The Present Status of Prolactin Assays in Clinical Practice*

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Interest in prolactin in reproductive endocrinology and in relation to world population problems is emphasized by the occurrence of physiological and pathological conditions in which amenorrhea and/or reduced fertility are associated with galactorrhea. The syndromes of Chiari–Frommel, Argonz–Del Castillo and Forbes–Albright are characterized by galactorrhea and amenorrhea, the latter sometimes occurring postpartum, and in some cases being associated with the presence of a pituitary tumour (Forbes et al, 1954). The overall incidence of galactorrhea in cases of amenorrhea has recently been reported to be approximately 21 per cent (Shearman and Smith, 1972). It is generally agreed that postpartum lactation induces a prolonged amenorrhea associated with a substantial degree of infertility. These facts have been recently confirmed by El-Minawi and Sadek Foda (1971) and by Perez et al (1972). One must, however, point out that ovulation occurred in a few of their patients despite persistent breast-feeding.

Since the existence of a pituitary prolactin in the human remained controversial until recently, evidence favouring its existence will first be presented. The biological activities of prolactin in animals will then briefly be reviewed before discussing the present knowledge with respect to the physiopathology of the hormone in man. Presently available assays for human prolactin will be described, and finally their use in clinical practice will be considered. It was felt that an extensive discussion of this new area would be necessary for the clinician to realise what he can currently expect from prolactin assays. It is, however, probable that with the passage of time the sphere of applicability of prolactin assays in human subjects will increase.

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EVIDENCE FOR A HUMAN PITUITARY PROLACTIN

Prolactin (PRL) of pituitary origin has been defined in mammals as a hormone exhibiting mammotropic and luteotropic activities. Its existence in the human, as a pituitary hormone separate from growth hormone (GH) has been previously contested. Indeed, reports that even highly purified human GH possessed strong lactogenic activity (Chadwick, Folley and Gemzell 1961; Ferguson and Wallace 1961; Hartree, Kovacic and Thomas, 1965), and failure to isolate from human pituitaries collected at postmortem a prolactin distinctly distinct from GH (Wilhelmi, 1961; Tashjian, Levine and Wilhelmi, 1965) led many investigators to question the existence of prolactin in human subjects (see Bewley and Li, 1970).

There was however, a considerable amount of physiological data favouring the existence of human prolactin; these included the occurrence of lactation in dwarfs with isolated GH deficiency (Rimoin et al, 1968), normal and even low serum GH levels in cases of non-puerperal galactorrhoea (Benjamin, Casper and Kolodny, 1969), during pregnancy and postpartum (Spallac and Buhi, 1969; Spallac, Buhi, and Birk 1970 a, b; Varma et al, 1971), the existence of pituitary tumours rich in prolactin (Takatani et al, 1967) in the absence of an increase in blood and pituitary GH (Peake et al, 1969). In addition, the tetrachrome staining of Herlant (1960) applied to histological sections of the pituitary permitted one to distinguish prolactin cells from somatotropes in the human (Herlant and Pasteels, 1967). They later found such PRL cells to be very scarce except during pregnancy and lactation. Goluboff and Ezrin (1969) later confirmed this finding; it explains previous failures to isolate prolactin from pituitary glands obtained at postmortem since such glands were usually not derived from pregnant or lactating women. Another convincing argument in favour of the existence of human PRL had been obtained by use of in vitro cultures of human adult and fetal pituitary glands. Pasteels (1962) found that such pituitaries secreted increasing amounts of a pigeon-crop-sac-stimulating material, while GH secretion in the tissue culture media dramatically declined with time (Brauman, Brauman and Pasteels, 1964). Furthermore, antisera raised against a protein extract, obtained from these culture media, and biologically active in the pigeon crop-sac, have been found to neutralise the lactogenic activity contained in sera from lactating women (Pasteels, Robyn and Hubinont, 1965). Use of these reagents allowed Greenwood et al (1971) to develop for the first time a radioimmunological procedure applicable to the detection of human prolactin in serum samples (Bryant et al, 1971).

However, the existence of the human of prolactin as a separate molecule was not universally and unequivocally accepted until demonstration of its biosynthesis (Friesen, Guyda and Hardy, 1970) and isolation (Lewis et al, 1971; Hwang, Guyda and Friesen, 1972). The sequence of the first 20 amino acids from the amino terminal (Niall, 1972), and a tentative structure deduced from peptide mapping (Lewis, Singh and Seavey, 1972) indicate that human prolactin is very closely related to ovine prolactin but not to human growth hormone. This explains the extensive immunological cross reaction found between human and ovine prolactins. This cross reaction has been the
basis of the development of heterologous (Guyda, Hwang and Friesen, 1971; Jacobs, Mariz and Daughaday, 1972) and homologous (L’Hermite and Midgley, 1971)\(^1\) radioimmunoassays for human prolactin. This cross reaction has been also used by Pasteels et al (1972) to localise by immunofluorescent staining the prolactin cells on histological sections of human adenohypophyses.

### BIOLOGICAL ACTIONS AMONGST VERTEBRATES

Prolactin is a uniquely versatile hormone. Nicoll and Bern (1972) reviewed 84 different reported actions among cyclostomes, teleosts, amphibians, reptiles, birds and mammals. Prolactin had previously been regarded as a mammotrophic or even solely as a gonadotrophic hormone (luteotrophin) until Riddle et al (1933) emphasised that it should be regarded as having metabolic properties. In the phylogeny of this hormone, there has been no propensity for the regulation of any single physiological process. Nicoll and Bern (1972) proposed to classify its numerous actions under the following five heads:

1. Related to reproduction.
2. Affecting water and electrolyte balance (osmoregulation).
3. Involving growth promotion (and growth hormone-like metabolic actions).
4. On integumentary (ectodermal) structures.
5. Involving synergism with steroid hormones or on organs which are also influenced by steroids.

Table 1 shows, under these headings, the actions of prolactin described in mammals, regardless of the animal species. It should be noted that a single effect can sometimes be classified in several categories. For example, effects on mammary development and lactation involve growth of an ectodermal structure which is also a reproductive structure, requiring synergism with steroids, and the secretory activity of which produces problems of osmoregulation.

