but occur as reported in 2-3% of a series of 249 patients by Landolt (1980). Serious complications are death by cerebral radionecrosis (0.8%), blindness,

motor hemiparesis, extrapyramidal motor symptoms by damage of the temporal lobes, personality changes and development of sarcomas (Landolt 1980, Sheline and Tyrrell 1984). Histologically the vascular changes with partial to complete fibrinoid necrosis are the most striking. Small hemorrhages occur in the area of necrotic vessels accompanied by a inflammatory reaction. There is an increasing risk for complications in patients treated with total doses above 50 gray or after irradiation with fraction of more than 2 gray as occurred in one of our patients irradiated elsewhere. Symptoms appear generally 1-2 years after the radiotherapy (Landolt 1980).

More common but often not recognized is a stepwise increasing loss of recent memory, sometimes many years after the radiotherapy, as observed in 15 % of the patients in our own series (Chapter VIII). Other complications that are not uncommon consist of transient headache and of permanent hormonal insufficiency caused by radiation damage of the hypothalamus and/or the normal pituitary tissue (Chapter VIII, Eastman et al 1979, Snyder et al 1986).

Proton beam irradiation is a specific form of external radiotherapy and only applied in a few centres in the world. Headache and also hormonal insufficiency are reported as side effects.

3.d.3. Dopaminergic drugs

In our experience the most common side effects of dopaminergic drug such as bromocriptine are nausea and postural hypotension, especially shortly after the initiation of therapy. Robbins (1986) reported the occurrence of nausea in at least 50% of all patients during the first few weeks and vomiting in 5-10%. Nasal congestion seems to be most prominent 2 to 3 hours after taking the drug and may last up to 6 hours (Robbins 1986). Furthermore, in our experience cold-sensitive digital vasospasm is a not uncommon side effect especially in acromegalic patients during the winter time. Thorner (1980b) reported an incidence of 30% in acromegalic patients. A lot of other side effects have been reported, as indicated in Table 9. One or more side effects may occur in 27-63% of the patients (Landolt 1985b), but persisting side effects are found in not more than 13% (Johnston et al

Table 9. Reported side effects of bromocriptine

Nausea, vomiting
Postural hypotension
Nasal stuffiness
Exertional headaches
Cold-sensitive digital vasospasm
Dyspepsia, constipation, gastrointestinal bleeding
Alcohol intolerance
Increased arousal state, insomnia
In Parkinson patients : dyskinesia
erythromelalgia
Neuropsychiatric disorders, depression, blurring of vision
Photosensitive skin rashes
In rats: Increased incidence of endometrial and myometrial tumours

1983), while in only 3% of the patients bromocriptine treatment has to be stopped (Landolt 1985b) because of persisting side effects. The chance on (initial) side effects such as nausea and postural hypotension can be decreased by starting with a low dose (1.25 mg per day) taken before sleeping time. Subsequently the drug may be taken with meals, increasing the dose gradually in one week to 2 times 2.5 mg. Also a new injectable form of bromocriptine may be helpful in patients with pronounced nausea (Landolt 1984b, Rolland et al 1984). Relatively late side effects of dopaminergic drugs are neuropsychiatric disorders. Turner et al (1984) describes psychotic reactions in 8 out of 600 patients. Oversuppression of PRL secretion may impair fertility (Moult 1984b). For a more detailed description of side effects I refer to other reports (Parkes 1979, Thorner et al 1980b, Thomas and Hall 1983, Moult 1984b, Vance et al 1984, Harris 1984, Robbins 1986).

Other dopaminergic drugs (such as mesulergine, pergolide, lisuride, terguride) have more or less the same PRL lowering effects (Kendall-Taylor et al 1983, Lamberts et al 1984b, Eskildsen et al 1984, Grossman et al 1985a, Thomas and Hall 1983, Dallabonzana et al 1985). No difference in side effects between lisuride or metergoline and bromocriptine have been reported (Stracke et al 1985, Thomas and Hall 1983). Grossman et al (1985a) observed that mesulergine and pergolide caused generally similar side-effects compared to bromocriptine. Pergolide showed more side effects according to Boyd (1982), but it has the advantage of being dosed once daily (in the evening). Furthermore, some patients who are intolerant to bromocriptine may accept pergolide (Franks et al 1983b) or as in our experience mesulergine (Lamberts et al 1984b). More clinical experience is needed before the value of these new drugs is established.

3.e. Recovery of vision

Both subfrontal and transsphenoidal decompression can result in recovery of vision (Post 1980c, Teasdale 1983). The improvement in visual deficits after transcranial removal of pituitary adenomas is

comparable to that achieved by the transsphenoidal route; however, there is some risk that as many as 7 % of patients will have worse vision following manipulation of the optic nerves (Post 1980c). Indeed, sometimes a rapid improvement is noticed immediately after operation. Visual evoked potentials (VEPs) have been measured in patients undergoing removal of adenomas, with demonstration that there is immediate improvement in the VEPs during decompression (Feinsod et al 1976). Marked improvement is more likely when the history is short and when the loss of vision before surgery is not extensive. Visual improvement can be expected in up to 85% of the patients (Fahlbush 1980, Post 1980c). In patients with prolactinomas also a rapid recovery of vision within a few days can occur during medical treatment (Thorner 1980a). Visual improvement has been reported to occur in 50–90% (see section 3.c.3.f).

Results of transsphenoidal surgery with regard to vision in patients operated upon in our university hospital have been reported by Blaauw et al (1985 and 1986). Sixty out of 204 transsphenoidally operated patients with a pituitary tumour had a disturbed visual function before operation. Visual acuity and visual fields measured both before surgery and about one year after surgery were compared. By preoperative examination a significant correlation appeared between the disturbances of visual acuity and of visual fields. Seriously decreased visual acuity occurred mainly in patients with more than 50% loss of the visual fields. After surgery the eyesight normalized in 16 patients (27%). In 45 (63%) out of 72 eyes with diminished visual acuity an improvement and in 7% a deterioration of the visual function was observed. Visual field loss was present in 102 eyes and this improved in 71 eyes (70%). Improvement of either visual acuity and/or visual field was found in 84 eyes (78%). In general, patients with less visual disturbances preoperatively had a better chance of normalization, while patients with extensive visual disturbances showed more frequently no improvement or even deterioration. So it appeared that patients with a score of less than 30% preoperatively had complete recovery of the visual disturbances. On the other hand the results of surgical treatment seemed unpredictable in patients with serious visual disturbances (more than 30% loss of function)

before treatment, some of them showing rapid (complete) improvement other no improvement. The kind of response was not correlated with age, sex, duration of symptoms, extent of suprasellar expansion or bilateral occurrence of disturbances.

3.f. Histological examination

Pituitary tumours are mainly adenomas. Carcinomas of the pituitary gland and metastases occur rarely (Myles et al 1984, Scheithauer et al 1985, Gasser et al 1985, Landgraf et al 1985).

Prolactinomas are mostly chromophobe adenomas, GH producing tumours acidophyl and ACTH producing tumours basophyl adenomas. However chromophobe adenomas or chromophobe tumour components (mixed adenomas) occur in about half of the patients with acromegaly (Chapter IV and VII) and in a significant part of patients with Cushing's disease. On the other hand 3 of 25 operated patients with a prolactinoma appeared to have by histological examination a mixed acidophyl-chromophobe adenoma, one patient a basophyl adenoma, another a mixed chromophobe-basophyl adenoma and one a neuroectodermal tumour, while 19 patients showed a chromophobe adenoma (2 cystic). Non-functioning tumours are mostly also chromophobe adenomas or oncocytic adenomas. In the last 10 years immunohistochemical examination of pituitary adenomas appeared to be more important than classic morphologic examination (Chapter VII, Lamberts et al 1982c, 1983, 1985a, 1986a, Nieuwenhuijzen Kruseman 1983).

Pituitary tumours are often circumscript (micro)adenomas, but tumours can be invasive (especially in men and in patients with large tumours) or diffuse in 10-35% of the cases (Saeger 1983, Randall et al 1986). In the latter situation transsphenoidal adenomectomy will be insufficient (Lamberts et al 1980f).

3.d. Present management of pituitary tumours

A number of factors play a role in the decision on the treatment of patients with pituitary tumours (Table 10). Important factors are treatment efficacy, side effects, tumour size, age, hormonal

Table 10. Factors, which has to be considered in the choice of therapy for patients with pituitary tumours.

1.a. Treatment efficacy.
  b. Side effects and risks of treatment.
  c. Previous treatment.
2. Age and general health of the patient.
3. Tumour size and location.
   Extrasellar extension with or without visual disturbances.
   Time period of visual loss.
   Presence of CSF leakage or pituitary apoplexia.
4. Hypersecretory status of tumour (PRL, GH, ACTH, TSH, LH, α-subunits).
5. Presence or absence of hormonal insufficiency.
   Infertility or fertility.
6. Type of the tumour (cystic, solid).
   Need for histology in patients with uncertain diagnosis.
7. Diagnosis by chance or because of symptoms.
8. Therapeutic goals: a. reversal of hypersecretory syndrome
   b. preservation of vision
   c. preservation of fertility and normal pituitary function
   d. control of tumour growth

hypersecretion, and last but not least the therapeutic goals. Therapeutic goals may be preservation of vision, control of tumour growth, reversal of hypersecretory syndrome, preservation of normal pituitary function and fertility. All these factors being more or less related with each other have to be considered in choosing the optimal treatment for one particular patient. On the other hand, each centre uses its own general treatment guidelines depending on the historical background of the institution, the number of modes of treatment available and its present fields of research interest. The decision to utilize a particular form of primary therapy varies from centre to centre. Today a practical problem is still the lack of randomized studies comparing the efficacy and side effects of two or more kinds of treatment. Therefore, the choice of treatment is mostly based on the results of non-randomized studies with one treatment modality in certain centres. For an overview of the general considerations in the treatment of pituitary tumours we would like to refer to some overviews (Kohler 1980, von Werder 1984, Quabbe 1984, overviews of Belchetz 1984, Sterman and Schlechte 1986).

In a considerable proportion of patients with prolactinomas cure can be obtained by surgery. This is especially valid in patients with microprolactinomas. However, postoperatively about 40% of these patients have still elevated plasma PRL levels, while 30% of the patients with normal postoperative PRL levels show recurrence within 5-10 years after surgery. One of the problems of surgical treatment can be the fact that after exploration of the sella turcica a circumscipt adenoma is sometimes not visible or that nests of tumour cells or hyperplasia may occur in the paraadenomatous tissue; in addition an adenoma can be also invasive. On the other hand medical treatment with bromocriptine causes prompt normalization of PRL levels and restoration of gonadal function and fertility in the majority of the patients with microadenomas. In patients with macroprolactinomas bromocriptine can cause significant reduction of tumour size within a few days without serious side effects or damage of normal pituitary function. However, the treatment is not sufficiently effective in all patients and has to be continued for long-term control of tumour growth. On the other hand, thus far no serious side effects of

treatment with bromocriptine for over 10 years have been reported and persisting suppression of prolactin secretion after withdrawal of long-term treatment with dopamine agonists has been observed in some patients. Short-term pretreatment with bromocriptine may facilitate surgery by tumour size reduction but according to some authors long-term pretreatment may jeopardize the results of subsequent surgery by changes in mechanical properties of the tumour. However, our surgeons did not observe specific problems in bromocriptine-pretreated patients. Primary surgery remains the first choice of treatment in patients with cystic or very large tumours with neurologic disturbances. The choice of surgical approach depends on the size and location of the tumour. External radiotherapy is less effective in patients with prolactinomas. Only in a few patients normal plasma PRL levels were reached mostly years after irradiation. Therefore radiotherapy is certainly not suitable for obtaining short-term effects as for induction of pregnancy. However, conventional external radiotherapy is an excellent adjunctive therapy for preventing recurrence of tumour or for long-term tumour growth control, especially in patients in whom surgical or medical treatment proved to be insufficient. In addition the area of irradiation can be smaller after (partial) shrinkage of the tumour induced by bromocriptine. Ultimately, no treatment can be proposed in patients with microprolactinomas without serious complaints or infertility, especially in patients above 40 years of age, because most microprolactinomas do not progressively increase in size or activity. Bone mineral content has than to be checked for development of osteoporosis.

In Rotterdam we prefer to use bromocriptine as primary mode of treatment for patients with prolactinomas with the exception of patients with very large tumours and neurologic disturbances, while external radiotherapy is used as adjunctive treatment especially in patients with macroprolactinomas.

In patients with acromegaly microadenomas without enlargement of the sella turcica are rare, but the macroadenomas extend less frequently extrasellarly than other macroadenomas (prolactinomas and non-functioning tumours). Therefore and because it would appear that

the disease rarely spontaneously "burns out" as suggested in the older literature, cure by transsphenoidal selective adenomectomy still is the main goal of treatment. However, using strict criteria (normal TRH-test, and a value of GH less than 2 ng/ml after an oral glucose load) complete cure occurred in only 8% of the patients. But at present transsphenoidal surgery in combination with postoperative external radiotherapy is the most effective way of treatment certainly with respect to long-term results and survival. It should be pointed out that the results with heavy-particle or proton-beam therapy for intrasellar tumours are nearly comparable with the surgical results and that lowering of plasma GH levels occurs more rapidly than after conventional external radiotherapy. Although medical treatment with bromocriptine causes a significant decrease of plasma GH concentration in the majority of the patients, bromocriptine usually does not reduce GH levels to normal. An escape phenomenon (increase of plasma GH concentration after initial decrease) or even an initial increase of plasma GH during treatment may occur in a few patients. In general bromocriptine or other dopaminergic drugs are given as adjunctive therapy in patients who continue to have elevated GH levels after surgery and radiotherapy. External radiotherapy as single primary treatment is less effective in rapidly lowering the plasma GH levels than combined therapy and does not prevent or combat cardiovascular disease effectively. However, in older patients with surgical risks and relatively low GH levels external radiotherapy can be applied as a primary form of treament. It may be concluded that 20-40% of the patients still have GH levels of more than 5 ng/ml after 10 years of treatment and that complete cure of acromegaly is probably not the rule with present forms of treatment, even when applied in combination. Treatment with somatostatin analogues may resolve this problem.

In Rotterdam, acromegalic patients are generally treated with transsphenoidal surgery followed by irradiation and with bromocriptine as adjunctive therapy. Recently it appeared in our hands that primary medical treatment with somatostatin analogues is an very effective treatment, but his form of treatment has at present the disadvantage that it has to be administered by multiple subcutaneous injections

daily. After future introduction of slow release depotpreparations treatment with somatostatin analogues, possibly in combination with a dopamine agonist, may be expected to become the primary form of treatment.

Patients with Cushing's disease mostly have microadenomas and selective transsphenoidal adenomectomy is the first choice of treatment. However, in a substantial number of patients additional external pituitary irradiation and/or bilateral adrenalectomy appeared necessary because of postoperatively persisting elevated cortisol secretion rates or late recurrences. Medical treatment with bromocriptine, serotonin antagonists as cyproheptadine or adrenal blockers (metyrapone, aminoglutethimide, ketoconazol etc.) are only of temporary value in a few patients.

Patients with "non-functioning" tumours mostly have macro-adenomas frequently with extrasellar extension. Surgical debulking of tumour mass followed by radiotherapy is the treatment usually chosen. Subtotal removal followed by radiation therapy is often safer and just as effective as an attempt to complete surgical removal. Incidental non-functioning tumours may become surgical candidates if there is evidence of progressive growth. Radiotherapy is very important with respect to preventing recurrences and for long-term control of tumour growth. Medical treatment is of less value in the treatment of non-functioning tumours. Preoperative radiation does not preclude surgical intervention, but it can make selective removal of an adenoma more difficult because of scarring and increased difficulty in distinguishing adenoma tissue from the normal gland.

In conclusion: Surgery, mostly in combination with postoperative irradiation, is the first choice of treatment in patients with acromegaly, Cushing's disease, and non-functioning tumours, while primary medical therapy has become generally accepted in patients with prolactinomas. Unfortunately, no single form of therapy is uniformly successful in all patients. Therefore multidisciplinary treatment is very important. It has to be pointed out that, although more sophisticated hormonal and radiological diagnostic procedures have resulted in the detection of pituitary tumours at an earlier stage and despite impressive improvements in operative, radiation and drug

therapy over the last 15 years, complete permanent cures are still not achieved in some patients with prolactinomas and in a significant number of patients with Cushing's disease and acromegaly. Therefore, it is to be hoped that new modes of treatment, especially medical ones, will be developed.

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CHAPTER III

THE IMPORTANCE OF PITUITARY TUMOUR SIZE IN PATIENTS WITH HYPERPROLACTINAEMIA IN RELATION TO HORMONAL VARIABLES AND EXTRASELLAR EXTENSION OF TUMOUR

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SUMMARY

In sixty-two patients with hyperprolactinaemia and a pituitary tumour (without growth hormone excess) we studied the importance of the size of the sella turcica plus extrasellar tissue (if present) in relation to the prevalence of extrasellar extension and impaired hormonal reserve. A lateral tumour area of 3 cm^2 turned out to be a critical value both in the development of extrasellar extension of the pituitary adenoma as well as in the development of insufficiency of the pituitary-gonadal, - thyroidal and -adrenal axis. Extrasellar extension occurred in 44% of the patients. Below the value of 3 cm^2 ($n = 36$) there was only one patient with radiologically detectable significant suprasellar extension. Above the value of 3 cm^2 twenty-four out of twenty-six patients had significant extrasellar extensions at radiological and perimetrical examination. Taking the size of the sella only, extrasellar extension occurred in one third of the cases with a sellar size between 2 and 3 cm^2. There was a strongly positive correlation between (log) tumour size and (log) basal prolactin level ($P < 0{\cdot}0005$). LH, FSH, TSH and ACTH secretion were evaluated by the consecutive administration of LHRH, TRH and metyrapone. Negative correlations were observed between (log) pituitary tumour size and (log) basal LH, FSH and TSH (respectively $P < 0{\cdot}005$, $P < 0{\cdot}005$, $P < 0{\cdot}025$) and between (log) tumour size and (log) Δ LH, Δ FSH, Δ TSH and the plasma Compound S concentration after metyrapone (respectively $P < 0{\cdot}0005$, $P < 0{\cdot}005$, $P < 0{\cdot}01$, $P < 0{\cdot}025$). Again the

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value of the index of tumour size (3 cm^2) proved to be critical. Above 3 cm^2 a highly significant increase in the incidence of insufficient responses to the stimuli mentioned was observed. Pituitary tumour size may be one of the most important indices used in the management of patients with hyperprolactinaemia and a pituitary tumour.

The incidence of extrasellar extension and hormonal insufficiency is higher among patients with large rather than small adenomas. It is not known however, whether there is a critical tumour size above which these problems are more likely to occur. In the galactorrhoea-amenorrhoea syndrome associated with hyperprolactinaemia, one distinguishes patients with a normal sized sella (with or without localized abnormalities) and an enlarged sella (Child et al., 1975; Kleinberg et al., 1977; Boyd et al., 1977; Asfour et al., 1977). We know of no previous data, however, on the quantitative aspects of the relationship between tumour size and the incidence of extrasellar extension or endocrine deficiencies.
We have studied sixty-two patients with hyperprolactinaemia and a pituitary tumour investigating the significance of tumour size in relation to extrasellar extension, plasma prolactin levels and anterior pituitary hormone secretory reserve.

PATIENTS, MATERIALS AND METHODS

Patients
Sixty-two consecutive patients with hyperprolactinaemia and evidence of a pituitary tumour (without growth hormone excess) were investigated. They were referred to our endocrine clinic directly or indirectly by specialists in internal medicine (29%), gynaecology (35%), neurology or neurosurgery (26%) and ophthalmology (10%). In fifty-eight patients the sella turcica was enlarged or asymmetrical on plain lateral and antero-posterior radiography and lateral polytomography, while in four female patients hyperprolactinaemia, galactorrhoea and secondary amenorrhoea were not accompanied by radiological evidence of a pituitary adenoma. Patients with primary hypothyroidism and/or circulating thyroid antibodies were excluded. All patients were untreated at the time of investigation. In thirty the presence of a pituitary tumour was later proven by surgery.

Radiological and ophthalmological examination
All patients were investigated by plain radiography, lateral polytomography (hypocycloidal, sections of 2 mm) and complete ophthalmological evaluation, including visual fields. Further radiological examination consisted of air-encephalotomography and in most cases angiography. Antero-posterior polytomography was only carried out in exceptional circumstances.

For practical, as well as theoretical reasons, we have chosen the largest lateral area of the sella in combination with that of extrasellar tissue (if present) as a measure of tumour size instead of a volume measurement. Direct surface measurement with a planimeter is easily carried out and remains reliable even in cases of large irregular tumour masses. A disadvantage of volume measurement is that on plain frontal radiograms, the sellar floor which is commonly used as a measure of sellar width, is not always clearly visible, either because of problems of projection or because of bone destruction. Furthermore, in patients with sellar asymmetry it turned out to be difficult to estimate consistently the width of the sellar floor because of marked variation in the configuration. In the case of large adenomas, the largest width of the tumour may not coincide with the width of the sellar floor or dorsum. On the other hand, the largest lateral area of the sella and extrasellar tumour tissue was always easily assessable by plain lateral radiography and air-encephalography. In the case of large adenomas the largest lateral area is good measure of the tumour size, because the size of the remaining normal pituitary tissue may be disregarded in relation to the size of the adenoma. Although a microadenoma often influences the outline of the sella by local bulging, it does not fill up the whole sella and the size of the sella therefore does not correspond with that of the microadenoma. For this category of patients we had to compromise and we used the largest lateral area of the sella.

Description of measurements

When there was no extrasellar extension present on the air-encephalogram (AEG) we measured the largest lateral area of the sella turcica on the plain lateral X-ray of the skull, after verification of the true sellar lining by means of lateral polytomography. The upper limit of the sellar content was taken as a straight line between the tuberculum sellae and the tip of the dorsum sellae. In the case of extrasellar extension, measurements were made on the plain lateral X-ray of the skull, obtained at air- encephalography. On this X-ray, the circumference of the tumour was outlined after verification of the true lining of the sella, and the suprasellar and/or infrasellar tumour mass, by means of the tomograms of the AEG. We measured the area of the sella and extrasellar tumour tissue (EST) directly with a planimeter (manufactured by A. Ott, Western Germany, type 30·115), going clockwise with the planimeter from the tip of the dorsum or the tuberculum sellae along the circumference of the sella, and in the case of extrasellar extension, the extrasellar tissue. The measurement of this area was carried out in all patients at least six times and the average coefficient of variation of this estimation was 3·4%. In 100 consecutive patients having neurological investigations for non-endocrine indications the 95% range of sellar area was between 0·7 and 1·4 cm^2. The magnification factor on the plain X-ray varied from 1·05 – 1·1 (distance from focus to film 1 m and from focus to object as large as possible).

Endocrine investigations and assays

Plasma prolactin (PRL) concentrations were determined according to Kwa et al. (1973). Normal basal PRL levels are up to 12 ng/ml in men and up to 15 ng/ml in women. The dynamics of the secretion of TSH, LH, FSH and ACTH were investigated by TRH-, LHRH- and metyrapone-tests. Only the result obtained in untreated patients are reported (in de majority of the remaining patients stimulation tests were not carried out because of the urgency of surgical intervention).

The pituitary-thyroidal axis

This was investigated in forty-one untreated patients (age between 15 and 44 years with the exception of one 67-year-old female) by measuring the response of plasma TSH to the intravenous administration of TRH (400 μg, Hoechst) and the measurement of basal thyroxine and free thyroxine index. Blood for determination of TSH was collected immediately before and 20, 30, 60 and 120 min. after the intravenous administration of TRH. The absolute difference between the highest value for TSH during the test and the basal value (maximal increment) was taken as Δ TSH. Serum TSH was measured with a double-antibody radioimmunoassay technique using the materials supplied by Calbiochem AG Lucerne, Switzerland. TSH was iodinated with the lacto-peroxidase method described by Mukhtar et al. (1975), using 0·4 mol/l instead of 4 mol/l acetate buffer pH 5·4. Preparation MRC 63/38 was used as a human TSH standard. Normal basal values of TSH are lower than 4·9 mU/l (95% range of seventy-one controls). In our laboratory Δ TSH was between 8·1 and 38·7 mU/l in ten females aged 20-40 years, between 7·1 and 16·0 mU/l in eight females aged 40-60 years and between 4·9 and 14·0 mU/l in seven females aged 61-72 years. In eight normal men Δ TSH varied between 6·1 and 13·1 mU/l.

The pituitary-gonadal axis

This was tested in forty untreated patients by intravenous administration of LHRH (100 μg, Hoechst). The maximal increment of LH and FSH during the test was taken as Δ LH and Δ FSH. LH and FSH were measured by double-antibody radioimmunoassay with materials purchased from KABI AB (Stockholm) using preparation MRC 69/104 as a standard (Bangham et al., 1973). Ten units FSH and twenty-five units LH of preparation MRC 60/104 are equivalent ot 0·5 mg LER 907. In our laboratory basal LH levels were between 0·6–2·8 U/l and basal FSH levels were between 1·2–4·2 U/l in the early to mid-proliferative phase of the menstrual cycle of normal women, while Δ LH in response to 100 μg LHRH varied between 3–12 U/l and Δ FSH between 2–8 U/l. In nine normal men basal levels of LH and FSH varied from 0·6–2·5 U/l and from 0·7–3·3 U/l respectively, while Δ LH varied between 3·7 and 8·8 U/l and Δ FSH between 1·3 and 5·5 U/l. Oestradiol-17 β was measured by radioimmunoassay without chromatography (de Jong et al., 1973).

The pituitary-adrenal axis

This was evaluated in forty-six untreated patients by means of a metyrapone test (750 mg every 4 h for 24 h orally). Four hours after the last dose of metyrapone, blood was taken for determination of plasma 11-desoxycortisol (Compound S) with the method of Meikle et al. (1969); who reported normal values of 11-desoxycortisol of 10-30 μg/dl after 6 x 750 mg metyrapone. Our normal values after metyrapone exceeded 15 μg/dl, while the range of 10-15 μg/dl may be regarded as borderline. In ten patients the integrity of the pituitary-adrenal axis was judged by the increase of urinary 17-OHCS only (Liddle et al., 1959). A response to the administration of metyrapone of plasma 11-desoxycortisol of less than 10·0 μg/dl and/or less than a doubling of the urinary 17-OHCS (with an absolute increase of less than 10 mg/24 h) was considered abnormal.

Statistical analysis

Statistical analysis of the data was performed using regression analysis, two-tailed Student's *t*-test or χ^2-test. Partial correlation coefficients were calculated according to Snedecor & Cochran (1967).

RESULTS

Epidemiological and clinical evaluation

Of our group of sixty-two patients twenty-four were male (39%). The mean age of the male patients was 35 years (median = 35 years, range 15−19 years) and of the female patients 32 years (median = 29 years, range 17−67 years). There was a fairly even distribution of the male patients with regard to age in contrast to a preponderance of female patients between 25−35 years.

The referral patterns and clinical features of these patients are summarized in Table I.

Most frequent were complaints concerning menstrual disorders coupled with infertility and headache. Galactorrhoea was (or had been) present in twenty-nine of the thirty-eight females (76%) and in five of the twenty-four males (21%). If the galactorrhoea had been noted by the patient, the mean duration of the galactorrhoea for the female patients was 4·25 years, for the male patients, 6·5 years. The mean duration of the secondary amenorrhoea or oligomenorrhoea, present in twenty-seven patients, was 8 years. Three females had primary amenorrhoea, two menstruated regularly, four were postmenopausal and two had previously a hysterectomy. Because of documented insufficiency of thyroid, adrenal and/or gonadal function, substitution treatment was eventually instituted in 17, 18 and 14 out of sixty-two patients, respectively. In all of them a sellar plus EST size of 3 cm^2 or more was observed with the exception of a 14-year-old boy with a mainly suprasellar-located chromophobe adenoma.

TABLE 1. Referred pattern and clinical features in sixty-two consecutive patients with hyperprolactinaemia and a pituitary tumour.

	Mode of presentation		Overall prevalence	
	n	%	n	%
Menstrual disorders and infertility	20	32	30	79*
Galactorrhoea	3	5	24†	39
Masopathia cytica	2	3	5	
Male infertility	2	3	2	8‡
Impotence	2	3	9	38‡
Headache	9	15	35	56
Visual disturbances	10	16	12	19
Complaints related to endocrine deficiencies	7	11	12	19
Paresis (eye muscles excl.)	3	5	3	5
Cerebrospinal fluid rhinorrhoea;			1	2
Via examination of family with MEN 1 ‖	1	2		
Increase of weight§	4	6	7	11
Total	62	100		

* % of the female patients
† twenty-two female patients (see text)
‡ % of the male patients
§ without hypothyroidism
‖ MEN 1 = Type 1 multiple endocrine neoplasia syndrome

Radiological evaluation

A normal sella was present in 6% (mean sellar size: 1·1 cm^2), sellar asymmetry without enlargement in 19% (mean sellar size: 1·2 cm^2), and an enlarged sella without extrasellar extension in 31% of the patients (mean sellar size: 2·3 cm^2). In 44% of the patients extrasellar extension was present as judged by radiological and perimetrical examination (mean sellar plus EST size: 7·3 cm^2). The lateral area of the sella and EST varied between 1 and 25 cm^2. In our male patients the average sellar and EST size was considerably larger than in the female patients (6·08 ± 5·15 cm^2 as opposed to 2·88 ± 2·74 cm^2; mean ± SD; $P < 0·01$). No correlation was observed between age and sellar and EST size.

Only one of the thirty-six patients with a sellar plus EST size of less than 3 cm^2 showed radiological evidence of suprasellar extension of the tumour (Fig. 1). This 14- year-old boy, to whom we referred above, turned out to have a chromophobe adenoma that was located mainly above the diaphragma sellae while on plain radiography only minor abnormalities of the sella turcica was observed. Apart from this patient, who had bitemporal hemianopsia, three others out of thirty-six patients with a sellar size of less than 3 cm^2, showed slight visual field defects without radiologically evident suprasellar extension. One of these three patients had a partially empty sella.

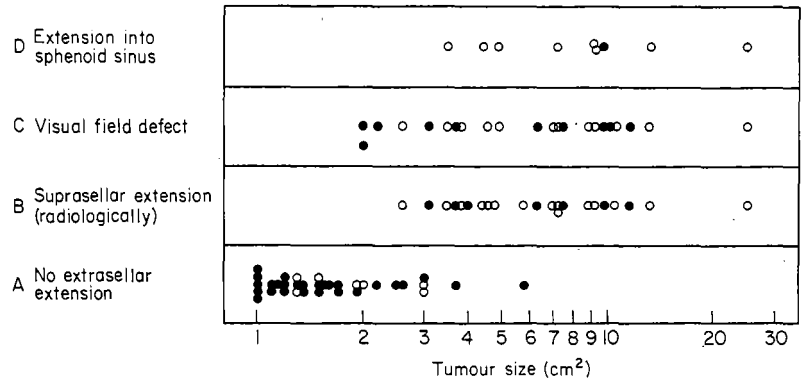

Fig. 1. Tumour size (=lateral area of the sella plus extrasellar tissue) in relation to the presence of extrasellar extension by radiological and perimetrical criteria ($n = 62$). It may be noted that there is some overlap of the groups of patients represented in the upper three panels (B, C, D). A patient may be represented in one, two or three of these panels, while all patients represented in the lowest panel by definition do not occur in one of the other three. Closed symbols represent female patients.

Twenty-four out of the twenty-six patients (92%) with a lateral area of the sella plus EST above 3 cm² showed extrasellar extension (suprasellar and/or into the sphenoid sinus) as shown by radiography and examination of the visual fields. Two out of these twenty-four patients (sellar plus EST size: 4·9 and 10·1 cm²) showed only impairment of the visual fields without radiologically detectable suprasellar extension of the tumour. One patient with a sellar plus EST size of 9·1 cm² showed infrasellar extension of the tumour only. Twenty-one patients had radiological evidence of suprasellar extension of the pituitary tumour, in five cases without impairment of the visual fields. Eight of the twenty-one patients also showed extension of the tumour into the sphenoid sinus.

When we take into consideration the size of the sella only (i.e. without the area of EST if present) six patients with a tumour size of more than 3 cm² turned out to combine extrasellar extension with a sellar size of less than 3 cm² (five of them between 2−3 cm²). This means that of the forty-two patients with a sellar size of less than 3 cm², seven had evidence of extrasellar extension (17%), in contrast to eighteen out of twenty patients with a sellar size exceeding 3 cm² (90%).

In a control group of 100 patients without evidence of pituitary pathology, the sellar floor was not visible on plain frontal radiographs in seventeen cases. In the other eighty-three individuals in this control group there was a good correlation ($r = +0{\cdot}69$, $P < 0{\cdot}0005$) between the results of our area measurement and those of the volume calculation according to Di Chiro & Nelson (½ x length x height x width) (1962). In thirty-six patients with a pituitary tumour and hyperprolactinaemia, in whom we have performed volume measurements, there was a stronger correlation between (log) area and (log) volume ($r = +0{\cdot}96$, $P < 0{\cdot}0005$) than in the control group. In these patients there was a signifi-

cant positive correlation between (log) area and (log) sellar width ($r = +0{\cdot}63$, $P < 0{\cdot}0005$), while in the control group ($n = 83$, $r = -0{\cdot}179$, $P < 0{\cdot}05$) this correlation was lacking.

Endocrinological evaluation

Prolactin levels

Basal PRL levels varied between 17−5000 ng/ml in forty-seven as yet untreated patients, and between 17−27000 ng/ml in fifteen treated patients. In the untreated patients a positive correlation existed between (log) sellar plus EST area and (log) basal PRL (Fig. 2; $n = 47$, $r = +0{\cdot}68$, $P < 0{\cdot}0005$). The same applied to the (log) volume and (log) basal plasma prolactin ($n = 24$, $r = +0{\cdot}66$, $P < 0{\cdot}0005$).

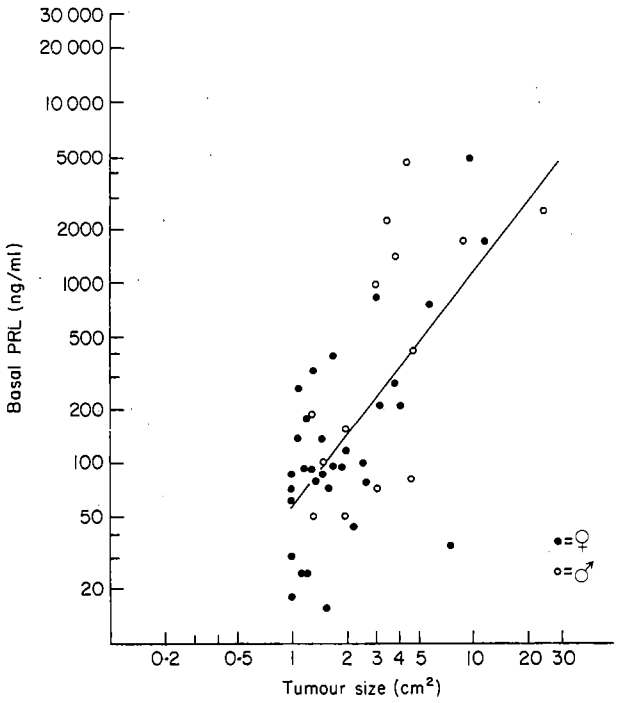

Fig. 2. Correlation between (log) tumour size (=lateral area of the sella plus extrasellar tissue) and (log) basal prolactin level in forty-seven untreated patients ($r = 0{\cdot}68$, $P < 0{\cdot}0005$). Closed symbols represent female patients.

TSH

Basal TSH (log) showed a negative correlation with (log) sellar plus EST size ($P < 0{\cdot}025$). In forty-one untreated patients a negative correlation was shown between (log)

sellar plus EST size and (log) Δ TSH ($P < 0{\cdot}01$; Fig. 3). At sellar plus EST sizes of 3 cm² or more twelve of the sixteen patients (75%) had an impaired response in relation to normal males and females (Table 2) in contrast to six of twenty-five patients with a value less than 3 cm² (24%). Taking a value of Δ TSH of 5 mU/l as the normal lower limit (Hershman, 1978; McLaren et al., 1974) subnormal responses occurred in eight of sixteen patients (50%) with a sellar plus EST size of 3 cm² or more against in only one of twenty-five patients (4%) with an area of less than 3 cm² ($P < 0{\cdot}0025$).
In this group of patients a tumour size of 3 cm² also appeared critical with respect of the circulating thyroid hormone concentrations in plasma (Klijn et al., 1979). Decreased or low normal values of T4 and T3 occurred only in patients with a tumour size above 3 cm² (57 and 62% respectively).

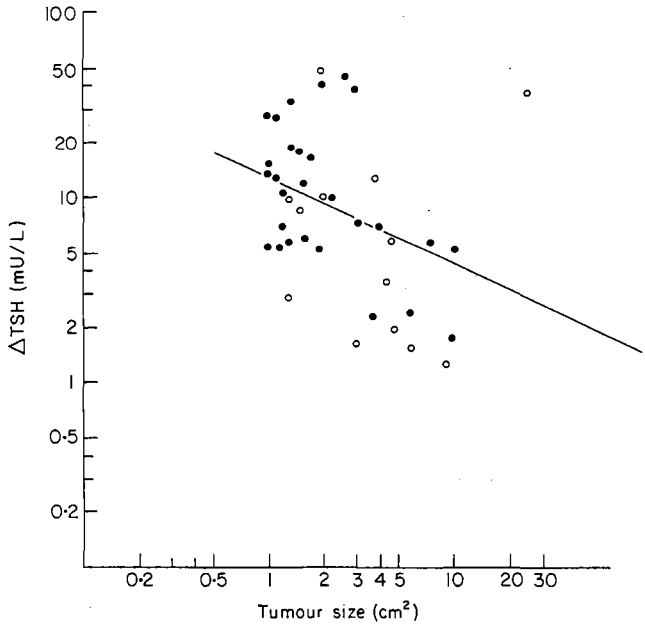

Fig. 3. Relationship between (log) tumour size (=lateral area of the sella plus extrasellar tissue) and (log) Δ TSH in the TRH test in forty-one untreated patients ($r = 0{\cdot}36$, $P < 0{\cdot}01$). Closed symbols represent female patients.

Gonadotrophins

Basal plasma LH and FSH levels (log) showed a negative correlation with (log) sellar plus EST size (both $P < 0{\cdot}005$). A negative correlation existed also between (log) Δ LH as wel as (log) Δ FSH (Fig. 4A) and (log) sellar plus EST size: $P < 0{\cdot}0005$ and $P < 0{\cdot}005$, respectively. In the female patients, (log) oestradiol was negatively correlated with (log) sellar plus EST size ($n = 21$, $r = -0{\cdot}45$, $P < 0{\cdot}025$). The partial correlation coefficient for the relationship between sellar plus EST size and Δ LH after

TABEL 2. Relationship between tumour size (= sella+extrasellar tissue size) and the prevalence of endocrine deficiencies

	< 3 cm^2	$\geqslant 3$ cm^2	*
Decreased ΔTSH (in relation to matched controls)	6/25 (24%)	12/16 (75%)	$P< 0.025$
Decreased ΔTSH (< 5 mU/l)	1/25 (4%)	8/16 (50%)	$P< 0.0025$
Decreased ΔLH	5/25 (20%)	8/15 (53%)	$P< 0.05$
Decreased ΔFSH	4/25 (16%)	11/14 (79%)	$P< 0.0025$
Impaired metyrapone test	1/30 (3%)	7/16 (44%)	$P< 0.0025$

* Significance of difference assessed using χ^2-test

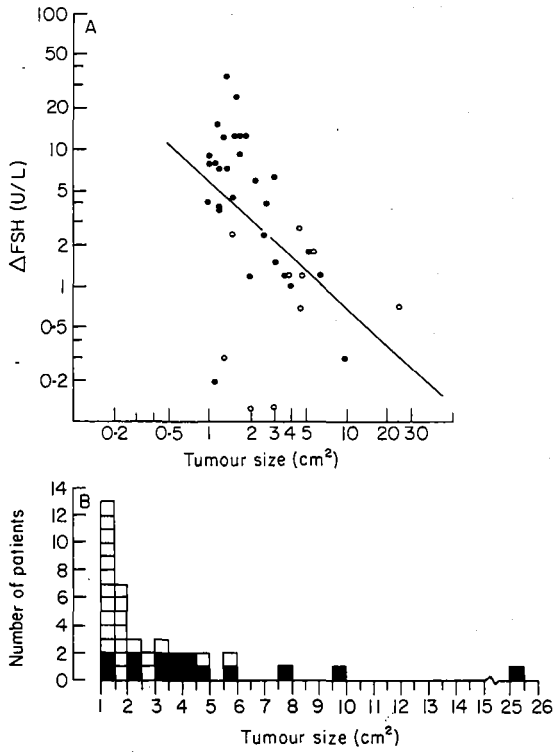

Fig. 4. **A.** Relationship between (log) tumour size (lateral area of the sella plus extrasellar tissue) and (log) Δ FSH in the LHRH test in thirty-eight untreated patients ($r = -0{\cdot}47$, $P < 0{\cdot}005$). Closed symbols represent female patients. **B.** A histogram concerning the relationship between tumour size and the incidence of an impaired FSH response to LHRH. Solid boxes indicate an impaired response. Difference between patients with a tumour size above and below 3 cm^2: $P < 0{\cdot}0025$ (χ^2-test).

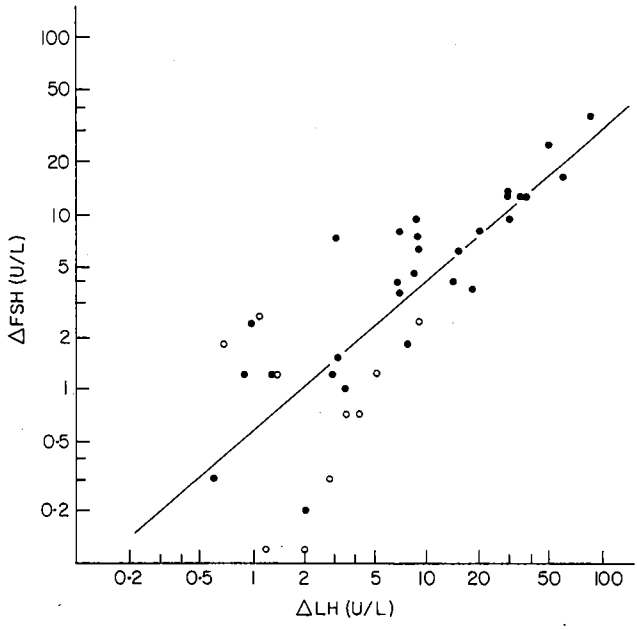

Fig. 5. Relationship between (log) Δ FSH and Δ LH in thirty-nine untreated patients with hyperprolactinaemia and a pituitary tumour ($r = +0{\cdot}79$, $P < 0{\cdot}0005$).

exclusion of the influence of oestradiol was $-0{\cdot}63$ ($P < 0{\cdot}005$). On the other hand the partial correlation coefficient for the relationship between sellar plus EST size and oestradiol after exclusion of the influence of Δ LH was $-0{\cdot}1171$ (n.s.).

A subnormal increase of LH after administration of 100 μg LHRH i.v. was seen in eight out of fifteen patients (53%) with a sellar plus EST size of 3 cm^2 or more against five out of twenty-five patients (20%) with a sellar plus EST size of less than 3 cm^2 (Table

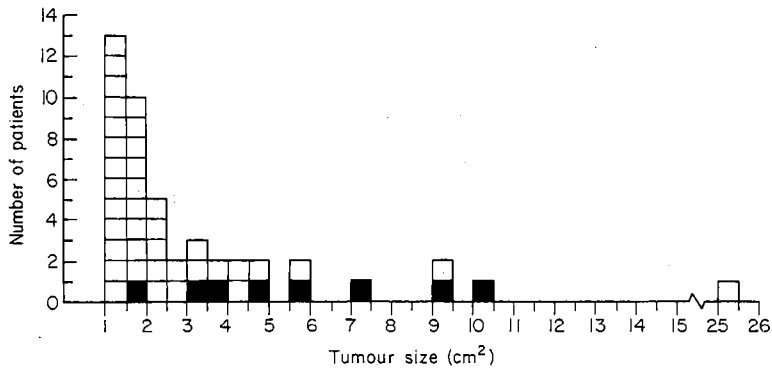

Fig. 6. Frequency distribution of impairment of the metyrapone test in forty-six untreated patients in relation to tumour size (lateral area of the sella plus extrasellar tissue). Solid boxes indicate an impaired metyrapone test.

2, χ^2-test, $P < 0{\cdot}05$). A subnormal response of FSH was observed in eleven out of fourteen patients with a sellar plus EST size of 3 cm² or more (79%) against four out of twenty-five (16%) patients with a sellar plus EST size below this value (Fig. 4B, χ^2-test $P < 0{\cdot}0025$). At tumour sizes between 2 and 3 cm² a decreased LH and FSH response occurred in three and two out of the five patients respectively. A very close relationship was noted between (log) Δ LH and (log) Δ FSH (Fig. 5; $P < 0{\cdot}0005$).
Hyper-responses of FSH and LH occurred only in women with a sellar size of less than 2·5 cm² (50 and 60% respectively). The mean age of these female patients with a hyper-response (29 years) was not significantly different from that of the other female patients (33 years).

ACTH

A negative correlation was also observed between sellar plus EST size and plasma desoxycortisol after metyrapone in thirty-six patients ($P < 0{\cdot}025$). Of the forty-six patients in whom the integrity of the pituitary-adrenal axis was judged either by the plasma desoxycortisol level or the increase of urinary 17-OHCS after metyrapone or both, sixteen patients had a sellar plus EST size of 3 cm² or larger (Fig. 6). Seven of these patients (44%) had an impaired response, in contrast to only one patient with a slight impairment of the metyrapone test in the thirty patients with a sellar size below 3 cm² (χ^2-test, $P < 0{\cdot}0025$).

DISCUSSION

In patients with hyperprolactinaemia and a pituitary tumour we investigated the relationship between morphological and functional indices of the pituitary and the tumour. Although an exact volume measurement of the pituitary tumours would be ideal, we have used the largest lateral area of the sella turcica plus EST as an estimate of the tumour size, instead of a volume measurement. A planimetric area determination is more accurate than measuring two linear dimensions (Di Chiro & Nelson, 1962; Hurxthal, 1947). Although in patients without a pituitary tumour a small lateral area of the sella turcica can be accompanied by a large width of the sella (Fischer & Di Chiro, 1964) and although in 'normal' adults there is a tendency to an inverse correlation between the area and the width of the sella turcica (Seki, 1965), in our patients with a pituitary tumour we found a significant positive correlation between lateral sellar or tumour area and sellar width. In a number of the patients it was ultimately not possible to calculate the sellar and tumour volume with Di Chiro's formula. In those patients in whom a calculation of sellar EST volume could be performed an excellent correlation between both indices was found. So, in patients with a pituitary tumour the area of the sella (+EST) is a useful measure for tumour size.
In the literature a distinction is generally made between micro- and macroadenomas, with and without extrasellar extension (Hardy et al., 1978; Chang et al., 1977; Wilson

& Dempsey, 1978; Jaquet et al., 1978). Microadenomas are defined as adenomas having a diameter of less than 10 mm. Newton (1978) found that in 100 patients with suspected prolactin-secreting pituitary adenomas, all patients with intracapsular adenomas of more than 10 mm in diameter (at operation) had abnormal sellar volume and all patients with intracapsular tumours less than 10 mm in diameter had normal sellar volume. Venzina (1978) never found suprasellar extension in patients with grade 1 microadenomas (that is exhibiting discrete modifications of the sellar floor only). Studying the incidence of extrasellar extension in relation to the accurately measured tumour area in patients with a pituitary tumour and hyperprolactinaemia, we found that an area of 3 cm^2 is rather critical. Below this value (microadenomas and smaller macroadenomas) extrasellar extension of the tumour (either supra- or infrasellar) rarely occurs. When tumour size exceeded 3 cm^2, however, the chance of extrasellar extension by radiological and perimetrical examination, increased sharply to 92% of the patients. Looking at the area of the sella only, the presence of extrasellar extension increases already at sellar sizes between 2 and 3 cm^2 (namely to five out of fifteen). Below a sellar size of 2 cm^2 the incidence is two out of twenty-seven patients. Extrasellar extension of the tumour is uncommon in patients with a normally sized sella with localized alterations or a double contour.

Therefore, we have now decided to omit air-encephalography in this group of patients with hyperprolactinaemia, provided a CAT-scan is normal, excluding the presence of a suprasellar tumour. However, at sellar sizes above 2 cm^2 (or when plasma prolactin levels exceed 400 ng/ml) we judge an examination with regard to suprasellar extension (CAT-scan, potentially followed by a AEG) necessary. When the sellar area exceeds 3 cm^2 we consider air-encephalography on a routine base.

With regard to the relationship between the size of the sella turcica and plasma prolactin levels, Child et al. (1975) described a positive correlation between (log) sellar area ($<$ 3 cm^2) and (log) basal prolactin level ($<$ 1000 ng/ml). Our data confirms this correlation for a larger group of untreated patients with a wider variation in tumour size (1$-$25 cm^2) and in basal prolactin level (17$-$5000 ng/ml). A practical consequence of this finding is the urge to look carefully for extrasellar extension when PRL levels are disproportionately high in relation to the size of the sella. In the group of patients with a microadenoma plasma prolactin levels varied between 17 and about 400 ng/ml and no correlation between sellar size and prolactin levels was found. This might be explained by the discrepancy between sellar and adenoma size and relative importance of peaks of secretion from normal lactotroph cells or adenoma cells in this category of patients. In cases, however, where the size of the microadenomas had been established (Bernasconi et al., 1978) after surgical intervention (diameter 5$-$10 mm) there was no observed correlation between this size and prolactin levels. This may also be explained by difference in secretory activity of the adenoma cells from patient to patient.

We also studied the relationship between sellar plus EST size and the occurrence of se-

condary endocrine deficiencies. We found that a tumour size of 3 cm^2 is not only important with regard to the incidence of extrasellar extension of the tumour but also with regard to the incidence of loss of pituitary functional reserve. We demonstrated that above a sellar plus EST size of 3 cm^2 disturbances in the responses to TRH, LHRH and metyrapone occur with a rather striking increase in frequency. Pituitary insufficiency may be associated with intrasellar as well as with suprasellar processes. In our series, a diminished hormonal reserve could be found in patients without suprasellar extension of the tumour, whereas a normal reserve was not uncommon in patients with suprasellar extension. This makes the evaluation of the relative importance of anterior lobe cell destruction on the one hand, and of destruction of the hypothalamo-pituitary connection on the other hand, very difficult as causes of function loss.

The TSH response to TRH is related to age and sex (Snyder & Utiger, 1972; Sowers et al., 1976; Hershman, 1978). In comparison with a control group matched with respect to age and sex, marginally low responses of TSH occurred both above and below a sellar plus EST size of 3 cm^2, but definite abnormal responses of TSH (less than 5 mU/l) mainly occurred above a sellar plus EST size of 3 cm^2.

In patients with pituitary tumours accompanied with hyperprolactinaemia the response of LH and FSH to LHRH may either be normal, excessive or subnormal (Zarate et al., 1973; Mortimer et al., 1973; Thorner et al., 1974; Glass et al., 1975; Franchimont et al., 1975; Archer et al., 1976; Chang et al., 1977; Campenhout et al., 1977). We found a significant difference between the incidence of an impaired response of LH and FSH to LHRH in patients with a sellar plus EST size above and below 3 cm^2, respectively. Above a sellar plus EST size of 2 cm^2 the incidence of an impairment of the pituitary-gonadal axis increases (LH and FSH, $P < 0{\cdot}05$ and $< 0{\cdot}0025$, respectively). The significant partial correlation between tumour size and Δ LH, after exclusion of the influence of oestradiol-17β, shows that tumour size, not lack of oestradiol-17β, is the causal factor in this diminished Δ LH.

According to several authors the response of FSH to LHRH tends to be more exaggerated than the LH response (Asfour et al., 1977; Lachelin et al., 1977; Kletzky et al., 1977). However, Healy et al. (1977) found hyper-responses of both FSH and LH not only in patients with hyperprolactinaemic secondary amenorrhoea but also in patients with normoprolactinaemic secondary amenorrhoea. We observed parallel hyper-responses of FSH and LH to LHRH only in female patients, and only when tumour size is less than 2·5 cm^2. An increased incidence of FSH hyper- responsitivity relative to that of LH was not found.

With regard to the pituitary-adrenal axis we found that the ACTH reserve was preserved somewhat better than TSH and gonadotrophin reserve. Impairment was almost totally restricted to patients with sellar plus EST size of more than 3 cm^2.

In patients with a pituitary tumour and hyperprolactinaemia, the rather sharply increased incidence of supra- or intrasellar extension of the tumour and of anterior lobe hormone

deficiencies at sellar plus EST sizes above 3 cm^2, is of great importance for determining the correct management of these patients. We have previously showed that an impaired response of LH and FSH to LHRH in patients with prolactin secreting pituitary tumours, greatly diminishes the possibility of obtaining ovulatory cycles after normalization of circulating PRL levels by bromocriptine therapy (Lamberts et al, 1978). We consider a sellar (+EST) area of more than 3 cm^2 as 'high risk' and a sellar area between 2 and 3 cm^2 as 'potential risk'. On the basis of the data presented here it appears necessary to perform a trans-sphenoidal adenomectomy before the sellar size has exceeded 2−3cm^2. With respect to the problems that may arise during pregnancy, an increased incidence of extrasellar extension and hormonal insufficiency during pregnancy is to be expected in female patients with a sellar size above 2−3 cm^2 before pregnancy.

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CHAPTER IV

INTERRELATIONSHIPS BETWEEN TUMOUR SIZE, AGE, PLASMA GROWTH HORMONE AND INCIDENCE OF EXTRASELLAR EXTENSION IN ACROMEGALIC PATIENTS

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SUMMARY

In 44 consecutive acromegalic patients we studied the interrelationships between tumour size, age, incidence of extrasellar extension, growth hormone levels and 'tumour growth'. These parameters were compared with results from a previous study in 62 prolactinoma patients. It appeared that the incidence of extrasellar extension in acromegalic patients was lower than in the prolactinoma patients (32 versus 44%). In acromegalic patients extrasellar extension occurred on the average at a lateral sellar and tumour area of almost 1 cm^2 larger than in prolactinoma patients (with respect to sellar size generally above 3 cm^2 versus 2 cm^2, with respect to tumour size generally above 4 cm^2 versus 3 cm^2).

