PROLACTIN AND LIVER DISEASE

prolactine en leverziekte

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CONTENTS

CHAPTER I

INTRODUCTION

Cirrhosis of the liver is associated with profound endocrinological disturbances. Until recently it was thought that these disturbances were caused mainly by ineffective elimination of hormones by the diseased liver. It is now known that the pathogenesis of disturbed hormonal function in liver cirrhosis is rather more complex, as, in many instances, it involves disturbed secretion and feedback mechanisms as well. In fact, in liver disease the metabolic clearance rate of sex-steroids for instance is not significantly altered (1,2). The most striking hormonal syndrome associated with cirrhosis of the liver is the feminization process which occurs in up to 40-50% of male patients (1,3). This syndrome is characterized by gynecomastia, a feminine distribution of hair, palmar erythema, the formation of spider nevi, disturbed gonadal function, impotence and infertility. The role of changes in androgen and estrogen metabolism in causing this syndrome has been the subject of extensive research in recent years and has been elucidated to a large extent (4,5,6,7,8,9,10,11,12). It is by no means certain, however, that the disturbed androgen and estrogen metabolism accounts for all the signs and symptoms found in this syndrome. It seemed interesting in this respect to look at possible disturbances in the synthesis, release and metabolism of prolactin (PRL). Prolactin, a polypeptide hormone, secreted by the anterior pituitary, plays a physiological role in breast development and lactation. When produced in excess it may lead to sterility, amenorrhea and loss of libido. In recent years sensitive radioimmunoassays for the determination of prolactin in plasma have become available, thus facilitating the study of prolactin metabolism and function in health and disease.

This thesis describes an attempt to elucidate the effect of liver disease on the synthesis, release and metabolism of prolactin.

REFERENCES

1. Baker, H.W.G., et al(1976)
   A study of the endocrine manifestations of hepatic cirrhosis.
   Quarterly J of Med. XLV, 145-178.
2. Olivo, J., Gordon, G.G., Rafii, F.et al (1975).
   Estrogen metabolism in hyperthyroidism and cirrhosis of the liver.
   Steroids 26, 47-56.
3. Lloyd, C.W., Williams, R.H. (1948). Endocrine changes associated with Laennec´ s cirrhosis of the liver. Amer. J.Med. 4, 315.
4. Adlercreutz, H (1974)
   Hepatic metabolism of estrogens in health and disease.
   New. Eng. J.Med. 290, 1081-1083.
5. Kley, H.K., Wiegelmann, W., Nieschlag, E.et al (1976).
   Leberzirrhose und hyperöstrogenismus. Dtsch.Med. Wschrift. 101, 1295 - 1298.
6. Galvao- Teles, A., Anderson, D.C., Burke, C.W. et al (1973).
   Biologically active androgens and estradiol in men with chronic liver disease. Lancet I, 173.
7. Gordon, G.G., Olivo, J., Rafii, F. et al (1976). Conversion of androgens to estrogens in cirrhosis of the liver. J.Clin.End.Metab. 40, 1018-1026.
8. Van Thiel, D.H., Lester, R., Sheriss, R.J.(1974). Hypogonadism in alcoholic liver disease: Evidence for a double defect.
   Gastroenterology 67, 1188-1199.
9. Chopra, I.J., Tulchinsky, D., Greenway, F.L. (1973).
   Estrogen-androgen imbalance in hepatic cirrhosis. Ann. Int. Med. 79, 198-203.
10. Green, J.R.B. (1977). Mechanism of hypogonadism in cirrhotic males. Gut 18, 843-853.

11. Lester, R., Eagon, P.K., Van Thiel, D.H.(1979). Feminization of the alcoholic: the Estrogen: Testosterone Ratio (E/T). Gastroenterology 76, 416-418.
12. Lourens, J. (1-73). Androgeen en œstrogeen metabolisme in cirrhose van de lever. Proefschrift : Utrecht.

CHAPTER 2

PROLACTIN : A REVIEW OF THE LITERATURE.

In 1928 Stricker and Grueter (1) reported that injection of cow pituitary extract induced lactation in pseudo-pregnant rabbits. Later, it was established by Riddle and Bates (2) that the adenohypophysis was responsible for crop milk production in pigeons and for mammary gland secretion in mammals.

In 1941 Astwood (3) demonstrated that the effectiveness of pituitary extracts in maintaining functional corpora lutea in rats was due to a substance, which they called lutetrophin. Although knowledge of its structure and physiological role in mammals steadily advanced, it is only since 1970 that this hormone has been definitely indentified and measured in human blood (4) as a hormone with lactogenic properties which is distinct from human growth hormone. In 1971 Friesen et al (5) developed the first radioimmunoassay of human prolactin and measured its serum concentration. Since then the study of the physiology and pathology of human prolactin has increased steadily.

In 1977 Shome and Parlow (6) finally established the complete amino acid sequence of prolactin.

BIOLOGIC ACTIONS OF PROLACTIN IN MAN.

More than a hundred different physiologic effects of prolactin have been described in vertebrates (7). These effects can be divided into five main categories:

- effects on the breast and on lactation
- effects on gonadal function
- effects on fluid and electrolyte balance
- effects on growth
- effects on adrenal function

Although a wealth of knowledge on the function of prolactin in vertebrates has been accumulated, knowledge about its specific functions in man is still deficient. As many possible actions of prolactin remain highly speculative, I will confine myself to those actions that have now been established with some certainty in man.

Effects on breast and lactation.

In contrast to the situation in several other species prolactin does not seem to be essential for the normal development of the ductal system of the human breast (8). However, prolactin, together with gonadal steroids and probably with adrenal steroids, insulin and thyroid hormones, is essential for the initiation and maintenance of lactation in humans (8,9). During pregnancy prolactin levels steadily increase (10). Breast enlargement takes place under the influence of the increased plasma concentrations of prolactin, estrogens, progesterone and human placentary lactogen. The estrogens and progesterone produced by the placenta are eliminated post partum, and this results in an acute change in the prolactin/sexhormone ratio. It is this change that leads to milk secretion (11). Lactation can be inhibited by both estrogens and the prolactin inhibitor, bromocriptine, in the post partum period (12). There is a good relationship between lactation and the prolactin response to suckling in the first days after parturition. In a group of rural African women, who

traditionally breast feed their children for 2 years or until the arrival of the next child, basal prolactin levels fall in the first 3 months, then remain stable for approximately 12 months, after which they fall to normal concentrations (13). It is probably through these elevated but slowly decreasing prolactin levels that breast feeding leads to a relative infertility and spacing of children (14), although a direct effect of the nursing stimulus is also important.

Effects on the gonadal function in the human male.
Prolactin receptors have been found in the testis, epidydimis, prostate and seminal vesicles (15,16,17). Prolactin has been suggested to influence testosterone secretion through inhibition of the conversion of testosterone to dihydrotestosterone by the enzyme 5 x reductase (18). Although not effective on its own, prolactin combined with gonadotropins was reported to enhance testosterone production (19,20) and combined with testosterone to regulate prostatic and seminal vesicular function (21). In the human male the actions of prolactin are dependent on its concentration : low or normal prolactin levels are necessary for normal testosterone secretion, but elevated prolactin levels reduce it (22). Elevated prolactin levels are associated with loss of libido and impotence, which can be succesfully treated by reducing these prolactin levels with bromocriptine (23),whereas bromocriptine has no effect on impotence without elevated prolactin levels (24).

Effects on gonadal function in the human female.
Prolactin receptors have been identified in the ovaries of different species, including humans (25). The site of action seems to be situated at the LH receptor (26). In vitro studies in human cultured granulosa cells have shown that high levels of prolactin decrease progesterone

production, and that neutralisation of prolactin with rabbit antihuman prolactin serum leads to a significant decrease in progesterone production (27). This is another example of a bell shaped dose response curve in which low/ normal concentrations of prolactin are necessary for normal granulosa cell functions but excess prolactin production leads to a block of progesterone production. Increasing levels of prolactin in the circulation result at first in a short luteal phase, then in irregular cycles and finally in amenorrhea (28). Prolactin levels are slightly higher in women than in men (29). Although many women do not show clear menstrual cycle-related changes in the plasma prolactin concentration, evidence is now accumulating that such a relationship does exist. Although the levels can be very variable because of pulsatile release, prolactin concentrations tend to be lowest in the early follicular phase of the menstrual cycle, increase in the late follicular phase to a peak around ovulation and then decrease in the luteal phase to levels which are still above follicular phase levels (30,31). These changes in PRL concentrations seem to be related to similar changes in estrogen levels but not to changes in the plasma levels of progesterone, LH or FSH. In fact, women on sequential contraceptives show elevated prolactin levels in the estrogen phase which normalize in the combined estrogen/progestagen phase (32).

Prolactin levels rise steadily during pregnancy, reaching a maximum in the third trimester (10). These increasing levels are most likely caused by the high circulating estrogen levels during pregnancy. After parturition,basal prolactin levels return to prepartum levels and, without suckling, are back to normal within two weeks. Apart from increases in serum prolactin levels very high prolactin concentrations (36-1800 ng/ml) are found in the amniotic fluid (10,33). The amniotic fluid PRL levels increase rapidly from the 12th to the 24th week of pregnancy and

tend to decrease in the last 6 weeks of pregnancy, although they remain about 10 times higher than the maternal plasma levels. There is evidence that prolactin in the amniotic fluid is secreted by decidual tissue (34), which may also explain the very high prolactin levels found in the plasma of patients with hydatiform moles. The regulation of amniotic prolactin secretion seems to be different from that of plasma prolactin, as bromocriptin during pregnancy suppresses both maternal and fetal plasma levels but does not influence amniotic fluid levels (35). The function of this amniotic fluid prolactin remains unclear, although a possible role for prolactin in the amniotic fluid osmolar balance has been suggested (36).

Effects on fluid and electrolyte balance.

Although prolactin is of great importance in the maintenance of fluid and electrolyte homeostasis in many lower vertebrate animals (7), its effect on fluid and electrolyte balance in man is unclear and controversial. Prolactin receptors in the kidney of rats and mice are concentrated in the proximal tubular cells (37,38), which may indicate a possible renal action of prolactin on sodium and water metabolism. Earlier studies indicated a possible antidiuretic effect of prolactin in humans through retention of sodium and water (39,40,41). Later studies, however, failed to show an effect of prolactin on tubular sodium and water metabolism either directly (42,43,44) or through interference with aldosterone secretion (45,46).

Effects on growth.

About half of the reported actions of prolactin in all classes of vertebrates, including mammals, deal with the effects on growth and metamorphosis of various tissues (7). In fact the action of prolactin on growth is similar to that of growth hormone itself, albeit weaker.
In humans a possible effect of prolactin on growth is much

less clear. Patients with hyperprolactinemia do not develop acromegaly, while prolactin does not seem to play a role in somatomedin production. It is therefore unlikely that prolactin plays an important role in growth in man except for a possible effect of prolactin on fetal lung maturation and surfacant production (48). It was reported that the incidence of respiratory distress syndrome was much higher in babies whose umbilical cord prolactin levels were less than 200 ng/ml (48). Other workers however have shown that normal lung maturation is compatible with very low fetal plasma prolactin levels at birth (49).

Effects on adrenal function.

The adrenals have also been found to have a very high affinity for prolactin (50). Forbes et al (51) reported in 1954 that the 24 hour urinary ketosteroid excretion was often increased in patients with the galactorrhoea-amenorrhoea syndrome. Later it was shown that a correlation exists between basal plasma prolactin and dihydroepiandrosterone-sulfate (DHEAS) levels (52). Prolonged prolactin elevation together with normal ACTH production leads to enhanced DHEAS production by the adrenal cortex (53). However no effect of prolactin suppression during pregnancy on plasma DHEAS values has been found both in mothers and newborns (54), which may indicate that prolactin is not an important regulatory factor in the synthesis of androgen precursors by the renal adrenal gland.

Prolactin probably does not play a role in the regulation of aldosterone secretion. Bromocriptine failed to prevent the potassium- induced aldosterone rise between dialysis in patients with renal insufficiency (55). Bromocriptine also did not influence aldosterone responses to changes in posture (35). The suppression of prolactin secretion by L-dopa or the stimulation of prolactin secretion with TRH did not influence aldosterone secretion (46), while

metoclopramide elevated prolactin levels but did not change aldosterone levels (56).
Prolactin has corticotropic effects in lower animal forms and is an important factor in steroidgenesis in lower vertebrates (57). However in man evidence for an effect of prolactin on cortisol secretion is scanty. Ingvarsson (58) claimed that ovine prolactin restored the adrenal responsiveness of a woman to exogenous ACTH in a patient after steroid therapy, while it has been shown that prolactin potentiates the effect of ACTH on steroid release by cultured adrenal cells and partially restores corticosterone synthesis in hypophysectomised animals (59). On the other hand shortterm administration of corticosteroids can partially suppress plasma prolactin levels in man (60), probably through interference of corticosteroids with prolactin release at a supra hypophyseal level (61).
In Cushing's disease, however, prolactin levels have been reported to be either normal (62) or slightly elevated (63).

Miscellaneous effects.
Evidence has been put forward that prolactin may play a role in the regulation of vitamin D metabolism in the kidney (64). Spanos et al found that in the chicken prolactin stimulated 1.25 dihydroxy cholecalciferol production by enzyme induction (65), while 1.25 dihydroxy cholecalciferol levels are elevated in the lactating rat (66). It is attractive to speculate about a role for prolactin in the changes in calcium absorption which take place during pregnancy and the puerperium. although direct evidence is still lacking. In this respect it is interesting that parathyroid hormone can elevate plasma prolactin when infused in normal subjects (67), while calcitonine administration (68) and calcium administration (69) have been shown to inhibit prolactin release. The latter has been suggested to occur through stimulation of hypothalamic

dopamin release (69).

Behavioural effects of prolactin.
Although a fair amount of evidence is available for the existence of a " parental behaviour " inducing effect in the fowl and in rodents (70,71) no such evidence is available at present in larger mammals or in man.

THE NATURE OF PROLACTIN
Physical properties and patterns of secretion.
In 1971 Guyda and Friesen were the first to separate monkey prolactin from growth hormone by a chromatographic method (72). Shortly thereafter the isolation and purification of human prolactin from the pituitary gland by Sephadex gel filtration and disc electrophoresis, was reported (73). Radioimmunoassays for prolactin were subsequently developed (5), which were so sensitive that reliable measurements of plasma prolactin concentrations became possible both in physiological and pathological circumstances (74).
Although the structure of animal prolactin has already been known for more than 10 years, it was only in 1977 that Shome and Parlow reported the entire linear aminoacid sequence of human prolactin, comprising 198 residues (6). The molecular weight is about 21.000 dalton and the structure shows a 73% identity of amino acid sequence when compared to ovine PRL, 77% compared to porcine and 60% compared to rat prolactin. Although prolactin has approximately the same molecular weight as human growth hormone large differences exist in the amino acid sequence as only 32 of the 198 residues are the same (16%). Gelfiltration and electrophoresis studies of purified prolactin preparations reveal substantial heterogeneity (75,76,77).
It appears that both in plasma (78) and in the pituitary (79) three molecular size forms of prolactin are present. The most important is the monomeric form which represents

approximately 75-80% of total prolactin. The second form is probably a dimer and has a molecular weight of approximately 40.000. It can be detected both in plasma and in the pituitary as up to 10-15% of total prolactin. Finally a polymeric "big,big" prolactin can be detected representing approximately 5% of the total amount in most instances. It appears that the monomeric fraction in plasma and in the pituitary increases in response to stimuli of prolactin secretion (TRH, pregnancy, puerperium) (78), while the "big,big" compound levels decrease after TRH stimulation (79). Although the monomeric form is usually predominant in prolactinomas, it appears that some tumours may preferentially secrete "big,big" prolactin (80). It is not known wheter this "big,big" form is merely a polymeric form (81) or wheter it should be considered a prohormone (79).

All forms appear to have similar receptor binding and immunological properties suggesting that the biochemical and the clinical significance of this heterogeneity is probably low.

Normal levels and patterns of secretion.

Normal levels.

At birth prolactin levels are as high as 200-300 ng/ml. They rapidly fall to a plateau of approximately 100 ng/ml which is maintained for several weeks postpartum before further declining to about 10 ng/ml at 1 year (29).

Before puberty prolactin levels are the same in males and females and do not differ from male adults (29,83). However, during late puberty a significant increase in serum prolactin levels takes place in girls probably because of the stimulatory effect of estrogens (84). In premenopausal women prolactin levels than remain (29) but tend to decrease after the menopause. Furthermore prolactin levels in both males and females decline with advancing age (85), while the prolactin response to TRH also tends to decline (86).

Levels during the menstrual cycle.
It has been reported that normally menstruating women show a consistent midcycle peak of prolactin with slightly higher prolactin levels in the luteal phase compared to the follicular phase (87). These changes are probably secondary to fluctuations in estradiol secretion and without functional meaning.

24 hour secretory pattern.
Like other pituitary hormones prolactin secretion follows a nycthemeral rhythm. The increase in prolactin secretion is not related to a day and night rhythm, but to sleep (88). Prolactin levels rise after falling asleep and fall in the morning. Furthermore intrasleep fluctuations in prolactin secretion exist and prolactin levels are lowest during rapid eye movement (REM) sleep (89), thus suggesting a causal relationship between REM sleep and the release of factors influencing prolactin secretion. Suppression of prolactin secretion during sleep does not disturb REM sleep, which suggests that prolactin does not influence normal sleep processes (90).
Apart from this diurnal secretory rhythm prolactin secretion has a pulsatory nature, characterised by the presence of frequent peaks, which increase in amplitude during sleep (88,91).

Other physiological stimuli of prolactin secretion.
Probably the strongest physiologic stimulus for prolactin secretion is suckling and nipple manipulation (4,5,92). This reflex is clearly important for the iniation and maintenance of lactation and is effected by a neuroendocrine reflex. Self stimulation of the nipples in non puerperal women usually does not elevate prolactin levels although stimulation by a sexual partner may elevate prolactin levels (92). There are some reports that prolactin levels may rise in women during sexual intercourse with

orgasm (93). A second strong physiologic stimulus for prolactin release is found by both physical and psychological stress (93,94,95) including anesthesia and minor and major surgical interventions (93,96).

CONTROL OF PROLACTIN SECRETION.

Introduction.

In contrast to other pituitary hormones, prolactin secretion is under tonic inhibitory control by the hypothalamus. In 1954 L.Desclin and Everett (93.96) demonstrated that secretion of all pituitary hormones is reduced after disconnection of the pituitary gland from the hypothalamus except for prolactin which is secreted in excess. Pituitaries transplanted under the renal capsule stop all hormone production except that of prolactin which is released. Exogenous prolactin injected in rats leads to a decrease in the circulatory prolactin concentrations, but not in animals with a pituitary gland transplanted under the renal capsule (99). When the original pituitary was left in situ in rats with pituitary transplants, it decreased in weight and prolactin content (100). This suggests that a feedback mechanism exists which is mediated through the hypothalamus. Apart from this hypothalamic factor inhibiting prolactin secretion (PIF) evidence has been presented that hypothalamic factors may lead to increased PRL secretion (PRF) (101,102). In addition high estrogen levels, wheter from exogenous or endogenous sources, stimulate prolactin secretion both in animals and in man (95,103,104) through an effect at the hypothalamic and/or at the pituitary level (105,106,107). Finally, there is increasing evidence for the existence of a neurohormonal negative feedback loop in which prolactin regulates its own synthesis and release through an effect on the activity of hypothalamic dopaminergic neurons (104,129).

The nature of the prolactin inhibiting factor(s).

Pituitary stalk transection increases prolactin secretion in man (98). As early as 1963 it had been found that the rat pituitary gland in culture continues to secrete prolactin (108), while coincubation with hypothalamic fragments or extracts decreases prolactin secretion (109,110). This suggests that the hypothalamus secretes a prolactin inhibiting factor. Later it was found that prolactin release could be inhibited in vitro by coincubation with both dopamine (DA) and also to some extent with norepinephrine (111,112,113,114), and that this effect could be prevented by DA receptor blocking drugs.

It was shown that hypothalamic dopamine is directly released into the pituitary portal capillary bed (115,116, 117) and that dopamine receptors are present on prolactin secreting cells in the pituitary gland (118,119,120). Beside these in vitro studies several in vivo studies using continuous infusions of dopamine have been performed. Dopamine infused at a rate of 4 ug/kg/min induced an immediate suppression of circulating prolactin in normal men and women (121). In a recent study by de Greef and Neill (122) it was found that pituitary stalk dopamine concentrations, within the physiologic range, significantly inhibited prolactin secretion. These and other reports (104) leave little doubt that dopamine is the most important PIF.

In 1976 Schally et al found after extraction of half a million pig hypothalami, that the fraction with the highest PIF activity contained dopamine and norepinephrine but was devoid of other polypeptides, thus suggesting that dopamine may be the only PIF (123). However other studies have provided evidence that hypothalamic extracts devoid of catecholamines, may still have PIF activity (124). Schally, using extracts from porcine hypothalami, presented evidence that gamma amino butyric acid (GABA) may also have PIF activity (125). They found that

both natural and synthetic GABA inhibited prolactin release in vitro in a dose- dependent way and that this inhibition could not be blocked by perphenazine, a dopamine agonist which reverses the prolactin inhibiting effect of dopamine. However these findings have not been confirmed in other studies in which no (126) or only a slight PIF effect (127,128) of GABA was found.

The nature of the prolactin releasing factors (PRF).
Apart from a diminished inhibition by dopamine, increased prolactin secretion may also be due to an increased secretion of hypothalamic prolactin releasing factor(s) (PRF). Valverde et al (129) extracted a porcine hypothalamic fraction able to stimulate prolactin secretion. At the same time it was discovered that thyrotropin releasing factor (TRH) stimulated prolactin release in vitro (130), thus suggesting that TRH might be a PRF. In humans TRH also leads to an immediate increase in prolactin secretion. During suckling serum PRL rises but not TSH (131), which suggests that TRH cannot be the only PRF.
TRH receptors are present on both thyrotropic and lactotropic cells in the pituitary gland (132) and administration of antiserum against synthetic TRH leads to a 50-70% decrease of both TSH and prolactin levels in rats (133). Thus, although not the only PRF, TRH seems to be important in the control of prolactin secretion. Serotonin and estrogens have also been suggested to be physiologically important PRF´s and will be discussed later. Other hypothalamic peptides and neurotransmitters implicated as stimulators of prolactin secretion, are vasoactive intestinal polypeptide (VIP), bombesin (134), substance P (135), neurotensin (136), endorphin (135,137) and enkephalins (138). As none of these substances (with the exception of VIP) has been shown to exert a stimulatory action in vitro, the stimulatory effects of these substances are probably indirect via a hypothalamic action. VIP stimulates prolac-

tin secretion after systemic administration (139) and has been found in hypophysial stalk blood (140). Reports of a prolactin-release stimulating effect are contradictory (139,141,142), although in one study the VIP concentration necessary to stimulate prolactin release in vitro was within the physiologic range (142). Therefore, VIP is possibly a physiological PRF in addition to TRH.

Serotonin.

It appears that serotonin stimulates prolactin release in both rat and man (101, 143,144). In the rat no effect of serotonin on prolactin release has been found in vitro (145).

Inhibition of serotonin synthesis by parachlorophenylalanine blocks the prolactin peak following lactation in rats (146). From these and other studies (145,147)it seems reasonably certain that in rat and perhaps also in man serotonin influences prolactin release at a supra hypophyseal level, probably the hypothalamus. Research in this field has provided a lot of conflicting data using serotonin antagonists.

However the specificity of action of many of these compounds remains doubtful (145). Thus Lamberts and McLeod (145) were able to show that methysergide , a compound used as a serotonin antagonist in many studies, can act in different ways : as a serotonin antagonist, a dopamine agonist and a dopamine antagonist.

Systemic injection of serotonin does not lead to PRL release, presumably because it cannot pass the blood brain barrier. Also, administration of the serotonin precursors L-tryptophan and 5-hydroxy-tryptophan (5-HTP) in humans led to conflicting results. Although some studies failed to find a modification of basal prolactin secretion by L-tryptophan and 5- HTP given orally (148,149), others provided evidence that intravenous administration of L-tryptophan (150) and 5-HTP (151,152) does elevate plasma prolac-

tin levels. It thus seems likely that the mode of administration influences the results. Although some influence of serotonin in human prolactin metabolism now seems certain, its relative importance as a PRF is still to be established.

Estrogens.

In 1909 Erdheim and Stumme (153) showed that the increase in pituitary weight during pregnancy, was entirely due to an increase of lactotropic cells. Later is was found that prolonged administration of estrogens led to pituitary enlargement (154) and to an increased mitotic rate of the lactotropic cells (104), while this effect could be blocked by the dopamine agonist bromocriptine.

It has also been established that estrogens stimulate prolactin release in male and female agonadal subjects (95, 103) and elevate basal prolactin levels in normal menstruating women (155), while the antiestrogen tamoxifen decreases plasma prolactin levels in normal women (156). In vitro, animal and human studies have provided evidence that estrogens significantly enhance the stimulating effect of TRH on prolactin secretion (101,157,158,170). This suggests that estrogens exert their effect in part via a direct influence on the receptor for TRH on the lactotropic cells. It was also shown that estradiol-17^{B} has a direct effect on prolactin secretion in rat pituitary cell cultures (159,160). Finally estradiol receptors have been found on rat pituitary lactotropic cells (161). All these findings support the proposition that estrogens influence prolactin release in part through a direct effect on the pituitary itself, perhaps through an estrogen induced reduction in the capacity to incorporate dopamine into prolactin secretory granules (168) or an estrogen induced reduction in the capacity of dopamine to stimulate lysosomal activity in the anterior pituitary gland (169). However, strong evidence also indicates an effect

of estrogens on prolactin release at a higher hypothalamic level (162,163). In rats the estrogen-induced prolactin rise can be blocked by bromocriptine administration (104), while incubation of rat anterior pituitary cells with estradiol-17B leads to the reversal of the inhibitory effect of ergocornine, a dopamine agonist (105). Estrogens also induce a fall in hypophysial stalk plasma dopamine levels (164). An excessive rise of prolactin after TRH and ether stress in estradiol-primed rats has been described (165), probably as a result of a lowered dopamine secretion from the hypothalamus (165,166,167). These findings suggest that the stimulation of PRL release by estrogens and the enhancement of the TRH-induced prolactin release by estrogens is effected by interference with the inhibitory effect of hypothalamic dopamine.

Conclusion:
It appears that prolactin secretion is governed by many factors. A constant release of PIF normally prevents prolactin release. This PIF is almost certainly dopamine; furthermore, mating, suckling and estrogens lead to a sustained prolactin release through a not completely understood mechanism although more and more evidence points to an effect at the hypothalamic level, probably through interference with the dopaminergic inhibition of prolactin release. In contrast minor stress and also the administration of TRH lead to relatively small and short lived prolactin bursts, probably by overriding PIF by prolactin releasing factors, one of which may be TRH.

DRUGS WHICH AFFECT PROLACTIN SECRETION.
A great many drugs have been found to influence prolactin secretion in vivo, either positively or negatively (table I). In many instances the stimulation of prolactin release is effected by blocking dopamine action, interfering with catecholamine/dopamine synthesis or depletion of

catecholamine stores.

Drugs which stimulate prolactin release.

Phenothiazines. (chlorpromazine, perphenazine).

Serum prolactin levels rise immediately after i.v. injection of perphenazine (171,172). This rise is caused by a blockade of the dopamine induced inhibition of prolactin release (110), either through an effect at the hypothalamic level (171,172), or directly on dopamine receptors at the pituitary gland (110).

Butyrophenones (haloperidol, pimozide).

Both haloperidol and pimozide block neurotransmission at dopaminergic receptors through competition with dopamine. It appears that the effect is dose related. In a low dosage both drugs stimulate prolactin release in vivo but not in vitro. In higher dosages the in vitro effect increases (173). In still higher dosages both drugs seem to block the binding of dopamine or its receptor completely and start to function as dopamine agonists themselves (173,174).

Procaine derivates (sulpiride, metoclopramide).

Sulpiride.

When administrated intravenously sulpiride quickly leads to a sustained increase in serum prolactin levels (6), probably through a direct blocking effect on the activity of dopamine on the pituitary lactotropic cells (176).

Metoclopramide.

Metoclopramide is widely used as an antiemetic and peristalsisstimulating drug. Like sulpiride it leads to a prompt and sustained rise in prolactin levels (178) by a specific blocking effect on dopamine receptors in the pituitary gland (179,180).

Antihypertensives (alpha methyldopa, reserpine).

Alpha methyldopa administration leads to increased prolactin levels by competitive inhibition of dopamine synthesis at the hypothalamic level, while reserpine increa-

ses prolactin levels indirectly through depletion of catecholamine stores in the central nervous system.

Hormones and hormone antagonists.

As has been discussed earlier thyreotropin releasing hormone and estrogens both cause hyperprolactinemia through different mechanisms. The effect of sex steroids and their antagonists has also been investigated. The antiestrogens lead, as can be expected, to a fall in prolactin levels and thus will be discussed later. It has been found that testosterone has no effect on prolactin release but its antagonist cyproterone acetate causes hyperprolactinemia in normal individuals (181) and in precocious puberty (182) through a still unclarified mechanism.

Finally, chronic use of spironolactone (aldactone) can be associated with gynecomastia and elevated prolactin levels perhaps as a result of increased estrogen levels (183).

Opiates.

Morphine (184) and brain opiates (185) stimulate prolactin release in man, while this effect can be blocked by the opiate receptor blocker naloxone (Narcan) (137,186). It has been found that morphine acetate does not influence prolactin release by the pituitary in vitro (187,188), thus suggesting an action at the hypothalamic level. The endogenous opioids B- endorphin, methionine-enkephaline and leucine-enkephaline have also been reported to elevate prolactin levels in rats (137,189).

Despite reports to the contrary (190) endogenous opioids do not appear to influence prolactin release directly in vitro (137,187) and probably act at an higher CNS level.

Histamine-2 receptor blocking agents (metiamide, cimetidine).

Recently it has been found that metiamide (191) and cimetidine (192,193,194) significantly increase prolactin levels in man. Although the mechanism of action is unknown it has been suggested that they may act through a blockade of brain H2 histamine receptors (194). In support of these

findings treatment with cimetidine can be accompanied by gynecomastia in a small percentage of patients (195).

Drugs which inhibit prolactin release.

Dopamine receptor stimulating drugs.

L-Dopa.

In rats with pituitary grafts, parenterally administered L-Dopa leads to a decrease in serum prolactin levels (196). The manner in which L-Dopa exhibits this effect is still a subject of controversy. L-Dopa may either inhibit prolactin secretion through a direct effect on the pituitary (197,198) or through an effect on the pituitary after decarboxylation to dopamine (199). There is some evidence that L-Dopa inhibits prolactin release but not its synthesis by the pituitary gland (200). Finally some reports haven't claimed that L-Dopa decreases serum prolactin levels by stimulating the uptake of the hormone in peripheral tissues (201,202).