It is obvious that some of the actions described can be only secondary, produced by interference with other endocrine or metabolic processes. In addition, some actions could be obtained only under pharmacological and not under physiological conditions. Potentially all the actions must be taken into consideration when studying the physiopathology of prolactin secretion in the human. However, one cannot assume that prolactin has any of these effects in the human unless they have been previously tested. This opens up a large field of possible clinical interest for prolactin, but most of the possibilities suggested require further investigation. Amongst the numerous actions claimed for prolactin among vertebrates, no single common denominator is obvious. Prolactin can be considered simply as the hormone of the pituitary gland which has been used by vertebrates to regulate a variety of ‘emerging’ physiological processes important in adaptation. Indeed the proposal has

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\(^1\)First presented by A. R. Midgley at a Prolactin Workshop, held in January 1971 at the National Institutes of Health (Bethesda, Maryland, U.S.A.).
been made that a common denominator for the widely differing effects of prolactin is that the hormone 'conditions' or modifies the responsiveness to the trophic influence of other endocrine factors (see Nicoll and Bryant, 1972).

<table>
<thead>
<tr>
<th>Reproduction</th>
<th>Growth Promotion</th>
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<tr>
<td>Mammary development</td>
<td>Mammary development</td>
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<tr>
<td>Lactation</td>
<td>Male accessory sex gland development</td>
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<tr>
<td>Luteotropic action</td>
<td>Luteotropic action</td>
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<tr>
<td>Fertility control</td>
<td>Spermatogenesis</td>
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<td>Luteolytic action</td>
<td>Erythropoietic effect</td>
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<tr>
<td>Advanced puberty</td>
<td>Hair growth</td>
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<tr>
<td>Decreased copulatory activity</td>
<td>Sebaceous and preputial gland growth</td>
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<tr>
<td>Parental behaviour</td>
<td>Synergism with steroids</td>
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<td>Vaginal mucification</td>
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<tr>
<td>Effect on male accessory sex glands</td>
<td>Mammary growth</td>
</tr>
<tr>
<td>Preputial gland size and activity</td>
<td>Milk secretion</td>
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<tr>
<td>Increased androgen binding in prostate</td>
<td>Advanced puberty</td>
</tr>
<tr>
<td>Increased cholesterol in testis</td>
<td>Luteotropic action</td>
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<tr>
<td>Increased glucuronidase activity in testis</td>
<td>Spermatogenesis</td>
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<td>Osmoregulation</td>
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<td>Lactation</td>
<td>Renal retention of Na⁺ and water</td>
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<td>Increased retention of Na⁺ and water</td>
<td>Vaginal mucification</td>
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<tr>
<td>Ectodermal structures</td>
<td>Effects on male accessory sex glands</td>
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<td>Mammary development</td>
<td>Sebaceous and preputial glands</td>
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<td>Lactation</td>
<td>Secretion</td>
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<td>Effects on sebaceous and preputial glands</td>
<td>Hair growth</td>
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<td>Hair maturation</td>
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ASSAYS OF HUMAN PROLACTIN

Pigeon crop-sac test
Classically prolactin activity has been estimated by use of the pigeon crop-sac bioassay. The original systemic procedure described in 1933 by Riddle, Bates and Dykshorn has been later modified to increase its sensitivity: the most sensitive tests use local morphological changes in the crop epithelium, but they are still too insensitive for clinical use. It is advisable to extract prolactin from serum (Simkin and Goodart, 1960) but non-specific substances contained in urine or serum can cause inflammatory reactions thus producing non-specific interference (Lahr, Bates and Riddle, 1943), even when corticosteroids are administered to minimise such reactions (von Berswordt-Wallrabe, Herlyn and Jantzen, 1965; Apostolakis, 1968). Finally, it must not be forgotten that growth hormone itself promotes lactogenesis in this test (Forsyth, Folley and Chadwick, 1965).
In vitro bioassays using mammary tissue explants

New bioassay procedures for the measurement of prolactin in unextracted human serum or plasma have been recently developed. They use short-term cultures (48 hours to 5 days) of mammary tissue taken from pregnant animals, either rabbits (Forsyth, 1969) or mice. Tissue culture medium 199 contains insulin (5 to 10 μg/ml), corticosteroids (1 to 20 μg/ml) and human serum or plasma at concentrations of 10 to 30 per cent in an atmosphere of 95 per cent O₂ and 5 per cent CO₂. Two of these procedures determine the secretory activity of the explants histologically and record it by use of an arbitrary grading system (Forsyth and Myres, 1971; Frantz and Kleinberg, 1970). Loewenstein et al (1971) used as their endpoint the induction of an enzyme, N-acetyllactosamine synthetase. This enzyme, which catalyzes the reaction: N-acetylgalactosamine + UDP-galactose → N-acetyllactosamine + UDP was estimated radiochemically following incubation of the homogenates with N-acetylgalactosamine and UDP-galactose-¹⁴C. Turkington (1971a) preferred to use as endpoint the synthesis of ³²P-casein. These last two methods have the advantage of being quantitative. All such assays utilised ovine prolactin standards, and lactogenic activity was expressed in 'ovine equivalents'. In addition, the standard was usually diluted in pooled plasma from normal males. This procedure cannot be recommended since radioimmunoassays have demonstrated that normal male plasma contains appreciable amounts of prolactin. This could therefore explain discrepancies between bioassay and radioimmunoassay results, especially when serum had been added to the reaction mixture in order to improve the linearity of the dose-response relationship (Turkington, 1971a).

Human growth hormone and human chorionic somatomammotrophin (HCS or HPL for human placental lactogen) both exhibit considerable lactogenic activity in these bioassays. Such interference with prolactin

Table 2. Comparison of the characteristics of various in vitro bioassays for human prolactin using mammary tissue explants

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal used</th>
<th>Duration of incubation</th>
<th>Nature of endpoint</th>
<th>Means of quantification</th>
<th>Sensitivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forsyth (1969)</td>
<td>Rabbit</td>
<td>5 days</td>
<td>Histological secretory response</td>
<td>Arbitrary grading</td>
<td>100</td>
</tr>
<tr>
<td>Frantz and Kleinberg (1970)</td>
<td>Mouse</td>
<td>4 days</td>
<td>Histological secretory response</td>
<td>Arbitrary grading</td>
<td>15</td>
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<tr>
<td>Loewenstein et al (1971)</td>
<td>Mouse</td>
<td>48 hours</td>
<td>Formation of N-acetyllactosamine synthetase</td>
<td>Counting of ¹⁴C radioactivity</td>
<td>4—40</td>
</tr>
<tr>
<td>Turkington (1971a)</td>
<td>Mouse</td>
<td>48 hours</td>
<td>Synthesis of ³²P—casein</td>
<td>Counting of ³²P radioactivity</td>
<td>2</td>
</tr>
</tbody>
</table>

*Expressed as the usual minimum detectable prolactin activity, in ng/ml (equivalents to N.I.H.—P.—S.8).
measurements can, however, be completely avoided by preincubation of the sample with a specific anti-HGH serum. The sensitivity of these assays (see Table 2) has varied considerably, ranging from 2 to 100 ng/ml. As a consequence, most assays could not detect basal prolactin levels in males and premenopausal non-pregnant women (Forsyth, 1972). Precision was low but was enhanced when a biochemical parameter was used as endpoint.