Log tumour size and log basal growth hormone level were positively correlated ($P < 0{\cdot}0005$). In the acromegalic patients there was a negative correlation between the size of the pituitary tumour and the age of the patient ($P < 0{\cdot}005$) in contrast to the absence of such a relationship in the prolactinoma patients. In the group of acromegalic patients mean tumour size decreased gradually from the third to the sixth decade (5.0, 3.8, 3.0 and 2.3 cm^2, respectively). The interval between the time of appearance of symptoms and the time of diagnosis was significantly shorter in younger patients and in women. The restriction of large tumours (lateral area > 5 cm^2) to young patients ($<$ 35 year) and the short period between the appearance of symptoms and the time of diagnosis in these patients indicate that growth hormone secreting pituitary tumours generally grow more rapidly in younger patients.

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In patients with a pituitary tumour extrasellar extension of the tumour constitutes one of the major complications. In a previous study we demonstrated that in 62 patients with hyperprolactinaemia and a pituitary tumour a sellar size or a 'tumour size' of 2-3 cm^2 - as measured directly with a planimeter on the lateral X-ray of the skull - represented a critical limit for the presence of extrasellar extension of the pituitary adenoma (Klijn et al. 1980b). A positive correlation between tumour size and the plasma prolactin (Prl) level was found. In acromegalic patients such a correlation between tumour size and basal growth hormone (GH) level is not well established up to now (Quabbe 1980; Giovanelli et al. 1980). Pituitary tumours occur at all ages from puberty onwards, but little is known about the influence of age on tumour size and growth.
The aim of the present study was to answer the following questions. 1) Is the incidence of extrasellar extension in acromegalic patients different from that in patients with Prl secreting tumours? 2) Are tumour size and basal plasma GH concentration related? 3) What is the influence of the age of the individual on tumour growth rate in acromegaly and on the chance of extrasellar extension?

MATERIALS AND METHODS

Patients

Forty-four consecutive acromegalic patients (21 women and 23 men) were investigated. They were referred to our endocrine clinic by specialists in internal medicine (n = 16), gynaecology (n = 5), neurology or neurosurgery (n = 6), ophthalmology (n = 2), rheumatology or orthopaedy (n = 6), urology (n = 3) or - incidentally - other specialists. All patients were untreated at the time of study. In 32 of them the presence of a pituitary tumour was proven by surgery. The others were not operated upon. The results of this study were compared with those of a previous investigation of 62 patients (38 women and 24 men) with hyperprolactinaemia and a pituitary tumour without growth hormone excess (Klijn et al. 1980b and in press).

Methods and materials

All patients were investigated by plain radiography, lateral polytomography (hypocycloidal, sections of 2 mm) and complete ophthalmological evaluation, including perimetry. Further radiological examination consisted of air-encephalography (AEG) and - in most cases - carotid angiography. As a measure of tumour size we have chosen the largest lateral area of the sella in combination with that of extrasellar tissue (EST), if present. The largest lateral area of the sella +EST was measured on a plain lateral X-ray after verification of the true sellar and extrasellar tissue outlines by means of lateral polytomography and the lateral tomograms of the AEG. Direct surface measurements were carried out with a planimeter as described in detail before (Klijn et al. 1980b). Normal values for sellar size are between 0.7 and 1.4 cm^2 (95% range in 100 patients without

TABLE 1. Referred pattern and clinical features in 44 consecutive acromegalic patients.

	Mode of presentation		Overall prevalence	
	n	%	n	%
Change of appearance/soft tisue swelling/ acral growth	6	14	44	100
Weight gain	—	—	20	45
Gigantism	—	—	1	2
Acroparaesthesias	3	7	31	70
Facial neuralgias	—	—	3	7
Arthropathy	2	5	13	30
Back pain	1	2	9	20
Tiredness	1	2	25	57
Hyperhidrosis	1	2	36	82
Thirst (without diabetes insipidus)	1	—	6	14
Hypertrichosis	—	—	14	32
Ear/nose/throat complications	1	2	14	32
Hypertension	4	9	9	20
Cardiac complications	2	5	10	23
Impaired glucose tolerance	—	—	13	30
Manifest diabetes mellitus	—	—	1	2
Abdominal pain	3	7	14	32
Inguinal or umbilical hernia	—	—	10	23
Renal stones	3	7	8	18
Goitre			18*	41
Headache	6	14	23	52
Hypersomnolence	—	—	4	9
Loss of concentration and/or memory	—	—	4	9
Visual impairment	2	5	6	14
CSF-rhinorrhoea	—	—	1	2
Oligo-, amenorrhoea/infertility	5	24**	11	52**
Impotence	1	4***	10	43***
Loss of libido	—	—	11	25
Galactorrhoea	1	2	10	48**
Cystic mastopathy	—	—	4	19**
Diagnosis by chance	2	5	2	5

* One patient with a papillary carcinoma.
** Percentage of the female patients.
*** Percentage of the male patients.

pituitary pathology). The area of the sella (+EST) was also measured by means of square mm paper.

Plasma GH levels were measured by a homologous radioimmunoassay (IRE, Belgium), and Prl according to Kwa et al. (1973). Normal basal Prl values are up to 15 and 12 ng/ml for women and men, respectively. The biochemical diagnosis of acromegaly was made on the basis of the absence of a suppression of plasma GH concentrations to less than 5 ng/ml in response to an oral glucose load (100 g).

Statistical analysis of the data was performed using regression analysis, two-tailed Student's *t*-test or X^2-test.

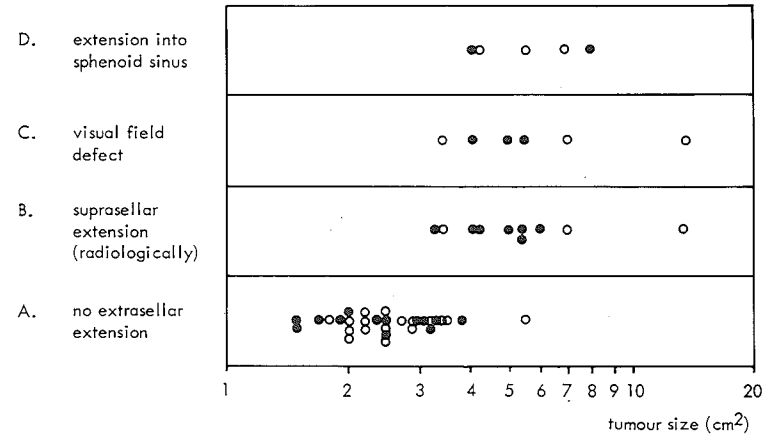

Fig. 1. Tumour size (=lateral area of the sella + extrasellar tissue) in relation to the presence of extrasellar extension by radiological and perimetrical criteria in 44 untreated acromegalic patients. There is some overlap of the groups of patients represented in the upper three panels (B, C, D). A patient may be represented in one, two or three of these panels. Closed symbols represent female, open symbols male patients.

RESULTS

Epidemiological and clinical evaluation

The mean age of the 21 female patients was 39 years (median 40 years, range 19-74 years) and of the male patients 40 years (median 41 years, range 21-71 years). There was a preponderance of patients between 20 and 50 years. Histological examination of tumour material of the 32 operated patients revealed the presence of a chromophobe adenoma in ten cases, an acidophil adenoma in nine cases and a mixed adenoma in nine cases. In four patients a precise histological diagnosis was not obtainable.

The referral pattern and clinical features are summarized in Table 1. One of the acromegalic women menstruated regularly, nine were (post) menopausal and 11 had secondary oligo- or amenorrhoea. The mean duration of the secondary menstrual disorder was 4.5 years (range ¼-15 years). Amenorrhoea occurred in three patients after cessation of oral contraceptive treatment and in one patient after childbirth. In 10 of these 11 patients with

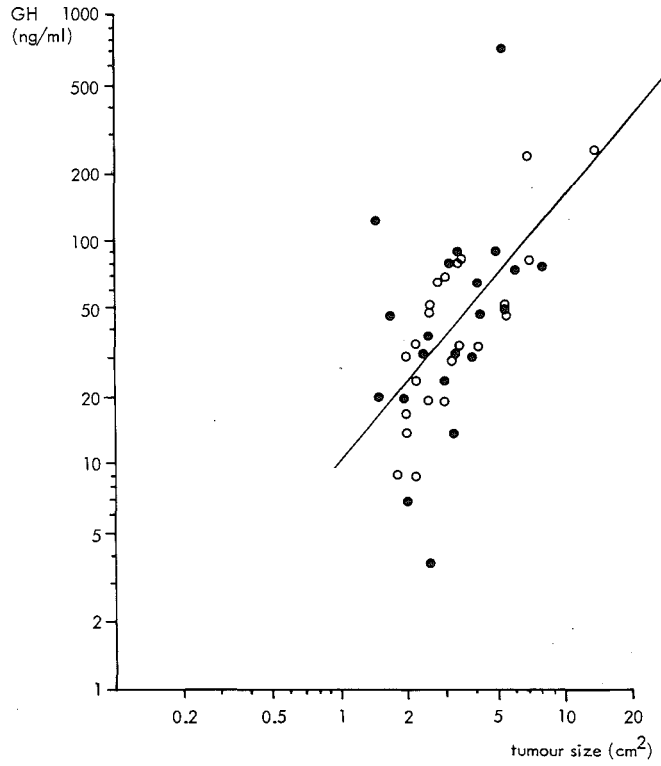

Fig. 2. Correlation between log tumour size (=lateral area of the sella + extrasellar tissue) and log basal growth hormone in 44 untreated acromegalic patients ($r = 0.59$, $P < 0.0005$). Closed symbols represent female patients.

oligo- or amenorrhoea the plasma Prl level was estimated. Three of them had a high basal Prl level (137, 174 and 183 ng/ml, respectively), six a mild hyperprolactinaemia (range 19-43 ng/ml) and one a normal Prl level (4 ng/ml). Galactorrhoea occurred in 10 out of 44 patients, all women (mean age 30 years, none of them older than 50 years). All but one had hyperprolactinaemia.

Radiological and ophthalmological evalution

In all patients the sella turcica was enlarged. The lateral area of the sella +EST varied between 1.5 and 13.6 cm^2 with a mean of 3.64 cm^2. There was no difference in tumour size between women and men (3.50 ± 1.69 cm^2 versus 3.76 ± 2.63 cm^2; mean $\pm$SD). In 14 patients (32%) extrasellar extension of the tumour was present radiologically (mean sellar +EST size: 5.9 cm^2). Ten patients (23%) had suprasellar extension, six (14%) of them with visual field defects and one of them with infrasellar extension. Four patients showed infrasellar extension of the tumour only (Fig.1). In the 30 patients without extrasellar extension mean sellar size was 2.6 cm^2. Extrasellar extension occurred only at tu-

mour sizes of more than 3 cm^2, namely in 14 out of 22 patients with a tumour size of more than 3 cm^2 (64%). However, only two out of nine patients with a tumour size between 3 and 4 cm^2 showed extrasellar extension, while at a tumour size of above 4 cm^2 extrasellar extension was present in 12 out of 13 patients (92%). Besides the increase in size the sella turcica of 37 patients was more or less asymmetrical. In 20 cases the left side was larger, in 17 cases the right side.

When we take into consideration the size of the sella only (i.e. without the area of EST, if present) two patients with a *tumour* size of more than 3 cm^2 turned out to combine extrasellar extension with a *sellar* size of less than 3 cm^2 (2.5 and 2.4 cm^2). This means that of the 23 patients with a sellar size of less than 3 cm^2 only two had evidence of extrasellar extension (9%) in contrast of 12 out of 21 patients with a sellar size exceeding 3 cm^2 (57%). Taking the group of patients with a sellar size between 2 and 3 cm^2 only two out of 16 patients had extrasellar extension (13%).

There was good agreement between the results of the two different methods of area measurements of tumour ($n = 44$; $r = +0.993$; $P < 0.0005$). The slope of the regression line was 1.004, the intercept -0.14 cm^2.

Endocrinological evaluation

Growth hormone was measured in all 44 patients before treatment. The basal GH level (mean of 2 or more estimations) varied between 7 and 720 ng/ml with a mean of 81 ng/ml in the female patients and 59 ng/ml in the male patients. There was a positive correlation between the logarithm of the sellar +EST area and the logarithm of the (average) basal GH level (Fig. 2). Furthermore a positive correlation was found between log tumour volume (½ x H x D x W according to Di Chiro & Nelson 1962) and log basal GH in those patients in whom the width of the sella floor was measurable on an anteroposterior plain X-ray ($n = 36$, $r = 0.48$; $P < 0.01$). A good correlation appeared to exist between the area and volume measurements of the tumours ($n = 36$, $r = 0.955$, $P < 0.0005$) as previously also found in patients with hyperprolactinaemia and a pituitary tumour (Klijn et al. 1980b).

Hyperprolactinaemia was observed in 13 (54%) out of 24 untreated patients. Prolactin levels of more than 100 ng/ml were found in only three of these patients.

Data with regard to pituitary-target organ function in these patients before treatment have been published before (Klijn et al., in press). Hypothyroidism was encountered in only one patient. This is to be compared to the occurence of a low TT4 level ($< \mu g/100$ ml) in 31% of prolactinoma patients with the same range of tumour size as found in the acromegalic patients ($\geqslant 1.5$ cm^2). An abnormal metyrapone test was found in four out of the 36 investigated acromegalic patients (11%; tumour sizes: 2.7, 4.1, 6.9 and 7.0 cm^2). In the group of prolactinoma patients with an enlarged sellar area ($\geqslant 1.5$ cm^2) an impaired metyrapone test was found in eight out of 33 patients (24%). A decreased gonadotrophin secretion was present in 12 out of 42 (29%) investigated acromegalic patients

(in contrast to about 50% of the prolactinoma patients with the same range of tumour size). All these 12 patients had tumour sizes of more than 2.5 cm^2 (only two of them below 3 cm^2).

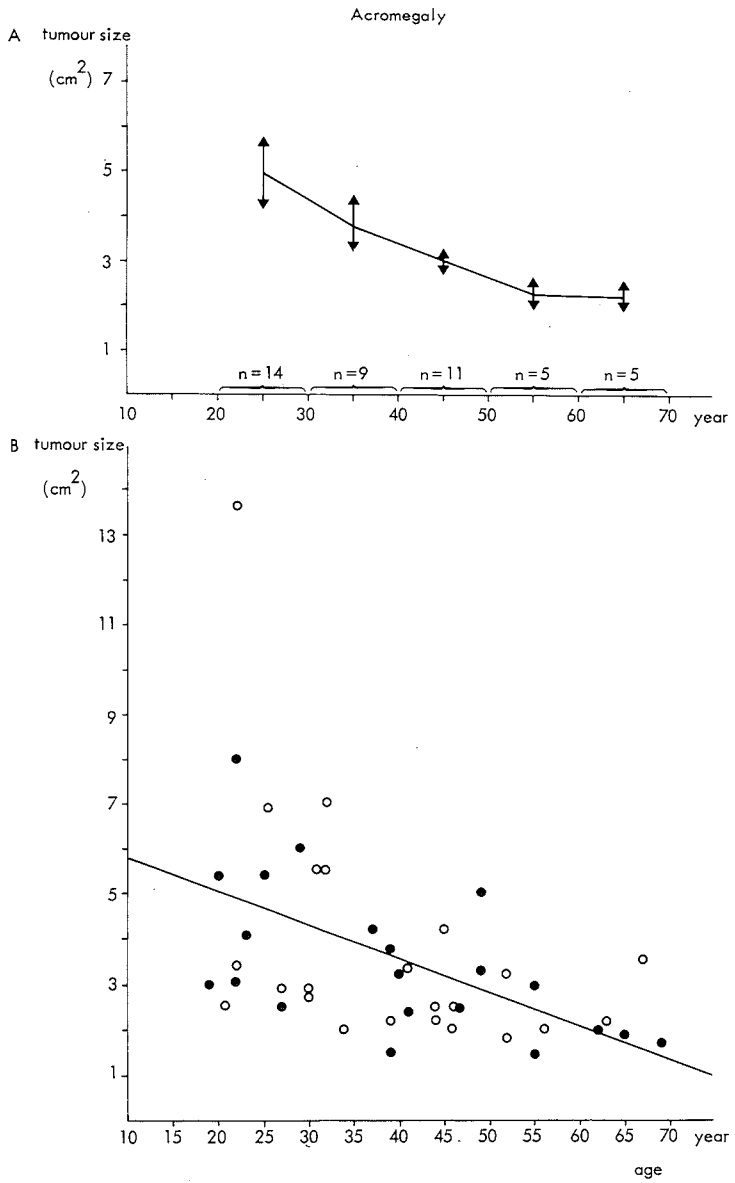

Fig. 3. Correlation between age and tumour size (= lateral area of the sella + extrasellar tissue) in 44 untreated acromegalic patients ($r = 0.48$, $P < 0.005$). In panel A the arrows represent SEM. Closed symbols indicate female patients.

Relation of age to tumour size and tumour growth rate

In the acromegalic patients we found an evident negative relationship between tumour size and age (Fig. 3A and B). The mean tumour size decreased gradually from the third to the sixth decade (4.98 ± 3.04; 3.82 ± 1.89; 3.02 ± 0.92; 2.30 ± 0.75; mean ± SD). This contrasts with the findings in our group of prolactinoma patients (Fig. 4). The nine acromegalic patients with the largest tumours (> 5 cm^2) were all younger than 35 years. Likewise 10 out of the 14 patients with extrasellar extension were less than 35 years old. On the other hand 15 out of 18 patients with a tumour size below 2.5 cm^2 were more than 35 years old. All three histological tumour types (acidophil, chromophobe, or mixed) were present in the group of patients with large tumours.

As to the mean period between the time of onset of symptoms of acromegaly and the time of diagnosis, two points are remarkable (Table 2). Firstly this delay was found to be shorter in younger patients and secondly there was a significant difference between women and men of all ages (4.1 year and 8.6 year, respectively, $P < 0.001$.).

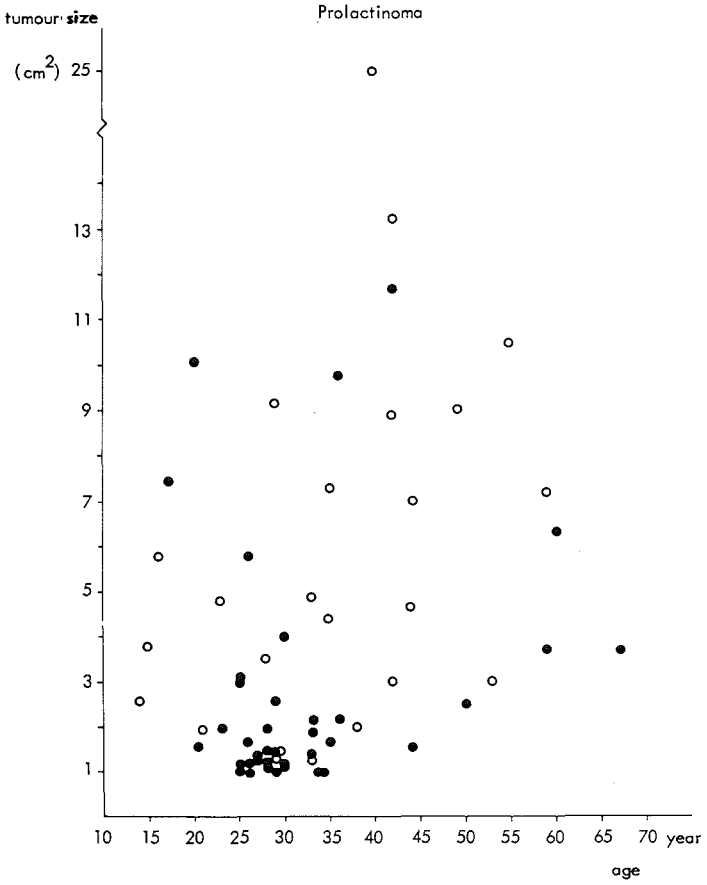

Fig. 4. Relationship between age and tumour size in 62 untreated patients with hyperprolactinaemia and a pituitary tumour (without GH excess). Closed symbols represent female patients.

TABLE 2. Mean delay (in years) between start of symptoms and time of diagnosis.

Age group (years)	Women	n	Men	n	Total	n
19 - 30	2.1	8	4.2	5	2.9	13
31 - 40	5.0	3	9.3	7	8.0	10
41 - 50	7.2	5	10.1	6	8.8	11
51 - 70	3.6	5	10.4	5	7.0	10
All patients	4.1±3.1*years	21	8.6±5.1*years	23	6.5 years	44

* $P < 0.001$

DISCUSSION

The frequency of the various clinical manifestations of acromegaly in our patients is on the whole in reasonable agreement with previous reports (Davidoff 1926; Davis 1941; Kellgren et al. 1952; Wright et al. 1970; Hirsch et al. 1969; Daughaday 1974). The high frequency of tiredness without hormonal insufficiency might be explained by myopathy (Mastaglia et al. 1970), abnormal joint mechanics (Kellgren et al. 1952), peripheral neuropathy caused by soft tissue swelling and perineural or endoneural fibrous proliferation (Daughaday 1974) and/or cardiac disease (Hirsch et al. 1969). Remarkable was a relatively high frequency of inguinal hernia in our acromegalic men (30%).

The incidence of extrasellar extension in the acromegalic patients (32%) was lower than in the prolactinoma patients (44%), whose data we reported before (Klijn et al. 1980b). With respect to pituitary *tumour* size extrasellar extension is uncommon below a size of 3 cm^2 in both groups of patients. However, above a tumour size of 3 cm^2 the frequency of extrasellar extension is lower in the acromegalic (64%) than in the prolactinoma patients (92%), as there appears to be a cluster of acromegalics with a tumour size of between 3 and 4 cm^2 without extrasellar extension (Fig. 1). However, at tumour sizes of more than 4 cm^2 extrasellar extension occurred in 92% of the acromegalic patients also.

Using the lateral *sellar* area (i.e. without the area of EST) a lower incidence of extrasellar extension of the tumour was again observed in acromegaly as compared to prolactinoma patients. In the patients with a sellar size of less than 3 cm^2 radiological evidence of extrasellar extension was found more frequently in the prolactinoma patients than in the acromegalics (17% versus 9%), as especially reflected in the relatively high incidence of extrasellar extension in prolactinoma patients with a sellar size between 2 and 3 cm^2 (33% versus 13%). In the patients with a sellar size of more than 3 cm^2 the frequency of extrasellar extension was also higher in the prolactinoma patients than in the acromegalics (90% versus 57%). In summary it appeared that on the average in acromegalic patients extrasellar extension occurred at sellar and tumour sizes of 1 cm^2 larger than in prolactinoma patients (for tumour size 4 cm^2 versus 3 cm^2, for sellar size 3 cm^2 versus

2 cm^2). In our experience ballooning of the pituitary fossa occurred frequently in acromegaly as also reported by Pribram & du Boulay (1971). We think that the lower frequency of extrasellar extension and the occurrence of this complication at *larger* tumour sizes in acromegalic patients may be caused by a (indirect) GH effect on the sella turcica wall itself. Growth hormone was found to be 'the most effective bone growth stimulating pituitary hormone' (Thorngren & Hansson 1977). We propose the following hypothesis. Acromegalic patients have an increased bone tissue turnover rate with increased periosteal bone formation (Roelfsema 1972; Daughaday 1974). The pituitary fossa bone wall may therefore adapt more easily to the pressure of an expansive pituitary tumour than when a pituitary tumour is present without GH excess. It is also possible that in acromegalic patients the pituitary fossa tends to increase in size anyhow just as the frontal, mastoid and ethmoid sinuses may enlarge considerably. However, with further increasing tumour size the intrasellar pressure will ultimately be sufficiently high to cause extrasellar extension, but at *larger* tumour sizes in acromegalic patients than in patients with other pituitary tumour types.

Hypothyroidism, decreased gonadotrophin secretion and insufficient pituitary adrenal function occurred also less frequently in the acromegalic patients than in our group of patients with hyperprolactinaemia and a pituitary tumour, especially when the same range of tumour size is considered (i.e. after exclusion of the microprolactinomas). In acromegalic patients, however, a high incidence of an impaired TSH response to TRH has been noted (Hall et al. 1972; Klijn et al., in press) and a discrepancy between LH and FSH secretion (Lindholm et al. 1977). This might be explained by an effect of GH on thyroid, gonads and adrenals (for example development of goitre with suppression of TSH secretion) or on hypothalamic function.

We found a positive correlation between tumour size and basal plasma GH level, as we reported before for tumour size and the basal plasma Prl level in patients with a prolactinoma (Klijn et al. 1980a,b). In the group of acromegalic patients with a small adenoma (< 3 cm^2) such a relationship was not present. This is in agreement with the findings of Giovanelli et al. (1980), who found no significant correlation between plasma GH concentration and tumour size in 57 acromegalic-patients with small intrahypophyseal GH-secreting tumours (less than 13 mm in diameter at operation). It is noteworthy that is our group of patients there may be a wide range of plasma GH levels for a certain tumour size. Quabbe (1980) found also that small tumours may be accompained by relatively high plasma GH concentrations, while larger tumours may have relatively low secretory activity. However, our data indicate that plasma GH levels of more than 50 ng/ml are associated predominantly with a tumour size of more than 2.5 cm^2.

Pituitary tumours may become manifest at an early age or later in life. The declining incidence of microprolactinomas in older patients (Fig. 4) may be explained by a lack of symptomatology. The true incidency may however be inferred from the frequency of microadenomas in routine autopsies, reported as 2.7% (Hardy 1978), 9% (McCormick

& Halmi 1971), and 22.5% (Costello 1935), the frequency of microprolactinomas 6% (Kovacs et al. 1979) and the observation of Kovacs et al. (1979) that 8% of a group of geriatric patients had an elevated plasma Prl level.

We know of no previous data concerning a negative correlation between pituitary tumour size and age. In our series of patients with acromegaly large tumours above 5 cm^2 appeared to be uncommon in older patients but were frequent (about 50%) in patients younger than 35 years. A second interesting finding is the relatively short period intervening between the onset of symptoms of acromegaly and the time of diagnosis in the younger patients. These two observations indicate that on the whole GH-secreting pituitary tumours grow more rapidly in the younger than in the older patients. Gonadal steroids might be implicated in this respect. The longer delay between the clinical appearance of acromegaly and the time of diagnosis found in men as compared to women might be explained by the fact that men are not so rapidly alarmed by changes in their appearance than women. One the other hand photographs taken over the year often show that hypersomatotropism must have been present years before complaints are noted. Therefore, some of the GH secreting pituitary tumours, arisen in younger patients, grow rapidly and others grow more slowly as tumours do in older people. On the basis of our findings treatment of acromegaly should be more aggressive in younger than in older patients.

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CHAPTER V

THE VALUE OF THE THYROTROPIN-RELEASING HORMONE TEST IN PATIENTS WITH PROLACTIN-SECRETING PITUITARY TUMORS AND SUPRASELLAR NON-PITUITARY TUMORS

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SUMMARY

The prolactin (PRL) response to thyrotropin-releasing hormone (TRH) (ΔPRL) was normal in 7 (18%) of 38 patients with clinical evidence of a prolactinoma. A negative percentage correlation between basal PRL and ΔPRL was found ($P < 0.05$), but a percentage correlation between tumor size and ΔPRL was absent. In a survey of literature concerning 548 patients, ΔPRL after TRH administration amounted to 100% or more of the basal value in 11% of patients with clinical evidence of a prolactinoma and in 9% with an adenoma proven by surgery. Hyperprolactinemia was also present in 12 of our 21 patients (57%) with suprasellar tumors not related to pituitary tumors. In 7 of 11 of these hyperprolactinemic patients (64%) the PRL response to TRH was decreased. In conclusion, the TRH test may be helpful but is not decisive in the diagnostic work-up of hyperprolactinemic patients.
Fertil Steril 35:155, 1981

The diagnostic value of the thyrotropin-releasing hormone (TRH) test in the differential diagnosis of hyperprolactinemia is still under discussion.[1-8] Hyperprolactinemic patients with a normal sella turcica or only minor alterations as shown by sellar tomography, referred to previously as "functional hyperprolactinemia," have been found to harbor mi-

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croprolactinomas at operation.[8-10] A distinction has been made between patients with microprolactinomas (prolactin [PRL]-secreting pituitary tumors less than 1 cm in diameter, sella not enlarged) and macroprolactinomas (tumors more than 1 cm in diameter, enlarged sella). Some authors have reported a diminished PRL response to TRH (ΔPRL) in all patients with micro- or macroadenomas,[2, 5, 8, 10-15] but other authors have reported a normal response in a minority of their patients (in larger series ranging between 6% and 33%).[4, 7, 16-20] We investigated the relationship between the response of PRL to TRH and the lateral area of the sella or of the pituitary tumor (in case of extrasellar extension) in 38 patients with clinical evidence of a PRL-secreting pituitary tumor. The relationships between ΔPRL and basal PRL, sex, age, thyroid-stimulating hormone (TSH) response to TRH, T_4, T_3, and suppressibility of PRL by bromocriptine, respectively, were evaluated. Furthermore, the incidence of a decreased PRL response to TRH was investigated in patients with hypothalamic disorders caused by suprasellar non- pituitary tumors.

MATERIALS

Patients. The PRL response to TRH (400 μg intravenously) was measured in 38 untreated hyperprolactinemic patients (28 women) with radiologic evidence of a pituitary tumor with the exception of 4 women with a normal sella turcica but with the typical galactorrhea-amenorrhea syndrome. In 11 patients (8 women) the presence of a pituitary adenoma was subsequently proven by surgery. Plasma PRL levels were also measured in 21 patients (13 women) with suprasellar tumors not arising from the pituitary (5 with an ectopic pinealoma or germinoma, 4 with a craniopharyngioma, 4 with a dermoid cyst, 6 with a meningioma, one with an astrocytoma, one with leukemia). In 18 of these patients a TRH test was carried out. Patients with primary hypothyroidism, renal insufficiency, or liver disease or who were undergoing drug treatment were excluded.

Diagnostic Testing. In eight patients with hyperprolactinemia and an abnormal sella (five women), in seven acromegalic patients (three women) with normal or slightly increased PRL level (< 35 ng/ml), and in eleven infertile oligospermic men (only one with a slightly increased PRL level) TRH tests were performed with two different doses (400 and 200 μg intravenously) in an at-random order. The two tests were conducted at an interval of 2 to 5 days. In all patients, plain radiography, lateral polytomography of the sella, and complete ophthalmologic evaluation, including assessment of the visual fields, were performed. Additional radiologic examinations, when indicated, consisted of air encephalography, and/or computerized tomography, and/or carotid arteriography. The largest lateral areas of the sella and of extrasellar tissue, if present, were used as a measure of pituitary tumor size. Estimation of plasma PRL levels and direct measurements of the surface of the sella turcica were carried out as described previously.[21] Normal values for sellar size ranged from 0.7 to 1.4 sq·cm (in 95% of 100 patients without pituitary abnormalities).

PRL Response. The PRL response to TRH was measured in untreated patients only. Blood samples were obtained through an indwelling venous catheter before and 10, 20, 30, 60 and 120 minutes after the intravenous administration of 400 μg of TRH (Hoechst). The differences between the highest value for PRL during the test and the basal value (ΔPRL), and the percentage increase in plasma PRL above the baseline level (%ΔPRL), were used as indices of response. The maximal TSH response to TRH (ΔTSH), plasma T_4, the free T_4 index, and plasma T_3 were also estimated, the latter not in all patients. In 15 patients with a PRL-secreting pituitary tumor, plasma PRL levels were measured every hour for 12 hours and again 24 hours after an oral dose of 2.5 mg of bromocriptine.

Statistical analysis of the data was performed using regression analysis.

Normal Values. Normal basal plasma PRL values are $\leqslant$ 15 ng/ml in women (mean 7.1 ng/ml) and $\leqslant$ 12 ng/ml in men (mean 4.6 ng/ml). In 22 female control subjects we found a range for ΔPRL between 11.2 and 86.4 ng/ml (mean $\pm$ standard deviation 43.6 $\pm$ 20.7 ng/ml) and in 7 normal men a range between 8.6 and 57.6 ng/ml (mean $\pm$ standard deviation 22.9 $\pm$ 17.6 ng/ml). In all controls ΔPRL was more than 100% (for women, mean 741%, range 361% to 2260%; for men, mean 638%, range 102% to 1646%). The peak PRL value occurred in 7 cases at 10 minutes, in 17 at 20 minutes, in 4 at 30 minutes, and in 1 at 60 minutes after the injection of TRH.

RESULTS

Single TRH Test (400 μg Intravenously)

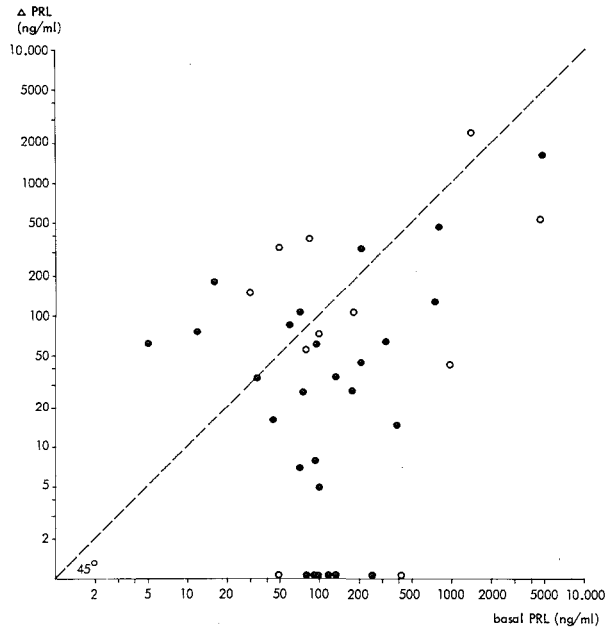

Fig. 1. Log-log plot of basal PRL levels against absolute PRL responses to TRH in 38 untreated patients with clinical evidence of a prolactinoma. ●, Female patients. No correlation was demonstrated.

Patients with Hyperprolactinemia and a Pituitary Tumor. The highest plasma PRL values were observed in six cases at 0 minute, in eight at 10 minutes, in eleven at 20 minutes, in eight at 30 minutes, in two at 60 minutes, and in three at 120 minutes after TRH administration. In 11 of 38 patients (29%) the maximal increment of PRL in response to TRH was more than 100% of the basal value (Figs. 1 and 2). A normal PRL response on percentage basis (in relation to our control population) occurred in 7 of the 38 patients (18%). The range of tumor size in these patients varied between 1.0 and 3.8 sq cm. In 16 patients (42%) with a wide range in basal PRL levels a PRL response of less than 20% occurred (Fig.2). Of the 11 patients with a surgically proven pituitary adenoma, 3 had a ΔPRL of more than 100%. There was no correlation between basal PRL levels and ΔPRL as expressed in nanograms per milliliter (Fig. 1), but there was a negative correlation between the basal PRL level and the percentage ΔPRL (Fig. 2; $P < 0.05$). No correlation was found between percentage ΔPRL and tumor size (Fig. 3) or ΔTSH. Also no correlation was found between ΔPRL or percentage ΔPRL and age, T_4 or T_3. One dose of 2.5 mg of bromocriptine suppressed the basal PRL level in all 15 patients

tested to 15.2% ± 3.2% (mean ± standard error of the mean) of the basal value with a range between 4% and 38% (maximal suppression after 7 hours). There was no correlation between the extent of the PRL response to TRH and the decrease in PRL response to bromocriptine.

Patients with Suprasellar Tumors Not Arising from the Pituitary. Of the 21 patients with primarily suprasellar tumors, 12 (57%) had elevated basal plasma PRL levels (range 22 to 182 ng/ml). In 11 of these 12 patients a TRH test was carried out. In seven of them (64%), ΔPRL was less than 100%. In contrast, in only one of seven normoprolactinemic patients of this group (14%) in whom a TRH test was carried out, the PRL response was decreased. No hyper-responders (ΔPRL > 2000%) were present. Six of the twelve hyperprolactinemic patients and six of the nine normoprolactinemic patients had an enlarged sella.

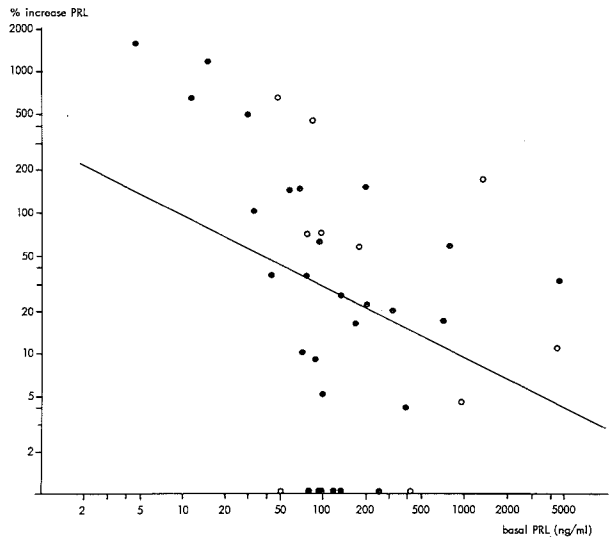

Fig. 2. Relationship between basal PRL levels and the percentage increase in PRL levels after TRH administration (both in log scale) to 38 untreated patients with clinical evidence of a prolactinoma ($r = -0.38$; $P < 0.05$). ●, Female patients.

Comparison of the Effects of 200 and 400 μg of TRH

Good agreement between the PRL responses to 200 and 400 μg of TRH was found in seven acromegalic patients and eleven oligospermic infertile men (Fig. 4: $r = +0.80$, $P < 0.001$). However, in eight patients with a prolactinoma no statistically significant correlation between the two responses was observed. In six patients the first test resulted in the highest response to TRH regardless of the dose used. One patient did not show a response in either test and in one patient (with the largest tumor of all) the second dose of 200 μg of TRH resulted in a higher response than the first one.

DISCUSSION

The diagnostic value of the TRH test in the differential diagnosis of hyperprolactinemia has been uncertain. In general, two groups of reports can be distinguished: those recording an impaired PRL response in all patients with a prolactin-secreting pituitary adenoma and those describing a normal (mostly low-normal) percentage ΔPRL in only a minority of patients suspected of having a prolactinoma and also in patients with a surgically proven adenoma (Table 1).

Most authors have reported a mean ΔPRL in normal women 3 to 6 times the mean basal value and approximately 3 times the mean basal value in normal men. Although the lower limit of the normal range for ΔPRL after TRH administration varies from author to author, in general the lower limit of the normal increment is taken as 100% of the basal value. Our lower limit of the percentage ΔPRL in normal women largely exceeded 100%.

We found normal responses in 18% of our patients and a response of more than 100% in 29%. Comparable observations have been reported by Ayalon et al.,[6] Jeske,[7] and Guinet et al.[16] We compiled individual data on hyperprolactinemic patients with normal-sized (microadenomas) or enlarged sellas (macroadenomas) from 35 reports in-

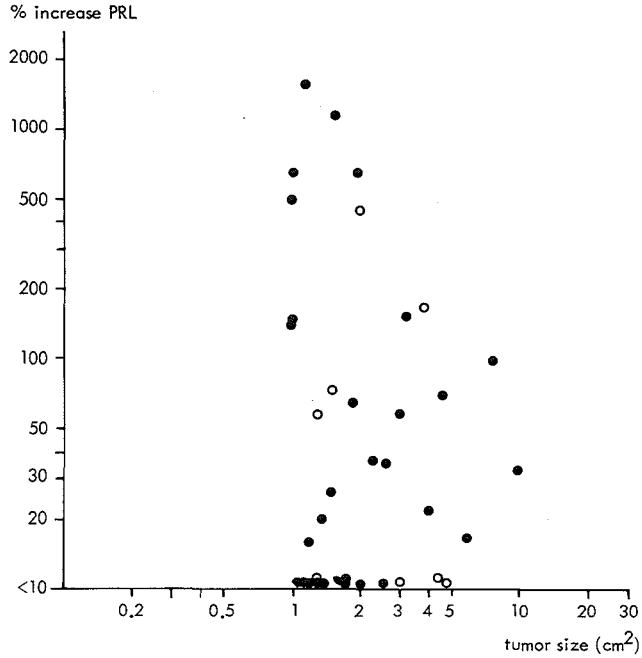

Fig. 3. Relationship between tumor size and percentage increase in ΔPRL after TRH administration (both in log scale) to 38 untreated patients with clinical evidence of a prolactinoma. ●, Female patients. No correlation was demonstrated.

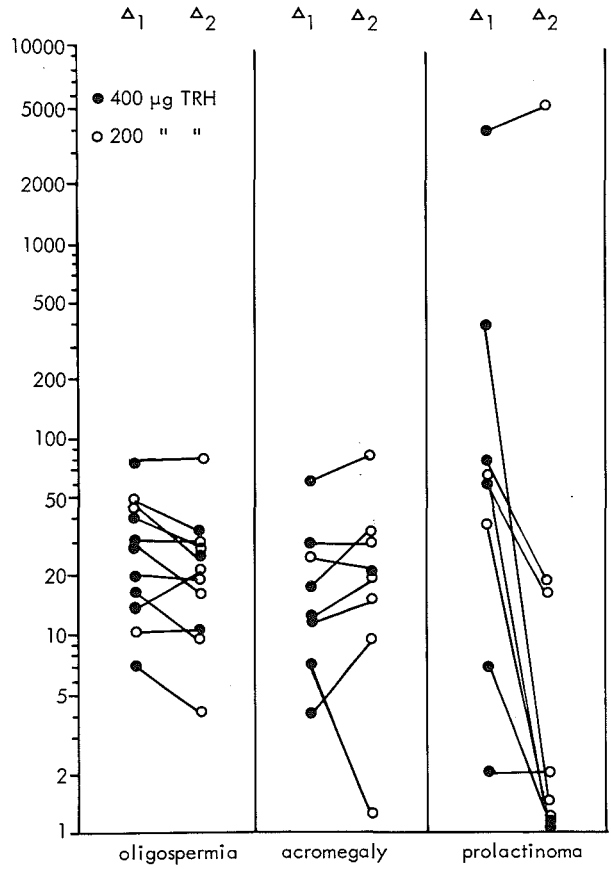

Fig. 4. Comparison of PRL responses (log scale) to 400 and 200 μg of TRH in patients with oligospermia, acromegaly, or a prolactinoma. Δ_1, Response to the first test; Δ_2, response to the second test.

sofar as these data were presented (Table 1). Of 548 patients investigated we counted 61 patients (11%) with a ΔPRL of more than 100%; such a PRL response was also found in 13 of the 144 patients (9%) with a surgically proven pituitary adenoma. The fact that some authors found only blunted responses in their patients with a prolactinoma whereas others observed normal responses in a minority of their patients may be explained by the selection of patients and/or the size of the group of patients investigated. On the basis of the data on these 548 patients it may be concluded that roughly 1 of 10 patients with clinical evidence of a prolactinoma will have a PRL response to TRH of more than 2 times the basal value (''false- positive responder'').

The dosage of TRH used in the various studies was 200 μg or more, whereas with 100 μg a maximal PRL response is found in normal subjects.[37] In our patients with a prolactinoma in whom we carried out a TRH test with 400 μg as wel as with 200 μg of TRH, the first test performed resulted in the highest response to TRH regardless of the dose

used. It is known that, in vitro, the secretion of PRL by pituitary tumor cells is much higher than the secretion by normal lactotrophs and is also higher in relation to the PRL content of the cells.[38] The chromophobe aspect of the tumor cells is probably caused by a low hormone storage[39]; thus exhaustion of the cells may be the cause of the lower or absent PRL response to TRH in the second test performed. This conclusion is further underlined by the observations by Lachelin et al.,[10] who also showed that in patients with a prolactinoma the basal plasma PRL concentration was diminished after repeated administration of TRH.

Values for ΔPRL lower than 100% after TRH administration have also been reported in some normal hyperprolactinemic women during pregnancy and after delivery[40, 41] and in several patients with hyperprolactinemia caused by drugs[42] or during hypoglycemia induced by an insul-intolerance test.[31] Therefore, in hyperprolactinemic states caused by stimuli in the presence of a normal PRL-secreting system, a decreased percentage response of PRL to TRH in relation to that of a control group is also possible.

In contrast to Ayalon et al.,[6] we found a negative correlation between the basal PRL level and the percentage ΔPRL. This finding may be explained by the higher incidence of positive responders in our patients with only slightly increased basal plasma PRL levels. Jeske,[7] Ayalon et al.,[6] and Kleinberg et al.[17] also reported a higher incidence of positive responses in patients with only slightly increased basal PRL levels and a normal-sized sella. However, positive responses may also occur in patients with macroadenomas. Indeed, in our series a correlation between tumor size and the percentage ΔPRL could not be demonstrated.

The use of L-dopa or dopaminergic agonists as a test in the differential diagnosis of hyperprolactinemia appears to be of no value because these agents invariably cause suppression of plasma PRL levels.[7, 8, 12, 16, 17] The absence of an increase in the PRL level after the administration of dopamine antagonists (chlorpromazine, metoclopramide, or sulpiride) as well as the absence of a nocturnal PRL increase are of diagnostic value in addition to the TRH test,[6-8, 28, 33] but normal responses to antagonists[16] and normal nocturnal increases in PRL levels have been reported in patients with a prolactinoma.

In the differential diagnosis of hyperprolactinemia with an impaired response of PRL to TRH, hypothalamic disorders not caused by suprasellar extension of a pituitary tumor must be considered.[1, 25] Snyder et al.[1], Schwinn et al.,[25] and Woolf et al.[44] together reported a lowered PRL response (< 100%) in nine of seventeen hyperprolactinemic patients with hypothalamic disorders. We found also a decreased PRL response to TRH in seven of eleven cases. Thus in approximately 60% of hyperprolactinemic patients with suprasellar disorders, a blunted PRL response to TRH is present (''false-negative responders'').

We conclude that the TRH test may be a useful adjunct in identifying hyperprolactinemic patients with a prolactinoma. However, a positive response does not exclude the presence of a prolactinoma, whereas a negative response with or without an abnormal sella tur-

cica does not exclude primarily suprasellar abnormalities. It is advisable to carry out a computerized tomographic scan (or air encephalography) in most if not all patients with unexplained hyperprolactinemia.

TABLE 1. Survey of 35 Reports Concerning 548 Hyperprolactinemic Patients with Gonadal Insufficiency (with Enlarged or Normal-Sized Sellas)

Reference	Year	TRH dose	Microadenoma		Macroadenoma		Total		Reported surgically proven adenoma	
		μg	No.[a]	%	No.[a]	%	No.[a]	%	No.[a]	%
Jacobs and Daughaday[22]	1973	400			1/7	14	1/7	14		
Del Pozo[23]	1973	250	0/6	0	1/6	17	1/12	8		
Snyder et al.[1]	1974	400				2/11	18			
Archer et al.[24]	1974	500	3/5	60			3/5	60		
Schaison et al.[9]	1975		1/8	12			1/8	12	1/8	12
Schwinn et al.[25]	1975	500			0/5	0	0/5	0	0/5	0
Guinet et al.[16]	1975				3/9	33	3/9	33		
Zarate et al.[26]	1975	400			0/7	0	0/7	0	0/1	0
Aono et al.[27]	1976	500	1/10	10			1/10	10		
Hirvonen et al.[3]	1976	200	1/10	10	0/1	0	1/11	9		
Fossati et al.[28]	1976	200	0/4	0	0/2	0	0/6	0		
Jacobs et al.[2]	1976	200	0/17	0	0/12	0	0/29	0		
Glass et al.[29]	1976	200					0/9	0		
Lamberts et al.[4]	1976	400	3/8	37	4/14	29	7/22	32	3/10	30
Antunes et al.[30]	1977	500					0/4	0		
Kleinberg et al.[17]	1977	500	2/6	33	1/16	6	3/22	14		
Lachelin et al.[10]	1977	200	0/18	0			0/18	0	0/5	0
Boyd et al.[18]	1977	500	0/7	0	2/7	29	2/14	14	2/5	40
Kletzky et al.[31]	1977	500					2/15	13		
Healy et al.[5]	1977	200	0/16	0	0/2	0	0/18	0		
Samaan et al.[32]	1977	500			3/4	75	3/4	75	3/4	75
Tolis[11]	1977						0/11	0	0/2	
L'Hermite et al.[12]	1977	200					0/41	0		
Faglia et al.[13]	1977	200					0/29	0	0/25	0
L'Hermite et al.[19]	1978	200	3/10	30			3/10	30		
Jaquet et al.[33]	1978	200					2/14	14	2/14	14
Silvestrini et al.[34]	1978	200					3/33	9	0/16	0
Cowden et al.[8]	1979	200	0/7	0			0/14	0	0/14	0
Barbarino et al.[14]	1979	200					0/20	0	0/20	0
Ayalon et al.[6]	1979	200					6/18	33	0/2	0
Jeske[7]	1979	200	4/13	31	3/18	17	7/31	23		
Eversmann et al.[35]	1979	200	0/8	0	0/3	0	0/11	0		
Assies[20]	1979	200	3/21	14	5/26	19	8/47	17	2/11	18
Murray et al.[15]	1979	500	0/4	0	0/6	0	0/10	0		
Volpe et al.[36]	1979	200	2/13				2/13	15		
Total			23/191	12	23/145	16	61/548	11	13/144	9

[a] Number to the left indicates number of patients with a positive TRH test (ΔPRL >100%); to the right, number of patients investigated.

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CHAPTER VI

THE FUNCTION OF THE PITUITARY-THYROIDAL AXIS IN ACROMEGALIC PATIETS V. PATIENTS WITH HYPERPROLACTINAEMIA AND A PITUITARY TUMOUR

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SUMMARY

The function of the pituitary-thyroidal axis was examined in fifty-three of sixty-two patients with hyperprolactinaemia and a pituitary tumour and in forty of forty-four acromegalic patients, in whom one or more indices of the pituitary-thyroid function were determined before treatment. In the patients with hyperprolactinaemia and a pituitary tumours, sellar + extrasellar tissue (EST) size showed a significant negative correlation with the response of TSH to TRH (ΔTSH) as well as with the circulating T4 and T3 levels. These correlations were not present in the acromegalic patients. In the prolactinoma group a sharp decrease in mean serum T4 and T3 levels was found at sellar + EST sizes exceeding 3 cm^2. In twenty-three patients with a sellar + EST size of 3 cm^2 or more, thirteen (57%) showed a T4 level of less than 6 μg/dl against none of twenty-eight patients with a sellar + EST size of less than 3 cm^2. For T3, using a limit of 120 ng/dl, the corresponding numbers were eight out of thirteen (62%) and none of ten patients respectively. A positive correlation was observed between ΔTSH and the T3 levels but not between ΔTSH and T4, while in the acromegalic patients there was no correlation between TSH reserve and T3 or T4. In the patients with hyperprolactinaemia and a pituitary tumour positive correlations between basal TSH and ΔTSH as well as between T4

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and T3 levels were observed. These correlations were not found in the acromegalic patients.

In conclusion: (1) Thyroid function appears to be independent of pituitary tumour size in patients with acromegaly but not in patients with hyperprolactinaemia and a pituitary tumour. (2) In acromegalic patients the high incidence of an impaired TSH response (without hypothyroidism and independent of tumour size) may be caused by suppression of TSH secretion rather than by destruction of thyrotrophic cells.

In a previous study (Klijn et al., 1980) we showed that in patients with hyperprolactinaemia and a pituitary tumour, a sellar size or a 'tumour size' [area of sella + extrasellar tissue (EST), if present, on the lateral X-ray of the skull] of 2-3 cm^2 is a critical value with regard to the incidence of extrasellar extension of the tumour and loss of anterior pituitary hormone reserve. The TSH response to TRH (ΔTSH) was negatively correlated with tumour size, while in patients with tumour sizes exceeding 3 cm^2 a sharply increased incidence of an impaired TSH response was seen. Since the importance of the pituitary TSH reserve with respect to the thyroid function in patients with a pituitary tumour is not clear, we examined the significance of tumour size and ΔTSH in relation to circulating thyroid hormone levels in the group of patients we reported on before (Klijn et al., 1980), comparing the results to those obtained in a group of acromegalic patients.

PATIENTS, MATERIALS AND METHODS

Patients

Sixty-two patients (thirty-eight women) with hyperprolactinaemia and a pituitary tumour (without growth hormone excess) were investigated as well as forty-four patients (twenty-one women) with acromegaly. In the group of patients with hyperprolactinaemia and a pituitary tumour the mean age of the female patients was 32 years (median 29 years, range 16-67 years) and of the male patients 35 years (median 35 years, range 15-59 years). In the acromegalic patients the mean age of the women was 39 years (median 40 years, range 19-74 years) and of the men 40 years (median 41 years, range 21-71 years). All patients were untreated.

Radiological and ophthalmological examination

All patients were investigated by plain radiography, lateral polytomography (hypocycloidal, section of 2 mm) and complete ophthalmological evaluation, including visual fields. Further radiological examination consisted of air-encephalography (AEG) and in most cases angiography of the carotid artery. As a measure of tumour size we have chosen the largest lateral area of the sella in combination with that of extrasellar tissue (EST), if present (Klijn et al., 1980).

Endocrine investigations and assays

In forty-one untreated patients with a pituitary tumour and hyperprolactinaemia (thirty

females, eleven males, age range 15-67 years; the age of only one patient exceeded 44 years) and in twenty-seven untreated acromegalic patients (fifteen women, twelve men, age range 19-65 years; six patients above 50 years) the TSH response to TRH (400 μg, Hoechst) was measured as described before (Klijn et al., 1980).

Before any treatment basal thyroxine and triiodothyronine levels were determined in fifty-two and twenty-three of the sixty-two patients with hyperprolactinaemia and a pituitary tumour as well as in forty and eighteen of forty-four acromegalic patients, respectively. (In this retrospective study T4 and T3 were not determined before operation in a number of patients). In addition, in most of the patients, thyroxine binding proteins were assessed by means of the resin-uptake test (triosorbkit Abbott Laboratories). Prolactin, thyroxine (T4), triiodothyronine (T3) and TSH were measured using previously described radioimmunoassay techniques (Kwa et al., 1973, Visser et al., 1975, Docter et al., 1972, Klijn et al., 1980). Plasma GH levels were measured by a homologous radioimmunoassay (IRE, Belgium). Thyroid antibodies were investigated in forty-four patients with hyperprolactinaemia and a pituitary tumour and in twenty-seven acromegalic patients, especially when T4 was low or basal TSH at the upper limit of normal. Circulating thyroid antibodies were found in a 28-year-old acromegalic woman (T4 = 9.5 μg/dl, basal TSH = 4.2 mu/l, ΔTSH = 13.8 mu/l) and in a 64-year-old acromegalic man (T4 = 9.7 μg/dl, basal TSH = 1.6 mu/l, ΔTSH = 4.7 mu/l).