Ergot drugs.

Although ergotamine itself (203) does not influence prolactin levels other ergot derivates like ergocornine and ergocryptine do decrease prolactin levels significantly (203,204), by direct inhibition of prolactin release by the pituitary gland (205,206). This effect can be blocked completely by dopamine receptor blocking agents (207). The long-acting synthetic ergot derivate 2 bromo-alpha-ergocryptine (bromocryptine) is so succesful in lowering prolactin levels that it has become the drug of choice in the treatment of hyperprolactinemia.

In patients with prolactinoma s or functional hyperprolactinemia, prolactin levels can be normalized in more than 90% of cases by low doses (10 mg/24 hours) of bromocriptine. The newer dopamine receptor agonists peribedil (208) lisuride (hydrogen maleate) (209) and lergotrile mesylate (210) all directly inhibit prolactin release through an effect at the dopamine receptor level in the pituitary approximately in the same way as bromocriptine.

Table I

List of drugs known to influence prolactin release in vivo.

Stimulators	Inhibitors
Neuroleptics	L-Dopa
Phenothiazines	
Butyrophenones	Ergot drugs
Procaine derivatives	Bromocriptine
Metoclopramide	Lergotrile
Sulpiride	Lisuride
	Metergoline
Antihypertensives	Non Ergot drugs
x-methyl dopa	Apomorphine
reserpine	Peribedil
Hormones and antagonists	Anti Estrogens
TRH	Tamoxifen
Estrogens	Clomifen
Cyproterone acetate	Vit B_6 (pyridoxine)
aldosterone antagonists	
Opiates	
Morphine	
Brain opioids	
Others	
Cimetidine	
Metiamide	

Non ergot drugs.

Apomorphine.

Apomorphine inhibits prolactin release in vivo and vitro directly at the pituitary level through dopamine receptor stimulation (211) in both normal controls and in patients with hyperprolactinemia (212).

Antiestrogens (clomiphene, tamoxifen).

The effect of Clomiphene, an estrogen receptor blocking agent, on prolactin release is unclear. Reports on a inhibiting effect of this drug on puerpueral lactation are contradictory (213,214,215).

The nonsteroidal antiestrogen tamoxifen lowers basal but especially TRH stimulated prolactin levels in both animals (216,217) and man (156,218,219) and has been shown to inhibit lactation in puerperal women (219). The antiestrogenic effect is exerted by competition at the estrogen receptor (220) and estrogen levels may even rise during administration (156). In rats an inhibiting effect of tamoxifen on pituitary tumor growth and prolactin synthesis by the pituitary gland has been demonstrated (216,217), probably due to an inhibiting effect at the pituitary estrogen receptor (221). It has been shown in vitro in dispersed pituitary tumor cells that tamoxifen does not influence prolactin secretion directly but enhances the sensitivity of these cells to dopamine and to bromocriptine (222). This enhancing effect can be prevented by coincubation with estradiol-17B. Tamoxifen in addition prevents the stimulatory effect of TRH on prolactin secretion (222).

PERIPHERAL METABOLISM OF PROLACTIN AND METABOLIC CLEARANCE.

Studies with iodinated prolactin in animals (mainly rats) have shown specific binding of prolactin in many tissues including the ovary, mammary gland, uterus, kidney, liver, muscle, adrenal cortex, pituitary and brain (17,37,223,

224,225,226,227).
Surprisingly the tissues showing the highest binding were the liver and the kidney (17,37,225,226). In the kidney radioactivity was concentrated in the proximal tubular epithelial cells (17,37,38,228), while in the liver radioactivity was distributed diffusely throughout the liver lobule. Within the hepatocyte there appeared to be a special preference for the Golgi apparatus (262). The liver receptors for prolactin were found to be separate from the receptors for growth hormone (229). Both receptor binding and a significant clinical effect of Prolactin have been observed in the mammary gland, the ovary and the adrenals. The highest affinity for prolactin, however, has been found in the liver and the kidney, organs on which a clinical effect of prolactin is less obvious.
However it does not follow automatically from these binding studies that prolactin exerts a specific effect on these tissues and it may merely indicate that both the liver and the kidney are important in prolactin degradation. However it does not seem likely that prolactin receptors sites in the liver are only associated with the degradation of the prolactin molecule (226,230), as prolactin may be involved in the synthesis of RNA (231), free fatty acids (232), somatomedin production (233) and maintenance of its own receptor levels (234). Finally, in contrast to growth hormone, no significant elimination by the liver of prolactin was found in normal men during hepatic vein catheterization studies (240).
Prolactin receptors in the kidney have been studied less well. Prolactin seems to be concentrated in the proximal tubules. It appears that prolactin gains access to the epithelial cells via the glomerular filtrate (228). Although a specific action of prolactin on kidney metabolism and osmoregulation has been claimed in man (41), these results have not been confirmed (43,45,235,238); however, specific prolactin receptors in the kidney have

recently been described in the rat (38).
The presence of specific binding sites for prolactin in the kidney again does not prove that the hormone has a physiological effect on osmoregulation and may merely indicate that the kidney is important for the clearance of prolactin from the circulation, in the same way as has been shown for placental lactogen (236), parathormone (253), insuline, C Peptide (254), gastrin (255), glucagon (256) and vasopressine (257). Further support for this concept is provided by the fact that in renal disease, hyperprolactinemia only occurs when renal function is impaired and by the fact that the degree of increase in prolactin levels correlates directly with the degree of impairment of renal function (237,258), while prolactin levels can be normalized by transplantation (259). Chronic hemodialysis, however, does not seem to influence prolactin levels (260,261). Since only minute amounts of prolactin can be detected in the urine (239) even in patients with prolactinomas and very high serum prolactin levels (Bauer et al, unpublished observations), it is probable that prolactin is broken down in the proximal tubular cells after resorption from the glomerular filtrate.

Metabolic clearance rate.

Hormone levels in the circulation are determined both by production and clearance rates. Usually metabolic clearance rates (MCR) are determined by the constant infusion to equilibrium technique (241). In this way the MCR of other polypeptides hormones like growth hormone (242,243), luteinizing hormone(244), follicle stimulating hormone (245), thyrotropin (246) and gonadotropin releasing hormone (247), have been determined.
Because native human prolactin was not available in the past, studies of the metabolic clearance rate of prolactin in humans have only been performed recently. Using this

technique however, several studies in rats established MC rates in that animal (248,249,250,251).Kock et al (248) found no difference in MCR in male and female rats. They also did not find an influence of ovariectomy, hypophysectomy, or the stage of the estrous cycle on the MCR of prolactin. These findings were later confirmed by Jacobi and Lloyd in male rats (249). Grosvenor and Whitworth (250) also did not observe a difference in MCR between lactating and nonlactating animals. The same authors described an increase of the metabolic clearance of prolactin when the infusion rate was increased (251), suggesting an ability to clear greater amounts of prolactin as the plasma concentration increases. In 1979 Cooper et al (252) finally described the MCR of prolactin in humans. The MCR in women was 45 $\pm$ 1 ml/min per m^2 and 44 $\pm$ 3 ml/m^2 in men, and was highly correlated with body mass. They also found that the MCR was slightly lower in the hyperprolactinemic patients (40 $\pm$ 5 ml/min per m^2) and that dopamine infusion did not substantially alter the prolactin MCR.

PRL production rates (PR) were not significantly different in males and females (187 $\pm$ 44 ug/day per m^2, and 211 $\pm$ 74 ug/day per m^2 respectively) while the PR was extremely high in hyperprolactinemia due to prolactinoma (PR = 31.000 $\pm$ 29.000 ug/day per m^2, n = 9). Dopamine infusion decreased prolactin PR from 270 to 66 ug/day per m^2, which indicates that its effect is on secretion rather than clearance.

HYPERPROLACTINEMIA.

Hyperprolactinemia may be one of the most common endocrine disorders in clinical practice. In unselected patients with secondary amenorrhea the incidence of hyperprolactinemia is between 13 and 20% (263,264,265). Similar figures have been reported on the incidence of hyperprolactinemia in anovulatory syndromes (264,265,266,267,268). Patients

may present with such diverse symptoms as visual field defects, headaches, amenorrhea, uterine bleeding, galactorrhea, infertility with or without amenorrhea and impotence and may thus be seen by specialists in many non-endocrine fields.

Clinical presentation in the female.

The clinical presentation of hyperprolactinemia in the female is related to the disturbed hypothalamic pituitary gonadal axis, with irregular menstruation, short luteal phase, anovulation, or amenorrhea as the presenting symptoms. As mentioned earlier, the incidence of hyperprolactinemia in amenorrheic and anovulatory syndromes may be in the order of 20%. In these patients ovulation and menstruation may be restored by treatment with the prolactin secretion inhibiting drug bromocriptine (269). The next common clinical feature of hyperprolactinemia is galactorrhea,either spontaneous or only on breast pressure (270,271). Galactorrhea has been found in up to 40% in patients with hyperprolactinemia (263,265).

Clinical presentation in the male.

Impotence, decreased libido, infertility, galactorrhea and delayed puberty may be the presenting symptoms in male patients with hyperprolactinemia. Elevated serum PRL levels were found in 17% of patients with impotence (272), while Thorner et al found impotence in allmost all hyperprolactinemic men studied by them (273).

In the former study the beneficial effect of bromocriptine treatment was not higher than that of placebo.In long standing hyperprolactinemia plasma testosterone levels may be suppressed (273,274), while the serum PRL levels in a large group of infertile men were found to be significantly higher than in infertile controls (275). In this study hyperprolactinemia was found to interfere with spermatogenesis. Others have reported normalization of sperma-

togenesis after bromocriptine treatment (276).Finally, Thorner et al reported galactorrhea in one third of men with hyperprolactinemia (273), but gynaecomastia was less frequent.

Causes of hyperprolactinemia.

Increased serum PRL levels are found in a number of clinical conditions (table II). The most common causes are the use of psychotropic and anti-hypertensive drugs as discussed earlier and pituitary (micro) adenomas, either through autonomous prolactin secretion or pressure of an upward growing tumor on the pituitary stalk. Infiltrative processes of the hypothalamus also may lead to hyperprolactinemia through interference with the hypothalamic-hypophyseal portal circulation and thus to disruption of the tonic inhibition of prolactin secretion. Primary hypothyroidism may also cause hyperprolactinemia (277), which can be normalized by treatment with thyroxine (267). When prolactin levels do not return to normal after thyroxine treatment a concomittant PRL secreting pituitary adenoma should be suspected. However, since abnormalities of the sella may regress under a thyroxine treatment, the diagnosis of such a tumor may be difficult (278,279,280).
The hyperprolactinemia in chronic renal disease and in chronic liver disease will be discussed in separate chapters.

Table II

Causes of elevated serum PRL concentrations.

- prolactin secreting pituitary tumors
- non-prolactinomas pressing upon the pituitary stalk
- infiltrative processes in the hypothalamus
  - histiocytosis, sarcoidosis
    metastatic tumors, leukemia
- target endocrine gland failure
  - hypothyroidism
  - hypoadrenalism
- polycystic ovarian syndrome
- chronic renal failure
- chronic liver failure
- drugs

Hyperprolactinemia and chronic renal disease.

The kidney may be an important clearing organ for prolactin (237). In patients without renal disease a 16% transrenal arterio-venous prolactin concentration difference was established. It is therefore not surprising that several studies reported the occurence of hyperprolactinemia in chronic renal disease (95,237,238,259,260,281). Furthermore a significant relationship between plasma creatinine and prolactin concentrations was found (237), while elevated prolactin levels return to normal after kidney transplantation (259).

Hemodyalisis does not influence prolactin levels (238, 261) and a disturbance of prolactin secretion may also exist (259,282,283), in view of the fact that in chronic renal disease both the responsiveness to TRH stmulation as well as the suppression by L-dopa and dopamine infusions is diminished (258,259,261,283).

Hyperprolactinemia and chronic liver disease.

After a report that the number of prolactin secreting cells in the anterior pituitary was increased in approximately a half of patients (286) with Laennec's cirrhosis, several studies reported elevated prolactin levels in this condition (287,288,289,290,291,292,293,294).

The hyperprolactinemia is usually moderate (290,291,292) and in fact plasma prolactin levels may be normal (292, 295,296,297,298,299,300). On the other hand many, although not all, studies report exaggerated prolactin responses to TRH (289,290,291,292,294,300,301). The interpretation of these contrasting findings is further complicated by the fact that several drugs, often prescribed in such patients, are able to stimulate prolactin release. Several reports do not differentiate between alcoholic cirrhosis and alcoholism per se (288) or between alcoholic and non-alcoholic liver cirrhosis (290). Both elevated (302,303) and normal (304) plasma prolactin levels have been des-

cribed after acute and chronic alcohol intake. Some authors report higher plasma prolactin concentrations in alcoholic liver cirrhosis compared to liver cirrhosis of other etiology (291); these differences are more prominent after TRH stimulation (289,291,294,300). Morgan et al (292) however did not find a statistically significant differenbe between plasma prolactin levels in alcoholic and non-alcoholic liver cirrhosis. Generally prolactin levels seem to be higher in relation to the extent of the liver parenchymal damage, although this is not invariably so. Morgan et al (292) found hyperprolactinemia in 4% of patients with alcoholic steatosis and in 16% of patients with alcoholic hepatitis and cirrhosis. Wernze et al (291) found hyperprolactinemia in 27% of patients with cirrhosis without ascites but in 47% of patients with ascites, while van Thiel et al (293) found a tendency towards a relationship between liver function parameters and basal plasma prolactin levels. Langer et al (301) found higher plasma prolactin concentrations in decompensated liver disease than in compensated liver disease, while the response to TRH was significantly increased. Others, however, failed to find a direct relationship between liver function parameters and circulating prolactin levels (292,294). Serum prolactin concentrations are higher in cirrhotic patients with hepatic encephalopathy (305) compared to cirrhosis without encephalopathy and they are also found to be elevated in the hepatic encephalopathy of Reye´s syndrome (306). The relationship between hyperprolactinemia, liver cirrhosis and gynecomastia is also controversial. Some studies report a direct relationship between plasma prolactin levels and gynecomastia (288,289); others however, could not confirm these results (291,292,296). Certainly not all patients with gynecomastia have elevated prolactin levels (292) nor do all patients with hyperprolactinemia develop gynecomastia. It seems likely therefore that hyperprolactinemia plays a role in the

development of gynecomastia only in cooperation with other factors, such as a disturbed estrogen/testosterone balance, which has been found to correlate positively with gynecomastia in chronic liver disease (307,308,309). It is interesting in this respect that a disturbed estrogen/testosterone balance with increased estrogen levels and decreased testosterone levels has been found to increase PRL receptor activity (284,285). The role of prolactin in gonadal insufficiency occurring in liver cirrhosis is also not clear. Hyperprolactinemia leads to hypo or amenorrhea and libido disturbancies irrespective of gonadotropin secretion (310), while long-term hyperprolactinemia leads to a decrease of plasma testosterone levels (311).
In conclusion, plasma prolactin levels may be mildly elevated in liver cirrhosis and often show an exaggerated response after TRH stimulation. Hyperprolactinemia seems to be correlated with the severity of liver parenchymal function loss and is especially notable in patients with cirrhosis and ascites and in patients with hepatic encephalopathy. Hyperprolactinemia occurs more readily in alcoholic cirrhosis than in other forms and can be caused by alcoholism alone. Although possibly a factor in the development of gynecomastia and hypogonadism of liver cirrhosis, its exact role remains unclear.

The mechanism of hyperprolactinemia in liver cirrhosis.
Elevated hormone levels may be caused by a decreased metabolic rate, and increased secretion rate or both. Very little is known about the metabolic clearance rate of prolactin in liver cirrhosis. However, the kidney seems to be important in prolactin clearance (237) while in normal man no hepatic extraction of prolactin is found (240); This suggests that liver disease may not greatly affect prolactin clearance. Also, the exaggerated rise of prolactin after TRH and the loss of the diurnal changes in plasma prolactin levels (213) primarily indicate an abnormali-

ty in the control of prolactin secretion. Prolactin secretion is mainly regulated by tonic hypothalamic inhibition through dopamine and seems to be further subject to the stimulatory influences of hypothalamic releasing factors and estrogens. Circulating estrogens are elevated in liver cirrhosis both through an increased peripheral aromatization of testosterone via androstenedione (309,314,315) and to a lesser extent through a decreased elimination by the liver (316). As estrogens stimulate prolactin release both through interference with dopamine from the hypothalamus, and through a direct effect on the anterior pituitary (103,104,105,313), it is possible that hyperprolactinemia in liver cirrhosis is caused by elevated estrogen levels. On the other hand, the increased prolactin levels may be caused by a disturbance of the hypothalamic inhibitory and stimulating factors. It is interesting in this respect that plasma prolactin concentrations are more elevated in cirrhosis with hepatic encephalopathy. In this condition changes in amino acid balance may lead to changes in central neurotransmitter systems.

Several authors have suggested that in hepatic encephalopathy false neurotransmitters (e.g.octopamine instead of dopamine) might be formed due to a change in the ratio of plasma aromatic amino acids to branched chain amino acids (317,318). This theory is based on evidence that in hepatic encephalopathy aromatic amino acids (e.g. tryptophan and tyrosine) are increased in relation to the branched chain amino acids. As these amino acids all compete for the same carrier through the blood-brain barrier (319), this will result in an excess of aromatic amino acid/neuro transmitter precursors in the brain. Because tyrosine is the precursor for catecholamine synthesis and tryptophan the precursor for serotonin synthesis these changes are expected to influence neurotransmitter levels substantially. It appears that excess tyrosine in the brain cannot be handled in the normal way because of rate

limiting enzymes, and octopamine is formed instead of dopamine (317). On the other hand elevated levels of 5-hydroxyindole acids in the cerebrospinal fluid indicate an increased production and turnover of serotonin (320,321) A relationship has been established in liver cirrhosis between free plasma tryptophan levels and prolactin levels (290). It is therefore likely that both decreased dopamine levels and/or increased serotonin levels play a role in the hyperprolactinemia of liver cirrhosis, especially in hepatic encephalopathy. This is further substantiated by the fact that the administration of both the dopamine agonists bromocriptine (322) and L-dopa (323) have been found to result in a dramatic clinical improvement of hepatic encephalopathy, with disappearance of coma.
In conclusion, although not much is known about the metabolic clearance rate of prolactin in liver cirrhosis, it is expected that the MCR will not be greatly affected in liver cirrhosis. On the other hand the regulation of prolactin secretion seems to be disrupted, probably through the combined effect of increased estrogen levels, increased hypothalamic serotonin levels and decreased hypothalamic dopamine levels.

This present study was undertaken to bring more clarity in the mechanism of hyperprolactinemia in chronic liver disease. In order to do this we formulated the following 5 questions.

1. what is the relative importance of the liver in the elimination/degradation of prolactin in liver disease and is prolactin degradation decreased in chronic liver disease.
2. Is prolactin degradation in chronic liver disease different from that of hormones of comparable molecular weight.

3. Is there evidence of a disturbance of central prolactin release and if so what is the relative importance of increased estrogens and/or impaired dopamine inhibition.
4. Is the hyperprolactinemia of liver cirrhosis caused by the decreased parenchymal function or by the concomittant portal hypertension with porto-systemic shunting?
5. Is the hyperprolactinemia of compensated liver cirrhosis caused by factor´s different from those that cause the prolactinemia of hepatic encephalopathy ?

REFERENCES

1. Stricker, P., Grueter, R. (1928).Action du lobe antérieur de l´hypophyse sur la montée laiteuse. Compt. Rend. Soc. Biol.99, 1978-1980.
2. Riddle, O., Bates, R.W. (1939).The preparation, assay and actions of lactogenic hormone. In: Sex and internal secretions. Ed: A.E.Williams, Wilkins (Baltimore) 1088-1117.
3. Astwood, E.B. (1941). The regulation of corpus luteum function by hypophysial lutetrophin. Endocrinology, 29, 309-319.
4. Frantz, A.G., Kleinberg, D.L. (1970). Prolactin: evidence that it is separate from growth hormone in human blood. Science : 170: 745-747.
5. Hwang, P., Guyda, H., Friesen, H. (1971). A radioimmunoassay for human prolactin. Proc. Nath.Acad. Sci. U.S.A.68, 1902-1906.
6. Shome, B., Parlow, A.F. (1977).Human pituitary prolactin : the entire linear amino acid sequence. J.Clin. Endocrin.Metab. 45, 1112-1115.
7. Nicoll, C.S. (1974).Handbook of physiology. Section 7 Endocrinology, vol.IV. The pituitary gland part II. American Physiological society, Washington.pp 253-291
8. Jacobs, L.S. (1977). The role of prolactin in mammogenesis and lactogenesis. Adv.Exp.Biol.Med.80, 173-191.
9. Schanss, D (1976). Hormonal control of lactation. Ciba Foundation Symposium 45, 27-48.
10. Biswas, S., Rodeck, C.H. (1976). Plasma prolactin levels during pregnancy. Br.J.Obstet.Gynaecol. 83, 683-687.
11. Aono, T., Shioji, T.,Shoda, T.et al (1977). The initiation of human lactation and prolactin response to suckling. J.Clin.End. Metab. 44, 1101-1106.

12. Del Re, R.B., Del Pozo, E., De Grandi, P. et al (1973) Prolactin inhibition and suppression of puerperal lactation by a BR-ergocryptine (CB154), a comparison with Estrogen.
Obstet. Gynaecol. 41, 884
13. Delvoye, P., Delogne-Desnoeck, J., Nyampeta, U.et al (1977). Time-course of physiological hyperprolactaemia during two years lactation. Clin.Endocrinol.7, 257-259.
14. Thomson, M.O., Hytten, F.E., Black, A.E. (1975). Lactation and reproduction. Bull.WHO 1975, 52, 337-349.
15. Aragona, C., Bohnet, H.G., Friesen, H.G. (1977). Localisation of prolactin binding in prostate and testis: the role of serum prolactin concentration on the testicular LH receptor.
Acta Endocrinol.84, 402-409.
16. Charreau, F.H., Athamal, A., Torjeron, P.A. et al (1977). In: The testis in normal and infertile man. Ed:Troen, P. en Nankin, H.R. Raven Press, New York, p. 387.
17. Costlow, M.E., McGuire, W.L. (1977). Autoradiographic localization of the binding of ^{125}I-labelled prolactin to rat tissues in vitro. J.Endocrinol.75, 221-226.
18. Magrini, G., Ebiner, J.R., Burckhardt, P.et al (1976). Study on the relationship between plasma prolactin levels and androgen metabolism in man. J.Clin. End. Metab. 43, 944-947.
19. Bohnet, H.G., Friesen, H.G. (1976). Effect of prolactin and growth hormone on prolactin and LH receptors in the dwarf mouse.
J.Reprod.Fert. 48, 307-311.
20. Hafiez, A.A., Bartke, A., Lloyd, D.W. (1972). The role of prolactin in the regulation of testis function. The synergic effects of prolactin and luteinizing hormone on the incorporation of ^{14}C-acetate into testosterone and cholesterol by testes from hypophysectomized rats

in vitro.
J.Endocrinol. 63, 223-230.
21. Negro-Vilar, A., Saad, W.A., McCann, S.M. (1977). Evidence for a role of prolactin in prostate and seminal vesicle growth in immature male rats. Endocrinol. 100, 729-737.
22. Horrobin, D.F. (1978). Prolactin : effects and clinical significance. Annual research reviews, volume 6. Churchill : Livingstone.
23. Wass, J.A.H., Thorner, M.O., Morris, D.V. et al (1977) Long term treatment of acromegaly with bromocriptine. Br. Med. J. I, 875-879.
24. Cooper, A.J.(1977). Bromocriptine in impotence. Lancet 2, 567.
25. Saito, T., Saxena, B.B. (1975). Specific receptors for prolactin in the ovary. Act. Endocrinol.80, 126-137.
26. Holt, J.A., Richards, J.S., Midgley, A.R. et al (1976) Effect of prolactin on LH receptor in rat luteal cells. Endocrinol.98, 1005-1013.
27. McNatty, K.P., Sawers, R.S., McNeilly, A.S.(1974). A possible role for prolactin in control of steroid by the human graffian follicle. Nature 250, 653-655.
28. Robijn, C.M., Vekemans, M., Delvoye, P.et al (1976). Prolactin and fertility control in women. In: Growth hormone and related peptides. Ed: Pecile and Müller. Excerpta Medica, Amsterdam, p. 396-406.
29. Guyda, H., Friesen, H.G. (1973) Serum prolactin levels in humans from birth to adult life. Ped. Res. 7, 534-540.
30. Franchimont, P., Dourcy, C., Legross, J.J. et al (1976) Prolactin levels during the menstrual cycle. Clin. Endocrinol. 5, 643-650.
31. Vekemans, M., Delvoye, P., L'Hermite M., et al(1977). Serum prolactin levels during the menstrual cycle. J.Clin.End. Metab. 44, 989-993.

32. Reymond, M., Lemarchaud-Berand, T. (1976).Effects of oestrogens on prolactin and thyrotrophin responses to TRH in women during the menstrual cycle and under oral contraceptive treatment. Clin. Endocrinol. 5, 429-437.
33. Clements, J.A., Reyes, F.I., Winter, J.S.D. et al (1977). Studies on human sexual development IV. Fetal pituitary and serum, and amniotic fluid concentrations of prolactin.
J.Clin.End.Metab. 44, 408-413.
34. Riddick. D.H., Kusmick, W.F. (1977) Decidua: a possible source of amniotic fluid prolactin. Am.J.Obstet. Gynaecol. 127, 187-190.
35. Del Pozo, E., Danagh, A., Lancranjan, I.et al (1977). Effect of Bromocriptine on the endocrine system and fetal development.
Clin.Endocrinol. 6, suppl. I, 47-56S.
36. Jasimovick, J.B. (1977) In: prolactin and human reproduction. Eds: Crosignani, P.G., Robijn, C. Academic Press, London-New York, p 27.
37. Rajaniemi, H., Oksanen, A., Vanka-Pertulla, T.(1974) Distribution of ^{125}I-prolactin in mice and rats. Studies with wholebody and microautoradiography. Horm. Res. 5, 6-20.
38. Mountjoy, K., Cowden, E.A., Dobbie, J.W. et al (1980). Prolactin receptors in the rat kidney. J.Endocrinol. 87, 47-54.
39. Horrobin, D.F., Lloyd, I.J., Lipton, A. et al (1977). Actions of prolactin on human renal functions. The Lancet I , 352-354.
40. Buckman, M.T., Peake, G.T. (1973) Osmolar control of prolactin secretion in man. Science 181, 755-757.
41. Buckman, M.T., Peake, G.T., Robertson, G. (1976). Hyperprolactinemia influences renal function in man. Metabolism 25, 509-516.
42. Adler, R.A., Noël, G.L., Wartofsky, L. (1975). Failure of oral water loading and intravenous hypotonic saline

to suppress plasma prolactin in man. J.Clin.End. Metab. 41, 383-389.

43. Del Pozo, E., Ohnhaus, E.E. (1976). Lack of effect of acute prolactin suppression on renal water, sodium and potassium excretion during sleep. Horm.Res.7, 11-15.
44. Baumann, G., Marynick, S.P., Winters, S.J.et al (1977). The effect of osmotic stimuli on prolactin secretion and renal water excretion in normal man and in chronic hyperprolactinemia. J.Clin.End.Metab.44, 199-202.
45. Baumann, G., Loriaux, D.L. (1976). Failure of endogenous prolactin to alter renal salt and water excretion and adrenal function in man. J.Clin.End.Metab.43, 643-649.
46. Holland, O.B., Gomez-Sanchez, C.E., Kern, D.C.et al (1977). Evidence against prolactin stimulation of aldosterone in normal human subjects and patients with primary aldosteronism, including a patient with primary aldosterone and a prolactin producing pituitary microadenoma.
J.Clin.End.Metab.45, 1064-1076.
47. Gluckman, P.D., Holdaway, I.M. (1976). Prolactin and somatomedin studies in the syndrome of growth hormone-independent growth. Clin. Endocrinol.5, 545-549.
48. Hauth, J.C., Parker, C.R., McDonald, P.C.et al (1978). A role of fetal prolactin in lung maturation. Obstet. Gynec.51, 81.
49. Bigazzi, M., Ronga, R., Lancranjan, I. et al (1979). Pregnancy in an acromegalic women during bromocriptine treatment; effect on growth hormone and prolactin in the maternal, fetal and aminiotic compartments. J.Clin.End.Metab. 49: 847.
50. Kelly, P.A., Ferland, L., Labrie, F. (1978). Endocrine control of prolactin receptors. In: Progress in prolactin physiology and pathology Eds: Robijn and Harten. Elsevier-North Holland, New York, p.59-68.

51. Forbes, A.P., Henneman, P.H., Griswold, G.C. et al (1954). Syndrome characterized by galactorrhoe, amenorrhoe and low urinary FSH : comparison with acromegaly and normal lactation.
J.Clin.End. Metab. 14, 265-271.
52. Carter, J.N., Tyson, J.E., Warne, G.L. et al (1977). Adrenocortical function in hyperprolactinemic women. J.Clin.End. Metab. 45, 973-980.
53. Winters, A.J., Colston, C., McDonald, P.et al (1975). Fetal plasma prolactin levels. J.Clin.End. Metab. 41, 626-629.
54. Del Pozo, E., Schulz, K.D., Wyss, H. et al (1978). Effect of prolactin on the mechanisms of ovulation and on pregnancy. In: Progress in prolactin physiology and pathology.
Eds: Robijn and Harter. Elsevier/North Holland, New York, p. 281-292.
55. Ølgaard, K., Hagen, C., Madsen, S. et al (1977). Lack of effect of prolactin inhibition by alpha-bromoergocriptine on plasma aldosterone in anephric and non-nefrectomised patients on regular haemodyalisis. Acta Endocrinol. 85, 587-594.
56. Ogihara, T., Matsumura, S., Onishi, T. et al (1977). Effect of metoclopramide-induced prolactin on aldosterone secretion in normal subjects. Life Sci. 20, 523-526.
57. Armstrong, D.T., Knudsen, K.A., Miller, C.S. (1970). Effects of prolactin upon cholesterol metabolism and progesterone biosynthesis in corpora lutea of rats hypophysectomised during pseudo pregnancy. Endocrinol. 86, 634-641.
58. Ingvarsson, C.G. (1969). Acta Rheum Scand.15: 18-20. The action of prolactin on the adrenocortical function.
59. Lis, M., Gilardeau, C., Chrètien, M. (1974). Clin.Res. 1974: 21 : 1027.
Effect of prolactin on corticosterone production by

rat adrenals.
60. Copinscki, G., L´Hermite, M., Leclerq, R.et al (1975). Effects of glucicortocoids on pituitary hormonal response to hypoglycaemia. Inhibition of prolactin release. J.Clin. End. Metab. 40, 442-449.
61. Lamberts, S.W.J., de Quyada, M., Visser, T.J. (1981). Regulation of prolactin secretion in patients with Cushing´s disease.
Neuroendocrinol. 32, 150-154.
62. Krieger, D.T., Howanitz, P.J., Frantz, A.G. (1975). Absence of nocturnal elevation of plasma prolactin concentrations in Cushing´s disease. J.Clin.End. Metab. 42, 260-274.
63. Lamberts, S.W.J., Timmermans, H.A.T., de Jong, F.H. et al (1977). The role of dopaminergic depletion in the pathogenesis of Cushing´s disease and the possible consequences for medical therapy. Clin.Endocrinol.7, 185-194.
64. Editorial (1977), vitamin D and the pituitary. Lancet 1 : 840.
65. Spanos, E., Pike, J.W., Haussler, M.R. et al (1976). Circulating 1x , 25-dihydroxy vitamin D in the chicken: enhancement by injection of prolactin and during egg laying.
Life Sci. 19, 1751-1756.
66. Boass, A., Toverud, S.U., McCain, T.A. et al (1977). Elevated serum levels of 1 x 25 dihydrocholecalciferol in lactating rats. Nature 267, 630-632.
67. Isaac, R., Merceron, R.E., Caillens, G. et al (1978). Effect of parathyroid hormone on plasma prolactin in man.
J.Clin.End.Metab.47, 18-23.
68. Isaac, R., Merceron, R.E., Caillens, G. et al (1980). Effects of calcitonin on basal and thyrotropin releasing hormone stimulated prolactin secretion in man. J.Clin.End.Metab. 50, 1011-1015.

