Successful laboratory tests for early pregnancy utilize the endogenous production and excretion of chorionic gonadotrophin (CG). There is wide variation in sensitivity and specificity of various biological and commercially available immunochemical methods for determining urinary CG levels. Normal values for immunochemically reactive CG during the first trimester of pregnancy are given.

Serial determinations of CG have diagnostic value in assessing various abnormal pregnancy states. An abnormally increased urinary output of CG is observed in some neoplasms—e.g., choriocarcinoma, and a rapidly rising CG level is strongly suggestive of molar pregnancy. Urinary CG levels falling below the normal range may indicate an ectopic pregnancy or an inevitable, incomplete, or "missed" abortion.

The immunochemical CG determination also offers a sensitive, simple, and convenient routine method for following patients who have been treated for hydatidiform mole or chorionepithelioma.

The folklore of ancient primitive tribes contains many references to devices for the accurate diagnosis of early pregnancy. No less strong today is the desire to diagnose pregnancy at the earliest possible moment.

Recently, the thalidomide tragedy has focused attention on the problem of drug therapy during the gestation period. Because many other drugs may have effects known and unknown on the fetus, it is apparent that any test which indicates early pregnancy (normal or abnormal) will be important from the medical as well as the sociological point of view.

It has long been known that the only successful pregnancy tests are those which utilize the endogenous production and excretion of chorionic gonadotrophin (CG). Pioneer studies on CG by Wide and colleagues (1, 2) have now made it practical to examine CG production as a placental function test during the first trimester of pregnancy in the same way that pregnanediol and estrogen determinations can be used as a measure of placental efficiency during the latter half of pregnancy.

From the Department of Laboratories, Royal Columbian Hospital, New Westminster, British Columbia, Canada.

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Human CG is a glycoprotein of molecular weight 25,000–30,000 (3). Relatively high concentrations of this protein (30–300 mg./day) are found in the blood and urine during the ninth week of pregnancy. At this period the CG level in the early morning urine may even be sufficient to give a positive indicator strip test\* for protein. However, the output of CG in very early pregnancy is so small that it cannot currently be measured by classical chemical analysis. For many years a low concentration of CG in urine could be assayed only by biological methods. These utilized the weight of the prostate, seminal vesicles, uteri, and ovaries of rats or mice after injection of urine preparations. Alternatively, depletion of ascorbic acid in the rat ovary was used as the response parameter. A review of the various bioassays for CG is given by Loraine (4). Advances in immunochemistry, especially the development of better immunization methods and of technics for purifying protein antigens, have now made available a superior means of measuring CG.

**Principle of the Immunochemical Method**

Interaction between CG and its specific antibody can be made visible in several ways. One method\* employs the agar diffusion technic. The “electroprecipitin” method of Watson and Whinfrey (5), in which the antigen-antibody reaction takes place during electrophoresis, is another approach. Recently, a radioimmunoassay for human CG has been described (6).

In contrast to these direct methods are the indirect agglutination inhibition methods. A typical example of the latter can be carried out on a microscope slide by mixing 1 volume of anti-CG (antiserum), 1 volume of urine, and 2 volumes of a suspension of CG-coated polystyrene latex particles. If the antiserum contains sufficient anti-CG to neutralize (for example) 0.1 μg. CG, then if the urine contains less than 0.1 μg. of CG, some antisera will remain available to cause agglutination of the latex-CG particles. On the other hand, if the urine contains more than 0.1 μg. of CG per volume, all of the anti-CG will be neutralized and no agglutination of the latex-CG particles will occur.

