Measurement of hormone concentrations in the blood

Most hormones are present in the blood in extremely minute quantities; some concentrations are as low as one billionth of a milligram (1 picogram) per milliliter. Therefore, it was very difficult to measure these concentrations by the usual chemical means. An extremely sensitive method, however, was developed about 40 years ago that revolutionized the measurement of hormones, their precursors, and their metabolic end products. This method is called radioimmunoassay.

Radioimmunoassay

The method of performing radioimmunoassay is as follows. First, an antibody that is highly specific for the hormone to be measured is produced. Second, a small quantity of this antibody is (1) mixed with a quantity of fluid from the animal containing the hormone to be measured and (2) mixed simultaneously with an appropriate amount of purified standard hormone that has been tagged with a radioactive isotope. However, one specific condition must be met: There must be too little antibody to bind completely both the radioactively tagged hormone and the hormone in the fluid to be assayed. Therefore, the natural hormone in the assay fluid and the radioactive standard hormone compete for the binding sites of the antibody. In the process of competing, the quantity of each of the two hormones, the natural and the radioactive, that binds is proportional to its concentration in the assay fluid. Third, after binding has reached equilibrium, the antibody-hormone complex is separated from the remainder of the solution, and the quantity of radioactive...
hormone bound in this complex is measured by radioactive counting techniques. If a large amount of radioactive hormone has bound with the antibody, it is clear that there was only a small amount of natural hormone to compete with the radioactive hormone, and therefore the concentration of the natural hormone in the assayed fluid was small. Conversely, if only a small amount of radioactive hormone has bound, it is clear that there was a large amount of natural hormone to compete for the binding sites. Fourth, to make the assay highly quantitative, the radioimmunoassay procedure is also performed for “standard” solutions of untagged hormone at several concentration levels.

Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assays (ELISAs) can be used to measure almost any protein, including hormones. This test combines the specificity of antibodies with the sensitivity of simple enzyme assays. Figure 74–10 shows the basic elements of this method, which is often performed on plastic plates that each have 96 small wells. Each well is coated with an antibody (AB1) that is specific for the hormone being assayed. Samples or standards are added to each of the wells, followed by a second antibody (AB2) that is also specific for the hormone but binds to a different site of the hormone molecule. A third antibody (AB3) is added that recognizes AB2 and is coupled to an enzyme that converts a suitable substrate to a product that can be easily detected by colorimetric or fluorescent optical methods. Because each molecule of enzyme catalyzes the formation of many thousands of product molecules, even very small amounts of hormone molecules can be detected. In contrast to competitive radioimmunoassay methods, ELISA methods use excess antibodies so that all hormone molecules are captured in antibody-hormone complexes. Therefore, the
amount of hormone present in the sample or in the standard is proportional to
the amount of product formed. The ELISA method has become widely used in
clinical laboratories because (1) it does not employ radioactive isotopes, (2)
much of the assay can be automated using 96-well plates, and (3) it has proved
to be a cost-effective and accurate method for assessing hormone levels.

EXPLORATION OF THYROID GLAND

Thyroid gland secreted thyroid hormones, which are synthetized in follicular colloid through
iodination and condensation of tyrosine molecules binding with thyroglobulin.

1. DIRECT METHODS OF EXPLORATION
1.1. PLASMA DETERMINATION. We can determine through RIA method the following:

Plasma concentration of total thyroid hormones, with the normal value:
- Total T₃ (TT₃): 77–135 ng/dl
- Total T₄ (TT₄): 5.4–11.7 μg/dl

Plasma concentration of free thyroid hormones, with the normal value:
- FT₃: 2.4–4.2 pg/ml
- FT₄: 0.8–1.7 ng/dl

Plasma TSH, with the normal value between 0.34–4.25 μIU/ml

Plasma iodine, with the normal value: 4-8 μg/dl

Plasma thyroglobulin (Tg), with the normal value: 0.5–53 ng/ml, which is increased in
thyroid cancer.

1.2. THYROID IODIDE UPTAKE TESTS (I¹³¹ fix)

We administered 10-30 μCi I¹³¹ and we measured thyroid uptake at 2-6-24 h. Radioactive
iodine thyroid uptake is 50-70% after 24 hours. The following graphs present the distribution
of radioactive iodine, in plasma and thyroid tissue, in normal, hyper- and hypothyroid
individuals (Fig.1 A, B, C).

![Graphs showing thyroid iodide uptake](image)

**Fig. 1.** Distribution of radioactive iodine, in plasma and thyroid tissue

1.3. DYNAMIC TESTS

TSH stimulation test – is used for differential diagnosis of myxedema (primary,
secondary). Normally the test is positive (+) – increase TT₄ and iodine uptake.

