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Development of an Assay for a Biomarker of Pregnancy and Early Fetal Loss

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Human chorionic gonadotropin (hCG) is a glycoprotein hormone, secreted by the syncytiotrophoblast cells of the fertilized ovum, that enters the maternal circulation at the time of endometrial implantation. It is composed of two nonidentical subunits; α and β, with molecular weights of 14 kD and 23 kD, respectively. Its α subunit is identical in primary structure to its glycoprotein homologs, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH). Human chorionic gonadotropin binds to the same receptor as hLH and displays the same biological response, namely, to stimulate the declining function of the corpus luteum to produce progestins and estrogen late in the menstrual cycle. The differences in the structures of hCG and hLH have been exploited to develop antibodies that can measure hCG specifically in the presence of hLH.

Two-site antibody binding assays have been developed, based on a surface immunological concept of hCG epitopes, that involve four distinct regions to which antibodies against hCG can bind simultaneously. Antibody cooperative effects, in conjunction with kinetic advantages derived from the concentration factors by use of the sandwich assay technique (immunoradiometric assay, IRMA), have enabled development of extremely sensitive and specific measurement protocols for urinary hCG. The assay described herein permits the detection of pregnancy on an average 25.4 days after the first day of the preceding menses, as opposed to 29.5 days for conventional radioimmunoassay techniques. In addition, the greater sensitivity and specificity of this assay method has permitted the detection of episodes of fetal loss not detected by radioimmunoassay of urine specimens. A large scale epidemiological study is in progress using this assay technique as a way to identify pregnancies that are lost before becoming clinically apparent. This methodology provides a valuable tool for the determination of the rate of early fetal loss.

Introduction

Human chorionic gonadotropin (hCG) is a glycoprotein hormone that is secreted by the syncytiotrophoblast and appears to enter the maternal circulation at the time of endometrial implantation of the fertilized ovum (*1*). Its principal known role is to act at the ovarian corpus luteum to stimulate further the secretion of progestins in support of the growth of the endometrium. Its level in blood and in urine rises rapidly to reach a maximum approximately 12 weeks after the onset of the last menstrual period; thereafter, it slowly declines until the fetus and placenta are delivered.

This paper describes the development and application of an assay for this biomarker that can be used in field studies of clinically inapparent, early fetal losses. The text has been designed to provide those interested in such epidemiologic studies with a brief review of the chemistry and immunochemistry that led to a new assay for this hormone; knowledge of these features is generally essential to improvement in assay design.

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Early Assays of the Pregnancy Hormone, Human Chorionic Gonadotropin (hCG)

It has been known for some time that hCG, in contrast to many other plasma proteins, is excreted in the urine at a concentration that is approximately equal to that in blood. The studies by Marshall et al. (*2*) showed this to be the case for individuals throughout the first trimester, and more recent studies have documented that the same relationship appears to exist even when minute quantities of hCG are circulating in blood (*3*). Thus, urine specimens have always been a ready source for both assay and purification of this hormone. However, it is likely that urine specimens also contain partially degraded hormone fragments and measurement of a serum specimen has sometimes been preferred for diagnostic interpretation.

Biological assays for hCG were first developed by Aschheim and Zondek in the late 1920s (the A-Z test) as a method to detect pregnancy several weeks after the first missed menstrual period (*4*). In the 1960s immunologic assays for hCG began to replace the more cumbersome bioassays (*5,6*). These also had the feature

of increased sensitivity and reproducibility, leading to earlier diagnoses of pregnancy (*7*). However, polyclonal (usually rabbit) antibodies raised against hCG always cross-reacted to some extent with the human luteinizing hormone (hLH) in the maternal circulation or with this hormone's components in urine. This is due to the considerable degree of homology between hCG and hLH. Thus, sensitivity of these assays for the early detection of pregnancy was limited by the inevitable presence of hLH immunoreactivity in maternal serum and also by either the intact hLH hormone or its fragments in urine.

In the early 1970s the relationship of all the homologous glycoprotein hormones became apparent with the discovery that each was composed of a single α subunit and a single β subunit (*8*). While the amino acid sequences of the α subunits of hLH and hCG were shown to be identical, there were minor differences in the structures of the β subunits as illustrated in the next section (*9–19*). Vaitukaitis, Braunstein, and Ross found that occasionally, a rabbit, when immunized with the purified β subunit of hCG, made antibodies that were predominantly directed against this hormone and had limited cross-reactivity with hLH (*20*). This led to the development of radioimmunoassays that could routinely detect 1 ng/mL of hCG in serum in the presence of circulating hLH. Radioimmunoassays with urine specimens were more complex because of interfering substances and the presence of degradation products. Many manufacturers of diagnostic kits took advantage of this assay methodology, however, and developed home testing kits for hCG in urine specimens that could detect pregnancy with moderate reliability (*21*) during the first 9 days after the first missed menstrual period.