Normal values

Normal values for sellar size are between 0.7 and 1.4 cm^2 (95% range in 100 patients without pituitary pathology). Normal basal values of TSH are less than 4.9 mu/l (95% range of 71 controls). In our laboratory the maximal increment of plasma TSH in response to stimulation with 400 μg TSH is between 8.1 and 38.7 mu/l in eleven females aged 20-40 years, between 7.1 and 16.0 mu/l in eight females aged 40-60 years and between 4.9 and 14.0 mu/l in seven females aged 61-72 years. In eight normal men ΔTSH varied between 6.1 and 13.1 mu/l. The mean value $\pm$ SD of T4 is 8.6 $\pm$ 1.95 μg/dl in fifty controls (95% between 4.7 and 12.5 μg/dl). The mean value $\pm$ SD of T3 is 145 $\pm$ 30 ng/dl (95% range in 180 controls between 85 and 205 mg/dl). There was no sex difference in the thyroid hormone concentrations.

Statistical analysis

Statistical analysis of the data was performed using regression analysis, two-tailed Student's t test or χ^2-test. Partial correlation coefficients were calculated according to Snedecor & Cochran (1967).

RESULTS

Relationship between tumour size and ΔTSH

In contrast to observations made in patients with hyperprolactinaemia and a pituitary tu-

mour there was no correlation between sellar + EST size and ΔTSH in acromegalic patients ($n = 27$, r = 0.03). Impaired TSH responses to TRH appeared to be independent of tumour size (Fig. 1**b**, Table 1), age, basal GH ($n = 21$, r = 0.19) or GH response to TRH ($n = 13$, r = 0.35). The first finding is in sharp contrast with the significantly increased incidence of a subnormal TSH reserve in patients with hyperprolactinaemia and a pituitary tumour at sellar + EST sizes of 3 cm² or more (Fig. 1**a**). This was true using control groups divided according to sex and age (see normal values) as well as using a standard lower limit of ΔTSH of 5 mu/ml (Table 1).

No correlation between basal 17β-estradiol and ΔTSH was present in the amenorrhoeic patients with a prolactinoma ($n = 13$, r = −0.32) or acromegaly ($n = 7$, r = +0.13).

Relationship between tumour size and circulating thyroid hormone levels

In the patients with hyperprolactinaemia and a pituitary tumour, sellar + EST size showed a negative correlation with the circulating T4 and T3 levels in serum (Figs. 2**a** and 3**a**, both $P < 0.005$). A sharp decrease in mean plasma T4 and T3 levels was found at sellar + EST sizes exceeding 3 cm². Lowered or low normal levels of T4 (< 6 μg/dl) and T3 (< 120 ng/dl) were present only in patients with a sellar + EST size of 3 cm² or more (57% for T4 and 62% for T3; $P < 0.005$ and $P < 0.01$ respectively). Slight (ΔTSH: 5-7 mu/l; $n = 5$) or more marked lowering (ΔTSH < 5 mu/l; $n = 1$) of TSH reserve in patients with tumours smaller than 3 cm² was never accompanied by low T4 or T3 levels.

In contrast to these findings we observed no correlation between sellar + EST size and circulating plasma T4 or T3 levels in the acromegalic patients (Figs. 2**b** and 3**b**). Low values of T4 (less than 6 μg/dl) were not found. However, one patient had a borderline free thyroxine index on the basis of a decrease in T3 resin uptake.

Relationship between ΔTSH and circulating thyroid hormone levels

Although in the patients with hyperprolactinaemia and a pituitary tumour a tendency to lower T4 levels existed with decreasing values of ΔTSH, no significant correlation between ΔTSH and serum T4 levels was found (Fig. 4**a**). A positive correlation, however, was observed between ΔTSH and the circulating T3 levels (Fig. 5**a**, $P < 0.01$). In the group of patients with hyperprolactinaemia and a pituitary tumour partial correlation coefficients were calculated for the relationships between tumour size, ΔTSH and T4 or T3. The partial correlation coefficient for the relationship between tumour size and T4 after exclusion of the influence of ΔTSH ($n = 39$) was −0.4018 ($P < 0.05$); between ΔTSH and T4 after exclusion of the influence of tumour size 0.1213 (not significant = n.s.) and between ΔTSH and tumour size after exclusion of T4 −0.4508 ($P < 0.01$). The partial correlation coefficients for the same relationships with T3 instead of T4 ($n = 19$) were −0.3387 (n.s.), 0.4041 (n.s.) and −0.3775 (n.s.)

In the acromegalic patients T4 as well as T3 was not correlated to ΔTSH (Figs. 4**b** and 5**b**).

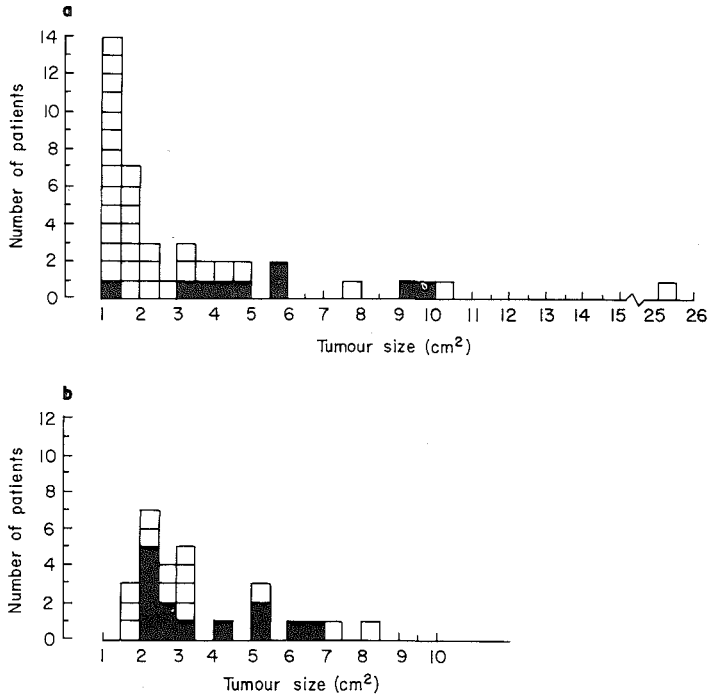

Fig. 1. Frequency distribution of impairment of the TSH response to 400 μg TRH in forty-one untreated patients with hyperprolactinaemia and a pituitary tumour (**a**) and in twenty-seven untreated acromegalic patients (**b**) with respect to tumour size. Solid boxes indicate an impaired TSH response (ΔTSH < 5 mu/l). **a** Difference between patients with a tumour size above and below 3 cm²: $P < 0.0025$ (χ^2test). **b:** n.s.

Relationship between T4 and T3

In the patients with hyperprolactinaemia and a pituitary tumour a positive correlation between T4 and T3 levels was found ($n = 23$, r = +0.42, $P < 0.05$), but low levels of T3 can be accompanied by normal levels of T4 and vice versa. In the acromegalic patients a correlation between T4 and T3 was absent ($n = 16$, r = +0.23, n.s.).

TABLE 1. Incidence of pituitary-thyroid hypofunction in untreated patients with pituitary tumours (% of cases investigated).

	Prolactinoma			Acromegaly		
	<3 cm²	>3 cm²	Whole group	<3 cm²	>3 cm²	Whole group
ΔTSH ↓ (<5 mu/l)	4	50	22	50	46	48
ΔTSH ↓ (control groups)	24	75	44	57	54	56
T4 <6 μg/dl	0	57	25	0	0*	0*

* One patient with a borderline value of the F.T.I.

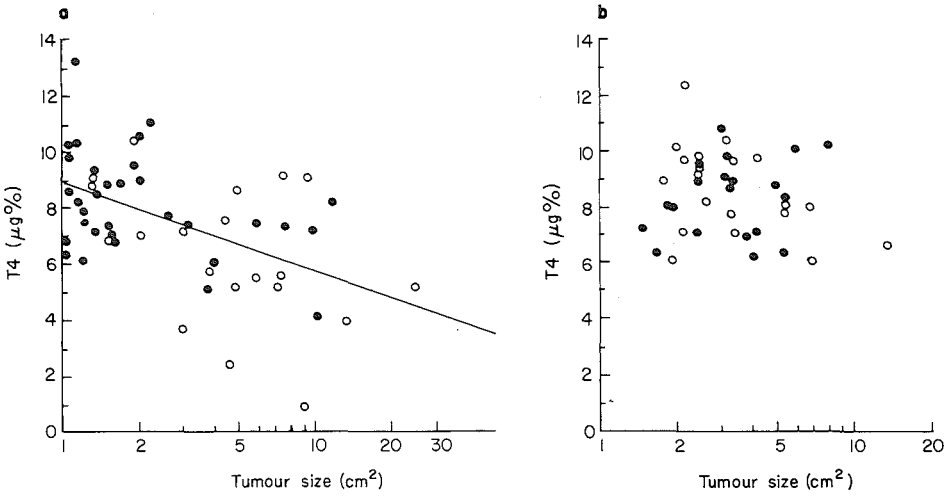

Fig. 2. Relationship between (log) tumour size and serum T4 levels in fifty-two untreated patients with hyperprolactinaemia and a pituitary tumour (**a:** r = −0.50; $P < 0.0005$) and in forty untreated acromegalic patients (**b:** r = +0.14, n.s.). ● = Women; ○ = men.

Significance of basal TSH

In the patients with hyperprolactinaemia and a pituitary tumour a negative correlation was found between log sellar + EST size and log basal TSH ($P < 0.025$). There was a positive correlation between log basal TSH and log ΔTSH ($P < 0.001$; Fig. 6a). Only one out of twenty-six patients with a basal TSH of 1 mu/l or more showed a ΔTSH of

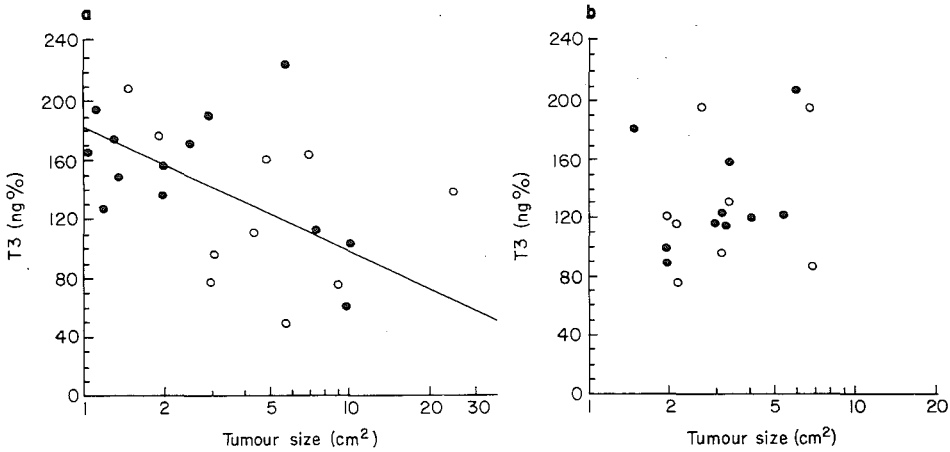

Fig. 3. Relationship between (log) tumour size and serum T3 levels in twenty-three untreated patients with hyperprolactinaemia and a pituitary tumour (**a:** r = −0.60; $P < 0.005$) and in eighteen untreated acromegalic patients (**b:** r = +0.80; n.s.). ● = Women; ○ = men.

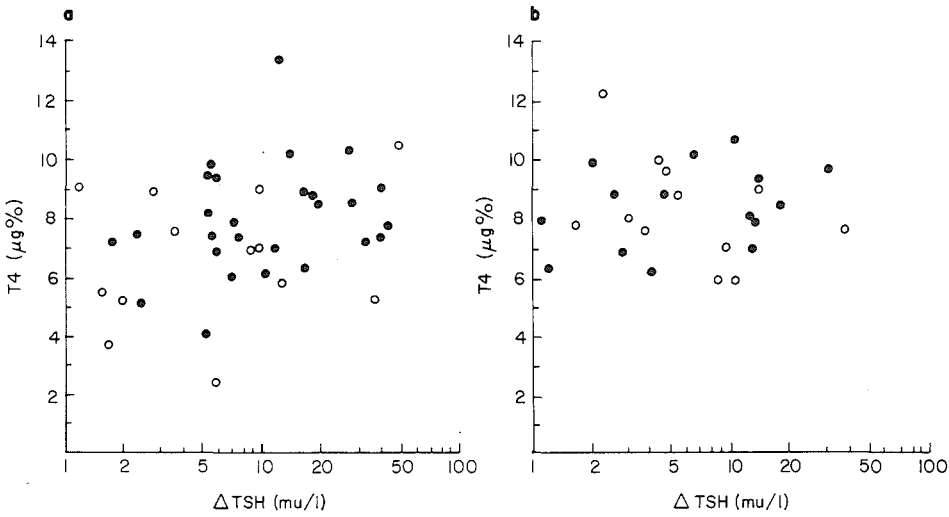

Fig. 4. Relationship between (log) ΔTSH and serum T4 levels in forty untreated patients with hyperprolactinaemia and a pituitary tumour (**a:** r = +0.31; $0.10 > P > 0.05$) and in twenty-seven untreated acromegalic patients (**b:** r = +0.02; n.s.). ● = Women; ○ = men.

less than 5 mu/l. In the acromegalic patients correlations between basal TSH and sellar + EST size (n = 27, r = 0.006) or ΔTSH (Fig. 6**b**) were absent.

In both groups of patients correlations between the basal TSH level and circulating levels of T4 or T3 were absent because of the incidence of normal T4 and T3 levels in the presence of undetectable basal TSH.

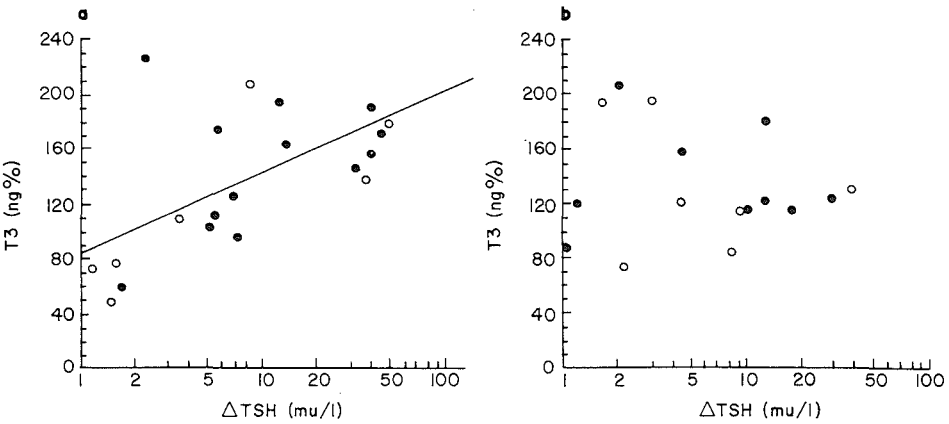

Fig. 5. Relationship between (log) ΔTSH and serum T3 levels in twenty untreated patients with hyperprolactinaemia and a pituitary tumour (**a:** r = +0.62; $P < 0.01$) and in sixteen untreated acromegalic patients (**b:** r = +0.01; n.s.). ● = Women; ○ = men.

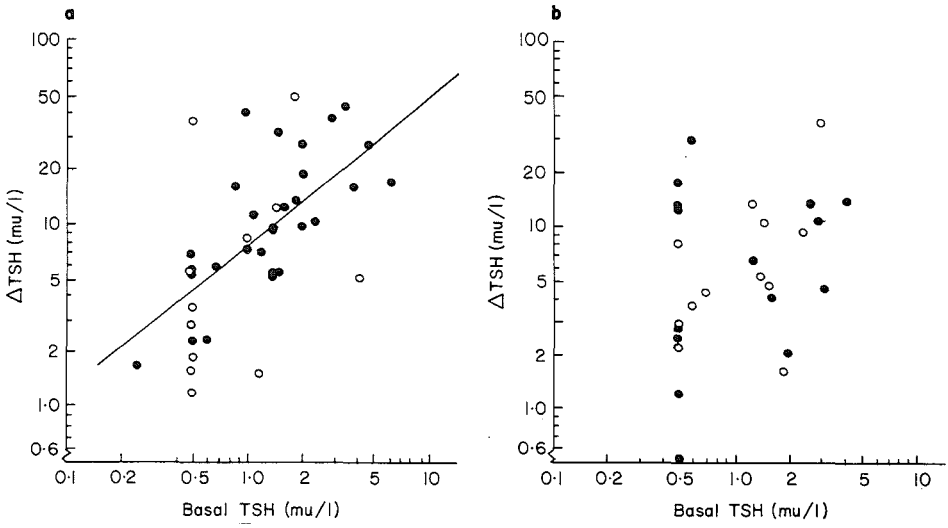

Fig. 6. Relationship between (log) basal TSH and (log) ΔTSH in the TRH test in forty-one untreated patients with hyperprolactinaemia and a pituitary tumour (**a:** r = +0.61; $P < 0.001$) and in twenty-seven untreated acromegalic patients (**b:** r = +0.35; $P < 0.10$). • = Women; ○ = men.

DISCUSSION

In patients with hyperprolactinaemia and a pituitary tumour we have previously shown that there is a sharply increased incidence of loss of anterior pituitary hormone reserve above a radiological sellar + EST area of 3 cm² (Klijn et al., 1980). The results of the present study indicate that in these patients an area of 3 cm² might also be critical with regard to the function of the target organ, the thyroid gland. It is not clear to what extent the TSH secretion has to be impaired before secondary hypothyroidism ensues. In patients with hyperprolactinaemia and a pituitary tumour we did not find a positive correlation between ΔTSH and serum T4 levels. Such a relationship could be absent because of the possible inclusion of patients with tertiary hypothyroidism: low normal or slightly decreased T4 levels in combination with a normal or a hyper-response of TSH to TRH. Another cause might be the production of an abnormal TSH molecule which could be responsible for subnormal or low normal thyroid function (Faglia et al., 1975; Petersen et al., 1978). A third mechanism could be an increased sensitivity of the thyroid to TSH in partial pituitary failure (Sluiter, 1979). The finding of the combination of low thyroid hormone levels and decreased TSH reserve (instead of the expected increased TSH reserve in the case of an intact pituitary-thyroidal axis) indicates destruction of thyrotrophs in at least some of the patients with hyperprolactinaemia and a large pituitary tumour. In cases with extensive pituitary destruction an impaired conversion of T4 to T3 may also be involved (Balsam et al., 1978).

In the acromegalic patients the lack of correlation between tumour size, basal TSH,

ΔTSH, T4 and T3 is striking. The discrepancy observed between the very low incidence of hypothyroidism and the frequent occurrence of a decreased TSH reserve (independent of tumour size) which is in contrast to the findings in our prolactinoma patients, indicates suppression of TSH secretion rather than destruction of thyrotrophs in some acromegalic patients. This suppression of TSH secretion may be caused by an autonomous thyroid function (as in hyperthyroidism) as suggested by Hall et al. (1972). Goitre has been found in a considerable number (25-50%) of acromegalic patients (Davidoff, 1926; Davis, 1941; Daughaday, 1974). Our findings in acromegalic patients show a certain similarity to those patients with euthyroid multinodular goitre in whom ΔTSH is inversely related to thyroid weight and not related to basal serum T4 or T3 concentrations (Smeulers et al., 1977). The observation that GH administration may reduce the TSH response to TRH in pituitary dwarfs (Kanatsuka et al., 1979) as well as the finding that the TSH reserve improved after blocking the release of GH in acromegaly (Fanghanel-S et al., 1978) suggest increased somatostatin secretion in response to high GH levels as an alternative cause of suppressed TSH secretion. However, in our acromegalic patients we found no relationship between GH levels on the one hand and TSH reserve on the other.

In is concluded that the cause of an impaired TSH response to TRH in acromegalic patients may differ from that observed in patients with prolactinomas.

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CHAPTER VII

THE ROLE OF PROLACTIN IN THE INHIBITORY ACTION OF BROMOCRIPTINE ON GROWTH HORMONE SECRETION IN ACROMEGALY

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SUMMARY

Abstract. Bromocriptine treatment results in clinical improvement and inhibition of plasma GH levels in only part of the acromegalic patients. The possible role of the simultaneous presence of Prl and GH in GH-secreting pituitary adenomas was investigated with regard to the inhibitory action of bromocriptine on GH secretion and the paradoxical increase of GH release in reaction to TRH.

Surgically obtained pituitary tumour tissue from 35 consecutive acromegalic patients was studied immunohistochemically. In 21 patients no Prl was present in the tumour tissue. These patients had normal plasma Prl levels. In the other 14 patients Prl was present in the tumour tissue. Hyperprolactinaemia was found in 10 of these 14 patients. Plasma GH levels from 2 till 10 h after the administration of 2.5 mg bromocriptine measured before operation were significantly more suppressed in the patients with mixed GH/Prl-containing than in those with pure GH-containing pituitary adenomas, being 38 $\pm$ 4% and 65 $\pm$ 4% of basal values, respectively ($P < 0.01$). The response of GH to TRH, however, did not differ significantly between the two groups. Conclusions: 1. In about 70% of patients with 'mixed' GH/Prl containing adenomas, hyperprolactinaemia is present. 2. The simultaneous presence of Prl and GH in a GH-secreting pituitary tumour increases the sensitivity of GH secretion to bromocriptine. 3. The plasma Prl level is of value to predict which patients with acromegaly are likely to respond to bromocriptine with an inhibition of GH secretion.

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Acute administration of dopaminergic drugs paradoxically suppresses growth hormone (GH) secretion in part of the patients with acromegaly (Liuzzi et al. 1972, 1974a,b). Chronic bromocriptine therapy was reported to lower plasma GH levels and to have beneficial clinical effects in a lot of acromegalic patients (Sachdev et al. 1975; Belforte et al. 1977; Wass et al. 1977). In other studies however, no or hardly any positive effect was reported (Summers et al. 1975; Dunn et al. 1977; Lindholm et al. 1981). No clear explanation for the discrepancy between these results of the treatment with bromocriptine has been offered so far (Thorner et al. 1981; Köbberling et al. 1982).

We recently studied the relationship between the plasma prolactin (Prl) concentration of 79 untreated acromegalic patients and the response of GH to bromocriptine (Lamberts et al. 1982). Hyperprolactinaemia was present in 42% of these patients, and it was shown that an increased plasma Prl level is accompanied in most acromegalic patients by high sensitivity of GH secretion to bromocriptine in the acute test (Lamberts et al. 1982), but also during chronic therapy (Lamberts et al. 1979).

In the present study the immunohistochemical presence or absence of Prl in pituitary tumour tissue of 35 transsphenoidally operated acromegalic patients was correlated with the in vivo sensitivity of GH secretion to bromocriptine and TRH.

PATIENTS AND METHODS

Thirty-five untreated acromegalic patients (16 males and 19 females) aged 23-62 years were studied. The diagnosis of acromegaly was made by the combination of the presence of clinical signs and symptoms of increased GH secretion with elevated fasting plasma GH levels, which could not be suppressed to less than 5 ng/ml after oral administration of 100 g of glucose. Plasma levels of GH were determined by a double antibody radioimmunoassay method using the C.E.A. kit (CIS, France). Normal values in both sexes are 1-5 ng/ml. Plasma Prl levels were determined by the same method with a kit from IRE (Antwerp, Belgium). In both assay systems 1 ng of the standard employed is equivalent to 1 ng of the standard VLS = 1 of the NIH. The upper limit of normal plasma Prl levels is 12 ng/ml in men and 15 ng/ml in women. Inhibition curves of standard human GH were repeatedly compared with inhibition curves of patient plasma and were always found to be parallel. No cross- reactivity of GH was found in the Prl-assay under GH levels of 150 ng/ml and vice versa.

In the study of GH secretion in response to bromocriptine, the patients were studied in the morning in basal conditions. All studies were done between 3-14 days before operation. Serial blood specimens were collected through an indwelling polyethylene catheter placed in an antecubital vein. At least 4 blood samples were taken before 2.5 mg bromocriptine was administered at 8 a.m. and additional samples were drawn hourly up till 12 h following the drug and after 24 h. The TRH test was carried out on a separate day at 2 p.m. Four-hundred μg TRH (Hoechst, Wiesbaden, Germany) was administered iv

for 30 s, and apart from at least two baseline blood samples, additional samples were obtained at 20, 30, 60 and 120 min.

In these patients no medication that might account for an elevated plasma Prl level was taken and the existence of primary hypothyroidism was excluded. Suprasellar extension of the pituitary tumour was investigated by CT-scanning of the suprasellar region.

Paraplast-embedded tissue blocks from pituitary adenomas of acromegalic patients obtained by transsphenoidal surgery were investigated by light microscopic and immunohistochemical methods. Pathological investigations were done blindly, without knowledge about the endocrine characteristics of these patients. All tissues had been fixed for 6-8 h in 4% phosphate-buffered neutral formaldehyde and processed routinely. Tumour histology was studied by haematoxylin and eosin, PAS and Masson staining methods. For the immunohistochemical identification of GH and Prl producing cells in the adenomatous tissues, an direct immunohistoperoxydase method (Nakane & Pierce 1967) was used as described in detail elsewhere (Nieuwenhuijzen Kruseman et al. 1975). The rabbit anti-human GH antibodies were purchased from Behring Werke (Western Germany; lot no. 1896, OTXIX) and Wellcome (Beckenham, England). The rabbit anti-human Prl antibodies were a kind gift from Dr. P.K. Nakane (Denver, USA) or were obtained from Calbiochem- Behring Corp.

Statistical evaluation was done by analysis of variance.

RESULTS

Specimens of pituitary tumour tissue obtained at transsphenoidal operation from 35 consecutive acromegalic patients were investigated (Table 1). In all patients immunoreactive GH was present in the tumour tissue, while no Prl immunoreactivity was observed in the tumour tissue of 21 patients ('pure GH containing pituitary adenomas'). In 14 patients both immunoreactive GH and Prl were present in the tumour tissue ('mixed GH/Prl containing pituitary adenomas'). The prevalence of acidophilic, chromophobic and mixed acidophilic/chromophobic staining pattern was similar in both groups of patients (Table 1).

In 10 of the 14 patients in whom Prl was shown to be present in the pituitary tumour, elevated circulating plasma Prl levels were present, while no additional hyperprolactinaemic acromegalics were found in the group of patients with 'pure GH- containing adenomas' (Table 1). Suprasellar extension of the pituitary tumour was present in 10 patients (5 patients with pure GH- and 5 with mixed GH/Prl- containing adenomas; Table 1).

No statistically significant difference was seen between plasma GH concentrations between both groups of patients (Table 1). The percentage inhibition of GH levels after bromocriptine as measured over 24 h (Fig. 1) was from 2 till 10 h after a dose of 2.5 mg statistically significantly more pronounced in the 14 patients with mixed tumours than in the 21 patients with pure GH-containing tumours ($P < 0.01$ after 3, 4, 5, 6, 7, 8

TABLE 1. Histology, immunohistochemistry, circulating GH and Prl levels in 35 acromegalic patients operated transsphenoidally.

Patient	M/F	Age years	Histology	Immunohistochemistry Prl present	Plasma Prl[1] (ng/ml)	Plasma GH[1] (ng/ml)	Plasma GH after bromocriptine[2] %	Plasma GH after TRH[3] %	Suprasellar extension of the tumour
1	M	33	acid+chrom	−	4±3	80±6	90±2	−(122)	+
2	M	36	acid	−	10±3	23±8	57±3	+(366)	−
3	M	57	acid	−	9±1	24±3	80±5	+(356)	−
4	M	33	acid+chrom	−	7±3	69±5	71±3	−(80)	+
5	M	48	acid	−	8±2	33±8	51±3	+(129)	−
6	M	23	acid	−	6±3	43±4	60±4	+(1500)	−
7	M	47	acid	−	8±2	57±13	57±4	+(252)	−
8	F	52	acid	−	11±3	12±3	96±7	−(10)	−
9	F	32	acid+chrom	−	10±1	35±6	65±6	−(27)	−
10	M	55	acid	−	9±4	15±4	62±5	−(8)	−
11	M	61	acid+chrom	−	9±1	23±4	82±5	+(225)	−
12	M	50	acid	−	11±3	16±3	78±6	−(72)	−
13	F	46	acid+chrom	−	7±2	28±2	52±3	−(67)	−
14	F	47	acid	−	3±2	51±6	91±4	−(0)	−
15	F	63	acid+chrom	−	12±1	11±2	36±7	+(412)	−
16	F	47	acid+chrom	−	2±1	102±10	49±4	+(144)	+
17	M	45	acid	−	6±2	29±2	84±3	−(50)	−
18	F	47	acid	−	10±3	17±4	52±3	−(67)	−
19	F	52	acid	−	4±2	8±3	102±2	−(0)	+
20	M	37	acid	−	3±4	11±1	86±4	−(40)	−
21	F	28	acid+chrom	−	9±2	107±12	79±5	−(44)	+
22	F	44	acid+chrom	+	160±39	16±3	17±5	+(2300)	−
23	F	36	chrom+acid	+	69±6	54±7	50±1	not done	−
24	M	50	chrom	+	145±46	244±63	33±2	+(135)	+
25	M	62	acid+chrom	+	173±16	88±5	35±5	+(118)	+
26	F	31	acid+chrom	+	141±21	142±47	33±7	+(183)	+
27	M	53	acid+chrom	+	27±4	24±3	40±3	−(67)	−
28	F	49	acid	+	9±2	44±4	52±6	+(800)	−
29	M	49	acid	+	5±2	18±3	31±4	+(414)	−
30	M	42	acid	+	2±3	35±4	31±2	−(75)	−
31	F	26	acid+chrom	+	19±3	247±11	58±20	−(13)	+
32	M	62	acid+chrom	+	175±36	117±22	19±4	+(1350)	+
33	F	31	acid	+	24±8	36±5	37±6	−(18)	−
34	F	53	acid+chrom	+	20±3	12±2	27±2	+(963)	−
35	F	56	acid	+	5±2	18±4	44±3	+(525)	−

1 Plasma Prl and GH are mean ± SEM of at least 5 determinations on different days.

2 Plasma GH measured in 8 samples taken hourly from 2 till 10 h after the administration of 2.5 mg bromocriptine as a percentage of two basal values.

3 Maximal increment of plasma GH after TRH as percentage of the basal level (+ = increment) of more than 100%; the percentage maximal increase is given in parentheses.

acid = acidophilic; chrom = chromophobic.

and 9 h and $P < 0.05$ 2 and 10 h after bromocriptine). The individual responses of GH secretion in these 35 patients are shown in Table 1. Bromocriptine induced an inhibition of GH release of more than 50% in 2 of 21 patients with pure GH-containing tumours and in 11 of 14 patients with mixed tumours.

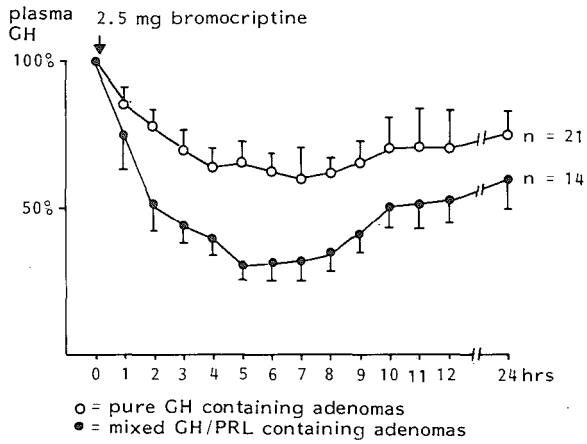

Fig. 1. The response of plasma GH to the administration of 2.5 mg bromocriptine at 8 a.m. followed for 24 h in 21 acromegalic patients (o) with 'pure' GH-containing pituitary ademonas and 14 acromegalic patients (•) with presumably 'mixed' GH/Prl containing adenomas. A significantly higher suppression of plasma GH was observed in the latter group from 2 till 10 h after administration of bromocriptine: $P < 0.01$ after 3, 4, 5, 6, 7, 8 and 9 h and $P < 0.05$ after 2 and 10 h. The vertical bars represent SEM.

A paradoxical increment of plasma GH after TRH administration of more than 100% of the basal value was seen in 9 of the 21 patients with pure GH-containing adenomas and in 9 of the 13 patients examined with mixed tumours. This difference is not statistically significant. A homogeneity in the responses of GH to bromocriptine and TRH (i.e. the presence or absence of both a decrease of GH of more than 50% after bromocriptine and an increase of GH of more than 100% of the basal value after TRH) was observed in 23 of the 34 acromegalic patients (68%). This homogeneity in responsiveness of GH to TRH and bromocriptine in patients with acromegaly was also present if the percentage increase of GH after TRH was correlated with the percentage decrease of GH from 2-8 h after bromocriptine ($r = 0.48$; $P < 0.01$ in the whole group of 34 patients and $r = 0.59$; $P < 0.05$ in the 13 patients with mixed GH/Prl containing tumours).

DISCUSSION

Medical treatment with dopaminergic drugs of patients with acromegaly is still controversial, because of the differences reported in the sensitivity of GH secretion to bromocriptine and the variability of the clinical improvement (Belforte et al. 1977; Dunn et al. 1977; Lindholm et al. 1981; Sachdev et al. 1975; Summers et al. 1975; Wass et al. 1977). Liuzzi et al. (1974b) showed that in general those acromegalic patients whose GH levels are inhibited by dopaminergic drugs also react to TRH with a paradoxical increase of GH secretion, while patients unresponsive to dopaminergic stimuli were also unresponsive to TRH. This homogeneity in the responsiveness of GH to bromocriptine and TRH in acromegaly was seen in 86% (Liuzzi et al. 1974b), 76% (Lamberts et al. 1982)

and 68% (present study) of the patients. This resemblance between the characteristic qualities of GH secretion in some acromegalics with those of normal Prl secretion, led several groups to suggest a role for Prl in the response of acromegalics to bromocriptine (Chiodini et al. 1974; Lamberts et al. 1976, 1979; Werner et al. 1978). In a recent study (Lamberts et al. 1982) elevated plasma Prl levels were found in 42% of 79 untreated acromegalic patients. Bromocriptine (2.5 mg) inhibited GH secretion by more than 50% in 22 % of the normoprolactinaemic patients and in 70% of the hyperprolactinaemic patients ($P < 0.001$).

Elevated plasma Prl levels were found in 32% of 73 untreated acromegalic patients in another report (De Pablo et al. 1981). A subject of speculation has been whether normal or tumour cells are reponsible for the hypersecretion of Prl. The GH- producing tumour may interfere in the transport of hypothalamic Prl-inhibting factor (PIF) resulting in hypersecretion of Prl by the normal lactotroph which is no longer restrained by hypothalamic PIF. The immunohistochemical investigation in the present study indicates, however, that in the majority of the cases the elevated plasma Prl level is a result of secretion of Prl by a mixed GH/Prl containing adenoma. Histological examination of pituitary tumour tissue from our acromegalic patients did not allow a prediction as to the presence or absence of Prl in the GH- secreting pituitary adenoma, because histological staining procedures are not specific for the nature of the hormones present in the cells. There was suprasellar extension of the pituitary adenoma in 5 of the 14 patients with mixed tumours and in 5 of the 21 patients with pure GH-secreting adenomas. This series of patients seems too small to exclude a role of tumour mass in hyperprolactinaemia observed in the 5 patients with mixed tumours.

From the results observed in this group of 35 acromegalic patients and the 79 patients reported before (Lamberts et al. 1982) we conclude that the plasma Prl concentration may be of value in the prediction of which patients would be likely to react with a paradoxical inhibition of GH secretion to bromocriptine. A parallel was observed between the presence of clearly increased circulating Prl concentrations and the presence of Prl in the tumour in only 10 of 14 patients. So hyperprolactinaemia cannot be used as the sole parameter in order to predict whether an acromegalic patient is likely to react to bromocriptine with an inhibition of GH secretion. We suggest that the findings in the present study might explain at least in part the discrepant results in the medical treatment of acromegaly (Belforte et al. 1977; Dunn et al. 1977; Lindholm et al. 1981; Sachdev et al. 1975; Summers et al. 1975; Wass et al. 1977).

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CHAPTER VIII

LONG-TERM FOLLOW UP AFTER EXTERNAL PITUITARY IRRADIATION OF PITUITARY ADENOMAS

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Introduction

Surgery, medical treatment and radiation therapy are the three main types of therapy for pituitary tumors. Radiation therapy was first applied in the management of acromegaly. In 1907 successful radiotherapy was carried out by Gramegma (1) in a 45-year-old acromegalic female and by Béclère (2) in a 16-year-old girl with gigantism. After 75 years of experience, there is no longer doubt that radiotherapy plays a major role in the management of pituitary adenomas especially because of its efficacy in longterm tumor control (3-7). External radiotherapy can be used as a single therapy or after surgery as an additional treatment and for preventing tumor recurrences. However, there are hardly any data on the long-term effects of radiotherapy (without or with surgery) especially on survival. Therefore, the aim of the present retrospective study was measurement of the long-term effects of conventional external radiotherapy in combination with surgery or as a single treatment. Patients (n = 336) with a pituitary tumor treated since 1950 in Rotterdam were studied with regard to 1) hypersecretion, especially growth hormone secretion; 2) anterior pituitary functions; 3) recurrence rate and 4) survival.

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PATIENTS AND METHODS

The records of 336 patients (169 women) with a pituitary tumor treated since 1950 were reviewed. It concerns 117 patients with acromegaly (group I) and 219 patients with a macroprolactinoma or a ''non-functioning'' tumor (group II). Of group II 47 were certain macroprolactinomas and in 172 cases it was impossible to retrospectively decide whether it concerned a ''non-functioning'' or a prolactin (PRL) secreting tumor. The mean age of group I was 43 year (range 14-72 year) and of group II 46 year (range 16-82 year). The cumulative percentage to age is nearly the same in both groups of patients (fig. 1). Thirty patients in group I and 33 in group II needed a second surgical and/or radiotherapeutical treatment because of recurrence or a disturbing residue of the tumor. The occurrence of a recurrence was determined by radiological (pneumoencephalography, computertomography), ophthalmological and/or hormonal examinations.
Eighty two patients were treated with orthovoltage (40 as a single therapy and 42 in combination with surgery) and 238 patients with megavoltage (53 as a single therapy and 185 in combination with surgery) irradiation, while 16 did not receive any kind of radiotherapy. In total 111 patients were operated by the transsphenoidal route.

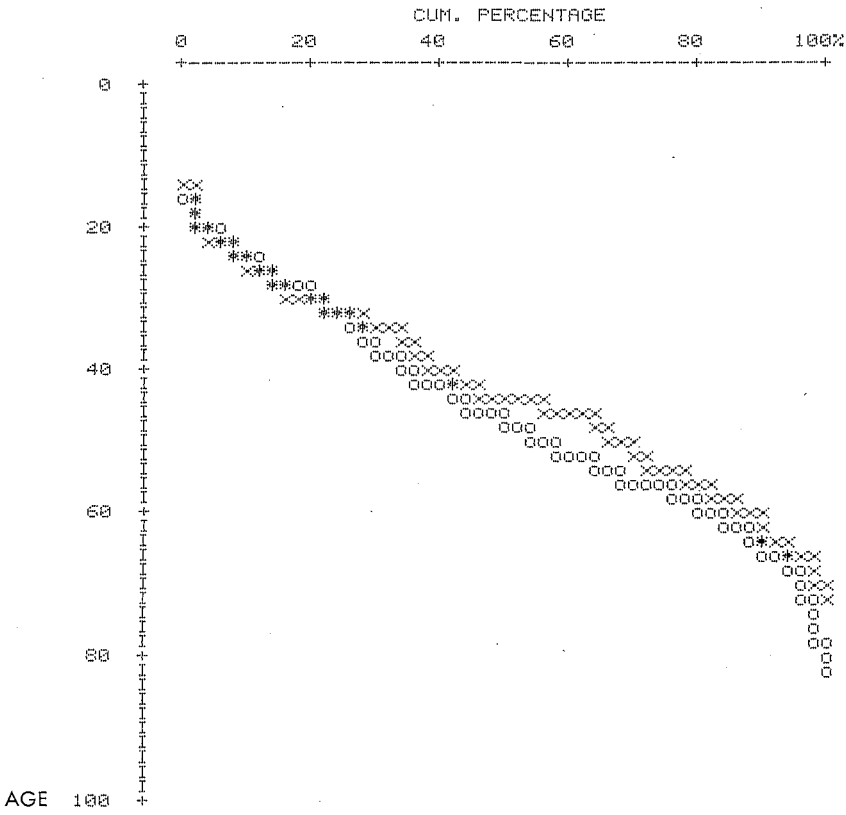

Fig. 1. The cumulative percentage to age for group 1 (xxxx) and group II (oooo): see text.

Plasma concentrations of GH, PRL, TSH, thyroxine, gonadotropins, sex steroids, cortisol and 11-desoxycortisol as well as urinary 17-OHCS were determined as described before (8). Determination of plasma GH was available since 1965, while most of the other hormones were measured in plasma since the beginning of the seventies. For measurement of normal pituitary function we have used also the TRH-, LHRH- and metyrapone test.
Statistical analysis of the data was performed using two-tailed students t-test or x^2- test.

Radiotherapeutic procedure
Radiotherapy was given within 1-2 months after operation in 23 doses of 200 rad, four to five doses a week. Before 1966 the patients were irradiated with an orthovolt apparatus of 250 KV (3000 rad in 18-22 days, 200 rad per dose). After 1966 the patients have been treated with 4 or 6 MV linear accelerator. A coronal arc rotation technique was used with an arc angle of 220° through the top of the skull and with a radiation source-to-skin distance of 80-100 cm. The isodose curves were computer- generated with the tumorvolume (indicated by the neurosurgeon on the x-rays) within the 95% isodose line. In the case of very large tumors radiation therapy was carried out with a parallel opposed-field technique via a pair of temporal fields. In this set-up the total dose was also 4600 rad, 5 doses of 200 rads per week.

RESULTS

Acromegaly
In a study concerning 64 acromegalic patients, which were examined in a more detailed manner after 1969 we have investigated the effects of conventional external radiotherapy as a single treatment (n = 19) in comparison with those of combined treatment with transsphenoidal surgery (n = 45) on plasma GH levels and anterior pituitary function.

I) Single treatment with radiotherapy
The individual results of primary conventional radiotherapy in 19 patients are shown in fig. 2. In general a satisfactory decrease of GH levels was obtained during the years after irradiation. Nine patients (47%) reached a plasma GH level of less than 10 ng/ml; 7 of them of (less than) 5 ng/ml. Especially the patients with a pretreatment plasma GH level of less than 50 ng/ml reached in general the normal range. Five patients subsequently underwent transsphenoidal surgery and four were treated with bromocriptine because of an insufficient decrease of plasma GH levels.

2) Combined treatment
The individual short-term effect of transsphenoidal surgery on basal plasma GH levels and the effect of additional radiotherapy in 45 operated patients is shown in fig. 3. In seven out of 43 patients (16%) with preoperative GH levels above 5 ng/ml plasma GH

levels were normalised (GH < 5 ng/ml) after transspehnoidal surgery. However, four out of five of these 7 "cured" patients, in whom a TRH test was carried out after operation, showed a persisting pathological response of GH to TRH, while the fifth patient without increase of the GH level after TRH postoperatively had shown also no response to TRH before surgery. Ten out of the 39 patients (26%) with preoperative GH levels above 10 ng/ml had postoperatively GH levels of less than 10 ng/ml. The results of transsphenoidal surgery appeared dependent on preoperative GH levels. Patients with preoperative GH levels of more than 31 ng/ml never reached GH levels of less than 5 ng/ml

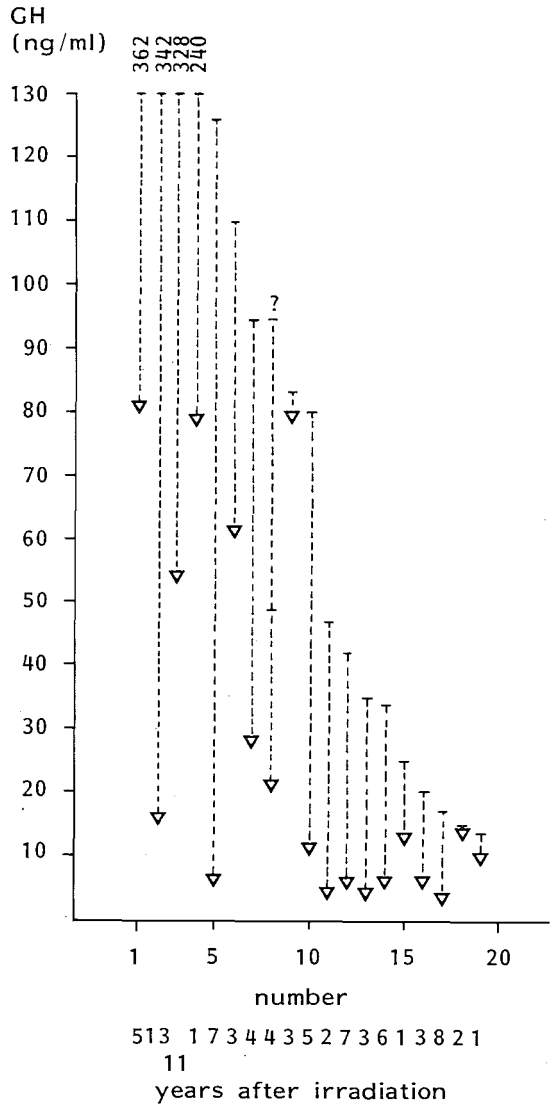

Fig. 2. The individual results of primary conventional radiotherapy in 19 patients with acromegaly.

postoperatively, while patients with GH levels of less than 10 ng/ml after operation, always had GH levels of less than 42 ng/ml preoperatively. However, in the years after postoperative radiotherapy the majority of the patients reached normal plasma GH levels. In fig. 4 the same results are presented in chronological order as a percentage of the preoperative GH level. In 11 patients the decrease in GH level was not more than 30%. In the first 15 patients the results were worse (six without important decrease in GH level) than in the remaining 30 patients. Additional radiotherpy caused a substantial further decrease in plasma GH.

3) Comparison of single radiotherapy with combined surgical and radiotherapy

By surgery alone the mean plasma GH level decreased immediately to 52% of the preoperative value (fig. 5). After postoperative irradiation mean plasma GH level decreased gradually further to 5-10% of the preoperative value with a maximal decrease after 7 years; subsequently mean plasma GH remained constant.

Radiotherapy alone caused a decrease in plasma GH levels of 40% in the first year (fig. 5). After 1½-2 years the same effect as by transsphenoidal surgery (a decrease of about

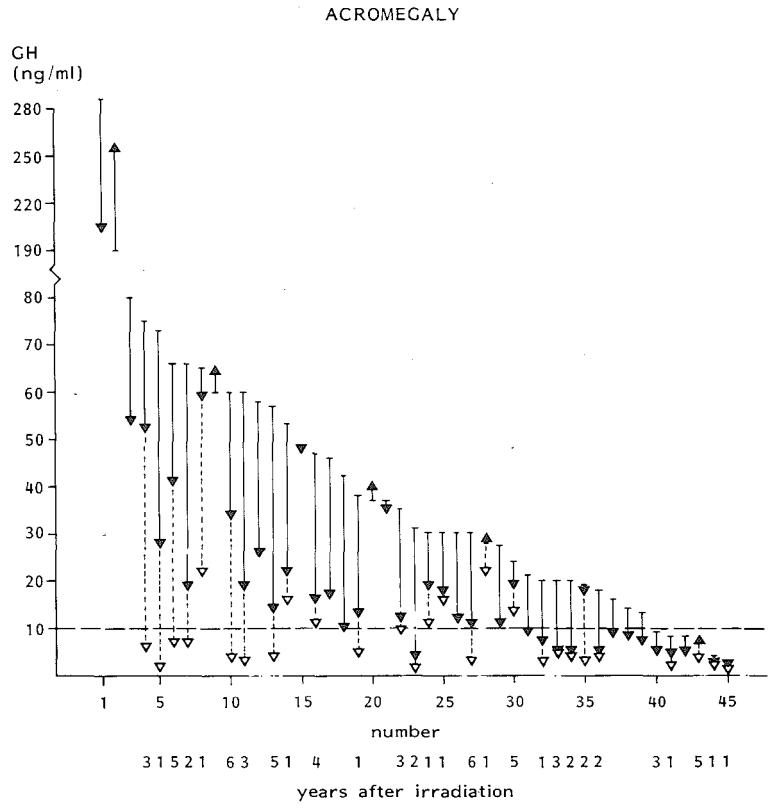

Fig. 3. The effect of transsphenoidal surgery (continued lines) and additional radiotherapy (dotted lines) on plasma GH concentration.

50%) was reached. After 3 years of treatment no significant difference was seen between the decrease in the GH levels after irradiation alone or after transsphenoidal surgery followed by irradiation. However, a subgroup of 34 patients (76%) with a relatively successful surgical intervention showed over 7 years a significantly larger percental decrease of plasma GH levels than a subgroup of the remaining 11 (24%) unsuccessfully operated patients (decrease of plasma GH < 30%). This last group with the same mean plasma GH level pre- and postoperatively – showed the same curve of percental decrement as the patients treated with radiotherapy alone (fig. 6).

4) Effect on basal plasma prolactin in acromegalic patients

Hyperprolactinemia was found in 54% of our acromegalic patients. In these hyperprolactinemic patients the mean plasma PRL level decreased by surgery to 47% of the preoperative value.In the normoprolactinemic patients the mean decrease was only to 82% of the preoperative value. In general, radiotherapy caused in the hyperprolactinemic acromegalic patients, a decrease of the plasma PRL concentration by about 50%.

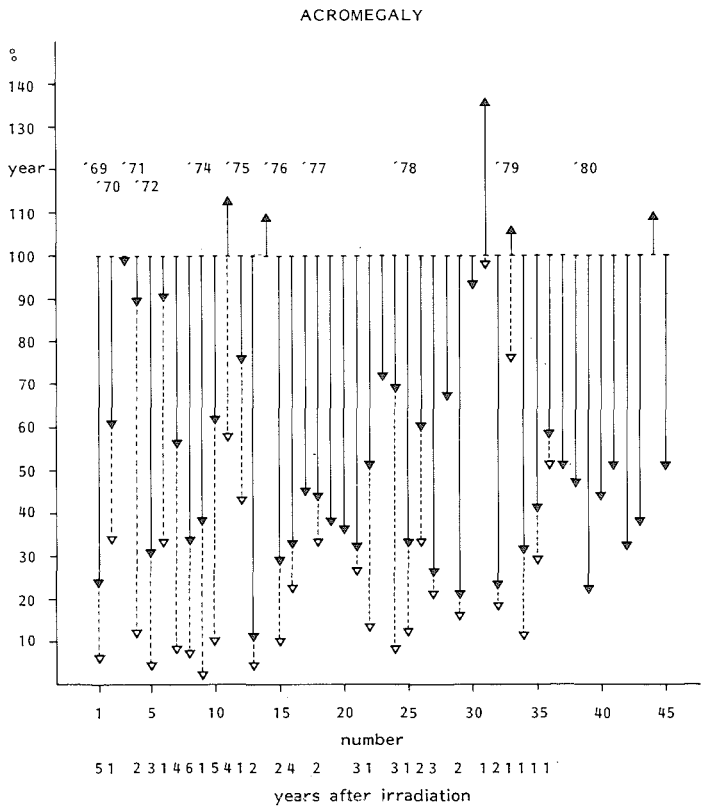

Fig. 4. The effect of transsphenoidal surgery (continued lines) and additional radiotherapy (dotted lines) on plasma GH as a percentage of the preoperative value.

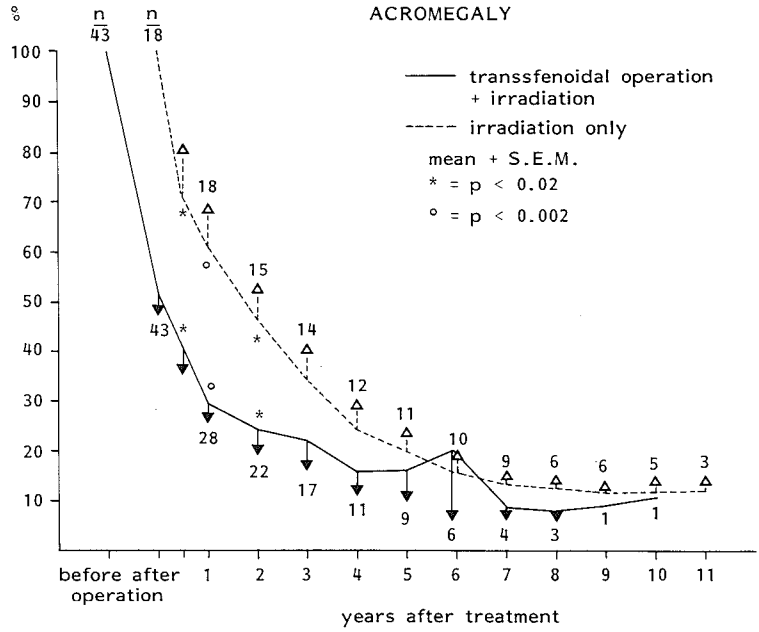

Fig. 5. The effect of transsphenoidal surgery and irradiation (continued lines) versus radiotherapy alone (dotted lines) with regard to percental decrease of plasma GH.

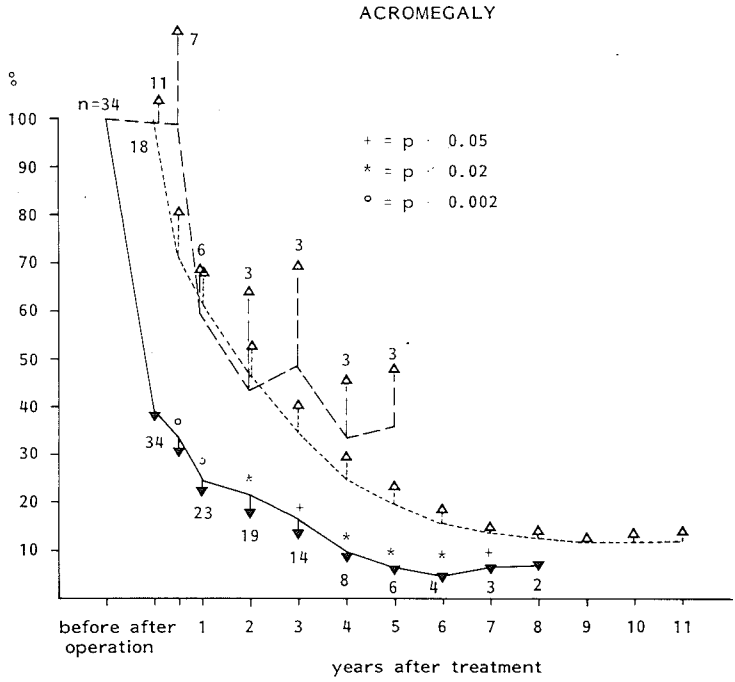

Fig. 6. The effect of transsphenoidal surgery plus irradiation in 34 relatively successfully operated patients (continued lines) and in 11 unsuccessfully operated patients (----) versus the effect of radiotherapy alone in 18 acromegalic patients (.....) with regard to percental GH decrease in plasma.