Pathological variations
- If the test is negative (-) _ primary myxedema.
- If the test is very positive (+++) _ secondary myxedema.

TRH stimulation test – normally determines an increase in T₄ and iodine uptake, by TSH stimulation

T₃ suppression test – normally determines a decrease in T₄ and iodine uptake. Normally the test is positive.

Pathological variations: Negative (-) tests typical for hyperfunctional autonomy in hyperthyroidism.

1.4. IMAGING THYROID GLAND EXPLORATION for thyroid gland hypertrophy diagnosis (thyroid goiter).

Simple radiography of neck. Is used in disorders caused by compression, calcifications;

Ultrasound examination of the thyroid. Allowed thyroid volume measurement; thyroid relation with cervical anatomic structures; nodular thyroid changing; thyroid function appreciation;

Thyroid scintiscanning. We administrated radioactive iodine 50 µCi and we recorded thyroid radiation. We obtained data about gland localization and dimension; it shows the uniform or uneven attachment of radioactive iodine in thyroid; intensive uptake (warm areas) or absence of uptake (cold areas).

Computed tomography is another exploration method of the thyroid gland.

1.5. IMMUNOLOGICAL EXPLORATION

Thyroid autoimmunity markers. We can determinate circulating antibodies against thyroglobulin, thyroid peroxidase, and anti TSH receiver, which appear in thyroid autoimmune diseases (Hashimoto thyroiditis).

1.6. CITOLOGIC AND ANATOMO-PATHOLOGICAL EXPLORATION allows differential diagnosis between benign or malign lesions. It is represented by:

- Cytology examination thought aspiration biopsy
- Extemporaneously histopathology examination on ice
- Histopathology examination in paraffin
- Immunohistochemical methods

2. INDIRECT METHODS OF EXPLORATION

There are tests that examine the response of tissues and receiver structures at the action of thyroid hormones.

- Plasma cholesterol, with the normal values: 140–190 mg/dl, which is increased in
hypothyroidism and decreased in hyperthyroidism.

- **Blood glucose**, with the normal values: 70-110 mg/dl, which is increased in hyperthyroidism and decreased in hypothyroidism
- **Plasma muscles enzymes**, with the normal values:
  - LDH (lactic deshydrogenase) = 100–190 IU/l
  - myoglobin – man = 19–92 μg/l, female = 12–76 μg/l.
They have increased values in hyperthyroidism.

**Hematological determination.** We determine complete blood count and leukocyte formula; in hyperthyroidism polycytemia, neutropenia, eosinophilia and thrombocytopenia are described.

**Achilian reflexogramme.** It represents graphic recording of achilian reflex. We measure the distance from the percussion artifact to the point of hemirelaxation, which is determined by the following method: draw the perpendicular from the highest point of the curve on the baseline. We measure half of this perpendicular and we unit it with a horizontal line to the downward slope of the curve. The distance obtained is converted into time, knowing the speed of the paper (60 mm/sec)

**Normal value:** 260-340 msec.

**Pathological variations.** The duration of achilian reflexogramme is short in hyperthyroidism and longer in hypothyroidism.

**EKG.** In hyperthyroid patients: tachycardia, extrasystoles and other forms of cardiac arrhythmias; in hypothyroid patients: bradycardia.

**Cardiac ultrasound.** Systolic times and diastolic ventricular function (assessed by the isovolumetric relaxation time) are short in hyperthyroidism and longer in hypothyroidism;

**Bone and dental radiography** present the following aspects:
- in infantile hypothyroidism late skeletal maturation, dental eruption and calcification can be observed
- in the case of a hyperthyroid child bone maturation is accelerated
- in the case of a hypothyroid adult there is a bone hyperdensity
- in the case of a hyperthyroid adult osteoporosis occurs.

**Determined basal metabolic rate (BMR)** varies between 90% and 115% of ideal values and it is expressed through the limits of normal, respectively -10% and +15%. In hyperthyroidism BMR increases and it decreases in hypothyroidism.

**THYROID HORMONE SECRETION DISORDERS**

3.1. Adult thyroid hypofunction may be:
- **primary** – thyroid or iodine deficiency (endemic goiter)
- **secondary** – pituitary with TSH secretory insufficiency
- **tertiary** – hypothalamic with TRH secretory impairment
Hypothyroidism in adults is manifested by decreased basal metabolism, trophic skin disorders (dry skin and thickened - myxedema), fattening trend, physical and mental slowness, sleepiness, cold intolerance, and bradycardia.

3.2. **Congenital hypofunction** – congenital myxedema or thyroid dwarfism characterized by a slowed growth, with disturbances in the ossification process, dentition development disorders, nervous system development disorder (goiter imbecility).