Radioimmunoassays

Principles. These are based on the ‘competition’ for a fixed amount of antiserum between a fixed amount of labelled tracer and a variable amount of antigen contained in the standard or in the unknown sample. A characteristic of radioimmunoassays is that the specificity of the measurement does not require a specific antiserum but rather depends on the combination of antiserum and tracer. This explains how it is possible to develop a reasonably specific assay for a hormone by use of an antiserum obtained against a crude material, in combination with a highly purified preparation of the hormone as the labelled tracer.

While bioassays have generally been valid for measurement of a hormone irrespective of animal species, this has not been true for radioimmunoassays of glycoprotein hormones, for which assays are usually species-specific. An assay will be called ‘homologous’ when the hormone used for immunisation and for labelling are of the same animal species, and ‘heterologous’ where one is using an antiserum of one species in combination with a labelled hormone of a second species. The use of such heterologous radioimmunoassays has been found of value in obviating problems of extensive cross reactivity between related hormones, such as in the case of luteinising hormone, follicle-stimulating hormone, thyroid-stimulating hormone and human chorionic gonadotrophin (L’Hermite and Midgley, 1971; Midgley, 1971; L’Hermite et al, 1972c).

Human prolactin assays. The first attempts utilised material prepared from tissue culture media of fetal pituitary glands; this was employed both for the production of antiserum and for labelling (Bryant et al, 1971). Such systems gave some indication of prolactin secretion, but did not appear to measure the endogenous hormone alone (Greenwood, 1972). In fact, the heterologous approaches proved to be the most successful in this area (Guyda, Hwang and Friesen, 1971; Jacobs, Mariz and Daughaday, 1972). The cross-reaction of primate prolactins with ovine prolactin was employed to isolate monkey prolactin and to use it subsequently in the assay for human prolactin as the labelled hormone in combination with an anti-HGH serum (Hwang, Guyda and Friesen, 1971). The latter contained anti-human prolactin antibodies, probably due to contamination of the HGH preparation used for immunisation. Final isolation and purification of human prolactin (Lewis, Singh and Seavy, 1971; Hwang, Guyda and Friesen, 1972) enabled investigators to develop reliable, specific and sensitive homologous radioimmunoassays for this hormone (Friesen et al, 1972c; Sinha et al, 1973).

As it can be seen in Table 3, several different procedures have been described. Besides typical homologous and heterologous assays, three assays were classified as ‘homogeneous’, since the material used for labelling and
<table>
<thead>
<tr>
<th>Type of assay</th>
<th>Material used for production of antiserum</th>
<th>Material used for labelling</th>
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<tr>
<td>Heterologous assays</td>
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<tr>
<td>Guyda, Hwang and Friesen</td>
<td>Monkey prolactin</td>
<td>Ovine prolactin</td>
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<td>(1971)</td>
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<td>Hwang, Guyda and Friesen</td>
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<td>(1971)</td>
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<td>Jacobs, Mariz and</td>
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<td>Porcine prolactin</td>
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<td>Daughaday (1972)</td>
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<td>Midgley and Jaffe (1972)</td>
<td>Ovine prolactin</td>
<td>Human prolactin</td>
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<td>'Homogeneous' assays</td>
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<tr>
<td>L'Hermite et al (1972b)*</td>
<td>Ovine prolactin</td>
<td>Ovine prolactin</td>
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<tr>
<td>Hwang, Guyda and Friesen</td>
<td>Monkey prolactin</td>
<td>Monkey prolactin</td>
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<tr>
<td>(1971)</td>
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<tr>
<td>Josimovich, Bocella and</td>
<td>Carbamidomethyl human chorionic somatomam-</td>
<td>Human chorionic somatomammatrophin</td>
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<td>Levitt (1971)</td>
<td>somatotrophin</td>
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<td>Homologous assays</td>
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<tr>
<td>Bryant et al (1971)</td>
<td>Extract from tissue cultures of human fetal pituitary glands</td>
<td>Extract from tissue cultures of human fetal pituitary glands</td>
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<tr>
<td>Friesen et al (1972c)</td>
<td>Human prolactin</td>
<td>Human prolactin</td>
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<tr>
<td>Sinha et al (1973)</td>
<td>Human prolactin</td>
<td>Human prolactin</td>
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<tr>
<td>Cole and Boyns (1973)</td>
<td>Extract from human amniotic fluid</td>
<td>Human prolactin</td>
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*First reported by Dr A. R. Midgley at the Prolactin Workshop Conference held in January 1971 at the National Institutes of Health (Bethesda, Maryland, U.S.A.).

for immunisation was of the same species but either not human (L'Hermite et al, 1972b; Hwang, Guyda and Friesen, 1971) or not prolactin (Josimovich, Bocella and Levitt, 1971).

In the radioimmunoassays, as distinct from the bioassays previously described, there has been no cross reaction with either growth hormone or human chorionic somatomammotrophin. All the assays used gave comparable results in terms of physiopathological findings, but the absolute values and the relative variations may differ slightly, suggesting that these assays do not all bind at the same immunological sites of the prolactin molecule. There is a clamant need for wider availability of international standards and reagents, because the supply of research standards by the British Medical Research Council is insufficient, and therefore it is not possible for investigators to compare results obtained by different methods on a large scale.

PHYSIOPATHOLOGY OF HUMAN PROLACTIN SECRETION

Basal levels

Prolactin levels were not detectable by bioassay in normal males and non-pregnant females either before or after the menopause (Kleinberg and Frantz, 1971; Turkington, 1971a; Forsyth, 1972). On the other hand, radioimmuno-
assays enabled workers to detect prolactin in the majority of subjects (Hwang, Guyda and Friesen, 1971; Jacobs, Mariz and Daughaday, 1972; Noel et al, 1972; L’Hermite et al, 1972b). Mean basal levels were found to be slightly higher in women (mean = 9-0 to 14-0 ng/ml) than in men (6-2 to 13-0 ng/ml). However, this sex difference was statistically significant only in the studies of Jacobs, Mariz and Daughaday (1972) and Nokin et al (1972). Friesen et al (1972a) found that prolactin concentrations were similar in normal children and adults.