Two developments opened the way for new hCG assays with enhanced sensitivity and specificity. One was the production of monoclonal antibodies against the hCG-β subunit so that urine could be extracted efficiently by immunoaffinity adsorption. The second development was the finding that preparations of the unique β-COOH-terminal peptide region of hCG could be employed as immunogens to make high-affinity polyclonal antibodies (*22*). These are summarized in the following section.

The Chemistry and Immunochemistry of hCG: Similarity to hLH

Structural Considerations

The amino acid sequences of the glycoprotein hormones thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and chorionic gonadotropin (CG), all closely resemble each other and probably evolved from a common ancestral gene (*17,23*). Each is composed of two nonidentical subunits, designated α and β. In the human there is only one gene for the α subunit (*9*), so all of the hormones have the identical α subunit amino acid sequence, although carbohydrate differences exist (*15,17*). Hence, the structures of the β subunits are responsible for the different target organ specificities, and these have been shown to induce different conformational alterations when combined with their complementary α subunits (*24–26*).

hLH and hCG act at the same receptor. hLH is secreted by the pituitary in a cyclic fashion during the female reproductive years, with a pulsatile surge in blood level just preceding ovulation, while hCG is the chemical message secreted by the fertilized ovum for the purpose of rescue of the corpus luteum from its declining phase, just prior to menstruation. This latter action serves to sustain a favorable endometrial environment for the implanted, fertilized ovum. hCG and hLH have very similar receptors specifying β subunits whose structural similarities have been preserved during evolution. The amino acid sequences of these β subunits, that have been derived from both protein chemical and genetic analyses (*9,11,27*), are shown in Figure 1. The high degree of homology for the first 113 amino acids, especially the alignment of the 12 half-cystine residues, reflects their nearly identical receptor-specifying structures. The pairing of the disulfide bridges is still not definitively proven, although two bonds appear firmly established for each subunit (*17*).

The location and structures of the carbohydrate portions of hCG have been defined (Fig. 2). The carbohydrate structures of hLH differ from those of hCG in that hLH contains a significant amount of sulfate-terminating sugar moieties as compared to the terminal sialic-acid groups present in hCG (*28,29*). The carbohydrate heterogeneity of the hCG-β-COOH-terminal region is also of interest (*30*), although this area of the molecule is probably more related to the prolonged circulating half-life of hCG, as compared to hLH, and not to hormone action at the receptor.

The studies by Fiddes et al. (*31*) suggest that an evolutionary accident occurred at the terminator codon for the β subunit of hCG, permitting the addition of approximately 30 amino acids to that structure, which are not present in hLH β. This so-called β-COOH-terminal peptide region (β-CTP) of hCG thus provides a unique epitope by which immunochemical recognition of hCG can be achieved with absolute specificity, i.e., the β-CTP structure does not exist in hLH.

Immunochemical Considerations

Our group and many others have raised preparations of antibodies that recognize hCG and hLH to varying degrees (*32*). Of particular interest with regard to the topic discussed here are those antibodies which exhibit a high degree of selectivity for hCG, i.e., have very low reactivity with hLH. The following discussion primarily outlines results from our own research group, but the reader will also be referred to other studies that have yielded comparable results.

Figure 3 depicts the α and β subunits of hCG. In this figure we have defined four regions, shown by the cross-