**Sensitivity of CG Assays**

Table 1 indicates the minimum levels of CG which could be detected by each of 4 biological and 8 immunochemical assays. The data shown were obtained by making tests with serial dilutions of the First and Second International Standards for human CG (7). It is clear that the

\*Albustix, Ames Co., Elkhart, Ind.
\*Immunoplate, Hyland Laboratories, Los Angeles, Calif.
Table 1. Sensitivity of Some Pregnancy Tests Based on CG Detection

<table>
<thead>
<tr>
<th>Urine used (ml.)</th>
<th>CG with prescribed urine vol. (I.U./ml.)</th>
<th>CG per vol. urine tested (µg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BIOLOGICAL TESTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse uterine reaction (Aschheim &amp; Zondek)</td>
<td>4 × 0.4</td>
<td>2–4</td>
</tr>
<tr>
<td>Rabbit ovulation (Friedman &amp; Lapham)</td>
<td>15</td>
<td>3–7</td>
</tr>
<tr>
<td>Rat ovarian hyperemia (Zondek, Sulman &amp; Black)</td>
<td>1</td>
<td>1–3</td>
</tr>
<tr>
<td>Toad spermiation (Galli-Mainini)</td>
<td>10</td>
<td>10–15</td>
</tr>
<tr>
<td><strong>HEMAGGLUTINATION INHIBITION TESTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepuerin (Burroughs Wellcome, 1964)</td>
<td>1.0 of 1:10</td>
<td>4</td>
</tr>
<tr>
<td>Pregnosticon (Organon, 1963)</td>
<td>0.1</td>
<td>1–2</td>
</tr>
<tr>
<td>UCG (Denver, London, 1963)</td>
<td>0.25 of 1:3</td>
<td>15</td>
</tr>
<tr>
<td>UCG (Denver, Montreal, 1965)</td>
<td>0.25 of 1:3</td>
<td>2</td>
</tr>
<tr>
<td><strong>LATEX CLUMPING INHIBITION TESTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy Tube Test (Ortho, 1963)</td>
<td>0.5</td>
<td>10–11</td>
</tr>
<tr>
<td>Gravindex (Ortho, 1964)</td>
<td>0.05</td>
<td>5–6</td>
</tr>
<tr>
<td>Brevindex (Ortho, 1965)</td>
<td>0.05</td>
<td>4–5</td>
</tr>
<tr>
<td>HCG Test (Hyland, 1965)</td>
<td>0.05</td>
<td>2–3</td>
</tr>
</tbody>
</table>

*One International Unit of human CG equals 0.42 µg.

The minimum amount of CG which can be detected will vary widely according to the type of test. In the biological tests, sensitivity to CG depends on the nature and strain of the animal as well as the volume of urine employed. For example, the toad (*Bufo marinus*) spermiation test is about 1/50 as sensitive as the rat ovarian hyperemia test.

The sensitivity of the immunochemical test depends on the volume of urine tested and, inversely to a degree, on the strength of the antisera. A twofold increase in sensitivity in 1 latex-agglutination inhibition test may be achieved simply by using 2 volumes of urine instead of 1 (8). Detection of smaller amounts of CG requires that the urine be concentrated. A tenfold concentration may be made by the kaolin-calcium phosphate method of Brown and Loraine (9). Another method (10) is to add a small amount of albumin to the urine, to precipitate and remove urine protein with sodium tungstate and sulfuric acid, and finally to dissolve the precipitate in a measured small volume of an alkaline buffer at pH 8.4.

In order to determine larger amounts of CG than the minimum detectable, tests are commonly made on a number of serial dilutions of urine in water. However, the results from this laboratory, reported
here, have mostly been obtained by direct application of the reaction to ultramicro volumes of fluid using pipets containing 1, 2, and 5 μl.

**Accuracy of CG Assays**

Attempts to assess "accuracy" of CG testing have been made by many workers. So-called "percentage accuracy" is as strongly influenced by the gestation period of the patient as it is by the sensitivity of the test. Any uncertainty about this accuracy will end when the actual CG output per day is considered in relation to the period of time after the last normal menstrual period.

If accuracy is defined as the nearness with which the analytical estimate approaches the true value for CG, then the situation closely resembles that existing for another protein hormone—insulin. Both CG and insulin assays show consistent discordances when immunochemically and biologically determined levels are compared. It seems too, that in common with some other proteins, CG can be chemically or physically degraded while still preserving immunological identity with intact CG. Such treatment as heating to 80°, storage in an acid medium, or enzymic action of amylase decreases the biological activity but does not appreciably affect the immunochemically reactive parts of the CG molecule (1, 10). A small loss of immunochemically reactive CG does, however, occur when the urine is stored at room temperature for more than a day. If there must be a delay, urine should be stored at below 5° and preferably frozen until the estimation can be undertaken.