3.3. **Adult thyroid hyperfunction** may be primary, secondary or tertiary and is manifested by exophthalmia goiter (Basedow–Graves goiter), increases in the metabolic rate, warm and wet skin, weight loss, tremors, insomnia, hyperemotivity, heat intolerance, tachycardia.

**EXPLORATION OF PARATHYROID GLANDS**

Parathormone (PTH) is the most important hypercalcium hormone, secreted by the main cells
of parathyroids. When the extracellular fluid concentration of calcium ions falls below normal, the nervous system becomes progressively more excitable, because this causes increased neuronal membrane permeability to sodium ions, allowing easy initiation of action potentials. At plasma calcium ion concentrations about 50 per cent below normal, the peripheral nerve fibers become so excitable that they begin to discharge spontaneously, initiating trains of nerve impulses that pass to the peripheral skeletal muscles to elicit tetanic muscle contraction. Consequently, hypocalcemia causes tetany. Tetany ordinarily occurs when the blood concentration of calcium falls from its normal level of 9.4 mg/dl to about 6 mg/dl, which is only 35 per cent below the normal calcium concentration, and it is usually lethal at about 4 mg/dl.

When the level of calcium in the body fluids rises above normal, the nervous system becomes depressed and reflex activities of the central nervous system are sluggish. Also, increased calcium ion concentration decreases the QT interval of the heart and causes lack of appetite and constipation, probably because of depressed contractility of the muscle walls of the gastrointestinal tract. These depressive effects begin to appear when the blood level of calcium rises above about 12 mg/dl, and they can become marked as the calcium level rises above 15 mg/dl. When the level of calcium rises above about 17 mg/dl in the blood, calcium phosphate crystals are likely to precipitate throughout the body.

1. CLINICAL INVESTIGATIONS
1. Facial sign (Chvostek's sign). It consists in percussion of the facial nerve, at the halfway between tragus and labial corner. Normally it is negative, it is positive only in the case of neuro-muscular hyperexcitability (hypoparathyroidism) (causes uni- or bilateral contraction of lip orbicularis muscle, eyelids);
1. Trousseau's sign. It consists in moderate compression with the tensiometer cuff or with the tourniquet for 3 min. Normally it is negative, it is positive only in the case of neuromuscular hyperexcitability (hypoparathyroidism) (causes the spasm of the metacarpophalangeal joint or "obstetrician hand ").
2. PARACLINICAL INVESTIGATIONS
Show the neuro-muscular hyperexcitability signs that appear in hypoparathyroidism.

1. EMG: repetitive electrical activity of the type doublets, triplets, multiplets biphasic potentials that occur in resting muscle, spontaneously or after activation through hyperpnoea or through arm compression with tourniquet (Alajouanine method).

2. EKG: ST segment and QT interval elongation, shortened T wave duration.

3. EEG: diffuse irritative route.

3. PLASMA DETERMINATIONS
Determine parameters are schematized in Table I.

### Table I. Main plasma levels and pathological changes seen in the parathyroid glands

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Normal values</th>
<th>Primary hyperparathyroidism</th>
<th>Primary hypoparathyroidism</th>
<th>Pseudo-hypoparathyroidism (renal diseases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium</td>
<td>9-11 mg%</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Serum phosphorus</td>
<td>3-4.5 mg%</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Urine calcium</td>
<td>100-300 mg/24 h</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Urine phosphorus</td>
<td>400-1300mg/24h</td>
<td>↑</td>
<td>↓</td>
<td>N</td>
</tr>
<tr>
<td>Serum parathyroid hormone</td>
<td>8-51 pg/ml</td>
<td>↑</td>
<td>N or ↓</td>
<td>↑</td>
</tr>
</tbody>
</table>

4. PROVOKED TESTS
1. Hypocalcemia provoked test. We administrate EDTA-Na i.v. and then we determine the serum calcium level in dynamics. After 2 hours the serum calcium level decreases with 25% towards initial value and after 12 hours it is over 90%. In parathyroid insufficiency the serum calcium level recovery takes longer than 12 hours, and this recovery is under 90% of the initial value;

2. Hypercalcemia provoked test. We administrate calcium i.v. and we follow up the serum phosphorus level. Normally calcium administration inhibits PTH secretion and determines increase of the serum phosphorus level and decrease of the urine phosphorus level;
3. **PTH test.** It investigates renal receiver response at PTH action. We determine the urine phosphorus level/hour and then we administrate 200 U PTH. Then we follow up for 3-4 hours the urine phosphorus level. At healthy subjects the urine phosphorus level increases 5-6 times. In hypoparathyroidism it increases 10 times, and in hyperparathyroidism it increases 2 times.