5) Overall effect

Virtually all the patients treated with orthovoltage irradiation had their primary treatment before the time (1965), that the radioimmunological plasma GH estimation was available. So, it is impossible to compare the results of this type of radiotherapy with that of megavoltage irradiation. The overall cumulative ''curation'' rate in 84 evaluable acromegalic patients, treated with MeV radiation therapy (with or without surgery) was found to be 74% using 10 ng/ml and 60% using 5 ng/ml as the normal upper limit for plasma GH after 10 years (fig. 7). In 79 patients surgery in combination with radiotherapy (N = 47) appeared more effective in causing ''curation'' (GH < 5 ng/ml) than radiotherapy alone: 78% versus 43% after 10 years of follow up (fig. 8). In parallel it can be stated that complaints concerning hyperhydrosis, acroparaesthesias and soft tissue swelling disappeared quicker after surgery in combination with irradiation than after irradiation alone.

Macroprolactinomas and hormonally inactive tumors

In 16 transsphenoidally operated patients with a macroprolactinoma the mean plasma PRL concentration decreased directly by surgery to 67 ± 9% (mean ± SEM) of the preoperative value. Postoperative irradiation exerted a great variation in response; the mean

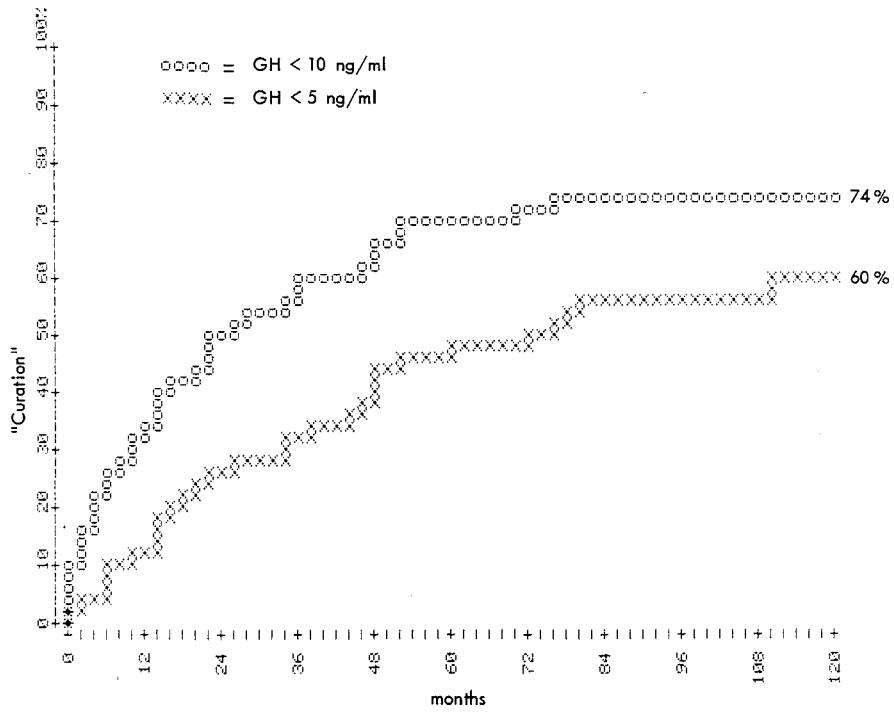

Fig. 7. Overall ''curation'' rate in 84 acromegalic patients treated with MeV radiation therapy (with or without surgery).

plasma PRL level decreased to 62 ± 17% after one year and to 27 ± 11% of the presurgical value after two years. The maximal decrease was reached after 3 years (to 10 ± 3%). However, some of the patients were excluded for evaluation later on during the follow up because of additional treatment with bromocriptine.

Only four patients were treated with radiotherapy alone. One patient reached normal PRL levels seven years after the radiotherapy and menses returned spontanously.

In the patients with ''non-functioning'' tumors there is no valuable tumor marker. Objective response to treatment or recurrence of the pituitary tumor can only be evaluated by radiological and sometimes by ophthalmological examinations. Therefore it was difficult to differentiate between complete or partial (residue) ''curation''.

Side effects

1) In general

Locally loss of hair was sometimes a short-term side effect. The incidence of persisting side effects is indicated in Table I. Headache occurred in 15% of the patients without headache before treatment; this varied between a heavy feeling on the head and severe migrainic pain. On the other hand in patients with headache before treatment this complaint sometimes disappeared after treatment. Ten percent of the patients complained about memory loss during the follow up. Pituitary hypofunction, caused by radiotherapy

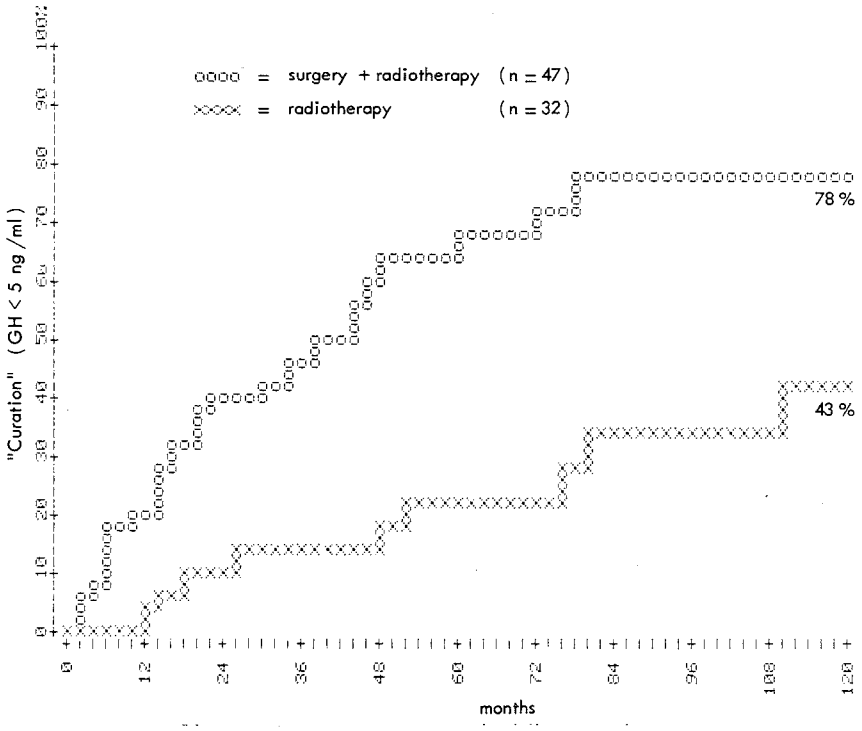

Fig. 8. ''Curation '' rate in 79 acromegalic patients treated with MeV irradiation with or without surgery.

alone, occurred in up to 21% of the patients (see below). There were no deaths or pituitary sarcomas in the follow up. One patient, who was overtreated elsewhere after normal conventional treatment, became blind.

TABLE 1. Persisting side effects of radiation therapy.

start or increase of headache	15%
Partly loss of memory	10%
Loss of normal pituitary function up to	21%
Visual loss*	0%

** with the exception of one patient, treated additionally with 4700 R (10 doses) in an other hospital (1959) after conventional treatment with 3000 R ½ year before.*

2) Effect on pituitary function

In 3 groups of patients with a pituitary tumor the effect of surgical and/or radiotherapeutical treatment on normal pituitary function was studied. Group A consisted of 45 acromegalic patients treated by transsphenoidal surgery and radiotherapy, group B consisted of 22 acromegalics treated with radiotherapy alone, group C consisted of 37 operated (25 x transsphenoidal, 12 x subfrontal) and subsequently irradiated patients with a prolactinoma as well as 28 patients with a ''non-functioning'' tumor treated in the same way (21 x transsph., 7 x subfrontal).

In groups A and C *surgical treatment* did not cause a significant decrease of basal TSH, LH and FSH or the reaction of desoxycortisol to metyrapone. In contrast, a significant decrease of the pituitary reserve of TSH ($p < 0.002$), LH ($p < 0.01$) and FSH ($p < 0.01$) occurred. A recovery of the pituitary adrenal axis has been observed in 4 out of 9 acromegalic patients with a disturbed metyrapone test before operation. In case of normal pituitary function before therapy surgical treatment disturbed most frequently gonadotropin secretion (23%), while the pituitary-adrenal function was frequently disturbed by *irradiation* both as single treatment as well as postoperatively (Table IIA, fig. 9). Hypothyroidism did not occur after radiotherapy alone, but did after postoperative irradiation. In group C a significant increase of Δ TSH ($p < 0.02$) in the presence of a significant decrease of plasma T4 ($p < 0.01$) was remarkable after postoperative radiotherapy indicating hypothalamic insufficiency. The cumulative incidence of hormonal insufficiency before and after both kinds of therapy is shown in Table IIB.

Recurrence-free interval and survival

1) Recurrence rate

The cumulative recurrence rate is indicated in Fig. 10. After 20 years of follow-up 88% (zie 2 en 3) of the acromegalics was recurrence-free and 76% of the patients with a macroprolactinoma or a ''non-functioning'' tumor.

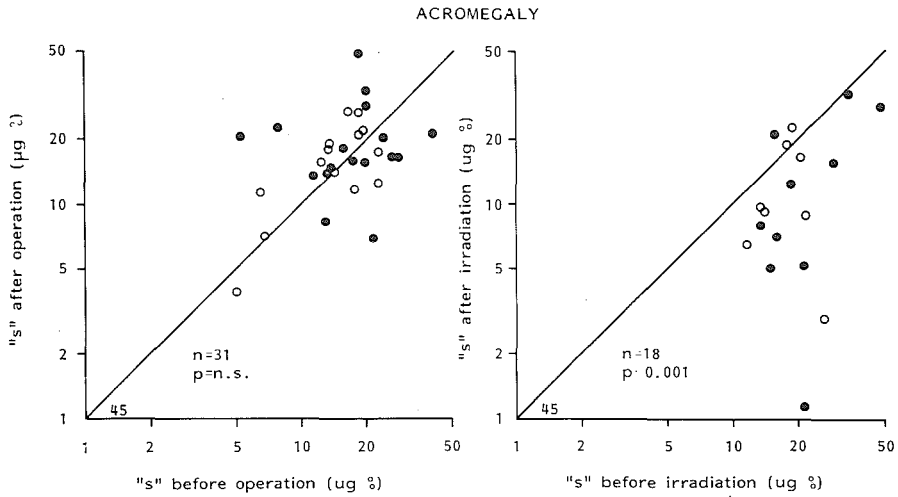

Fig. 9. The effect of transsphenoidal surgery (left) and postoperative irradiation (right) on pituitary- adrenal function (''S'' = desoxycortisol in plasma after 6x 750 mg metyrapone orally).

TABLE 2A Incidence of hormonal insufficiency caused by therapy (group A and B)

Function	Caused by surgery only (= group A)	Caused by radiotheraphy after surgery (= group A)	radiother. only (= group B)
— Hypothyroidism	5%	17%	0%
— Gonadotropin secretion ↓	23%	13%	8%
— Corticotropin secretion ↓	12%	44%	21%

TABLE 2B Cumulative incidence of hormonal insufficiency before and after surgery, and after subsequent radiotherapy in 45 acromegalic patients (group A).

	Before surgery	After surgery	After radiotherapy
— Hypothyroidism	7%	11%	29%
— Gonadotropin secretion ↓	42%	54%	61%
— Corticotropin secretion ↓	20%	21%	59%

2) Survival

Ten years after treatment 83% of the acromegalics were alive and 82% of the patients with macroprolactinomas or ''non-functioning'' tumors. This contrasts with 92% and 90% respectively for 2 control groups matched for sex and age and living in the time period between 1965-1969 (Fig. 11). Twenty years after treatment 71% of group I and 66% of group II were alive. The overall survival after therapy appeared better in the

group of patients treated with the combined surgical and radiotherapeutical therapy than with radiotherapy alone (Fig. 12). In general, the difference in survival is about 10% from the third year onwards.

3) Causes of death

Cardiovascular- and cerebrovascular disease, respiratory insufficiency and malignant tumors occurred more frequently in the acromegalic patients (Table III). Also in 3 other acromegalic patients benign polyps in the colon were found. In the group of patients with macroprolactinomas and ''non-functioning'' tumors death by postoperative complications was more frequent than in the group acromegalics, possibly because of the fact that the mean tumor size and the incidence of suprasellar extension was larger in this group of patients.

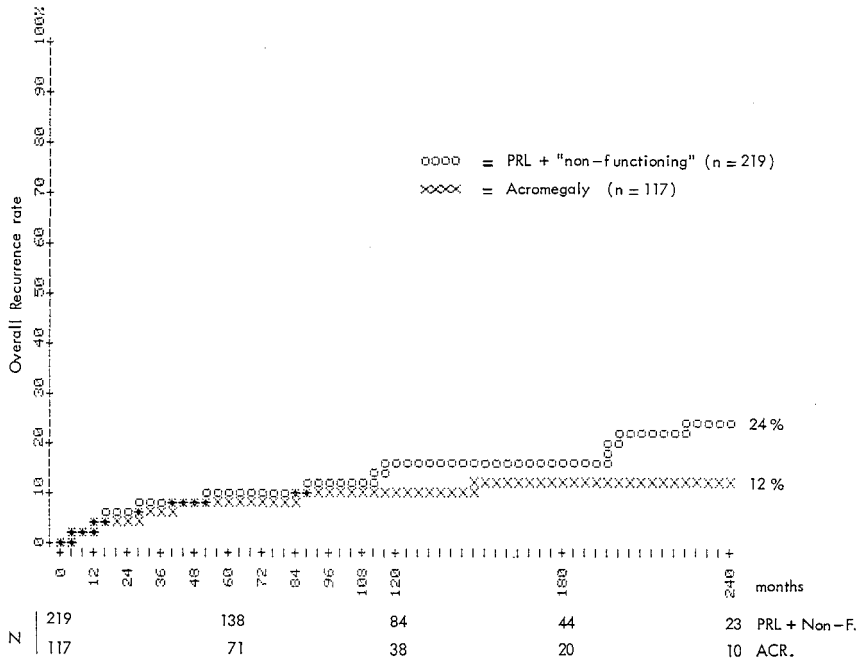

Fig. 10. Cumulative recurrence rate in 336 patients with a pituitary tumor treated since 1950. xxxx = Acromegaly (n = 117) oooo = macroprolactinomas and ''non-functioning'' tumors (n = 219).

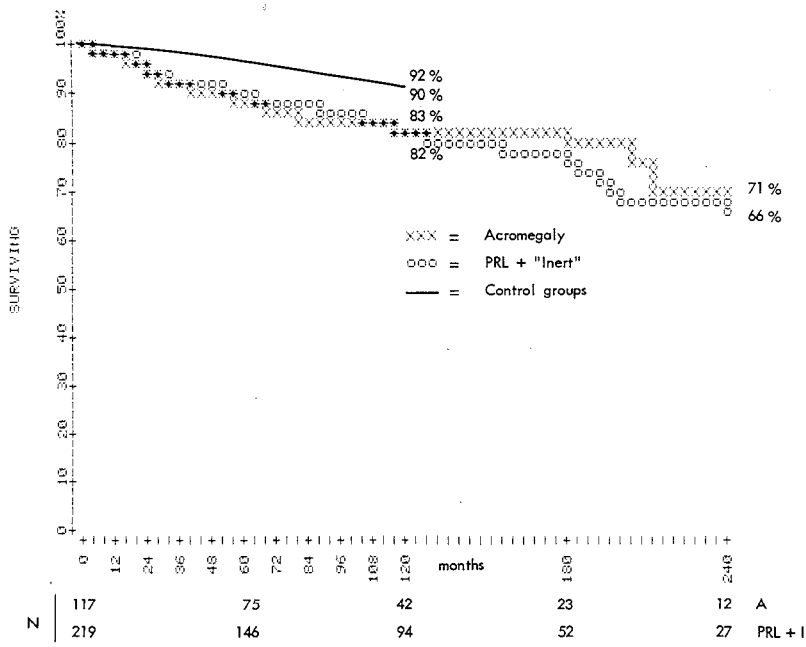

Fig. 11. Survival rate in 117 patients with acromegaly (xxxx) and in 219 patients with macroprolactinomas and "non-functioning" tumors (oooo) in comparison with 2 normal groups (continued lines).

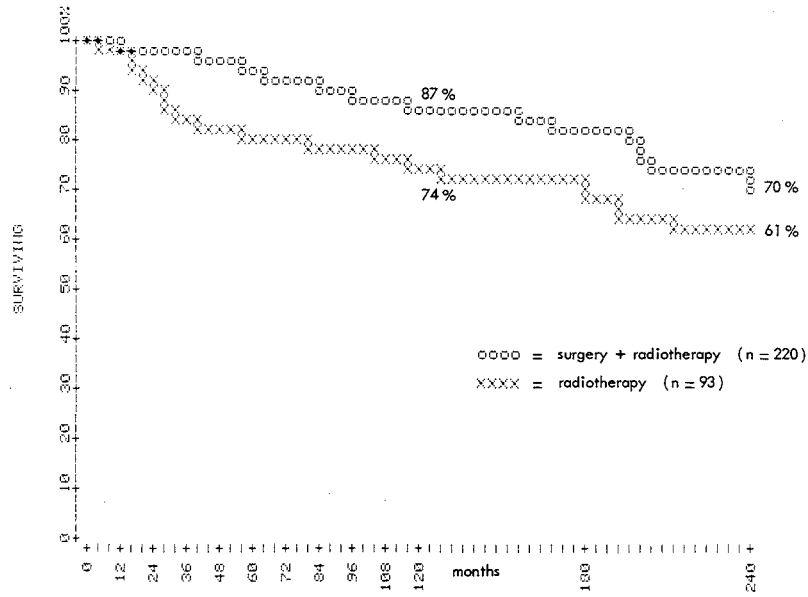

Fig. 12. Overall survival after first therapy in 313 patients, treated with conventional external radiotherapy (with or without surgery) since 1950.
(oooo) = surgery + radiotherapy (n = 220).
(xxxx) = radiotherapy (n = 93).

TABLE 3. Causes of death.

	Macroprolactinomas and "non-functioning" tumors (n = 219)			Acromegaly (n = 117)	
	n	%		n	%
— cardiovascular disease	8	17		6	26
— cerebrovascular disease	4	8		4	17
— respiratory — insufficiency	1	2		2	9
— embolism	4	8		0	0
— malignant neoplasms					
— stomach	1			0	
— colon	0			2	
— renal	0	4	8	1+1	5	22
— breast	2			1	
— skin	1			0	(1)*	
— thyroid	0			0	(1)*	
— tumor progression	3	7		0	0
— postoperative	5	11		1	4
radiation damage	0	0		1	4
accident	1	2		0	0
others	4	8		2	9
unknown	14	29		2	9
total	48	100%		23	100%

* Present tumors not related to death.

DISCUSSION

Conventional external radiotherapy is an important part of the therapy of pituitary adenomas especially because of its efficacy of long-term tumor control (3-7). A disadvantage of irradiation is the delayed effect. On the other hand surgery is rarely curative in macroadenomas and even in 30-40% of microadenoms (9-12). Recurrences occur in the majority of macroadenomas (3, 5, 7) and in some microadenomas if surgery is not combined with radiotherapy (13). Medical treatment with dopamine agonists as bromocriptine is effective in the majority of prolactinomas (12, 14), but these tumors recur shortly after stopping the treatment (14); so long-term medical treatment is often necessary.

Although the combination of surgery and radiotherapy is as effective as radiotherapy alone in preventing recurrences (5), the combined treatment causes a more rapid decrease of GH and PRL secretion and a higher "curation" rate. In this study it appeared for the first time that the combined treatment was also superior to radiotherapy alone with respect to long-term survival. The better survival may at least partly be caused by a shorter time period of exposure to high plasma GH levels in the acromegalic patients. The causes of death in our patients are in general comparable with those found in the studies of Wright (15) and Alexander (16).

A disadvantage of the combined therapy is the higher incidence of hormonal insufficiency compared with the direct effect of surgery and the long-term effect of radiotherapy. However, the gain of preventing recurrences, a higher chance of more rapidly occurring curation with less complaints and longer survival seems more important than life long medical treatment of hypopituitarism. Even with respect to fertility the possibilities of treatment are improving. On the other hand, in old patients with small tumors and/or relatively low plasma levels of GH – which small tumors indeed occur more frequently in older patients than in young ones as we reported before (17) – treatment with external radiotherapy alone will be sufficient in most of them.

In conclusion

1) Conventional external radiotherapy plays a major role in the management of pituitary (macro) adenomas, especially with respect to efficacy of long-term tumor control.
2) Combined surgical and radiotherapeutic treatment is more effective then single therapy with respect to ''curation'' and survival.
3) a After radiotherapy or the combined therapy the corticotrophic function was more frequently disturbed than the other functions.
   b Pituitary insufficiency caused by radiotherapy occurred more frequently after postoperative irradiation than when irradiation was used as a single treatment.
4) The overall survival of treatment patients with pituitary (macro)adenomas is about 10% less than that of a normal control group (about 80 vs about 90% after a follow up of 10 years), which means a death rate of twice the expected number.

ACKNOWLEDGEMENTS

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CHAPTER IX

SUMMARY AND CONCLUSIONS

The main aims of this thesis were to investigate 1) the value of diagnostic procedures 2) the relationship between symptoms, tumour size, age, hormonal hypersecretion, hormonal insufficiency, and extrasellar extension of tumour 3) the results of surgery, irradiation and medical treatment.

After a historical introduction in chapter I, our results are reported and discussed together with recent literature data in chapter II with reference to our own publications (chapter III-VIII).

The occurrence of pituitary gland tumours with different symptomatology has been recognized long before 1900. Surgical and radiotherapeutical treatment started in the first decade of this century. Prominent pioneers were especially Hirsch and Cushing. Later on in the 1960s Guiot and Hardy acquired fame by the introduction of microsurgical techniques for transsphenoidal surgery. In the 1960s also the irradiation techniques improved. The endocrinology of the pituitary and its target organs started really in the 1920s, but the unraveling of the structure of most pituitary and hypothalamic hormones with the introduction of numerous radioimmunoassays in the 1970s caused a break-through in the diagnostics of pituitary tumours. These revolutionary developments in endocrinologic evaluation together with important advances in neuroradiological techniques have made possible early diagnosis of small pituitary tumours since 1970 resulting in improvement of therapeutical results. With respect to medical treatment major achievements have been made by the development of dopaminergic drugs as bromocriptine and of somatostatin analogues as Sandostatin, apart from developments of substitution therapies in case of hormonal insufficiency. With respect to the latter therapies the discovery in 1950 of adrenal steroids for pre-, intra- and postoperative care is one of the important historical events because it strikingly decreased the death rate related to pituitary surgery.

Symptoms and patient characteristics are described in Chapter II section C.1., and Chapters III and IV. The main symptoms in patients with prolactinomas are gonadal dysfunction and galactorrhoea caused by the hyperprolactinaemia per se. Microprolactinomas are mainly found in young women, while postmenopausal women and men have predominantly presenting symptoms caused by macroprolactinomas (hypopituitarism, neurologic and visual disturbances). In patients with acromegaly the spectrum of symptoms is much broader than in patients with prolactinomas, because hypersecretion of GH and indirectly of somatomedin C affects all organs and tissues. Most frequent symptoms are soft tissue swelling, acral growth, hyperhidrosis, acroparaesthesias and headache. Cardiovascular complications, and maybe a slightly increased incidence of malignant tumours, are causes of a decreased life-expectancy. In general, young acromegalic patients have larger and more rapidly growing tumours than older patients necessitating more agressive treatment in younger patients. Patients with non-functioning tumours generally have very large tumours and symptoms of local tumour extension resulting in destruction of surrounding tissues.

Radiological and ophthalmological evaluation is described in Chapter II, section C.2.a. and Chapters III and IV. In our material the incidence of extrasellar extension was lower in acromegalic patients than in patients with prolactinomas (32 vs 44%). It appeared that extrasellar extension occurred at a critical size of the pituitary tumour and sella turcica. In acromegalic patients extrasellar extension occurred on the average at a lateral sellar and tumour area of almost 1 cm^2 larger than in prolactinoma patients (with respect to sellar size generally above 3 cm^2 versus 2 cm^2, with respect to tumour size generally above 4 cm^2 versus 3 cm^2). We found a striking negative correlation between pituitary tumour size and age in acromegalic patients in contrast to the absence of such a relationship in the prolactinoma patients.

Endocrine diagnosis is described in chapter II, section C.2.b., and Chapters III-VII. Although for a certain tumour size basal plasma PRL and GH concentrations will vary and vice versa, there appeared a strong correlation between tumour size and PRL or GH levels (Chapter

III and IV, respectively). A number of dynamic tests are available for the diagnosis of hypersecreting pituitary tumours. The TRH-test is most commonly used in patients with prolactinomas and acromegaly in addition to an oral glucose tolerance (suppression) test in acromegaly. Patients with prolactinomas show a decreased response of PRL (by less than 100% of the basal value) to TRH in 90% of the patients (Chapter V). In contrast acromegalic patients show a paradoxical increase of GH secretion in reaction to TRH. Hyperprolactinaemia was also present in 57% of patients with suprasellar tumours not arising from the pituitary with a PRL response of less than 100% to TRH in 64% of these patients (Chapter V). In acromegaly a paradoxical increase of GH to TRH (by more than 100% of the basal value) was observed in 44% of the normoprolactinaemic, in 59% of the midly hyperprolactinaemia and in 75% of the clearly hyperprolactinaemic patients (Chapter II, section C.3.b.3.a.3., and Chapter VII).

The occurrence of hormonal insufficiency is related to pituitary tumour size and type of pituitary tumour (Chapter II section C.2.b.2., Chapters III, IV and VI). In patients with prolactinomas we found a negative correlation between tumour size and basal as well as stimulated pituitary functions. A prolactinoma size of 3 cm^2 turned out to be a critical value not only for the occurrence of extrasellar extension, but also for the occurrence of hypopituitarism (Chapter III). Decreased pituitary hormone reserve was observed in 3-24% of the patients with a tumour size less than 3 cm^2 in contrast to 44-79% in patients with a tumour size of more than 3 cm^2. Acromegalic patients show much less frequently hormonal insufficiency than prolactinoma patients (Chapter IV and VI). Hypothyroidism is rare in acromegalic patients, but we found a high incidence (about 50%) of an impaired TSH response to TRH independent of tumour size. These observations suggest that an impaired TSH response may be caused by suppression of TSH secretion rather than by destruction of thyrotrophic cells (Chapter VI).

Hyperprolactinaemia occurs in 40-55% of acromegalic patients. We found a clear relationship between the basal PRL level, the presence of a defined GH response to TRH and the presence of a defined GH response

to a test dose of 2.5 mg bromocriptine (Chapter II section C.3.b.3.a.3. and Chapter VII). Acromegalic patients with hyperprolactinaemia show a higher sensitivity to TRH (increase of GH) and one dose of bromocriptine (more pronounced decrease of GH) than normoprolactinaemic patients. A homogeneity in the responses of GH to TRH and bromocriptine was observed in 74% of all acromegalic patients and in 92% of hyperprolactinaemic acromegalic patients, but there was no significant correlation between the percentage increase of GH after TRH and the percentage decrease after bromocriptine. On the basis of immunohistochemical examination of pituitary tumours (Chapter VII) hyperprolactinaemia appeared to be present in 70% of the patients with mixed GH/PRL containing adenomas. Plasma GH levels were significantly more suppressed by bromocriptine in patients with mixed GH/PRL-containing than in those with pure GH-containing adenomas, but the response of GH to TRH did not differ significantly between the two subgroups of acromegalic patients. The plasma PRL concentration and the GH response to TRH and one test dose of bromocriptine appeared of predictive value with respect to the result of chronic treatment with bromocriptine. Acromegalic patients with hyperprolactinaemia and/or with a high response of GH to TRH and/or with especially a strong suppression of GH in the acute bromocriptine test, showed more pronounced suppression of GH during chronic bromocriptine treatment than other patients.

Unfortunately, no single form of therapy is uniformly successful in all patients. Therefore an multidisciplinary approach is important. In patients with acromegaly, Cushing's disease and non-functioning tumours surgery mostly in combination with irradiation postoperatively is generally the first choice of treatment, while primary medical therapy has become generally accepted in patients with prolactinomas. Medical treatment with bromocriptine is often given as an adjunctive therapy in patients with acromegaly. Somatostatin analogues maybe applied in the near future as a first-line treatment in these patients, especially when slow-release depotpreparations will be available. Conventional external irradiation is sufficiently effective in strongly lessening tumour recurrences and in long-term control of tumour growth, but not for obtaining rapid therapeutic effects.

However, it has to be stated that, although more sophisticated hormonal and radiological diagnostic procedures have resulted in the detection of pituitary tumours at an earlier stage, and despite important improvement in operative, irradiation and drug therapy in the last 15 years, complete permanent cures can still not achieved in some patients with prolactinomas and in a sifnificant number of patients with acromegaly or Cushing's disease.

SAMENVATTING EN CONCLUSIES

De belangrijkste doelstellingen van dit proefschrift waren te onderzoeken, ten eerste de waarde van diagnostische technieken, ten tweede het verband tussen symptomen, tumorgrootte, leeftijd, hormonale hypersecretie, hormonale insufficiëntie en extrasellaire uitbreiding van de tumor, ten derde de resultaten van chirurgische, radiotherapeutische en medicamenteuze behandeling.

Na een inleiding betreffende de historie in hoofdstuk I, beschrijven en bediscussiëren wij de eigen resultaten in het licht van recente literatuurgegevens in hoofdstuk II met verwijzing naar onze eigen publikaties (hoofdstuk III-VIII).

Het voorkomen van hypophysetumoren met verschillende symptomatologie is reeds lang voor 1900 herkend. De toepassing van chirurgische en radiotherapeutische behandeling begon in de eerste decade van deze eeuw. Vooraanstaande pioniers waren vooral Hirsch en Cushing. Later in de 60-er jaren verwierven Guiot en Hardy roem door de introduktie van microchirurgische technieken voor de transsphenoidale operatie. In de 60-er jaren vond ook een verbetering plaats van de bestralingstechnieken. De endocrinologie van de hypophyse en zijn doelorganen begon feitelijk in de 20-er jaren maar de ontrafeling van de struktuur van de meeste hypophysaire en hypothalame hormonen tezamen met de introduktie van talrijke radio-immunoassays in de 70-er jaren veroorzaakte een doorbraak in de diagnostiek van de hypophysetumoren. Deze revolutionaire ontwikkelingen in endocrinologische evaluatie tezamen met belangrijke verbeteringen in neuroradiologische technieken heeft een vroege diagnose van kleine hypophysetumoren mogelijk gemaakt sinds 1970, resulterend in verbetering van de behandelingsresultaten. Met betrekking tot medicamenteuze therapie werd belangrijke vooruitgang geboekt door de ontwikkeling van dopamine-agonisten als bromocriptine en van somatostatine-analogen, zoals Sandostatine, los van ontwikkelingen betreffende substitutietherapie, in geval van hormonale insufficiëntie. Met betrekking tot deze laatste vorm van therapie, kan gesteld worden dat de ontdekking van corticosteroiden in 1950 en de toepassing bij de peri-operatieve zorg een van de belangrijkste

historische gebeurtenissen is geweest omdat de sterfte bij en na operatie daardoor sterk afnam. De symptomen en kenmerken van de patiënten zijn beschreven in hoofdstuk II sectie C.1., en de hoofdstukken III en IV. De belangrijkste symptomen in patiënten met prolactinomen zijn gonadale dysfunktie en galactorrhoe, veroorzaakt door de hyperprolactinaemie als zodanig. Microprolactinomen worden voornamelijk gevonden in jonge vrouwen terwijl postmenopausale vrouwen en mannen zich overwegend presenteren met symptomen veroorzaakt door macroprolactinomen (hypophysaire insufficiëntie, neurologische en visus-stoornissen). In patiënten met acromegalie is het spectrum van symptomen veel uitgebreider dan in patiënten met prolactinomen omdat hypersecretie van groeihormoon en indirekt van somatomedine-C alle organen en weefsels aantast. De meest voorkomende symptomen zijn weke delen zwelling, groei van de lichaamsuiteinden, abnormaal sterke transpiratie, tintelingen en hoofdpijn. Cardiovasculaire complicaties, en misschien een licht toegenomen incidentie van maligne tumoren, zijn oorzaken van een verminderde levensverwachting. Jonge acromegale patiënten hebben in het algemeen grotere en sneller groeiende tumoren dan oudere patiënten, hetgeen een meer aggressieve therapie bij jonge patiënten noodzakelijk maakt. Patiënten met niet-functionerende tumoren hebben over het algemeen zeer grote tumoren en symptomen veroorzaakt door lokale uitbreiding van de tumor resulterend in destructie van omgevende weefsels.

Het radiologisch en oogheelkundig onderzoek is beschreven in hoofdstuk II, sectie C.2.a., en hoofdstukken III en IV. In ons patiëntenbestand kwam extrasellaire uitbreiding van de hypophysetumor bij acromegale patiënten minder frequent voor dan bij patiënten met prolactinomen (32 vs 44 %). Het bleek dat extrasellaire uitbreiding optrad bij een kritische grootte van de hypophysetumor en sella turcica. Bij acromegale patiënten trad extrasellaire uitbreiding gemiddeld op bij een lateraal oppervlak van sella en tumor van ongeveer 1 cm^2 grotere omvang dan bij patiënten met prolactinomen (met betrekking tot sellagrootte over het algemeen boven 3 cm^2 vs 2 cm^2, met betrekking tot tumorgrootte over het algemeen boven 4 cm^2, vs 3cm^2). Wij vonden een opvallend negatieve correlatie tussen de grootte van de hypophysetumor en de leeftijd bij acromegale patiënten in

tegenstelling tot de afwezigheid van zo'n relatie bij de patiënten met prolactinomen.

De endocriene diagnostiek is beschreven in hoofdstuk II, sectie C.2.b. en in hoofdstukken III-VII. Hoewel voor een bepaalde tumorgrootte de basale plasma prolactine en groeihormoonconcentraties variëren en omgekeerd, bleek er een sterke correlatie te bestaan tussen tumorgrootte en de plasmaconcentraties van prolactine of groeihormoon (resp. hoofdstuk III en IV). Een aantal testen met stimulatie of remming van de hormoonsecretie zijn beschikbaar voor het diagnostiseren van hypersecernerende hypophysetumoren. De TRH-test wordt veelvuldig toegepast bij patiënten met prolactinomen en acromegalie tezamen met een glucose-tolerantie-test bij patiënten met acromegalie. Patiënten met prolactinomen vertonen een verminderde respons van prolactine (minder dan 100% van de basale waarde) op TRH in 90% van de patiënten (hoofdstuk V). Daarentegen vertonen acromegale patiënten een paradoxe toename van groeihormoonsecretie in reactie op stimulatie met TRH. Hyperprolactinaemie was ook aanwezig bij 57% van de patiënten met suprasellaire tumoren niet van hypophysaire origine en een prolactine respons van minder dan 100% kwam voor bij 64% van deze patiënten. Bij acromegalie werd een paradoxe toename van groeihormoon op stimulatie met TRH (meer dan 100% van de basale waarde) waargenomen bij 44% van de normoprolactinaemische, bij 59% van de licht hyperprolactinaemische en bij 75% van de duidelijk hyperprolactinaemische patiënten (hoofdstuk II, sectie C.3.b.a.3., en hoofdstuk VII).

Het voorkomen van hormonale insufficiëntie staat in verband met de grootte van de hypophysetumor en het type hypophysetumor (hoofdstuk II, sectie C.2.b.2., hoofdstukken III, IV en VI). Bij patiënten met prolactinomen vonden wij een negatieve correlatie tussen tumorgrootte en zowel de basale als de gestimuleerde hypophysefuncties. Een tumorgrootte van 3 cm^2 bleek bij prolactinomen niet alleen een kritische waarde te zijn ten aanzien van het voorkomen van extrasellaire uitbreiding maar ook met betrekking tot het voorkomen van hypophysaire insufficiëntie (hoofdstuk III). Verminderde hypophysaire hormoonreserve werd vastgesteld bij 3 tot 24% van de patiënten met een tumorgrootte minder dat 3 cm^2, in tegenstelling tot

44-79% bij patiënten met een tumorgrootte van meer dan 3 cm^2. Patiënten met acromegalie vertonen veel minder frequent hormonale insufficiëntie dan patiënten met een prolactinoom (hoofdstuk IV en VI). Hypothyreoidie komt zelden voor bij acromegale patiënten, maar wij vonden een hoge frequentie (ongeveer 50%) van een verminderde TSH-respons op TRH onafhankelijk van de tumorgrootte. Deze waarnemingen suggeren dat een verminderde TSH respons eerder wordt veroorzaakt door suppressie van de TSH-secretie dan door destructie van thyreotrope cellen (hoofdstuk VI).

Hyperprolactinaemie komt voor bij 40-55% van acromegale patiënten. Wij vonden een duidelijke relatie tussen de basale prolactine-concentratie in het plasma, de aanwezigheid van een gedefinieërde groeihormoon-respons op TRH en de aanwezigheid van een gedefinieërde groeihormoon-respons op een testdosis van 2.5 mg bromocriptine (hoofdstuk II, sectie C.3.b.3.a.3. en hoofdstuk VII). Acromegale patiënten met hyperprolactinaemie vertonen een grotere gevoeligheid voor TRH (in de zin van een toename van groeihormoon) en voor een eenmalige dosis van bromocriptine (meer uitgesproken daling van groeihormoon) dan normoprolactinaemische patiënten. Een homogeniteit in de response van groeihormoon op TRH en bromocriptine werd waargenomen bij 74% van alle acromegale patiënten en bij 92% van de hyperprolactinaemische acromegale patiënten maar er was geen significante correlatie tussen de percentuele toename van groeihormoon na TRH en de percentuele afname na toediening van bromocriptine. Bij 70% van de patiënten met gemengde (groeihormoon- en prolactine-bevattende) adenomen (hoofdstuk VII) bleek hyperprolactinaemie aanwezig. Plasma groeihormoon-concentraties werden bij patiënten met gemengd groeihormoon/prolactine-bevattende adenomen significant sterker onderdrukt met behulp van bromocriptine dan bij patiënten met alleen groeihormoon-bevattende adenomen, maar de respons van groeihormoon op TRH verschilde niet significant tussen de 2 subgroepen van patiënten met acromegalie. De plasma prolactine-concentratie, de groeihormoon-respons op TRH en op een testdosis van bromocriptine bleken van voorspellende waarde met betrekking tot de resultaten van chronische behandeling met bromocriptine. Acromegale patiënten vertoonden een meer uitgesproken onderdrukking van de groeihormoon-

secretie tijdens chronische behandeling met bromocriptine in vergelijking tot andere patiënten wanneer er sprake was van hyperprolactinaemie en/of een sterke respons van groeihormoon op TRH en/of met name een sterke daling van groeihormoon tijdens de acute bromocriptine-test.

Helaas bestaat er geen enkelvoudige vorm van behandeling welke op uniforme wijze effectief is bij alle patiënten. Daarom is een multidisciplinaire benadering belangrijk. Bij patiënten met acromegalie, de ziekte van Cushing en niet-functionerende tumoren, is operatie, meestal in combinatie met post-operatieve bestraling gewoonlijk de eerste keuze van therapie, terwijl bij patiënten met prolactinomen over het algemeen medicamenteuze therapie met bromocriptine wordt toegepast als primaire behandeling. Bij patiënten met acromegalie wordt deze medicamenteuze therapie dikwijls toegepast als een aanvullende behandeling. In de nabije toekomst zullen mogelijk somatostatine-analogan worden aangewend als een eerste vorm van behandeling bij deze patiënten, vooral wanneer depotpreparaten beschikbaar zullen komen. Conventionele uitwendige bestraling is voldoende effectief met betrekking tot het drastisch verlagen van het aantal recidieven en het remmen van de tumorgroei op lange termijn, maar deze therapie is niet geschikt voor het verkrijgen van een snel therapeutisch effekt. Opgemerkt moet echter worden dat, hoewel meer geavanceerde endocriene, radiologische en diagnostische technieken hebben geresulteerd in opsporing van hypofysetumoren in een vroegere fase, complete definitieve genezing nog steeds niet kan worden bereikt bij sommige patiënten met prolactinomen en bij een belangrijk aantal patiënten met acromegalie of met de ziekte van Cushing, ook ondanks de belangrijke verbeteringen in operatieve, radiotherapeutische en medicamenteuze behandeling in de laatste vijftien jaar.

PART II

MANIPULATION OF CANCER WITH HYPOTHALAMIC HORMONES

CHAPTER X

LONG-TERM PEPTIDE HORMONE TREATMENT WITH LHRH-AGONISTS IN METASTATIC BREAST CANCER: A REVIEW

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1. Introduction

Different steroid hormones, peptide hormones, growth factors and other trophic substances are involved in growth regulation of breast cancer cells (23). Endocrine therapy of breast cancer consists of a variety of both medical and surgical treatment modalities including ovariectomy and hypophysectomy. In general medical treatment usually concerns (anti)steroidal agents. Recently, a new approach in the treatment of hormone-dependent tumors has been applied, namely manipulation of the hypothalamic-pituitary function by analogs of hypothalamic hormones. Neuropeptides might play a more important role in cancer treatment because of the absence of serious side effects. Especially analogs of LuteinizingHormone-Releasing-Hormone (LHRH) are of great interest (33). In addition, recently we observed also breast cancer growth inhibitory effects of an analog of another hypothalamic hormone i.e. somatostatin (21,35). Moreover analogs of these both neuropeptides appear to act also directly on breast cancer cells in an inhibitory fashion (1,2,18,26,27).

Since the unravelment of the structure of LHRH and elucidation of its physiology by Schally and Guilemin (1971), numerous analogs with

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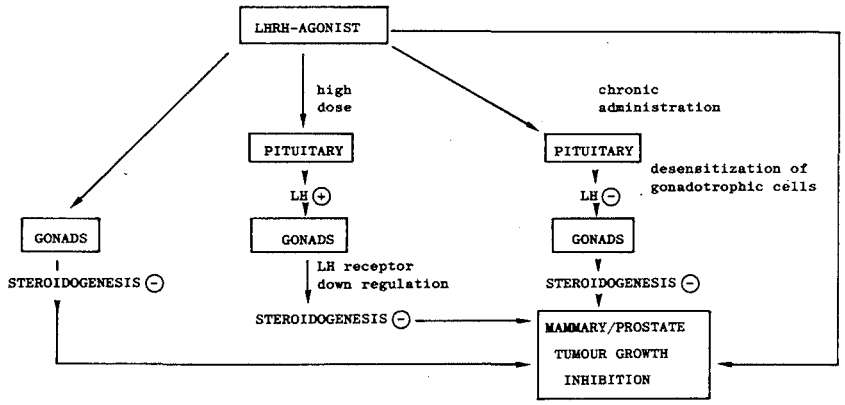
pronounced and long-term effects have been synthesized and tested (3,8,32,33). Most of these agents appeared to have a paradoxical antifertility effect during long-term treatment with sufficiently high (supraphysiological) doses. The most potent ones of these agents have been applied in experimental and clinical studies. In 1976 De Sombre et al. reported for the first time regression of rat mammary tumors effected by a LHRH analog (5). Since then a number of experimental studies on endocrine and growth inhibiting effects in rodents with different kinds of tumors have been published (3,8,33). Summarizing these data, chronic LHRH agonist treatment with pharmacological doses appears to cause: 1) "partial hypophysectomy" with decreased gonadotropin and prolactin secretion, 2) "chemical castration" with a striking fall in plasma sex steroids followed by a reduction in weight of accessory sex organs, 3) inhibition of enzymes involved in steroidogenesis, 4) direct effects on extrapituitary tissues such as gonadal tissue and both breast and prostate tumor cells. LHRH-like receptors have been found in a prostate tumor (33) and in breast cancer cells (6,26,27). Inhibition of tumor growth by LHRH analogs have been observed in rats with experimental mammary, prostate, pituitary, bone and pancreatic tumors (33).

Since 1981 a large number of clinical phase II and a few comparative studies have established the value of LHRH agonists in prostate cancer treatment (22,31,32,33), as also in our hands (4,16,17,34). With respect to breast cancer, in 1982 we reported for the first time tumor regression in premenopausal patients with metastatic breast cancer using the LHRH agonist buserelin (12). From October 1981 till January 1985 we have treated 32 premenopausal patients with a minimal follow-up of 1.5 year. In this report we will present a summary of our updated results and those reported in the literature during the last few years. Furthermore we will present some data on our experimental studies in vitro (MCF-7 cells) and vivo (DMBA rat mammary tumor).

2. Mechanism of action

The possible mechanisms of action of LHRH analog treatment are summarized in Figure 1. There are both indirect

POSSIBLE MECHANISMS OF ACTION OF LHRH-ANALOGS



acute and chronic endocrine effects via the pituitary and ovaries as well as direct effects on tumor cells. The main mechanism of action is medical castration as a result of suppression of the hypothalamo-pituitary-gonadal function. This is a consequence of desensitization via down-regulation of pituitary LHRH receptors (32) while also gonadal gonadotropin receptor levels decrease after a short peak of plasma gonadotropin concentration on the first day of treatment. Furthermore, besides pituitary and gonadal desensitization a direct effect of LHRH analogs on gonadal tissue of animals has been described while there is no agreement about such effect occurring in humans. Ultimately, these effects on pituitary and gonads lead to decrement of LH secretion and inhibition of steroidogenesis and thus to castrate plasma concentrations of estradiol and progesterone during chronic treatment. The importance of the direct growth inhibitory effects on tumor cells observed in vitro is not clear but is probably

minor compared to those of medical castration. We will describe the reported direct and indirect effects below.

2.a. Direct antitumor effects

Dose-dependent effects of the LHRH agonist buserelin on human breast cancer cells (MCF-7) have been described by us (1,2,7,18) and by others (26,27,37). Especially the stimulated growth with low doses of estradiol (E2) appears to be inhibited. These doses of E2 used in in vitro studies are comparable with castrate or postmenopausal E2 plasma concentrations. With higher E2 doses the inhibitory effect of the LHRH agonist decreased. This suggests that a certain balance between E2 and buserelin concentrations is a prerequisite to obtain inhibiting effects of buserelin at the cellular level. Furthermore, Wiznitzer and Benz (37) observed also direct inhibition of the prolactin stimulated growth of other mammary carcinoma cells (T47D). In contrast to their experience with this cell line, we found that the addition of tamoxifen to buserelin abolished partly the inhibitory effects of buserelin on E2 induced growth of MCF-7 tumor cells and vice versa (7). The inhibitory action of LHRH agonists is specific because little effects are observed in cultures of other breast cancer cell lines and a "normal" breast cell line (27).

In search for the mechanism of action we found that, with 30 pmol/l E2 as mitogen, buserelin did not change the patterns of secreted proteins (analyzed by SDS-PAGE followed by fluorography) or amounts of secreted polypeptides with epidermal growth factor (EGF)-like activity (7). Interestingly, while we did not observe effects of buserelin administration on MCF-7 cytoplasmic and nuclear E2 receptor (ER) levels, the E2-induced progesterone receptor (PgR) synthesis was strongly (87%) inhibited. Interference of buserelin and tamoxifen on E2-induced PgR synthesis might partly explain their antagonistic effects in vitro as mentioned above.

2.b. Receptors for LHRH and steroids

As in an experimental prostate tumor (33) LHRH-like receptors have been demonstrated in experimental and primary human breast carcinoma

(6,26,27). LHRH agonists may, however, exert their direct effects not only via LHRH receptors but also via modulation of steroid and peptide hormone receptors (see section 2a) or via influencing secretion of autocrine or paracrine glycoproteins and growth factors.

In clinical studies the most beneficial effects of LHRH agonist therapy have been observed in patients with ER-positive tumors (53% response), while tumor regression was observed in only a few patients with ER-negative tumors (20). During chronic LHRH agonist treatment of rats with mammary tumors, ER and PgR levels were strongly decreased as was observed after surgical castration (19).

2.c. Indirect (endocrine) antitumor effects

The influences of chronic LHRH agonist treatment on gonadotropin, prolactin and steroid hormone secretion in patients with metastatic breast cancer are extensively described by us (12-15,18-20) and by others (9,24,28,29).

After a short-term increase during a few hours on the first treatment day plasma gonadotropin levels decreased below pretreatment levels within 2-3 weeks for LH and within 3 days for FSH (14,15). Thereafter plasma gonadotropin concentrations can be kept low (especially biologically active LH) for many years during treatment. No ovulatory peaks were observed.

Anovulation as indicated by persisting low plasma progesterone levels occurred in all patients even with low doses of LHRH agonists intranasally (i.n.) (13-15,18). During treatment with relatively low doses (see section 3) recurrent peaks of E2 may occur followed by menstruation. However, with high doses subcutaneously (s.c.) ($\geq$ 1 mg per day) complete suppression of ovarian follicular function can be reached in all patients with a striking fall of plasma E2 concentration to castration values within 2-4 weeks (Figure 2) as observed for plasma testosterone in men with prostate cancer (4,16,17).

Similarly serum estrone and estrone sulfate are profoundly suppressed, whereas no constant changes were observed in the serum levels of androstenedione and cortisol (9,24). On the first treatment day a

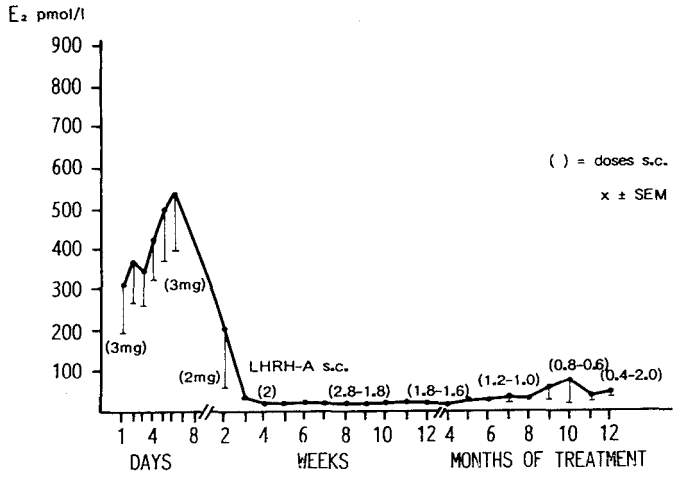


Figure 2. Mean plasma E2 concentrations during chronic single treatment with decreasing dosages of buserelin subcutaneously (s.c.) in 11 patients.

small rise in plasma prolactin occurs, but disappears during continuing treatment (18). All authors found no significant effect on basal prolactin levels. However, we observed a decrease of the natural night peak of prolactin during chronic subcutaneous treatment (18).

3. Which dose for use?

There appeared a great interindividual variation in endocrine response (13-15,18). While there is relatively less difference in potency between the commonly used LHRH analogs, some patients need 100 times higher dosages than others to reach complete medical castration. In general, amenorrhea has been observed in 15-20% of the patients treated with a daily dose of 400-600 ug i.n. ($\sim$ 8-12 ug s.c.), in 40% treated with 1200 ug i.n. ($\sim$ 25 ug s.c.), in $\pm$ 75% treated with 100-1000 ug s.c. and in 100% of the patients treated with 1000 ug s.c. or more respectively. In our study (20), decreasing gradually the initial dose of 3 mg to 0.4 mg s.c. per day, we observed that plasma E2 can increase to above castrate levels when dosages below 1 mg were used (Figure 2). Therefore we advice a dose of at least 1 mg per day, as is also used by others (9,24). Relatively lower daily dosages between 50-300 ug can reach medical castration in nearly all (90-100%) patients when administered continuously per infusion or slow release formulations. Furthermore, the highest dosages have the advantage of potential direct antitumor effects in vivo.

4. Clinical treatment results

In 9 (39%) out of 23 premenopausal patients with metastatic breast cancer we observed an objective tumor response (20). Four other studies (9,10,11,24,25,28,29) with a shorter followup show response rates (CR+PR) varying between 31 and 47% (Table 1). Overall an objective remission was found in 44 out of 116 patients (38%). The reported durations of remission are comparable with those occurring during common kinds of endocrine therapy. The longest duration of remission until now is more than 54 months (20). An objective response has been observed in 30 out of 57 patients with an ER-positive

Table 1

RESULTS OF LHRH-AGONIST TREATMENT IN PREMENOPAUSAL METASTATIC BREAST CANCER (Updated 1986)

Author	LHRH-agonist	n	CR + PR
1) Klijn et al.	Buserelin	23	9 (39%)
2) Nicholson et al.	Zoladex	45	14 (31%)
3) Harvey et al.	Leuprolide	25	11 (44%)
4) Mathé et al.	D-Trp-6-LHRH	8*	3 (38%)
5) Höffken et al.	Buserelin	15	7 (47%)
In total		116	44 (38%)

* pretreated

Overall response rate of ER-positive tumors: 30/57 (53%)

Table 2

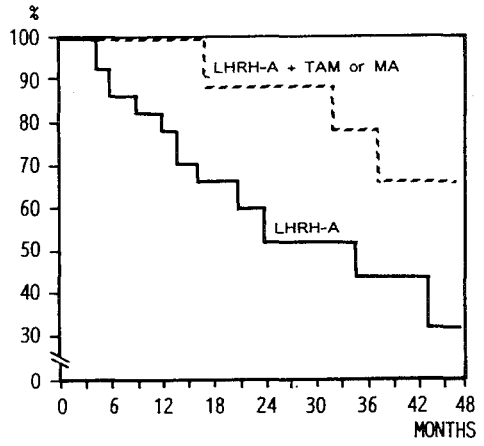
RESULTS OF LHRH-AGONIST TREATMENT IN POSTMENOPAUSAL METASTATIC BREAST CANCER (Updated 1986)

	Author	LHRH-agonist	n	CR + PR
1)	Harvey et al.	Leuprolide	41*	4 (10%)
2)	Mathe et al.	D-Trp-6-LHRH	15*	3 (20%)
3)	Plowman	Zoladex	10	2 (20%)
4)	Waxman	Buserelin	18	0 (0%)
In total			84	9 (11%)

* pretreated

tumor (53%) while remissions in patients with ER-negative tumors occur rarely. The median survival appeared in our study 3 years (Figure 3), which is at least as good as has been reported for other kinds of endocrine therapy. In another study (29) responding patients showed a clear longer survival than the failures . As shown in Table 2 objective remissions are also observed in a minority (11%) of postmenopausal patients with breast cancer (9,10,25,30,36). Because no change in plasma postmenopausal steroid hormone concentrations has been found (28,36), these tumor regressions might be explained by the reported (see section 2.a.) direct antitumor effects of LHRH analogs.