**Specificity of CG Assay Methods**

Bioassay of CG may be affected by seasonal variation in the animal's response. Some biological procedures—e.g., the rat ovarian hyperemia test, give "false" positive reactions to metabolites of drugs such as promazine or to excessive amounts of estrogens which may be present in the urine tested. Another disadvantage of biological methods is the frequent need for detoxification of the urine when toxic substances are present in such amounts that the animals fail to respond to CG, or die.

The chemical CG assays do not suffer from these limitations and no drug, medication, or pathological urinary constituent has yet been shown to produce a detectable delay or acceleration of agglutination inhibition in the assay of CG. Nevertheless, it can readily be demonstrated that one or more of the common constituents of urine from both male and nonpregnant females inhibits both the chemical and the biological recoveries of CG from solution. A solution which contains 2 μg./ml. human CG in saline inhibits agglutination in a latex sys-
tem;* the same concentration in normal urine does not. There is thus no doubt that the present CG assays measure the excess of CG over the amount of CG that has been inactivated by a urinary constituent.

Biological testing systems for CG may respond to the natural estrogens, pituitary luteinizing hormone (LH, ICSH), and follicle-stimulating hormone (FSH). Immunological cross-reaction occurs between purified CG antiserum and human LH (1) but not human FSH† (10). In this laboratory, 1 commercially available human CG antiserum (Gravindex) has failed to detect LH in random untreated urine specimens collected from 57 postmenopausal women. After extraction giving rise to a tenfold concentration, urine from 2 of these subjects reacted with the CG antiserum. Apparently, the levels of pituitary gonadotrophins that are normally present in urine do not interfere with this latex agglutination inhibition technic for human CG determination.

Most immunochemical tests for CG detect between 1 and 4 I.U./ml. (Table 1) and the same tests also detect similar concentrations of the Second International Preparation of human menopausal gonadotrophin, containing a mixture of ICSH and FSH. The ICSH level in urine is normally 0.01-0.02 I.U./ml. HMG and rises to 0.25-1.5 I.U./ml. HMG in the postmenopausal woman (11). Conditions in which increased ICSH production and excretion may be confused with CG by the current clinical methods are (1) tumors of the anterior pituitary; (2) tumors of the testicle such as embryonal cell carcinoma and choriocarcinoma; (3) acute loss of functioning testicular tissue arising from a condition such as a scrotal abscess. Here, interference with the testosterone feed-back mechanism—a temporary depression of blood testosterone—may cause increased liberation of gonadotrophins from the pituitary (12); and (4) medication with certain psychotropic drugs such as chlorpromazine (13).

On the other hand, the hemagglutination tests occasionally suffer from reactions from nonspecific agglutination inhibitors (8); one substance which may cause difficulty is found in the urine of about 1 in 10 postmenopausal women (14). This interference may be due to the use of antiserum prepared from impure human CG.

**Urinary CG in Pregnancy**

*Normal Pregnancy*

CG has been found in serum and urine as early as Day 24 of pregnancy, dated from the beginning of the last menstrual period. Its

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*Gravindex, Ortho Pharmaceutical Corporation, Raritan, N. J.
†Human FSH was prepared and given to us by Dr. J. B. Brown, Royal Women's Hospital, Melbourne, Australia.
presence in urine at an even earlier date, 9 days after artificial insemination, has also been reported (4). However, detection in clinical practice requires that a level of 0.4–2.0 μg./ml. (1–5 I.U./ml.) be present, and this concentration is normally reached 10 days after the missed period or on about Day 38 of pregnancy.

We have found the concentration of CG in urine to vary widely with the time of collection. In women 50–60 days pregnant, the early (first) morning specimen contained 4–100 μg./ml., while most afternoon specimens from these women contained less than 4 μg./ml. The urine of 2 subjects was also tested on different occasions at intervals during the morning. No consistent pattern of excretion emerged, and it was concluded that an apparent fluctuation in CG output is best avoided by pooling specimens to provide a 24-hr. assay value.