69. Kruse, K., Kracht, U. (1981). Inhibitory effect of calcium on serum prolactin. Acta Endocrinol. 98, 339-344.
70. Riddle, O. (1963). Prolactin or progesterone as key to parental behaviour : a review. Animal Behaviour 11, 419-432.
71. Riddle, O., Lahr, E.L., Bates, R.W. (1942). The role of hormones in the initiation of maternal behaviour in rats.
Am.J.Physiol. 137, 299-317.
72. Guyda, H.J., Friesen, H.G. (1971). The separation of monkey prolactin from monkey growth hormone by affinity chromatography. Biochem. Biophys.Res. Commun. 42, 1068-1076.
73. Lewis, K.J., Singh, R.N.P., Seavy, B.K. (1971). Human prolactin, isolation and some properties. Biochem. Biophys. Res. Commun. 44, 1169-1175.
74. Friesen, H.G., Tolis, G., Shiu, R. et al (1973). Studies on human prolactin : chemistry, radioreceptor-assay and clinical significance.
In: Human prolactin. Eds: Pasteels,J.C., Robijn, C. Excerpta Medica, Amsterdam, p. 11-23.
75. Lewis, U.J., Singh, R.N.P. (1973).
In: Human prolactin. Eds: Pasteels, J.C., Robijn, C. Excerpta Medica, Amsterdam, p. 1-11.
76. Hwang, P., Robertson, M., Guyda, H. et al (1973). The purification of human prolactin from frozen pituitary glands.
J.Clin.End. Metab. 36, 1110-1118.
77. Hummel, B., Brown, G., Hwang, P. et al (1975). Human and monkey prolactin and growth hormone: separation of polymorphic forms by isoelectric focusing. Endocrinol. 97, 855-867.
78. Suh. H.K., Frantz, A.G. (1974). Size heterogeneity of human prolactin in plasma and pituitary abstracts. J.Clin.End.Metab. 39, 928-935.

79. Guyda, H. (1975). Heterogeneity of human growth hormone and prolactin secreted in vitro : immunoassay and radioreceptor assay correlations.
J.Clin.End.Metab. 41, 953-967.
80. Farhouk, N.H., Packer, M.G., Frantz, A.G. (1977).
Progr. 59th Ann. Meeting Endocrine. Society. Chicago Illinois, Abstract 135.
81. Vescovi, G., Belleville, F., Paysant, P.et al (1977).
Study of molecular forms of ovine and human prolactin.
C.R.Soc.Biol. (Paris) 171, 437-440.
82. Pensky, J., Marray, R.M.J., Mozaffarian, G.et al (1976). In: Prolactin and carcinogenesis. Eds: Boyns, A.R. and Griffiths, K.
Alpha. Omega, Alpha publishing Cardiff, p.24.
83. Franks, S., Brook, C.E.D. (1976). Basal and stimulated prolactin levels in childhood. Horm.Res. 7, 65-70.
84. Ehara, Y., Yen, S.S.C., Siler, T.M.(1975). Serum prolactin levels during puberty. Am.J.Obstet. Gynaecol. 121, 995-997.
85. Del Pozo, E., Hiba, J., Lancranjan, I.et al (1977).
Prolactin measurements throughout the lifecycle : endocrine correlation.
In: Prolactin and human reproduction. Eds: Crosignani and Robijn. Academic Press, London/New York p.61-69.
86. Jacobs, L.S., Snijder, P.J., Utiger, R.D. et al (1973)
Prolactin response to thyreotropin releasing hormone in normal subjects. J.Clin.End.Metab.36, 1069-1073.
87. Robijn, C., Delvoye,P., van Exter, C. et al (1977).
Physiological and pharmalogical factors influencing prolactin secretion and their relation to human reproduction.
In: Prolactin and human reproduction. Eds: Crosignani, P., Robijn, C. Academic Press, New York/London p.71-96.
88. Sassin, J.F., Frantz, A.G., Weitzman, E.D.et al (1972).
Human prolactin : 24 hour pattern with increased release during sleep. Science 177, 1205-1207.

89. Parker, D.C., Rossman, L.G., van der Laan, E.F.(1974). Relation of sleep entrained human prolactin release to REM non REM cycles. J.Clin.End.Metab. 38, 646-651.
90. Del Pozo, E., Brownell, J. (1979). Prolactin 1: Mechanism of control, peripheral actions and modification by drugs. Horm.Research 10 : 143-172.
91. Boyer, R.M., Kapen, S., Finkelstein, J.W. et al (1974). Hypothalamic-pituitary function in diverse hyperprolactinemic states. J.Clin.Invest. 53, 1588-1598.
92. Noel, G.L., Suk, H.K., Frantz, A.G.(1974). Prolactin release during nursing and breast stimulation in postpartum and nonpostpartum subjects. J.Clin.End,Metab. 38, 413-423.
93. Noel, G.L., Suk, H.K., Stone, J.G. et al (1972). Human prolactin and growth hormone release during surgery and other conditions of stress. J.Clin.End.Metab. 35, 840-851.
94. Neill, J.D. (1970). Effect of stress on serum prolactin and luteinizing hormone levels during the estrous cycle of the rat.
Endocrinol.87, 1192-1197.
95. Neill, J.D.(1974). Prolactin : its secretion and control.
In: Handbook of physiology, section 7, Endocrinol. vol.Iv. American Physiological Society. Washington DC, p. 469-488.
96. Frantz, A.G., Kleinberg, D.L., Noel, G.L. (1972). Studies on prolactin in man. Rec.Prog.Horm.Res. 28, 527-590.
97. Everett, J.W. (1954). Luteotrophic function of autograft of the rat hypophysis. Endocrinol. 54, 685-690.
98. Turkington, R.W., Underwood, L.E., van Wijk, J.J. (1971). Elevated serum prolactin levels after pituitary stalktransection in man. New. Engl. J.Med. 285, 707-710.

99. Sinha, Y.N., Tucker, H. (1968). Pituitary prolactin content and mammary development after chronic administration of prolactin.
Proc.Soc.Exp.Biol. 128, 84-88.
100. Chen, C.L., Minagushi, H., Meites, J. (1967). Effects of transplanted pituitary tumors on host pituitary prolactin secretion.
Proc. Soc. Exp. Biol. Med. 126, 317-320.
101. Nicoll, C.S., Fiorindo, R.P., McKenna, C.F.et al (1970). Assay of hypothalamic factors which regulate prolactin secretion. In : Hypophysiotropic hormones of the hypothalamus assay and chemistry. Ed.: Meites, J., William Wilkins, Baltimore, p.115-150.
102. Valverde, C., Chieffo, V., Reichlin, S. (1972). Prolactin releasing factor in porcine and rat hypothalamic tissue.
Endocrinol. 91, 982-993.
103. Meites, J. (1973) Control of prolactin secretion in animals. In: Human prolactin. Eds: Pasteels and Robijn.
Excerpta Medica, Amsterdam, p. 105-118.
104. MacLeod, R.M. (1976). Regulation of prolactin secretion.
In: Frontiers in neuroendocrinology. Eds: Martini, L., Ganong, W.F. Raven Press, New York 4, 169-194.
105. Yen, S.S.C., Ehara, Y., Siler, T.M.(1974). Augmentation of prolactin secretion by estrogen in hypogonadal women.
J.Clin.Invest. 53, 652-655.
106. Lloyd, H.M., Meares, J.D., Jacobi, J. (1975). Effects of Oestrogen and Bromocriptine on in vivo secretion and mitosis in prolactin cells.
Nature 255, 497- 498.
107. Raymond, V., Beaulieu, M. Labrie, F. (1978). Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release.

Science 200, 1173-1175.

108. Meites, J., Nicoll, C.S., Talwalker, P.K. (1963). The central nervous system and the secretion and release of prolactin.
In: Advances in neuroendocrinology. Ed.: Nalbandov. University of Illinois press, Urbana USA.

109. Pasteels, J.L. (1961). Sécrètion de prolactin par l'hypophyse en culture de tissue. C.R.Acad.Sci. (Paris) 253, 2140-2142.

110. Talwalker, P.K., Ratner, A., Meites, J. (1963). In vitro inhibition of pituitary prolactin synthesis and release by hypothalamic extracts. Am.J.Physiol. 205, 213-218.

111. MacLeod, R.M. (1969). Influence of Norepinenefrine and catecholamine depleting agents on the synthesis and release of prolactin and growth hormone. Endocrinol. 85, 916-923.

112. MacLeod, R.M., Lehmeyer, J.E. (1974). Studies on the mechanism of the dopamine mediated inhibition of prolactin secretion.
Endocrinol. 94, 1077-1085.

113. Birge, C.A., Jacobs, C.S., Hammer, C.T.et al (1970). Catecholamine inhibition of prolactin secretion by isolated adenohypophyses.
Endocrinol. 86, 120-130.

114. Shaar, C.J., Clemens, J.A. (1974). The role of catecholamines in the release of anterior pituitary prolactin in vitro.
Endocrinol. 95, 1202-1212.

115. Fuxe, K., Hökfelt, T. (1969). Catecholamines in the hypothalamus and pituitary gland.In:Frontiers in neuroendocrinology. Eds: Ganong, W.F., Martini, L. Oxford University Press, New York, p. 47-96.

116. Ben Jonathan, N., Olivaer, C., Weiner, H,J, et al (1977).
Dopamine in hypophysial portal plasma of the rat

during the estrous cycle and throughout pregnancy.
Endocrinol. 100, 452-458.
117. Gudelsky, G.A., Porter, J.C. (1979). Release of newly synthesized dopamine into the hypophysial portal vasculature of the rat.
Endocrinol. 104, 583-587.
118. Brown, G.M., Seeman, P., Lee,T. (1976). Dopamine/neuroleptic receptors in basal hypothalamus and pituitary.
Endocrinol.99, 1407-1410.
119. Carson, M.G., Raymond, V., Lefkowitz, R.J.et al (1977) Identification of dopaminergic receptors in anterior pituitary: correlation with the dopaminergic control of prolactin release.
Fed.Proc. 36, 278 (abstr).
120. Calabro, M.A., MacLeod, R.M. (1978). Binding of dopamine to bovine anterior pituitary gland membranes.
Neuroendocrinol. 25, 32-46.
121. Leblanc, H., Lachelin, G.C.L., Abu Fadil, S. (1976). Effects of dopamine infusion on pituitary hormone secretion in humans.
J.Clin.End.Metab. 43, 668-674.
122. De Greef, W.J., Neill, J.D. (1979). Dopamine levels in hypophysial stalk plasma of the rat during surges of prolactin secretion induced by cervical stimulation.
Endocrinol. 105, 1093-1099.
123. Schally, A.V., Dupont, A., Arimura, A.et al (1976). Purification of a catecholamine-rich fraction with prolactin releaseinhibiting factor (PIF) activity from porcine hypothalami.
Acta Endocrinol. 82, 1-14.
124. Enjalbert, A., Priam, M., Kordon, C. (1977). Evidence in favour of the existence of a dopamine free prolactin inhibiting factor (PIF) in rat hypothalamic extracts.Eur.J.Pharmacol. 41, 243-244.

125. Schally, A.V., Redding, T.W., Arimura, A. et al (1977) Isolation of gamma amino butyric acid from pig hypothalami and demonstration of its prolactin release inhibiting (PIF) activity in vivo and in vitro. Endocrinol. 100, 681-692.
126. Mioduszewski, R., Grandisan, L., Meites, J. (1976). Stimulation of prolactin release in rats by GABA. Proc.Soc.Exp.Biol.Med. 151, 44-46.
127. Rivier, C., Vale, W., (1977). Effects of γ-amino-butyric acid and histamine on prolactin secretion in the rat. Endocrinol. 101, 506-511.
128. Lamberts, S.W.J., MacLeod, R.M. (1978). Studies on the mechanisms of the GABA mediated inhibitions of prolactin secretion. Proc.Soc.Exp.Biol.Med.
129. Valverde, R., Chieffo, V., Reichlin, S.(1972). Prolactin releasing factor in porcine and rat hypothalamic tissue. Endocrinology 91: 982-983.
130. Tashjian, A.H., Barowsky, N.J., Jensen, D.K. (1971). Thyrotropin releasing hormone : direct evidence for stimulation of prolactin production by the pituitary cells in culture. Biochem. Biophys. Res. Comm. 43, 516-523.
131. Gautvik, K.M., Tashjian, A.H., Kourides, I.A.et al (1974). Thyrotropin releasing hormone is not the sole physiologic mediator of prolactin release during suckling. N.Engl.J.Med. 290, 1162-1165.
132. Labrie, F., Bardeu, N., Poirier, G.et al (1972). Binding of thyrotropin releasing hormone to plasma membranes of bovine anterior gland. Proc.Nat.Acad.Sci.USA, 69, 283-287.
133. Koch, Y., Golhaber, G., Fireman, I. et al (1977). Suppression of prolactin and thyrotropin secretion

in the rat by antiserum to thyrotropin releasing hormone.
Endocrinol. 100, 1476-1478.

134. Rivier, C., Rivier, J., Vale, W. (1978). The effect of bombesin and related peptides on prolactin and growth hormone secretion in the rat.
Endocrinol. 102, 519-522.

135. Chihara, K., Arimura, A., Coy, D.H. et al (1978). Studies on the interaction of endorphins, substance P and endogenous somatostatin in growth hormone and prolactin release in rats.
Endocrinol.102, 281-290.

136. Maeda, K., Frohman, L.A. (1978). Disassociation of systemic and central effects of neurotensin on the secretion of growth hormone, prolactin and thyrotropin.
Endocrinol. 103, 1903-1909.

137. Rivier, C., Vale, W., King, N. et al (1977). Stimulation in vivo of the secretion of prolactin and growth hormone by endorphin.
Endocrinol. 100, 238-241.

138. Vale, W., Rivier, C., Brown, M. (1977).Regulatory peptides of the hypothalamus.
Ann. Rev. Physiol. 39, 473-527.

139. Vijayan, E., Samson, W.K., Said, S.E. et al (1979). Vasoactive intestinal peptide : Evidence for a hypothalamic site of action to release growth hormone, luteinising hormone and prolactin in conscious ovariectomised rats. Endocrinol. 104, 52-57.

140. Said, S.I., Porter, J.C. (1979). Vasoactive intestinal polypeptide : release into hypophyseal portal blood. Life Sci. 24, 227-230.

141. Kato, Y., Iwasaki, Y., Iwasaki, J. et al (1978). Prolactin release by vasoactive intestinal polypeptide in rats. Endocrinol. 103, 554-558.

142. Ruberg, M., Rotszteijn, W.H., Arancibia, S. et al

(1978). Stimulation of prolactin release by vasoactive intestinal peptide. Eur.J.Pharmacol. 51, 319-320.

143. Kamberi, I.A., Mical, R.S., Porter, J.C. (1971). Effects of melatonin and serotonin on the release of FSH and prolactin.
Endocrinol. 88, 1288-1296.

144. Turkington, R.W., MacIndoe, J.H. (1972). Stimulation of human prolactin secretion by intravenous infusion of L-tryptophan.
J.Clin. Invest. 51, 323 (abstract).

145. Lamberts, S.W.J., MacLeod, R.M. (1978). The interaction of the serotonergic and dopaminergic systems on prolactin secretion in the rat. The mechanism of action of the "specific" serotonin receptor antagonist methysergide.
Endocrinol. 103, 287-295.

146. Kordon, C., Blake, C.A., Terkel, J. et al (1973). Participation of the 5HT containing neurons in the suckling induced rise in plasma prolactin levels in lactating rats.
Neuroendocrinol. 13, 213-223.

147. Clemens, J.A., Sawyer, B.D., Berimele, B. (1977). Further evidence that serotonin is a neurotransmitter involved in the control of prolactin secretion.
Endocrinol. 100, 692-698.

148. Wiebe, R.H., Handwerger, S., Hammond, C.B. (1977). Failure of L-Tryptophan to stimulate prolactin secretion in man.
J.Clin.End. Metab. 45, 1310-1312.

149. Beck-Peccoz, P., Ferrari, C., Rondena, M. et al (1976) Failure of oral 5-hydroxy tryptophan administration to affect prolactin secretion in man.
Horm. Res. 7, 303-307.

150. MacIndoe, J.H., Turkington,R.W. (1973). Stimulation of human prolactin secretion by intravenous infusion of L-tryptophan.

J.Clin.Invest. 52, 1972-1978.
151. Kato, Y., Nahai, Y., Imura,H.et al (1974). Effects of 5-hydroxy tryptophan (5-HTP) on plasma prolactin levels in man. J.Clin.End.Metab. 38, 695-697.
152. Lancranjan, I., Wirz-Justice, A.,Pühringer,W.et al (1977).Effect of 1-5 hydroxy tryptophan infusion on growth hormone and prolactin secretion in man. J.Clin.End.Metab. 45, 588-593.
153. Erdheim, J., Stumme,E. (1909). Uber Hypophyse veränderungen bei der Schwangerschaft. Beitr.Path. 46, 1-132.
154. El Etreby, M.F., Günzel, P. (1973). Prolactinzelltumoren im Tierexperiment und beim Menschen. Arzneimittel-Forsch.23, 1768-1790.
155. Vekemans, M., Robijn, C. (1975). The influence of exogenous estrogen on the circadian periodicity of circulating prolactin in women. J.Clin.End.Metab. 40, 886-889.
156. Groom, G.V., Griffiths, K. (1976). Effect of the antiestrogen tamoxifen on plasma levels of luteinizing hormone, follicle stimulating hormone, prolactin, oestradiol, and progesterone in normal premenopauzal women. J.Endocrinol. 70, 421-428.
157. DeLean, A., Ferland, L., Drouin, J. et al (1977). Changes of pituitary thyrotropin releasing hormone (TRH) receptor level and prolactin response to TRH during the rat estrous cycle. Endocrinol. 100, 1505 - 1510.
158. Stevens, R.W., Lawson, D.M. (1977). The influence of estrogen on plasma prolactin levels induced by thyrotropin releasing hormone (TRH), clonidine and serotonin in ovariectomized rats. Life Sci. 261-266.

159. Brunet, N., Gourdji, G., Moreau, M.F. et al (1977). Effect of 17B Estradiol on prolactin secretion and thyroliberin responsiveness in two rat prolactin continuous cell lines : Definition of an experimental model.
Ann. Biol.Anim. Biochim. Biophys. 17, 413-424.

160. Haug, E., Gautvik, K.M. (1976). Radioimmunoassay of rat prolactin and its use in measuring prolactin production by cultured pituitary cells.
Acta Endocrinol. 82, 282-297.

161. Keefer, D.A., Strumpf, W.E., Petrusky, P. (1976). Quantitative autoradiographic assessment of 3H-estradiol uptake in immunocytochemically characterized pituitary cells.
Cell Tissue Res. 166, 25-35.

162. Caligaris, L., Taleisnik, S. (1976). The role of hypothalamic afferents in the release of prolactin induced by ovarian steroids.
Neuroendocrinology 21, 139-145.

163. Cardinali, D.P., Gomez, E. (1977). Change in hypothalamic noradrenaline, dopamine and serotonin uptake after oestradiol administration to rats.
J.Endocrinol. 73, 181-182.

164. Cramer, O.M., Parker Jr, C.R., Porter, J.C. (1979). Estrogen inhibition of dopamine release into hypophysial portal blood.
Endocrinol. 104, 419-421.

165. Piercy, M., Shin, S.H. (1980). Comparative studies of prolactin secretion in Estradiol-primed and normal male rats induced by ether stress, pimozide and TRH.
Neuroendocrinol. 31, 270-275.

166. Norman, R.L., Quadri, S.K., Spier, H.G. (1980). Differential sensitivity of prolactin release to dopamine and thyrotropin releasing hormone in intact and pituitary stalk sectioned rhesus monkey's.
J.Endocrinol. 84, 479-487.

167. De Greef, W.J., Visser, T.J.(1981). Evidence for the involvement of hypothalamic dopamine and thyrotropin releasing hormone in suckling-induced release of prolactin.
J.Endocrinol. 91, 213-223.
168. Gudelsky, G.A., Nausel, D.D., Porter, J.C. (1981). Role of estrogen in the dopaminergic control of prolactin secretion.
Endocrinol. 108, 440-444.
169. Nansel, D.D., Gudelsky , G.A., Reymond, M.J. et al (1981). Estrogen alters the responsiveness of the anterior pituitary gland to the actions of dopamine on lysosomal enzyme activity and prolactin release.
Endocrinol. 108. 903-907.
170. Carlson, H.E., Jacobs, L.S., Daughaday, W.H. (1973). Growth hormone, thyrotropin and prolactin responses to thyrotropin releasing hormone following diethylstilbestrol pretreatment.
J.Clin.End.Metab. 37, 488-490.
171. Lu, K.H., Amenomori, Y., Chen, C.L. et al (1970). Effects of central acting drugs on serum pituitary prolactin levels in rats.
Endocrinol. 87, 667-670.
172. Ben David, M., Danon, A., Sulman, F.G. (1970). Acute changes in blood and pituitary prolactin after a single injection of perphenazine.
Neuroendocrinol. 6, 336-342.
173. MacLeod, R.M., Lamberts, S.W.J. (1978). The biphasic regulation of prolactin secretion by dopamine agonists-antagonists.
Endocrinol. 103, 200-203.
174. Lawson,D.M., Gala, R.R.(1975). The influence of adrenergic, dopaminergic, cholinergic and serotonergic drugs on plasma prolactin levels in ovariectomized estrogen treated rats.
Endocrinol. 90, 313-318.

175. Mancini, A.M., Guitelman, A., Vargas, C.A. et al (1976). Effect of sulpiride on serum prolactin levels in humans.
J.Clin.End. Metab. 42, 181-184.
176. MacLeod, R.M., Robijn, C. (1977).
Mechanism of increased prolactin secretion by sylpiride.
J. Endocrinol. 72, 273-277.
177. Mc-Neilly, A.S., Thorner, M.O., Volans, G. et al (1974). Metoclopramide and prolactin.
Br.Med.J 2 : 729.
178. Judd, S.J., Lazarus, L., Smythe, G. (1976).
Prolactin secretion by metoclopramide in man.
J.Clin.End. Metab.43, 313-317.
179. Carlson, H.E., Briggs, J.E., McCallum, R.W.(1977).
Stimulation of prolactin secretion by metoclopramide in the rat.
Proc.Soc.Exp.Biol.Med. 154, 475-478.
180. Yamauchi, J., Takahara, J., Ofuji, T. (1977).
Effect of metoclopramide on rat prolactin secretion in vivo.
Life Sci. 20, 1581-1583.
181. Gräf, K.J., Schmidt-Gollwitzer, M., Koch, K.J. et al (1978). Hyperprolactinemia induced by cyproterone acetate in human subjects.
Acta Endocrinol. 87, suppl. 215, 96.
182. Fonzo, D., Angeli, A., Sivieri, R. et al (1977).
Hyperprolactinemia in girls with idiopathic precocious puberty under prolonged treatment with cyproteroneacetate.
J.Clin.End.Metab. 45, 164-168.
183. Rose, L.I., Underwood, R.H., Newmark, S.R. et al (1977). Pathophysiology of spironolactone induced gynaecomastia.
Ann. Int.Med. 87, 398-403.
184. Tolis, G., Hickey, J., Guyda, J. (1975).

Effects of morphine on serum growth hormone, cortisol, prolactin and thyroid stimulating hormone in man. J.Clin.End. Metab. 41, 797-800.

185. Von Graffenried, B., Del Pozo, E., Roubicek, J. et al (1978). Effects of the synthetic enkephalin analogue FK33-824 in man. Nature, 272, 729-730.

186. Cocchi, D., Santagostino, A., Gil-Ad, I. et al (1977). Leuenkephalin-stimulated growth hormone and prolactin release in the rat : comparison with the effect of Morphine. Life Sci.20, 2041-2046.

187. Schaar, C.J., Frederikson, R.C.A., Dinniger, N.B. et al (1977). Enkephalin analogues and naloxone modulate the release of growth hormone and prolactin-evidence for regulation by a endogenous opioid peptide in the brain. Life Sci. 21, 853-860.

188. Rivier, C., Brown, M., Vale, W. (1977). Effect of neurotensin, substance P and morphine sulphate on the secretion of prolactin and growth hormone in the rat. Endocrinol. 100, 751-754.

189. Dupont, A., Cusan, L., Labrie, D. et al (1977). Stimulation of prolactin release in the rat by intraventricular injection of B endorphin and methionine-enkephalin. Biochem.Biophys.Res. Comm. 75, 76-82.

190. Enjalbert, A., Ruberg, M., Fiore, L. et al (1979). Effect of morphine on the dopamine inhibition of pituitary prolactin release in vitro. Eur. J.Pharm. 53, 211-212.

191. Arakelian, M.C., Libertun, C. (1977). H_1 and H_2 histamine receptor participation in the brain control of prolactin secretion in lactating rats.Endocrinol. 100, 890-895.

192. Carlson, H.E., Ippoliti, A.F. (1977).
Cimetidine and H_2 antihistamine stimulates prolactin secretion in man.
J.Clin.End. Metab. 45, 367-370.

193. Sharpe, P.C., Melvin, M.A., Mills, J.G. et al (1980).
Prolactin responses to histamine H_2 receptor antagonists.
Acta Endocrinol. 95, 308-313.

194. Ferrari, C., Caldara, R., Barbieri, C. et al (1979).
Prolactin release by intravenous cimetidine in man : evidence for a supra pituitary locus of action.
Clin. Endocrinol. 11, 619-623.

195. Dell Fave, G.F., Tamburrano, G., De Majestris, L. et al (1977). Gynecomastia with cimetidine.
Lancet 1, 1319.

196. Lu, K.H., Meites, J. (1972). Effects of L-Dopa on serum prolactin and PIF in intact and hypophysectomised pituitary-grafted rats.
Endocrinol. 91, 868-872.

197. Diefenbach, W.P., Carmel, P.W., Frantz, A.G. et al (1976). Suppression of prolactin secretion by L-dopa in the stalk-sectioned rhesus monkey.
J.Clin.End. Metab. 43, 638-643.

198. Wiggins, J.F., Fernstrom, J.D. (1977). L-Dopa inhibits prolactin secretion in preoestrous rats.
Endocrinol. 101, 469-472.

199. Donoso, A.O., Banzan, A.M., Barcaglioni, (1974).
Further evidence on the direct action of L-dopa on prolactin release.
Neuroendocrinol. 15, 236-239.

200. Leblanc, H., Yen, S.S.C. (1976). The effect of L-Dopa and chlorpromazine on prolactin and growth hormone secretion in normal women.
Am.J.Obstet. Gynecol. 126, 162-164.

201. Van der Gugten, A.A., Sahuleka, P.C., Van Galen, G.H. et al (1976).

Prolactin inhibition test with L-dopa. Decrease and restoration of plasma prolactin levels in the rat by a peripheral process.
J.Endocrinol. 68, 369-381.

202. Manku, M.S., Horrobin, D.F., Zinner, H. et al (1977). Dopamine enhances the action of prolactin on rat blood vessels. Implications for dopamine effects on plasma prolactin.
Endocrinol. 101, 1343-1346.

203. Meites, J., Clemens, J. (1972).Hypothalamic control of prolactin secretion. Vitam. Horm. 30, 165-221.

204. MacLeod, R.M., Lehmeijer, J.E. (1973). Suppression of pituitary tumor growth and function by Ergot Alkaloids.
Can.Res. 33, 849-855.

205. Lu. K.H., Kock, Y., Meites, J. (1971). Direct inhibition by ergocornine of pituitary prolactin release.
Endocrinol. 89, 229-233.

206. Nicoll, C.S., Yaron, Z., Nutt, N.et al (1970). Effects of ergotamine tartrate on prolactin and growth hormone secretion by rat adenohypophysis in vitro.
Biol. Reprod. 5, 59-66.

207. MacLeod, R.M., Lehmeijer, J.E. (1972). Regulation of the synthesis and release of prolactin. In: Lactogenic Hormones. Eds: Walstenholme, G.E.W. and Knight, J. Churchill Livingstone. London and Edinburgh, p.53-82.

208. MacLeod, R.M., Kimura, H., Login, I. (1976). Inhibition of prolactin secretion by dopamine and peribedil (ET-495).
In: Growth hormone and related peptides. Eds.: Pecile, A., and Mueller, E.E. Excerpta Medica, Amsterdam,

209. MacLeod, R.M.(1977). Influence of dopamine, serotonin

and their antagonists on prolactin secretion.
Prog.Reproduc.Biol. 2, 54-68.

210. Sinka, Y.N., Salocks, C.B., van der Laan, W.P. (1976). A comparison of the effects of CB 154 and lergotrile mesylate on prolactin and growth hormone secretion in mice.
Horm. Metab. Res. 8, 332-336.

211. MacLeod, R.M., Lehmeijer, J.E. (1974). Reciprocal relationship between the in vitro synthesis and secretion of prolactin and growth hormone.
Proc. Soc. Exp.Biol. Med. 145, 1128-1131.

212. Martin, J.B., Lal, S., Tolis, G. et al (1974). Inhibition by apomorphine of prolactin secretion in patients with elevated serum prolactin.
J.Clin.End. Metab. 39, 180-182.

213. Zuckerman, H., Carmel, S. (1973). The inhibition of lactation by clomiphene.
J.Obstet. Gynaecol. Br. Common. 80, 822-823.

214. Weinstein, D., Ben David, M., Polishuk, W.Z. (1976). Serum prolactin and the suppression of lactation.
Br. J.Obstet. Gynaecol. 83, 679-682.

215. Canales, E.S., Lasso, P., Soria, J. et al (1977). Effect of clomiphene on prolactin secretion and lactation in puerperal women.
Br. J.Obstet. Gynaecol. 84, 758-759.