Figure 3. SURVIVAL OF 23 PATIENTS WITH METASTATIC BREAST CANCER TREATED WITH BUSERELIN ALONE AND 9 PATIENTS TREATED WITH BUSERELIN + TAM OR MA



5. Side effects

No side effects occurred with the exception of those caused by the intended hypogonadism, especially hot flushes (20). A few patients treated with subcutaneous injection had more or less short-term urticarial skin irritation which did not cause pain or discomfort.

6. Combination treatment

In analogy to the intended complete androgen blockade by combination therapy in patients with metastatic prostate cancer (17,22), complete estrogen blockade by using LHRH analogs in combination with antiestrogens may cause more rapid relief of complaints (during single LHRH agonist treatment it takes several weeks) and hopefully an improvement in response rate, duration of response and survival. Potential disadvantages of this combination might be both abolishment of the suppressive effect of LHRH analogs on pituitary-ovarian function by the stimulating effect of tamoxifen, at least during intranasal buserelin therapy (13-15,18) and abolishment of the direct inhibitory effect of LHRH analogs on tumor cells (see section 2.a) (7). On the other hand we observed objective responses in 5 of 9 patients during combination therapy with buserelin and tamoxifen or megestrol acetate (20). The survival of these 9 patients appeared slightly better than the group of patients treated with buserelin alone (Figure 3), but the numbers are too small for analysis and for making definite conclusions.

7. Treatment strategy in endocrine dependent breast cancer

Because of the absence of serious side effects and the observed efficacy which is comparable to that of surgical castration or tamoxifen treatment, LHRH analogs are suitable first-line agents in the treatment of premenopausal breast cancer. Especially when long-acting sustained release formulations (injected once per month) indeed will appear to be as effective as daily administration of the drug (29), this kind of treatment may be of great advantage. Ovariectomy as second line treatment after LHRH agonist treatment seems less effective (29) and mainly effective in a few patients with insufficiently suppressed ovarian function. Tamoxifen can be effective in patients not responding to LHRH agonist therapy (13,14), but its value for these patients is not clearly defined. More logical is the use of progestins in the second line (after LHRH agonist therapy) because of their antigonadotropic properties, their continuous

suppression of ovarian function, and their potential antitumor effects in patients with ER-negative tumors. However, this kind of treatment causes more side effects.

In postmenopausal patients LHRH agonist treatment is of less value because of the low response rate and short-term duration of response (9,24). Maybe, in very old patients with low drug compliance and slowly growing tumors, the use of long-acting depot preparations might be of any value.

8. Conclusions

1. In premenopausal metastatic breast cancer chronic LHRH agonist treatment appears as effective as other common kinds of endocrine therapy with respect to response rate, duration of response and survival.
2. No serious side effects have been observed.
3. Although intranasal administration appeared to be a pleasant and sufficiently effective way of treatment in some patients, high subcutaneous dosages of LHRH-agonists (at least 1 mg per day) are needed for reaching optimal and rapid suppression of the pituitary-gonadal function within 3 weeks in all patients. Probably sustained release formulations administered once per month will be sufficient in most patients too.
4. The reported tumor remissions in some postmenopausal patients (10%) and the demonstration of LHRH binding sites in some human breast cancers may support the occurrence of direct antitumor effects in vivo as observed in vitro.

9. Future prospects

- Controlled randomized prospective studies with LHRH agonists versus ovariectomy or tamoxifen or other LHRH agonist formulations have to be performed in the near future.
- Application of more long-acting sustained release preparations, which can be injected once per month, and later possibly once per 3-6 months.

- Use of LHRH antagonists which may cause medical castration within one day and thus a more rapid tumor response.
- More effective combination treatments.
- Application in old postmenopausal patients?
- Use of adjuvant therapy?
- Application in hormonal synchronization regimens before chemotherapy.

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CHAPTER XI

EFFECTS OF SOMATOSTATIN ANALOG (SANDOSTATIN) TREATMENT IN EXPERIMENTAL AND HUMAN CANCER

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INTRODUCTION

New approaches to the therapy for some known hormonedependent but also unknown "hormone-dependent" tumors are being developed on the basis of experimental studies in animal models. For instance such a new approach is the use of recently produced analogs of hypothalamic hormones, especially of Luteinizing-Hormone-Releasing-Hormone (LHRH) and somatostatin (30,31). In the last few years the value of LHRH analog treatment has been established by a number of clinical studies concerning metastatic breast and prostate cancer (see this book). However, only a few studies are reported about the application of somatostatin analogs in the treatment of malignant tumors (2,24,25,28,31,36).

Somatostatin is a cyclic tetradecapeptide and was first isolated by Brazeau et al. (1973) from the hypothalamus (5). It is believed that somatostatin acts primarily locally, with paracrine effects, but it

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can act also endocrine, autocrine and as a lumone (9,26,27). The natural hormone has a short half-life and a short duration of action. To circumvent this short duration of action and to develop organ-selective compounds, extensive attempts to synthesize analogs have been made (1,24-28, 31). Several somatostatin analogs have been shown in experimental animals to possess greater potency, longer duration of action and a different spectrum of biological actions compared to the parent somatostatin molecule. They appeared to suppress the secretion of pituitary hormones (GH, PRL, TSH), insulin, glucagon and several other gastrointestinal hormones, some of which (gastrin, secretin, cholecystokinin) being involved in the growth regulation of gastrointestinal tumor cells (15,20, 25,27,34,35, Lamers this book). Furthermore, plasma concentrations of some growth factors as somatomedin C (IGF-1) (16, 17) and epidermal growth factor (EGF) (10), which factors stimulate the growth of some types of tumor cells (mammary, pancreatic), may decrease during chronic treatment. On the basis of the mechanism of actions as mentioned above, it is conceivable that somatostatin analogs may have direct and indirect inhibiting actions even on the growth of unknown "endocrine-related" tumors as showed by Redding and Schally (24,25,31). Very recently, sufficient quantities of somatostatin analogs came available for long-term (pre)clinical studies. In this article we will summarize our results of chronic somatostatin analog treatment in various tumor models using the analog Sandostatin (SMS-201-995), kindly provided by Sandoz, Basel. Furthermore we will present preliminary data of a clinical study in patients with malignant gastrointestinal tumors.

RESULTS

Pancreatic tumors

A number of pancreatic tumors appears to contain high levels of steroid hormone receptors and enzymes involved in steroid metabolism (14, Johnson this book) indicating potential hormone dependency.

Furthermore the secretion of some gastrointestinal hormones, which are involved in the growth regulation of pancreatic (tumor) cells, can be suppressed by somatostatin. These data and those reported by Redding and Schally (25) prompted us to examine the effects of Sandostatin, a new potent somatostatin analog, in rats with a transplantable pancreatic acinar tumor (Prof. Longnecker). We have investigated the characteristics of this tumor(model) and the mechanism of action of Sandostatin. Furthermore we have compared the effects of different dosages of Sandostatin on tumor growth and plasma concentrations of some hormones and growth factors with those of other endocrine measures.

Tumors were kindly provided by Dr. A.G. Bogden, E.G. and Mason Research Institute. About 100 microliter of tumor suspension was injected subcutaneously (s.c.) in the left- and right side of male inbred Lewis rats. Two weeks after the tumor transplantation 95% of the rats already had a detectable tumor mass. With respect to other tumor characteristics we have demonstrated in these tumors the presence of EGF receptors and low levels of progesterone receptors (PgR) in the absence of estradiol receptors (ER), while Reubi and Maurer (Sandoz, Basel) were able to show the presence of specific binding sites for the somatostatin analog by autoradiography.

Subcutaneous treatment was usually started right from tumor transplantation (prophylactic treatment) or 2 weeks after transplantation (treatment groups). The treatment groups (n=8) were compared with control groups with tumor and supercontrols without tumor. The rats were treated twice daily with 5 dosages of Sandostatin (0.05, 0.2, 1.0, 5.0, and 20 ug), the LHRH agonist buserelin (5 ug), and the aromatase inhibitor aminoglutethimide (0.5 and 2.0 mg).

Tumor bearing control rats showed lower body weight than rats without transplanted tumors (32). Somatostatin analog treatment had no or minor effect on the body weight of tumor bearing rats. Untreated tumor bearing rats had lower plasma concentrations of GH, IGF-1 and EGF compared to rats without tumors. This might be explained by the worse condition of rats with tumors

accompanied by delayed body growth. In tumor bearing rats treatment with Sandostatin decreased plasma concentrations of GH (±40%), IGF-1 (±15%), EGF (20-80%), and tumor PgR levels (30-50%). Rats treated right from time of tumor inoculation showed pronounced inhibition of tumor growth after 2 weeks (4.1. ±6.6 mm2 (s.d.) vs. 45.8 ± 34.4 mm2 in controls) and 55% inhibition after 6-9 weeks. In the average a growth inhibition of 35% was reached in the non-prophylactic treatment groups after 6-9 weeks of treatment with the different dosages of Sandostatin used. A dose of 1.0 ug twice daily seems slightly more effective than the other dosages when used prophylactically, but we did not observe a difference in growth inhibiting effects between the 5 different dosages of Sandostatin used in the treatment groups with tumors. Treatment with 2x2 mg aminoglutethimide caused comparable tumor growth inhibition (50%) while buserelin appeared somewhat less effective (Fig. 1).

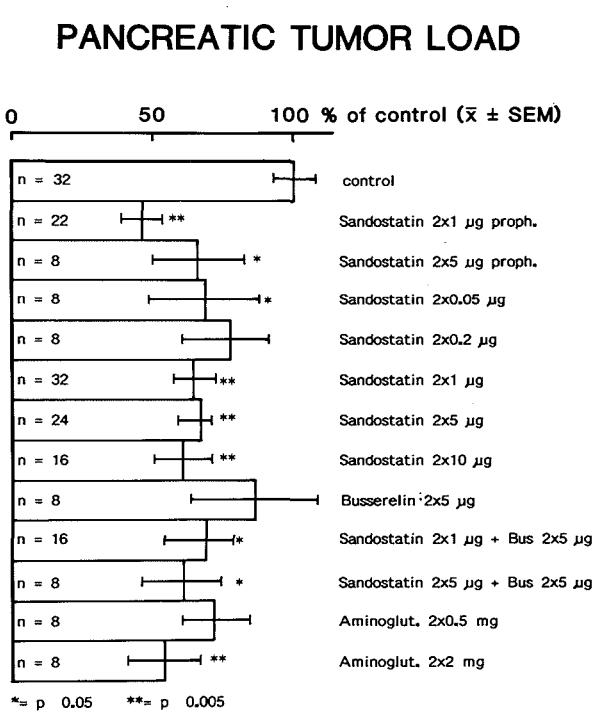

Fig. 1 Tumor growth inhibiting effects of different kinds of treatment in rats with transplantable pancreatic tumors.

Rhabdomyosarcomas

The observation that some sarcomas can produce growth factors as insulin-like growth factor II (IGF-2) and secondly that IGF-1 is involved in the growth regulation of muscle cells, led us to study the effect of Sandostatin treatment on the growth of transplantable rhabdomyosarcomas in WAGRIJ rats. However, no growth inhibiting effect could be observed (Fig. 2).

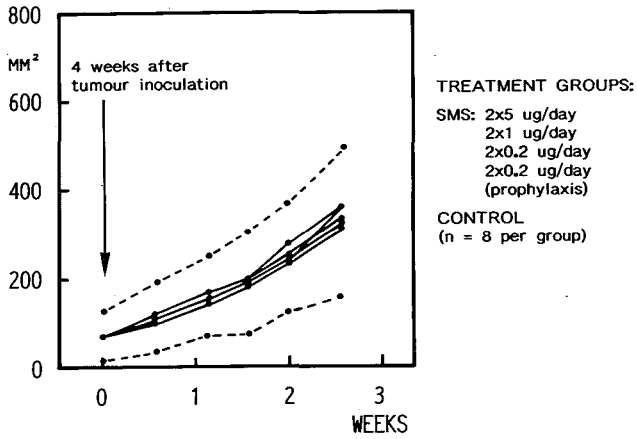

Fig. 2. No effect of various dosages of Sandostatin on tumor load in rats bearing rhabdomyosarcoma on the back (results are mean values with indication of the maximal S.D. values calculated).

DMBA rat mammary tumors

Rats with DMBA induced mammary tumors were treated twice daily with the same 5 dosages of Sandostatin as described before (i.e. 0.05, 0.2, 1.0, 5.0, and 20 ug) for 3 weeks. We observed a bell-shaped curve of dose-response relationship for single Sandostatin treatment (Fig.3, Table 1). Maximal tumor growth inhibition (82% as compared to controls i.e. 10% vs. 57% growth) was reached with a dose of 2 x 0.2 ug per day. Lower and higher dosages of Sandostatin appeared to cause less or no growth inhibition. There was even a tendency to an increased tumor growth rate in rats treated with the highest dose.

Treatment with buserelin showed equal results as ovariectomy causing clear tumor regression, while single somatostatin analog treatment caused only inhibition of tumor growth. The best results were obtained with the combination of LHRH and somatostatin agonists i.e. 77% regression (Table 1).

Sandostatin treatment with the highest dosages caused no

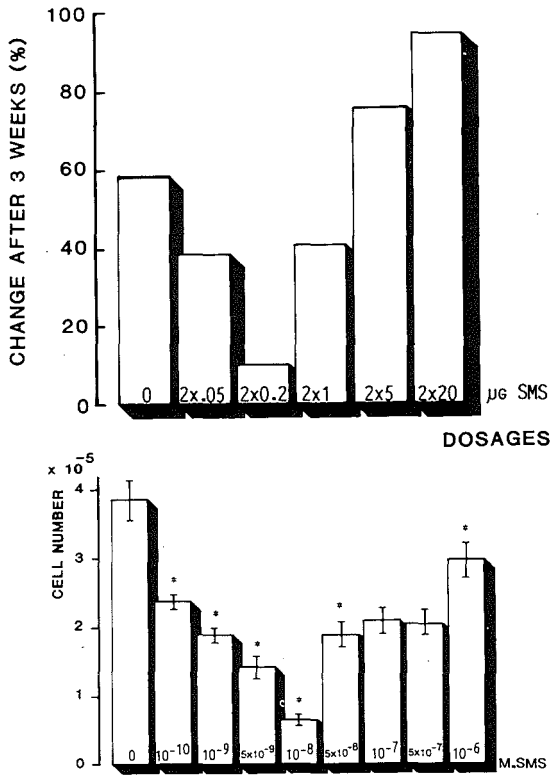

Fig. 3. Effects of different dosages of Sandostatin on rat mammary tumor growth in vivo (upper part) and on MCF-7 tumor cell growth in vitro (lower part).

effects on the weight of the pituitary, adrenals, ovaries and uterus. Using the lowest dosages sometimes a small but significant decrement of the weight of these organs was observed. Treatment with Buserelin

caused highly significant decrement of the weight of the uterus and ovaries. Plasma hormone studies are in progress. No binding sites for Sandostatin could be demonstrated by autoradiography (Maurer and Reubi, Basel) using a technique described in detail before (29).

Human breast cancer cells (MCF-7)

Looking for possible direct effects at the level of tumor cells we have studied the effects of 9 different concentrations

Table 1. Comparison of the relative effects on rat mammary tumor load of various doses of Sandostatin compared to those of other endocrine treatments

	CHANGE (%)
CONTROL	+57/+57
2x 0.05µg SMS	+38
2x 0.2µg SMS	+10
2x 1µg SMS(L)	+38/+42
2x 5µg SMS	+76
2x 20µg SMS(H)	+94
2x 5µg Buserelin	-40/-49
Buserelin + SMSL	-77
Buserelin + SMSH	-27
ovariectomy	-51

of Sandostatin between 10^{-6} and 10^{-10} M in cultures of MCF-7 tumor cells both in the absence and presence of estradiol and/ or insulin. The known stimulating effects of estradiol and insulin were confirmed. But, we showed for the first time that a somatostatin analog directly inhibits the growth of breast cancer cells in vitro. In all four conditions tested Sandostatin appears to inhibit tumor cell growth. Based on 3 consecutive experiments the most profound inhibition (73 $\pm$ 18%; x $\pm$ SEM) measured by cell number (Fig.3), DNA and protein content of the cultures was observed in cultures with insulin and without

added estradiol ($p < 0.005$). With respect to dose response relationship, strikingly again a bell-shaped curve was observed as in the in vivo experiments with rat mammary tumors. Maximal suppression of the growth of the cultures was obtained at a sharply defined amount of Sandostatin (10^{-8} mol); at lower and higher dosages the inhibition of cell growth was less striking.

The observation that Sandostatin had a direct inhibitory effect in vitro on MCF-7 cell growth prompted us to investigate the possible presence of somatostatin receptors. We have measured the kinetics of uptake of the Sandostatin derivative ^{125}I-SMS 204-090 in MCF-7 cell cultures and indeed specific binding was observed namely 3 x 10^4 molecules per cell (32).

Patients with pancreatic and gastrointestinal adenocarcinomas

Patients with metastatic pancreatic (n=7), gastric (n=2) and colorectal tumors (n=9) were chronically treated with 3 x 200 ug Sandostatin s.c. per day. Plasma IGF-I decreased in nearly all patients. IGF-1 concentrations in plasma (measured directly and by acid extraction) decreased both in patients with pancreatic tumors and colorectal tumors but an increase occurred to pretreatment levels after 8-13 weeks of treatment (Fig.4).

Acid-extracted IGF-1 concentrations were much lower in pancreas carcinoma patients than in colorectal carcinoma patients and appeared in the first group not different from direct assayable IGF-1 plasma concentrations. This might be explained by the fact that most patients with (liver) metastases of pancreatic cancers were cachectic and had no appetite.

With respect to possible antitumor effects, no objective remissions were observed but 3 patients with metastatic colorectal tumors showed stable disease during 3-9 months. No serious side effects were observed. Nearly all patients had increased fecal fat loss, but significant loss of body weight did not occur.

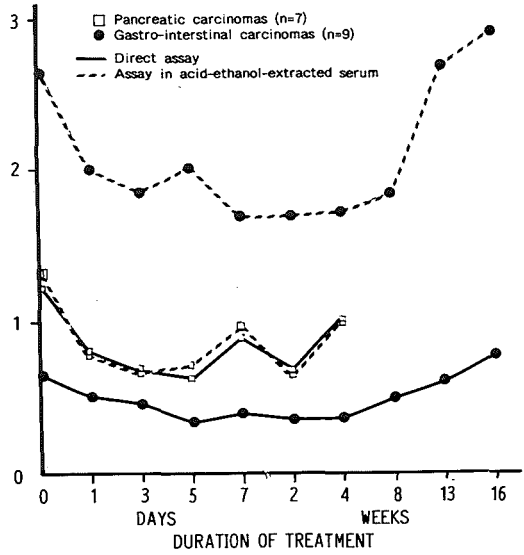

Fig. 4. Mean serum IGF-1 concentrations during chronic treatment with Sandostatin in patients with metastatic pancreatic and gastrointestinal carcinomas.

DISCUSSION AND CONCLUSIONS

Somatostatin analog treatment appeared to cause clear (dose dependent) growth inhibiting effects in experimental pancreatic and breast cancer. From our experience the dose used appeared very critical. Higher dosages than the optimally suppressive dose are less effective, maybe as a consequence of desensitization of the cells during treatment.

With regard to mechanism of action somatostatin analogs have direct and indirect effects on tumor cells. In Lewis rats with pancreatic tumors growth inhibition might be caused both via the demonstrated somatostatin receptors and/or decrement of hormone and growth factor secretion especially of EGF.

Sandostatin can act on mammary tumor cells also by different ways. Based on our observation of growth inhibition in vitro of tumor cells

(MCF-7) bearing binding sites for Sandostatin and of mammary tumors in vivo without somatostatin receptors the presence of direct and indirect effects is probable. Somatostatin and analogs may exert their action directly by interacting with its specific receptor or by modulation of other hormone receptors, and indirectly by a decrease in the secretion of pituitary hormones (GH, PRL) and growth factors such as IGF-1 and EGF. With respect to indirect actions Murphy et al. (23) described that human GH may be a potent ligand for the lactogenic receptor in human breast cancer cells, while Shiu and Iwasiow (33) observed induction of specific proteins by GH and prolactin (PRL) in human breast cancer cells. The observations that increased plasma GH levels in breast cancer patients occur (8) and that hyperprolactinemia is an unfavorable prognostic factor (6) might be of importance.

In addition somatostatin may act as a paracrine or autocrine regulator of tumor cell proliferation by influencing secretion of autocrine or paracrine growth factors. Recently IGF-1 appeared to be one of the autocrine growth factors for various breast tumor cell lines and to stimulate the growth of MCF-7 tumor cells (18,19). The interaction of mammogenic peptide hormones (GH, PRL, insulin), steroids and EGF with modulation of their respective receptors (7,11-13,22,23) in addition to the possible inhibiting effects of Sandostatin on the secretion of EGF and IGF-1, suggests a very complex mechanism through which somatostatin and its analogs act in vivo and in vitro. It is interesting to note that analogs of LHRH (3,4,21) and somatostatin (this report) have direct inhibitory effects on tumor cell growth antagonizing in vitro biological effects of those steroid (estradiol) and peptide hormones (GH, insulin) which secretion in vivo is suppressed by pharmacological doses of the same neuropeptide analogs.

In conclusion

1. In experimental pancreatic and breast cancer models somatostatin analog treatment appears to cause clear tumor growth inhibitory effects in vivo and in vitro.

2. With respect to dose-response relationship a bell-shaped curve was observed.

3. Specific binding sites for a iodinated derivative of Sandostatin appear to be present in MCF-7 cells and pancreatic tumors.

4. No antitumor effect was observed in rats with rhabdomyosarcomas.

5. In the majority of patients with gastro-intestinal tumors chronic Sandostatin treatment decreased plasma IGF-1 levels; the clinical data with respect to tumor growth inhibition are thus far too scarce for definite conclusions.

6. More studies are needed to define the optimal dose and mode of administration in patients with carcinomas.

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SOMATOSTATIN ANALOG TREATMENT IN RATS WITH TRANSPLANTABLE PANCREATIC TUMORS.

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INTRODUCTION

Somatostatin has mainly been detected in the central nervous system and the gastrointestinal tract. Somatostatin and its analogs inhibit the secretion of growth hormone (GH), some growth factors, and most gastrointestinal hormones, of which some are involved in the growth regulation of pancreatic cells. Furthermore, a number of pancreatic tumors appears to contain a high content of steroid hormone receptors and enzymes involved in steroid metabolism indicating potential hormone dependency. These data and those previously reported by Schally et al. led us to assume that somatostatin analogs may influence the growth of different experimental pancreatic tumors.

AIMS OF THE STUDY

1) To test whether the somatostatin analog SMS 201-995 (Sandostatin) inhibits the growth of a transplantable acinar pancreatic tumor in rats (Prof. Longnecker).

2) Determination of the characteristics of the tumor (model).

3) Investigation of the mechanism of action of the somatostatin analog.

4) Investigation of the effects of combined treatment and comparison with other endocrine measures.

METHODS

Animals
Tumors were kindly provided by Dr. A.E. Bogden, Eg & G Mason Research Institute. About 100 microliter of tumor suspension was injected s.c. in the left-and right side of the hind flanks of male inbred Lewis rats. The rats were divided into groups of 8 animals. After randomization each group had about the same average body weight. Two weeks after the tumor transplantation 95% of the rats already had a detectable tumor mass. Subcutaneous treatment was usually started right from tumor transplantation (prophylactic treatment) or two weeks after transplantation (treatment groups). The treatment groups were compared with control groups (with tumor) and supercontrols (without tumor).

Treatment – different dosages Sandostatin (0.05, 0.2, 1, 5, and 20 µg twice daily).
- Buserelin: an LHRH agonist (2 x 5 µg per day).
- Aminoglutethimide (2 x 0.5 and 2 x 2.0 mg/day).

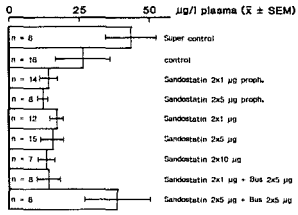
Hormones : GH and IGF-1 (Somatomedin C) by radioimmunoassay. EGF like activity by radioreceptor assay.

Hormone receptors : - ER and PgR according EORTC guidelines.
- EGFr by scatchard analysis.
- Somatostatin receptors with ^{125}J Sandostatin and autoradiography (by Maurer and Reubi, Basel).

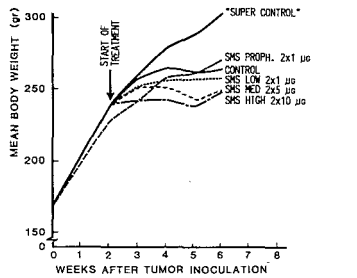
CHARACTERISTICS OF THE PANCREAS TUMOR

Histology	:	acinar adenocarcinoma
Estradiol receptor	:	-
Progesterone receptor	:	+
EGF receptor	:	+
Somatostatin receptor	:	+

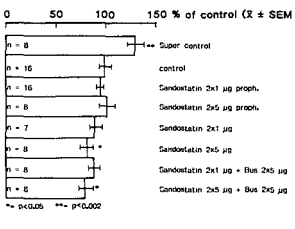
PLASMA LEVELS OF GROWTH HORMONE



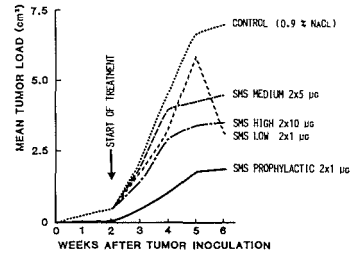
EFFECT OF SOMATOSTATIN ANALOG TREATMENT ON BODY WEIGHT OF RATS WITH TRANSPLANTABLE PANCREATIC TUMORS



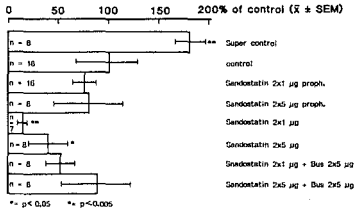
SOMATOMEDINE C IN RATS WITH PANCREATIC TUMORS



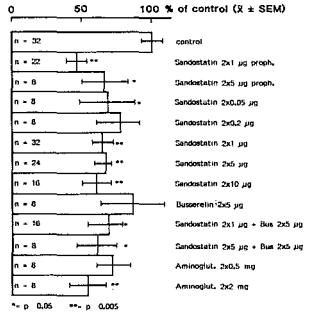
EFFECT OF SOMATOSTATIN ANALOG TREATMENT ON MEAN TUMOR LOAD OF TRANSPLANTED PANCREATIC TUMORS IN RATS



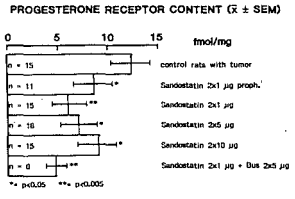
PLASMA EGF IN RATS WITH PANCREATIC TUMORS



PANCREATIC TUMOR LOAD



PROGESTERONE RECEPTOR CONTENT (x̄ ± SEM)



CONCLUSIONS

1) Tumor bearing rats showed lower body weight than rats without transplanted tumors. Somatostatin analog treatment had no or minor effect on the body weight of tumor bearing rats.

2) - Tumor bearing rats had lower plasma concentrations of GH, Somatomedin C (IGF-1) and EGF compared to rats without tumor.
- Somatostatin analog treatment decreased plasma concentrations of GH (± 40%), Somatomedin C (± 15%), EGF (20-80%), and tumor PgR content (30-50%).
- Somatostatin analog treatment (6-9 weeks) inhibited pancreatic tumor growth (35%) with the best results (55% inhibition) when used directly from tumor inoculation.

side-effects.[8] This safety is believed to be due to the drug's selective dopaminergic blocking action in the limbic forebrain with virtually no such effect in the nigrostriatal system,[9] although it is also possible that its high inherent anticholinergic activity may protect against parkinsonian side-effects.[10]

Although extrapyramidal side-effects with melleril are alleged to be caused only by high doses,[5] severe parkinsonism developed in patient 3 when he was taking only 25 mg daily. We detected that patients 1 and 2 were taking thioridazine only after some time and after apparently effective levodopa therapy had been introduced. In patient 4 prochlorperazine therapy was discovered only at a systematic review of current medication at a home visit by the geriatrician. These cases amply confirm the statement by Lader[11] that the more diligent the search, the greater will be the yield of adverse drug reactions.

In all cases of apparent drug-induced parkinsonism it is uncertain whether the condition is entirely iatrogenic or whether mild parkinsonism has been aggravated by the dopamine antagonism of the offending drug.[4] In this small series all four patients returned to normal when treatment with the phenothiazine was stopped. Duvoisin[12] has emphasised, however, that subclinical parkinsonism may become overt during a period of psychotropic therapy and may apparently subside when the offending therapy is stopped, only to re-emerge in the ensuing year or two.

The practical lessons of these findings are clear. Phenothiazines, even the "safe" thioridazine, may cause severe parkinsonism in elderly patients. Parkinsonism thus induced may persist for a considerable time. C. D. Marsden (personal communication) maintains that it may persist for up to 2 years. Even with good standards of general practice and a specific inquiry about current medication, it may be difficult to determine accurately whether a patient is receiving (or has recently received) a phenothiazine. Special inquiry is necessary. Phenothiazines should therefore be prescribed as seldom and as sparingly as possible, and a most meticulous check should be made of drugs being taken (or recently taken) in all patients diagnosed as having parkinsonism. Perhaps the only foolproof safeguard is a careful "drug holiday" every 6 months in parkinsonian patients in whom a drug effect is seriously considered. This time off therapy would reveal whether the condition had been entirely or partly drug induced (Marsden: personal communication).

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Preliminary Communication

TREATMENT WITH A LUTEINISING-HORMONE-RELEASING-HORMONE ANALOGUE (BUSERELIN) IN PREMENOPAUSAL PATIENTS WITH METASTATIC BREAST CANCER

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Summary Four premenopausal women with metastatic breast cancer were treated with a potent luteinising-hormone-releasing-hormone (LHRH) analogue as a single first-line agent. Buserelin (6-D-ser-(bu^t)-LHRH-(1–9)-ethylamide) was given in very high doses intravenously and then intranasally; ovarian function did not decrease within 1–2 weeks, but after this period low to very low (postmenopausal) plasma oestradiol levels were reached in all patients, accompanied by flushes. Two patients, however, subsequently showed transient peaks of plasma oestradiol, probably due to a persistent follicle, which lasted for some weeks without a rise in plasma luteinising hormone, follicle-stimulating hormone, or progesterone. In two patients there was no significant change in the tumour, whereas in the other two patients there was remarkable improvement.

INTRODUCTION

MANY luteinising-hormone-releasing-hormone (LHRH), analogues with pronounced and long-term effects have been synthesised and tested.[1–4] Most of these agents have a paradoxical antifertility effect in male and female rats during long-term treatment with sufficiently high (supra-physiological) doses. Inhibition of steroidogenesis is followed by weight loss of the gonads and secondary sexual organs. Plasma oestradiol levels fall to the concentrations found in oophorectomised rats;[3] thus LHRH analogues can elicit chemical oophorectomy.

In human beings administration of LHRH analogues initially leads to stimulation and subsequently to suppression of pituitary and gonadal function.[5] Short-term treatment both during the follicular phase[6] and after midcycle shortens the menstrual cycle with luteolysis.[7,8] When an LHRH analogue was administered throughout the menstrual cycle there was no ovulation.[9] This hormonal oophorectomy might be important in the treatment of hormone-dependent tumours. No results of treatment with LHRH analogues in patients with metastatic breast cancer have yet been reported, but several studies in animals indicate that LHRH agonists produce regression of spontaneous or dimethylbenz-(a)anthracene-induced mammary tumours.[4,10-12] The results are similar to those obtained with oophorectomy[10,12] and tamoxifen studies.[12] No serious side-effects have been reported.[9] We have treated four premenopausal women with metastatic breast carcinoma with one of the most potent LHRH analogues, buserelin (6-D-ser [bu^t]-LHRH [1–9] ethylamide), as a single first-line agent; we initially gave very high doses intravenously and then smaller doses intranasally.

PATIENTS AND METHODS

Four premenopausal women (aged 44, 41, 48, and 44, respectively) with metastatic breast carcinoma gave informed

consent for this new form of hormonal treatment. All menstruated regularly once every 4 weeks. They had undergone surgical treatment and (with the exception of patient 2) local radiotherapy for primary breast cancer 1½, 5, 1½, and 3½ years previously. Patients 1–3 were treated with an 8 h infusion of 3 mg buserelin from 9.00 A.M. until 5.00 P.M. on 7 successive days. Blood samples for measurement of luteinising hormone (LH) and follicle-stimulating hormone (FSH) were taken by way of an intravenous catheter twice before, then 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, and 8 h after the start of the infusion. Patient 4 was treated with a continuous infusion of 3 mg/day buserelin for three days, and blood samples for LH and FSH measurement were taken every 4 h. In all patients plasma oestradiol and progesterone were measured every day before the infusion and on days 1 and 7 in the first three patients 1 h, 4 h, and 8 h after the start of the infusion. After the intravenous treatment the daily therapy was changed to three doses per day of 200 μg buserelin intranasally (3×400 μg in patient 4). During this treatment blood samples were taken weekly for measurement of LH, FSH, oestradiol-17β, and progesterone. Each patient was asked to make a basal temperature curve.

LH, FSH, oestradiol-17β,[13] and progesterone[14] concentrations were measured as reported previously. Results for LH are now expressed in terms of the World Health Organisation standard 68/40 rather than standard 69/104. The normal range for basal LH is 3·5–12·6 IU/l. and for basal FSH 1·2–4·2 IU/l.

RESULTS

Endocrine Effects

Patient 1 had, before treatment, high basal plasma concentrations of LH and FSH and low plasma oestradiol and progesterone levels, suggesting that she was perimenopausal (see accompanying figure). In patients 2 and 3 basal plasma LH and FSH were high, but oestradiol levels were also high (figure). Ovulation had probably occurred 1 or 2 days earlier as shown by rising plasma progesterone concentrations. In patient 4 (figure) basal LH and FSH were normal before treatment, and plasma progesterone showed only a small and slow increase during the week after treatment started.

On the first treatment day peak values for plasma LH and FSH were reached after 3 to 8 h. In patients 2 and 3 plasma LH and FSH fell to the starting level within 1 day, whereas in patients 1 and 4 high plasma levels persisted for more than 1 week (figure). Ultimately, plasma gonadotropin levels fell below the pretreatment values in all patients.

Patients 2, 3, and 4 began menstruating on days 24, 28, and 25, respectively, of the cycle in which therapy was started, but patient 1 did not menstruate. The duration of the luteal phase was normal in patients 2 (14 days) and 3 (at least 12 days). After 2 weeks' therapy plasma oestradiol concentrations were 66–206 pmol/l, whereas plasma progesterone was low and remained low (<1·5 nmol/l) in the next few weeks of follow-up. The basal temperature curves showed an irregular (patients 1 and 3) or monophasic pattern (patients 2 and 4). Within 7 weeks all patients had very low plasma oestradiol concentrations (33–103 pmol/l) and experienced hot flushes for the first time in their lives. However, patients 1 and 3 subsequently showed transient peaks of plasma oestradiol without increases in plasma LH, FSH, or progesterone.

The very high doses of buserelin caused no side-effects which the four patients could recall or which could be shown by physical and routine laboratory examinations.

Effects on Tumour Growth

Patient 1 had cytologically proven costal-bone metastases which were causing bone pain. During treatment the pain varied and decreased after 7 weeks. Radiographic examination of the skeleton showed no change. Physical and laboratory examinations also showed no signs of progression.

Patient 2 had increasing bone pain and sciatica with immobility caused by a partially destroyed and compressed lumbar vertebra (L2) due to progressive metastatic disease. This patient, who asked for active euthanasia 1 week after the start of therapy, improved remarkably. She is now free of pain and works full-time. A suspect hard axillary lymph-node (1·8×1·5 cm) disappeared completely.

Patient 3 had cytologically proven skin, lymph-node, and bone metastases. After 7 weeks' treatment there was no significant change in the extent of the metastases, although there was a tendency to progression of the skin metastases.

Patient 4 had a pleural effusion histologically proven to be carcinomatous. The effusion disappeared in 6 weeks without intrapleural cytostatic therapy or discharge of the pleural effusion, and her complaints of coughing ceased.

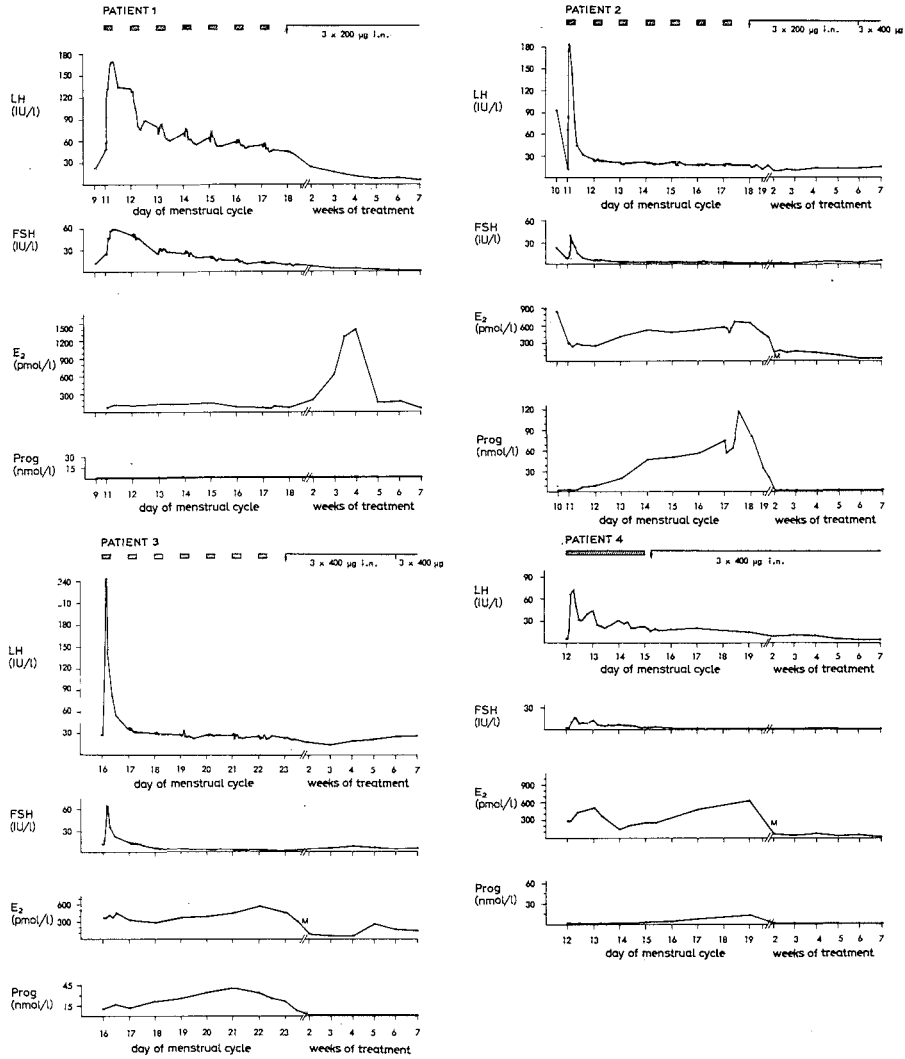
DISCUSSION

Long-term treatment with large doses of LHRH agonists causes pituitary exhaustion and desensitisation,[2,4–6] inhibition of prolactin secretion,[11,15,16] and down-regulation of gonadal gonadotropin receptors with decreased steroidogenesis;[2,4,17,18] a direct effect of LHRH analogues at the ovary has also been demonstrated.[19,20] Inhibition of oestradiol secretion seems to be the major determinant for an antitumour effect in animals with mammary tumours.[4] Also, the recently reported ability of LHRH analogues to antagonise the biological actions of sex steroids[21,22] may be important for hormone-dependent tumours such as breast cancer.

Plasma LH and FSH fell below pretreatment values after about 2 weeks' treatment and then rose slightly in some of our patients. Very low plasma LH levels (1 or 2 IU/l) were not reached. The paradoxical reaction of plasma LH to the LHRH analogue in patient 1 during the 1st week of treatment is interesting. From the 2nd day plasma LH fell, after a slight rise, during the infusion period, but during the night LH levels rose. This finding indicates increased release and production of LH outside the period of intravenous therapy. Although shortening of the menstrual cycle with luteolysis has been reported when treatment was started after midcycle,[7,8] in patients 2 and 3 the duration of the luteal phase of the cycle in which treatment began was normal. Patient 2 menstruated 4 days early probably because of an early ovulation rather than luteolysis. In patient 4 corpus luteum insufficiency is a plausible cause of the moderate increment in plasma progesterone and the early onset (3 days) of menstruation.

All four patients became anovulatory after the menstruation at the end of the menstrual cycle in which buserelin treatment began. Plasma oestradiol decreased further to postmenopausal values 6–7 weeks after treatment started. The high plasma level of oestradiol (without a rise in progesterone) for some weeks in patient 1, possibly due to a persistent follicle, was disappointing in terms of the aims of this therapy. It seems the pituitary is not able to respond to the increased levels of oestradiol by releasing the mid-cycle peak of LH and FSH (i.e., the positive feed-back is disturbed).

Follow-up was short for the determination of effects on the tumours. In patients 1 and 3 after 7 weeks' treatment there were no significant clinical, chemical, or radiological signs of regression or progression of the tumour. However, in patients 2 and 4, who showed the most favourable endocrine



Effects of long-term buserelin treatment on plasma LH, FSH, oestradiol-17β(E_2), and progesterone (Prog) in patients 1 to 4.
M = menstruation. Hatched area represents period of intravenous treatment.

effects of treatment, there was remarkable improvement.

The results of this therapy with the LHRH analogue buserelin are not the best but offer hope. The problem of inhibiting luteal steroidogenesis within a few days remains, but most hormonal treatments for breast cancer need 1 or 2 weeks before they are effective. Also we must be aware that a persistent follicle or a number of active small follicles may develop. We believe, however, that LHRH analogues will ultimately be useful in the treatment of metastatic breast cancer, certainly in combination with other hormonal treatment.

ADDENDUM

14 and 9 weeks respectively after the start of the treatment patients 2 and 4 were still in remission with continuous "oophorectomised" levels of plasma oestradiol (about 45 pmol/l), while patient 1's condition was stable at 14 weeks with very low plasma oestradiol (about 45 pmol/l). Because of slowly growing metastases in the skin and because of a second oestradiol peak, we decided to add anti-oestrogenic therapy in patient 3.

We thank the medical department, Hoechst Pharma Holland, for supplies of the LHRH agonist, our colleagues of the hospital breast cancer group, the

nurses for carefully collecting blood samples, Dr R. Docter for measurement of LH and FSH, H. Portengen, D. M. van Gool, and H. G. Bierings for technical assistance, C. Hoeijmans and G. van den Bos for administrative help, and J. Marselje for preparing the prints.

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Hypothesis

CHOLERA AND ESTUARINE SALINITY IN CALCUTTA AND LONDON

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Summary Laboratory investigation has shown that *Vibrio cholerae* requires a salinity of at least 0·01% to survive beyond 24 h. This requirement, when coupled with the observation that water becomes unpalatable at salinities above approximately 0·1%, suggests that waterborne transmission of cholera is unlikely to take place when water salinity falls outside the range 0·01–0·1%. A hypothesis is proposed that this constraint on waterborne transmission, together with the seasonal fluctuations of estuarine salinities, may explain the seasonality of cholera in estuarine cities.

INTRODUCTION

UNTIL the mid-1970s *Vibrio cholerae* was regarded as an organism with only a limited ability to survive in the environment.[1] However, isolation of the O1 serotype of *V. cholerae* from aquatic environments in areas believed to be free of clinical cholera has challenged this view and raised the possibility that the organism is a normal inhabitant of specific aquatic habitats.[2,3] Even if this is not the case, these environmental isolations suggest that *V. cholerae* can survive for lengthy periods in certain aquatic environments.[4]

V. CHOLERAE SURVIVAL AND SALINITY

All of the recent isolations of the O1 serotype of *V. cholerae* in areas non-endemic for cholera have been made from brackish waters. In Chesapeake Bay, U.S.A., for example, *V. cholerae* was only isolated from waters of salinity of 0·4–1·7%.[5] We have measured the effect of salinity on *V. cholerae* survival to explore whether salinity might be a dominant controlling factor in the aquatic distribution of the organism.

Six Bangladesh isolates of O1 *V. cholerae* El Tor Inaba were suspended in a range of sea salt solutions prepared from sterile distilled water and natural sea salt containing no additives. The pH was standardised at 8·0 and the solutions were stored in the dark at room temperature (about 25°C). Bacterial counts were made by means of a spread plate technique. We inoculated 0·1 ml of $\log_{10}$ dilutions of the bacterial suspension on to trypticase soy agar plates. The plates were incubated overnight at 37°C and the dilution chosen for the count was that which gave between 30 and 300 colonies. Each count was carried out in duplicate.

The minimum level of salt required for survival for 1 day at 25°C was 0·01%, whilst the optimum level for survival fell in the range 0·5–3·0% (fig. 1). This may explain the discrepancy between the reported short survival of *V. cholerae* in potable water and the extended survival of the vibrio in estuarine waters of appreciably higher salinity.[4]

SALINITY OF DRINKING WATER

Water becomes unpalatable at salinities above 0·1%.[6] Therefore, if cholera in man is associated with drinking water in which there is a *V. cholerae* population, transmission is most probable when water salinities fall in the range 0·01–0·1%. This may in part explain the historical association between human cholera and cities with poor sanitation located on estuaries.

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APPENDIX PAPER 2.

LONG-TERM LHRH-AGONIST TREATMENT IN METASTATIC BREAST CANCER AS A SINGLE TREATMENT AND IN COMBINATION WITH OTHER ADDITIVE ENDOCRINE TREATMENTS

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(*Received for publication* 13 *July* 1983)

Twenty-one premenopausal patients with metastatic breast cancer (unselected for receptor status) were treated during 3–14 months with the potent LHRH-agonist Buserelin (Hoe 766) as a first-line therapy. In one-third of 12 patients treated with Buserelin alone an objective tumour response was observed in the absence of side effects. The longest duration of response occurred in 2 patients with complete remissions (14^+ and 13^+ months). After addition and in combination with tamoxifen this LHRH-agonist treatment caused an objective response in about half (8/17) of the patients. A problem appears to be the great variation in hormonal response especially during the combination treatment with tamoxifen, which was not found during combination treatment with megestrol acetate in 4 patients. Ultimately, of the whole group of 21 patients, 9 patients (43%) showed an objective remission, 6 stable disease and 6 progression of tumour growth.

Key words: **Breast cancer, LHRH.**

INTRODUCTION

Many analogues of luteinizing-hormone-releasing-hormone (LHRH) with marked and prolonged effect in comparison with the natural hormone have been synthesized and tested.[1–4] A lot of them appeared to have a paradoxical antifertility effect in male and female rats during chronic treatment in sufficiently high (supra-physiological) doses. Such long-term treatment with large doses of LHRH-agonists causes: (1) 'partial hypophysectomy' with decreased gonadotrophin[1–5] and prolactin secretion;[4–7] (2) 'chemical castration' with decreased steroidogenesis and reduction in weight of the gonads and of secondary sexual organs;[1, 4–8] (3) Possibly a direct antisteroidal effect at the target organs.[9–11]

These endocrine effects are important with regard to the treatment of hormone-dependent tumours. In experimental studies such chronic LHRH-agonist treatment appeared to inhibit the growth of mammary,[4, 6, 12, 13] prostate[14] and pituitary tumours.[7]

In view of these findings we started in 1981 a study in premenopausal patients with metastatic breast carcinoma. Recently the preliminary results have been published.[15, 16] Meanwhile we have experience with long-term endocrine and anti-tumour effects of chronic treatment with the LHRH-agonist Buserelin (Hoe 766) as a first-line agent in a larger group of patients. The longest duration of treatment is by now more than 14 months. Further we studied the effects of combination therapy with the LHRH-agonist and tamoxifen as well as megestrol acetate.

PATIENTS, METHODS AND TREATMENT

Twenty-one premenopausal patients with metastatic breast cancer gave informed consent to be treated with the potent LHRH-agonist Buserelin as a single agent or in combination with other additive hormonal treatment. All patients had not previously been treated for their metastatic disease and were unselected for receptor status. Twelve patients were treated with Buserelin alone (1) and totally 14 patients with the combination of the LHRH-agonist with 2 × 20 mg tamoxifen (2) as indicated in Table 1. In addition, we treated recently 4 other patients with the combination of Buserelin and 4 × 45 mg megestrol acetate per day (3).

Blood samples for the measurement of LH, FSH, oestradiol (E2) and progesterone (prog) were taken daily in the first week and later on weekly during chronic

Table 1. Data of 17 patients treated with Buserelin alone or in combination with tamoxifen

Group A

1. Twelve patients (median age = 40 yr) treated with a daily dose of 3 mg Buserelin per infusion or subcutaneously during 3–7 successive days followed by 3 X 400 μg per day intranasally.

2. In 9 of these patients tamoxifen 20 mg twice daily was added later on because of tumour progression or recurrent peaks of plasma oestradiol

Group B

Five patients (median age = 37 yr) treated with the combination of Buserelin and tamoxifen from the start of treatment

Disease-free interval: x = 2.5 yr (range 0.5–5.0 yr)

Oestradiol receptor: 4 X positive; 2 X negative; 11 X unknown

Start of treatment during the luteal phase: 11 X

intranasal therapy. Plasma concentrations of these hormones were measured by radioimmunoassay as described previously.[17] The normal range for basal LH is 3.5–12.6 I.U./l and for basal FSH 1.2–4.2 I.U./l. Measurements of tumour response were performed according to UICC criteria.

RESULTS

1. *Single treatment with Buserelin*

An objective tumour response occurred in 4 patients and stable disease in 4 further patients, while progression was shown in the remaining 4 patients (Table 2). The longest duration of response is more than 14 months. No side effects were observed with the exception of hot flushes.

An ovulation as indicated by persisting low plasma progesterone levels was reached in all patients during chronic treatment with Buserelin alone, but recurrent peaks of oestradiol occurred in 6 out of 10 evaluable patients (Table 3). In 4 patients a complete castration effect with persisting postmenopausal values for plasma oestradiol was found (for instance Fig. 1). In 2 patients the chronic effect on ovarian function could not be evaluated because the treatment had to be changed within 6 weeks due to rapid progression in tumour growth. Objective remissions occurred especially in patients with complete 'chemical castration' (3X), but it appeared also in one patient with recurrent peaks of oestradiol.

2. *Combination therapy with Buserelin and tamoxifen*

In 9 of the 12 patients, treated with Buserelin alone, tamoxifen was added later on because of recurrent peaks of oestradiol or tumour progression. In addition to the 4

Table 2. Anti-tumour effects during treatment with Buserelin alone and in combination with tamoxifen

Group	Type of response[a]		Duration (months)
A (1)	CR : 2X	4/12	14^{+}–13^{+}
	PR : 2X		3–5
	NC : 4X		4.5–3–3^{+}–3
	F : 4X		
A (2)	After addition of TAM: 2X PR		
B	CR : 1X		3^{+}
	PR : 1X		5^{+}
	NC : 1X		5^{+}
	F : 2X		
In total	CR : 3X	8/17	
	PR : 5X		
	NC : 4X		
	F : 5X		

[a]CR = complete remission, PR = partial remission, NC = no change, F = failure.

Table 3. Endocrine effects in 17 patients treated with Buserelin alone and in combination with tamoxifen

Sex steroids	Effect		Occurrence	
1. Buserelin alone ($n = 12$)	(a) 'Castration'		: 4X	
	(b) Rec. E_2 peaks		: 6X	
	Not evaluable		: 2X	
2. Buserelin + TAM (group A + group B = 9 + 5 = 14)	(a) 'Castration'	5 + 1 =	6X	
	(b1) Rec. E_2 peaks without progesterone		1X	8X
	(b2) Rec. E_2 peaks with progesterone secretion	3 + 4 =	7X	

Complete 'chemical castration' in 40% of the patients.

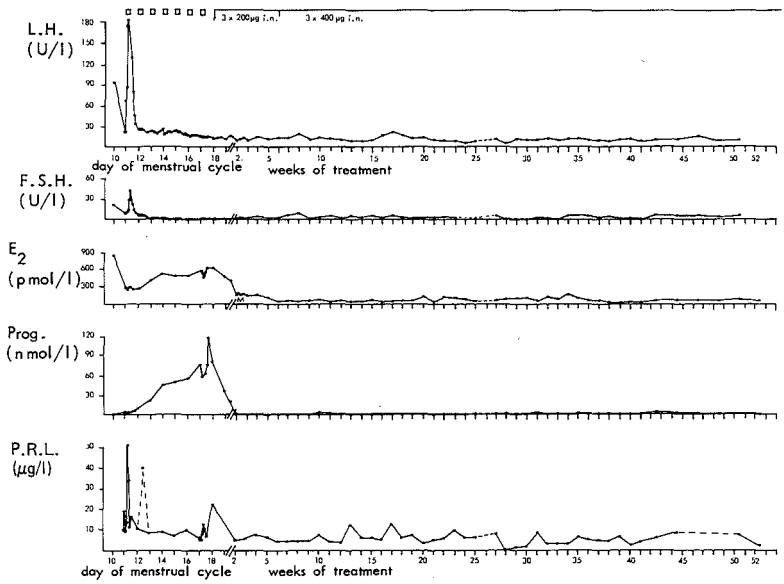

Fig. 1. Changes of plasma hormone concentrations during chronic LHRH-agonist treatment in a patient with chemical castration (group A).

objective responders during chronic Buserelin treatment two more patients showed a partial response during the combination therapy (one had stable disease and one progression during Buserelin treatment). So, in total, 6 of the 12 patients of group A reached ultimately an objective response.

In the 5 patients (group B), treated with the combination therapy from the start of treatment, we observed two objective remissions, one stable disease and two failures (Table 2).