In the eighth week of a normally progressing pregnancy, urinary excretion of CG rises very rapidly to reach a concentration of 30–70 mg. per day. During the eleventh and twelfth weeks there is a less rapid fall to a level which is usually below 30 mg. per day (Fig. 1). The fall in the

![Graph](image-url)  
**Fig. 1.** Urinary CG excretion (mg./24 hr.) in first trimester of pregnancy. High, low, and mean values in 390 normal pregnancies.
Vol. 12, No. 9, 1966

CHORIONIC GONADOTROPHIN

583

CG level may be delayed in a multiple pregnancy (1, 15). Output of CG in the first trimester has been studied by 3 groups of workers who have obtained similar but not identical results (Table 2). The considerably lower values obtained by Fairweather and Loraine (16) are in line with the finding of Wide (1) that a CG level estimated biologically is about 0.5–0.8 of the level obtained immunochemically.

Our mean values for 390 urinary CG analyses during the first trimester are shown in Fig. 1; they were obtained over a period of 3 years with Gravindex, a latex agglutination inhibition technic (8). The levels were generally midway between the values found by Wide (1), with a hemagglutination method, and those recently reported by Noto et al. (17). As can be seen from Table 2, the scatter of values about the mean at any given period of gestation is considerably reduced when the analyses are performed on 24-hr. urine specimens rather than on early morning or random urine samples.

Abnormal Pregnancy

Extrauterine Pregnancy

Wide (1) studied 6 women suspected of having an ectopic pregnancy, a condition subsequently confirmed by laparotomy. Five had CG outputs significantly below the normal level. In our series of 7 similar patients, there were 3 with persistently subnormal levels and 4 with normal but falling CG outputs.

Table 2. Urinary CG Output in Normal Early Pregnancy

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Time of collection</th>
<th>No. of tests</th>
<th>Days after LNMP (μg./ml., mean and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50–60</td>
</tr>
<tr>
<td>Wide (1)</td>
<td>Hemagglutination inhibition</td>
<td>Early morning</td>
<td>229</td>
<td>21(4–80)</td>
</tr>
<tr>
<td>Fairweather and Loraine (16)</td>
<td>Rat prostate enlargement</td>
<td>24-hr.</td>
<td>53</td>
<td>10(5–20)</td>
</tr>
<tr>
<td>Noto et al. (17)</td>
<td>Latex agglutination inhibition Random midnight</td>
<td>295</td>
<td></td>
<td>10(2–65)</td>
</tr>
<tr>
<td>Present work</td>
<td>Latex agglutination inhibition Early morning</td>
<td>520</td>
<td></td>
<td>19(4–100)</td>
</tr>
<tr>
<td>Present work</td>
<td>Latex agglutination inhibition 24-hr.</td>
<td>18</td>
<td></td>
<td>17(2–45)</td>
</tr>
</tbody>
</table>
Abortion

Weekly urinary CG assays were made on 89 women in their first trimester of pregnancy who were hospitalized for threatened abortion or because of previous abortions. Thirty-five aborted spontaneously and of these, 32 had subnormal CG excretion for at least 1 week before the abortion. Three had persistently normal CG levels. No special distinguishing feature was observed in the CG excretion pattern of the habitual aborters. Our experience resembles that of other investigators using different methods (14, 15); it can therefore be concluded that patients who show evidence of a poorly functioning cytotrophoblast usually but not invariably show subnormal CG production.

In several of the cases, the rat ovarian hyperemia assay was run in parallel with the immunochemical analyses (8). Owing to their different sensitivities the former assays revealed CG present 36–48 hr. after fetal death in utero, while the 3 most sensitive immunochemical methods (Table 1) detected CG up to 4–5 days after fetal death.

Neoplasms

The urinary excretion of CG by 6 women with a hydatidiform mole in situ was, in each case, outside the upper limit of the range for normal pregnancy. The highest level we have observed was 3600 mg. per day (6000 I.U./ml.).

Five patients with chorionepithelioma also excreted more than 450 mg. (750 I.U./ml.) CG per day. A precipitous fall in the urinary CG level began between 1 and 3 days after the destruction of the choriocarcinoma cells with methotrexate.

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