216. Nagy, I., Valdenegro, C.A., Login, I.S. et al (1979). Antiestrogens decrease prolactin synthesis and enzyme activities in the anterior pituitary of rats.
Endocrinol. 105, A116.

217. De Quyada, M., Timmermans, H.A.T., Lamberts, S.W.J. (1980). Tamoxifen suppresses both the growth of prolactin secreting pituitary tumours and normal prolactin synthesis in the rat.
J.Endocrinol. 86, 109-116.

218. Willis, K.J., London, D.R., Ward, H.W.C. et al (1977). Recurrent breast cancer treated with the antiestrogen

tamoxifen : correlation between hormonal changes and clinical course.
Br. Med. J 1, 425-428.

219. Masala, A., Delitala, G., Lo Dico, G. et al (1978). Inhibition of lactation and inhibition of prolactin release after mechanical breast stimulation in puerperal women given tamoxifen or placebo.
Br. J. Obstet. Gynaecol. 85, 134-137.

220. Powell- Jones, W., Jeuner, D.A., Blamey, R.W,et al (1975). Influence of anti-oestrogens on the specific binding in vitro of (^{3}H) oestradiol to cytosol of rat mammary tumours and human breast carcinomata.
Biochem. J. 150, 71-75.

221. Lamberts, S.W.J., Uitterlinden, P., Zuiderwijk-Roest, J.M. et al (1981). Effects of a luteinizing hormone-releasing hormon analog and tamoxifen on the growth of an estrogen-induced prolactin secreting rat pituitary tumor and its influence on pituitary gonadotropins.
Endocrinol. 108, 1878-1883.

222. De Quyada, M., Timmermans, H.A.T., Lamberts, S.W.J. et al (1980). Tamoxifen enhances the sensitivity of dispersed prolactin secreting pituitary tumor cells to dopamine and bromocriptine.
Endocrinol. 106, 702-706.

223. Roth, J. (1973). Peptide hormone binding to receptors: a review of direct studies in vitro.
Metabolism 22, 1059-1073.

224. Shiu, R.P.C., Kelly, P.A., Friesen, H.G. (1973). Radioreceptor assay for prolactin and other lactogenic hormones.
Science 180, 968-971.

225. Kelly, P.A., Posner, B.I., Isushima, T. et al (1974). In: Advances in human growth hormone research.Eds.: Raiti, S.,General Printing Office, Washington DC, p. 567-584.

226. Posner, B.I., Kelly, P.A., Shiu, R.P.C. et al (1974). Studies on insulin, growth hormone and prolactin binding, tissue distribution species variation and characterization.
Endocrinol. 95, 521-531.
227. Frantz, W.C., McIndoe, J.H., Turkington, R.W. (1974). Prolactin receptors : characteristics of the particulate fraction binding activity.
J.Endocrinol. 60, 485-497.
228. Donatsch, P., Richardson, B. (1975). Localization of prolactin in rat kidney tissue using a double-antibody technique.
J. Endocrinol. 66, 101-106.
229. Postel-Vinay, M.C. (1976). Binding of human growth hormone to rat liver membranes lactogenic and somatotropic sites.
FEBS Lett. 69, 137-140.
230. Kelly, P.A., Posner, B.I., Tsushima, T. et al (1974). Studies of insulin, growth hormone and prolactin binding: Ontogenesis effects of sex, and pregnancy.
Endocrinol. 95, 532-539.
231. Chen, H.W., Hamer, D.H., Heiniger, H.J et al (1972). Stimulation of hepatic RNA synthesis in dwarf mice by ovine prolactin.
Biochem. Biophys. Acta. 287, 90-97
232. MacLeod, R.M.,Bass, M.B., Huang, S.C.et al (1968). Intermediary metabolism in the liver and adipose tissue of rats with hormone-secreting pituitary tumors.
Endocrinol. 82, 253-265.
233. Francis, M.J., Hill, D.J. (1975).
Prolactin stimulated production of somatomedin by rat liver.
Nature 255, 167-168.
234. Costlow, M.E., Buchow, R.A., McGuire, W.C. (1975). Prolactin stimulation of prolactin receptors in rat

liver.
Life Sci. 17, 1457-1465.

235. Berl, T., Brautbar, N., Ben David, M. et al (1976).
Osmotic control of prolactin release and its effect on renal water excretion in man.
Kidney International 10, 158-163.

236. Reddy, S., Watkinds, W.B. (1975).
Uptake of ^{125}I labelled human placental lactogen and human placental lactogen by the tissues of normal and lactating rats.
J.Endocrinol. 65, 183-194.

237. Cowden, E.A., Ratcliffe, W.A., Ratcliffe, J.G. et al (1978).
Hyperprolactinemia in renal disease.
Clin. Endocrinol. 9, 241-248.

238. Sciarra, F., Sparano, F., Giaquinto, G. et al (1977).
Prolactinemia during hemodyalisis : lack of correlation with sodium, potassium, and renine aldosterone system.
Horm.Res. 8, 129-138.

239. Nader, S., Mashiter, K., Joplin, G.F. (1975).
Urinary prolactin excretion.
Lancet 1, 928.

240. Bratusch- Marrain, P., Björkman, O., Waldhäusl, W., et al (1979).
Hepatic disposal of endogenous growth hormone and prolactin in man.
Europ. J.Clin. Invest. 9, 257-260.

241. Tait, J.F. (1963). Review : the use of isotopic steroids for the measurement of production rates in vivo.
J.Clin.End. Metab. 23, 1285-1297.

242. Taylor, A.L., Finster, J.L., Mintz, D.H. (1969).
Metabolic clearance and production rates of human growth hormone.
J.Clin.Invest. 48, 2349-2358.

243. Mac Gillivray, M.H., Frohman, L.A., Doe, J. (1970). Metabolic clearance and production rates of human growth hormone in subjects with normal and abnormal growth.
J.Clin,End. Metab. 30, 632-638.
244. Kohler, P.O., Ross, G.T., Odell, W.D. (1968). Metabolic clearance and production rates of human luteinizing hormone in pré- and post-menopausal women.
J.Clin.Invest. 47, 38-47.
245. Cobble, Y.D., Kohler, P.O., Cargille, C.M. et al (1969). Production rates and metabolic clearance rates of human stimulating hormone in premenopausal and postmenopausal women.
J.Clin. Invest. 48, 359-363.
246. Ridgway, E.C., Weintraub, B.D., Maloof, F. (1974). Metabolic clearance and production rates of human thyrotropin.
J.Clin.Invest. 53, 895-903.
247. Pimstone, B.L., Epstein, S., Hamilton, S.M. et al (1977). Metabolic clearance and plasma half disappearance time of exogenous Gonadotropin releasing hormone in normal subjects and in patients with liver disease and chronic renal failure.
J.Clin.End. Metab. 44, 356-360.
248. Koch, Y., Chow, Y.F., Meites, J. (1971). Metabolic clearance and secretion rates of prolactin in the rat.
Endocrinol. 89, 1303-1308.
249. Jacobi, J.M., Lloyd, H.M. (1978). Prolactin clearance rate in male rats.
Horm. Metab. Res. 10, 451-452.
250. Grosvenor,C.E., Whitworth, N.S. (1979). Secretion rate and metabolic clearance rate of prolactin in the rat during mid- and late lactation.
J.Endocrinol. 82, 409-415.

251. Grosvenor, C.E., Mena, F., Whitworth, N.S. (1977). Metabolic clearance of rat prolactin in the lactating and nonlactating rat.
Endocrinol. 73, 1-10.
252. Cooper, D.S., Ridgway, E.C., Kliman, B. et al (1979). Metabolic clearance rate and production rates of prolactin in man.
J.Clin.Invest. 64, 1669-1680.
253. Hruska, K.A., Kopelman, R., Rutherford, W.E. et al (1975). Metabolism of immuno reactive parathyroid hormone in the dog : the role of the kidney and the effects of chronic renal disease.
J.Clin. Invest. 56, 39-48.
254. Katz, A.I., Rubenstein, A.H. (1973). Metabolism of proinsulin, insulin and C-peptide in the rat.
J.Clin. Invest. 52, 1113-1121.
255. Grace, S.G., Davidson, W.D., State, D. (1974). Renal mechanisms for removal of gastrin from the circulation.
Surg. Forum. 25, 323-325.
256. Emmanouel, D.S., Jaspan, J.D., Kuhu, S.F. et al (1976) Pathogenesis and characterization of hyperglucagonemia in the uremic rat.
J.Clin.Invest. 58, 1266-1272.
257. Rabkin. R., Share, L., Payne, P.A. et al (1979). The handling of immunoreactive vasopressin by the isolated perfused rat kidney.
J.Clin.Invest. 63, 6-13.
258. Ramirez, G., O'Neill, W.M.Jr., Bloomer, A. et al (1977). Abnormalities in the regulation of prolactin in patients with chronic renal failure.
J.Clin.End. Metab. 45, 658-661.
259. Lim, V.S., Kathpalia, S.C., Frohman, L.A. (1979). Hyperprolactinemia and impaired pituitary response to suppression and stimulation in chronic renal failure.

Reversal after transplantation.
J.Clin.End.Metab. 48, 101-107.
260. Nagel, T.C., Freinkel, N., Bell, R.H.et al (1973). Gynecomastia, prolactin and other peptide hormones in patients undergoing chronic hemodialysis. J.Clin.End.Metab.36, 428-432.
261. Ølgaard, K., Hagen, C., McNeilly, A.S. (1975). Pituitary hormones in women with chronic renal failure : the effect of chronic intermittent haemo- and peritoneal dyalisis. Acta Endocrinol. 80, 237-246.
262. Josepberg, Z., Posner, B.I., Patel, B. et al (1979). The uptake of prolactin in female rat liver, concentration of intact hormone in the Golgi apparatus. J.Biol.Chem. 254, 209-214.
263. Bohnet, H.B., Dahlen, H.G., Wuttke, W.et al (1976). Hyperprolactinemic anovulatory syndrome. J.Clin.End.Metab. 42, 132-143.
264. Glass, M.R., Williams, G.W., Butt, W.R. et al (1976). Basal serum prolactin values and responses to the administration of TRH in women with amenorrhoe. Br.J.Obstet. Gynaecol. 82, 495-501.
265. Franks, S., Murray, M.A.F., Jequier, A.M. et al (1975) Incidence and significance of hyperprolactinemia in women with amenorrhoe. Clin. Endocrinol. 4, 597-607.
266. Ruggere, M., Tolis, G. (1977). On the incidence of hyperprolactinemia in amenorrhoic patients. Clin.Res.25, 301.
267. Tolis, G. (1975). Inappropriate prolactin secretion in galactorrhea amenorrhoe associated with primary hypothyroidism. Clin. Res. 23, 618.
268. Van Loon, P.F.A., McNeilly, A.S., Hunter, W.M. et al (1977). The role of prolactin in secondary amenorrhea.

In: Prolactin and human reproduction. Eds.:
Crosignani, P., Robijn, C.
Academic Press, London, New York, San Francisco,
p. 217-224.

269. Friesen, H.G., Tolis, G. (1977).
The use of bromocriptine in the amenorrhoea galactorrhea syndromes. The canadian cooperative study.
Clin. Endocrinol. 6, 91-99.

270. Tolis, G. (1977). Galactorrhoea amenorrhoea and hyperprolactinemia Pathophysiological aspects and diagnostic tests.
Clin. Endocrinol. 65, 81-90.

271. Tolis, G., Somma, M., van Campenhout, J. et al (1974).
Prolactin secretion in 65 patients with galactorrhoea.
Am. J.Obstet. Gynaecol. 118, 91-101.

272. Ambrosi, B., Bara. R., Travaglini, P. et al (1977).
Study of the effects of bromocriptine on sexual impotence.
Clin. Endocrinol. 7, 419-421.

273. Thorner, M.O., Edwards, C.R.W., Hanker, J.P. et al (1977). Prolactin and gonadotropin interactions in the male.
In: The testis in normal and infertile men.Eds.:
Troen, P., Nankin, H.R.,Raven Press, New York, p. 351-366.

274. Faglia, G., Travaglini, P., Ambrosi, B. et al (1977).
Some aspects of prolactin influence on gonadal function in humans .
Prog. Reprod. Biol. 2, 256-260.

275. Segal, S., Polishuk, W.Z., Ben David, M. (1976).
Hyperprolactinemic male infertility.
Fertil Steril. 27, 1425-1427.

276. Boucher, D., Hermabessiere, H., Doly, M. (1977).
Prolactin secretion in infertile man before and after treatment with bromocriptine.
Ann.Biol.Anim.Biochem.Biophys. 17, 483-498.

277. Vagenakis, A.G., Dole, V., Braverman, L. (1976). Pituitary enlargement, pituitary failure, and primary hypothyroidism.
Ann.Int.Med. 85, 195-198.
278. Tolis, G., Hoyte, K., McKenzie, J.M., et al (1978). Clinical, biochemical and radiological reversibility of hyperprolactinemic galactorrhea- amenorrhea and abnormal sella by thyroxine in a patient with primary hypothyroidism.
Am.J.Obstet. Gynaecol. 131, 850-858.
279. Keye, W.R., Yuen, B.H., Knopf, R.F. et al (1977). Amenorrhea, hyperprolactinemia and pituitary enlargement secondary to primary hypothyroidism.
Am.J.Obstet.Gynaecol. 48, 697-702.
280. Stockigt, J.R., Essex, W.B., West, R.A. et al (1976). Visual failure during replacement therapy in primary hypothyroidism.
J.Clin.Endocrinol.Metab. 43, 1094-1100.
281. Chirito, I., Guyda, A., Friesen, H.G. (1972). Prolactin in renal failure.
Clin. Res. 20, 423.
282. Czernichow, P., Dauzt, M.C., Broyer, M. et al (1976). Abnormal TSH, PRL and GH response to TSH releasing factor in chronic renal failure.
J.Clin.End.Metab. 43, 630-637.
283. Leroith, D., Danovitz, G., Trestan, S. et al (1979). Dissociation of prolactin response to thyrotropin-releasing hormone and metoclopramide in chronic renal failure.
J.Clin.End.Metab. 49, 815-817.
284. Aragona, C., Friesen, H.G. (1975). Specific prolactin binding sites in the prostate and testis of rats.
Endocrinol. 97, 677-684.
285. Posner, B.I., Kelly, P.A., Friesen, H.G. (1974). Induction of a lactogenic receptor in rat liver : influence of estrogen and the pituitary.

Proc.Nat.Acad.Sci. USA 71, 2407-2410.
286. Jung, Y., Russfield, A.B. (1972).
Prolactin cells in the hypophysis of cirrhotic patients.
Arch.Pathol. 94, 265.
287. Horrobin, D.F., Manku, M.S., Nassar, B.A. (1974).
Hepatorenal syndrome and prolactin.
New Engl.J.Med. 290, 408.
288. Van Thiel, D.H., Gavaler, J.S., Lester, R. et al
(1975). Plasma estrone, prolactin, neurophysin and
sex steroid-binding globulin in chronic alcohol men.
Metabolism 24, 1015-1019.
289. Zanoboni, A., Zanoboni-Muciaccia, W. (1975).
Gynaecomastia in alcoholic cirrhosis.
The Lancet II, 876.
290. Panerai, A.E., Salerno, F., Manneschi, M. et al (1977).
Growth hormone and prolactin responses to thyrotropin
releasing hormone in patients with severe liver
disease.
J.Clin.End.Metab. 45, 134-140.
291. Wernze, H., Schmitz, E. (1977).
Plasma prolactin and prolactin release in liver cir-
rhosis.
Acta. Hepato-Gastroenterol. 24, 97-101.
292. Morgan, M.Y., Jakobovits, A.W., Gore, M.B.R. et al
(1978). Serum prolactin in liver disease and its
relationship to gynaecomastia.
Gut. 19, 170-174.
293. Van Thiel, D.H., McClain, C.J., Elson, M.C. et al
(1978). Hyperprolactinemia and thyrotropin-releasing
factor (TRH) responses in men with alcoholic liver
disease.
Alcoholism: Clin. Exp.Res. 2, 344.
294. Hasselbach, H.C., Beck, K., Eskildsen. P.C. (1981).
Serum prolactin and thyrotropin responses to thyro-
tropin-releasing hormone in men with alcoholic cirrho-
sis. Acta Med. Scand. 209, 37-40.

295. Lindholm, J., Hagen, C., Fabricius-Bjene, N. et al (1977). Gonadal function and serum prolactin in males with chronic alcoholism.
Acta Endocr. 85, 209.

296. Turkington,R.W. (1972).
Serum prolactin levels in patients with gynaecomastia.
J.Clin.Endocrinol. 34, 62.

297. Neubauer, M., Lemisch, K., Althoff, P. et al (1976).
Störungen des Sexualfunction und veränderungen der Sexualhormone bei Männern mit chronischen leberkrankheiten.
Vndl.Dtch.Ges.Inn.Med. 82, 318.

298. Nunziata, V., Ceparano, G., Mazzacca, G. et al (1978).
Prolactin secretion in nonalcoholic liver cirrhosis.
Digestion 18, 157-161.

299. Decoulx, M., Racadot, A., Fourlinnie, J.C.L. et al (1980). Prolactine et fonction gonadotrope dans 35 cas masculins d´hepatite alcoholique.
La Revue de Med. Int. I, 173-179.

300. Galvao-Teles, A., Monteiro, E., Goncalves, L. et al (1980). Male hypogonadism in alcoholic patients with and without cirrhosis.
IASL Abstract of papers Nov., abstract 1-12A, p.12.

301. Langer, M., Alagna, S., Madeddu, G. (1981).
Alterations in thyrotropin (TSH) prolactin (PRL) and growth hormone (GH) secretion in chronic alcoholism and in liver cirrhosis.
Abstract, Symposion: Hormones and the liver, Rome.

302. Majumdar, S.K. (1978).
Hyperprolactinemia in chronic alcoholics treated with promazine.
Lancet II, 101.

303. Ylikahri, R.H., Huttunen, M.O., Harkonen, M. (1977).
Effect of alcohol on anterior pituitary secretion of trophic hormones.
Lancet II, 1353.

304. Toro, G., Kolodny, R.C., Jacobs, C.S. et al (1973). Failure of alcohol to alter pituitary and target organ hormone levels. Clin.Res. 21, 505.

305. McClain, C.J., Kromhout, J.P., Van Thiel, D. (1979). Serum prolactin concentration in portal systemic encephalopathy. Gastroenterol. 76, 1292.

306. Newman, S.L., Caplan, D.B., Camp, V.M., et al (1979). Prolactin and the encephalopathy of Reye´s syndrome. Lancet II, 1097-1100.

307. Chopra, I.J., Tulchinsky, D., Greenway, F.L.(1973). Estrogen-androgen imbalance in hepatic cirrhosis. Ann. Int.Med. 79, 198-203.

308. Green, J.R.B., Mowat, N.A.G., Fisher, R.A. et al (1976). Plasma estrogen in men with chronic liver disease. Gut. 17,426-430.

309. Mowat, N.A.G., Edwards, C.R.W., Fisher, R. et al (1976). Hypothalamic-pituitary-gonadal function in men with cirrhosis of the liver. Gut. 17, 345-350.

310. Thorner, M.O., McNeilly, A.S., Hagan, C. et al (1974). Longterm treatment of galactorrhea and hypogonadism with bromocriptine. Brit.Med.J. 2, 419.

311. Thorner, M.P., Besser, G.M.(1977). Hyperprolactinemia and gonadal functions : results of bromocriptine treatment. In: Prolactin and human reproduction. Eds.: Crosignani, P.G., Robijn, C. Proceedings of Serono Symposia. Academic Press, London 11, 285.

312. Tarquini, B., Gheri, R., Anichini, P. et al (1977). Circadian study of immunoreactive prolactin in patients with cirrhosis of the liver. Gastroenterology 73, 116-119.

313. Piercy, M., Shin, S.H. (1980).
Comparative studies of prolactin secretion in Estradiol-primed and normal male rats induced by ether stress, pimozide and TRH.
Neuroendocrinol. 31, 270-175.

314. Gordon, G.G., Olivo, J., Rafii, F. et al (1975).
Conversion of androgens to estrogens in cirrhosis of the liver.
J.Clin.End. Metab.40, 1010-1026.

315. Thijssen, J.H.H., Laurens, J.,Donker, G.H. (1973).
Increased oestrogen production by peripheral conversion of androgens in male patients with cirrhosis of the liver.
J.Endocrinol. 57, 49.

316. Adlercreutz, H. (1974).
Hepatic metabolism of estrogens in health and disease.
New.Engl.J.Med. 290, 1081-1083.

317. Fisher, J.E., Baldessarini, R.J. (1971).
False neurotransmitters and hepatic failure.
Lancet 1, 75-79.

318. Manghani, K.K., Lunzer, M.R., Billing, B.H. (1975).
Urinary and serum octopamine in patients with portal systemic encephalopathy.
Lancet II, 943-946.

319. Partridge, W.M. (1977).
Kinetics of competitive inhibition of neutral amino acid transport across the blood-brain barrier.
J.Neurochem. 28, 103-108.

320. Knell, A.J., Davidson, A.R., Williams, B.D. et al (1974). Dopamine and serotonin metabolism in hepatic encephalopathy.
Brit.Med.J.1, 549-551.

321. Lal, S., Young, S.N., Sourkes, T.L. (1975).
5-Hydroxytryptamine and hepatic coma.
Lancet II, 979.

322. Morgan, M.Y., Jacobovits, A.W., James, I.M.et al (1980). Succesful use of bromocriptine in the treatment of chronic hepatic encephalopathy. Gastroenterol. 78, 663-670.
323. Parkes, J.D., Sharpstone, P., Williams, R. (1970). Levodopa in hepatic coma. Lancet II, 1341.

CHAPTER 3

THE KIDNEY IS THE MAIN SITE OF PROLACTIN ELIMINATION IN PATIENTS WITH LIVER DISEASE.

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ABSTRACT. Basal and TRH-stimulated PRL levels have been reported to be increased in patients with severe liver disease. To elucidate the relative role of the liver and kidney in the elimination of PRL in such patients, plasma PRL levels were measured before and after TRH in peripheral, hepatic, and renal vein samples taken from eight patients undergoing diagnostic hepatic vein catheterization. In two patients, arterial PRL levels, effective renal plasma flow, and estimated hepatic plasma flow were measured. All patients had evidence of portal hypertension with parenchymal liver cell insufficiency varying from mild to severe. There was no significant difference among basal arterial, peripheral, hepatic, and renal venous PRL levels. After TRH administration, the mean renal PRL levels were significantly lower than the mean peripheral levels in six of eight individual patients and lower when mean renal venous PRL levels and mean peripheral levels of all patients taken together were compared ($P < 0.001$). The two patients in whom no significant difference was found were the only patients who also had evidence of disturbed kidney function. Mean hepatic venous levels after TRH stimulation were slightly lower than mean peripheral levels, although the difference was significant in only one patient. The difference between mean hepatic and peripheral venous PRL levels of all patients taken together was also significant ($P < 0.025$). In two patients, effective renal plasma flow measured 468 and 324 ml/min, respectively, and estimated hepatic plasma flow measured 657 and 815 ml/min. From these data renal extraction of PRL was calculated to be 186.0 and 94.0 μg during the 60 min, while liver extraction of PRL amounted to only 16.4 and 11.4 μg, respectively.

The finding that hepatic elimination was not markedly affected by the severity of liver disease suggests that the kidney is more important than the liver in the removal of PRL. In patients with liver disease the kidney is the main site of PRL elimination. (*J Clin Endocrinol Metab* **51:** 70, 1980)

ALTHOUGH current knowledge of PRL secretion and metabolism is steadily increasing, relatively little is known about PRL elimination. Recent reports (1–3) show elevated plasma PRL levels in patients with either liver or kidney failure. To elucidate the relative role of the liver and kidney in the elimination of PRL in patients with liver disease, we measured plasma PRL levels before and after TRH injection in peripheral, hepatic, and renal vein samples taken from patients undergoing diagnostic hepatic vein catheterization.

Materials and Methods

Eight consecutive patients with liver disease with evidence of portal hypertension in whom wedged hepatic vein pressure measurements were indicated were asked to participate. The clinical and laboratory findings are shown in Table 1. The diagnosis of liver disease was based on percutaneous liver biopsy and laparoscopy in all instances. The function of the liver parenchyma, as measured by plasma albumin, Thrombotest (Nyegaard, Oslo, Norway), Normotest (Nyegaard), and bilirubin determination, ranged from normal to severely disturbed (Table 1). Renal function was monitored by plasma creatinine.

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Wedged hepatic vein pressure measurements were performed by the Seldinger technique via the femoral vein. This procedure was followed by iv injection of 400 μg TRH (Relefact, Hoechst, Amsterdam, The Netherlands). At 0, 10, 20, 30, 40, 50, and 60 min, blood samples were drawn selectively from the cubital, renal, and hepatic veins, while in patients 7 and 8 samples were taken from an indwelling needle in the radial artery at the same time. The renal and hepatic venous samples were taken by means of single catheter which was rapidly moved between the two veins. Samples were taken within 90 sec of of each other. All samples were immediately centrifuged, and the plasma was stored at −20 C until assay. Duplicate PRL levels were measured by immunoassay (normal value, up to 12 ng/ml in males and up to 15 ng/ml in females). The intraassay variation of determinations was 4.5% (50 duplicates; 2–50 ng/ml), and the interassay variation was 3.8% (mean, 14 ng/ml; n = 12). In patients 7 and 8, estimated hepatic plasma flow and effective renal plasma flow measurements were made at the same time during hepatic and renal vein catheterization. Estimated hepatic plasma flow was measured by continuous infusion of indocyanine green (4); effective renal plasma flow was measured by continuous infusion of [^{131}I]hippuran (5). Renal and hepatic removals of PRL in these two patients were calculated during the 60 min after TRH by the following equation, assuming a constant hepatic and renal plasma flow during the procedure: renal PRl removal = $(\bar{A} - \bar{R}) \times ERPF \times 60$ and hepatic PRL removal = $(\bar{A} - \bar{H}) \times EHPF \times 60$, where $\bar{A}$ is the mean arterial

TABLE 1. Clinical particulars and laboratory results of the eight patients undergoing catheterization

Patient no.	1	2	3	4	5	6	7	8
Pathology	Alcoholic cirrhosis	Steatosis hepatis	Incomplete septal fibrosis	Macronodular cirrhosis + chronic active hepatitis	Alcoholic hepatitis	Alcoholic cirrhosis	Idiopathic portal hypertension	Hepatic schistosomiasis
Age (yr)	44	67	68	67	46	54	64	27
Sex	M	F	M	M	M	M	F	M
BW (kg)	67	62	68	80	67	61	104	66
Clinical portal hypertension	+	±	+	+	+	+	+	±
Wedged HVP-free HVP (mm Hg)	17	3	19	16	25	12	13	1
Bilirubin (mg/dl)	9.4	3.0	1.2	2.6	11.7	0.9	1.3	1.4
Normotest (%)	19	140	73	57	25	68	86	106
Thrombotest (%)	16		85	79		66	34	91
Albumin (g/liter)	28	32.6	45	42.5	29	27.5	40	52
Creatinine (mg/dl)	1.2	0.5	1.1	1.0	0.7	1.9	0.8	1.3

HVP, Hepatic vein pressure.

PRL level, $\bar{R}$ is the mean renal venous PRL level, $\bar{H}$ is the mean hepatic venous PRL level, ERPF is the effective renal plasma flow, and EHPF is the estimated hepatic plasma flow.

Statistical analysis

The statistical significance of peripheral-renal vein and peripheral-hepatic vein differences was assessed by Student's paired t test. Both in the group as a whole and in each individual patient, all of the post-TRH PRL values (at all time points) were considered together in calculating the statistics.

Results

There was no significant difference among basal peripheral, renal, and hepatic venous PRL levels in the eight patients. After TRH stimulation, the mean renal venous PRL levels in each individual were significantly lower than mean peripheral PRL levels in six of the eight patients (Figs. 1 and 2; patients 1, 2, and 7, $P < 0.01$; patient 5, $P < 0.005$; patients 3 and 4, $P < 0.001$). In patient 8, the difference was not significant. This patient had a slightly elevated serum creatine level, while the effective renal plasma flow was below normal. Patient 6 (Fig. 3) showed a different pattern, with a delayed progressive rise of PRL after TRH and no significant difference among the peripheral, renal, and hepatic venous levels. This patient had severely disturbed kidney function due to chronic glomerulonephritis in addition to his liver disease. The difference between mean peripheral and mean renal venous levels of all patients taken together was highly significant ($P < 0.001$). Mean hepatic venous PRL levels were significantly lower than peripheral levels in only one patient (patient 5; $P < 0.05$); however, when the hepatic and peripheral venous values of all patients were combined, the difference between mean hepatic and peripheral levels was significant ($P < 0.025$).

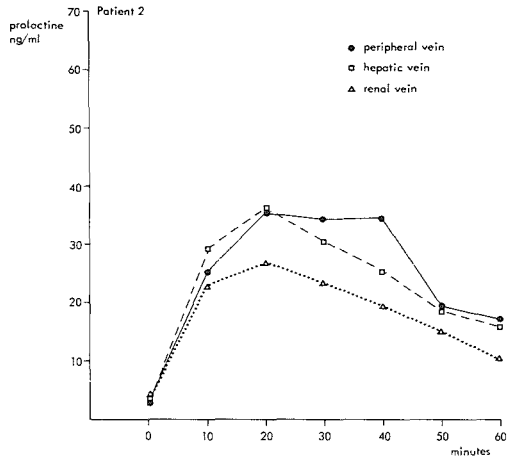

FIG. 1. Peripheral, hepatic, and renal venous PRL levels before and after TRH in patient 2.

In patients 7 and 8, arterial and peripheral venous levels were not found to differ significantly. The mean post TRH difference in PRL levels between hepatic vein and peripheral vein and between renal vein and peripheral vein did not correlate with the severity of liver failure, as judged by the albumin and clotting factor levels. In patients 7 and 8, effective renal plasma flows of 468 and 324 ml/min, respectively, were calculated assuming constant renal plasma flow during the duration of the catheterization. PRL levels of 186 and 94 μg, respectively, were determined to have been extracted by the kidney. In the same patients, estimated hepatic plasma flow rates

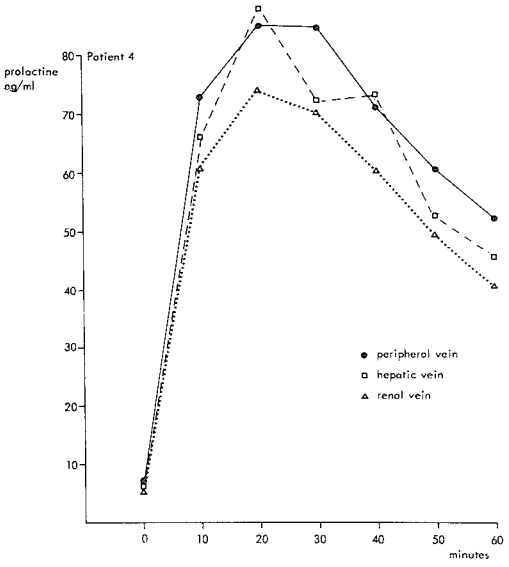

FIG. 2. Peripheral, hepatic, and renal venous PRL levels before and after TRH in patient 4.