The endocrine response during the combination therapy was strongly variable. Addition of tamoxifen to Buserelin treatment can cause disappearance of oestradiol peaks, but also recurrence of ovarian progesterone secretion (Table 3, Fig. 2). In one case hyperstimulation of the ovaries was observed (without progression of tumour growth).

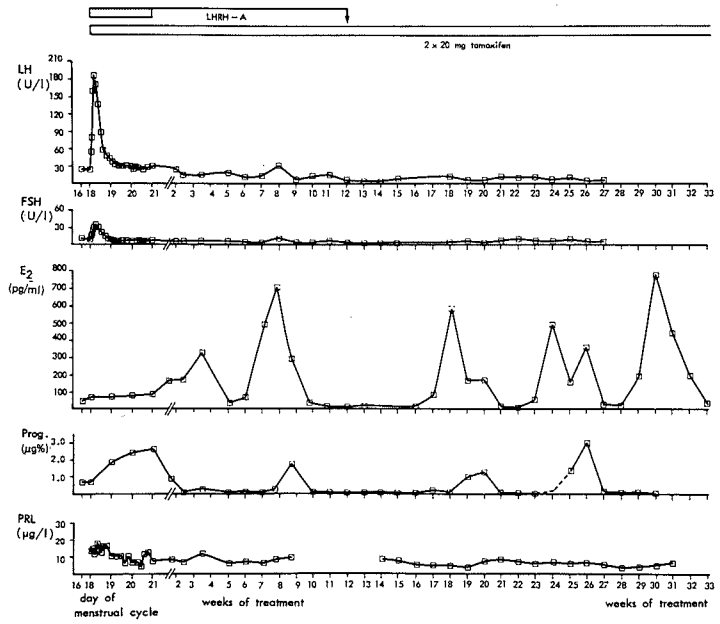

Fig. 2. Changes of plasma hormone concentrations during combination therapy with Buserelin and tamoxifen in a patient of group B.

3. *Combination therapy with Buserelin and megestrol acetate*

Recently we treated 4 patients with this drug combination from the start of treatment. All patients showed a complete castration effect with continuously postmenopausal values for plasma oestradiol (Fig. 3). With regard to tumour response we observed one objective remission, two times stable disease and one progression.

Relationship with receptor status

In only 6 of the 17 patients of group A + B was the receptor status of the primary tumour known (Table 4), so it is very difficult to make conclusions. Four patients with an ER-positive tumour showed 3X an objective response and 1X stable disease during combination treatment, while 1 of the 2 patients with an ER-negative tumour showed a partial remission during Buserelin treatment and the other progression.

DISCUSSION

In this study chronic single treatment with the LHRH-agonist Buserelin has been proven to be effective in one-third (4/12) of unselected premenopausal patients with metastatic breast cancer without causing side effects. After addition of tamoxifen an objective response has been ultimately observed in about half of the patients (8/17). Thus, the response rate of tumour growth inhibition appears as good as other types of ablative or additive endocrine therapy. Although direct antisteroidal actions of LHRH-agonists[9–11] are described and prolactin secretion decreased,[4–7] the main mechanism of anti-tumour action seemed to be a 'chemical castration'.[4, 18] Also in our study the patients with a castration effect showed the best responses. So, another advantage of LHRH-agonist treatment together with the absence of side effects might be the possibility for the future to select patients, who will respond to oöphorectomy.

However, the problem remains the great variation in hormonal response during treatment with Buserelin alone (as well as in combination with tamoxifen), which is in contrast with the findings in rats[1–8] and with the relatively uniform endocrine response in patients treated with LHRH-analogues for prostate carcinoma.[19–21] In our patients with breast cancer unpredictable recurrence of plasma peaks of oestradiol was found in 60% of the

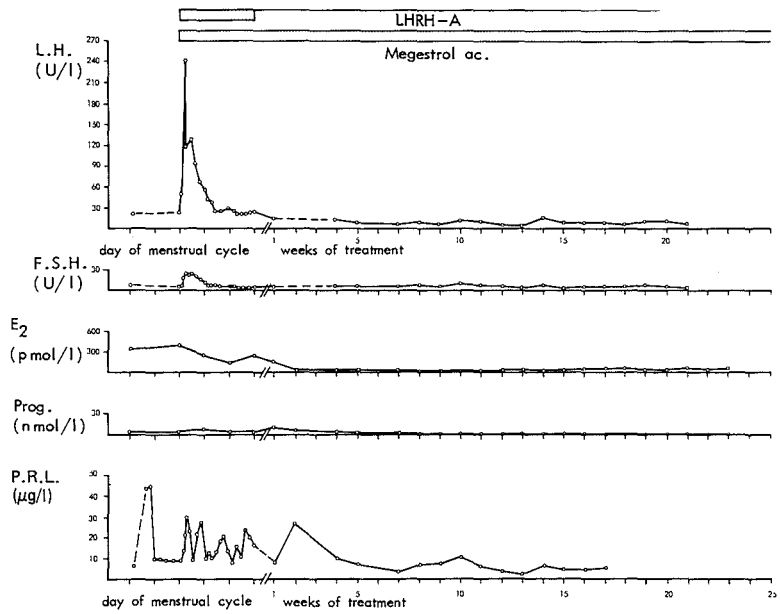

Fig. 3. Changes of plasma hormone concentrations during combination therapy with Buserelin and megestrol acetate.

Table 4. Relationship with receptor status in 17 patients treated with Buserelin alone and in combination with tamoxifen

E_2-receptor		Treatment	
		LHRH-A alone (group A) $n = 12$	LHRH-A + TAM (group A + B) $n = 14$
ER +	CR + PR	–	3/4
	NC	1/2	1/4
	F	1/2	–
ER –	CR + PR	1/2	–
	NC	–	–
	F	1/2	2/2
ER unknown	CR + PR	3/8	1/8
	NC	3/8	2/8
	F	2/8	5/8

patients. The reason for this variation in endocrine response is unclear and has to be resolved. Maybe even higher dosages of Buserelin are necessary to reach a castration effect in all patients.

During treatment with the combination with tamoxifen an (in)sufficient corpus luteum function was found in half of the patients (7/14, Table 3) as proven by postovulatory plasma progesterone levels. This indicates that tamoxifen may overcome the suppressive effect on hypothalamo-pituitary function by chronic LHRH-agonist treatment. Therefore we combined Buserelin with high-dose megestrol acetate treatment because of its antigonadotropic properties. From the endocrine point of view this combination appeared more suitable than the combination of Buserelin with tamoxifen. However, not all patients responded with tumour growth

inhibition, and side effects as body weight increase with a cushingoid face occurred after about 3 months of treatment.

In conclusion, chronic intranasal Buserelin treatment was shown to be effective in premenopausal women with metastatic breast cancer without causing side effects. Further clinical investigations are necessary to find the most suitable dose and drug combination.

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APPENDIX PAPER 3.

Breast Cancer Research and Treatment, 4, 209–220 (1984)

Report

Anti-tumor and endocrine effects of chronic LHRH agonist treatment (Buserelin) with or without tamoxifen in premenopausal metastatic breast cancer

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Keywords: breast cancer, Buserelin, LHRH agonist, premenopausal women, tamoxifen

Summary

Seventeen premenopausal women with metastatic breast cancer were treated with the potent Luteinizing Hormone Releasing Hormone (LHRH) agonist Buserelin as a first-line agent. Twelve patients (group A) were treated with Buserelin alone and five patients (group B) with the combination of Buserelin and tamoxifen from the start of treatment. In nine patients of group A tamoxifen was added to Buserelin later on because of tumor progression or recurrent peaks of plasma estradiol (E_2). Chronic intranasal therapy with Buserelin alone, preceeded by parenteral administration, caused an objective remission in four patients ($2 \times$ C.R., $2 \times$ P.R.) and stable disease in four further patients without causing side effects. The longest duration of response until now is more than 29 months. After addition of tamoxifen a partial response occurred in two more patients of group A. Anovulation with suppressed progesterone secretion was reached in all patients treated with Buserelin alone, but transient peaks of E_2 occurred in the majority (60%) of the patients. Addition of tamoxifen to Buserelin treatment caused disappearance of E_2 peaks in 2 patients, but also reappearance of progesterone secretion with recurring E_2 peaks in 3 other patients; in one case hyperstimulation of the ovaries was observed without progression of tumor growth. In group B only one woman showed a complete castration effect, while in four patients progesterone secretion was not (completely) suppressed. In two of these five patients an objective response occurred. In conclusion, Buserelin appears effective in the treatment of premenopausal women with metastatic breast carcinoma, but with the regimen used close control of endocrine parameters is necessary because of the variation in hormonal response with a risk of (hyper)stimulation of the ovaries, especially during combination therapy with tamoxifen.

Introduction

In animals chronic treatment with supraphysiological doses of LHRH agonists causes: 1) 'partial hypophysectomy' with decreased gonadotropin (1, 2) and prolactin secretion (2–5); 2) 'chemical castration' with a striking fall in plasma sex steroids followed by a reduction in weight of secondary sexual organs (1–6); and 3) inhibition of the biological actions of sex steroids at their target organs (7–9). At least some of these endocrine effects have been suggested to be important in the treatment of

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hormone-dependent tumors such as breast (3, 5, 10–12) and prostatic cancer (12, 13) induced in animals.

Recently the preliminary results of treatment with LHRH agonists in patients with advanced breast (14, 15) and prostatic cancer (16–19) have been reported. The aim of the present study was the evaluation of long-term effects of single treatment with the LHRH agonist Buserelin (Hoe 766; 6-D-Ser-(bu^t)-LHRH-(1-9)-ethylamide) on tumor growth and hormone secretion in premenopausal patients with metastatic breast cancer treated during a period of 3–20 months after our 'Preliminary Communication' (15) on 2 months follow-up. In addition, the effect of a combination therapy of the LHRH agonist with tamoxifen was investigated, especially with respect to the endocrine response. It was not our intention to compare the anti-tumor efficacy of the two treatments.

Patients, methods and treatment

Patients

Seventeen consecutive menstruating women (unselected for receptor status; mean age 40 yr, range 28–53 yr) with previously untreated metastatic breast carcinoma gave informed consent for this new form of hormonal treatment. The mean disease-free interval between treatment of primary and secondary breast cancer was 2.5 years (range 0.5–5.0 yr). The receptor status of the primary tumor was known in 6 patients (Table 1). Various kinds of metastases occurred in this group of patients (Table 1); patients with clear liver metastasis were excluded from this study.

Before treatment all patients were examined radiologically by total skeletal survey, bone-scan, and scintigraphy or CT-scan of the liver. Physical and routine laboratory examinations (hematology and chemistry) were performed. The patients were

Table 1. Pretreatment data and outline of the treatments in 17 premenopausal patients with metastatic breast cancer.

Patient	Age (yr)	Parenteral LHRH agonist treatment			Dose of chronic i.n. treatment	Menstrual cycle day at start of treatment	Localization of metastasis	ER (fmol/mg)	PR
		daily dose	by	days					
Group A									
1	44	3 mg	8 hr inf.	7	3 × 400 μg	11	B		
2	41	3 mg	8 hr inf.	7	3 × 400 μg	11	B, Ln		
3	48	3 mg	8 hr inf.	7	3 × 400 μg	16	B, Ln, S	20	80
4	44	3 mg	24 hr inf.	3	3 × 400 μg	12	P		-
5	44	3 mg	24 hr inf.	5	3 × 400 μg	24	L		
6	43	3 mg	24 hr inf.	7	3 × 400 μg	12	Lu, S		
7	40	3 mg	3 × 1 s.c.	7	3 × 400 μg	26	S		
8	35	3 mg	3 × 1 s.c.	7	3 × 400 μg	15	B, P, Lu, Li, Ln		
9	35	3 mg	3 × 1 s.c.	7	3 × 400 μg	25	Ln, (B?), thyroid	0	0
10	44	3 mg	3 × 1 s.c.	7	3 × 400 μg	postovulatory	S, (Ln?)		
11	35	3 mg	3 × 1 s.c.	7	3 × 400 μg	9	Ln, P	0	0
12	35	3 mg	3 × 1 s.c.	7	3 × 400 μg	21	B, P, Ln	23	-
Group B									
13	53	3 mg	24 hr inf.	3*	3 × 400 μg*	16	Ln, S	25	0
14	28	3 mg	24 hr inf.	3*	3 × 400 μg*	18	B		
15	30	3 mg	24 hr inf.	3*	3 × 400 μg*	9	B	40	-
16	41	3 mg	24 hr inf.	3*	3 × 400 μg*	15	B		
17	37	3 mg	24 hr inf.	3*	3 × 400 μg*	6	P, Lu, Ln		

B = bone; P = pleura; Lu = lung; Li = liver; S = skin; Ln = lymph node; inf. = infusion; s.c. = subcutaneously; i.n. = intranasal; * = plus 2 × 20 mg tamoxifen daily.

seen every three weeks after the start of treatment for recording of complaints, physical examinations, and routine laboratory investigations. Bone or lung metastases were evaluated every 3–6 weeks during the first half year of treatment (later on at least every 3 months in case of long-term regression or stable disease). Total skeletal survey and bone-scan were repeated at least once every half year; echosound or/and CT-scan or/and scintigraphy of the liver were repeated if indicated. Measurements of tumor response were performed according to UICC criteria.

Treatment

Twelve patients (group A) were treated with Buserelin as a first-line single agent; in 9 of these patients tamoxifen 20 mg twice daily was added later on because of tumor progression or recurrent peaks of plasma estradiol. A second group (group B) of 5 patients was treated with the combination of Buserelin with tamoxifen from the start of treatment.

After parenteral (intravenous or subcutaneous) administration of 3 mg Buserelin per day for 3–7 days, the therapy was changed to three doses of 400 μg Buserelin per day intranasally (Table 1). In eight patients of group A and in three of group B the treatment was started during the luteal phase.

Hormone and receptor measurements

During the parenteral treatment period, blood samples for the measurement of luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin (PRL) were taken frequently (6–9 times per day). Plasma estradiol-17β (E_2) and progesterone were measured daily during this period. During intranasal therapy blood samples were taken weekly for measurement of LH, FSH, PRL, E_2, and progesterone. Plasma concentrations of these hormones were measured by radioimmunoassay as described previously (15, 20). The normal range for basal LH is 3.5–12.6 IU/l, for basal FSH 1.2–4.2 IU/l, and for basal PRL up to 15 μg/l. Luteal phase plasma progesterone concentrations exceed 3 nmol/l while follicular phase E_2 levels are larger than 150 pmol/l. Steroid receptors were assayed in the primary tumors by the method recommended by the EORTC Breast Cancer Cooperative Group (21).

Significances of differences between mean values at various time points within treatment groups were assessed by Student's paired *t*-test.

Results

The individual endocrine effects concerning the presence or absence of chemical castration and effects on tumor growth are listed in Table 2. Typical examples of endocrine responses to treatment with these doses of Buserelin are shown in Figs. 1A–D.

Endocrine effects

Gonadotropins

Group A. On the first day of treatment, peak values for plasma LH (186.7 ± 20.3 IU/l; mean ± SEM) and FSH (31.8 ± 5.0 IU/l) were reached about 4–6 hours after Buserelin administration. Within one day LH and FSH dropped rapidly (Fig. 2). Mean plasma LH concentrations decreased below pretreatment levels after 2–3 weeks and continued to decrease slowly. Mean plasma FSH concentrations fell to the pretreatment level within 3 days, decreased below the pretreatment level during the first week, and remained thereafter at the same level (2.0–3.0 IU/l) during more than one year (Fig. 2). No ovulatory peaks were observed. Subnormal levels occurred rarely.

After addition of tamoxifen in 9 patients, later on during treatment, mean plasma LH and FSH did not change significantly. Mean plasma LH did tend to increase 6–7 weeks after the addition of tamoxifen, but this might be caused by incidental peaks of LH and FSH in two of the patients. In patient 5 a clear increase of plasma LH and FSH was observed after discontinuation of hormone therapy (Fig. 1C), maybe caused by the following chemotherapy.

Group B. The mean plasma LH and FSH concen-

Table 2. Summary of endocrine and anti-tumor effects of chronic Buserelin treatment with or without tamoxifen.

	Effects of Buserelin treatment only					Effects of Buserelin + tamoxifen				
Patient	Chemical castration	E_2 peaks	Prog. 'peaks' (>3.0 nmol/l)	Tumor response	Duration of response (months)	Chemical castration	E_2 peaks	Prog. 'peaks' (>3.0 nmol/l)	Tumor response	Duration of response (months)
Group A										
1	–	+	–	N.C.	4.5	–	+	+	P?	–
2	+	–	–	C.R.	20+					
3	–	+	–	N.C.	3	+	–	–	P.R.	10
4	+	–	–	C.R.	19+					
5	–	+	–	P	–	+	–	–	P	–
6	–	+	–	P.R.	3	–	+	–	N.C.	2
7	–	+	–	N.C.	3+	–	+	+	N.C.	1+
8	+	–	–	N.C.	3	+	–	–	P	–
9	+	–	–	P.R.	5	+	–	–	P	–
10	–	+	–	P	–					
11	?	?	–	P	–	+	–	–	P	–
12	?	?	–	P	–	–	+	+	P.R.	4
Group B										
13						–	+	+	P.R.	5+
14						–	+	+	C.R.	3+
15						+	–	–	N.C.	5+
16						–	+	+	P	–
17						–	+	+	P	–

C.R. = complete remission; P.R. = partial remission (decrease of more than 50% of size of tumor lesions); N.C. = no change; P = progression; Prog = (cyclic) ovarian progesterone secretion; E_2 = estradiol-17β.

tration showed the same curve during combination therapy as during long-term treatment with the LHRH agonist alone (Figs. 3A and 3B). After two weeks of treatment there was a tendency to somewhat higher levels in group B, but a significant difference was found only at 5 weeks for LH ($p<0.01$).

During the combination therapy with Buserelin and tamoxifen we observed 5 times (3× in group A, 2× in group B) a plasma LH peak between 27 and 44 IU/l. In 3 cases (patients 1, 7 and 14) such a peak was followed by a clear increase of plasma progesterone, indicating corpus luteum function. Other patients showed increased progesterone secretion though peaks of secretion of LH and FSH were not detected (Table 2).

Sex steroids

Group A. In the first weeks of treatment no increase of plasma progesterone concentrations was detected in patient 1 (perimenopausal) and patient 12 (one month hospitalization and treatment with morphine and metoclopramide causing hyperprolactinemia before Buserelin therapy). Nine patients finished their ongoing cycle after normal duration of the luteal phase with normal plasma progesterone levels. Only in patient 4 (Fig. 1B) there was an indication of corpus luteum insufficiency.

During chronic therapy with $3 \times 400\,\mu g$ Buserelin intranasally, anovulation occurred in all patients as indicated by persisting low progesterone levels (Table 2). In four of the 10 patients who were treated with Buserelin alone for a period long enough for evaluation of the effect on ovarian function, a complete 'chemical castration' was found with persisting low plasma estradiol levels between <20 and 75 pmol/l during follow-up periods of 3 to

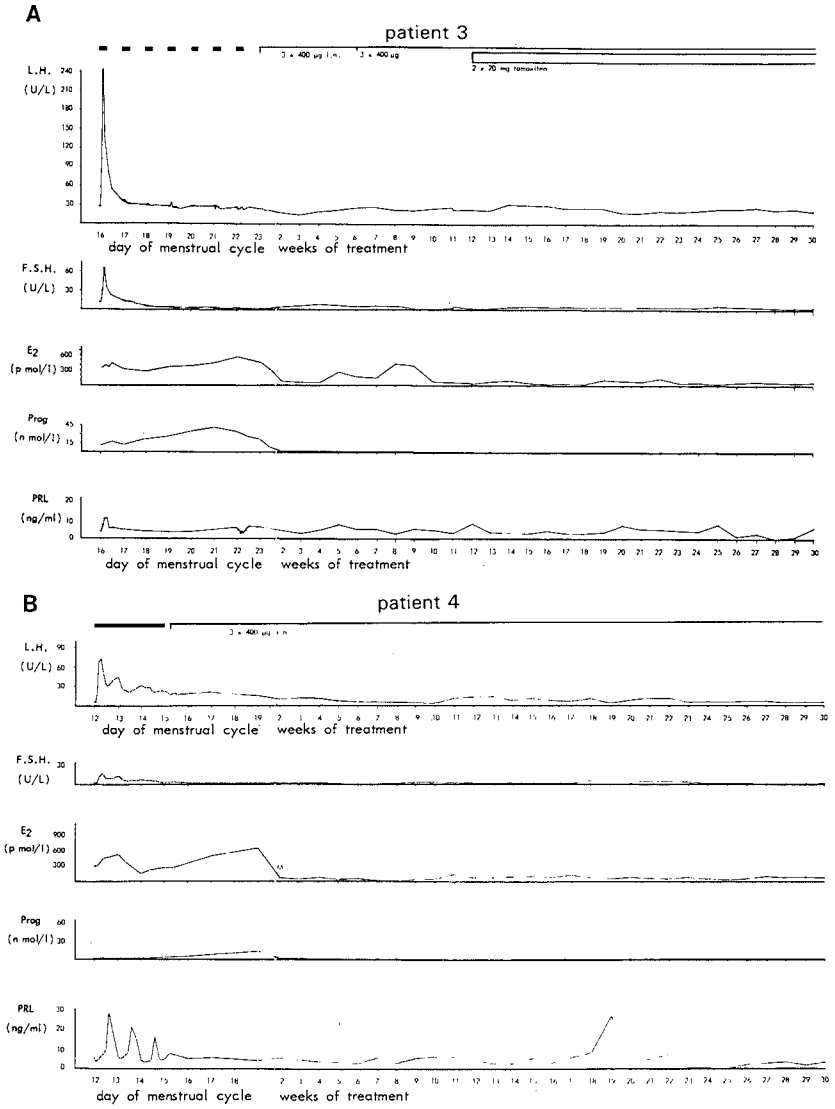
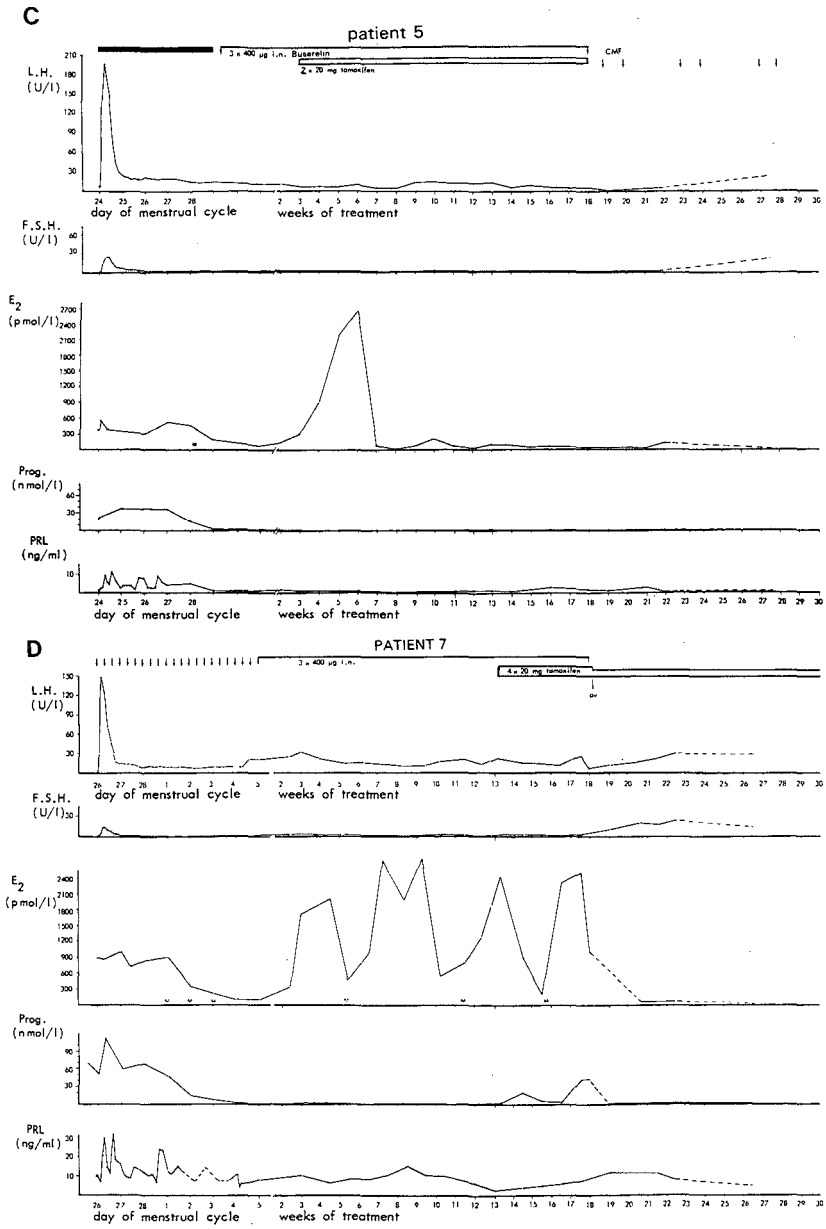


Fig. 1A–D. Effects of long-term Buserelin treatment without or with tamoxifen on plasma LH, FSH, estradiol-17β (E_2), progesterone (Prog), and prolactin (PRL) in patients 3 (A), 4 (B), 5 (C), and 7 (D). M = menstruation; ov = ovariectomy; ■■ = intravenous Buserelin treatment; ↓ ↓ ↓ ↓ ↓ = subcutaneous injections with Buserelin; ↓‾↓ = period of intranasal Buserelin treatment; □□ = period of oral treatment with tamoxifen.


C
patient 5
3 x 400 µg i.n. Buserelin
2 x 20 mg tamoxifen
CMF
L.H. (U/l)
day of menstrual cycle
weeks of treatment
F.S.H. (U/l)
E2 (pmol/l)
Prog. (nmol/l)
PRL (ng/ml)
day of menstrual cycle
weeks of treatment
D
PATIENT 7
3 x 400 µg i.n.
4 x 20 mg tamoxifen
L.H. (U/l)
day of menstrual cycle
weeks of treatment
F.S.H. (U/l)
E2 (pmol/l)
Prog. (nmol/l)
PRL (ng/ml)
day of menstrual cycle
weeks of treatment

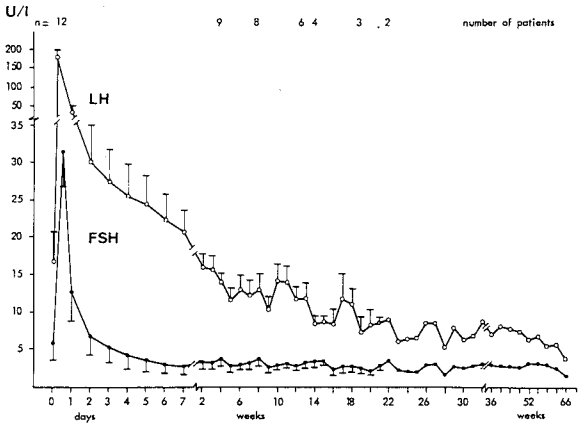


Fig. 2. Acute and chronic effects of long-term treatment with Buserelin alone on plasma LH (○——○) and FSH (●——●) concentrations in 12 patients (group A).

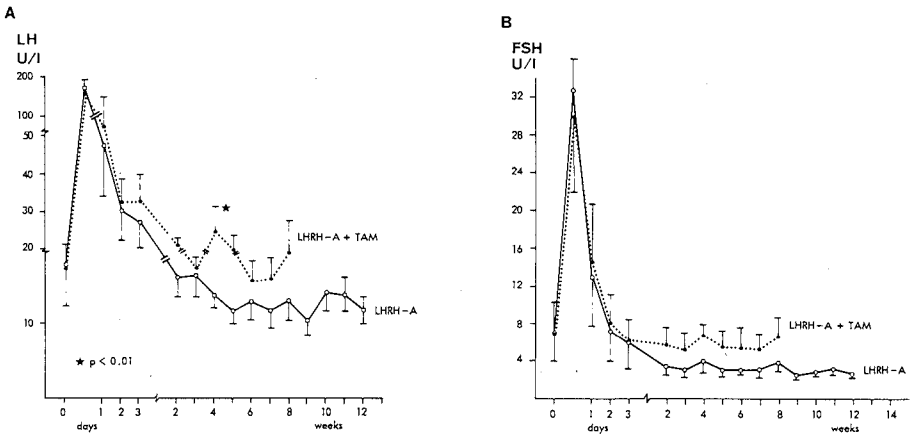


Fig. 3. Acute and chronic effects of long-term treatment with Buserelin alone (○——○) on plasma LH (Fig. 3A) and FSH (Fig. 3B) concentrations compared with those during chronic treatment with the combination of Buserelin and tamoxifen (●.....●) in 8 and 4 patients respectively, all of them treated for at least 12 and 8 weeks respectively.

20 months (for example patient 4, Fig. 1B). However, the remaining six patients showed transient peaks of plasma estradiol without increases in plasma LH, FSH, or progesterone (Table 2).

Hyperstimulation of the ovaries occurred in one patient (patient 7, Fig. 1D). One week after addition of a high dose of tamoxifen (80 mg daily) ovulatory progesterone secretion preceded by a small LH peak returned in this patient. Although the histologically proven skin metastases showed no change either before or after addition of tamoxifen to Buserelin treatment, we decided to perform

surgical castration. At operation both ovaries showed multiple follicles with a ruptured follicle containing blood in one ovary. Tumor size showed no change before or during more than 9 months after the ovariectomy.

In 9 patients tamoxifen was later added to Buserelin treatment because tumor progression or plasma estradiol peaks occurred. Four patients (Table 2) still showed estradiol peaks during the combination therapy, and in 3 of these 4 patients even ovarian progesterone secretion returned at 1, 4 and 7 weeks after the addition of tamoxifen (see also Fig. 1D). On the other hand, the estradiol peaks which occurred during treatment with Buserelin alone were no longer observed after addition of tamoxifen in patient 3 and patient 5 (Fig. 1A and 1C).

In summary, the endocrine response to Buserelin alone (with the doses used), as well as in combination with tamoxifen, is very variable. Ultimately 'chemical castration' was reached in 7 patients (58%). In 3 (maybe 4) of 6 patients consecutively treated with combined chemotherapy (CMF), plasma estradiol and progesterone remained low after discontinuation of hormonal therapy (with exception of continuing tamoxifen in patient 10 and 12).

Group B. In three patients (13, 15 and 17) we observed a clear corpus luteum insufficiency with short-term and decreased progesterone secretion, while two patients (14 and 16) showed normal corpus luteum function. Ovulation occurred about 1 and 3 weeks later than normal in patients 15 and 17 during the ongoing cycle at the start of treatment. After the end of the ongoing cycle only patient 15 showed a complete castration effect subsequently, while in the other four patients estradiol peaks occurred in the presence of progesterone secretion (Table 2); low luteal progesterone secretion was observed in 3 of these patients.

Prolactin secretion

In general, during long-term treatment there were no impressive changes in plasma prolactin concentrations (Fig. 1A–D). Detailed data during parenteral and intranasal therapy will be published separately.

Effects on tumor growth

Single treatment

Buserelin alone appeared effective in four patients (two complete and two partial remissions) and caused stable disease in four more, while progression occurred in the remaining four patients (Table 2). The longest remission until now is more than 20 months. This patient had a pleural effusion histologically proven to be carcinomatous. The effusion disappeared in 6 weeks without intrapleural cytostatic therapy or discharge of the pleural effusion. Her complaints of coughing ceased and she is still normally functioning as housewife without any complaints or side effects of the treatment except a few flushes per day. In three of the four patients in whom a 'chemical castration' was attained, we observed an objective response, while in the fourth patient (patient 8 with stable disease) temporary improvement occurred with less complaints. The fourth objective response occurred in patient 6 in spite of recurrent peaks of estradiol.

Combination therapy with tamoxifen

Group A. After addition of tamoxifen to the treatment with Buserelin, two more patients (patient 3 and 12) showed an objective response. In patient 3 extensive diffuse skin metastases disappeared completely, while bone pain disappeared and plasma alkaline phosphatase decreased. However, after 10 months a pleural effusion developed, in which tumor cells were found. She showed again an objective response during treatment with megestrol acetate, indicating hormone sensitivity. Although in patient 5 (Fig. 1C) a complete chemical castration was reached after addition of tamoxifen, further progression of lung metastases occurred indicating the possibility of a hormone-independent tumor. These lung metastases disappeared after chemotherapy, but they recurred after seven months.

Group B. Two patients showed an objective response, one stable, and two progressing disease (Table 2). Patient 13 had a partial regression of skin and lymph node metastases; patient 14 showed recalcification of osteolytic bone lesions although chemical castration was not obtained.

Relationship with receptor status

It appears very difficult to make conclusions because receptor measurements were performed in only 6 tumors. One of the 2 patients with a receptor-negative tumor showed a partial remission for 5 months during treatment with Buserelin alone (patient 9, Table 2).

Side effects

Hot flushes and decreased libido occurred in the patients with 'complete chemical castration'. Hot flushes occurred also in other patients between peaks of estradiol. In 3 of 6 patients who were treated in the first week with subcutaneous injections of Buserelin, urticarial skin irritation occurred a few minutes after injection at the injection site lasting 1–2 hours. Antibodies against Buserelin could not be identified in plasma obtained between a few weeks and a few months after the start of treatment (estimations performed by the Department of Pharmacology Hoechst, Frankfurt). Other side effects such as cardiovascular complications were not observed.

Discussion

In animal studies, LHRH analogues have been shown to act directly and indirectly at the levels of the pituitary (1, 7, 22, 23), the gonads (1, 7, 22–31), and target organs of sex steroids (7–9). In this study the response of metastatic breast carcinoma to Buserelin alone or in combination with tamoxifen was investigated. The effect of Buserelin alone might be ascribed to its castrating and possibly intrinsic anti-estrogenic effect, while by the combination therapy the castration effect of Buserelin might be enhanced by inhibition of the effect of the already low plasma estradiol concentration on the tumor by tamoxifen. Chronic intranasal Buserelin therapy was shown to be effective in one third (4/12) of premenopausal women with metastatic breast cancer without causing side effects (except hot flushes or decreased libido). In total, in 8 of 17 patients an objective response was observed after treatment with Buserelin, alone or in combination with tamoxifen. One patient (no. 9) with an estrogen receptor (ER)-negative tumor showed an objective response. Similar observations were made in rats with ER-negative mammary tumors (11), although in the latter system the effect might be mediated through decreased prolactin levels.

The main problem to be resolved remains the great variation in hormonal response to treatment with Buserelin alone as well as in combination with tamoxifen. The hormonal response varied from a complete castration effect to ovarian hyperstimulation. Treatment with tamoxifen alone has been found also to cause a great variation in hormonal response (32–34). Plasma E_2 concentrations varied between postmenopausal levels and a three-fold increase as compared to normal menstrual cycles. In contrast to treatment with tamoxifen, treatment with the LHRH agonist alone prevents the development of corpora lutea (as indicated by the absence of progesterone secretion in all patients) probably by suppression of the positive feedback of estradiol on the preovulatory LH and FSH peak. With regard to the effects of tamoxifen on gonadotropin secretion, the reported results are conflicting. Sherman et al. (34) found no significant increase of plasma gonadotropin levels during two cycles, but Manni and Pearson (32) observed an increase of plasma gonadotropin levels during longer treatment. In our study, long-term treatment with Buserelin alone caused suppression of gonadotropin secretion to about 40–50% of the pretreatment value, while addition of tamoxifen did not increase the plasma concentrations significantly. However, during the combination therapy we sometimes observed peaks of LH and FSH, followed in some patients by ovarian progesterone secretion, so that the stimulatory effect of tamoxifen on the pituitary gonadal axis (32) may overcome the suppressive effect of the doses of LHRH agonist used. A point of concern is the potential fertility of patients on this combined endocrine treatment; addition of tamoxifen may annihilate the advantage of contraception caused by single treatment with Buserelin (35–37), unless the endometrial changes caused by long-term tamoxifen treatment (34) prevent gravidity.

Ovarian 'hyperstimulation' was not reported to

occur during treatment with tamoxifen alone (in contrast to treatment with clomid (33)), but in our study the combined tamoxifen and LHRH agonist treatment caused clear ovarian 'hyperstimulation' with enlarged ovaries containing multiple follicles in one of our patients (patient 7). Nevertheless, tamoxifen appeared able to cause an objective remission of the tumor in spite of recurring peaks of estradiol as observed in patients 12, 13 and 14, which observations confirm the findings of Manni and Pearson (32).

Intriguing is the phenomenon that in two patients estradiol peaks disappeared after addition of tamoxifen without a clear change in basal gonadotropin levels. This might be caused by a direct effect of tamoxifen on the ovary via ovarian estradiol receptors, which have been demonstrated in normal ovaries (38).

In order to find the most effective way to start treatment we have used several schemes of parenteral therapy in subgroups of patients (Table 1). We did not find a clear difference in endocrine response between the subgroups. After an initial peak of plasma gonadotropins we observed an absent or reduced pituitary responsiveness (desensitization) during chronic treatment with the LHRH agonist Buserelin, which might be explained by a decrease or abnormal breakdown of pituitary LHRH receptors (22). In contrast with the complete suppression of ovarian estradiol secretion in rats (5), an unpredictable recurrence of plasma peaks of estradiol was found in the majority (60%) of our patients during chronic treatment with a moderately high dose of 1200 μg Buserelin intranasally. Nevertheless, ovulation did not occur in these patients, as indicated by the absence of progesterone secretion. The recurrence of estradiol secretion was also found in about 75–85% of the women in studies where the efficacy of lower doses of LHRH analogues was investigated with respect to contraception (35–37). In those studies progesterone secretion appeared not completely suppressed in all women (10–15% of treatment cycles) during chronic treatment with lower intranasal doses of 400–600 μg per day. Suppression of gonadal function may have been caused both indirectly via the initial LH burst with subsequent down-regulation of gonadal receptors and interference with enzymes involved in steroid biosynthesis, as well as by a direct effect on the ovary (1, 7, 22–31). In animals, LHRH binding proteins are found in ovarian tissue (25, 29, 30), while LHRH analogues also appear to have a direct in vitro effect on granulosa cells in the presence of gonadotropins (28, 31). However, in women a direct effect on the gonads is uncertain (39–41). The possible absence of a direct effect of LHRH and its analogues on the human ovary could be a cause for the difference in gonadal response between rats and women. Another cause might be the change of the very high parenteral dose of 3 mg per day after the first week to a relatively low dose of about 25 μg resorbed intranasally (about 2% resorption of the total intranasal dose of 1200 μg). This dose might be at the lower limit of the effective range, causing complete castration in only some of the women. Possibly higher doses are necessary for complete inhibition of ovarian function in all patients. Indeed, preliminary data of an ongoing study about the effects of very high subcutaneous doses of LHRH agonist indicate that complete chemical castration can be reached in all patients with sufficiently high doses (J.G.M. Klijn et al., unpublished data).

Recently some authors found that LHRH analogues inhibit the action of sex steroids on their target organs (7–9); these observations are in conflict with those of Furr and Nicholson in castrated immature rats (5). An indication for a direct temporary antitumor action of LHRH analogues was found by Corbin (12), who showed a direct dose-related effect on the growth of mouse mammary tumor cells in vitro after 4 days of treatment. Also we found a weak anti-estrogenic effect of Buserelin on MCF-7 cells (42). Further, the finding of an objective response after 10 weeks in 16% of postmenopausal women (14) treated chronically with very high doses of a LHRH analogue after prior other therapy, may be an argument for a direct antitumor effect, because ovariectomy is not effective in postmenopausal patients. In our clinical study we found no clear indication for a direct anti-tumor effect of the Buserelin dose used, because the best responders had a complete castration indicating that 'pharmacological castration' may be the main

mechanism of action in inhibiting tumor growth. However, one patient (6) showed an objective partial response in spite of the presence of peaks of estradiol.

In conclusion, Buserelin was shown to be effective in the treatment of premenopausal women with metastatic breast cancer, but with the doses used the hormonal response appeared not uniform as found in patients with prostatic carcinoma. It is not possible to predict the type of hormonal response for an individual patient. So, although the response rate of tumor growth inhibition appears as good as that of other types of endocrine therapy (for instance ovariectomy) in the absence of side effects, further investigations with other dose regimens and modes of application or combinations with other drugs are necessary to show that LHRH agonists will be valuable in treating patients with breast cancer.

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In addition

At the time of revision of the manuscript, patient 4 was still in remission after more than 29 months of treatment, while patient 2 showed tumor progression after 24 months of treatment. Patient 15 had a change from stable disease to partial remission and has already been treated for more than 18 months.

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APPENDIX PAPER 4.

LHRH-agonist Treatment in Metastatic Prostate Carcinoma

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Abstract—*Three patients with metastatic prostatic cancer were treated for* 10, 6 *and* 2 months *with the potent LHRH-agonist Buserelin (Hoe* 766*) as a first-line agent. All showed a fall of elevated prostatic acid phosphatase levels (nearly undetectable after treatment in* 2 *patients) parallel to plasma testosterone with a relief of complaints after* 3–4 weeks *of treatment. Two patients had an increment of appetite and body weight. In one patient radiological evidence for objective tumour regression was found by CT scan of the prostate (decrease of* 41% *in prostate volume), skeletal X-rays and bone scan. In this patient plasma alkaline phosphatase showed a transient increase parallel to disappearance of osteolytic bone lesions (indicating new bone formation) followed by a normalization. It is concluded that LHRH-agonist treatment is effective in patients with metastatic prostatic carcinoma in the absence of serious side-effects.*

INTRODUCTION

LUTEINIZING - hormone - releasing - hormone (LHRH) is a hypothalamic decapeptide that induces release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary. These gonadotropins stimulate gonadal steroid production. However, LHRH has a short-lived effect on gonadotrophin secretion. It is therefore not suitable for long-term therapy. To date, many LHRH analogues with marked and prolonged effect have been synthesized and tested [1–4]. A lot of them appeared to have a paradoxical antifertility effect in male and female rats during chronic treatment with pharmacological doses. Effects observed were at the level of the pituitary [1–7], gonads [8, 9] and the target organs of sex steroids [10–12].

Firstly, in the pituitary such long-term treatment with large doses of LHRH analogues causes exhaustion and desensitisation of the gonadotrophic cells [1–4] and further inhibition of prolactin secretion [5–7]. Secondly, after a short-term increase of gonadotrophin secretion down-regulation of gonadal gonadotrophin receptors with decreased steroidogenesis occurred [2, 4, 13, 14]. Further, a direct extrapituitary effect of LHRH at the gonad has also been demonstrated [8, 9]. Clayton *et al.* found a direct inhibition of testicular function by LHRH receptors in interstitial cells [9]. Locally produced LHRH-like peptides in the testis appear to have a regulating function for the testosterone secretions [15, 16]. A third possible important way of action is the recently reported ability of LHRH analogues to antagonize the biological actions of sex steroids [10–12]. The antiandrogenic action of LHRH-agonists appears to be different from cyproterone acetate [11]. Sundaram *et al.* [10] demonstrated that these peptides could block the growth-promoting effect of testosterone on the rat prostate and seminal vesicles. An *in vitro* study suggests that the antiandrogenic activity of LHRH analogues is not due to its ability to compete with androgens for their intracellular receptors [11]. On the other hand, Furr and Nicholson have been unable to show anti-androgenic effects of two analogues in castrated immature rats [17].

The striking fall in plasma sex steroid levels to post-castration values and reduction in weight of secondary sexual organs during chronic treatment with LHRH-analogues [2, 4, 7, 18, 19]

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suggested a treatment for hormone-dependent tumours [4, 17]. In animals a regression of prostatic [18], mammary [4, 5, 17, 20], pituitary [7] and cartilage tumours [21] (chondrosarcoma) has been shown.

Recently, the first data of LHRH-agonist treatment in small series of patients with prostate [22–28] and breast cancer [29, 30] have been published. After our reports about the results in patients with breast cancer [29, 30], we present in this study detailed data about the effects of long-term LHRH-agonist treatment in three patients with prostate cancer.

MATERIALS AND METHODS

Three patients with metastatic prostate carcinoma gave informed consent to be treated with an LHRH-agonist (Buserelin = Hoe 766). Their relevant data are summarized in Table 1. Patient 3 had received primary radiotherapy and total body irradiation (6 Gy) 3 yr before the occurrence of metastasis, but the patients had undergone no further treatment for either primary or metastatic disease.

They were treated with 3 × 0.5 mg Buserelin subcutaneously for 6 days followed by 3 × 400 μg daily per intranasal spray. In the first week blood samples were taken frequently (Figs 1 and 2) for measurement of LH, FSH, prolactin (PRL), testosterone (T), oestradiol (E_2), alkaline phosphatase, acid phosphatase and specific prostatic acid phosphatase (PAP). Blood sampling has been performed daily before the morning treatment injection, and for LH, FSH and PRL on some days (2–5) 4 hr after the injections. During the follow-up (10, 6 and 2 months respectively) they were measured weekly during the first months, later every 3 weeks. Plasma hormone concentrations were measured by radioimmunoassay as described previously [29, 31]. Alkaline phosphatase was assayed in glycine–NaOH buffer of pH 9.6 at 37°C (normal values 15–38 U/l) and total acid phosphatase in acetate buffer of pH 5.5 at 37°C (normal up to 12.0 U/l). PAP was determined using a solid-phase enzyme immunoassay [32] (normal up to 1.6 U/l).

Before treatment all patients were examined radiologically by total skeletal survey, bone scan and abdominal CAT scan apart from physical and routine laboratory examinations. They were seen every 3 weeks after the start of treatment for physical examinations and recording of complaints. Local bone lesions were evaluated every 6–12 weeks by X-rays and by skeletal survey after 6 months. CT scan of the prostate was repeated after 3 and 6 months.

RESULTS

Endocrine effects

After stimulation of gonadotrophin secretion on the first treatment day plasma LH and FSH levels decreased gradually to below pretreatment values with a plateau level after about 5–8 weeks. After the second treatment day there was no increase of the gonadotrophins in the plasma 4 hr after the subcutaneous Buserelin injections, indicating desensitisation of the pituitary.

Plasma testosterone showed a slight increment in the first week (with peak levels of 158, 148 and 122% of the basal value after 2–3 days of treatment) followed by a clear decrease to near castration levels in 3–9 weeks (1.1–2.4 nmol/l) (Fig. 1). Plasma oestradiol concentration showed changes in parallel with those of plasma

Table 1. Pretreatment patient dates

	Patient 1	Patient 2	Patient 3
Age (yr)	72	65	82
Stage of tumour	$T_2N_xM_1$	$T_2N_0M_1$	$T_{2-3}N_xM_1$
Tumour differentiation	?	high	moderate to poor
Symptoms at presentation	disturbed diabetes mellitus, bone pain, prostatism	prostatism	bone pain, pancytopenia
Weight loss (kg)	8	0	4
Prostatic acid phosphatase (U/l)	32.2	3.2	27.6
Alkaline phosphatase (U/l)	47	23	43
Bone scan	abnormal	abnormal	abnormal
α skeletal-survey	metastasis	arthrosis, metastasis?	metastasis
CT scan (abdomen)	hydronephrosis, enlarged lymph nodes	no lymph nodes	no lymph nodes
Duration of treatment	10 months	6 months	2 months

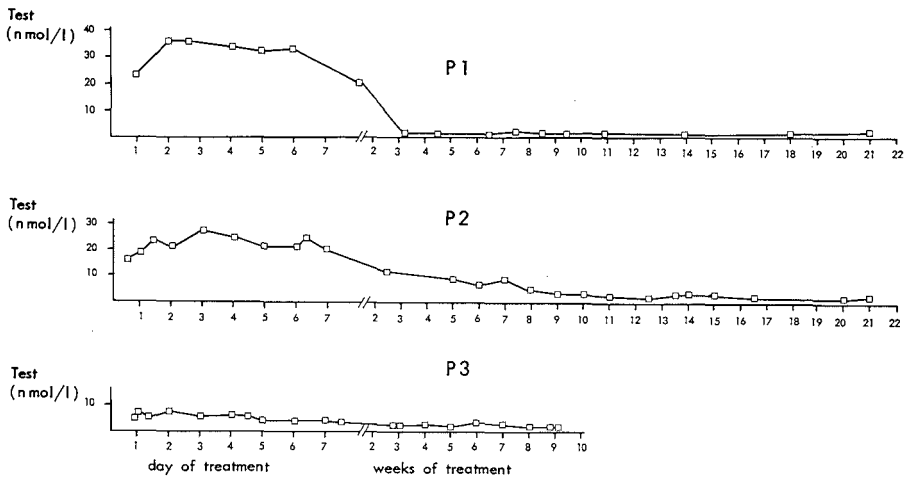

Fig. 1. Changes of plasma testosterone levels in 3 patients during chronic LHRH-agonist (Hoe 766) treatment (p = patient).

testosterone. Mean plasma oestradiol decreased from 117 before treatment to 14 pmol/l after 2 months of treatment. In patient 1, whose detailed data are shown in Fig. 2, plasma prolactin increased in the first week with only a slight decrease later on; the other two patients did not show a decrement.

It has to be noted that patient 3 already had subnormal plasma testosterone values before treatment (5.7 and 7.2 nmol/l) in the presence of elevated LH (54 IU/l) and FSH (43 IU/l) levels. During treatment plasma testosterone decreased to 2.0 nmol/l and the gonadrotrophins to 12.4 and 14.6 respectively.

Antitumour effects

Patient 1. Complaints of prostatism diminished dramatically within 3–4 weeks of treatment. He was again able to make long trips by coach without stopping every half-hour as before treatment. Bone pain and the disturbance of the regulation of his diabetes disappeared; it was no longer necessary to use analgesics. During treatment the appetite increased followed by an increase of 13 kg in body weight.

An objective response has been proven by recalcification of an osteolytic lesion (after 3 months) in the right femur neck (Fig. 3), a clear improvement of the bone scan (Fig. 4), rapid normalisation of acid phosphatase and PAP (Fig. 2), and a decrement in prostatic volume with 41% from 76.0 to 44.6 cm^3 after 3 months (Fig. 5). Thereafter no further significant decrease was observed. Enlarged parailiacal lymph nodes decreased in size. A cloudy osteoblastic vertebral lesion disappeared. On the other hand, a sharp round osteoblastic lesion became visible in the neck of the left femur (Fig. 3), while before treatment the bone scan showed a hot spot locally.

Patient 2. This patient had a more gradual improvement of his prostatism—probably in relation with the slower decrease in plasma testosterone levels—although the CAT scan showed no significant decrement of the prostate volume. A slightly increased PAP before treatment became nearly undetectable (from 3.2 to 0.1 U/l) during treatment, with a normalisation within 6 weeks. The bone scan showed an improvement without changes of the X-rays, which also showed spondylarthrosis of the vertebral column. Faint muscle pain disappeared. His appetite improved and his weight increased by 3.3 kg. He is now free of complaints and feeling well.