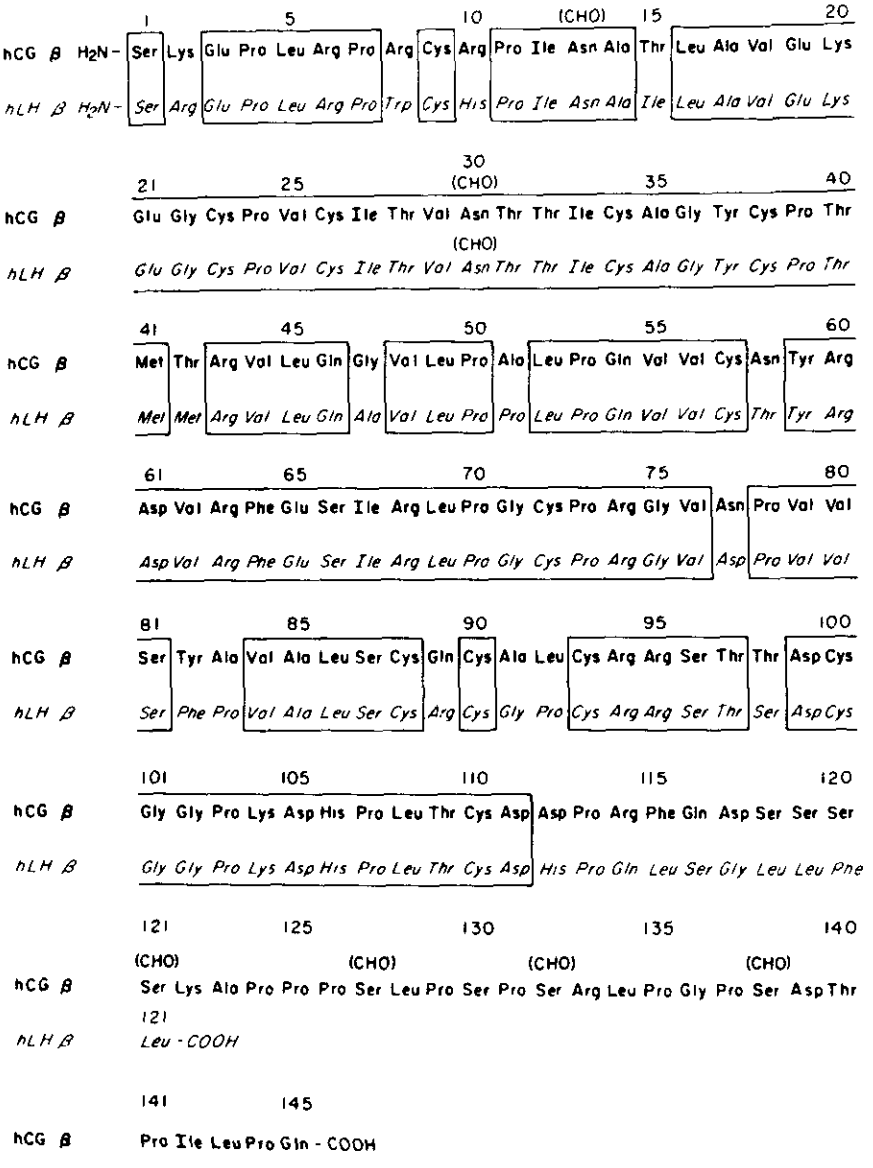

FIGURE 1. The amino acid sequence of the β subunit of hCG (*10–12*) as compared to that of hLH; the latter was determined by DNA sequencing (*27*). The hLH sequence has over 80% homology to hCG-β, being different mainly in the COOH-terminal portion of each hormone. Carbohydrate groups are indicated by (CHO) above the amino acid to which it is attached.

hatched circles, that appear to give rise to antibodies that can have a high degree of selectivity for hCG in relation to hLH, i.e., they must have epitopes that reflect some of the differences in structure that are shown in Figure 1. The following is a brief description of each of the four regions shown in Figure 3.

Region I consists of the β-CTP of hCG. This has been isolated, coupled to carrier proteins for immunization, and shown to give rise to rabbit antibodies that are both sensitive and specific for this structure (*22*). While the binding constants of antibodies raised against the synthetic peptide from this region have generally been low (*33*), we have found that the presence of the carbohydrate side-chains on this peptide immunogen can give rise to antibodies with considerable sensitivity and even carbohydrate selectivity (*22,34*). These antibodies are absolutely specific for hCG, since they are directed to a structure absent in hLH (*34*). Interestingly, this region appears to be equally reactive in the native hormone, in the free β subunit, and in the reduced and alkylated β subunit, suggesting that it is a structure that is loosely arranged at the surface of the hormone molecule and that the antibody combining sites recognize elements of primary structure. Chen, Matsuura, and colleagues have studied this phenomenon extensively and suggest that most of the antibodies bind to the last 15 COOH-terminal residues (*35,36*). Since the only antibodies that we have ever obtained to this region with high affinities were polyclonal antisera raised in rabbits, the example shown is an antiserum from rabbit