TABLE 2. PRL levels (nanograms per ml) before and after TRH in peripheral, hepatic, and renal vein samples (mean ± SEM)

	Time (min)						
	0	10	20	30	40	50	60
Peripheral vein							
Mean	7.7	46.8	49.8	48.6	44.8	39.9	33.2
SEM	1.3	6.5	6.5	6.4	4.6	5.1	5.1
Hepatic vein							
Mean	7.6	43.2	52.0	45.2	41.6	38.1	33.4
SEM	2.3	4.9	6.8	6.2	5.9	4.9	4.2
Renal vein							
Mean	7.1	40.4	44.9	41.1	37.7	34.3	29.5
SEM	1.0	4.9	5.8	5.3	4.6	4.8	5.2

of 657 and 815 ml/min were found. Assuming a constant hepatic plasma flow, 16.4 and 11.4 μg PRL, respectively, were calculated to have been extracted by the liver during the same 60-min period.

Discussion

The organs involved in the inactivation of PRL have been studied in several animal species. Raganiemi *et al.* (6) observed a rapid accumulation of iv administered ^{125}I-labeled ovine PRL in the livers and kidneys of both mice and rats. With microautoradiography of the liver, a diffuse distribution of [^{125}I]PRL in the liver suggested that both the parenchymal and Kuppfer cells may be involved

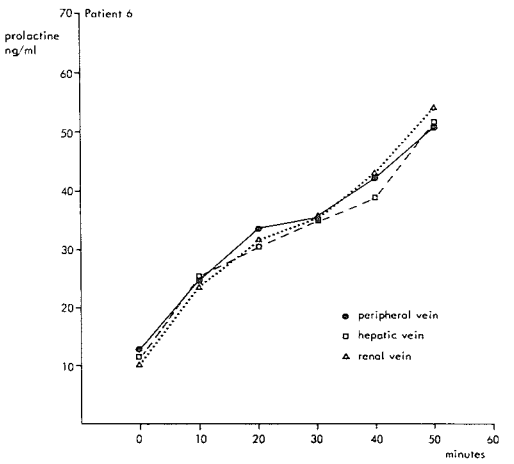

FIG. 3. Peripheral, hepatic, and renal venous PRL levels before and after TRH in a patient with cirrhosis and chronic glomerulonephritis (patient 6).

in the inactivation of PRL. In the kidney, PRL was almost exclusively localized in the proximal tubular cells. Donatsch and Richardson (7) confirmed, with a fluorescein-labeled double antibody technique, that 30 sec after iv injection, the ovine PRL could be visualized in the lumina of the proximal tubular; these findings suggested that the hormone gained access to these cells via the glomerular filtrate.

Cowden *et al.* (3) found elevated PRL levels in 32% of patients with renal disease. A significant correlation was observed between PRL and creatine concentration in these patients, while PRL reverted toward normal after successful renal transplantation. A significant arteriovenous PRL concentration difference across the kidney was found under basal conditions in seven patients with nonrenal, nonendocrine disease, suggesting that the kidney plays an important role in PRL metabolism.

In contrast, Modlinger and Gutkin (8) reported that the mean renal venous PRL concentration did not differ from peripheral venous concentrations obtained simultaneously during renal vein catheterization. TRH stimulation, however, was not used.

Lim *et al.* (2) observed a diminished responsiveness to TRH in chronic renal failure. In these patients the PRL response was less than that in controls, and the time of response was delayed 30 min. Panerai *et al.* (1), on the other hand, found an exaggerated and sustained PRL rise after TRH stimulation in subjects with liver disease whose mean basal plasma PRL levels were within normal range.

In our study no difference was found in basal periph-

eral, hepatic, and renal venous PRL levels. After TRH stimulation, however, significantly lower PRL levels were found in the renal vein compared with peripheral PRL levels in six of eight patients, while the difference between renal and peripheral levels of PRL in all patients taken together was also highly significant. The two patients in whom no significant difference was found were the only patients who also had impaired renal function. Patient 8, in whom the difference was not significant, had a slightly elevated serum creatinine level and a diminished effective renal plasma flow. Patient 6 had severely disturbed renal function due to chronic glomerulonephritis. In this patient, the reaction to TRH stimulation was of the type described by Lim (2) in chronic renal failure. Hepatic vein PRL levels were somewhat lower than peripheral levels in seven of eight patients, although this difference reached significance in only one patient (patient 5). However, when the hepatic and peripheral venous PRL levels of all patients taken together were compared, the difference was found to be statistically significant ($P < 0.025$).

In two patients, renal extraction of PRL was found to be 186 and 94 μg, respectively during a 60-min period compared to a liver extraction of 16.4 and 11.4 μg, respectively, during the same 60-min period. These results suggest that the kidney is the main site of PRL elimination in patients with hepatic disease, while the liver plays only a minor role. For obvious reasons, a control group could not be established. However, the fact that there was no relation between hepatic elimination of PRL and degree of liver failure suggests that even in the presence of normal hepatic function, the kidney is more important than the liver in PRL elimination. This point is further substantiated by a recent report by Bratusch-Marrain *et al.* (9), who did not find a significant uptake of endogenous PRL by the liver in 12 healthy patients. The mechanism by which PRL is removed by the kidney is not clear. In view of the molecular weight of PRL, it is likely that it passes into the glomerular filtrate. Since only a small amount has been detected in the urine (10) most of the PRL is probably taken up by the tubular cells.

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References

1. Panerai AE, Salerno F, Manneschi N, Cocchi D, Müller EE 1977 Growth hormone and prolactin responses to thyrotropin releasing hormone in patients with severe liver disease. J Clin Endocrinol Metab 45:134
2. Lim VS, Kathpalia SC, Frohman LA 1979 Hyperprolactinemia and impaired pituitary response to suppression and stimulation in chronic renal failure: reversal after transplantation. J Clin Endocrinol Metab 48:101
3. Cowden EA, Ratcliffe WA, Dobbie JW, Kennedy AC 1978 Hyperprolactinemia in renal disease. Clin Endocrinol (Oxf) 9:241
4. Leevy CM, Mendenhall CL, Lesko W, Howard MM 1962 Estimation of hepatic blood flow with indocyanine green. J Clin Invest 41: 1169
5. Donker AJM, van der Hem GK, Sluiter WJ, Beekhuis H 1977 A radioisotope method for simultaneous determination of the glomerular filtration rate and the effective renal plasma flow. Neth J Med 20:97
6. Raganiemi H, Oksanen A, Vanha-Perttula T 1974 Distribution of ^{125}I prolactin in mice and rats. Studies with whole body and micro autoradiography. Horm Res 5:6
7. Donatsch D, Richardson B 1975 Localisation of prolactin in rat tissue using a double antibody technique. J Endocrinol 66:101
8. Modlinger RS Gutkin M 1978 Plasma prolactin in essential and renovascular hypertension. J Lab Clin Med 91:693
9. Bratusch-Marrain P, Björhman O, Waldhäusl W, Wahren J 1979 Hepatic disposal of endogenous growth hormone and prolactin in man. Eur J Clin Invest 9:257
10. Nader S, Mashiter K, Joplin GF 1975 Urinary prolactin excretion. Lancet 1:928

CHAPTER 4.

PERIPHERAL ELIMINATION OF GROWTH HORMONE IN CHRONIC LIVER DISEASE.

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ABSTRACT.

Chronic liver disease is associated with raised basal and TRH stimulated PRL and GH levels. In a recent study we found the kidney to be the main site of prolactin elimination in patients with liver disease. In order to determine whether this is specific for PRL or a more general mechanism for polypeptide removal we studied the elimination of GH, which resembles PRL in molecular weight and primary amino acid sequence, in 5 patients with portal hypertension and hepatic cirrhosis and 5 patients with non-cirrhotic portal hypertension. Plasma GH levels were measured before and after TRH in peripheral, hepatic and renal vein samples, taken during diagnostic hepatic vein catheterization. An excessive paradoxical increase of GH after TRH stimulation was found in 4 out of 5 cirrhotic patients but in none of the noncirrhotic individuals ($p < 0.025$). After TRH the mean hepatic venous levels were significantly lower than the peripheral venous levels in 4 out of 5 noncirrhotic patients but in only one of the 5 cirrhotic patients ($p < 0.05$). The mean renal vein GH levels were significantly lower than the peripheral levels in 3 out of 5 noncirrhotic patients and in none of the cirrhotic patients. In two patients in whom renal and hepatic plasma flow was measured, renal extraction of GH was found to be 0 ug and 6.4 ug, while liver extraction amounted to 22.1 ug and 34.7 ug of GH during the same 60 minute period. Despite the similarity in molecular weight and primary amino acid sequence between PRL and GH, GH appears to be mainly taken up by the liver while PRL is mainly eliminated by the kidney in this group of patients with portal hypertension. This suggests that the renal elimination of prolactin is not solely dependent on glomerular filtration. The selective hepatic removal of growth hormone is probably related to a specific action of growth hormone on liver metabolism.

INTRODUCTION.

Chronic liver disease is associated with raised basal and TRH stimulated levels of prolactin (PRL) and growth hormone (GH) (Panerai et al 1977). In a recent study we showed that the kidney was the main site of PRL elimination in patients with liver disease (Bauer et al 1980). It has been suggested that due to its molecular size, PRL passes into the glomerular filtrate and is then either taken up by the tubular cells or excreted in the urine (Nader et al 1975). In view of the resemblances in molecular weight and primary amino acid sequence between PRL (Shome and Parlow 1977) and GH (Niall et al 1973) we measured GH in plasma from peripheral, renal and hepatic veins from the same patients, to determine whether GH elimination occurs in a similar fashion to PRL elimination.

PATIENTS,MATERIALS AND METHODS.

Ten consecutive patients with clinical portal hypertension in whom wedged hepatic vein pressure measurements were indicated, were asked to participate. All patients had splenomegaly and presented with varying degrees of oesophagal varices and/or ascites.
Clinical and laboratory findings are summarized in table I. The patients could be subdivided into a group of five patients with histologically proven liver cirrhosis and a group of five patients with noncirrhotic portal hypertension. The diagnosis of liver disease was confirmed by percutaneous liver biopsy and laparoscopy in all instances. Liver parenchymal function, as assessed by plasma albumin, Thrombotest ^{R}(Nyegaard, Oslo,Norway), Normotest ^{R}(Nyegaard) and bilirubin determination, ranged from normal to severely disturbed (table I). Renal function was monitored by plasma creatinine. Wedged hepatic vein pressure measurements were performed by the Seldinger technique via the femoral vein.

Heparinised blood samples from hepatic, renal and antecubital veins before and at 10 minute intervals during one hour after the intravenous injection of 400 ug of TRH (Relefact ^{R}, Hoechst) were taken. The renal and hepatic venous samples were taken by means of a single catheter which was rapidly moved between the two veins. Samples were taken within 90 seconds of each other. In patients 7 and 8 arterial samples were obtained from an indwelling needle in the radial artery, and in addition estimated hepatic plasma flow and effective renal plasma flow measurements were made at the same time during hepatic and renal vein catheterization. Estimated hepatic plasma flow was measured by continuous infusion of Indocyanine Green (Leevy et al 1962); effective renal plasma flow was measured by continuous infusion of (^{131}I) Hippuran (Donker et al 1977).

Renal and hepatic GH removal during the catheterization in patients 7 and 8 were calculated as follows:

$$\text{Renal GH removal} = (A - R) \times ERPF \times 60$$

$$\text{Hepatic GH removal} = (A - H) \times EHPF \times 60$$

where A = mean arterial GH, R = mean renal vein GH, H = mean hepatic vein GH, ERPF = effective renal plasma flow and EHPF = estimated hepatic plasma flow.

Duplicate GH levels were measured by immunoassay (I.R.E.). Normal values under basal conditions are less than 5 ng/ml; the interassay variation of determinations was 5.3% (30 duplicates 1-25 ng/ml) and the interassay variation was 4.2% (x = 3.8 ng/ml; n = 8).

Statistical analysis: The statistical significance of differences in peripheral and renal vein, and peripheral and hepatic vein GH levels, was assessed by paired Student s t-test. In each individual patient and in the patient groups all of the post-TRH GH values were used in the analysis.

The significance of differences between the two patients

groups in the incidence of paradoxical GH increases after TRH and the occurence of significant peripheral - renal vein and significant peripheral - hepatic vein differences, was assessed by Fishers exact probability test.

RESULTS.

There was no significant difference between basal peripheral, renal and hepatic venous GH levels in the 10 patients, nor were there significant differences in the basal GH levels between the cirrhotic and the noncirrhotic group (table II). An excessive increase in GH after TRH stimulation was found in 4 out of 5 cirrhotic patients but in none of the noncirrhotic individuals ($p < 0.025$). The paradoxical increase of GH after TRH did not however correlate with serum albumin, a decrease in clotting factors as measured by the Normotest ^{R}, bilirubin or SGOT concentration, nor with renal function as measured by the serum creatinine level. After TRH stimulation the mean hepatic vein levels were significantly lower than the peripheral venous levels in 4 out of 5 noncirrhotic patients, but in only one of the 5 cirrhotic patients ($p < 0.05$). The mean renal vein GH levels were significantly lower than the peripheral levels, in 3 out of 5 noncirrhotic patients and in none of the cirrhotic patients. Examples of growth hormone levels in the renal, hepatic and peripheral veins from patients from each group are shown in fig. 1 and 2. The difference between all hepatic vein and all peripheral vein GH levels of each group when taken together was found to be statistically significant ($p < 0.001$). The difference between all renal vein and all peripheral vein GH levels was also significant for both groups when all individuals were taken together(cirrhotic group $p < 0.05$, noncirrhotic group $p < 0.001$). Mean values for all peripheral vein GH levels were 13.8 ng/ml for the cirrhotic patients and 4.2 ng/ml for the

noncirrhotic group, mean values for all hepatic vein GH levels were 12.9 ng/ml for the cirrhotics and 3.1 ng/ml for the non-cirrhotics; mean renal vein GH levels were 12.9 ng/ml and 3.6 ng/ml for the two groups.
In patients 7 and 8 arterial and peripheral vein levels did not differ significantly. In patient 7 renal extraction of GH could not be demonstrated. In patient 8 the difference in peripheral and renal vein GH levels was significant ($p < 0.05$). This patient had a renal plasma flow of 324 ml/min and assuming a constant renal plasma flow during the duration of the catheterization it was calculated that 6.4 ug of GH was extracted by the kidneys in this patient. In the same patients an estimated hepatic plasma flow of 657 ml/min and 815 ml/min was found. Again assuming a constant hepatic plasma flow it was calculated that 22.1 ug and 34.7 ug of GH were extracted by the liver during the same 60 minute period.

DISCUSSION

Both the liver and the kidney have been implicated in the peripheral elimination of GH, and it has been suggested that elevated GH levels in patients with liver cirrhosis might in part be due to decreased hepatic removal (Cameron et al 1972). In healthy man the liver has been shown to be responsible for approximately 50% of the total GH clearance (Bratusch-Marrain et al 1979). In this small series of patients we were able to confirm the work of others (Panerai et al 1977, Zanoboni and Zanoboni-Mucciacca 1977, van Thiel et al 1978) that liver cirrhosis is associated with a paradoxical rise in GH levels following TRH administration, and furthermore that hepatic removal of GH was lower in the cirrhotic group than in the non-cirrhotic group. Normal metabolic clearance rates have been reported in patients with liver cirrhosis (Owens et al 1973), and although the diminished uptake of GH by

the liver is probably in part responsible for the raised GH levels in cirrhosis, it seems unlikely that impaired extraction plays a major role in initiating a paradoxical increase. It is more likely that a paradoxical rise of GH after stimulation is caused by an abnormal GH secretion. Several possible mechanisms come to mind. The first possibility is an impaired feedback mechanism caused by a diminished somatomedin production in the liver. Elevated GH levels together with decreased somatomedin production in the liver have been reported by Schimpff et al (1978). Although increased somatomedin production took place in the kidney, peripheral vein somatomedin levels were significantly lower than in controls. A negative feedback mechanism by somatomedin is therefore a possibility.A relation between increased GH levels and elevated estrogen levels was reported (Hernandez et al 1969), however van Thiel et al (1978) found a significant increase of GH after TRH in cirrhotic patients but not in alcoholic patients without cirrhosis, while the estrogen levels in both groups were the same. However sex steroid binding protein concentrations were not measured. A third possibility is that the elevated GH levels in chronic liver disease may be related to impaired nutritional status. Basal plasma growth hormone is elevated in kwashiorkor, which is caused by a lack of protein, but not in marasmus. After refeeding GH levels return to normal (Raghunamulu and Jaya Rao 1974). In chronic liver disease (Wright et al 1968) a direct relationship between low plasma albumin and elevated growth hormone has been established. In our small patient group no such relation could be found. Both GH and PRL have a molecular weight of about 21.5000 and the primary amino acid sequence of these two hormones shows many similarities (Niall et al 1973). Despite these resemblances, in this group of patients with portal hypertension GH appears to be mainly taken up by the liver while PRL is eliminated mainly by the kidney (Bauer et al 1980). The mechanisms

whereby these two polypeptide hormones are removed from the circulation by the liver or the kidney remains unclear. Specific binding sites for GH have been demonstrated in human liver (Carr and Friesen 1976) but it is not known whether these are involved in the clearance of GH. The relative specificity of PRL removal by the kidney suggests that the process is not solely due to loss via glomerular filtration.

REFERENCES.

1. Bauer, A.G.C., Wilson, J.H.P. and Lamberts, S.W.J. (1980). The kidney is the main site of prolactin elimination in patients with liver disease. J.Clin.Endocrinol.Metab. 51: 70-73
2. Becker, M.D., Cook, G.C. and Wright, A.D. (1969). Paradoxical elevation of growth hormone in active chronic hepatitis. Lancet 2: 1035 -1039.
3. Bratusch-Marrain, F., Björkman, O., Waldhäusl , W., and Wahren, J. (1979). Hepatic disposal of endogenous growth hormone and prolactin in man. Europ.J.Clin.Invest. 9 : 257-260.
4. Carr, D. and Friesen, G. (1976): Growth hormone and insulin binding to human liver. J.Clin.Endocrinol. Metab. 42 : 484-493.
5. Cameron, D.P., Burger, H.G., Catt, K.J., Gordon, E. and Watts, J.H. (1972). Metabolic clearance of human growth hormone in patients with hepatic and renal failure, and in the isolated pig liver. Metabolsim 21 : 895 - 904.
6. Donker, A.J.M., van der Hem, G.K., Sluiter, W.J. and Beekhuis, H. (1977). A radioisotope method for simultaneous determination of the glomerular filtration and the effective renal plasma flow. Neth. J.Med. 20 : 97-99.
7. Hernandez, A., Zorilla, E. and Gerhberg, H.(1969). Decreased insulin production elevated growth hormone levels, and glucose intolerance in liver disease. J.Lab.Clin.Med. 73 : 25-33.
8. Leevy, C.M., Mendenhall, C.L., Lesko, W. and Howard, M.M. (1962).

Estimation of hepatic bloodflow with Indocyanine Green.
J.Clin.Invest. 41 : 1169 - 1172.

9. Nader, S., Mashiter, K. and Joplin, G.F. (1975).
Urinary prolactin excretion.
Lancet 1 : 928.

10. Niall, H.D., Hogan, M.L., Tregear, G.W., Segre, G.V., Hoang, P. and Friesen, H. (1973).
The chemistry of growth hormone and the lactogenic hormones.
Current Progress Horm.Res. 29 : 387.

11. Owens, D., Srivasrava, M.C., Tompkins, C.V., Nabarro, J.D.N. and Sönksen, P. (1973).
Studies on the metabolic clearance rate, apparent distribution space and plasma half-disappearance time of unlabelled growth hormone in normal subjects and in patients with liver disease, renal disease, thyroid disease and diabetes mellitus.
Europ.J.Clin. Invest. 3 : 284-294.

12. Panerai, A.E., Salerno, F., Manneschi, N., Cocchi, D. and Müller, E.E. (1977).
Growth hormone and prolactin responses to thyrotropin releasing hormone in patients with severe liver disease.
J.Clin.Endocrinol. Metab. 45 : 134- 140.

13. Raghunamulu, N. and Jayarad, K.S. (1974).
Growth hormone secretion in protein calorie malnutrition.
J.Clin.Endocrinol.Metab. 38 : 176 - 179.

14. Schimpff, R.M., Lebrec, D. and Bonnadieu, M. (1978).
Serum somatomedin activity measured as sulphation factor in peripheral hepatic and renal veins of patients with alcoholic cirrhosis.
Acta Endocrinol. 88 : 729 - 736.

15. Shome, B. and Parlow, A.F. (1977).
Human pituitary prolactin (h PRL) : the entire linear amino acid sequence.

J.Clin.Endocrinol. Metab. 45 : 1112 - 1115.

16. Van Thiel, D.H., Cavaler, J.S., Wight, C., Smith, W.I. and Abuid, J. (1978).
Thyrotropin releasing hormone (TRH) induced growth hormone (h GH) responses in cirrhotic man.
Gastroenterology 75 : 66-70.

17. Wright, A.D., Lowy, C., Russel Fraser T., Spitz, I.M., Rubenstein, A.K. and Bersohn, I. (1968).
Serum growth hormone and glucose intolerance in renal failure.
Lancet 2 : 798 - 800.

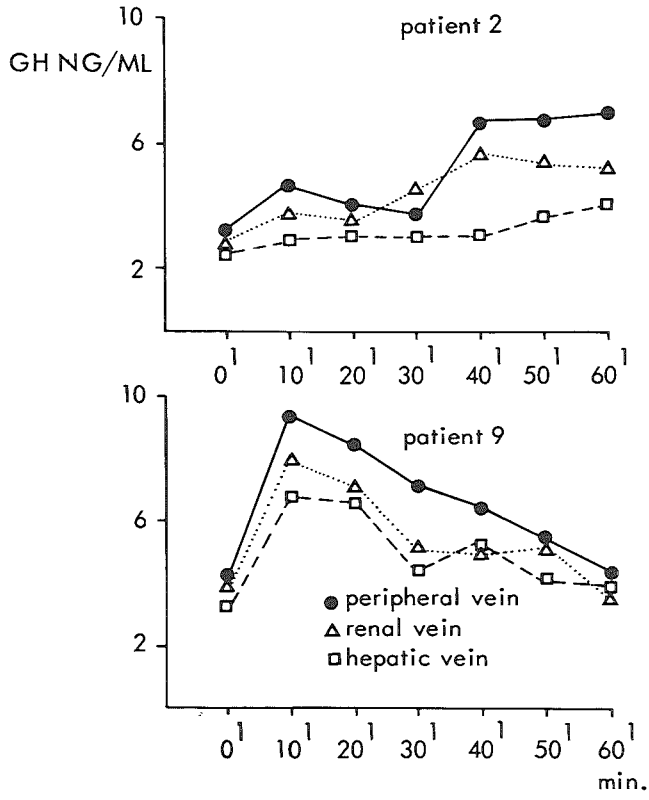

Fig.1: Peripheral vein, hepatic vein and renal vein GH levels before and after TRH in 2 patients with non-cirrhotic liver disease.

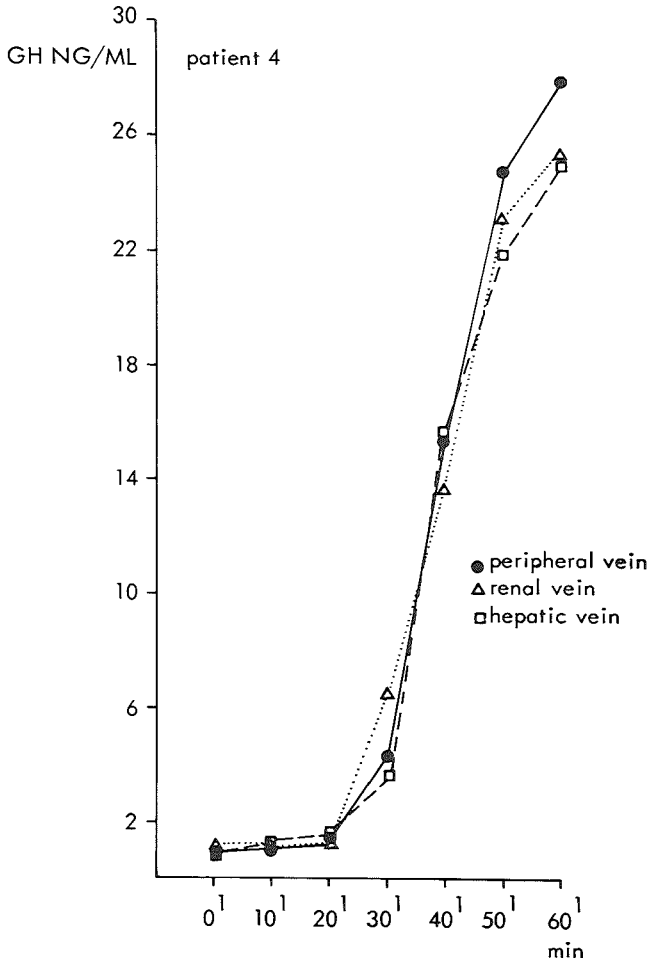

Fig.2: Peripheral vein, hepatic vein and renal vein GH levels before and after TRH in 1 patient with cirrhosis.

Table 1: Clinical particulars and laboratory results of the 10 patients undergoing catheterization.

	cirrhosis					noncirrhosis				
patient	1	3	4	5	6	2	7	8	9	10
pathology	alcoholic cirrhosis	incomplete septal fibrosis	macro nodular cirrhosis + chronic act.hep.	alcoholic cirrhosis	alcoholic cirrhosis	steatosis hepatis	idiopathic portal hyper-tension	hepatic schisto-somiasis	right heart failure	idiopathic portal hyper-tension
age (yrs)	44	68	67	46	54	67	64	27	65	67
sex	M	M	M	M	M	F	F	M	M	M
body weight (kg)	67	68	80	67	61	62	104	66	56	85
wedged H.V.P. free H.V.P. (mmHg)	17	19	16	25	12	3	13	1	3	22
bilirubin (n=12 umol/l)	80	10	22	100	8	26	11	12	20	10
normotest	19%	73%	57%	25%	68%	140%	86%	106%	82%	76%
albumin g/l	28	46	52.5	29	27.5	32.6	40	52	43	42
creatinine (n=60-110 umol/l)	108	100	87	64	170	48	72	115	130	99
basal GH (ng/ml)	9.0	0.8	1.6	1.6	6.6	4.1	1.1	5.2	5.2	3.2
max GH (after TRH) ng/ml	23.0	27.7	24.5	2.2	30.7	9.4	4.1	5.3	5.4	6.7

Table II: Mean growth hormone (ng/ml) S.E.M. at 0' and after 400 ug TRH

		0'	10'	20'	30'	40'	50'	60'
cirrhosis	v. per	3.9 ± 1.6	9.6 ± 3.4	13.5 ± 5.0	16.0 ± 5.6	18.8 ± 4.5	19.4 ± 4.6	15.2 ± 5.3
N = 5	v. hep	3.2 ± 1.2	9.2 ± 3.4	12.5 ± 4.7	15.7 ± 5.4	17.3 ± 4.3	17.6 ± 4.2	15.2 ± 5.0
	v. ren	4.1 ± 1.5	9.0 ± 3.4	13.2 ± 5.0	14.9 ± 4.4	16.6 ± 4.2	17.6 ± 4.5	14.8 ± 5.0
noncirrhosis	v. per	3.8 ± 0.8	5.3 ± 1.0	5.1 ± 0.8	4.0 ± 0.8	4.0 ± 1.0	3.6 ± 0.9	3.3 ± 1.0
N = 5	v. hep	3.2 ± 0.7	3.9 ± 0.8	3.7 ± 0.8	3.0 ± 0.4	2.9 ± 0.6	2.7 ± 0.6	2.5 ± 0.6
	v. ren	3.0 ± 0.8	4.2 ± 0.3	4.3 ± 0.7	3.8 ± 0.7	3.7 ± 0.9	3.5 ± 1.0	2.8 ± 0.7

Statistical significance cirrhosis VP - VH : $p < 0.001$
VP - VR : $p < 0.05$

noncirrhosis VP - VH : $p < 0.001$
VP - VR : $p < 0.001$

CHAPTER 5

HYPERPROLACTINEMIA OF PORTAL HYPERTENSION IN RATS.

Hyperprolactinemia of Portal Hypertension in Rats

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Plasma prolactin levels are often raised in patients with liver cirrhosis and portal hypertension. To obtain more insight into the underlying mechanisms we examined the synthesis and release of prolactin in male rats with partially ligated portal veins. Portal hypertension led to an increase in pituitary prolactin, plasma prolactin, and plasma 17β-estradiol, and a decrease in hypophyseal stalk dopamine levels. Castration decreased plasma prolactin levels and prevented the induction of hyperprolactinemia by portal hypertension. Administration of dihydrotestosterone to castrated animals did not affect prolactin levels in the pituitary gland or in the plasma. Plasma tryptophan and tyrosine concentrations did not change in portal hypertension. A low protein diet caused a decrease in plasma tryptophan and an increase in plasma tyrosine levels without affecting prolactin levels in either controls or portal hypertensive rats. The hyperprolactinemia of portal hypertension is probably caused by elevated estrogen levels which interfere with hypothalamic dopamine release. Changes in plasma amino acid levels are of little importance in the regulation of prolactin release in portal hypertensive rats.

Plasma prolactin (PRL) levels are often elevated in patients with cirrhosis of the liver (1–5). Since PRL in patients with liver disease is mainly eliminated by the kidney (6–8), it is unlikely that the observed higher PRL levels are caused by reduced peripheral elimination. These patients also have an exaggerated response of plasma PRL to thyrotrophin releasing hormone (TRH) (2,3,5) and lose the diurnal changes in plasma PRL levels (9,10). Thus elevated PRL levels in liver cirrhosis are likely caused by an abnormality in the control of PRL release.

PRL secretion by the anterior pituitary is tonically inhibited by the hypothalamus and this inhibition is mainly caused by dopamine (11–13). Thus the increased PRL release in liver cirrosis may be caused by changes in dopaminergic activity in the central nervous system (3). Plasma prolactin levels are much higher in cirrhotic patients with hepatic encephalopathy than in patients with cirrhosis (14). Several authors have suggested that in hepatic encephalopathy false neurotransmitters (e.g., octopamine instead of dopamine) might be formed due to a change in the ratio of plasma aromatic amino acids to branched chain amino acids (15,16). Administration of the dopamine agonist bromocriptine has been reported to result in improvement of the encephalopathy (16), thus providing evidence in favor of a decreased dopaminergic activity of the central nervous system in this type of patient. On the other hand estrogen levels are elevated in cirrhosis (17) and it is well known that estrogens can stimulate PRL release, probably through interference with dopamine secretion from the hypothalamus, and through a direct effect on the anterior pituitary (18–21).