Slight differences amongst absolute values reported by several authors could be due to the use of different standards and/or to intrinsic differences related to the type of assay. The latter explanation is likely to be especially relevant with basal values obtained with our ‘homogeneous’ ovine prolactin assay. In addition it should be pointed out that bioassay and radioimmunoassay results are not necessarily identical. Thus Frantz, Kleinberg and Noel (1972) reported a good correlation between both methods; on the other hand, Turkington (1971a) could not detect basal prolactin levels with a bioassay claimed to have a threshold sensitivity at 2 ng/ml.

**Pregnancy, post partum and suckling**

A major difference between animal and human physiology resides in the fact that prolactin levels rise gradually until term during human pregnancy (L’Hermite and Robyn, 1972; Tyson et al, 1972). This feature characteristic of humans has not been shown in monkeys in which prolactin remains low throughout pregnancy. Friesen et al (1972b) attributed this difference to different types of oestrogen metabolism in the two species. Basal prolactin levels were higher around delivery and then fell rapidly and progressively to reach, by the end of the first week, values just slightly higher than in the non-pregnant state (Hwang et al, 1971); they returned to non-pregnant levels 4 to 6 weeks postpartum. It is interesting to note that painful breast engorgement occurred usually on the third or fourth day, at a time where prolactin levels had already declined to half those found at delivery. Thus it is obvious that prolactin is not the only factor involved in the onset of lactation; otherwise, pregnant women should experience a continuous flow of milk. In nursing mothers, prolactin levels fluctuated greatly (Jaffe, L’Hermite and Midgley, 1972) with abrupt rises after each suckling period (Bryant et al, 1971; Hwang, Guyda and Friesen, 1971). Peak prolactin levels occurred usually within 30 minutes after beginning the nursing period (Frantz et al, 1972). While the rise of prolactin levels in response to suckling appeared to be modest during the first postpartum days, it was greater at the tenth day (Tyson et al, 1972; L’Hermite, Stavric and Robyn, 1972), with subsequently a progressive decline despite persistent lactation (Tyson et al, 1972). Reyes, Winter and Faiman (1972) reported that, although there were wide fluctuations in individual prolactin levels, overall prolactin secretion was higher in a successful lactator than in an unsuccessful one.

Frantz et al (1972) have shown that the stimulus causing the rise in prolactin during nursing was mechanical breast stimulation, and that psychic factors associated with the presence of the child had almost no effect. Further-
more, it has been reported that breast stimulation may also cause a modest prolactin elevation in women who are not postpartum and in men (Noel, Suh and Frantz, 1972).

Neonates and amniotic fluid

Serum prolactin concentrations in newborns are similar to maternal levels at term and then decline progressively to reach those of normal adults by 6 weeks (Hwang et al, 1971). Tyson et al (1972) found no striking differences between arterial and venous prolactin concentrations in the umbilical cord. They reported that amniotic fluid contained huge amounts of immunoreactive prolactin; in the first 20 weeks of gestation concentrations ranged from 1.2 to 7.0 μg/ml; thereafter they declined until term. There is no definite evidence concerning the nature and origin of this immunoreactive prolactin present in amniotic fluid. When labelled human prolactin has been injected into the amniotic sac, Friesen et al (1972b) found that the transfer of prolactin from the mother into the fetus was small. They also showed on the basis of incubation experiments that some prolactin was released into the medium by the amnion and chorion but not by placental tissue. Accordingly, the most likely source of the prolactin in amniotic fluid appears to be the chorion.

Circadian rhythm

Nokin et al (1972) described a circadian rhythm in serum prolactin concentrations, with peak values occurring between 1 and 5 a.m. in non-pregnant women, and at 5 a.m. in adult men. Sassin et al (1972) reported that prolactin concentrations began to rise 60 to 90 minutes after the onset of sleep, became progressively higher during the remaining hours of sleep and showed maximal concentrations in the early morning between 5 and 7 a.m.; levels fell rapidly during the hour immediately after awakening. However Vanhaeyst et al (1973) showed that these circadian discharges in prolactin were not necessarily related to the cycle of sleeping and waking. They demonstrated the occurrence of episodic prolactin release throughout the 24 hours, the lowest blood concentrations being between 10 a.m. and 12 noon.

Conditions of stress

Noel et al (1972) reported elevations of prolactin in a number of stressful situations. Thus, mean prolactin levels were significantly higher;

1. Immediately and 15 minutes after strenuous exercise.
2. Ten minutes after a gastroscopy, performed following the administration of atropine and diazepam (Valium); the latter drug was shown to have no effect on prolactin secretion.
3. Ten minutes after proctoscopy, performed without any premedication.
4. In women 10 and 30 minutes after completion of sexual intercourse.
5. During and after surgery under general anaesthesia.
6. Sixty minutes after intravenous injection of insulin (0.2 U/kg body weight).

In most but not in all of these stressful conditions, prolactin release was associated with growth hormone; however, the magnitude of secretion of the two hormones differed greatly depending on the type of stimulus.

Absolute prolactin changes were usually greater in women than in men,
although this sex difference has not been statistically significant in response to all stimuli.

Mean preoperative values for female patients were three times higher than for normal resting women, even in the absence of obvious pre-operative stress and excluding patients receiving drugs known to elevate prolactin secretion. Noel et al (1972) have ascribed these high prolactin values found pre-operatively in women to anxiety or psychic stress.

The rises in prolactin associated with surgery might be attributed to several factors, for example, drugs used for general anaesthesia, tracheal intubation, and the cutting and manipulation of tissues.

Daughaday and Jacobs (1972) reported that sexual intercourse can induce a modest increase in prolactin levels in men, while the studies of Noel et al (1972) suggest a correlation between prolactin and orgasm in women.

To attribute all these increases in prolactin levels to stress only reflects our ignorance regarding the physiological significance of the hormone. Also, it should be pointed out that most of these data have been obtained at a time at which the subjects studied were free of pain and did not appear severely stressed.

**Oestrogens, menstrual cycle and gynaecomastia**

Oestrogens are known to be potent stimulators of prolactin secretion in animals (see Meites, 1969). It appears that man is less sensitive than animals to this effect of oestrogens. Thus, it has been reported that high dosages of oestrogens caused an increase in prolactin levels, in postmenopausal women with advanced breast cancer (L'Hermite et al, 1972b) as well as in male patients (Frantz et al, 1972).

Studies of the urinary excretion of prolactin by bioassay had shown higher hormone levels in the luteal than in the follicular phase of the cycle (Coppedge and Segaloff, 1951). Hwang et al (1971) using a radioimmunoassay, failed to observe a similar pattern during the menstrual cycle in the plasma of nine subjects. With a different type of radioimmunoassay, Vekemans et al (1972) studied serum prolactin levels daily in 18 women with ovulatory cycles; they noted a modest peak of the hormone at midcycle and observed that prolactin secretion was significantly increased in the luteal phase. In spite of this finding by these investigators the question remains open as to whether prolactin is luteotropic in man as it is in animals.