Patient 3. This very arteriosclerotic patient had extensive bone metastasis, as indicated by multiple bone lesions and severe pancytopenia. A bone marrow aspiration showed tumour cells. An operation was contraindicated. Buserelin spray was administered every day by his wife or daughter. The acid phosphatase activity in the serum decreased within 9 weeks without significant changes on the X-rays. Concomitantly, the activity of PAP decreased from 27.6 to 5.7 U/l. An elevated plasma LDH activity decreased from 1160 to 459 U/l in the presence of normal plasma bilirubin. He needed a lot of analgesics (aspirin, indomethacin) because of bone pain, which

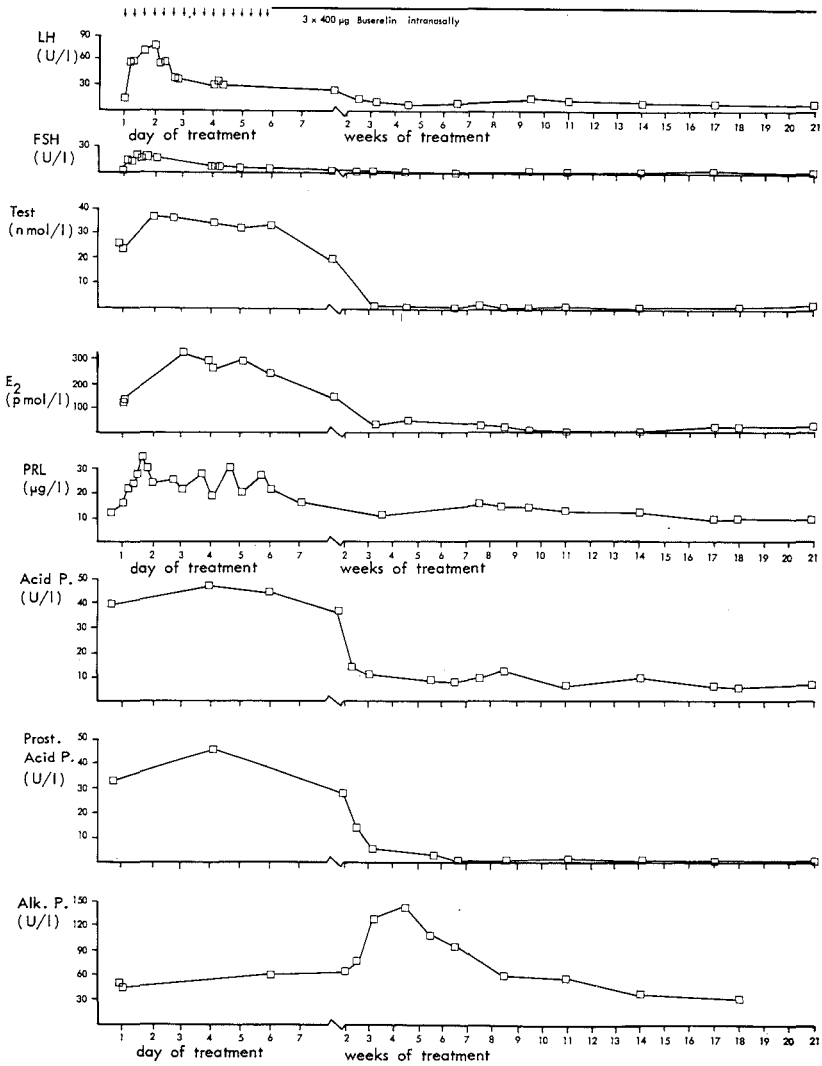

Fig. 2. Changes of plasma hormone levels, and serum alkaline and acid phosphatase activities in patient 1. ⫼ = *injections;* ⌐ = *intranasal administration.*

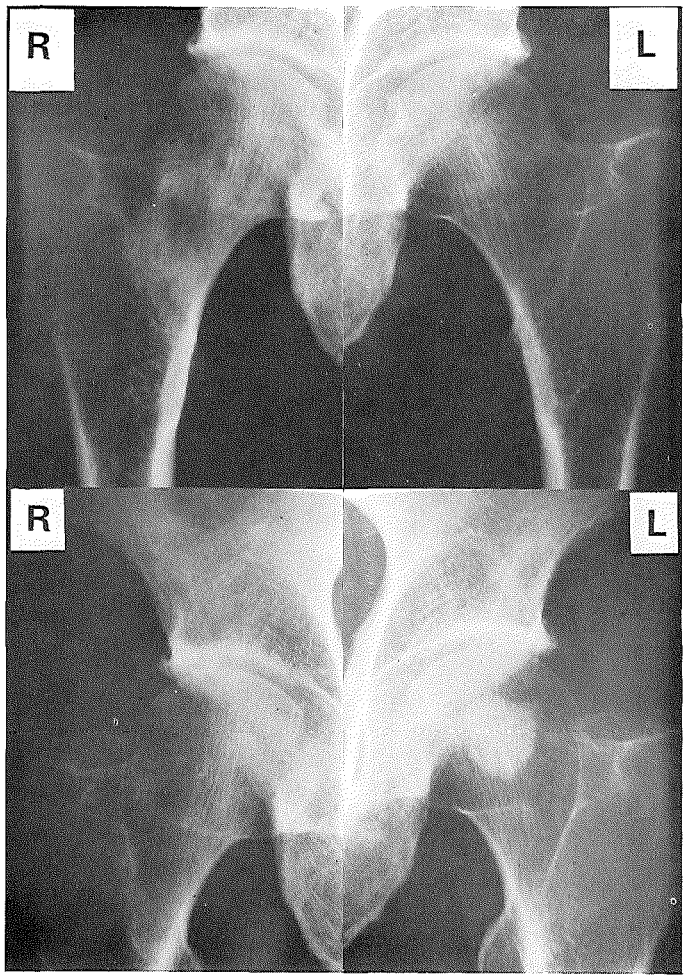

Fig. 3. (A) (upper part) An osteolytic lesion with an osteosclerotic surrounding in the right femur neck of patient 1 before treatment; a minimal sclerotic lesion in the head of the left femur; (b) (lower part) clear improvement in the right femur neck and the appearance of a dense sharp osteoblastic lesion in the left femur head after 3 months *of treatment.*

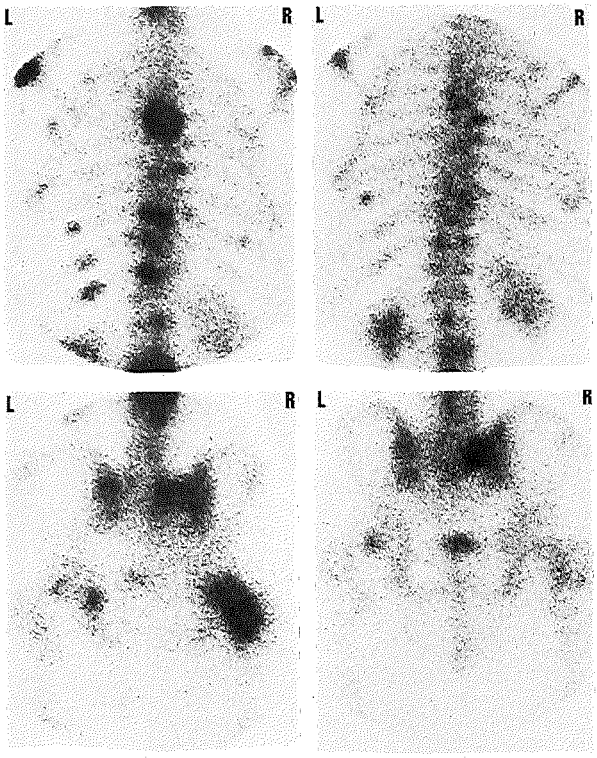

Fig. 4. *Bone scintigraphy before treatment (left) and* 6 months *after start of treatment (right) in patient* 1.

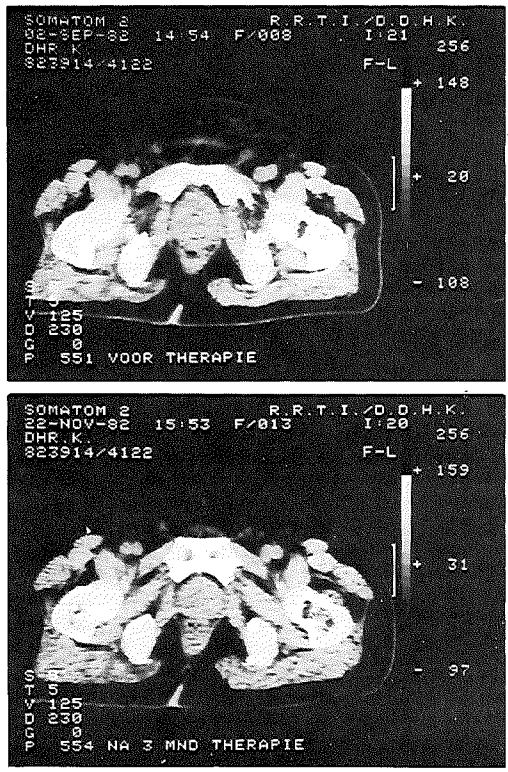

Fig. 5 CAT scan of the prostate before (upper part) and 3 months *after start of treatment (lower part) in patient* 1.

diminished slightly after 8 weeks. At this time he developed pain in the gastric area, which might have been caused by the analgesics. After 9 weeks he died subacutely, possibly by a gastrointestinal bleeding caused by the analgesics in combination with thrombocytopenia.

Side effects

Patient 1 experienced an increase of libido during the first week (no increase of bone pain) followed by a sharp decrease after 3 weeks. Also, in patient 2 libido and potency decreased. Both patients got hot flushes. Patient 3 already had a low sexual activity before treatment. None of the patients showed cardiovascular side-effects in the sense of hypertension, oedema, dyspnoea or thrombosis.

DISCUSSION

Several hormones are involved in the regulation of growth and functions of the prostatic gland, but androgens are the most important ones [33]. The most important androgen is testosterone, mainly (95%) produced in the testis. Other androgens such as androstenedione and dehydroepiandrosterone are also derived from the adrenals.

The major part of prostatic carcinomas are 'hormone-dependent' for their growth [34]. Bilateral orchidectomy appeared effective in the treatment of prostate cancer as well as high-dose oestrogens. The latter treatment, however, caused cardiovascular side-effects in about 40% of the patients [35]. The first preliminary studies with LHRH analogues have shown that this new kind of hormonal treatment, administered subcutaneously or intranasally, decreases plasma testosterone to (nearly) castration levels [22, 23, 27, 28]. Response rates of 70–90% are reported in the absence of serious side-effects. The main mechanism of action is probably a 'chemical castration' [17], but intrinsic antisteroidal effects [10, 11] and inhibition of enzymes (17-hydroxylase, 17-desmolases) involved in steroid synthesis are described [4]. Recently, chronic treatment with LHRH-agonist appeared superior to bilateral orchidectomy in regressing metastatic bone lesions and decreasing plasma PAP in spite of the absence of a significant difference in plasma testosterone levels [36]. This superior effect might be caused by the possible additional antisteroidal properties of LHRH-agonists or by decreased adrenal androgen synthesis. Further, a direct effect of the LHRH-agonist on the tumour cells cannot be excluded since LHRH-receptors were found to be present in an experimental prostatic tumour [37].

All our patients showed subjective improvement and decrement of serum acid phosphatase and PAP, of which the enzyme activity has been shown to be related with tumour mass [38]. Patient 1 also showed radiological evidence for objective tumour regression. Improvement with respect to prostatism can be explained by decrement of primary tumour size, but might be partly due to shrinkage of normal prostate tissue. Remarkable is the close relationship between plasma testosterone and PAP levels. In this patient bone scintigraphy appeared superior in early detection of bone lesions (head of left femur, ribs and spines). Plasma alkaline phosphatase showed a transient increase parallel to the disappearance of osteolytic lesions (indicating new bone formation) followed by a normalisation (Fig. 2). In the head of the left femur a circumscript pronounced osteoblastic lesion, which was scarcely visible on the X-ray before treatment, appeared at the same place as a hot spot was shown on the bone scan before treatment. This can be explained as an abnormal bone reaction that can occur after inhibition of the growth of a spot tumour cells. This sharp, round osteoblastic lesion did not change after 3 months of treatment. So, the events in this patient indicate that an increase of plasma alkaline phosphatase and/or appearance of new osteoblastic bone lesions during the first period of treatment are not a proof for tumour progression.

In conclusion, long-term treatment with the LHRH-agonist Buserelin appeared effective in patients with hormone-dependent metastatic prostate cancer without causing any serious side-effects. Our results are comparable with those reported by other centres using the same or other analogues. After the end of ongoing phase II studies in different countries phase III studies will be needed to indicate which treatment modality will be the most suitable one as a first-line therapy.

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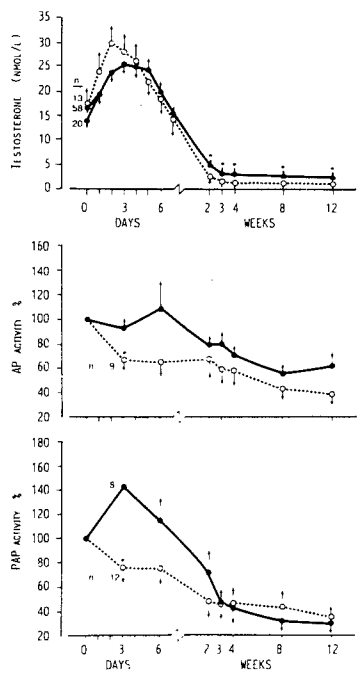
Letters to the Editor

COMBINED TREATMENT WITH BUSERELIN AND CYPROTERONE ACETATE IN METASTATIC PROSTATIC CARCINOMA

SIR,—Since establishment of the value of LHRH (luteinising hormone releasing hormone) agonists in prostatic cancer[1-5] clinical interest has focused on different formulations, on LHRH antagonists, and on combination treatments. Labrie et al[4] reported excellent results with a combination of an LHRH agonist and pure (non-steroidal) antiandrogens, both in preventing disease flare and in terms of survival, the effect of this treatment being "complete blockade" of testicular and adrenal androgens. Until now these promising results have not been confirmed.

We have treated thirteen previously untreated patients with metastatic prostatic cancer with the steroidal antiandrogen cyproterone acetate (CPA) at a dose of 3×50 mg daily, in combination with the LHRH agonist buserelin at a dose of 3×0·5 mg subcutaneously for 7 days followed by 3×0·4 mg daily intranasally. Plasma testosterone, LH, and cortisol concentrations and prostatic acid phosphatase (PAP) activity were compared with results in fifty-eight unselected patients being treated with buserelin alone in a multicentre study.

During the first week the addition of the steroidal antiandrogen CPA to LHRH agonist therapy prevented the LHRH-agonist-induced rise in PAP despite a temporary increase in plasma testosterone (figure). Thus the risk of "flare-up"[2,4] is remote with combination therapy. Furthermore, the addition of CPA to chronic LHRH agonist treatment seemed to cause a significantly more pronounced suppression of mean plasma testosterone concentration from the second week of treatment (decrease to 18% of pretreatment value, compared with 39% during treatment with buserelin alone after 2 weeks; $p < 0{\cdot}05$) and this difference was sustained for at least 3 months (figure). However, this difference in plasma testosterone levels did not lead to differences in plasma PAP activity in the longer term. Basal LH secretion and stimulated LH secretion (LHRH test) was more suppressed during combination therapy than it was in patients on buserelin done. We observed no difference and no significant change in basal and corticotropin stimulated cortisol concentration between those treated with buserelin alone and those given the combination, either before or after 3 months of treatment.



Testosterone and acid and prostatic acid phosphatase levels in patients on buserelin only (—) or buserelin plus CPA (····).

For plasma testosterone n=58 for patients treated with buserelin alone (n=20 for samples collected on days 1–6) and n=13 for those on buserelin plus CPA. Results as mean and SEM. *$p<0{\cdot}05$.

Whether complete blockade of androgens not only prevents the LHRH-agonist induced disease flare but also greatly improves survival remains uncertain. Indeed, besides two non-cancer (treatment) related deaths we have seen progressive disease in four out of thirteen patients treated with buserelin/CPA (mean follow-up 9 months, range 7–13 months). Twenty-four out of fifty-eight patients treated with buserelin alone have shown tumour progression within one year. Our preliminary data accord with those of Fauré et al, who used a combination of buserelin with a pure antiandrogen.[5] We recommend more comparative studies, and with that in view we have started a randomised study within the EORTC to investigate the possible advantages of the addition of an antiandrogen (CPA) to medical castration with an LHRH agonist.

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TRANSIENT NEONATAL HYPOTHYROIDISM IN VERY LOW BIRTHWEIGHT INFANTS: IS TREATMENT NECESSARY?

SIR,—Routine neonatal screening for hypothyroidism was introduced in the West of Scotland in 1979. Thyrotropin (TSH) is measured on Guthrie blood spots by an immunoradiometric method[1] on or soon after the 10th day of life. Preterm babies in our neonatal units are also screened by the same method irrespective of gestational age, although testing may be delayed beyond the 10th day until milk feeding is well established for the correct diagnosis of phenylketonuria and galactosaemia (which is included in the Guthrie test).

In both healthy and sick preterm infants low T3 and T4 values, which progressively increase to approach normal term values by 5–7 weeks, are well documented.[2,3] A high TSH is thought to distinguish those infants with congenital hypothyroidism. However, in the sick very low birthweight infant this type of

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APPENDIX PAPER 6.

LHRH-AGONIST TREATMENT IN CLINICAL AND EXPERIMENTAL HUMAN BREAST CANCER

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Summary—Thirty-one premenopausal patients with metastatic breast cancer were treated for 3–33 months with the potent LHRH-agonist Buserelin (Hoe 766) as a first-line therapy. Twelve women (group IA) were treated with a daily dose of 3 × 400 μg Buserelin intranasally and 10 women (group IB) with a daily dose of 2 × 1 mg subcutaneously after parenteral treatment with 3 mg per day in the first week. Nine patients (group II) were treated chronically with 3 × 400 μg Buserelin intranasally in combination with 2 × 20 mg tamoxifen ($n = 5$, group IIA) or 4 × 45 mg megestrol acetate ($n = 4$, group IIB). A great variation in hormonal response with recurrent peaks of E_2 in about half of the patients was observed in groups IA and IIA, while in group IB and IIB a "chemical castration" was reached in all patients with the most pronounced suppression of E_2 secretion in group IB. An objective tumour response was found in 13 (42%) and stable disease in 7 (23%) out of 31 patients. Nine out of 22 patients (41%) treated with Buserelin alone showed an objective response. In 8 of 17 patients (48%) with an estradiol receptor-positive tumour and in one of 2 patients with an ER-negative tumour we observed an objective remission. In an experimental study we found that Buserelin has a weak direct anti-estrogenic effect on the growth of human mammary tumour cells (MCF-7) *in vitro*. In conclusion medical treatment with high doses of Buserelin appears as effective as surgical castration in premenopausal metastatic breast cancer with an absence of serious side effects.

INTRODUCTION

Analogues of luteinizing-hormone-releasing-hormone (LHRH) are of increasing importance and of great promise in the treatment of different kinds of tumours [1–6]. In animals chronic treatment with pharmacological doses have been reported to cause exhaustion and desensitization of gonadotroph cells in the pituitary, inhibition of prolactin secretion, "chemical castration" with a striking fall in plasma sex steroids followed by a reduction in weight of accessory sex organs, inhibition of enzymes involved in steroidogenesis, and antagonism of biological actions of sex steroids [7, 8]. Furthermore, the findings of both LHRH-like receptors in an experimental prostate tumour [9] and the observation of an inhibitory effect of a LHRH analogue on the growth of mouse mammary tumour cells *in vitro* [2] suggest a possible direct effect of LHRH analogues at the level of hormone dependent tumour cells.

During the last 2–3 years analogues of LHRH have been shown to cause long-term remissions of prostate tumours in a number of clinical studies [1, 10–12]. However, reports on the effects in patients with breast cancer are limited [13]. Since 1981, we have been working on the antitumor effects of LHRH-agonist Buserelin (Hoe-766) for the treatment of metastatic breast cancer. In addition, the effect of a combination therapy of this LHRH agonist with tamoxifen and megestrol acetate was investigated especially with respect to the endocrine response. Based on our previous reports [13–16] we present this review on long-term LHRH agonist treatment (3–33 months) in 31 patients and describe results of laboratory experiments on the effect of Buserelin on a human mammary tumour cell line (MCF-7).

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PATIENTS, TREATMENT AND METHODS

Clinical studies

Thirty-one premenopausal patients with metastatic breast cancer gave consent for treatment with the potent LHRH-agonist Buserelin (Hoe 766) as a single agent or in combination with other agents like tamoxifen (TAM) or megestrol acetate (MA). All patients had not been treated previously for their metastatic disease. In total, 17 patients had an estradiol receptor (ER)-positive and two an ER-negative tumour, while in 12 patients the receptor status was unknown.

During the first week all subgroups of patients were treated with a daily dose of 3 mg Buserelin parenterally (for 3–7 successive days) as described before [15, 16]. Subsequently 12 patients (group IA) were treated chronically with 3 × 400 μg Buserelin intranasally (i.n.) and 10 patients (group IB) with 2 × 1 mg Buserelin subcutaneously (s.c.) decreasing this dose after 2 months with 2 × 0.1 mg per day for 1 month. Ultimately the patients in this subgroup IB have been treated with doses between 800 and 2000 μg per day s.c. Five women (group IIA) were

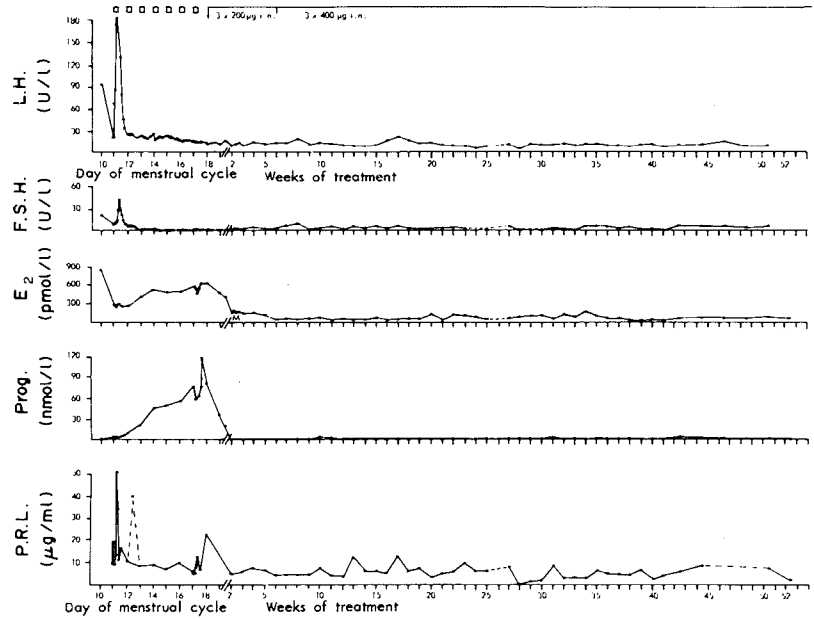

Fig. 1. Changes of plasma hormone concentrations during chronic LHRH-agonist treatment in a patient with chemical castration and a complete tumour remission during 2 years.

treated with $3 \times 400\ \mu g$ Buserelin i.n. in combination with 2×20 mg tamoxifen from the start of treatment, while in 9 out of the 12 patients of group IA tamoxifen was added later because of tumour progression or recurrent peaks of plasma estradiol (E_2). Four patients (group IIB) were treated with $3 \times 400\ \mu g$ Buserelin i.n. in combination with 4×45 mg megestrol acetate s.c.

Blood sampling, measurement of plasma luteinising hormone (LH) follicle stimulating hormone (LH) follicle stimulating hormone (FSH), estradiol, progesterone (Prog) prolactin (PRL) and estradiol receptor were done as described previously [17, 18]. Measurement of tumour response were performed according to the UICC criteria.

Significances of differences between mean values at various time points within treatment groups were assessed by Student's paired t-test.

Experimental studies

MCF-7 cells cultured at 37°C in an atmosphere of 5% CO_2 were trypsinized and seeded in T-25 flasks in "fully supplemented medium" to allow attachment of the cells to the culture flasks [19, 20]. Additions to the medium included estradiol, the LHRH agonist Buserelin, and synthetic LHRH (Relefact, Hoechst AG). These additives were given either alone or in combination at various concentrations.

In some experiments dextran-coated charcoal treated foetal calf serum (DCCFCS) was used. After the desired culture period the medium was removed and the cells were washed twice with 0.154 M NaCl. Thereafter, cells were dissolved in 1 ml 1 M NaOH at 50°C for 1 h. The protein concentration of the resulting solution and the DNA content were measured as described elsewhere [20]. An excellent correlation between DNA and protein contents was observed (0.863, $P < 0.001$).

RESULTS

Clinical studies

Endocrine effects

Gonadotropins. On the first treatment day peak values for plasma gonadotropins were reached about 4–6 h after the start of single treatment with Buserelin. Thereafter plasma LH and FSH dropped rapidly in spite of continuous infusion with Buserelin while during the following days no significant increase was observed (Fig. 1) indicating pituitary exhaustion and desensitisation.

In group IA mean plasma LH concentrations decreased below pretreatment levels after 2–3 weeks and continued to decrease slowly (Fig. 2). Mean plasma FSH concentration fell to the pretreatment level within 3 days, decreased further during the first week and remained thereafter at the same level during

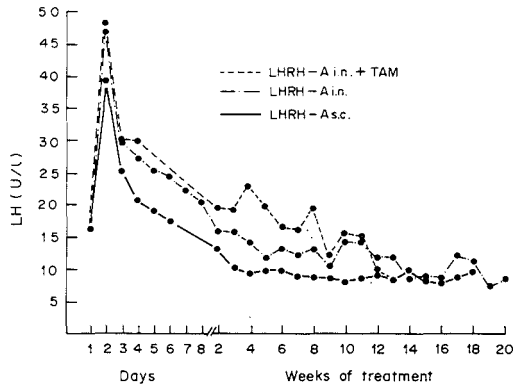

Fig. 2. Mean plasma concentrations of LH during chronic intranasal (group IA) and subcutaneous (group IB) treatment with Buserelin as a single treatment and in combination intranasally with tamoxifen (group IIA).

more than 1 year. During chronic subcutaneous treatment (group IB) the suppression of plasma LH (Fig. 2) and FSH (Fig. 3) was more pronounced than during intranasal administration. No ovulatory peaks were observed in both subgroups, while subnormal gonadotrophin levels occurred rarely.

During combination therapy with tamoxifen (group IIA) mean plasma gonadotrophin concentrations were slightly higher than during single treatment with Buserelin (Fig. 2) while preovulatory peaks of LH and FSH were observed in some patients. In group IIB (Buserelin s.c. + MA) plasma gonadotrophin concentrations were comparable with those observed in group IB.

Sex steroids. Anovulation as indicated by persisting low plasma progesterone levels occurred in all patients of groups IA, IB and IIB (Table 1); however, sub-normal peaks of progesterone were observed in 7 out of 14 patients treated with Buserelin intranasally in combination with tamoxifen.

During single intranasal therapy with Buserelin "complete medical castration" with persistent postmenopausal plasma E_2 concentrations, was reached in only 4 out of 10 evaluable patients, e.g. in the 2 patients with a complete tumour remisson during 24 (Fig. 1) and 33+ months. In the other, 6 evaluable patients had recurring E_2 peaks of various sizes. During chronic subcutaneous treatment plasma E_2 concentrations showed a striking fall to castration

Table 1. Relative changes in plasma hormone (LH, FSH, E_2, Prog) concentrations during single LHRH agonist treatment with Buserelin (group IA + IB) and in combination with tamoxifen (TAM) or megestrol acetate (MA) (i.e. groups IIA and IIB respectively)

	LH/FSH	E_2	Prog.
IA LHRH-A i.n.	↓	↓=↑	0
IB LHRH-A s.c.	↓↓↓	↓↓↓	0
IIA LHRH-A i.n. + TAM	↓=↑	↓=↑	↓=0
IIB LHRH-A i.n. + MA	↓↓	↓↓	0

↓-reduced, ↑-increased, = -equal, 0-no change.

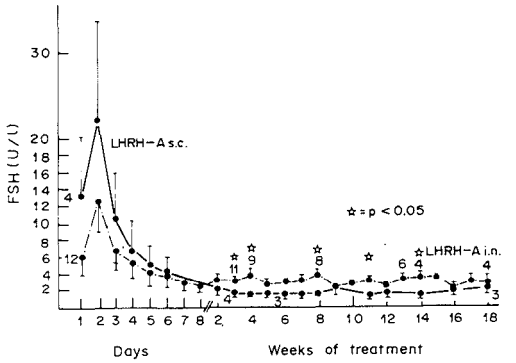

Fig. 3. Mean plasma concentrations (±SEM) of FSH during chronic intranasal (i.n.) and subcutaneous (s.c.) treatment with Buserelin alone (group IA and IB).

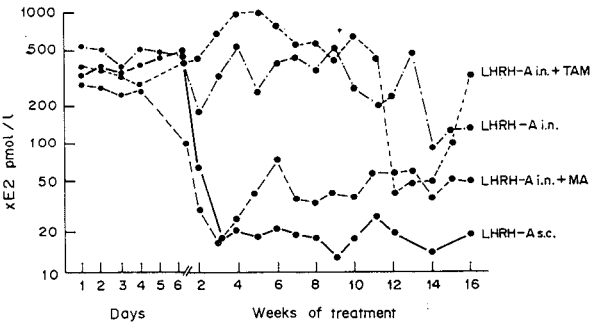

Fig. 4. Mean plasma E_2 concentrations during single treatment with Buserelin and in combination with tamoxifen (TAM) and megestrol acetate (MA); groups IA, IB, IIA and IIB.

levels within 2–3 weeks in all 10 patients (group IB) with a mean concentration of 19 pmol/l (=5 pg/ml) during long-term treatment (Fig. 4).

The response of E_2 concentrations during combination therapy with tamoxifen was very variable; from complete medical castration level to hyperstimulation level. Recurrent peaks of E_2 occurred in 8 out of 14 treated patients. In contrast, during combination therapy with megestrol acetate no peaks of E_2 were observed, while plasma E_2 concentrations were suppressed to a mean value of 45 pmol/l (Fig. 4).

Prolactin. On the first treatment day a moderate rise of plasma PR was seen in 10 out of 11 patients of group IA. Peak values between 7 and 51 μg/l were reached mostly at 6 h after start of parenteral treatment. The mean basal value of 7.5 ± 1.12 μg/l increased to a mean peak value of 18 ± 3.9 μg/l ($P < 0.05$). On the last day of parenteral single treatment with Buserelin plasma PRL levels remained unchanged and equivalent to the pretreatment values in 9 examined patients.

During parenteral therapy the mean night peak of PRL at 0100 decreased from 27.2 ± 4.6 to 15.9 ± 3 μg/l ($n = 6$, $P < 0.05$). During chronic intranasal treatment with 3×400 μg Buserelin the day–night rhythm remained intact (Fig. 5). Pituitary PRL reserve, as measured by the TRH-test, showed an increase in 7 of 9 investigated patients after 1 week of treatment ($n = 9$, 58.7 ± 13.3 to 76.6 ± 14.1, $P < 0.05$). After 3 months there was no significant difference with pituitary PRL reserve as measured before the start of treatment.

In the group of patients treated with Buserelin in combination with tamoxifen (group IIA) only a temporary small rise in plasma PRL was seen on the first treatment day (8.4 ± 1.98 to 13.5 ± 1.93 μg/l, $P < 0.05$), which did not occur on the last day of parenteral treatment. During chronic treatment the day–night rhythm was not present in 3 investigated patients (Fig. 5). In group IIB there was a tendency to an increase in basal plasma PRL levels probably caused by megestrol acetate, which was shown to increase prolactin secretion [21].

Effects on tumour growth

In group IA an objective tumour response was found in 4 out of 12 patients with a mean duration of 16^+ months (Table 2). The longest duration of response occurred in the 2 patients with a complete remission (CR). One had a recurrence of tumour after 24 months of treatment and did not respond to tamoxifen as a second line agent; the other patient is

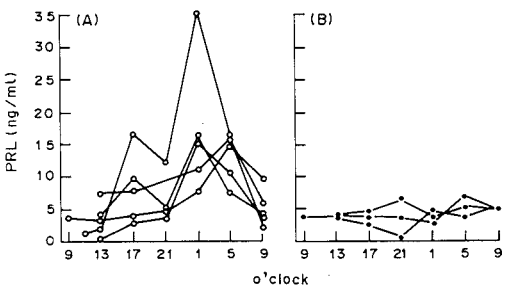

Fig. 5. 24-Hour profile of plasma prolactin (PRL) concentrations during intanasal therapy with Buserelin alone (A) and in combination with tamoxifen (B); groups IA and IIA.

Table 2. Antitumour effects in 31 premenopausal patients with metastatic breast cancer during single LHRH agonist treatment with Buserelin (group IA + IB) and in combination with tamoxifen (TAM) or megestrol acetate (MA)

Group	Treatment	CR + PR	No change	Failure	*n*
IA	Hoe 766 i.n.	4 × ($\bar{x}$ = 16⁺ m)	4 × (3–5 m)	4 ×	12
IB	Hoe 766 s.c.	5 × ($\bar{x}$ = 5⁺ m)	1 × (5 m)	4 × (2 × mixed)	10
IIA	Hoe 766 + TAM	3 × ($\bar{x}$ = 10⁺ m)	0 ×	2 ×	5
IIB	Hoe 766 + MA	1 × (19⁺ m)	2 × (17, 20⁺)	1 ×	4
Total		13 × (42%)	7 × (23%)	11 × (35%)	31

Objective response rate during single Hoe 766 treatment (IA + B) = 9/22 (41%). Longest duration of response: 33⁺ months.

still in complete remission after 33 months of treatment without any side effects. In group IB, with a relatively short mean duration of follow up, an objective response was observed in 5 out of 10 patients (2 × CR) with a longest duration of response of 9 months.

In the 5 patients treated with Buserelin in combination with tamoxifen (group IIA) 3 objective remissions were observed; the longest duration of response until now is more than 22 months. In the 4 patients treated with Buserelin in combination with megestrol acetate (group IIB) one partial response (19⁺ months) and two times stable disease (Table 2) occurred. On the whole an objective tumour response was observed in 13 of all 31 patients (42%) and in 9 of 22 patients (41%) during single treatment with Buserelin (group IA + B). No side effects occurred with the exception of those caused by the intended hypogonadism.

Antitumour effects in relation to receptor status

Only the patients of group IB were selected for receptor status. Five of these ten patients (50%) with an ER-positive tumour showed an objective response. In total, 17 out of the 31 patients had an ER-positive tumour and 8 of them (47%) showed an objective response. Of the 2 patients with an ER-negative tumour 1 had a partial remission during 5 months, while 4 of the other 12 patients with an ER-unknown tumour showed an objective response also.

EXPERIMENTAL STUDIES

At concentrations of 80 and 800 nmol/l, Buserelin did not affect the growth of the MCF-7 cells significantly (Fig. 6). Although the stimulatory effect of estradiol on the cells is rather small, addition of Buserelin combined with estradiol results in a significantly lower protein and DNA content than addition of estradiol alone. The inhibitory effect of Buserelin on the protein content of MCF-7 cultures in the presence of estradiol was dose dependent. Moreover, LHRH itself also showed a slight anti-estrogenic effect. In medium containing 10% DCCFCS Buserelin appeared to inhibit the estradiol-induced increase in cellular protein and DNA. In our experience this anti-estrogenic effect of Buserelin can be counteracted by an equimolar amount of a LHRH antagonist.

DISCUSSION

In our studies with the LHRH agonist Buserelin, the response rate of tumour growth inhibition appears at least as good as other known types of ablative or additive endocrine therapy with 50%

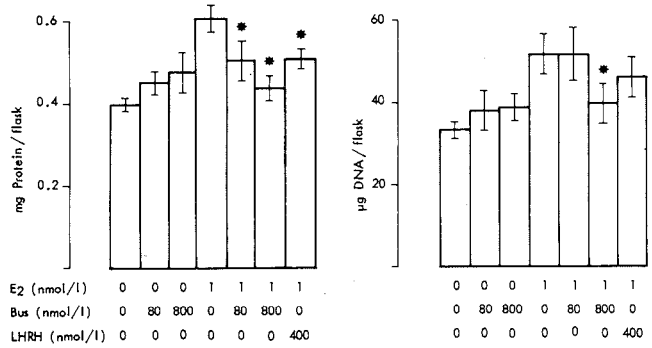

Fig. 6. Protein and DNA content of MCF-7 cultures grown in fully supplemented medium after five daily additions of estradiol (E_2) alone or combined with 80 or 800 nM Buserelin (Bus) or 400 nM synthetic LHRH. Results are given as means ± SD; n = 8–9; *$P < 0.01$ vs cultures kept in the presence of estradiol alone.

objective remissions in the ER-positive patients. Two recent preliminary reports concerning two studies with a relatively short follow-up confirmed our data. Harvey *et al.* [22] found an objective remission in 11 out of 25 premenopausal patients (44%) treated with 1–10 mg of the LHRH agonist Leuprolide by daily subcutaneous injection. Further, Walker *et al.*[23] reported an objective tumour response in 3 of 16 premenopausal patients treated with 250–1000 μg of the LHRH agonist Zoladex by daily subcutaneous injection.

The main mechanism of antitumour action of LHRH is probably by "chemical castration". In our clinical study the patients with a castration effect during single treatment with Buserelin showed the best responses, but it is still unclear how "complete" the chemical castration has to be. Our experimental studies with MCF-7 mammary tumour cells indicate that there may be an additional direct antitumour effect based on antagonism of the stimulatory action of estrogens remaining in the circulation after incomplete chemical castration by this new form of endocrine therapy.

With respect to the endocrine effects, the occurrence of complete chemical castration and suppression of gonadotrophin secretion appeared dose dependent. During intranasal treatment with $3 \times 400\ \mu$g Buserelin (2% resorption, i.e. comparable with 25 μg subcutaneously) a chemical castration occurred in 4 out of 10 evaluable patients (40%) as found by Hardt and Schmidt-Gollwitzer[24] in 5 out of 9 premenopausal women (55%). Hence, in total a chemical castration was reached in 9 out of 19 patients (47%). However, high doses of Buserelin subcutaneously (800–2000 μg daily) appeared to cause very low mean plasma E_2 concentrations (as after castration) in all 10 patients (100%). During treatment with the combination of Buserelin and tamoxifen an insufficient corpus luteum function was found in half (7/14) of the patients as proven by post-ovulatory plasma progesterone levels. In some patients pre-ovulatory LH and FSH peaks were observed. This indicates that Tamoxifen may overcome the suppressive effect on hypothalamo–pituitary function following chronic intranasal LHRH-agonist treatment. Therefore we combined Buserelin with high-dose megestrol acetate treatment because of its antigonadotropic properties. From the endocrine point of view this combination appeared more suitable than the combination of Buserelin with tamoxifen. However, side effects such as body weight increase with a cushingoid face occurred after about 3 months of treatment.

In conclusion treatment with high doses of buserelin appears as effective as surgical castration in premenopausal patients with metastatic breast cancer. Apart from the absence of serious side effects another advantage of LHRH-agonist treatment might be an additional weak anti-estrogenic effect as observed on MCF-7 mammary tumour cells. A dose of $3 \times 400\ \mu$g Buserelin i.n. (comparable with 25 μg s.c. per day) caused anovulation in all patients but suppression of follicular maturation occurred in only about half of the patients. However, high doses of Buserelin subcutaneously (2000–8000 μg) appeared to cause a striking suppression of E_2 secretion within 2–3 weeks in *all* patients.

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APPENDIX PAPER 7.

Direct Inhibitory Effect of a Luteinizing Hormone-releasing Hormone Agonist on MCF–7 Human Breast Cancer Cells*

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Abstract—*The effect of the LHRH agonist Buserelin on the MCF–7 human breast cancer cell line was studied. Cells were cultured in medium containing* 10% *untreated foetal calf serum or* 10% *steroid-depleted serum. In both media the DNA and protein content of cultures kept for* 3–5 days *in the presence of* 80–800 nM *Buserelin and* 1 nM *oestradiol were* 8–27% *lower than those of flasks cultured in the presence of oestradiol alone* ($P < 0.05$). *LHRH itself* (400 nM) *also displayed an antiproliferative effect on the MCF–7 cultures. At an equimolar concentration, the LHRH antagonist ORG* 30093*D abolished the antiproliferative effect of Buserelin. MCF–7 cells did not specifically take up radioiodinated LHRH. Our data are the first to indicate that LHRH analogues may inhibit the growth of MCF–7 cells to a limited extent. The antitumour activity of these compounds* in vivo *may, then, be due to the main pituitary and gonadal effects, resulting in a decrease of the concentration of oestrogen in the circulation and, in addition, a direct effect at the target cell level.*

INTRODUCTION

ANALOGUES of LHRH are currently being evaluated for use in the treatment of advanced breast [1, 2] and prostate cancer [3–5]. When administered over a long period and at a sufficiently high dose, these compounds elicit antifertility effects [6]. Two mechanisms have been suggested which would lead to 'medical gonadectomy'. Firstly, prolonged stimulation with pharmacological doses of LHRH agonists results in exhaustion and/or desensitization of the gonadotropic cells in the pituitary, which in turn leads to a reduced gonadotropin output [7, 8] and, hence, a decreased steroidogenesis. Secondly, direct effects of analogues of LHRH at the gonadal level may also cause a decreased steroidogenesis [9, 10]. In this respect it has been suggested that gonadal cells use a locally synthetized LHRH-like peptide for communication [11]. The direct effects of LHRH-like peptides at the gonadal level have been extensively investigated in the rat. They are thought to be mediated by receptors for LHRH on the membranes of the gonadal cells [9, 12–14]. By analogy with the rat, an LHRH agonist has been reported to inhibit the secretion of progesterone by cultured human granulosa cells [15]. In another study, however, no such effect was observed [16]. Similarly, the presence of receptors for LHRH-like peptides in the human gonads is still a matter of debate [17, 18].

In addition to causing medical gonadectomy, analogues of LHRH have been suggested to interfere with the action of steroid hormones on their target cells [19–21]. LHRH analogues can inhibit oestrogen-induced growth of the rat uterus [21, 22], oestrogen-induced increases in the activity of enzymes associated with uterine cell proliferation [23], androgen-induced growth of rat seminal vesicles and ventral prostate [22] and β-glucuronidase activity in the mouse kidney [20]. From these observations, the working hypothesis has been derived that, apart from impairing ovarian steroidogenesis, LHRH agonists may exert an additional antitumour effect based on interaction with the remaining (adrenal) steroids at the target cell level. The preliminary results of Corbin [19], who reported an inhibitory effect of an LHRH analogue on the growth of mouse mammary tumour cells *in vitro*, support this hypothesis. By contrast, Furr and Nicholson [24] did not observe an antioestrogenic effect of an LHRH analogue in ovariectomized immature rats.

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The present study was designed to study the existence of direct antitumour effects of LHRH agonists. In this respect, effects of Buserelin and oestradiol on the human breast cancer cell line MCF–7 and the binding of radioactive LHRH to these cells have been investigated.

MATERIALS AND METHODS

The human breast cancer cell line MCF–7 was obtained from EG&G Mason Research Institute, Worcester, MA, U.S.A., in its 219th passage. Cells were cultured at 37°C in an atmosphere of 5% CO_2 and air in Falcon T–75 culture flasks in RPMI–1640 medium, supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 0.1 mg/ml streptomycin (Gibco, Grand Island, NY, U.S.A.) and 10 ng/ml insulin (Organon, Oss, The Netherlands). This medium will be referred to as 'fully supplemented medium'. For experiments the cells were trypsinized and seeded in T–25 flasks in fully supplemented medium to allow attachment of the cells to the culture flasks. After one day the medium was changed for the experimental medium, which was changed daily unless indicated otherwise.

Experimental media

Additions to the medium included oestradiol, (Merck, Darmstadt, F.R.G.), tamoxifen (ICI-Farma, Rotterdam, The Netherlands), the LHRH agonist Buserelin (Hoechst-Pharma, Amsterdam, The Netherlands) and the LHRH antagonist [NAc-p-Cl-(D)Phe-1,2, (D)Trp-3, (D)Phe-6, (D)Ala-10]LHRH (ORG 30093D Organon International, Oss, The Netherlands) [25]. These additives were given either alone or in combination at concentrations indicated at the individual experiments. Oestradiol and tamoxifen were added to the medium as concentrated solutions in ethanol. The final concentration of ethanol in the medium never exceeded 0.2% (v/v). Corresponding amounts of ethanol were added to media which did not contain oestradiol or tamoxifen. Working solutions of Buserelin were prepared in Dulbecco's phosphate-buffered saline (Gibco, Grand Island, NY, U.S.A.), which was also added to the media which lacked Buserelin. A concentrated solution of the LHRH antagonist was prepared in 70% ethanol. Working solutions were prepared by dilution with phosphate-buffered saline. In one experiment synthetic LHRH (Relefact, Hoechst AG, Frankfurt-am-Main, F.R.G.) was used. This compound was used directly as supplied. In some experiments FCS was treated for 30 min at room temperature with 0.5% (w/v) Norit and 0.05% dextran T–70. Charcoal was removed by centrifugation for 30 min at 10,000 ***g***. The supernatant is referred to as dextran-coated charcoal-treated FCS (DCCFCS).

Termination of experiments

After the desired culture period the medium was removed and the cells were washed twice with 0.154 M NaCl. Thereafter, cells were dissolved in 1 ml 1 M NaOH at 50°C for 1 hr. The protein concentration of the resulting solution was estimated by the method of Bradford [26] using the kit from Bio-Rad (Richmond, CA, U.S.A.) and human serum albumin (KABI, Stockholm, Sweden) as a standard. DNA was measured with a fluorimetric assay [27]. Diaminobenzoic acid (Merck, Darmstadt, F.R.G.) and herring sperm DNA (Schuchardt, Munich, F.R.G.) were used as reagent and standard respectively. Cell numbers were not counted since it has been described that for the MCF–7 cell line changes in cell number show a consistent correlation with changes in DNA mass [28].

Binding studies with LHRH

Radio-iodinated LHRH was purchased from New England Nuclear (Dreieich, F.R.G.). Radiochemical purity was verified by thin-layer chromatography on cellulose plates (Merck, Darmstadt, F.R.G.) in the system *n*-butanol:water:ethyl acetate = 11:2:1 (v/v/v). In binding studies the cells were washed twice and incubated for 90 min at 4°C with approximately 300,000 cpm of tracer in 10 mM Tris–HCl buffer, pH 7.8, containing 1 mM dithiothreitol and 0.1% bovine serum albumin as described by Loumaye *et al.* [29]. After the incubation, cells were washed twice and dissolved in 1 M NaOH as described above. For comparative purposes, the binding of radioactive LHRH to 25,000 ***g*** membrane preparations [17] of rat pituitary and human breast and ovarian carcinoma tissue was also studied.

In the figures results are given as means ± standard deviation. The number of replicate observations is represented by *n*. Statistical analysis was performed with Wilcoxon's test. Differences were considered to be statistically significant when a P value of less than 0.05 was found.

RESULTS

Effects of Buserelin and tamoxifen

In early experiments we studied the effect of a single administration of Buserelin on the growth of MCF–7 cells. At concentrations of 10 and 100 ng/ml, Buserelin did not affect the protein content of the cells which were cultured for 1–3 days after addition of the drug. In further experiments the medium was changed daily in order to reduce the possibility that putative effects of Buserelin on the cells remain undetected as a result of rapid metabolism (and inactivation) of the peptide. The re-

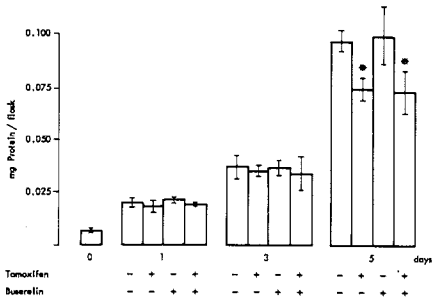

Fig. 1. Protein content of MCF-7 cultures grown in fully supplemented medium at different times during daily administration of 80 nM *Buserelin and/or* 1000 nM *tamoxifen. Data are given as means ± S.D.;* n = 5; *P < 0.05 *vs corresponding control.*

sults in Fig. 1 demonstrate that under the conditions used, i.e. in fully supplemented medium and after daily administration, Buserelin has no effect on the growth of the cells. Tamoxifen, on the other hand, significantly decreased the growth rate of the cells ($P < 0.05$), as evidenced by a lower protein content of the cultures. Buserelin did not alter the response of the cells to tamoxifen.

Comparison of protein and DNA content

To justify the use of the protein content of the cultures for the evaluation of the results of the present experiments, this parameter was compared to the DNA content of the cultures. An excellent correlation was found between these parameters. In 36 sets of data originating from four different experiments a correlation coefficient of 0.863 ($P < 0.001$) was found. The intercept of the regression line [DNA = 0.22 × protein − 2.02] was statistically not distinguishable from zero. Moreover, changes in the protein content of the cultures as a result of experimental manipulation of the cultures also showed a significant correlation with changes in the DNA content of the cultures ($r = 0.849$; $P < 0.001$). Again, the intercept of the regression line was not different from zero.

Combined effects of Buserelin and oestradiol

Subsequently, the possibility that the LHRH analogue interferes with the action of oestradiol on the cells was investigated. The results presented in Fig. 2 show that although the stimulatory effect of oestradiol on the cells is rather small, addition of Buserelin combined with oestradiol results in a significantly lower protein and DNA content than addition of oestradiol alone. The inhibitory effect of Buserelin on the protein content of MCF–7 cultures in the presence of oestradiol was dependent on the dose of the peptide (Fig. 3). Moreover, LHRH itself also showed a slight anti-proliferative effect (Fig. 3).

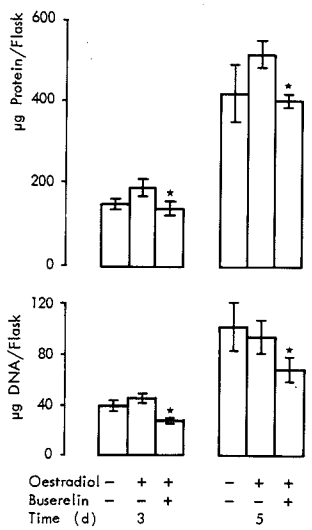

Fig. 2. Protein and DNA content of MCF-7 cultures grown in fully supplemented medium at different times during daily administration of 1 nM *oestradiol alone or in combination with* 80 nM *Buserelin. Results are means ± S.D.;* n = 4–6; *P < 0.02 *vs cultures kept in the presence of oestradiol only.*

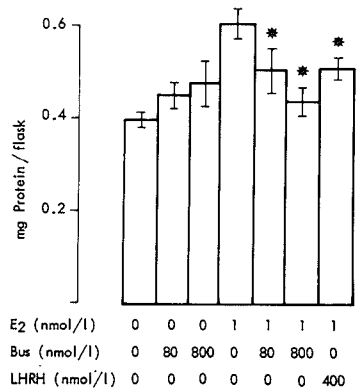

Fig. 3. Protein content of MCF-7 cultures grown in fully supplemented medium after five daily administrations of oestradiol (E_2) alone or combined with 80 *or* 800 nM *Buserelin (Bus) or* 400 nM *synthetic LHRH. Results are given as means ± S.D.;* n = 8–9; *P < 0.01 *vs cultures kept in the presence of oestradiol alone.*

Effect of an LHRH antagonist and steroid-depleted medium

The data in Fig. 4 demonstrate that the effect of Buserelin on oestrogen-stimulated MCF–7 cells can be counteracted by an equimolar amount of the LHRH antagonist ORG 30093D. This com-

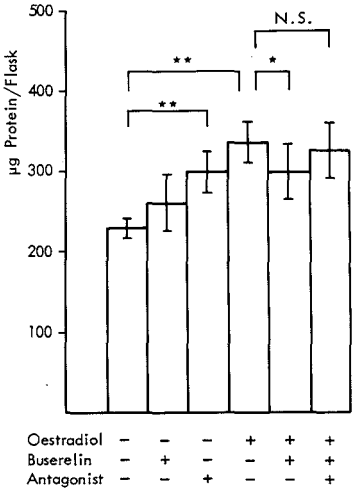

Fig. 4. Protein content of MCF-7 cells kept for 5 days *in fully supplemented medium containing oestradiol* (1 nM), *Buserelin* (800 nM) *or the LHRH antagonist ORG* 30093*D* (800 nM), *either alone or in combination. Culture medium was changed daily. Results are given as means ± S.D.;* n = 10; *P < 0.05; **P < 0.01.

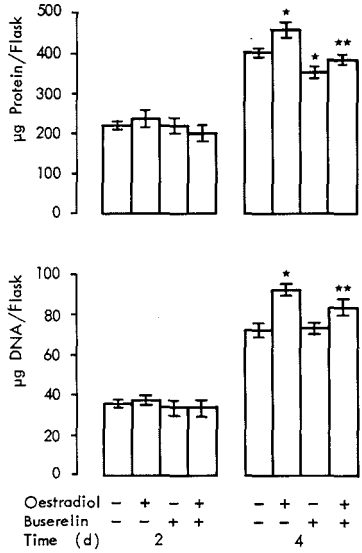

Fig. 5. Protein and DNA content of MCF-7 cultures kept in medium containing 10% *DCCFCS for different periods of time. Cells were trypsinized and transferred to T-25 flasks in fully supplemented medium. After* 1 day *(at day* 0*), this medium was replaced by the steroid-depleted medium containing* 1 nM *oestradiol and* 800 nM *Buserelin either alone or in combination. Results are given as means ± S.D.;* n = 7; *P < 0.01 *vs control cultures.* **P < 0.01 *vs cultures kept in the presence of oestradiol alone.*

pound also appeared to be able to stimulate the growth of the MCF-7 cells.

Effects of oestradiol and Buserelin may have been reduced by interference from compounds present in FCS. The results in Fig. 5 show that in medium prepared with steroid-depleted FCS Buserelin also inhibits the oestradiol-induced increases in cellular protein and DNA.

LHRH binding studies

The binding of radio-iodinated LHRH to MCF-7 cells and rat pituitary membranes is shown in Fig. 6. Pituitary membranes readily bound the tracer, which could be displaced from its binding sites by an excess of radio-inert LHRH or Buserelin. Scatchard plot analysis of the binding of radioactive LHRH to rat pituitary membranes revealed a binding capacity of 40 fmol/mg membrane protein and a dissociation constant of 0.06 nM. By contrast, the tracer bound to the MCF-7 cells to a much smaller extent, and the binding observed could not be displaced by an excess of radioinert ligand. No binding was observed to membranes prepared from solid human mammary and ovarian tumours.

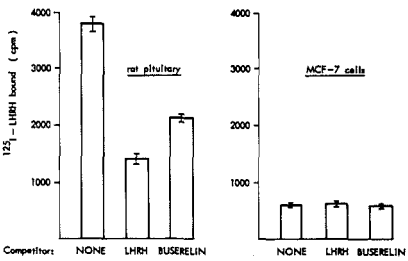

Fig. 6. Binding of [^{125}*I]-LHRH to rat pituitary membranes and MCF-7 cells and displacement by a* 1000*-fold excess of radioinert LHRH or an* 8000*-fold excess of Buserelin. Results are given as means ± S.D.;* n = 3 *for the pituitary membranes;* n = 5 *for the MCF-7 cells.*

DISCUSSION

The results described in the present paper are the first to demonstrate that an LHRH agonist can directly interfere with the proliferation of human breast cancer cells in culture. It appears from our data that Buserelin can antagonize the stimulatory action of oestrogens on these cells. LHRH itself was also capable of inhibiting the oestradiol-induced proliferation of the cells, whereas the LHRH antagonist ORG 30093D counteracted the effect of Buserelin. The limited magnitude of the effects of Buserelin on the cells, however, supports the current consensus that the main anti-tumour effect of LHRH analogues is exerted via a suppression of circulating levels of oestrogens.

The reason why only relatively small effects of

Buserelin were observed may very well reside in the fact that oestradiol also had limited effects. Yet the magnitude of the stimulatory effect of oestradiol on our MCF–7 cells is not different from that in other reports [30–32]. Other investigators have shown that the effect of oestradiol on these cells may depend on the source of serum [33]. It is conceivable that the anti-proliferative action of Buserelin is more pronounced in cells which show a higher increase in growth rate in response to oestradiol. Therefore the use of other sera is currently under investigation.

One attempt to increase the sensitivity of the cells to oestradiol was already undertaken in the present series of experiments. The presence of FCS in the culture medium may result in an oestrogen concentration which already causes a maximal or near-maximal proliferation rate of the cells. Alternatively, other mitogens present in FCS may stimulate the proliferation of the cells to such an extent that they cannot respond to addition of oestradiol with an increase in the rate of proliferation. Therefore experiments were also done with medium in which the FCS was replaced with DCCFCS. The rates of both the basal and the oestradiol-induced proliferation in medium prepared with DCCFCS (Fig. 5) decreased only marginally as compared with medium prepared with untreated FCS (Fig. 2), which led us to conclude that other factor(s) present in FCS and resistant to charcoal treatment must be responsible for maintaining the proliferation rate of the cells on a relatively high level. It could be argued that the action of Buserelin on the MCF–7 cells is directed against the stimulatory action of these other mitogens, rather than oestradiol. In our opinion, however, this possibility is not very likely since Buserelin had no effect in the absence of oestradiol.

Our data are in agreement with those of Corbin [19], who found that daily administration of the LHRH agonist Wy 40,972 temporarily retarded the growth of mouse mammary tumours cells in culture. In contrast to our results, however, Corbin observed an effect already 1 day after the first administration, and after 6 days cell numbers in cultures treated with the analogue were equal to those in control cultures. In our study the first effects could be shown only after 3–4 days. In view of the relatively high growth rate of the cells, effects could not be investigated for more than 6 days. Therefore we can offer no data on the duration of the suppression of cell growth. From the data in Figs 2 and 5, however, it appears that the effect of Buserelin on the MCF–7 cells is also transient. In the experiment reported in Fig. 2, for example, the protein content of control cultures and cultures kept in the presence of oestradiol or oestradiol plus Buserelin all increased by a factor of 2.7 between days 3 and 5. The inhibitory action of Buserelin thus appears to be manifested only after initial exposure of the cells to the peptide. The most obvious explanation for this observation is inactivation of the peptide by degradative enzymes. To circumvent this possibility, the culture medium was replaced daily in the present series of experiments. To further evaluate this possibility, an experiment was done in which the culture medium was not changed. Medium which is conditioned by MCF–7 cells for more than 5 days can still stimulate the secretion of LH and FSH by rat pituitary cells in culture. In this respect there was no difference between fresh and conditioned medium [F.H. de Jong, personal communication]. The presence of biological LHRH activity after prolonged exposure of the medium to MCF–7 cells virtually rules out the possibility that the transiency of the effect of Buserelin on the cells is caused by exhaustive degradation of the peptide.