In the present investigation the possible role of changes in dopaminergic control and of increased estrogen levels in inducing high levels of PRL were studied. In order to eliminate the possible effect of liver disease or alcoholism, both factors that may lead to elevated prolactin levels, a rat model of portal hypertension in which hepatic mass is preserved was used (22). Plasma 17β-estradiol and hypophyseal stalk plasma dopamine concentrations were measured in these and control animals. Some animals were placed on a protein-restricted diet in order to change the plasma levels of the amino acid precursors of dopamine and serotinine, whereas other

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Table 1. *Pituitary PRL Concent Without or After Incubation with Dopamine in 8 Portal Hypertensive and 8 Sham-Operated Rats*

	PRL (μg/mg pituitary)		Total pituitary PRL concent (μg)
	Medium	Pituitary gland	
Sham-operated controls	1.82 ± 0.29	1.62 ± 0.30	23.1 ± 1.8
+ dopamine (500 nm)	0.49 ± 0.10[a]	2.43 ± 0.45	
Portal hypertension	3.15 ± 0.24[a]	3.38 ± 0.29[a]	34.9 ± 3.1[a]
+ dopamine (500 nm)	0.36 ± 0.02[a]	3.38 ± 0.29[a]	

[a] $p < 0.01$ vs. control.

animals were castrated to cause lower peripheral estrogen levels (23).

Methods

Male Wistar rats (TNO, Zeist, The Netherlands) weighing 202 ± 23 g (mean ± SD) were used. Portal hypertension (PH) was induced by partial portal vein ligation (21); control rats underwent sham operation. Spleen weight served as a measure of the success of the operation on the portal circulation. Furthermore, portal vein pressure measurements were performed via cannulation of the mesenteric vein under ether anesthesia in 5 sham-operated and 5 portal hypertensive rats. The pressure in the inferior vena cava was taken as a reference. The portal vein pressure reported is the difference between portal vein pressure and inferior vena cava pressure.

Experiment 1

Eight portal hypertensive rats and 8 sham-operated rats were studied 3 wk after operation. The rats were killed by decapitation, and trunk blood was collected into heparinized tubes for 17β-estradiol assay. Plasma samples were stored at −20°C until assay. The pituitary glands were removed, weighed, incubated for 4 h in medium 199 (Gibco-Biocult, Glascow, Scotland), with or without dopamine, as described previously (12).

Experiment 2

Three groups of 8–10 portal hypertensive rats and 8–10 sham-operated rats were used. Group 1 rats were fed normal chow, group 2 rats, in addition to undergoing portal vein ligation or sham-operation, were castrated, and group 3 rats were put on a protein-restricted diet (5% caseine by weight, Hope Farms, Woerden, The Netherlands). Four weeks later all rats were decapitated. Plasma was stored at −20°C. The pituitary glands, livers, and spleens were removed and weighed. Plasma prolactin levels were measured in the pituitary glands. Plasma levels of 17β-estradiol, PRL, tyrosine, and tryptophan were determined.

Experiment 3

Hypophyseal portal blood was sampled in 8 rats with portal hypertension and 8 sham-operated control rats at a rate of 4–8 μl/min for 60 min using the method of Porter and Smith (24) with some modifications (11,24) while rats were under urethane (ethylcarbamate, 1.2 g/kg body wt) anesthesia.

Dopamine was measured in the hypophyseal stalk plasma samples. Just before cutting the hypophyseal stalk a peripheral blood sample was obtained via a cannula inserted in the right femoral artery to measure its PRL content.

Experiment 4

Two groups of 7 castrated portal hypertensive rats were established. Group 1 rats were injected subcutaneously with 150 μg dihydrotestosterone propionate in oil s.c. once daily, while the second group, injected with the same amount of oil (0.1 ml) served as controls.

Assays

Plasma prolactin levels were measured by a double antibody radioimmunoassay using materials and protocols supplied by the NIAMDD Rat PRL RP-1. Intraassay variation in male animals was 4.2% (n = 38, values varying between 10 and 30 ng/ml), interassay variation 6.7% (n = 6). 17β-Estradiol was measured by radioimmunoassay as described previously (23). Dopamine was measured by a high performance liquid chromatographic-electrochemical method (25) as previously described (26). The intra- and interassay variations of these determinations have been described in the references cited above (23,26). Tyrosine was determined by a fluorometric method (27). Tryptophan was measured by a modification of the fluorometric method of Denkla and Dewey (28).

Statistical Analysis

Results are expressed as mean ± SEM. Statistical analysis comprised the nonparametric Wilcoxon test and analysis of variance. When significant overall effects were obtained with the analysis of variance, comparisons between groups were made with Duncan's multiple range test. Differences were considered significant when $p < 0.05$.

Results

Partial ligation of the portal vein resulted in significant increases in mean spleen weight (88 ± 4.5 mg, n = 38) compared with 69.0 ± 3.2 mg in controls (n = 38, $p < 0.005$). In contrast liver weights remained unchanged (sham 9.68 ± 0.23 g, PH 9.22 ± 0.31 g NS). Mean portal pressure (portal vein pressure − inferior vena cava pressure) was 11.9 ± 1.0 mmHg in the rats with partial ligation of the portal vein (n = 5) as opposed to 1.6 ± 0.5 mmHg in the control animals (n = 5, $p < 0.005$).

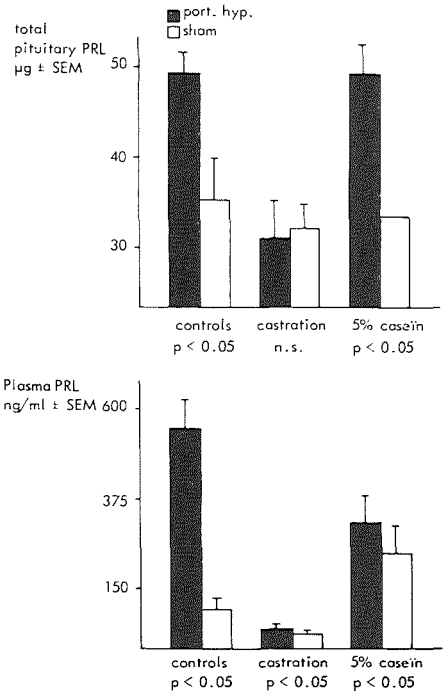

Figure 1. The effect of portal hypertension, castration, and a 5% casein diet on pituitary and plasma prolactin.

Experiment 1

The results are summarized in Table 1. Total pituitary PRL content was increased in the portal hypertensive rats. Dopamine inhibited PRL release by the pituitary gland in vitro of portal hypertensive rats to a similar extent as in control rats. Plasma 17β-estradiol levels were significantly elevated in the portal hypertensive rats (17.0 ± 1.6 pg/ml) compared with sham-operated controls (11.4 ± 1.9 pg/ml) ($p < 0.01$).

Experiment 2

Total pituitary PRL content and plasma prolactin was significantly higher in portal hypertensive than in sham-operated rats (group 1, normal chow) (Tables 1 and 2, Figure 1). However, castration eliminated the effect of portal hypertension on pituitary PRL content and on plasma PRL levels (group 2) (Table 2, Figure 1). Castration also lowered plasma PRL concentration in control rats (Figure 1). Plasma tyrosine and tryptophan levels did not differ between the portal hypertensive and sham-operated rats of groups 1 and 2. Neither portal hypertension nor castration affected plasma tyrosine and tryptophan levels (Table 2). In the rats on a protein-deficient diet (group 3) plasma PRL levels and the pituitary PRL content were similar to those of group 1 portal hypertensive and sham-operated controls (Table 2, Figure 1); however, plasma tyrosine levels were increased and plasma tryptophan levels were decreased significantly in this group of rats (Table 2). No difference in the levels of plasma tyrosine and tryptophan existed between portal hypertensive and sham-operated rats in group 3 (Table 2).

Experiment 3

Hypophyseal stalk plasma dopamine levels were significantly lower in the portal hypertensive group than in sham-operated controls (3.57 ± 0.87 ng/ml vs. 6.34 ± 0.93, $p < 0.05$). Plasma prolactin levels in this group of rats were lower than in experiments 1 and 2 while the differences between portal hypertensive and sham-operated rats were not significant (36.9 ± 11.3 ng/ml vs. 32.3 ± 6.7 NS), probably due to the urethane anesthesia (29).

Experiment 4

Daily substitution with 150 μg dihydrotestosterone (DTHP) in the castrated animals led to a significant decrease in pituitary weight compared

Table 2. The Effect of Portal Hypertension, Castration, and a 5% wt/wt Casein Diet on Body Weight, Pituitary Weight, Plasma Tyrosine, and Tryptophan

	Group 1		Group 2		Group 3	
	Sham ($n = 9$)	Portal hypertension ($n = 9$)	Sham + castration ($n = 10$)	PH + castration ($n = 8$)	Sham + 5% casein diet ($n = 10$)	PH + 5% Casein diet ($n = 10$)
Mean body weight (g ± *SD*)	252 ± 18	245 ± 21	234 ± 19	240 ± 22	200 ± 16	180 ± 20
Mean pituitary weight (mg ± *SD*)	6.94 ± 2.36	7.62 ± 0.81	9.84 ± 1.60[a]	10.05 ± 1.33[a]	5.11 ± 0.58[a]	5.23 ± 0.67[a]
Mean plasma tryptophan (*nmol/ml* ± *SEM*)	111.8 ± 4.3	109.1 ± 4.6	110.7 ± 5.6	101.7 ± 4.5	68.8 ± 4.2[a]	60.7 ± 4.5[a]
Mean plasma tyrosine (*nmol/ml* ± *SEM*)	91.2 ± 3.4	90.6 ± 3.4	78.9 ± 1.9	82.1 ± 5.1	123.1 ± 8.1[a]	116.5 ± 8.3[a]

[a] Difference significant ($p < 0.05$) compared with group 1 controls.

with controls (DTHP: 6.77 ± 0.34 mg; placebo: 9.01 ± 0.29 mg, $p < 0.005$), while the pituitary prolactin content (DTHP 34.8 ± 2.8 μg; placebo 35.7 ± 2.5 μg) was not altered. The plasma PRL levels did not differ between both groups. They were, however, higher than the groups of castrated rats who were not injected. Perhaps as a reaction to the injection stress large fluctuations in plasma PRL concentrations (DTHP 413.7 ± 136.8 ng/ml; placebo 120.5 ± 47.5 ng/ml NS) were noted.

Discussion

We have shown in the present study that portal hypertension without concomitant liver cirrhosis induces hyperprolactinemia in rats. This hyperprolactinemia was associated with increased plasma 17β-estradiol levels and decreased hypophyseal stalk plasma dopamine levels. Van Thiel et al. reported that plasma estrone levels are increased in rats with portal hypertension (22). The elevated estrogen levels found in portal hypertensive males are mainly caused by increased peripheral aromatization of testosterone via androstenedione (30–32) and partly by decreased elimination by the liver (33). Castration, which causes lower plasma levels of estrogens in the male rat (22), led to markedly decreased peripheral PRL levels and completely blocked the portal hypertension-induced hyperprolactinemia. Supplementation of castrated animals with dihydrotestosterone, a testosterone metabolite that cannot be transformed to estrogen in vivo, led to a significant decrease of the castration-induced increase in pituitary weight, but did not influence the castration-induced fall in pituitary prolactin and plasma prolactin levels. This suggests that the effect of castration on prolactin levels in the portal hypertensive rat is mediated by changes in estrogen levels and is not due to decreased testosterone levels. The increase in the pituitary weight of both portal hypertensive and sham-operated castrated rats therefore is probably caused by increased gonadotrophin production while prolactin production remains low. In rats, neurotransmitter levels in the brain are to a large extent dependent on the plasma levels of their aromatic amino acid precursors (34,35). This makes it possible to influence the concentration of neurotransmitters in the brain by diet-induced changes in plasma aromatic amino acids, e.g., tryptophan and tyrosine levels (36,37). In liver cirrhosis increased plasma aromatic amino acids compete with decreased branched chain amino acids for carrier bound transport through the blood–brain barrier (38,39). This leads to increased aromatic amino acid levels in the brain. Evidence has been presented that excessive levels of the aromatic amino acid and dopamine precursor tyrosine in the brain lead to the formation of the false neurotransmitter octopamine (15,40).

However in this rat model, portal hypertension did not lead to changes in either plasma tryptophan or plasma tyrosine, and the false neurotransmitter mechanism therefore is unlikely to be responsible for the hyperprolactinemia. In vitro experiments, furthermore, have shown that octopamine does not have an effect on prolactin release by the pituitary gland in vitro, nor does it influence the PRL-inhibiting effect of dopamine (S.W.J. Lamberts and R.M. MacLeod, unpublished observations). We confirm in the present study the observations that severe protein restriction in rats leads to a fall in plasma tryptophan and increase in plasma tyrosine concentrations (41,42). This should have caused a decrease in central nervous system serotonin (serotonin stimulates PRL secretion) and an increase of the prolactin inhibitor dopamine. Plasma and pituitary PRL levels, however, did not change after protein restriction and the portal hypertension-induced PRL rise remained intact.

The present results strongly suggest that the hyperprolactinemia induced by portal hypertension and the associated fall in hypophyseal stalk dopamine levels are not caused by a change in brain neurotransmitter precursor concentrations or formation of false neurotransmitters but are the result of increased plasma estrogen levels. It has been shown that estrogens stimulate PRL levels in humans (18). Studies in rats indicate that the estrogen-induced PRL rise can be blocked by bromocriptine administration (19), while incubation of rat anterior pituitary cells with 17β-estradiol leads to a reversal of the inhibitory effect of dihydroergocornine, a dopamine agonist (20). Estrogens also induce a fall in dopamine in hypophyseal stalk plasma (43). An excessive rise in PRL after TRH and ether stress in estradiol-primed rats has been found (21), perhaps as a result of the lowered hypothalamic dopamine secretion (26,44). These findings suggest that estrogens stimulate PRL release, probably through interference with the inhibitory effect of dopamine. We have shown that prolactin levels in the rat are elevated in portal hypertension without severe liver damage. We conclude that this increase is caused by elevated estrogen levels through interference with the hypothalamic release of dopamine. In this model, changes in plasma amino acid levels are of minor importance in PRL regulation.

References

1. Van Thiel DH, Gavaler JS, Lester R, et al. Plasma estrone, prolactin, neurophysin, and sex steroid-binding globulin in chronic alcoholic man. Metabolism 1975;24:1015–9.
2. Panerai AE, Salerno F, Manneschi M, et al. Growth hormone and prolactin responses to thyrotropin releasing hormone in

patients with severe liver disease. J Clin Endocrinol Metab 1977;45:134–40.
3. Wernze HF, Schmitz F. Plasma prolactin and prolactin release in liver cirrhosis. Acta Hepato-Gastroenterol 1977; 24:97–101.
4. Morgan MY, Jacobovits AW, Gore MBR, et al. Serum prolactin in liver disease and its relationship to gynecomastia. Gut 1978;19:170–4.
5. Van Thiel DA, MacClain CJ, Elson MC, et al. Hyperprolactinemia and thyrotropin releasing factor (TRH) responses in men with alcoholic liver disease. Alcoholism: Clin Exp Res 1974; 2:344.
6. Bratusch-Marrain P, Björkman O, Waldhäush W, Whren J. Hepatic disposal of endogenous growth hormone and prolactin in man. Eur J Clin Invest 1979;9:257–60.
7. Bauer AGC, Wilson JHP, Lamberts SWJ. The kidney is the main site of prolactin elimination in patients with liver disease. J Clin Endocrinol Metab 1980;51:70–3.
8. Cowden EA, Ratcliffe WA, Ratcliffe JG, et al. Hyperprolactinemia in renal disease. Clin Endocrinol 1978;9:241–8.
9. Tarquini B, Gheri R, Anichini P, et al. Circadian study of immunoreactive prolactin in patients with cirrhosis of the liver. Gastroenterology 1977;73:116–9.
10. Van Thiel DH, MacClain CJ, Elson MK, et al. Evidence for autonomous secretion of prolactin in some alcoholic men with cirrhosis and gynecomastia. Metabolism 1978;27:1778–84.
11. MacLeod RM. Regulation of prolactin secretion. In: Martini L, Ganong WF, eds. Frontiers in neuroendocrinology, Vol. 4. New York: Raven Press, 1976:69–94.
12. Gibbs DM, Neill JD. Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion in vivo. Endocrinology 1978;102:1895–1900.
13. Lamberts SWJ, MacLeod RM. The interaction of the serotonergic and dopaminergic systems on prolactin secretin in the rat. Endocrinology 1978;103:287–95.
14. MacClain CJ, Kromhout JP, Van Thiel D. Serum prolactin concentration in portal systemic encephalopathy. Gastroenterology 1979;76:1292.
15. Fisher JE, Baldessarini RJ. False neurotransmitters and hepatic failure. Lancet 1971;I:75–9.
16. Morgan MY, Jacobovits AW, James IM, et al. Successful use of bromocriptine in the treatment of chronic hepatic encephalopathy. Gastroenterology 1980;78:663–70.
17. Chopra IJ, Tulchinsky D, Greenway FL. Estrogen androgen imbalance in hepatic cirrhosis. Ann Intern Med 1973;79:198–203.
18. Yen SSC, Ehara Y, Siler TM. Augmentation of prolactin secretion by estrogen in hypogonadal women. J Clin Invest 1974;53:652–5.
19. Lloyd HM, Meares JD, Jacobi J. Effects of oestrogen and bromocriptine on in vivo secretion and mitosis in prolactin cells. Nature 1975;255:497–8.
20. Raymond V, Beaulieu M, Labrie F. Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. Science 1978;200:1173–5.
21. Piercy M, Shin SH. Comparitive studies of prolactin secretion in estradiol-primed and normal male rats induced by ether stress, pimozide and TRH. Neuroendocrinology 1980;31:270–5.
22. Van Thiel DH, Gavaler JS, Slone FL, et al. Is feminization in alcoholic men due in part to portal hypertension: a rat model. Gastroenterology 1980;78:81–91.
23. De Jong FH, Hey AH, van der Molen HJ. Effect of gonadotrophins on the secretion of oestradiol-17β and testosterone by the rat testis. J Endocrinol 1973;57:277–84.
24. Porter JC, Smith KR. Collection of hypophysial stalk blood in rats. Endocrinology 1967;81:1182–5.
25. Plotsky PM, Gibbs DM, Neill JD. Liquid chromatography electrochemical measurement of dopamine in hypophysial stalk blood of rats. Endocrinology 1978;102:1887–94.
26. De Greef WJ, Visser TJ. Evidence for the involvement of hypothalamic dopamine and thyrotropin releasing hormone in suckling-induced release of prolactin. J Endocrinol 1981 (in press).
27. Ambrose JA, Sullivan P, Ingerson A, et al. Fluorometric determination of tyrosine. Clin Chem 1969;15:611–20.
28. Bloxham DL, Warzen WH. Error in the determination of tryptophan by the method of Dunkla and Dewey. A revised method. Anal Biochem 1974;60:621–5.
29. De Greef WJ, Plotsky PM, Neill JD. Dopamine levels in hypophysial stalk plasma and prolactin levels in peripheral plasma of the lactating rat: effects of a stimulated suckling stimulus. Neuroendocrinology 1980;31:145–8.
30. Green JRB, Mowat NAG, Fisher RA, et al. Plasma oestrogens in men with chronic liver disease. Gut 1976;17:426–30.
31. Gordon GG, Olivo J, Rafii F, et al. Conversion of androgens to estrogens in cirrhosis of the liver. J Clin Endocrinol Metab 1975;40:1010–26.
32. Thijssen JHH, Lourens J, Donker GH. Increased oestrogen production by peripheral conversion of androgens in male patients with cirrhosis of the liver. J Endocrinol 1973;57:49.
33. Adlercreutz H. Hepatic metabolism of estrogens in health and disease. N Engl J Med 1974;290:1081–3.
34. Growden JH, Wurtman RJ. Dietary influences on the synthesis of neurotransmitters in the brain. Nutr Rev 1979;37:129–36.
35. Kato Y, Nakai Y, Imura H, et al. Effects of 5-hydroxy tryptophan (5-HTP) on plasma prolactin levels in men. J Clin Endocrinol Metab 1974;38:695–7.
36. Growdon JH, Cohen EL, Wurtman RJ. Treatment of brain disease with dietary precursors of neurotransmitters. Ann Intern Med 1977;86:337–9.
37. Clemens JA, Bennett DR, Fuller RW. The effect of a tryptophan free diet on prolactin and corticosterone release by serotonergic stimuli. Horm Metab Res 1980;12:35–8.
38. James JH, Escourrou J, Fisher JE. Bloodbrain neutral amino acid transport activity is increased after portocaval anastomosis. Science 1978;200:1395–7.
39. Partridge WM. Kinetics of competitive inhibition of neutral amino acid transport across the blood-brain barrier. J Neurochem 1977;28:103–8.
40. Manghani KK, Lunzer MR, Billing BH, et al. Urinary and serum octopamine in patients with portal systemic encephalopathy. Lancet 1975;II:943–6.
41. Dickerson JWT, Pao SK. The effect of a low protein diet and exogenous insulin on brain tryptophan and its metabolites in the weanling rat. J Neurochem 1975;25:559–64.
42. Rosen HM, Soeters PB, James JH, et al. Influences of exogenous intake and nitrogen balance on plasma and brain aromatic amino acid concentrations. Metabolism 1978;27:393–404.
43. Cramer OM, Parker Jr CR, Porter JC. Estrogen inhibition of dopamine release into hypophysial portal blood. Endocrinology 1979;104:419–21.
44. Norman RL, Quadri SK, Spier HG. Differential sensitivity of prolactin release to dopamine and thyrotrophin releasing hormone in intact and pituitary stalk sectioned rhesus monkeys. J Endocrinol 1980;84:479–87.

CHAPTER 6.

HYPERPROLACTINEMIA IN HEPATIC ENCEPHALOPATHY : THE EFFECT OF INFUSION OF AN AMINO ACID MIXTURE WITH EXCESS BRANCHED CHAIN AMINO ACIDS.

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ABSTRACT

Prolactin levels are elevated in patients with liver cirrhosis and hepatic encephalopathy. Patients with hepatic encephalopathy also have an abnormal plasma amino acid composition, with a relative excess of aromatic amino acids and a relative decrease in branched chain amino acid levels. In order to study the effect of the plasma amino acid composition on prolactin release, we measured plasma PRL at 0,10,20,30,40,50 and 60 minutes after 400 ug TRH both after infusion of a conventional amino acid mixture and after a branched chain amino acid enriched mixture (BCAA) in 5 patients with cirrhosis of the liver and hepatic encephalopathy.
After conventional amino acid infusion, a depressed branched chain / aromatic amino acid ratio was found in all patients, together with an increased PRL response to TRH. After the BCAA infusion the branched chain / aromatic amino acid ratio normalized. At the same time the excessive PRL response to TRH stimulation was significantly lower in all patients. This suggests that the elevated PRL levels in hepatic encephalopathy are caused by disturbance of hypothalamic neurotransmitter systems, due to altered amino acid-neurotransmitter precursor levels.

INTRODUCTION.

Plasma prolactin (PRL) levels may be elevated in patients with cirrhosis of the liver and portal hypertension (1-4). Even higher PRL levels have been reported in hepatic encephalopathy (5). In these patients an exaggerated reaction to thyrotropin releasing hormone (TRH) stimulation can also be found.

Since PRL elimination in liver cirrhosis is not decreased (6-8) the elevated PRL levels are probably caused by a disturbed PRL secretion. PRL secretion is mainly regulated by tonic inhibition by hypothalamic factors of which dopamin is the most important (9). It is therefore to be expected that a rise in PRL levels in patients with cirrhosis of the liver and hepatic encephalopathy is mediated through a loss of the dopaminergic control of prolactin secretion. Recently, evidence has been presented that hypothalamic dopamine secretion may indeed be abnormal in patients with cirrhosis of the liver and hepatic encephalopathy (10,11).

The hypothesis has been put forward that physiologic neurotransmitter systems in the brain may be disrupted and false neurotransmitters may be formed because of a relative increase of aromatic amino acids in relation to branched chain amino acids in the blood (12). This theory led to clinical trials with branched chain enriched amino acid mixtures (BCAA), designed to counteract this amino acid imbalance in hepatic encephalopathy. Several encouraging results have been reported, although not by all groups (13-16). It therefore seemed worthwhile to study the effect of such amino acid mixtures on PRL secretion in patients with cirrhosis of the liver and mild chronic hepatic encephalopathy and to compare the effect with those obtained by infusion of ordinary amino acid mixtures.

METHODS.

Five patients (3 men, 2 women) with cirrhosis of the liver and mild hepatic encephalopathy were studied. Two of these patients had surgical portosystemic shunts months and years before the study. Clinical and laboratory particulars are summarised in table I. The diagnosis of liver cirrhosis was verified by biopsy in all patients. The diagnosis of hepatic encephalopathy was based on the presence of the following symptoms: disturbed sleep pattern, inability to concentrate, slurred speach, flapping tremor, an abnormal Reitan trail test (17), and elevated plasma amonia levels. All patients were in a stable condition without signs of recent gastrointestinal hemorrhage. They were maintained on a 40-60 gram protein restricted diet and treated with lactulose and neomycine. None of the patients received drugs known to elevate PRL levels. The amino acid mixtures used were a mixture with excess branched chain amino acids (amino-steril Hepa 8%^{R}, Fresenius, Bad Homburg, West Germany) (table 2), and a mixture with a more conventional amino acid composition (Aminess ^{R} KabiVitrum, Stockholm, Sweden) (table 2). The volume infused was calculated to contain approximately the same amount of nitrogen (1000 ml Aminess ^{R}= 6.5 g N; 525 ml Aminosteril Hepa ^{R}= 6.7 g N). Both mixtures were infused intravenously over a 24 hour period with the aid of a peristaltic pump. Patients were randomized to start with either of these 2 mixtures and received the alternative 7 days later. Patients thus served as their own controls. After 24 hours infusion, heparinized, blood samples were taken at 0,10,20,30,40,50 and 60 minutes after the intravenous injection of 400 ug TRH (Relefact ^{R}), centrifuged and stored at -20^{o}C until assay. Normal values for basal PRL and maximal PRL after TRH were obtained from 14 healthy volunteers (7 males, 7 females). The amino acid composition of the blood was determined in plasma

samples taken immediately prior to the TRH injection after the BCAA-enriched amino acid infusion and after the conventional amino acid infusion. From these findings, a branched chain amino acid ratio (BCAA/AAA) was calculated according to the equation:

$$\frac{\text{valine + leucine + isoleucine}}{\text{tyrosine + phenylalanine}} \quad (12).$$

Normal values for the BCAA/AAA ratio for healthy adults were derived from Soeters (15).

ASSAYS.

PRL levels were measured in duplicate immunoassay (normal values, up to 12 ng/ml in males and up to 15 ng/ml in females). The intraassay varia tion of determination was 4.5% (50 duplicates; 2-50 ng/ml), and the interassay variation was 3.8% (mean 14 ng/ml, n = 12).
Plasma amino acid levels were determined with the aid of a Technicon TSMI amino acid analyser.

STATISTICAL ANALYSIS.

The difference between TRH stimulated PRL levels after BCAA-enriched amino acids infusions and conventional amino acid infusions was assessed by Student's paired t test in each individual and in the patient group as a whole.
The differences between basal PRL levels in normal controls, and in patients after BCAA-enriched amino acid infusion and after conventional amino acid infusions were assessed with the non parametric Mann-Whitney test. Differences between ΔPRL were assessed similarly in these three groups. The differences in amino acid composition of the blood after conventional amino acid infusion and after BCAA-enriched amino acid infusion were assessed by Student's paired t test for each individual amino acid. The differen-

ce between the BCAA to AAA ratio after both amino acid infusions were also assessed by Student's paired t test.

RESULTS.

The results are summarized in fig. 1 and table 3 and 4. Basal PRL levels were not elevated in these patients in comparison with normal controls and did not differ significantly between BCAA-enriched amino acid and conventional amino acid periods. After TRH, PRL levels were significantly higher following the conventional amino acid mixture compared to normal controls ($p < 0,05$) whereas in the BCAA-enriched treatment period this difference disappeared. The difference in PRL levels after TRH between the conventional amino acid and BCAA enriched treatment periods were significant in all five patients ($p < 0.001$ in 2 pts., $p < 0.005$ in 2 pts., $p < 0.05$ in 1 pt.) and in the group as a whole ($p < 0.001$). After conventional amino acid infusion the plasma amino acid pattern showed a relative excess of AAA and a relative decrease of BCAA in all patients (table 4). The BCAA/AAA ratio was depressed in all patients (15). After BCAA enriched infusion the AAA levels decreased (phenylalanine $p < 0.02$, tyrosine $p < 0.05$). The BCAA levels increased in 4 out of the 5 patients (valine $p < 0.05$, leucine n.s., isoleucine n.s.), while the BCAA/AAA ratio rose in all patients and normalized in 4/5 of the patients ($p < 0.05$).

DISCUSSION.

PRL levels are often elevated in cirrhosis of the liver and may be even higher in hepatic encephalopathy (1-5). In our small group of patients basal PRL levels were not elevated. However after TRH stimulation following infusion of a conventional amino acid mixture, PRL levels rose significantly higher than in normal controls. PRL

release is regulated by inhibitory and releasing hypothalamic factors in which the inhibiting effect of dopamine is the most important (9). To explain the excessive rise of PRL levels after TRH stimulation in hepatic encephalopathy it is therefore attractive to postulate a disturbance of the dopaminergic neurotransmitter system. Indirect evidence has been presented that hypothalamic dopamine release may indeed be disturbed in cirrhosis of the liver with hepatic encephalopathy (10,11,17). In hepatic encephalopathy changes in amino acid balance are thought to lead to changes in central neurotransmitter systems. This theory is based on evidence that in hepatic encephalopathy AAA (tryptophan, phenylalanine and tyrosine) are increased in relation to the BCAA (valine, leucine and isoleucine)) (12,14,19). Since these amino acids all compete for the same carrier through the blood-brain barrier (20) this will result in an excess of aromatic amino acid neurotransmitter precursors in the brain. Because tyrosine is the precursor for catecholamines (21) and tryptophan the precursor for serotonin (22) these changes should substantially influence neurotransmitter levels. It has been suggested that the production rate of dopamine in the brain is limited, and that excess tyrosine will be converted to the false neurotransmitter octopamine, instead of dopamine (10,11). On the other hand elevated levels of 5-hydroxy indole acids in the cerebro spinal fluid in cirrhosis indicate an increased production and turnover of serotonin (23,24). A relationship has been established in cirrhosis of the liver between free plasma tryptophan levels and PRL levels (2). PRL release can be stimulated by intravenous administration of L-tryptophan (25) and 5-hydroxy tryptophan (26,27). It is therefore likely that both decreased dopamine levels and/or increased serotonin levels play a role in the hyperprolactinemia of liver cirrhosis, especially in hepatic encephalopathy. In favour of this amino acid/neurotransmitter hypothesis is the

reported improvement in the level of consciousness of encephalopathic patients following administration of bromocriptine, a dopamine agonist (28) and BCAA-enriched amino acid mixtures (13,14,15).
In our study the plasma BCAA/AAA ratio was indeed found to be severely depressed after infusion of a conventional amino acid mixture, while infusion of a BCAA enriched amino acid mixture led to an improvement of the plasma amino acid pattern and an increase of the BCAA/AAA ratio. At the same time the PRL response to TRH stimulation was normalized or substantially lowered in all patients. We thus present indirect evidence that the elevated PRL levels found in patients with cirrhosis of the liver and hepatic encephalopathy are caused by a disturbance of hypothalamic dopamine and possibly also serotonin release, which is probably caused by a change in the amino acid-neurotransmitter precursor levels in the brain. Since octopamine does not have an effect on PRL release in vitro and also does not influence the PRL inhibiting effect of dopamine in vitro (S.W.J.Lamberts and R.M.MacLeod, unpublished observations), it seems less likely that the disturbance of prolactin secretion is caused by the formation of false neurotransmitters per se. It is however possible, that the abnormal amino acid levels are not the only factor contributing to the hyperprolactinemia of cirrhosis; since we have found in a study of portal hypertensive rats that the increased estrogen levels associated with portal hypertension lead to elevated PRL levels through decreased hypothalamic dopamine release (29), it seems likely that increased estrogen levels also play a role in the increased PRL levels in cirrhosis of the liver.