Turkington (1972a) failed to observe increased levels of biologically active prolactin in 23 patients presenting with gynaecomastia, the latter being associated with a variety of clinical conditions such as bronchogenic carcinoma and hepatic cirrhosis. The subjects were not receiving tranquillising drugs or reserpine. Similarly Frantz et al (1972) found normal levels of immunoreactive prolactin in six patients with uncomplicated gynaecomastia. Among their 16 subjects, 5 showed an abnormally large discrepancy between results obtained by bioassay and immunoassay, data by bioassay being higher. While this intriguing finding requires further study, it appears that elevated prolactin levels per se do not produce systematic breast development (e.g. in males), and that the hormonal sensitivity of the breast tissue might be an important factor in the development of gynaecomastia and of breast enlargement.
Osmoregulation
Horrobin et al (1971) demonstrated that injection of ovine prolactin in adult men resulted in a significant reduction of the renal excretion of water and sodium; there was an increase in plasma sodium levels and in osmolarity. Friesen et al (1972b) reported that 20 out of 100 patients with advanced renal failure had grossly elevated prolactin levels. Frantz et al (1972) also stated that renal failure appeared to be associated with a tendency toward elevated prolactin levels, as estimated both by bioassay and by radioimmunoassay.

One could ask therefore whether prolactin secretion could be involved in the circulatory changes and the deficiency in water metabolism occurring during pregnancy. It is also possible that prolactin could play a role in regulating the transport of sodium across the amniotic membrane.

Ectopic production
Turkington (1971b) reported ectopic prolactin production in a patient with undifferentiated bronchial carcinoma and in another subject with a hypernephroma.

<table>
<thead>
<tr>
<th>Table 4. Conditions characterised by elevated blood prolactin levels</th>
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<tr>
<td><strong>Physiological</strong></td>
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<tr>
<td>Nocturnal discharge (sleep)</td>
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<td>Luteal phase of menstrual cycle</td>
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<td>Pregnancy</td>
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<td>Postpartum period</td>
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<td>Suckling and breast manipulation</td>
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<td>Neonatal period</td>
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<td>Exercise</td>
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<td>Stressful situations</td>
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<td>Sexual intercourse</td>
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<td><strong>Pathological</strong></td>
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<tr>
<td>Syndromes characterised by amenorrhoea and galactorrhoea (Chiari–Frommel, Ahumada–Del Castillo, Argonz–Del Castillo, Fortes–Albright syndromes; oral contraceptive withdrawal)</td>
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<tr>
<td>Pituitary tumours secreting prolactin</td>
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<td>Pituitary stalk section with disturbance of hypothalamo–pituitary relationships, e.g. by suprasellar and infrasellar tumours</td>
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<td>Juvenile hypothyroidism (athyreosis)</td>
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<td>Renal failure</td>
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<tr>
<td>Ectopic production of the hormone by malignant tumours, e.g. undifferentiated bronchial carcinoma and hypernephroma</td>
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<td>Surgical stress</td>
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<td><strong>Pharmacological</strong></td>
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<tr>
<td>Insulin-induced hypoglycaemia</td>
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<tr>
<td>Administration of synthetic TRH</td>
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<td>High dosages of oestrogens</td>
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<td>Psychotropic drugs, e.g. phenothiazines, butyrophenones, sulpiride, etc.</td>
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<tr>
<td>Reserpine and α-methyldopa administration</td>
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<td>General anaesthesia</td>
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PHARMACOLOGICAL AGENTS ACTING ON PROLACTIN SECRETION

Stimulation

Insulin-induced hypoglycaemia has been reported to induce prolactin release, in addition to releasing growth hormone and ACTH (Frantz and Kleinberg, 1970; Copinschi et al, 1972a). The prolactin release has been systematically observed following the injection of 0.2 units of insulin per kg of body weight (Frantz, Kleinberg and Noel, 1972); the response was inconstant when a dosage of 0.05 U/kg was used. The time relationships for the prolactin release were similar to those for growth hormone, suggesting that both effects were mediated through the hypothalamus (Copinschi et al, 1972a). The finding that prolactin release was less marked after infusion than after injection of insulin (Wilson, Singhal and Percy-Robb, 1972) suggests that the rapidity of the fall in blood glucose might be a critical factor. It has been reported that pretreatment with dexamethasone inhibits the release of both growth hormone and prolactin (L’Hermite et al, 1973). However, in male subjects suffering from obesity the growth hormone but not the prolactin release was inhibited (Copinschi, Robyn and L’Hermite, 1973).

Numerous psychotropic drugs with different chemical structures have been reported to be mammotrophic in animals (see Sulman, 1970). In the human, therapy with some of these drugs has been reported to be associated with elevated prolactin levels and is occasionally accompanied by galactorrhoea (Apostolakis et al, 1972). The drugs most frequently used were phenothiazines and their derivatives (Frantz, Kleinberg and Noel, 1972; Turkington, 1972b), and sulpiride (Dogmatil) (L’Hermite et al, 1972b). All drugs sharing with chlorpromazine the property of depleting catecholamines in certain areas of the brain are likely to have a stimulatory action on prolactin secretion. It is of interest to note that, after cessation of therapy, serum prolactin levels declined very slowly to reach normal values only within 10 to 15 days. Long-term treatment with reserpine or α-methyldopa also caused markedly elevated prolactin levels (Turkington, 1972c).

Synthetic TRH has been found to induce prolactin as well as TSH release into the blood. As little as 10 μg of synthetic TRH given intravenously was active, and there appeared to be no difference in the threshold sensitivity with respect to prolactin and TSH (Bowers et al, 1971). The prolactin response was greater in women than in men (Bowers, Friesen and Folkers, 1972) and was statistically significant as early as five minutes after the injection (Jacobs et al, 1971; L’Hermite et al, 1972d). Synthetic TRH is likely to act directly on the prolactin cells of the pituitary gland, and its action is not inhibited by pretreatment with dexamethasone which inhibited the prolactin response to insulin-induced hypoglycaemia (Vanhaelst et al. 1972).