5. **OSTEOITIC ACTIVITY MARKERS**

1. **Serum alkaline phosphatase.**
   Serum alkaline phosphatase is an enzyme found in active osteoblasts, being involved in bone mineralisation.
   
   **Normal values:** 30-120 U/L.
   **Pathological variations:** increased values in patients with hyperparathyroidism, osteolytic metastases, in Paget's disease, rickets.

2. **Urine hydroxiproline** is produced by hydroxilation of the amino acid proline.
   
   **Normal values:** 38–500 μmol/day.
   **Pathological variations:** it is increased in hyperparathyroidism and assures differential diagnosis with idiopathic calciuria where its value is normal.

**PTH SECRETION DISORDERS**

1. **Hypoparathyroidism** - is characterized by hypocalcaemia and neuro-muscular and cortical hyperexcitability, which is called tetany (spasms of the smooth and striated muscle) cardiovascular disorders (palpitations, hypotension) and trophic disorders (skin, teeth).

2. **Hyperparathyroidism** - is characterized by hypocalcaemia due to mobilization of calcium from bones (demineralization - fibrocystic osteitis) followed by the deposit of excess calcium in soft tissues (kidney stones).

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**Pregnancy Test**

1. **Indications**
   1. Pregnancy diagnosis and monitoring
   2. Tumor monitoring: nonseminomatous germ cell tumor monitoring, hydatiform mole diagnosis and monitoring, **choriocarcinoma** diagnosis and monitoring

2. **Physiology**
   1. HCG is a glycoprotein hormone with subunits a and b
      1. Composed of 65% polypeptides by molecular weight
      2. Composed of 35% large sugar side chains (8 chains)
         1. Four are N-Linked (2 each on alpha and beta)
         2. Four are O-Linked (all 4 are on beta subunit)
   3. Forms of HCG found in blood and urine
      1. Intact HCG with alpha and beta subunits
      2. Nicked HCG and nicked free beta subunit
      3. Free beta subunit and free alpha subunit
      4. Hyperglycosylated free beta and free alpha subunits
      5. Beta core fragment (present only in urine)
   2. HCG shares the same alpha subunit with other hormones: **luteinizing hormone** (LH), **Thyroid Stimulating Hormone** (TSH)
   3. Urine and Blood HCG tests are specific for beta subunit
   4. Serum half life of HCG: 24 to 36 hours

3. **Interpretation:** Levels of bHCG in pregnancy
   1. Estimation in normal pregnancy for weeks 4 to 8
      1. Anticipate HCG doubling every 48 to 72 hours
   2. Chart of corresponding gestational age
1. Day 23 (3.3 weeks): 100 mIU/ml (correlates with blastocyst implantation)
2. Day 28 (4.0 weeks): 250 mIU/ml
3. Day 35 (5.0 weeks): 1000 mIU/ml
   1. bHCG 1800: **Gestational Sac** on Transvaginal U/S
   2. bHCG 3500: **Gestational Sac** on Transabdominal U/S
4. Day 42 (6.0 weeks): 4000 mIU/ml
5. Day 49 (7.0 weeks): 15000 mIU/ml
   1. bHCG 20,000: 5-10 mm **Embryo** with cardiac activity
6. Day 56 (8.0 weeks): 65000 mIU/ml
7. Decreases gradually after 8 weeks
8. Plateaus after 20 weeks

3. Ranges of bHCG over each Trimester
   1. First Trimester: 30,000 to 100,000 mIU/ml
   2. Second Trimester: 10,000 to 30,000 mIU/ml
   3. Third Trimester: 5,000 to 15,000 mIU/ml

4. Efficacy: Home Pregnancy Tests
   1. Variable efficacy after missed **Menses**
      1. Wide discrepancy between brands

5. Causes: Elevated bHCG in non-pregnant state
   1. HCG-producing tumors in women
      1. **Hydatidiform Mole** or Choriocarcinoma
   2. HCG-producing tumors in men
      1. HCG is usually undetectable in healthy males
      2. Nonseminomatous germ cell tumor (**Testicular Cancer**)
         1. Used with AFP for monitoring

6. Causes: False positive increased serum hCG
   1. Causes
      1. Heterophilic **Antibody** (most common)
      2. Human anti-mouse **Antibody** (HAMA)
      3. Nonspecific protein-binding hCG-like substances
      4. **Red Blood Cell** interference
      5. **Marijuana** use
      6. **Hypogonadism**
   2. Confirmation methods in non-pregnant conditions
      1. Qualitative urine hCG
      2. Serum hCG by different immunoassay method
      3. Serial dilutions of serum hCG sample