Table I. Clinical and laboratory particulars of 5 patients with cirrhosis of the liver and mild chronic encephalopathy.

patient	1	2	3	4	5
age	75	33	53	51	53
sex	F	F	M	M	M
diagnosis	cryptogenic micronod. cirrhosis	alcoholic micronod. cirrhosis	alcoholic micronod. cirrhosis	alcoholic micronod. cirrhosis	chronic hepatitis with cirrhosis
flapping tremor	-	-	+	+	+
sleep disturbancies	+	+	+	+	+
forget-fullness/concentration dis.	+	+	+	+	+
slurred speech	-	-	+	-	+
Reitan trail test 1 min.	+	+	+	+	+
Varices	-	+	+	+	+
portocaval shunt	+	-	-	+	-
albumin (g/l)	32	41	30	36	42
bilirubin umol/l, N 12	19	23	26	85	41
arterial ammonia umol/l n=10-30	84	52	60	66	120
SGOT (IE) n= 15-25	49	33	44	42	34

Table 2. Amino acid composition of Aminess^{R} and Aminosteril Hepa 8%^{R}.

Conventional amino acid mixture (Aminess^{R})		BCAA enriched amino acid mixture (Aminosteril Hepa 8%^{R})	
L-histidine	4.12 g	L-histidine	2.80 g
L-isoleucine	5.25 g	L-isoleucine	10.40 g
L-leucine	8.25 g	L-leucine	13.09 g
L-lysine	6.0 g	L-lysine	6.88 g
L-methionine	8.25 g	L-methionine	1.10 g
L-phenylalanine	8.25 g	L-phenylalanine	0.88 g
L-threonine	3.75 g	L-threonine	4.40 g
L-tryptophan	1.88 g	L-tryptophan	0.70 g
L-valine	6.0 g	L-valine	10.08 g
acetic acid	0.3 g	L-arginine	10.72 g
volume	1000 ml	L-cysteine hydro-chloride	0.75 g
Total N content	6.5 g/l	L-glycine	5.82 g
		L-alanine	4.64 g
		L-proline	5.73 g
		L-serine	2.24 g
		Acetic acid	7.25 g
		volume	1000 ml
		Total N content	12.9 g/l.

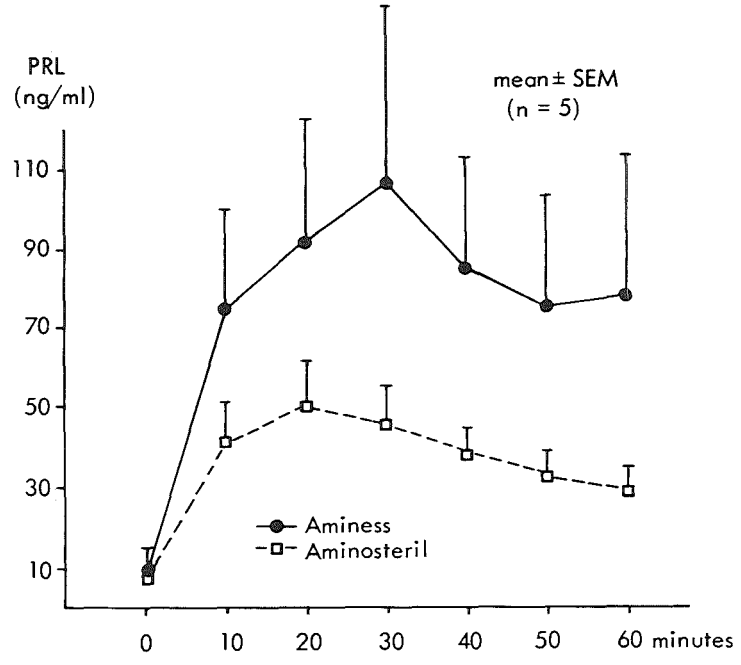
		0'	10'	20'	30'	40'	50'	60'	
Plasma prolactin (ug/l)									
patient 1.	conventional	16.9	68.5	74.8	82.5	63.7	63.3	56.1	$p < 0.005$
	BCAA enriched	13.7	46.6	55.5	38.0	41.9	32.2	32.2	
2.	conventional	7.9	25.6	29.3	26.4	25.3	26.6	20.2	$p < 0.001$
	BCAA enriched	8.0	19.4	24.5	21.6	20.7	20.9	12.8	
3.	conventional	10.4	75.6	93.0	77.9	77.0	46.8	60.5	$p < 0.05$
	BCAA enriched	11.8	70.4	85.3	75.2	59.0	44.5	39.1	
4.	conventional	6.0	166.4	210.8	289.2	204.3	188.8	217.3	$p < 0.001$
	BCAA enriched	3.7	48.6	58.6	64.2	48.6	44.9	41.5	
5.	conventional	4.8	38.0	50.9	57.6	58.5	51.8	39.6	$p < 0.005$
	BCAA enriched	1.8	23.0	27.0	28.2	23.0	18.3	19.6	
Basal PRL (mean ± S.D.)		Δ PRL							
mean ± S.D.	conventional	9.2 ± 4.8	n.s.					110.5 ± 102.8	$p < 0.001$
	BCAA enriched	7.8 ± 5.0		$p < 0.05$				51.5 ± 25.4	
controls	males (n=7)	4.6	n.s.					43.6 ± 20.7	
	females (n=7)	7.1						22.9 ± 17.6	

Table 3. PRL levels at 0,10,20,30,40,50 and 60 minutes after 400 ug TRH i.v., following 24 hour infusion of BCAA enriched and conventional amino acid mixtures in 5 patients with liver cirrhosis and hepatic encephalopathy.

	BCAA						AAA							
	valine		leucine		isoleucine		phenylalanine		tyrosine		BCAA/AAA ratio		methionine	
	conventional	BCAAenriched	conventional	BCAAenriched	conventional	BCAAenriched	conventional	BCAAenriched	conventional	BCAAenriched	conventional	BCAAenriched	conventional	BCAAenriched
pt 1	0.27	0.45	0.13	0.30	0.07	0.18	0.26	0.09	0.09	0.08	1.34	5.47	0.28	0.05
2	0.32	0.45	0.19	0.23	0.14	0.17	0.32	0.08	0.13	0.09	1.44	5.00	1.03	0.03
3	0.16	0.39	0.08	0.21	0.05	0.12	0.13	0.05	0.06	0.05	1.52	7.20	0.29	0.00
4	0.23	0.27	0.10	0.14	0.04	0.07	0.14	0.07	0.21	0.13	1.05	2.40	0.40	0.00
5	0.30	0.27	0.20	0.13	0.13	0.06	0.34	0.12	0.21	0.17	1.14	1.58	0.62	0.07
mean ± S.D.	0.26± 0.06	0.37± 0.09	0.14± 0.05	0.20± 0.07	0.09± 0.05	0.12± 0.05	0.24± 0.10	0.08± 0.02	0.14± 0.07	0.10± 0.05	1.29± 0.19	4.33± 2.30	0.52± 0.31	0.03± 0.03
	$p < 0.05$		n.s.		n.s.		$p < 0.02$		$p < 0.05$		$p < 0.05$		$p < 0.02$	
	threonine		serine		glycine		alanine		glutamine		proline			
pt 1	0.37	0.30	0.15	0.21	0.21	0.38	0.41	0.50	0.45	0.17	0.25	0.53		
2	0.35	0.28	0.11	0.19	0.20	0.34	0.41	0.37	0	0.15	0.19	0.32		
3	0.18	0.25	0.13	0.22	0.20	0.34	0.40	0.41		0.25		0.38		
4	0.28	0.23	0.23	0.24	0.22	0.28	0.33	0.36	0.21	1.45	0.28	0.52		
5	0.31	0.24	0.14	0.18	0.21	0.25	0.32	0.51	0.38	0.73	0.23	0.41		
mean ± S.D.	0.30± 0.07	0.26± 0.03	0.15± 0.05	0.21± 0.02	0.21± 0.01	0.32± 0.05	0.37± 0.04	0.43± 0.07	0.26± 0.20	0.55± 0.56	0.24± 0.04	0.43± 0.10		
	n.s.		$p < 0.01$		$p < 0.02$		n.s.							

Table 4. Plasma amino acids (umol/ml) in 5 patients with liver cirrhosis and hepatic encephalopathy after 24 hr infusion of BCAA-enriched and conventional amino acid mixtures.

Figure 1. Plasma prolactin levels following TRH stimulation, after infusion of a conventional mixture of amino acids (Aminess) or a branched chain amino acid enriched mixture (Aminosteril).



References.

1. Van Thiel DH, Gavaler DS, Lester R, Loriaux DC, Braunstein GD. (1975)
   Plasma estrone, prolactin, neurophysin and sex-steroid binding globulin in chronic alcoholic man.
   Metabolism 24: 1015.
2. Panerai AE, Salerna F, Mannischi M, Cocchi D, Müller EE, (1977.)
   Growth hormone and prolactin responses to thyrotropin releasing hormone in patients with severe liver disease.
   J.Clin. Endocrinol. Metab. 45 : 134.
3. Wernze HF, Schmitz F,(1977.)
   Plasma prolactin and prolactin release in liver cirrhosis.
   Acta Hepato-gastroenterol 24 : 97.
4. Morgan MY, Jacobovits AW, Gore MBL, Wills MR, Sherlock S, (1978.)
   Serum prolactin in liver disease and its relationship to gynecomastia.
   Gut 19 : 170.
5. Macclain CJ, Kromhout JP, Van Thiel D,(1979.)
   Serum prolactin concentration in portal systemic encephalopathy.
   Gastroenterology 76 : 1292.
6. Bratusch-Marrain P, Björkman O, Waldhaüsl W, Wahren J, (1979.)
   Hepatic disposal of endogenous growth hormone and prolactin in man.
   Eur.J.Clin.Invest. 9 : 257.
7. Bauer AGC, Wilson JHP, Lamberts SWJ,(1980.)
   The kidney is the main site of prolactin elimination in patients with liver disease.
   J.Clin.Endocrinol.Metab. 51 : 70.

8. Cowden EA, Ratcliffe WA, Ratcliffe JG, Dobbie JW, Kennedy AC, (1978)
Hyperprolactinemia in renal disease.
Clin. Endocrinol 9 : 241.
9. MacLeod RM, (1976)
Regulation of prolactin secretion.
In: Martini L, Ganong WF (eds)
Frontiers in neuroendocrinology, Raven Press, New York, vol 4 p 169.
10. Fischer JE, Baldessarini RJ, (1971)
False neurotransmitters and hepatic failure.
Lancet 2 : 75.
11. Mangh.ani KK, Lunzer MR, Billing BH, Sherlock S, (1975)
Urinary and serum octopamine in patients with portal systemic encephalopathy.
Lancet 2 : 943.
12. Fischer JE, Rosen HM, Ebeid AM, James JH, Keane JM, Soeters PB, (1976)
The effect of normalisation of plasma amino acids on hepatic encephalopathy in man.
Surgery 80 : 77.
13. Fiene R, Holm E, Dietl H, Mezitis N, Schuler A, (1977)
Parenterale Ernährung mit Aminosäuren bei Leber Insuffizienz.
Verh. Dtsch, Ges.Inn.Med. 83: 533.
14. Fischer JE, Yoshimura JE, Aguirre A, James JH, Cummings MG, Abel RM, Deindoerfer F, (1974)
Plasma amino acids in patients with hepatic encephalopathy ; effects of amino acid infusions.
Am.J.Surg. 127 : 40.
15. Soeters PB, (1979)
Hepatic encephalopathy; a new perspective for therapy.
MD Thesis, Maastricht, The Netherlands.
16. Michel H, Pomier-Layrargues G, Duhamel O, Lacombe B, Guilleret G, Bellet H, (1980.)
Intravenous infusion of ordinary and modified amino

acid solutions in the management of hepatic encephalopathy (controlled study, 30 patients).
Gastroenterology 79 : 1038.

17. Conn HO,(1977.)
Trailmaking and number-connection tests in the assessment of mental state in portal systemic encephalopathy.
Am.J.Dig.Dis. 22 : 541.

18. Dodsworth JM, James JH, Cummings MG, Fischer JE,(1974)
Depletion of brain norepinephrine in acute hepatic coma.
Surgery 75 : 811.

19. Daniel PM, Love ER, Moorehouse SR, Ratt OE,(1975)
Amino acids, insulin and hepatic coma.
Lancet 2 :179.

20. Partridge WM,(1977).
Kinetics of competitive inhibition of neutral amino-acid transport across the blood-brain barrier.
J.Neurochem. 28 : 103.

21. Wurtman RJ, Larin F, Mortafapour S, Fernstrom JD ,(1974)
Brain catechol synthesis : control by brain tyrosine concentration.
Science 185 : 183.

22. Fernstrom JD, Faller DV, Shabske Lowits H,(1975).
Acute reduction of brain serotonin and 5HIAA following food consumption : correlation with the ratio of serum trytophan to the sum of competing neutral amino acids.
J.Neural. Transm. 36 : 113.

23. Knell AJ, Davidson AR, Williams R, Kantamanei BD, Curzon G,(1974.)
Dopamine and serotonin metabolism in hepatic encephalopathy.
Br.Med. J. 1 :549

24. Lal S, Young SA, Sourkes TL,(1975)
5-Hydroxytryptamine and hepatic coma.
Lancet 2 : 979.

25. MacIndoe JH, Turkington RW,(1973.)
Stimulation of human prolactin secretion by intravenous infusion of l-tryptophan.
J.Clin.Invest. 52 : 1972.
26. Kato Y, Nahai Y, Imura H, Chihara K, Ohgo S,(1974.)
Effect of 5 hydroxytryptophan (5-HTP) on plasma prolactin levels in man.
J.Clin.Endocrinol.Metab. 38 : 695.
27. Lancranjan I, Wirz-Justice A, Pühringer W, del Pozo E, (1977.) Effect of 5-hydroxytryptophan infusion on growth hormone and prolactin secretion in man.
J.Clin.Endocrinol.Metab. 45 : 588.
28. Morgan MY, Jacobovits AW, James IM, Sherlock S,(1980).
Succesful use of bromocriptine in the treatment of a patient with chronic porta-systemic encephalopathy.
Gastroenterology 78 : 663.
29. Bauer AGC, de Groot WJ, de Jong FH, Wilson JHP, Lamberts SWJ,(1982).
Hyperprolactinemia of portal hypertension in rats.
Gastroenterology 82 : 179.

CHAPTER 7

THE EFFECT OF TAMOXIFEN ON PROLACTIN SECRETION IN CIRRHOSIS OF THE LIVER.

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SUMMARY.

In order to study the relationship between plasma prolactinlevels and the gynecomastia of liver cirrhosis, 7 male patients with cirrhosis of the liver with (4 patients) or without (3 patients) gynecomastia were studied before and after treatment with the anti estrogen, tamoxifen. Treatment with tamoxifen led to a significantly lower PRL response to TRH stimulation in all patients with gynecomastia but in none of the patients without gynecomastia ($p < 0.01$) irrespective of wether the original response to TRH stimulation was normal or exaggerated. Furthermore in 3 patients with gynecomastia the breasts became softer and less painful. This suggests that the absolute plasma PRL levels do not determine the role of PRL in the pathogenesis of gynecomastia in liver cirrhosis, but rather its relation to other factors, such as an increased estrogen/testosterone ratio.

INTRODUCTION.

The most striking hormonal syndrome associated with cirrhosis of the liver is the feminization process which occurs in up to 40-50% of male patients (1,2). This syndrome is characterized by a feminine distribution of hair, palmar erythema, the formation of spider naevi, disturbed gonadal function, impotence and infertility. In 25-50% of patients gynecomastia develops (1-3). The pathogenesis of these changes is not fully understood, although a disturbed estrogen/testosterone ratio has been implicated (4). On the other hand prolactin levels are often elevated in patients with cirrhosis of the liver (3,5,6), especially after TRH stimulation (6,7). Since prolactin plays a physiological role in both breast formation, lactation and normal gonadal function, it seems reasonable to expect a role for prolactin in the pathogenesis of gynecomastia.

However not all patients with cirrhosis of the liver have elevated prolactin levels, nor do all patients with hyperprolactinemia develop gynecomastia (3). It seems likely therefore that if hyperprolactinemia is indeed implicated in the pathogenesis of gynecomastia in liver cirrhosis it will be in coöperation with other factors, such as a disturbed estrogen / testosterone balance. It is not yet fully understood why prolactin levels are elevated in cirrhosis of the liver, although a disturbance of secretion is more likely than a disturbed elimination (9). We recently provided evidence (8) that in rats with portal hypertension elevated estrogen levels cause elevated prolactin levels through interference with hypothalamic dopamine release. Since elevated estrogen levels therefore seem to play a pivotal role both in stimulating prolactin release in portal hypertension and in the feminization process itself, we decided to study the release of prolactin before and after treatment with the anti estrogen tamoxifen in patients with cirrhosis of the liver with and without gynecomastia.

METHODS.

Seven men with chronic alcoholic liver cirrhosis (laboratory and clinical particulars table I) were studied (age 41-73). All had stopped alcohol consumption at least half a year previously and none showed evidence of hepatic encephalopathy. The diagnosis of cirrhosis was confirmed by biopsy in all patients. Although all patients showed testicular atrophy, palmar erythema and varying degrees of spider naevi formation, four of the patients had gynecomastia while three did not. None of the patients used drugs known to elevate prolactin levels or to cause gynecomastia.
Heparinized blood samples were taken at 0,10,20,30,40,50

and 60 minutes after 400 ug TRH (Relefact, Hoechst) i.v. both before and after treatment with tamoxifen (Nolvadex I.C.I.) 10 mg. twice daily during 1 week.

ASSAY.

Duplicate PRL levels were measured by radioimmunoassay (normal values, up to 12 ug/ml in males. The intra assay variation of determinations was 4.5%; (50 duplicates, 2-50 ug/ml, and the inter assay variation was 3.8% (mean 14 ug/ml; u = 12).

STATISTICAL ANALYSIS.

The difference between TRH stimulated PRL levels before and after treatment with tamoxifen was assessed by student´s paired t-test. The difference in prolactin release after treatment with tamoxifen in the group of patients with gynecomastia and the group without gynecomastia was assessed by Fisher´s exact propability test.

NORMAL VALUES.

Normal values for PRL were obtained from 7 healthy men: Normal basal plasma PRL values were less 12 ug/ml (mean 4.6 ug/ml) and maximal values after TRH stimulation between 8.6 and 57.6 ug/ml (mean + S.D. 22.9 $\pm$ 17.6 ug/ml).

RESULTS (table 2)

Basal PRL levels were normal in all patients studied. After TRH stimulation an exaggerated PRL reponse was found in 3 patients (2 with gynecomastia, 1 without gynecomas-

tia). Following treatment with tamoxifen the PRL response to TRH stimulation was significantly lowered in 4 out of 4 patients with gynecomastia ($p < 0.01$ in 1 pt, $p < 0.001$ in 2 pts, $p < 0.05$ in 1 pt.) but in none of the 3 patients without gynecomastia ($p < 0.01$ 2 tailed Fisher exact test) irrespective of the original normal or exaggerated PRL response to TRH before treatment with tamoxifen. In 3 patients with gynecomastia (pt 1,2 and 4) the breasts became softer and smaller and painfulness disappeared after one week of treatment with tamoxifen (20 mg/day).

DISCUSSION.

Estrogens stimulate PRL secretion in male and female agonadal subjects (9,10) and elevate basal prolactin levels in normal menstruating women (11). In vitro studies show that estrogens enhance the stimulating effect of TRH on PRL stimulation (12,13,14). Estradiol 17B has a direct effect on PRL secretion in rat pituitary cell cultures (15,16). Apart from this direct effect of estrogens on the pituitary, there is also evidence indicating an effect on prolactin release at a higher hypothalamic level (17,18) probably through interference with hypothalamic dopamine secretion (19).

In cirrhosis of the liver, estrogen levels are often elevated, mainly through increased peripheral aromatization of testosterone via androstenedione (20,21). Recently we found in rats that elevated estrogen levels induced by portal hypertension lead to increased PRL secretion through interference with hypothalamic dopamine release (22). It seems therefore reasonable to expect a role for estrogens in the elevated PRL levels sometimes found in cirrhosis of the liver, especially as estrogen levels are elevated in $\pm$ 60 % of patients with cirrhosis of the liver (2).

In this study we report the effect of the antiestrogen tamoxifen on prolactin release in 7 patients with cirrhosis of the liver, divided in a group of 4 patients with gynecomastia and 3 patients without gynecomastia. The non-steroidal anti-estrogen tamoxifen lowers basal but especially TRH stimulated PRL levels in both animals (23,24), and man (25,26,27).

The anti-estrogenic effect is exerted by competition at the estrogen receptor (28) and estrogen levels may even rise during administration (25). It has been shown in vitro that tamoxifen does not influence prolactin secretion by rat pituitary tumor cells directly but that it enhances the sensitivity of these cells to dopamine and bromocriptine (29). This enhancing effect can be prevented by coincubation with estradiol 17-B. In addition tamoxifen prevents the stimulatory effect of TRH on prolactin secretion (29). We found that tamoxifen significantly reduced the prolactin surge after TRH stimulation in all patients with gynecomastia but in none of the patients without gynecomastia, irrespective of whether the original PRL response to TRH before treatment was normal or exaggerated. In the responder (gynecomastia) group 2 patients had an exaggerated PRL surge, whereas in the non-responder (no gynecomastia) 1 patient showed an excessive rise after TRH. Furthermore in 3 patients with gynecomastia the breasts became softer and less painful. From these findings it appears that prolactin indeed plays a role in the pathogenesis of gynecomastia but that not the absolute PRL levels determine its effect but rather the relation to other factors.

It is interesting in this respect that a disturbed estrogen / testosterone balance with increased estrogen levels and decreased testosterone levels has been found to increase prolactin receptor activity (30,31). It seems worthwhile on these theoretical grounds, the observations made in some patients and the fact that no significant

side effects have been described, to conduct a trial of tamoxifen in gynecomastia (and impotence ?) associated with cirrhosis of the liver.

Table I.

PATIENT	1	2	3	4	5	6	7
AGE	50	44	73	54	41	62	45
P.A. DIAGNOSIS	Alcoholic cirrhosis	Alc. cirrh.	Alc. cirrh.	Alc. cirrh.	Alc. cirrh.	Alc. cirrh.	Alc. cirrh.
GYNECO-MASTIA	+	+	+	+	-	-	-
ERYTHEMA PALMARE	+	+	+	+	+	+	+
SPIDER NAEVI	++	++	$\pm$	+	++	++	+
ATROFIA TESTIS	+	+	+	+	+	+	+
SGOT u:l (N5-30)	40	25	33	48	45	48	98
BILIR (umol/l) N 2-12	24	16	13	11	34	13	50
ALB g/l	35	37	42.2	45	43	37.7	38.2

table I. clinical and laboratory particulars of 7 male patients with liver cirrhosis with (pt 1-4) and without (5-7) gynecomastia.

	PROLACTINE ug/L		0'	10'	20'	30'	40'	50'	60'	
GYNECOMASTIA	Patient 1	B	5.9	21.8	22.5	18.1	14.6	15.5	14.2	$p < 0.01$
		A	3.8	17.6	15.3	15.6	13.6	12.5	10.6	
	Patient 2	B	8.5	48.3	54.0	52.2	60.0	51.2	53.5	$p < 0.001$
		A	4.5	19.0	33.9	30.1	29.7	25.1	30.8	
	Patient 3	B	6.8	24.3	28.9	30.6	27.9	26.1	20.6	$p < 0.001$
		A	6.1	19.2	21.1	21.0	18.9	16.4	14.6	
	Patient 4	B	4.2	43.8	50.2	48.1	40.0	38.3	33.1	$p < 0.05$
		A	4.6	44.4	42.6	42.6	39.0	32.4	30.3	
NO GYNECOMASTIA	Patient 5	B	0.1	19.8	28.7	27.4	21.4	17.4	14.8	
		A	1.7	26.1	31.2	30.3	26.6	23.5	21.0	
	Patient 6	B	3.1	3.6	7.2	6.7	5.6	5.6	4.1	
		A	10.0	10.6	11.0	11.9	7.4	5.5	5.8	
	Patient 7	B	5.0	37.2	44.7	45.0	40.9	29.1	21.7	
		A	1.7	38.6	44.3	42.7	40.3	32.8	30.5	

TABLE 2. Prolactin levels (ug/l) at 0,10,20,30,40,50 and 60 minutes after 400 ug TRH iv before (b) and after (a) Tamoxifen twice daily during 1 week.

1. Baker H.W.G. (1976)
A study of the endocrine manifestations of hepatic cirrhosis ;
Quarterly J.Med., XLV, 145-178.
2. Lloyd, C.W., Williams R.H. (1948)
Endocrine changes associated with laennec's cirrhosis of the liver.
Amer.J.Med. 4 : 315-330
3. Morgan, M.Y., Jakobovits A.W., Gore M.B.R. et al (1978)
Serum prolactin in liver disease and its relationship to gynecomastia.
Gut 19 : 170-174
4. Lester R, Eagon P.K., Van Thiel D.H. (1979).
Feminization of the Alcoholic: the estrogen/testosterone ratio .
Gastro enterology 76 : 416-418.
5. Wernze, H., Burghardt, W. (1979).
Hyperprolactinämie bei lebererkrankungen.
Med. Klin. 74 : 1615-1623
6. Panerai, A.E., Salerno, F., Manneschi, M. et al (1977).
Growth Hormone and Prolactin responses to Thyrotropin releasing hormone in patients with severe liverdisease.
J.Clin.End.Metab. 45: 134-140
7. Zanoboni, A., Zanoboni-Muciaccia, W. (1975)
Gynecomastia in alcoholic cirrhosis.
Lancet 2 : 876
8. Bauer, A.G.C., Wilson, J.H.P., Lamberts S.W.J. (1980).
The kidney is the main site of the prolactin elimination in patients with liverdisease.
J.Clin.End.Metab. 51 : 70-73.
9. Neill, J.B. (1974).
Prolactin : its secretion and control.
In : handbook of physiology, section 7.
Endocrinol. American Physiological Society.
Washington D C pp. 469-488.

10. Meites, J. (1973)
Control of prolactin secretion in animals.
In human prolactin Eds.: Pasteels en Robijn
Exerpta Medica Amsterdam pp. 105-118.
11. Vekemans, M., Robijn, C. (1975).
The influence of exogenous estrogen on the circadian periodicity of circulating prolactin in women.
J.Clin. End. Metab. 40 : 886-889
12. De Lean, A., Ferland, L., Drouin, J. et al (1977).
Changes of pituitary thyrotropin releasing hormone (TRH) receptor level and prolactin response to TRH during the rat estrous cycle.
Endocrinol. 100 , 1505 - 1510.
13. Stevens, R.W., Lawson, D.M. (1977).
The influence of estrogen on plasma prolactin levels induced by thyrotropin releasing hormone (TRH), clonidine and serotonin in ovariectomised rats.
Life sci. 261-266
14. Carlson, H.E., Jacobs, L.S., Daughaday, W.H. (1973)
Growth hormone, thyrotropin and prolactin responses to thyrotropin releasing hormone following diethylstilbestrol pretreatment.
J.Clin.End.Metab. 37 : 488- 490.
15. Brunet R., Gamdji, Moreau M.F. et al (1977).
Effect of 17 B Estradiol on prolactin secretion and thyroliberin responsiveness in two rat prolactin continuous cell lines : definition of an experimental model.
Ann.Biol.Amin Biochim.Biophys. 17 : 413-424.
16. Haug,E., Gautvik, R.M. (1976)
Radio immuno assay of rat prolactin and its use in measuring prolactin productions by cultured pituitary cells.
Acta endocrinol. 82 : 282 - 297.
17. Caligaris, L., Taleisnik, S. (1976).
The role of the hypothalamic afferents in the release

of Prolactin induced by ovarian steroids.
Neuroendocrinology 21 : 139 - 145.

18. Cardinali, D.P., Gomez, E. (1977).
Change in hypothalamic noradrenaline, dopamine and serotonin uptake after oestradiol administration to rats.
J.Endocrinol. 73 : 181- 182

19. Cramer, O.M., Parker, Jr.C.R., Porter, J.C. (1979).
Estrogen inhibition of dopamine release into hypophysial portal blood.
Endocrinol. 104 : 419 - 421.

20. Thyssen, J.H.H., Laurens, J., Donker, G.H. (1973).
Increased oestrogen production by peripheral conversion of androgens in male patients with cirrhosis of the liver.
J.Endocrinol. 57 : 49

21. Gordon, G.G., Olivo, J., Raffi F. et al (1975).
Conversion of androgens to estrogens in cirrhosis of the liver.
J.Clin.End.Metab. 40 : 1010 - 1026

22. Bauer, A.G.C., de Groot, W.J., de Jong, F.H.et al (1982).
Hyperprolactinemia of portal hypertension in rats.
Gastro enterology in press.

23. Nagy, I., Valdenegro, C.A., Login, I.S et al (1979).
Anti estrogens decrease prolactin synthesis and enzym-activities in the anterior pituitary of rats.
Endocrinol. 105 : A 116.

24. De Quyada, M., Timmermans, H.A.T., Lamberts, S.W.J. (1980).
Tamoxifen suppresses both the growth of prolactin-secreting pituitary tumours and normal prolactin synthesis in the rat.
J.Endocrinol. 86 : 109 - 116.

25. Groom, G.V., Griffiths, K. (1976)
Effect of the anti estrogen tamoxifen on plasma levels

of luteinizing hormone, follicle stimulating hormone, prolactin, oestradiol and progesterone in normal premenopauzal women.
J.Endocrinol. 70 : 421 - 428.