Inhibition

Malarkey, Jacobs and Daughaday (1971) reported the acute inhibition of prolactin secretion by the oral administration of 0.5 g levodopa in women presenting with galactorrhoea and elevated serum prolactin levels. Marked
suppression (70 per cent to 97 per cent) occurred in all patients by 90 minutes, and thereafter levels rose again towards basal values. A similar effect was also found in normal female subjects. When this test was repeated on successive days, the inhibitory effect of levodopa was always observed, but the degree of suppression was often smaller, and sometimes rebound elevations of prolactin occurred after the first inhibition. It should be noted that levodopa exhibits an opposite effect on growth hormone secretion, causing increased levels 60 to 110 minutes after a single oral dose of 0.5 g of the drug (Kansal et al, 1972). In addition, levodopa suppresses the TSH response to TRH (Spaulding et al, 1972), and prevents the stimulatory effect on prolactin induced by chlorpromazine (Kleinberg, Noel and Frantz, 1971). Turkington (1972d) reported that six months' treatment with L-Dopa was successful in decreasing elevated prolactin levels towards normal and in stopping galactorrhea in 11 out of 14 patients. Two of the subjects who responded poorly to this form of treatment were subsequently found to have pituitary tumours.

It has been shown that ergot alkaloids, e.g. ergocornine, can inhibit prolactin secretion by a direct action on rat hypophysial cells (Pasteels and Ectors, 1970). A derivative, 2-bromo-D-ergocryptine (CB-154 (Sandoz)), has been used successfully in the treatment of galactorrhea (Copinschi et al, 1972b; Lutterbeck et al, 1971), acting presumably through an effective suppression of prolactin secretion (Copinschi et al, 1972b; Besser et al, 1972; del Pozo et al, 1972). This drug also completely inhibited puerperal lactation and breast engorgement and reduced elevated prolactin levels during the immediate postpartum period (del Pozo et al, 1972). Its duration of action was much longer than that of L-Dopa, the effect lasting for more than 12 hours after a single oral dose (del Pozo et al, 1972; Rozencweig et al, 1973).

INTERRELATIONSHIPS BETWEEN TSH AND PROLACTIN SECRETION

The finding that synthetic TRH consistently causes prolactin release as well as that of TSH (Jacobs et al, 1971; Bowers et al, 1971; L'Hermite et al, 1972d) led to the question whether this hypothalamic, hypophysiotropic hormone might play a physiological role in the control of prolactin secretion.

It is well known that menstrual disturbances are very often associated with hypothyroidism and hyperthyroidism (Rosenberg, 1969). Kinch, Plunkett and Devlin (1969) reported four cases of Chiari–Frommel syndrome which were associated with hypothyroidism and which had been rapidly and completely cured merely by thyroid replacement therapy. Primary hypothyroidism is a rare cause of galactorrhea, the latter being reversible by thyroid administration (Hennes, Wajchenberg and Ulhoa Cintra, 1960; Bayliss and Van 't Hoff, 1969; Edwards, Forsyth and Besser, 1971). Furthermore, in a woman with primary hypothyroidism who developed amenorrhea and galactorrhea during oral contraceptive therapy, prolactin levels, as assessed biologically, were elevated and then fell markedly following thyroxine treatment (Edwards et al, 1971; Forsyth et al, 1971). These data strongly suggested that the secretion of prolactin and TSH might be interrelated.
Moreover, this hypothesis is supported by the fact that in hypothyroid subjects the responses of both TSH and prolactin to the administration of TRH are similarly increased (Bowers et al., 1971; Jacobs et al., 1971).

However, abundant data do not confirm such a close interrelationship between TSH and prolactin. Thus, insulin-induced hypoglycaemia usually causes prolactin but not TSH release (Copinschi et al., 1972a). No relationship was found between the circadian rhythm of TSH and prolactin, which were in some cases completely dissociated in time (Vanhaeest et al., 1973).

In patients with diseases of the thyroid, such as primary hypothyroidism, hyperthyroidism, athyreosis and asymptomatic thyroiditis, basal levels of TSH and prolactin could not be correlated; indeed, only the athyreotic patients had significantly elevated basal prolactin levels together with high TSH values (L’Hermite et al., 1972c). In addition, but in contradistinction to the work of Bowers et al. (1971), the prolactin response to TRH was not abolished in hyperthyroid patients (L’Hermite et al., 1972c), and was not changed by pretreatment with tri-iodothyronine in euthyroid subjects (L’Hermite et al., 1973). These data are in good agreement with those reported in experiments on animals. Thus, in rats thyroid hormones promote prolactin secretion (Nicoll and Meites, 1963; Chen and Meites, 1969), and hypothalamic TRH is unaltered by thyroxine treatment (Sinha and Meites, 1965), while the existence of a positive feedback of thyroxine on the hypothalamic production of TRH has been suggested by the finding of increased TRH-synthetase activity in hypothalamic extracts from rats treated with thyroxine for four weeks (Reichlin et al., 1972). However, Guillemin (1972) has reported that thyroid hormones suppress the response of prolactin to TRH in cultures of rat pituitary tissue.

**HYPOTHALAMIC–PITUITARY DISEASES AND TUMOURS**

Prolactin-secreting pituitary chromophobe adenomas have been shown to cause syndromes characterised by amenorrhoea and galactorrhoea (Canfield and Bates, 1965; Pasteels 1967; Takatani et al., 1967; Peake et al., 1969; Friesen et al., 1972c; Naar et al., 1972; Turkington, 1972c). Other pituitary or suprasellar tumours may be associated with abnormally high prolactin levels, as is the case with craniopharyngiomas (Kleinberg and Frantz, 1971; Jacobs et al., 1972; Turkington, 1972c), ectopic pinealomas (Canfield and Bates, 1965; Turkington, 1972c), and Nelson’s syndrome (Jacobs et al., 1972; Turkington, 1972c; Copinschi, Robyn and L’Hermite, 1973). It is likely that these elevated prolactin levels result from an interruption of the pituitary portal venous system causing a decrease or suppression of the normal inhibitory control of prolactin secretion by the hypothalamus.

A similar mechanism is likely to be responsible for the persistence of detectable and even elevated prolactin levels after excision of a craniopharyngioma (Hwang et al., 1971) or of pituitary adenomas (Friesen et al., 1972c). It could also be the explanation for the occasional association of galactorrhoea and elevated prolactin levels with acromegaly (Frantz and Kleinberg, 1970; Hwang et al., 1971; L’Hermite et al., 1972b). An alternative hypothesis would be that there is an overproduction of both HGH and
prolactin in some acromegalics. Anyhow, it has now been demonstrated that pituitary-stalk section usually converts the human pituitary gland into a primarily prolactin-secreting organ (Turkington, Underwood and Van Wyk, 1971).