Other explanations for this phenomenon include transient effects on cell attachment, changes in the uptake of the peptide by the cells, the existence of several populations of cells, of which only a minority is sensitive to Buserelin, or the secretion of an LHRH-like peptide by the cells. The secretion of an endogenous LHRH-like regulatory peptide may, of course, obscure effects of exogenously added peptides. The presence of high concentrations of LHRH-like immunoreactive material has been documented in human milk [34, 35] and in ductal mammary carcinoma [36]. It remains to be investigated which, if any, of these possibilities accounts for the present observations.

In the present study the growth-inhibiting effect of Buserelin was tested at only one, relatively high concentration of oestradiol, i.e. 1 nM. This concentration was chosen because it is known to induce maximal stimulation of MCF–7 cells. It remains to be investigated whether the growth-inhibiting effect of Buserelin is dependent on the concentration of oestradiol. To answer this question it may be essential to use a system in which the response of the cells to oestradiol is much more pronounced.

Our observation that Buserelin and LHRH itself can act directly on MCF–7 cells is highly suggestive for the presence of specific receptors for LHRH-like peptides in these cells. A similar situation appears to prevail for the Dunning R 3327H rat prostatic carcinoma. This transplantable tumour regresses following administration of LHRH analogues [25]. Recently, this tumour was also found to contain receptors for LHRH-like peptides [37]. We have used commercially available iodinated LHRH in the search for the presence of such receptors in MCF–7 cells. No saturable binding of labelled LHRH to the cells was observed. By contrast, rat pituitary membranes showed specific binding of the peptide. This virtually rules out the possibility that the LHRH lost

its biological activity upon iodination, but indicates that the putative receptors for LHRH-like peptides in MCF-7 cells may have a very low affinity for LHRH itself. Alternatively, the observed effects of LHRH on the MCF-7 cells (Fig. 3) need not be mediated through receptors, or putative receptors may be occupied with endogenous LHRH-like material [34–36]. After submission of this paper, data were published by Miller *et al.* [38], which support the suggestion that the putative receptors for LHRH-like material have a low affinity for native LHRH. These authors found that iodinated LHRH-agonist was able to bind to MCF-7 cells, but that LHRH itself showed only a very limited cross-reactivity with this binding.

In summary, the data reported in the present paper support the hypothesis that LHRH agonists, apart from their main antitumour activities which are mediated by the pituitary and the gonads, may have an additional antitumour effect excerted directly on the tumour cells.

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APPENDIX PAPER 8.

COMBINED EFFECTS OF BUSERELIN, ESTRADIOL AND TAMOXIFEN ON THE GROWTH OF MCF-7 HUMAN BREAST CANCER CELLS IN VITRO

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SUMMARY The growth-stimulation of MCF-7 human breast cancer cells in vitro induced by 30 pM estradiol was inhibited both by the LHRH-agonist Buserelin and the anti-estrogen Tamoxifen used as single agents. Combined administration of both drugs was less effective in this respect. In the presence of estradiol Buserelin had no effect on the pattern of [^{35}S]methionine labelled secretory proteins when examined with one-dimensional gel electrophoresis, whereas the estrogen-induced progesterone receptor synthesis was inhibited. Thus with estradiol concentrations comparable to plasma values in medically castrated patients, the LHRH-agonist Buserelin can directly inhibit breast cancer cell growth in vitro.

Chronic treatment with pharmacological doses of LHRH agonists causes suppression of the pituitary-gonadal axis resulting in decreased gonadal steroidogenesis (1). This kind of therapy appeared to be effective in the treatment of hormone dependent tumors as breast (1-7) and prostatic cancer (1,8-11). Apart from its action on the pituitary-gonadal axis also direct effects of LHRH analogs on gonadal tissues have been observed (see for review ref. 12). Moreover, direct effects on extra-gonadal tissue as breast cancer cells have been previously reported by us and by others (5,13-16). Recently, specific binding sites for LHRH-agonists have been demonstrated in breast cancer cells (16,17). In search for the mechanism of action and to evaluate the possible clinical significance of direct anti-tumor effects of Buserelin, an LHRH agonist, we have studied the growth of human breast cancer cells in culture in the absence and presence of Tamoxifen with estradiol concentrations as found in vivo following medical castration.

MATERIALS AND METHODS

The human breast cancer cell line MCF-7 was obtained from EG&G Mason Research Institute, Worchester, MA, USA, in its 219th passage. Cells were maintained in

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culture in a humidified atmosphere of 5% CO_2 and air at 37°C in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, 0.1 mg/ml streptomycin and 10 ng/ml insulin. For experiments to study growth the cells were trypsinized and seeded in 25 cm^2 flasks. After one day the medium was changed for the experimental medium which was refreshed at day 3 and day 5.

Experimental media consisted of RPMI-1640 medium supplemented with 10% steroid-depleted male human serum (DC-MHS), 100 U/ml penicillin and 0.1 mg/ml streptomycin. Male human serum was obtained from healthy donors and heat-inactivated for 30 min at 56°C. Removal of sex steroids from sera was performed by 0.5% charcoal-0.05% dextran T-70 (w/v) absorption for 20 h at 4°C under continuous stirring. Additions to the medium included estradiol (Merck, Darmstadt, FRG), Tamoxifen (ICI-Farma, Rotterdam, The Netherlands), Buserelin (Hoechst-Pharma, Amsterdam, The Netherlands), and Org 30093D (Organon, Oss, The Netherlands).

After culturing for 7 days the experiments were terminated as described before (15), and protein or DNA-content of the cell cultures, which were shown to be equally applicable, were used as parameter to study the growth of the cultures.

Preparation of cytosol and nuclear extract

For measurement of estrogen and progesterone receptors cells grown in 75 cm^2 flasks were harvested with a rubber policeman in 5 ml phosphate buffered saline (PBS), pooled, and centrifuged for 5 min at 100xg. Pellets were resuspended in 3 ml 10 mM Tris-HCl buffer, pH 7.4, containing 1.5 mM EDTA, 10 mM monothioglycerol, and 10% (v/v) glycerol. Cells were homogenized with a Dounce glass-glass homogenizer with 30 strokes of a tight-fitting pestle. The supernatant obtained after centrifugation for 10 min at 800xg was further centrifuged for 40 min at 100,000xg. The 100,000xg supernatant fraction was defined as cytosol. The residual 800xg pellet was washed twice with 10 mM Tris-HCl buffer, pH 8.0, containing 3 mM $MgCl_2$, 1 mM monothioglycerol and 0.25 M sucrose, and following centrifugation for 10 min at 800xg nuclear extract was prepared as follows. Nuclear pellets were resuspended in 0.5 ml 10 mM Tris-HCl buffer, pH 8.0, containing 1.5 mM EDTA, 1 mM monothioglycerol, 10% glycerol and 0.6 M NaCl (buffer A), sonicated for 3x5 s with a MSE PG-104 sonicator at setting low 3, incubation for 1 h at 4°C and centrifugation for 15 min at 15,000xg.

Labelling and measurement of steroid receptors

Cytosolic estrogen and progesterone receptors were labelled by incubation for 18 h at 4°C with either 10 nM [H]estradiol or 10 nM [H]Org 2058 (Amersham, Houten, The Netherlands) in the absence and the presence of a 100-fold excess non labelled competitor to correct for non-specific binding. Nuclear receptors were labelled by incubation for 18 h at 4°C, followed by a further 3 h at 30°C for the estrogen receptor, and a further 3 h at 17°C for the progesterone receptor. Bound and free ligand were separated by G-25 gel-filtration on Sephadex PD-10 columns in buffer A, and 8-drop fractions were collected and counted for radioactivity. Specifically bound radioactivity in the void-volume fractions was considered to be receptor-bound.

RESULTS AND DISCUSSION

The LHRH-analog Buserelin appeared to have direct inhibitory effects on the growth of MCF-7 cells when cultured in medium supplemented with fetal calf serum (15,16). Since in our experiments the estradiol-stimulated growth was only approximately 10% (15), we have replaced fetal calf serum by male human serum, as applied by De Vleeshouwer et al. (18). In experiments in which

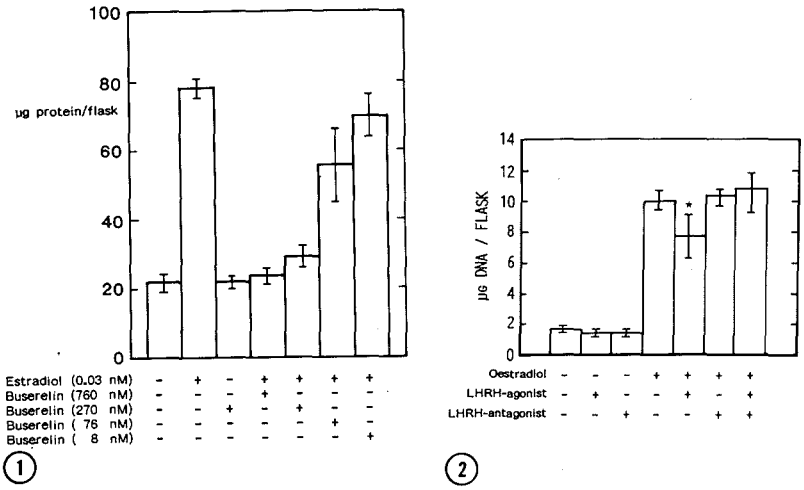

Figure 1. Effect of estradiol and Buserelin on the protein content of MCF-7 cell cultures grown in medium supplemented with DC-MHS. Results represent the mean ±SD of six incubations.

Figure 2. Effects of estradiol (30 pM), the LHRH-agonist Buserelin (0.8 µM) the LHRH-antogonist Org 30093D (0.6 µM) on the DNA content of MCF-7 cell cultures. Results represent the mean ±SD of six-fold incubations.
*Significantly different from all the other incubation conditions ($2p < 0.05$; Wilcoxon)

medium was supplemented with 10% DC-MHS, 10-100 pM estradiol already resulted in maximal stimulations (4-6 fold) of MCF-7 cell cultures when compared to cultures grown in the absence of estradiol (not shown). Similar observations were made in studies in which medium was supplemented with 10% female human serum (19).

When premenopausal patients are treated chronically with Buserelin a medical castration can be reached resulting in circulating concentrations of estradiol ranging from 5 to 50 pM (5). Postmenopausal women also have castrate values of estradiol. Therefore, we have used a comparable concentration of 30 pM estradiol in the first series of experiments looking for the effects of increasing dosages of Buserelin. It appeared that a dose-response relationship existed resulting in an almost complete inhibition (96%) of the stimulated growth with 0.8 µM Buserelin (Fig.1). Although it appeared that in subsequent

experiments, with different batches of male human serum used for the cultures, this effect was variable, the observed inhibitory effect of Buserelin could be blocked completely by addition of the LHRH-antagonist Org 30093D (Fig.2). This suggests the presence of specific receptors for LHRH on membranes of MCF-7 cells in culture (16). Combined effects of estradiol, Tamoxifen and Buserelin on the growth of MCF-7 cells were studied and it appeared that at different concentrations of estradiol (30 and 80 pM), an approximately 500-fold excess of Tamoxifen inhibited the estrogen-induced growth with 87% and 92%, respectively (Table 1). The additional presence of Buserelin during culturing resulted in less growth inhibition (32% and 67%) than was observed for Tamoxifen alone (Table 1).

To study whether the observed inhibitory effect of Buserelin on the growth of MCF-7 cell cultures might in some way be related to changes in steroid receptor concentrations, experiments were performed in which the cytosolic and nuclear estrogen and progesterone receptors were measured after culturing of the cells in the absence and the presence of 30 pM estradiol and 0.8 μM Buserelin. Buserelin appeared to have no effect on the amount of cytosolic estrogen receptors while a 380% increase in the amount of progesterone receptors, induced by estradiol, could be blocked for 87% when Buserelin was present

TABLE 1
INHIBITION OF ESTROGEN-STIMULATED GROWTH BY TAMOXIFEN AND BUSERELIN

	additions	inhibition of estrogen-stimulated growth (%)
experiment 1 (30 pM E_2)	Tamoxifen (13 nM)	87 ± 6
	Buserelin (270 nM)	87 ± 6
	Tamoxifen + Buserelin	32 ± 6
experiment 2 (80 pM E_2)	Tamoxifen (40 nM)	92 ± 4
	Buserelin (825 nM)	66 ± 14
	Tamoxifen + Buserelin	67 ± 14

Effects of Tamoxifen and Buserelin on the estradiol-stimulated growth of MCF-7 cell cultures. The growth-stimulations in experiments 1 (3.6-fold) and 2 (8.3-fold) were set at 100% for the calculation of the percentages inhibition by Tamoxifen and/or Buserelin. Data are obtained from the protein values of six-fold incubations.

TABLE 2
CYTOSOLIC AND NUCLEAR STEROID RECEPTORS

Conditions	estrogen receptor		progesterone receptor	
	cytosol (pmol/mg P)	nucleus (pmol/mg DNA)	cytosol (pmol/mg P)	nucleus (pmol/mg DNA)
Control	1.8	2.9	1.0	2.2
Buserelin	2.1	2.9	1.5	2.0
Estradiol	0.8	2.6	4.8	2.9
Estradiol + Buserelin	0.8	3.1	1.5	0.8

Effects of estradiol (30 pM) and Buserelin (0.8 µM) on the amounts of cytosolic and nuclear estrogen and progesterone receptor.

(Table 2). Also the progesterone receptor content measured in high salt nuclear extracts after stimulation with estradiol was reduced from 2.9 to 0.8 pmol/mg DNA by Buserelin (Table 2). It has been reported that although Tamoxifen below a concentration of 0.1 µM (20) and some other anti-estrogens (C1628M and U23,469M) (21) were able to increase cytoplasmic progesterone receptor levels, they as yet inhibited the growth of MCF-7 cell cultures. It is therefore uncertain whether the direct growth-inhibitory action of Buserelin can be attributed solely to its inhibiting effect on the estrogen-induced progesterone receptor synthesis, but interference at this level might explain why Tamoxifen and Buserelin partly abolish their mutual effects on MCF-7 tumor cell growth.

Apart from its effect on the synthesis of the progesterone receptor, estradiol affects the synthesis of specific secretory proteins (22) of which the most abundant (MW:52K) is believed to act as an autocrine mitogen (23). We have therefore investigated whether Buserelin could affect the synthesis of specific secretory proteins. Although estradiol at a concentration of 30 pM already significantly stimulates the synthesis of [^{35}S]methionine labelled specific secretory proteins, measured by fluorograms obtained after one-dimensional SDS-PAGE, we could not observe any effect of the addition of Buserelin (not shown). Effects on posttranslational events can not be excluded yet,

together with possible effects on the secretion of estrogen stimulated growth factors (24,25).

In conclusion, the LHRH-agonist Buserelin directly inhibits the estradiol-stimulated growth of MCF-7 human breast cancer cells in vitro. The direct anti-tumor effects are observed at concentrations of estradiol that prevail in chemically (as by LHRH agonist) castrated premenopausal breast cancer patients and in postmenopausal women. Use of LHRH-agonist therapy in clinical practice with the aim to obtain (additional) direct anti-tumor effects, may however depend on the availability of drug preparations which result in maintenance of sufficiently high plasma concentrations of circulating LHRH agonist. In this respect the observation of Miller et al (16) that concentrations of as low as 1 nM Buserelin, which can currently be obtained in vivo, already resulted in a net decrease of breast tumor cell growth in vitro, and secondly, the observation of objective tumor remissions in about 10% of postmenopausal patients (7,26-28) support the clinical significance of direct anti-tumor effects.

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APPENDIX PAPER 9.

LONG-TERM LHRH-AGONIST (BUSERELIN) TREATMENT IN METASTATIC PREMENOPAUSAL BREAST CANCER

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INTRODUCTION

Different steroid and peptide hormones, growth factors and other trophic substances are involved in the growth regulation of breast cancer cells (17). Especially oestrogens play an important role. Since the observation of tumour growth remission after surgical castration by Beatson (1896) various treatment modalities have been developed, which suppress gonadal, adrenal or peripheral oestrogen production or antagonize the stimulatory effects of oestrogens at the level of the tumour cells. Apart from castration by surgical and radiotherapeutical means it appeared possible to suppress pituitary-gonadal function by different kinds of medical treatment. During the last 5 years, it became apparent that LHRH analogues are of interest for suppression of pituitary gonadotrophin secretion and for reaching "medical castration", especially because of the absence of side effects (25). The effectiveness of this type of treatment has been established in patients with prostate cancer by a large number of investigators (see previous chapters and review, 25) as well as by our group (13,14,26) and the number of studies in breast cancer patients has been increasing since our first report in 1982 (9). Since that time we have treated 32 premenopausal patients with metastatic breast cancer with at present a minimal follow-up of 1.5 years. Part of the endocrine and clinical results have been published before in detail (9-12,15, 16). In this report we present our updated results and a review on this subject in the literature.

PATIENTS, TREATMENT AND METHODS

Thirty-two premenopausal patients with metastatic breast cancer gave consent for treatment with the potent LHRH-agonist Buserelin (Hoe 766) as a single agent or in combination with other agents like tamoxifen or megestrol acetate. The characteristics of this group of patients with respect to age, disease-free interval, oestradiol receptor status and follow-up are indicated in Table 1. All patients were unselected with the exception of the patients in group IB (see treatment scheme),

TABLE 1.

PATIENTS

- 32 premenopausal patients with metastatic breast cancer
- mean age: 40 yr (range 30 - 53 yr)
- disease-free interval: $\bar{x}$ = 2.5 yr (range 0.5 - 5.0 yr)
- oestradiol receptor: 18 x pos., 2 x neg., 12 x unknown
- mean follow-up: 3.5 yr (range 1.5 - 4.5 yr)

who were selected on the basis of having an oestradiol receptor positive tumour.

During the first week all subgroups of patients were treated parenterally (Table 2) with a daily dose of 3 mg Buserelin as described before (11). Subsequently 12 patients (group IA) were treated chronically with 3 x 400 µg Buserelin intranasally (i.n.) and 11 patients (group IB) with 2 x 1 mg Buserelin subcutaneously (s.c.), decreasing this daily dose after 2 months with 2 x 0.1 mg for every next month and switching to intranasal administration in the presence of continuous medical castration after one year of treatment. Ultimately the patients in this subgroup IB have been treated with doses between 2 x 1000 µg s.c. and 3 x 400 ug i.n. (∿25 µg s.c.) per day (Table 2). Five women (group IIA) were treated daily with 3 x 400 ug

TABLE 2.

PATIENTS AND TREATMENT SCHEME

May 1986

Group	n	first week	long-term treatment
IA	12	3 mg Hoe 766 i.v. or s.c.	3 x 400 µg i.n.
IB	11	3 mg Hoe 766 i.v.	2000 → 100 µg s.c. → i.n.
IIA	5	3 mg Hoe 766 i.v.	3 x 400 µg i.n. + 2 x 20 mg TAM*
IIB	4	3 mg Hoe 766 i.v.	3 x 400 µg i.n. + 4 x 45 mg MA*
Total	32	patients	

* TAM = tamoxifen

* MA = megestrol acetate

Buserelin i.n. in combination with 2 x 20 mg tamoxifen from the start of treatment, while in 9 out of the 12 patients of group IA tamoxifen was added later because of tumour progression or recurrent peaks of plasma oestradiol. Four patients (group IIB) were treated with 3 x 400 µg Buserelin i.n. in combination with 4 x 45 mg megestrol acetate s.c.

Blood sampling, measurement of plasma luteinising hormone (LH), follicle stimulating hormone (FSH), oestradiol (E2), progesterone (Prog), prolactin (PRL) and oestradiol receptor (ER) were done as described previously (4,8). Measurement of tumour response were performed according to the UICC criteria. Significances of differences between mean values at various time points within treatment groups were assessed by Student's paired t-test.

RESULTS

Endocrine effects

Gonadotrophins

Results have been described in detail before (11,12,15). During chronic subcutaneous treatment the suppression of gonadotrophin secretion was more pronounced than during intranasal administration. No pre-ovulatory peaks were observed with the exception of data in a few patients treated with Buserelin plus tamoxifen (group IIA), while subnormal immunoassayable gonadotrophin levels occurred rarely.

Sex steroids

Anovulation as indicated by persisting low plasma progesterone levels occurred in all patients of group IA, IB and IIB; however, subnormal peaks of progesterone were observed in 7 out of 14 patients treated with Buserelin i.n. in combination with tamoxifen (10,11).

During chronic subcutaneous treatment plasma E2 concentrations showed a striking fall to castration levels within 3 weeks with a mean concentration of 19 pmol/l (5 pg/ml) during 1-4 months after start of treatment (Fig.1). During subcutaneous treatment mean plasma E2 was much more suppressed than during intranasal treatment, during which a great variation in plasma E2 concentrations was observed as expressed by a large SEM. During intranasal treatment some patients (40%) reached medical castration values, others showed recurrent peaks of E2.

During gradual decrement of the daily subcutaneous dose, mean plasma E2 slowly increased after 5-12 months of treatment using Buserelin dosages of 0.6 - 1.4 mg per day. Plasma E2 levels reached the same range as observed in patients with medical castration during intranasal therapy (Fig.1) i.e. between 20 and 60 pmol/l. One patient (Fig.2) showed a clear escape of pituitary-gonadal suppression after 32-40 weeks of treatment s.c. while after 20 weeks plasma E2 levels were already

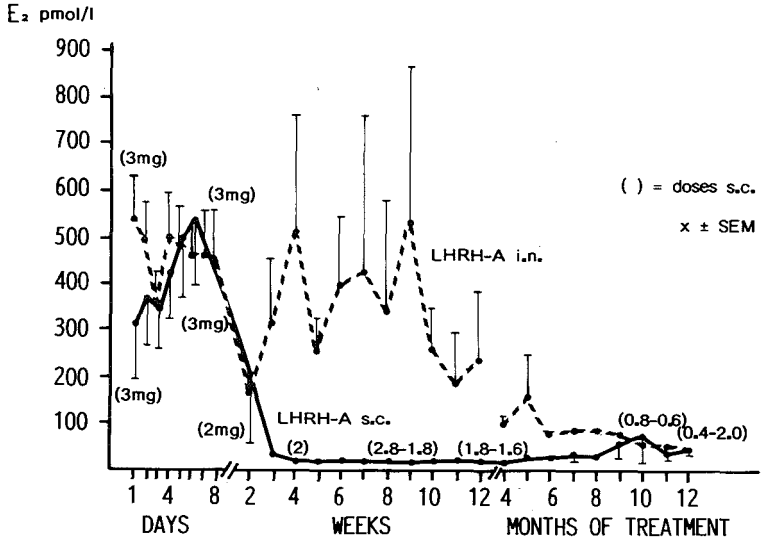

FIG. 1. Mean plasma E_2 concentrations during chronic single treatment with Buserelin intranasally (i.n.) and subcutaneously (s.c.) in 23 patients.

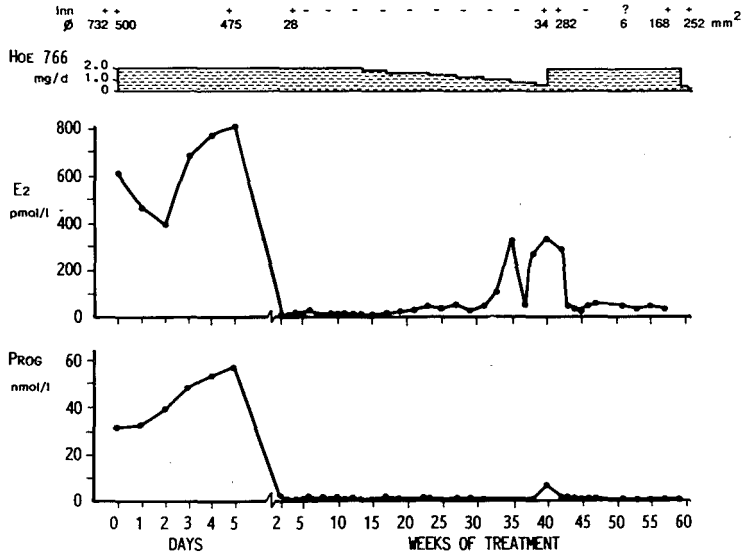

FIG. 2. Dose dependent effects on plasma E_2 and prog levels and lymph node metastasis (lnn) of subcutaneous treatment with Hoe 766 in the same patient.

slightly increased using dosages of 1.4 - 0.6 mg per day. Ultimately a small peak of progesterone occurred. After increment of the dose to 2 x 1 mg s.c. per day a medical castration was reached again. This patient showed a complete remission of her cervical lymph node metastases within 5 weeks after start of treatment, but a recurrence of these lymph node metastases occurred after 9 months, about 7 weeks after the time that plasma E2 increased above levels of 100 pmol/l. When medical castration was reached for the second time after dose increment a complete tumour remission occurred again indicating that the tumour was still hormone dependent. However, after one year of treatment hormone independency occurred with tumour progression in the presence of castration levels of plasma E2. On the other hand, in another patient it appeared possible to decrease the daily dose from 2000 µg s.c. to 1200 µg i.n. (∿25 µg s.c.) in the course of 1 year. In this patient continuous medical castration and nearly complete tumour regression for more than 2 years were observed.

Prolactin

Detailed data were reported in a previous report (15). The main observations were that basal and stimulated (TRH) plasma PRL levels increased during the first day and after one week of treatment respectively. Thereafter there appeared no significant difference with pretreatment values. During parenteral therapy the mean night peak of PRL, however, decreased significantly from 27.2 $\pm$ 4.6 to 15.9 $\pm$ 3 µg/l ($p < 0.05$).

Antitumour effects

The effects on tumour growth are summarized in Table 3. In the whole group 14 patients (44%) showed an objective remission with a mean duration of response of more than 19 months. Three patients are still under treatment. In addition 6 patients (19%) showed stable disease. An objective response during single Buserelin treatment was found in 9 out of 23 patients (39%) and in 5 out of 11 (45%) patients selected on the basis of an ER-positive tumour treated subcutaneously. Until now the longest duration of response is more than 53 months. At present, this patient has been treated with an LHRH analogue for the longest period reported. In total, 8 out of 18 patients with an ER-positive tumour responded objectively (44%).

Survival

The overall survival of the whole group of 32 patients is shown in Fig.3. Median survival is 3 years. Eight patients (25%) died within 1.5 years after start of treatment. Thirteen (54%) out of 24 patients, all of them with a follow-up of at least 3 years survived longer than 3 years. The 9 patients with combination treatment showed a somewhat better survival curve than the other patients, but the number is too small for definite conclusions. In general, these results are as good or

better than those reported in the literature.

TABLE 3.

ANTITUMOR EFFECTS IN 32 PATIENTS

May 1986

Group	Treatment	CR + PR	No Change	Failure	n
IA	Hoe 766 i.n.	4 x ($\bar{x}$ = 21^{+}m)	4 x (3-5m)	4x	12
IB	Hoe 766 s.c.	5 x ($\bar{x}$ = 14^{+}m)	1 x (5m)	5x(2x mixed)	11
IIA	Hoe 766 + TAM	3 x ($\bar{x}$ = 15^{+}m)	0 x	2x	5
IIB	Hoe 766 + MA	2 x (22,41^{+}m)	1 x (14m)	1x	4
Total		14 x (44%)	6 x (19%)	12x	32

- Objective response rate during single Hoe 766 treatment (IA+B) = 9/23 (39%)
- Objective response rate in patients with ER + tumors: 45%
- Longest duration of response: 53^{+} months

Side effects

No side effects occurred with the exception of those caused by the intended hypogonadism, i.e. hot flushes, decreased libido and in a few patients mental depression. Hot flushes were experienced 3-4 weeks after start of treatment at the time of medical castration. The frequency of the flushes varied between 3-30 times per day. During the years of treatment the intensity decreased. Some patients (about 15%) showed short-term (10-60 minutes) urticarial skin irritation at the injection site without pain or itching.

DISCUSSION AND CONCLUSIONS

In our study the objective response rate in premenopausal metastatic breast cancer is 39% during single LHRH agonist treatment. Recently, in 4 other studies (5-7,18,19,23) with a shorter follow-up and with application of 4 different LHRH agonists comparable response rates (between 31 and 47%, Table 4) have been reported. In total, an objective response was found in 44 (38%) out of 116 patients. The overall response rate in patients with ER-positive tumours appeared 53% (30/57). So, chronic LHRH-agonist treatment seems as effective as other common kinds of endocrine treatment in premenopausal breast cancer in the absence of serious side effects, but randomised

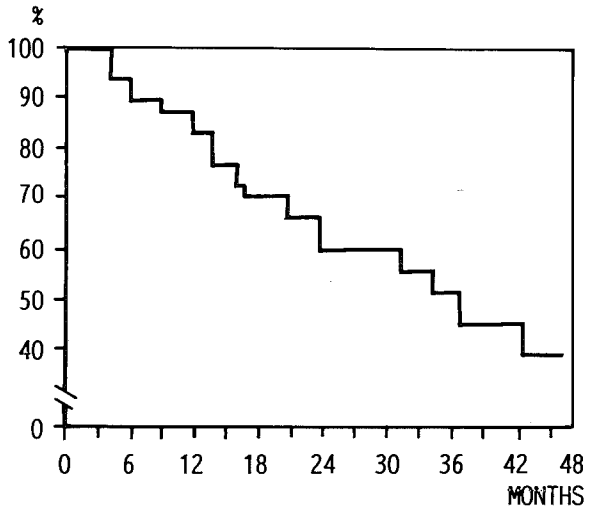

FIG. 3. Survival of 32 premenopausal patients with metastatic breast cancer treated with Buserelin (9 in combination with TAM or MA).

studies have still to be performed. On the basis of our experience and that of others (21,22), an escape of suppression of pituitary-ovarian function or non-complete medical castration may occur in some women treated with daily dosages of an LHRH agonist lower than 1 mg subcutaneously, especially after initiation of therapy during either the mid-cycle or luteal phase according to Nicholson et al. (21,22). Therefore we advise a daily dose of at least 1 mg s.c. to reach safely medical castration in all patients as also observed by Harvey and Manni et al. (5,6,18).

Although the main mechanism of action of LHRH agonist treatment is medical castration, direct antitumour effects at the level of tumour cells cannot be excluded. We reported before (1,2,15) and in this book (Foekens et al.) on direct antitumour effects of Buserelin using oestrogen stimulated breast cancer cell growth (MCF-7) in vitro in the presence of E2 concentrations as found in castrated or postmenopausal patients. Prolactin stimulated growth of breast cancer cells appeared inhibited too as found by Wiznitzer and Benz (28) using a prolactin responsive cell line (T-47-D). Direct effects were also observed by Miller et al. (20, this book) but not on all cell lines investigated. The suggestion of direct effects of the LHRH agonists in vitro is supported by the in vivo observation that LHRH agonist treatment can cause objective remission in postmenopausal metastatic breast cancer (5,6,19,24, Table 5) and by the presence of (low affinity) binding sites for LHRH agonists

TABLE 4.

RESULTS OF LHRH-AGONIST TREATMENT IN PREMENOPAUSAL METASTATIC BREAST CANCER
(Updated 1986)

Author	LHRH-agonist	n	CR + PR
1) Klijn et al.	Buserelin	23	9 (39%)
2) Nicholson et al.	Zoladex	45	14 (31%)
3) Harvey et al.	Leuprolide	25	11 (44%)
4) Mathé et al.	D-Trp-6-LHRH	8*	3 (38%)
5) Höffken et al.	Buserelin	15	7 (47%)
In total		116	44 (38%)

* pretreated

Overall response rate of ER-positive tumors: 30/57 (53%)

TABLE 5.

RESULTS OF LHRH-AGONIST TREATMENT IN POSTMENOPAUSAL METASTATIC BREAST CANCER
(Updated 1986)

	Author	LHRH-agonist	n	CR + PR
1)	Harvey et al.	Leuprolide	41*	4 (10%)
2)	Mathe et al.	D-Trp-6-LHRH	15*	3 (20%)
3)	Plowman	Zoladex	10	2 (20%)
4)	Waxman	Buserelin	18	0 (0%)
In total			84	9 (11%)

* pretreated

in breast cancer cells (3,20). However, the in vivo results could not be reproduced by all authors (27), and the relatively low overall response rate (11%) indicates that this kind of treatment will be of less value in postmenopausal patients. Maybe in some patients with bad drug compliance slow release preparations injected once per 1-3 months may be useful in the future.

In conclusion

1. In premenopausal metastatic breast cancer chronic LHRH-agonist treatment appears as effective as other common kinds of endocrine treatment in the absence of serious side effects.
2. Although intranasal administration appeared to be a pleasant and sufficiently effective way of treatment in some patients, high subcutaneous dosages of LHRH-agonists (at least 1.0 - 1.5 mg per day) are needed for reaching optimal and rapid suppression of the pituitary-gonadal function within 3 weeks in all patients.
3. The reported responses in some postmenopausal patients may indicate direct antitumour effects in vivo at the level of the tumour cells as was observed in vitro.
4. Sustained release preparations of LHRH agonist may be of great advantage if indeed medical castration can be reached in all patients.

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APPENDIX PAPER 10.

[CANCER RESEARCH 47, 1566–1570, March 15, 1987]

Direct Inhibitory Effects of Somatostatin (Analogues) on the Growth of Human Breast Cancer Cells[1]

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ABSTRACT

Various hormones and growth factors are involved in the growth regulation of breast (tumor) cells. In this report we show for the first time that an analogue of the neuropeptide somatostatin (Sandostatin) can also influence the proliferation of human breast cancer cells (MCF-7), namely, in an inhibitory fashion. With respect to dose-response relationship a bell-shaped curve was observed with the maximal inhibition of tumor cell growth at a sharply defined amount of Sandostatin (10 nM). The same effects were found with the natural hormone somatostatin-14 and another analogue (CGP 15-425). These results, together with the observation that high affinity binding sites for an iodinated derivative of Sandostatin are present in MCF-7 cells, support the conclusion that somatostatin and analogues act directly on breast cancer cells.

INTRODUCTION

Different steroid hormones (estrogens, progestins, androgens, glucocorticoids), peptide hormones (prolactin, growth hormone, insulin, calcitonin), growth factors (epidermal, transforming, and insulin-like growth factors), and other trophic substances (iodothyronines, vitamin D, retinoids) are involved in the growth regulation of breast cancer cells (1–10). Most of these factors are derived from endocrine glands such as the pituitary, gonads, and adrenals. Treatment of metastatic breast cancer is designed to decrease plasma concentrations of these hormones and factors or to antagonize the biological effects of these trophic substances directly at the level of the tumor cells.

Endocrine therapy of breast cancer consists of a variety of both medical and surgical treatment modalities including oophorectomy and hypophysectomy. Chronic treatment with analogues of LHRH,[3] a hypothalamic hormone, causes a "partial hypophysectomy" and medical castration resulting in breast tumor regression in about 40% of premenopausal patients (11–17). Recently, neuropeptides of another class, analogues of somatostatin, have become available. Somatostatin or its analogues appears to cause suppression of the secretion of a number of pituitary and gastrointestinal hormones (18–27) such as GH and insulin. In addition, it has been reported that treatment with somatostatin analogues can decrease plasma concentrations of some growth factors such as somatomedin C (*i.e.*, IGF-1) (25) and EGF (28). IGF-1 and EGF may also function as autocrine or paracrine growth factors for MCF-7 human breast cancer cells (8–10). The possibility of a 'complete medical hypophysectomy' by means of chronic treatment with potent long-acting somatostatin analogues in combination with other drugs [LHRH analogues, (anti)steroids, prolactin inhibitors] makes this class of peptides of potential value in the treatment of breast cancer. Furthermore somatostatin or somatostatin-like material (20–22, 29) and/or somatostatin receptors (30–32) have been found in tumors from several origins suggesting the possibility of direct effects of somatostatin on tumor cells. It has been shown that analogues of somatostatin are able to inhibit the growth of a number of classical endocrine (25–27, 33–37) and nonendocrine tumors (37–39). Somatostatin has endocrine effects, but it is believed that somatostatin can act locally as a paracrine or autocrine regulator of cell proliferation. Until now somatostatin has not been recognized as a hormone involved in the regulation of breast (tumor) cell growth. We have therefore studied whether the growth of human breast tumor cells can be influenced directly by an analogue of somatostatin. This is the first report showing that a somatostatin analogue inhibits the growth of human breast cancer cells.

MATERIALS AND METHODS

Chemicals and Materials. Estradiol was obtained from Merck, Darmstadt, West Germany. Nonradioactive Sandostatin (SMS201-995; D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol) and ^{125}I-labeled Tyr^3-Sandostatin (1770 Ci/mmol) were kindly provided by Sandoz, Basel, as was CGP 15-425 [cyclo-(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba)] by Ciba-Geigy AG, Basel, Switzerland. Somatostatin-14 (Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys) was purchased from Sigma, St. Louis, MO. All media, sera, and antibiotics used were purchased from Grand Island Biological Co. (Breda, The Netherlands). Insulin was obtained from Organon, Oss, The Netherlands.

Cell Culture. MCF-7 human breast cancer cells in their 219th passage were originally obtained from E.G. and G. Mason Research Institute, Worcester, MA. Cells were passaged weekly and were grown in plastic T-75 flasks in RPMI 1640 medium containing 5 μg/ml phenol red and supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), insulin (10 μg/ml), and 10% fetal calf serum that had been inactivated for 30 min at 56°C (complete growth medium). For experiments, cells growing exponentially were removed by treatment with 0.1% trypsin and 3 mM EDTA in 1 ml Dulbecco's phosphate-buffered saline free of Ca^{2+} and Mg^{2+} for 5 min at 37°C and were plated in T-25 flasks in 5 ml complete growth medium at a density as indicated in the legends to the figures. The following day the monolayers were washed twice with 5 ml 0.9% NaCl and 5 ml experimental medium were added. Experimental medium consisted of RPMI 1640 medium containing phenol red with or without somatostatin (analogues), estradiol, and/or insulin, and was supplemented with 5% fetal calf serum that had been inactivated and depleted of steroids by two 45-min incubations at 50°C with dextran-coated charcoal (1% charcoal-0.1% dextran). Estradiol was added from a concentrated stock solution in absolute ethanol. In media without estradiol the same amount of ethanol was added (<0.01%). Other vehicle controls consisted of 0.9% NaCl solution. Medium containing the respective supplements, including somatostatin (analogues), was refreshed as indicated in the legends to the figures. When indicated in the text RPMI 1640 medium without phenol red was used in the experimental medium.

Harvesting of Cells. Monolayer cells were harvested following two washes with 0.9% NaCl either by a 30 min incubation at 60°C in 1 ml NaOH or by incubation with 0.1% trypsin and 3 mM EDTA in Dulbecco's phosphate-buffered saline free of Ca^{2+} and Mg^{2+} for 10 min at ambient temperature. DNA and protein contents of the cell lysates

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[3] The definitions used are: LHRH, luteinizing hormone-releasing hormone; GH, growth hormone; IGF, insulin-like growth factor; EGF, epidermal growth factor; Tyr, tyrosine.

were estimated by a fluorimetric method as described before (40) and according to Bradford (41), respectively. Cells harvested by trypsinization were counted in a hemocytometer after preparing a single cell suspension by repeatedly forcing the cells through a 0.6-mm needle.

Determination of Sandostatin Binding Sites by Scatchard Analysis. MCF-7 cells were cultured in experimental medium in the absence of estradiol and in the presence of insulin. On day six, after medium refreshment every second day, monolayer cells were washed twice with RPMI 1640 medium containing 0.5% bovine serum albumin. After two subsequent incubations for 1 h at 37°C in medium with the same composition the cells were harvested with a rubber policeman and resuspended in ice-cold medium. Following centrifugation for 5 min at 100 × *g*, the cells were resuspended in receptor buffer consisting of 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.5) with 5 mM $MgCl_2$, 10 mg/ml bovine serum albumin, 0.02 μg/ml phenylmethylsulfonyl fluoride, 0.02 μg/ml Bacitracin, and 200 Kallikrein inhibiting units/ml Trasylol. Cells (3×10^6) were aliquoted in 1.5 ml conical polypropylene tubes and incubated with ^{125}I-labeled Tyr^3-Sandostatin (specific activity, 1770 Ci/mmol) ranging from 25 to 500 pM, in the absence and presence of a 200-fold excess nonlabeled Sandostatin to correct for nonspecific binding. After incubation for 2 h at 22°C, which appeared optimal in initial experiments with cells in suspension, the cells were pelleted by centrifugation for 5 min at 13,000 × *g*. Following one wash with 1 ml receptor buffer, radioactivity in the pellet fractions was estimated by gamma counting (efficiency, 88%).

Statistical Analysis. Statistical analysis was performed by the nonparametric method of Wilcoxon.

RESULTS

In initial experiments MCF-7 mammary tumor cells were cultured in medium supplemented with 5% steroid-depleted fetal calf serum and with increasing concentrations of the somatostatin analogue Sandostatin both in the absence and the presence of estradiol and/or insulin. Tested under these four conditions, Sandostatin at a concentration of 10 nM resulted in a significant inhibition ($P < 0.05$) of the growth of the cell cultures (Fig. 1). This inhibition was most profound when the cultures were grown in medium with insulin and without estradiol. Under this condition there was an 88% inhibition of cell growth based on DNA content of the cultures (Fig. 1*C*). Interestingly, maximal suppression of the growth of the cultures was obtained at a sharply defined amount of Sandostatin (10 nM); at lower and higher dosages the inhibition of cell growth was less striking. The known stimulating effects of estradiol and insulin on MCF-7 cell growth were confirmed from the differences in the amounts of DNA in the cultures grown in the absence of Sandostatin (Fig. 1, *left columns*). To evaluate whether the growth-inhibiting effect of Sandostatin also occurs under "complete estrogen-deprived" conditions, experiments were performed in medium lacking phenol red which was shown to have weak estrogenic effects (42). In such medium without phenol red and estrogens, 10 nM Sandostatin resulted in 25 ± 3% (SE; $n = 6$) inhibition of MCF-7 cell growth in the presence of insulin ($P < 0.02$).

In subsequent experiments with medium containing phenol red without estradiol and with insulin, the inhibiting effect of Sandostatin on MCF-7 cell proliferation appeared reproducible. It was found that in addition to its effect on the DNA content, Sandostatin at a concentration of 10 nM also caused a maximal decrease in the cell number and in the protein content of the cultures (Fig. 2). Based on DNA content of the cultures from three consecutive experiments, the mean inhibition of cell growth caused by the two most effective concentrations of Sandostatin were 44 ± 1% at 5 nM and 73 ± 18% ($n = 3$) at 10 nM Sandostatin.

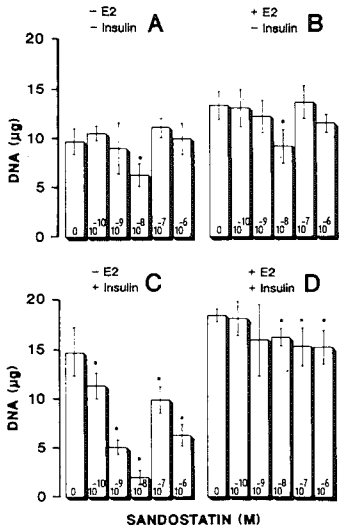

Fig. 1. Effect of Sandostatin on the growth of MCF-7 breast cancer cells. Cells were plated at a density of 4×10^4 cells/T-25 flask and were grown in the absence (*A*) or presence of added 30 pM estradiol (*E2*) (*B*), 10 μg/ml insulin (*C*), and 30 pM estradiol plus 10 μg/ml insulin (*D*). The medium was refreshed every second day, and after 6 days the cells were solubilized by incubation in 1 N NaOH as described in "Materials and Methods." Values are means ± SD (*bars*) ($n = 6$) of μg DNA per flask. *, Statistically significant difference ($P < 0.05$) from its respective control column.

In experiments in which medium was refreshed every day, it was found that a concentration of 5 nM Sandostatin caused a significantly higher inhibition of cell growth than did a concentration of 10 nM over a 6-day period (Fig. 3). This was in contrast to the observations in previous experiments when medium was refreshed every second day. This may be explained by instability or metabolism of Sandostatin during 2-day cultures or alternatively by the presence of other concentrations of autocrine growth factors. With the highest concentrations of Sandostatin used, a possible escape of cell growth inhibition is observed at day 5 (Fig. 3), suggesting desensitization of the cells.

To evaluate whether the observed inhibiting effects were not only restricted to the somatostatin analogue Sandostatin, additional experiments were performed with the natural hormone somatostatin-14 and with another analogue, CGP 15-425. Comparable effects were observed at 10 nM concentrations; 6-fold incubations with CGP 15-425 and somatostatin-14 showed 75 ± 2 and 76 ± 2% inhibition of cell proliferation measured by cell number (for both, $P < 0.02$). Again higher and lower concentrations, 1 μM and 0.1 nM, resulted in significantly less inhibition ($P < 0.02$).

In *in vivo* studies using dimethylbenzanthracene-induced mammary tumors in rats, we have also observed a bell-shaped curve of dose-response relationship, showing a critical concentration of Sandostatin which causes optimal inhibition of tumor growth. Rats were treated twice daily with five different dosages (0, 0.05, 0.2, 1, 5, and 20 μg) of Sandostatin for 3 weeks. A dose of 2 × 0.2 μg/day appeared to result in maximal inhibition (83%) of tumor growth. Dosages of Sandostatin above and

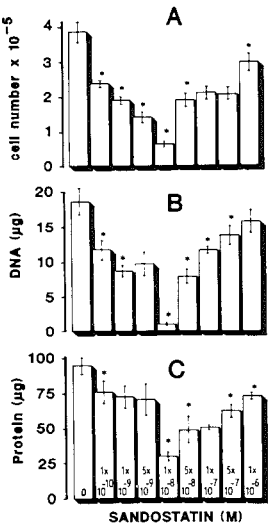

Fig. 2. Effects of increasing concentrations of Sandostatin on the proliferation of MCF-7 breast cancer cells. Cells were plated at a density of 4×10^4 cells/T-25 flask and were cultured for 6 days with medium refreshment every second day in the absence of estradiol and in the presence of added insulin (10 µg/ml). Values are means ± SD (*bars*) of 6-fold incubations. All doses of Sandostatin used caused significant ($P < 0.05$) inhibition of growth of cell cultures when expressed per cell number (*A*), DNA content (*B*), and protein content (*C*). •, statistically significant difference ($P < 0.05$) from its *left neighbor*.

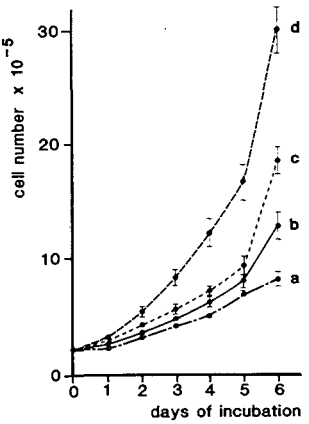

Fig. 3. Kinetics and dose-response relationships of Sandostatin on the proliferation of MCF-7 breast cancer cells. Cells were plated at a density of 2×10^5 cells/T-25 flask and were cultured with medium refreshment daily. Experimental medium consisted of RPMI 1640 supplemented with 5% steroid-depleted fetal calf serum and 10 µg/ml insulin. Cultures were grown in the absence (*d*) or presence of 5×10^{-9} (*a*), 1×10^{-8} (*b*), and 5×10^{-8} M (*c*) Sandostatin. For 6 consecutive days the cells were harvested and counted as described in "Materials and Methods." Values are means ± SD (*bars*) of triplicate incubations.

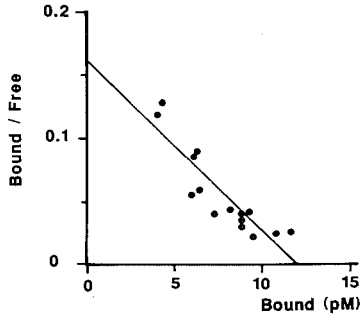

Fig. 4. Scatchard plot of ^{125}I-labeled Tyr^3-Sandostatin binding to MCF-7 breast cancer cells. Cells cultured as described in "Materials and Methods" to near confluency were incubated in suspension with increasing concentrations of ^{125}I-labeled Tyr^3-Sandostatin (25–500 pM) in the absence and presence of a 200-fold excess nonlabeled Sandostatin. Specifically bound ^{125}I-labeled Tyr^3-Sandostatin is plotted.

below 2×0.2 µg/day were less or not effective in this respect (43, 44).

The observation that Sandostatin has a direct inhibitory effect on the proliferation of MCF-7 cells in culture may imply the presence of specific receptors for somatostatin on MCF-7 cell membranes. We have therefore performed binding studies with an iodinated derivative of Sandostatin, ^{125}I-labeled Tyr^3-Sandostatin. Scatchard analysis of the binding data (Fig. 4) shows a single class of high affinity binding sites representing approximately 250 molecules/cell with a K_d of 73 pM.

DISCUSSION

In this report we show for the first time that somatostatin and two of its analogues can inhibit the growth of breast cancer cells. Because of the observed specific high-affinity binding of the Sandostatin derivative and the observation that a narrow concentration range of Sandostatin exists in which maximal inhibition of cell proliferation is achieved, it is tempting to speculate that within 1 week desensitization may occur by chronic administration of Sandostatin dosages above 10 nM as a result of downregulation of the somatostatin receptors. Recently it has been observed that desensitization of normal GH-secreting cells to Sandostatin can occur within 6–10 days in a dose-dependent manner (27). A similar phenomenon was observed with other somatostatin analogues (33, 39) and with respect to insulin secretion (25, 45). Preliminary experiments in our laboratory show indeed disappearance of the specific binding sites for ^{125}I-labeled Tyr^3-Sandostatin after culturing of MCF-7 cells for 6 days in the presence of Sandostatin (data not shown).

With regard to mechanism of action, somatostatin can act on mammary tumor cells by different ways. Somatostatin may exert its action by directly interacting with its specific receptor or by modulation of other receptors. Indirectly, it may act by decreasing the secretion of pituitary hormones and growth factors such as somatomedin C/IGF-1 and EGF. With respect to indirect actions of somatostatin via GH, interesting observations were made by Murphy *et al.* (46). Their results indicated that human GH may be a potent ligand for the lactogenic receptor in human breast cancer cells *in vitro*. Furthermore

Shiu and Iwasiow (47) showed induction of specific proteins by GH and prolactin in human breast cancer cells. The observation of increased plasma GH levels in breast cancer patients (48) may be of importance. Somatostatin may also act as a paracrine or autocrine regulator of tumor cell proliferation or by influencing secretion of autocrine or paracrine growth factors. The interaction of mammogenic peptide hormones (GH, prolactin, and insulin), steroids, and EGF with modulation of their respective receptors (27, 49–54) in addition to the possible inhibiting effects of somatostatin on the secretion of EGF and somatomedin C, suggests a very complex mechanism through which somatostatin acts *in vivo* and *in vitro*. Antagonizing effects of estradiol and somatostatin (analogues) at the level of autocrine growth factor secretion, for instance, somatomedin C, might explain our observation that the growth inhibiting effect of Sandostatin is less pronounced when estradiol is added to the medium.

In conclusion, proliferation of MCF-7 human breast cancer cells *in vitro* is inhibited by somatostatin (analogues) through direct action on the tumor cells. It is interesting to note that analogues of LHRH (14, 55–58) and somatostatin (this report) have direct inhibitory effects on tumor cell growth antagonizing *in vitro* especially the biological effects of those steroid (as estradiol by LHRH analogues) and peptide hormones (GH and insulin by somatostatin analogues) in which secretion *in vivo* is suppressed by pharmacological doses of the same neuropeptide analogues.

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LIST OF ABBREVIATIONS

TRH	= thyrotrophin-releasing hormone
LHRH	= luteinising hormone-releasing hormone
CRF	= corticotrophin-releasing factor
GRF	= growth hormone-releasing factor
TSH	= thyroid stimulating hormone
LH	= luteinising hormone
FSH	= follicle-stimulating hormone
ACTH	= adrenocorticotrophic hormone
PRL	= prolactin
GH	= growth hormone
T4	= thyroxine
T3	= triiodothyronine
E2	= oestradiol
Prog	= progesterone
(0)GTT	= (oral) glucose tolerance test
ITT	= insulin tolerance test
LVP	= lysin-vasopressin test

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CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 20 januari 1945 in Amsterdam. In 1964 behaalde hij het diploma gymnasium-β aan het Ignatius College te Amsterdam en begon zijn medische studie aan de Gemeentelijke Universiteit van deze stad. De co-assistentschappen werden doorlopen in het Wilhelmina Gasthuis, het Binnen Gasthuis en het Onze Lieve Vrouwe Gasthuis.

In mei 1972 werd de opleiding tot arts voltooid. Na het behalen van het artsexamen werd op 1 juli 1972 aangevangen met de opleiding tot internist in het ziekenhuis de Mariastichting te Haarlem (opleider J. Verwiel). Deze opleiding werd vanaf 1 april 1974 voortgezet op de afdeling Hematologie (hoofd Prof. Dr. J. Abels) en vanaf 1 mei 1975 op de afdeling Interne Geneeskunde III en Endocrinologie (hoofd Prof. Dr. J.C. Birkenhäger) van het Academisch Ziekenhuis "Dijkzigt" te Rotterdam. Op 1 juli 1977 volgde inschrijving als internist in het Specialisten Register. Daarna bleef hij tot 1 maart 1981 werkzaam als chef de clinique op de afdeling Interne Geneeskunde III en Endocrinologie. Sinds 1 maart 1981 is hij verbonden aan de Dr. Daniël den Hoed Kliniek te Rotterdam als hoofd van de afdeling Endocriene Oncologie (Endocrinologie en Biochemie) en bleef part-time (0.1) werkzaam op de afdeling Interne Geneeskunde III en Endocrinologie van het Academisch Ziekenhuis Rotterdam. Tussentijds functioneerde hij van oktober 1983 tot 1 januari 1986 tevens als tijdelijk hoofd van de Afdeling Interne Geneeskunde van de Dr. Daniël den Hoed Kliniek.