26. Willis, R.J., London, D.R., Ward, H.W.C. et al (1977)
Recurrent breast cancer treated with the anti estrogen Tamoxifen : correlation between hormonal changes and clinical course.
Br.Med.J. I: 425 - 428.

27. Masala, A., Delitala, G., Lo Dilo, G. et al (1978).
Inhibition of lactation and inhibition of prolactin release after mechanical breast stimulation in puerperal women given tamoxifen or placebo.
Br.J.Obstet, Gynecol. 85 : 134 - 137.

28. Powell-Jones, W., Jenner, D.A., Blamey, R.W. et al (1975)
Influence of anti oestrogens on the specific binding in vitro of (3 H) oestradiol to cytosol of rat mammary tumours and human breast carcinomata.
Biochem. J. 150 : 71- 75

29. De Quyada, M., Timmermans, H.A.T., Lamberts, S.W.J. et al (1980)
Tamoxifen enhances the sensitivity of dispersed prolactin secreting pituitary tumor cells to dopamine and bromocriptine.
Endocrinol. 106 : 702 - 706

30. Aragona, Friesen, H.G. (1975)
Specific prolactin binding sites in the prostate and testis of rats.
Endocrinol 97 : 667-684.

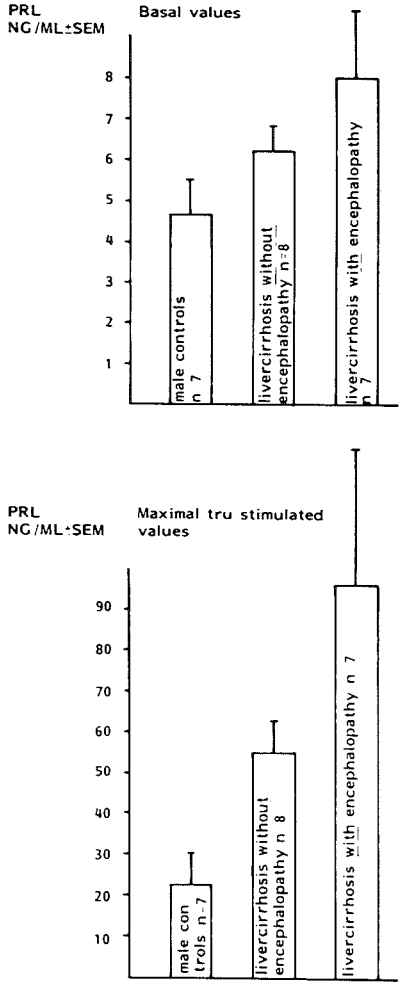
31. Posner, B.I., Kelly, P.A., Friesen, H.G. (1974)
Induction of a lactogenic receptor in rat liver : influence of estrogen and the pituitary.
Proc.Nat.Acad.Sci. U.S.A. 71 : 2407 - 2410.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS.

The review of literature has shown that basal prolactin levels in cirrhosis of the liver may be normal or mildly elevated, while prolactin levels may be excessively raised after TRH stimulation (1,2,3). In the present study we confirmed these findings. Basal prolactin levels were not significantly raised in cirrhotic patients compared to normal controls (table I). However after TRH maximal prolactin levels were significantly higher in the cirrhotic patients (table I). Furthermore prolactin levels were much higher in patients with cirrhosis with hepatic encephalopathy compared to cirrhosis without encephalopathy (table I). These findings by themselves already suggested a disturbance of prolactin secretion and synthesis in cirrhosis of the liver. Nevertheless a decrease of prolactin elimination by the diseased liver had to be excluded. In chapter 3 we described a study of catheterizations in patients with cirrhosis of the liver and portal hypertension, in whom arterio-venous differences across the liver and the kidney were studied. As both these organs show substantial uptake of radio-iodinated prolactin in rodents (4), it was expected that both organs are important in prolactin elimination. In cirrhotic patients a significantly larger arterio-venous prolactin deficit was found across the kidney, than across the liver, while these findings were not influenced by the degree of liver failure. In two patients without severe liver failure these catheterization studies were combined with hepatic and renal plasma flow studies. In this way a renal extraction of prolactin of 186 ug/60 min. and 94 ug/ 60 min., and a hepatic extraction of 16.4 ug/60 min, and 11.4 ug/60 min. respectively was found while liver and kidney perfusion were within the normal range. These findings correspond

Figure 1. Basal and maximal prolactin levels after TRH, in cirrhotic males with and without hepatic encephalopathy, and in healthy controls.



well with the prolactin production rates found in normal man by Cooper et al (males 187 ± 44 ug/d/m^2, females 211 ± 74 ug/d/m^2) and suggest that most circulating prolactin is eliminated by the kidney, both in cirrhotic and normal subjects. This is further substantiated by the fact that prolactin levels in kidney disease are inversily related to the creatinine clearance and normalise after kidney transplantation (6,7). In contrast no significant arteriovenous transhepatic prolactin difference has been found in normal man (8). It is thus highly unlikely that prolactin elimination in liver cirrhosis is substantially different from (or lower than) that in the normal situation. Definite proof, however, will be provided by MCR studies using purified human prolactin in patients with cirrhosis of the liver. In order to find out whether the way in which prolactin is eliminated in liver cirrhosis is specific for this hormone or is a more general way of polypeptide hormone removal, we also studied in the same manner the removal by the liver and the kidney of growth hormone, a hormone closely resembling prolactin in molecular weight. In this study it was found that growth hormone is removed in a different way, as it is mostly eliminated by the liver whereas no substantial growth hormone elimination takes place in the kidney. Growth hormone elimination by the liver is inversily related to the degree of liver failure in cirrhosis of the liver.
In fact the paradoxical rise of growth hormone after TRH stimulation in cirrhosis of the liver closely resembles the delayed reaction of prolactin secretion after TRH stimulation in patients with severe kidney disease (7). That the liver is very important in growth hormone elimination is further substantiated by the findings of Bratusch-Marrain et al (8) who reported that in normal man the liver accounts for approximately 50% of total growth hormone clearance. As growth hormone is implicated in somatomedin production by the liver the different metabolic

fate of human growth hormone and prolactin may mean that prolactin has a specific biologic effect on the kidney. It has been found that in the rat prolactin is mainly taken up by the proximal tubular cells from the glomerular filtrate (10). Since almost no prolactin is excreted in the urine (11), the hormone is probably broken down in the epithelium of the proximal tubules. In view of the effects of prolactin on water and electrolyte metabolism in lower animals, a specific effect of prolactin in volume homoiostasis has been suggested (12). However, as pointed out in the introduction, the evidence presented uptill now in man is scanty and inconclusive and a definite answer must be provided by further studies. On the basis of the observation that prolactin levels rise excessively after TRH stimulation (1,2,3) and that the normal diurnal prolactin secretion pattern may be lost in cirrhosis of the liver it is probable that the hyperprolactinemia of chronic liver disease is not caused by a decreased metabolic clearance rate but by an abnormality in the control of prolactin secretion. Prolactin secretion by the anterior pituitary is tonically inhibited by hypothalamic dopamine (14). Thus the increased prolactin release in cirrhosis of the liver could possibly be caused by changes in dopaminergic activity in the central nervous system. This might be effectuated by a disturbance of hypothalamic dopamine secretion through interference with neurotransmitter precursor/amino acid levels and/or the formation of false neurotransmitters (15,16), while dopamine secretion may also be influenced by the elevated plasma estrogen levels in cirrhosis of the liver (17,18). In addition estrogens have a direct stimulating effect on prolactin secretion by the pituitary (19). In order to test these various possibilities we used a rat model of portal hypertension in which hepatic mass was preserved and in which a possible prolactin stimulating effect of alcohol was eliminated. Prolactin levels were found to be significantly

raised in the rats. In this model, changes in plasma amino acids created by diet manipulation, did not influence prolactin secretion. However the induction of portal hypertension itself also did not influence plasma amino acid levels while it did raise prolactin levels. It thus seems possible that this model, in which hepatic integrity is maintained, cannot be used to test the neurotransmitter/amino acid hypothesis. Furthermore, prolactin secretion after TRH stimulation in hepatic encephalopathy was found to be higher than in cirrhosis of the liver without encephalopathy (20). Hepatic encephalopathy only occurs in man when liver function is severely impaired. Evidence has been put forward that in hepatic encephalopathy hypothalamic dopamine release may be disturbed, possibly through the formation of false neurotransmitters (15,16). This disturbance of central dopaminergic systems and the possible formation of false neurotransmitters are thought to be caused by a change in the ratio of plasma branched chain to plasma aromatic amino acids towards increased aromatic amino acids. Since both branched chain and aromatic amino acids compete for transport through the blood-brain barrier by the same carrier (21,22) this change will express itself in changed amino acids levels in the brain. Evidence in favour of this hypothesis is formed by the reported effectiveness of amino acid mixtures designed to counteract this deranged amino acid ratio (23,24). In our own study reported in chapter 6 we confirm the disturbed amino acid pattern found in chronic hepatic encephalopathy, while this pattern normalized after infusion of amino acid mixtures with excess branched chain amino acids.At the same time prolactin levels after TRH stimulation were either normalized or substantially lowered. We thus provide indirect evidence that in hepatic encephalopathy a disturbance in CNS neurotransmitters (probably dopamine) systems causes the increased prolactin secretion. In view of the finding that the false neurotransmitter octopamine

does not have an effect on prolactin release by the pituitary gland in vitro and also does not influence the prolactin secretion inhibiting effect of dopamine in vitro (Lamberts and MacLeod, unpublished observations), it seems less likely that the disturbance in CNS neurotransmitter systems responsible for the hyperprolactinemia in cirrhosis of the liver and encephalopathy includes the formation of false neurotransmitters. Although our rat model possibly was not suitable to test the amino acid neurotransmitter hypothesis it proved eminently suitable to test the elevated estrogen hypothesis, as both prolactin and estradiol-17B were found to be significantly raised in the portal hypertensive rats. Castration, which caused lower plasma levels of estrogens in the male rat, significantly decreased peripheral prolactin levels and completely blocked the portal hypertension-induced hyperprolactinemia, while suppletion with dihydrotestosterone did not influence the castration-induced fall in pituitary prolactin and plasma prolactin levels. This suggests that the effect of castration on prolactin levels is mediated by changes in estrogen levels rather than by the decreased testosterone levels. Furthermore we found that the elevated plasma estradiol-17B and plasma and pituitary prolactin levels found in the portal hypertensive rats were associated with significantly decreased hypophysial stalk plasma dopamine levels. Estrogen levels are very often raised in cirrhosis of the liver with portal hypertension, mainly because of increased peripheral aromatization of testosterone via androstenedione (25,26). It has been shown that estrogens stimulate prolactin levels in man and rats and that they enhance the effect of TRH stimulation on prolactin release through interference with hypothalamic dopamine release and an action on the pituitary dopamine receptor (18,19,27). We thus provide evidence that the elevated estrogen levels associated with portal hypertension and cirrhosis of the liver may

cause hyperprolactinemia through interference with the hypothalamic release of dopamine. That this mechanism probably also plays a role in cirrhotic patients can be concluded from the study described in chapter 7, in which it was found that the prolactin secretion after TRH stimulation in male cirrhotic patients with evidence of clinical hyperestrogenismus could be significantly lowered by the administration of the antiestrogen tamoxifen, a drug that blocks the effect of estrogens through competition at the estrogen receptor (28). Although the patients in this study were not followed for a long time, a remarkable clinical improvement of painful gynecomastia was found in some individual patients. This brings us to the question whether any clinical implications can be derived from these studies. On theoretical grounds, the observations made in some patients and the fact that uptill now no significant side effect of tamoxifen have been described, it seems to be worthwhile to conduct a trial of this drug in the gynecomastia and impotence associated with cirrhosis of the liver, provided that patients refrain from drinking alcohol, since alcohol may influence both prolactin secretion and testicular function directly (29). The gynecomastia of cirrhosis of the liver may be associated with normal or elevated prolactin levels (30) but is more often associated with a change in the estrogen/testosterone ratio occuring in this condition (31,32). It is however very interesting in this respect that an elevated estrogen/decreased testosterone ratio has been found to enhance prolactin receptor activity (33). It thus seems likely that a combination of various subtile hormonal changes lead to gynaecomastia. Since the elevated estrogen levels seem to play a pivotal role, the choice of tamoxifen to combat this condition seems appropriate. Finally it is highly unlikely that prolactin is the only pituitary hormone influenced in cirrhosis of the liver and possibly the disturbance of prolactin is an example

of a more general hypothalamic/pituitary disorder. It seems interesting to extend studies to the role of MSH in cirrhotic patients with increased skin pigmentation (34) and to the search for the elusive hypothalamic factor thought to play a role in aldosterone secretion (35), especially in patients with ascites and/or the hepatorenal-syndrome since in both cases dopamine has been implicated as a neurotransmitter. When we return to the questions we set out to answer we may conclude that:

1. The liver is not important in prolactin elimination and prolactin degradation in chronic liver disease is probably normal.
2. The degradation of prolactin is different from that of a comparable polypeptide hormone, growth hormone, as it is mainly eliminated by the kidney, while growth hormone is mainly eliminated by the liver. This different metabolic fate may be the consequence of a biologic action of these hormones on these respective organs.
3. Central prolactin release may be disturbed both by increased estrogens levels, which interfere with hypothalamic dopamine secretion and impaired CNS dopamine release through altered amino acid/neurotransmitter precursor levels.
4. The rat with portal hypertension but without concomitant liver disease has been found to exhibit many of the hormonal changes associated with cirrhosis of the liver including the hyperprolactinemia.
5. The hyperprolactinemia of compensated liver cirrhosis is probably caused by elevated estrogen levels, apart from a possible direct effect of alcohol on prolactin secretion, while the more excessive hyperprolactinemia of hepatic encephalopathy points to a more complicated disturbance of CNS neurotransmitter systems possibly in part caused by changed amino acid/neurotransmitter precursor levels.

1. Panerai, A.E., Salerno, F., Manneschi, M. et al (1977)
Growth hormone and prolactin responses to thyrotropin releasing hormone in patients with severe liver disease.
J.Clin.End. Metab. 45, 134 - 140.
2. Wernze, H., Schmitz, E. (1977).
Plasma prolactin and prolactin release in liver cirrhosis.
Acta Hepato-Gastroenterol. 24, 97 - 101.
3. Zanoboni, A., Zanoboni-Muciaccia, W. (1975).
Gynaecomastia in alcoholic cirrhosis.
The Lancet II, 876.
4. Rajaniemi, H., Oksanen, A., Vanka-Perttula, T. (1974).
Distribution of ^{125}I-prolactin in mice and rats.
Studies with whole body and micro autoradiography.
Hormone Res. 5, 6-20.
5. Cooper, D.S., Ridgway, E.C., Kliman, B. et al (1979)
Metabolic clearance and production rates of prolactin in man.
J.Clin.Invest. 64, 1669 - 1680.
6. Cowden, E.A., Ratcliffe, W.A., Ratcliffe, J.G.et al (1978).
Hyperprolactinemia in renal disease.
Clin. Endocrinol. 9, 241 - 248.
7. Lim, V.S., Kathpalia, S.C., Frohman, L.A.(1979).
Hyperprolactinemia and impaired pituitary response to suppression and stimulation in chronic renal failure : Reversal after transplantation.
J.Clin.End.Metab. 48, 101 - 107.
8. Bratusch- Marrain, P., Björkman, O., Waldhäusl, W. et al (1979).
Hepatic disposal of endogenous growth hormone and prolactin in man.
Eur.J.Clin.Invest. 9, 257 - 260.

9. Schimpff, R.M., Lebrec, D., Donnadieu, M.(1978).
Serum somatomedin activity measured as sulphation factor in peripheral, hepatic and renal veins of patients with alcohol cirrhosis.
Acta Endocrinol. 88, 729-736.
10. Donatsch, P., Richardson, B. (1975).
Localization of prolactin in rat kidney tissue using a double-antibody technique.
J.Endocrinol. 66, 101-106.
11. Nader, S., Mashiter, K., Joplin, G.F. (1975).
Urinary prolactin excretion.
Lancet I, 928.
12. Buckman, M.T., Peake, G.T., Robertson, C. (1976).
Hyperprolactinemia influences renal function in man.
Metabolism 25, 509-516.
13. Tarquini, B., Gheri, R., Anichini, P.et al (1977).
Circadian study of immunoreactive prolactin in patients with cirrhosis of the liver.
Gastroenterol. 73, 116-119.
14. MacLeod, R.M. (1976).
Regulation of prolactin secretion. In: Martini, L., Ganong, W.F., Eds. Frontiers in neuroendocrinology, vol.4. New York, Raven Press 69, 94.
15. Fisher, J.E., Baldessarini, R.J. (1971).
False neurotransmitters and hepatic failure.
Lancet I, 75-79.
16. Manghani, K.K., Lunzen, M.R., Billing, B.H. et al (1975).
Urinary and serum octopamine in patients with portal systemic encephalopathy.
Lancet II, 943 - 946.
17. Yen, S.S.C., Ehara, Y., Siler, T.M. (1974).
Augmentation of prolactin secretion by estrogen in hypogonadal women.
J.Clin.Invest. 53, 652-655.

18. Lloyd, H.M., Meares, J.D., Jacobi, J. (1975).
Effects of oestrogen and bromocriptine on in vivo secretion and mitosis in prolactin cells.
Nature 255, 497-498.
19. Raymond, V., Beaulieu, M., Labrie, F. (1978).
Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release.
Science 200, 1173-1175.
20. MacClain, C.J., Kromhout, J.P., Van Thiel, D. (1979).
Serum prolactin concentration in portal-systemic encephalopathy.
Gastroenterol. 76, 1292.
21. James, J.H., Escourrou, J., Fisher, J.E. (1978).
Blood brain neutral amino acid transport activity is increased after portocaval anastomosis.
Science 200, 1395-1397.
22. Partridge, W.M. (1977).
Kinetics of competitive inhibition of neutral amino acid transport across the blood-brain barrier.
J.Neurochem. 28, 103-108.
23. Fisher, J.E., Rosen, H.M., Ebeid, A.M. et al (1976).
The effect of normalization of plasma amino acids on hepatic encephalopathy in man.
Surgery 80, 77-91.
24. Fiere, R., Holm, E., Dietl, H. et al (1977).
Parenteral Ernährung mit Aminosäuren bei leberinsuffficienzy.
Vern.Dtsch.Ges.Inn.Med. 83, 533-537.
25. Gordon, G.G., Olivo, J., Rafii, et al (1975).
Conversion of androgens to estrogens in cirrhosis of the liver.
J.Clin.End.Metab. 40, 1010 - 1026.
26. Thijssen, J.H.H., Lourens, J., Donker, G.H. (1973).
Increased oestrogen production by peripheral conversion of androgens in male patients with cirrhosis of the liver.

J.Endocrinol. 57, 49.
27. Piercy, M., Shin, S.H. (1980).
Comparative studies of prolactin secretion in estradiol-primed and normal male rats induced by ether stress, pimozide and TRH.
Neuroendocrinol. 31, 270-275.
28. Jordan, V.C., Douse, L.J. (1976).
Tamoxifen as an antitumour agent: effect on oestrogen binding.
J.Endocrinol. 68, 297-303.
29. Van Thiel, D.H., Lester, R. (1976).
Alcoholism: its effect on hypothalamic pituitary gonadal function.
Gastroenterol. 71, 318-327.
30. Morgan, M.Y., Jacobovits, A.W., Gore, M.B.R. et al (1978). Serum prolactin in liver disease and its relationship to gynaecomastia.
Gut 19, 170-174.
31. Kley, H.K., Krüskemper, H.K. (1975).
Gynäkomastie.
Deutsche Med. Wochenschr. 100, 2612- 2617.
32. Carlson, H.E. (1980).
Gynaecomastia.
New. Engl.J.Med. 303, 795 - 799.
33. Posner, B.I., Kelly, P.A., Friesen, H.G.(1974).
Induction of a lactogenic receptor in rat liver : influence of estrogen and the pituitary.
Proc. Nat. Acad. Sci U.S.A. 71 : 2407.
34. Schmitt, G., Stoechel, M.E., Koch, B. (1981).
Evidence for a possible Dopaminergic control of pituitary alpha-MSH during actagenesis in mice.
Neuroendocrinol. 33, 306-311.
35. Mc Dougall, J.G., Scoggins, B.A., Butteus, A. et al (1981).
Dopaminergic modulation of aldosterone secretion ?
J.Endocrinol. 91, 271- 280.

SUMMARY.

Prolactin levels in liver cirrhosis may be elevated, especially after TRH stimulation. In chapter 3 we presented evidence that these elevated prolactin concentrations are not caused by diminished prolactin elimination, since this elimination takes place mainly in the kidney both in patients with and without liver disease, while in other studies no substantial prolactin elimination by the normal liver was found. The manner in which prolactin is eliminated in the kidney seems to be specific for this hormone, since growth hormone, a comparable polypeptide hormone, was found to be mainly removed by the liver, as described in Chapter 4. This different metabolic fate may reflect on different biological actions of both hormones on their respective target organs. From these studies, the excessive rise of PRL after TRH stimulation and the reported loss of the normal diurnal PRL secretion pattern it follows that the hyperprolactinemia of chronic liver disease is not caused by a decreased metabolic clearance but by an abnormality in the control of prolactin secretion. Since prolactin secretion is mainly regulated by a tonic inhibition of secretion by hypothalamic dopamine, a disturbance of central dopaminergic systems was postulated, either through elevated estrogen levels found in patients with cirrhosis of the liver or because of the deranged amino-acid neurotransmitter precursor levels found in these patients. In Chapter 5 a study in male portal hypertensive rats is described in which an increased prolactin secretion was found to be associated with elevated serum estradiol-17 B levels and decreased hypothalamic dopamine release. This increase in PRL secretion could be completely blocked by castration, which removes the major estrogen source in male rats. In chapter 7 we showed that elevated estrogen levels may also play a role in the hyperprolactinemia of livercirrhosis in man, especially when

gynecomastia is present. On the other hand the changes in aminoacid/neurotransmitter precursor levels associated with cirrhosis of the liver and hepatic encephalopathy may be important in causing the even higher prolactin secretion often found in this condition. In the portal hypertensive rat model described in chapter 5 no effect of dietary induced aminoacid changes on prolactin release could be found. However, in this model hepatic mass and function is preserved, whereas hepatic encephalopathy only occurs in man when liver function is seriously disturbed. Furthermore in chapter 6 we presented evidence that the disturbed aromatic amino acid/branched chain amino acid ratio found in hepatic encephalopathy is indeed associated with abnormal prolactin secretion and can be influenced by normalization of this abnormal ratio. From these findings we conclude that the elevated prolactin levels found in liver cirrhosis are caused by an abnormality in the secretion of Prolactin. This abnormal secretion is probably caused both by increased estrogen levels especially in patients with concurrent gynecomastia and by a disturbance in serum aminoacid/neurotransmitter precursor levels, especially in patients with concurrant hepatic encephalopathy, through interference with hypothalamic dopamine release.

SAMENVATTING.

Serum prolactine spiegels zijn vaak matig verhoogd bij patiënten met levercirrhose, vooral na stimulatie met TRH. In hoofdstuk 3 wordt aannemelijk gemaakt dat deze verhoogde prolactine concentraties niet worden veroorzaakt door een verminderde afbraak, aangezien de afbraak van prolactine voornamelijk in de nier blijkt plaats te vinden zowel bij patiënten zonder als bij patiënten met levercirrhose. Bovendien werd door anderen geen belangrijke afbraak van prolactine in de normale lever gevonden. De manier waarop prolactine door de nier wordt verwijderd lijkt specifiek te zijn, aangezien het vergelijkbare polypeptide hormoon groeihormoon juist wel voornamelijk door de lever wordt verwijderd, zoals beschreven in hoofdstuk 4. Dit verschil in eliminatie kan mogelijk wijzen op een verschillend biologisch effect van beide hormonen op hun respectievelijke eliminatie organen. Door deze studies, de excessieve stijging van prolactine na TRH-stimulatie en het door anderen aangetoonde verlies van het normale dagelijkse secretiepatroon van prolactine wordt het aannemelijk dat niet een verminderde metabolic clearance rate maar een toename van de secretie van prolactine de oorzaak is van de verhoogde prolactine spiegels. Aangezien de secretie van prolactine voornamelijk wordt gereguleerd door de tonische inhibitie van het door de hypothalamus afgegeven catecholamine dopamine, ligt een verstoring van de centrale dopamineafgifte voor de hand. Deze verstoring zou zowel veroorzaakt kunnen worden door de verhoogde oestrogeenspiegels die bij levercirrhose gevonden worden als door een verstoring van aminozuur/neurotransmitterprecursor spiegels, die vooral bij een ernstige verstoring van de leverfunctie optreedt. In hoofdstuk 5 wordt een studie in een model van portale hypertensie in de rat beschreven waarin een verhoogde prolactine secretie werd gevonden in associatie met verhoogde estradiol-17 B spiegels en een verlaagde hypo-

thalame afgifte van dopamine. Deze toename van de prolactine secretie kan volledig worden geblokkeerd door castratie, waardoor de belangrijkste oestrogeenbron in mannelijke ratten wordt verwijderd. In hoofdstuk 7 werd aangetoond dat verhoogde oestrogeenspiegels bij levercirrhose waarschijnlijk ook een rol spelen bij het veroorzaken van hyperprolactinemie bij de mens, vooral wanneer er eveneens gynecomastie bestaat. Daar tegenover zijn veranderingen in de serum aminozuur samenstelling zoals deze beschreven worden bij levercirrhose met hepatische encephalopathie mogelijk ook van belang voor het ontstaan van hyperprolactinemie. In het rattemodel van portale hypertensie beschreven in hoofdstuk 5 werd geen effect gezien op de prolactine secretie van manipulatie van de aminozuur samenstelling van het serum door beinvloeding van het dieet. In dit model blijft de leverfunctie echter grotendeels behouden terwijl in hepatische encephalopathie bij de mens de leverfunctie over het algemeen sterk gestoord is. Bovendien wordt in hoofdstuk 7 aannemelijk gemaakt dat een verstoorde aromatische aminozuur vertakte-keten-aminozuur verhouding inderdaad samengaat met een toegenomen prolactine secretie terwijl normalisering van deze verhouding door toediening van aminozuur oplossingen met overmaat vertakte ketens de prolactine secretie eveneens grotendeels normaliseert. Uit deze bevindingen werd de conclusie getrokken dat de verhoogde prolactine spiegels welke bij levercirrhose worden aangetroffen, veroorzaakt worden door een abnormale prolactine secretie. Dit abnormale secretie patroon wordt waarschijnlijk veroorzaakt zowel door toegenomen oestrogeenspiegels in het bloed, vooral bij patiënten met gynecomastie, als door een verstoring van de serum aminozuurspiegels, vooral bij patiënten met hepatische encephalopathie. Beide factoren beinvloeden de prolactine secretie door een remming van de hypothalame dopamine afgifte.

VERANTWOORDING.

In een periode van bezuiniging op algemeen maatschappelijk zowel als academisch terrein wordt de periode, beschikbaar voor het bewerken van een proefschrift, in veel gevallen beperkt tot de periode, die, in het kader van een specialistenopleiding, in een academisch milieu wordt doorgebracht. Dit betekent voor een internist in opleiding dat een onderzoek en de beschrijving hiervan in een periode van 5 jaar moet worden verricht. Dat lijkt lang, maar is in de praktijk kort. In feite moet alles meezitten. Men moet geluk hebben. De oorspronkelijke vraagstelling moet blijken zinnig geweest te zijn, bepalingen en protocollen moeten worden ontwikkeld en gestandariseerd, en de uiteindelijk bereikte resultaten moeten liefst ook nog houtsnijden.
Het zal duidelijk zijn, dat hulp op alle denkbare terreinen dan ook onontbeerlijk is; waarbij een zinnig en selectief gebruik maken van de aangeboden hulp misschien wel de meest noodzakelijke eigenschap van de jonge onderzoeker moet zijn. In mijn geval heeft het aan hulp niet ontbroken. Prof. Frenkel, die mij in opleiding nam en mij zodoende, naast mijn internistische vorming, gelegenheid gaf tot het verrichten van wetenschappelijk onderzoek, vervolgens Steven Lamberts en Paul Wilson die gezamenlijk, geholpen door grote kennis van zaken, dit " gat in de markt " ontdekten en vervolgens enthousiast en stimulerend geholpen hebben het te exploiteren. Zelden zal een promovendus een zo gemakkelijk, veelvuldig en vriendschappelijk contact met zijn promotoren hebben gehad. Vervolgens Wim de Greef en Frank de Jong en vooral Piet Uiterlinden, John Zuiderwijk, Ellen Bons, Theo Verleun en Marlijn Zandvoort van het laboratorium Interne III, die allen op uiterst nauwkeurige wijze essentiële bijdragen aan dit proefschrift hebben geleverd. Grote bewondering heb ik ook voor het uithoudingsvermogen van Wim van den Berg, Felix de Rooy en alle andere medewerkers van het laboratorium Interne II die hebben getracht

mij de beginselen van de laboratorium technologie bij te brengen, zonder daarbij het noodzakelijk gevoel voor humor en betrekkelijkheid te verliezen. Voorts dank aan de afdeling röntgenologie voor hulp bij de catheterisaties en aan René van Zanten voor hulp bij de ratteoperaties. Tenslotte heb ik voor de vormgeving van het uiteindelijke proefschrift veel te danken aan Marieke Horsch-Blecking en later aan Trudi van Campenhout-Pors, die nauwgezet al het noodzakelijke tikwerk hebben verricht. Ik realiseer me dat velen, die allen op hun wijze, direct of indirect, hebben bijgedragen aan het tot stand komen van dit proefschrift onvermeld zullen blijven. Hun bijdrage is hierdoor zeker niet minder belangrijk; uiteindelijk is de vriendschap en steun van velen op de achtergrond onontbeerlijk.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 4 november 1947 te Amsterdam. In Hilversum werd het lager en middelbaar onderwijs genoten. In 1965 werd het eindexamen Gymnasium B aan het Hilversums Gymnasium behaald. In 1965 werd de medicijnen studie aangevangen aan de Universiteit te Leiden, alwaar in 1971 het doctoraalexamen, en in 1973 het artsexamen werden behaald. In verband met uitzending naar de tropen werden in 1973 en 1974 assistentschappen gynaecologie, verloskunde en chirurgie gevolgd in het ziekenhuis St.Antoniushove te Leidschendam en aansluitend de nationale tropencursus voor artsen aan het Koninklijk Instituut voor de tropen te Amsterdam. In 1975 en 1976 was de schrijver District Medical officer in het Qachesnek-district in Lesotho. Na terugkeer uit Afrika werd de opleiding tot internist aangevangen onder leiding van Professor M.Frenkel. In december 1981 werd de schrijver in het specialistenregister ingeschreven. Hij is thans werkzaam als internist in het Havenziekenhuis te Rotterdam.