PROLACTIN AND HUMAN BREAST CANCER

The dependence of experimentally induced rat mammary cancer on prolactin is a well-documented fact; it has even been proposed as a model for man (Pearson et al, 1969). In the human, however, the available data involve apparent contradictions (Heuson, 1973). Surgical hypophysectomy, which is thought to abolish prolactin secretion, has been regarded as the most effective means of inducing tumour regression (Hayward et al, 1970), while the administration of ovine prolactin has been stated to increase breast cancer growth (McAllister and Welbourn, 1962). However, pituitary-stalk section, usually resulting in increased prolactin secretion (Turkington, Underwood and Van Wyk, 1971), can also produce regression in breast cancer patients (Elahi and Eckles, 1959).

Murray, Mozaffarian and Pearson (1972) found higher serum prolactin levels in postmenopausal women with metastatic breast cancer than in controls, but this has not been confirmed by others (Forrest, 1972; Boynt et al, 1973; L'Hermite, Heuson and Robyn, 1973). Administration of high dosages of oestrogens represents an effective means of treating some cases of advanced breast cancer; however, such treatment consistently caused an elevation in blood prolactin levels (L'Hermite, Heuson and Robyn, 1973). On the other hand, Heuson (1972) has reported in a preliminary clinical trial, that treatment with the drug CB-154 (known to inhibit human prolactin secretion) failed to induce a significant number of objective remissions in patients with advanced breast cancer.

The problem of the possible role of prolactin in carcinogenesis and the growth of breast cancer remains unclear and requires further investigation. However, parameters other than prolactin secretion will have to be taken into account. Thus Maas et al (1972) reported an association between the presence of receptors for oestrogens in the tumour tissue and remissions after regimes involving endocrine treatment. Salih et al (1972) have shown in vitro prolactin dependence in 32 per cent of human breast cancer patients studied, and therefore proposed to treat metastatic tumours on the basis on in vitro findings taken together with actual blood prolactin measurements.

CLINICAL PRACTICE

The newly described biological methods of assay appear to be sufficiently sensitive to measure increased human prolactin in small aliquots of blood; however they do not usually detect normal basal levels. The major point against their use in clinical practice lies in the fact that they do not permit the handling of numerous samples.

The radioimmunological procedures, on the other hand, are more sensitive, quite specific, precise and reproducible, and permit the handling of a great
number of samples in a short time. However, one must not forget that discrepancies can exist between biological and immunological activities. Although at present the radioimmunoassay represents the technique of choice, bioassay should (or could) still be used when unexpected results occur or when adequate facilities are not available for the handling of radioactive isotopes. At the time of writing, the availability of radioimmunoassays for prolactin is very limited because of shortage of supplies of reagents and of standards. It is, however, probable that this difficulty will be overcome in the near future, thus making it possible to conduct radioimmunoassays for prolactin on a wide, and even on a commercial scale.

In order to investigate prolactin secretion clinically, I suggest that the pharmacodynamic tests described in Table 5 should be used rather than depending on single isolated blood estimates. If this cannot be done, great care must be taken with respect to the conditions under which the isolated sample is collected. The best procedure is to insert an indwelling needle or plastic catheter at least 30 minutes before taking the blood sample; the latter should be taken between 10 a.m. and 12 noon.

It is obvious from the review of our present knowledge of the physiology and pathology of prolactin in the human that single determinations of this hormone will be of little assistance to the clinician. Thus, the finding of an elevated prolactin level must lead him to ask about the administration of drugs and to rule out the existence of a neoplasm inside or near to the sella turcica. Since galactorrhoea along with elevated prolactin levels can occur and can last for many years before a clinical diagnosis of pituitary tumour can be made, one might suspect that some prolactin-producing adenomas do not represent the primary condition but are a secondary consequence of hypothalamic disease. Thus, it is important:

1. If galactorrhoea and/or elevated prolactin levels could be attributable to drug therapy, to test the effect of withdrawal of the drug on the patient’s symptomatology and on the prolactin levels.

2. In any case of persistent galactorrhoea following cessation of drug administration or in any case of elevated prolactin levels not related to drug therapy, to perform (a) all investigations designed to reveal the presence or absence of a pituitary tumour (e.g. visual field examination, x-rays of skull with special reference to the sella turcica, pneumoencephalography, etc.); (b) clinical and biological investigation of the other types of endocrine function (e.g. thyroid); (c) dynamic tests listed in Table 5 in order to provide an assessment of the activity of the hypothalamo-pituitary axis in relation to prolactin secretion.

For example, tests with L-Dopa and CB-154 can reveal that an overproduction of prolactin is completely autonomous (Copinschi et al, 1972b) or is only partially controlled by the hypothalamus (Turkington, 1972d), thus suggesting the existence of a pituitary prolactin-producing adenoma rather than hyperplasia of the prolactin-secreting cells of the pituitary due to the hypothalamic dysfunction. Thus these tests will also give important information leading to the choice of the appropriate treatment. Stimulation tests will provide information concerning the effectiveness of hypophysectomy (Friesen et al, 1972c), and the presence or absence of functional pituitary
<table>
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<tr>
<th>Drug</th>
<th>Dose</th>
<th>Administration</th>
<th>Recommended sampling times&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Maximal action</th>
<th>Preferential level of action</th>
</tr>
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<tr>
<td>Stimulation</td>
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<tr>
<td>Insulin</td>
<td>0.2 U/kg</td>
<td>I.V.</td>
<td>-30; 0; +30; +60; +120; +240 min</td>
<td>+60 min</td>
<td>Hypothalamus</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>25 mg</td>
<td>I.M.</td>
<td>-30; 0; +30; +60; +120; +360 min</td>
<td>+1--2 h</td>
<td>Hypothalamus</td>
</tr>
<tr>
<td>Synthetic TRH</td>
<td>200 μg</td>
<td>I.V.</td>
<td>-30; 0; +15; +30; +60; +120 min</td>
<td>+15--30 min</td>
<td>Pituitary</td>
</tr>
<tr>
<td>Inhibition</td>
<td></td>
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<tr>
<td>L-Dopa</td>
<td>500 mg</td>
<td>per os</td>
<td>-30; 0; +30; +60; +90; +120; +180; +300 min</td>
<td>+90--120 min</td>
<td>Hypothalamus</td>
</tr>
<tr>
<td>2-Br-α-Ergocryptine</td>
<td>2.5 mg</td>
<td>per os</td>
<td>-30; 0 min; +2; +4; +10; +24 h</td>
<td>+4--24 h</td>
<td>Pituitary</td>
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<sup>a</sup>To be performed through an indwelling intravenous needle or plastic catheter.
tissue in cases of hypopituitarism, thus helping to locate the precise site of origin of such cells (Foley et al., 1972; Kaplan et al., 1972; Crabbe et al., 1973). The TRH test can sometimes be helpful in cases of prolactin overproduction. Thus Yen (1972) reported preliminary data indicating that the prolactin release was increased in patients with amenorrhea and galactorrhea resulting from pituitary adenomas. Using the chlorpromazine test, Turkington (1972b) described an isolated deficiency of prolactin secretion in two patients with failure of lactation. In such a case, direct stimulation of the pituitary by TRH should be performed in order to locate the cause of the defect and possibly to provide appropriate treatment.

It seems obvious that the dynamic tests will be as helpful for prolactin as has been the TRH test as an index of TSH secretion (Schalch et al., 1972). Such tests will be used to characterise the location and extent of lesions or diseases of the hypothalamo–pituitary axis and to provide a pointer to appropriate therapy.

As our knowledge of the physiology and pathology of prolactin grows, tests for this hormone might become of increasing clinical value in the following situations:
1. Male and female sterility.
2. Growth retardation and delayed onset of puberty.
3. Fetal growth.
4. Renal diseases, including pre-eclamptic toxemia.
5. Metabolic conditions, e.g. obesity.
6. Mammary disease in all its forms.

CONCLUSIONS

Although various clinical situations and experimental data were in favour of the existence in the human of prolactin as a pituitary hormone distinct from growth hormone, its presence remained contested until the recent in vitro demonstration of its biosynthesis, thereafter followed by its purification and isolation. Amongst animal species, prolactin exhibits many diversified biological effects, of which 84 have been described. These can be classified into five groups:
1. Actions related to reproduction.
2. Actions affecting osmoregulation.
3. Actions promoting growth and growth hormone-like metabolic effects.
4. Actions on intertegumentary structures.
5. Synergistic actions with steroids.

The common denominator of the effects of such a versatile hormone could be the 'conditioning' of tissues and organs to the action of other endocrine factors. Some or perhaps most of these biological actions could be physiological in the human, but such a suggestion awaits further investigation and requires a wide availability of purified human prolactin for metabolic studies. At the present time recently developed sensitive bioassay and radioimmunoassay procedures allow for the detailed study of the physiopathology of prolactin in man.
Prolactin is detectable in blood from normal adults and children. It was found to be elevated in pregnant women and neonates and in most subjects showing amenorrhoea and galactorrhoea. The following situations produced transitory rises of blood prolactin levels: suckling and breast manipulation, exercise, proctoscopy and gastroscopy, sexual intercourse, psychic stress, surgery, insulin-induced hypoglycaemia, treatment with a number of psychotropic drugs, and synthetic TRH administration. A circadian rhythm of serum prolactin concentrations has been shown to be present. Oestrogens appeared to be less marked stimulators of prolactin secretion in man than in animals, although it has been shown that serum prolactin levels followed a pattern similar to that of endogenous oestrogens during the normal ovulatory menstrual cycle. However, gynaecomastia was not usually associated with abnormally high prolactin levels. Although synthetic TRH produced release of prolactin into the blood, correlations between thyroid-stimulating hormone (TSH) and prolactin secretion were slight.

The study of the hypothalamo–pituitary axis for prolactin secretion requires dynamic tests which stimulate and inhibit the hormone. These tests are of major importance for the clinician in order to evaluate any disturbance of the axis. The measurement of prolactin secretion, together with a study of oestrogen receptors and of effective hormonal dependence in malignant mammary tissue, could be helpful in the management of breast cancer in human subjects. In addition to these direct clinical applications of prolactin assays, it is likely that many indications for estimations of the hormone will become apparent in the future when knowledge of the activities of prolactin in man becomes more precise. At present, prolactin assays remain of limited clinical value and are mainly used for research purposes.

NOTES ADDED IN PROOF

RADIORECEPTOR ASSAY. Shiu, Kelly and Friesen (1973) have recently developed a sensitive radioreceptor assay for lactogenic hormones: they are quantitated by their ability to inhibit the binding of $^{125}I$-prolactin to membrane receptors prepared from rabbit mammary glands. This method is much closer to bioassay than radioimmunoassay and provides a convenient assay for any hormone (regardless of its animal species) with lactogenic activity. As a corollary, this assay does not discriminate prolactin from other hormones exhibiting lactogenic activity (growth hormone and chorionic somatomamotrophin) and therefore cannot be considered as specific for prolactin measurement.

AVAILABILITY OF HUMAN PROLACTIN RADIOIMMUNOASSAY. The British Medical Research Council (Division of Biological Standards, National Institute for Medical Research, Hampstead Laboratories, Holly Hill, London, N.W.3) supplies very limited amounts of Research Standard A for Human Prolactin, 71/222; it is intended to serve the purpose of an international common reference standard and must thus be compared by each investigator to his own laboratory standard, this latter to be used in each assay (Cotes, 1973). The National Institute of Arthritis, Metabolism and Digestive Diseases (U.S.A.) distributes, to qualified investigators having previous experience in
radioimmunoassays, a human prolactin radioimmunoassay kit V.L.S. No. 1 (address requests to the Hormone Distribution Officer, National Institutes of Health, Building 31, Room 9A-47, Bethesda, Maryland, 20014, U.S.A.). The use of its reagents (antiserum to human pituitary prolactin and purified human pituitary prolactin for labelling) in a radioimmunoassay for human prolactin has been described by Sinha et al (1973).

CLINICAL PRACTICE. While galactorrhoea does not appear to be invariably associated with prolactin hypersecretion, there has been a roughly 30 per cent prevalence of hyperprolactinaemia in a group of patients with 'non-functional' pituitary tumours (Jacobs and Daughaday, 1973). Since these clinically non-functional pituitary tumours occurred much more frequently than those associated with acromegaly, galactorrhoea or Cushing's syndrome, the commonest endocrine manifestation of pituitary tumours is thus hyperprolactinaemia, for which a search should be made. A serum prolactin higher than 500 ng/ml is more likely indicative of the existence of pituitary tumour rather than functional hyperprolactinaemia (Jacobs and Daughaday, 1973). The use of the pharmacodynamic tests available for the investigation of the hypothalamopituitary axis in relation to prolactin secretion proved of less absolute value than anticipated in attributing hyperprolactinaemia to a pituitary tumour versus a functional disturbance (Jacobs and Daughaday, 1973; L'Hermite and Robyn, 1973). However, they were found to be very useful in investigation and classification of the patients otherwise considered to present with panhypopituitarism (Tolis, Goldstein and Friesen, 1973; L'Hermite and Robyn, 1973).

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