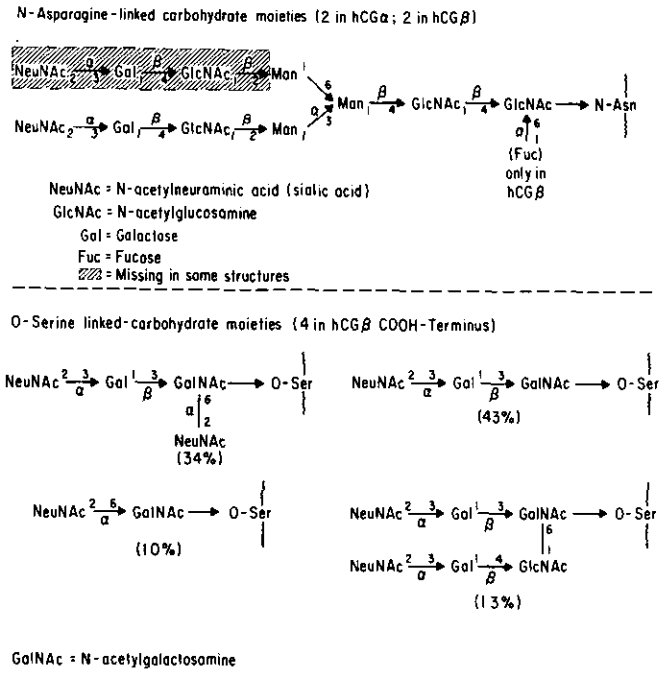

FIGURE 2. The structure of the carbohydrate moieties hCG-β. Upper portion of figure: *N*-asparagine-linked structures, showing heterogeneity in the biantennary structures. Lower portion of figure: the *O*-serine-linked carbohydrate moieties present on the β-CTP portion of hCG-β. Recent studies have demonstrated the heterogeneity in *O*-serine-linked oligosaccharides (*30*).

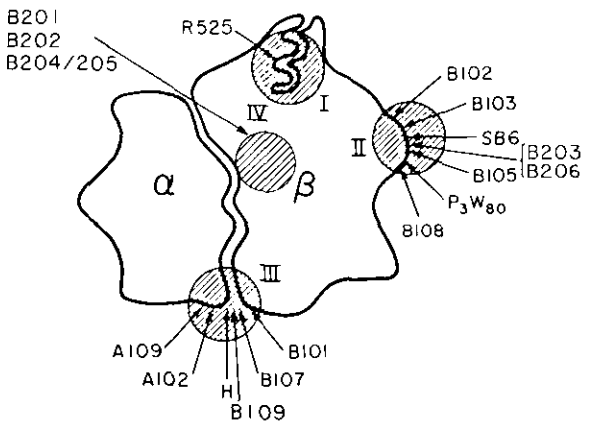

FIGURE 3. A schematic illustration of the orientation of epitope orientation in hCG. Determined as described in the text by competitive binding studies with monoclonal antibodies. Four sites are shown, based on the ability of antibodies to bind simultaneously: site I, the βCTP region; site II, a conformationally dependent region equally reactive with free β subunit and intact hCG; site III requires combination of α and β subunits for binding, as exemplified by human anti-hCG antibody H (*39*); site IV, the free β subunit and β fragment binding site.

525 (R525). Recently, Bellet and his associates (*37*), as well as Stevens and colleagues (*38*), have obtained monoclonal antibodies to this region.

Region II appears to contain the epitope for the antibody developed by Vaitukaitis, Braunstein, and Ross that was designated SB6 (*20*). Other monoclonal antibodies listed in the figure as recognizing some elements of this general region have been defined by virtue of their ability to compete with each other for binding (*32*). In addition, they all have the characteristics of binding relatively well to the β subunit, as well as to intact hCG, and each requires that the β subunit be folded in its native conformation for antibody binding.

Region III first became apparent to us when we had the opportunity to study some human autoantibodies to hCG and discovered that they reacted only with the intact hormone, exhibiting virtually no reactivity for either the isolated α or β subunits (*39*). This was a distinct contrast to the antibodies of region II. Subsequently, we have discovered several monoclonal antibodies (B107 and B109), which were raised against hCG and exhibit similar properties of recognizing only the intact hormone. These also compete for reactivity at the same site as the human antiserum. Monoclonal antibody B101 has been assigned to this site, because it also competes for binding with some of the antibodies shown there (*40*).

Region IV represents an epitope that is not readily reactive in native hCG but is present on the free β subunit and also on forms of the degraded hCG-β subunit that are excreted in human urine. For this reason we suspect that it is covered in the native hormone by the α subunit and have located it as shown in Figure 3. This is the region that has been most recently identified, and it affords the opportunity to distinguish between degraded hCG-β subunit fragments and the intact hormone. We have determined the complete structure of the degraded β-core fragment and have developed monoclonal antibodies that bind to region IV (*41,42*).

Taken together, these results indicate that it is possible to define four separate regions in hCG that can give rise to antibodies which may be employed in assays that will have a selectivity for hCG over hLH. Each of these regions presumably reflects a structural change resulting from amino acid differences shown in Figure 1. Several other research groups have also developed epitope maps of hCG. For example, Schwartz et al. have published a three-dimensional epitope map that was produced by permutations of simultaneous binding studies of 231 monoclonal antibodies (*43*). Bellet's group has also published an epitope map of the β-CTP-terminal region (*44*).

These details have been presented here largely to define the nature of some of the reagents available for the assay of this biomarker. One additional point underscores the importance of these four discrete regions of the hCG molecule. Immunoradiometric assays (sandwich assays) use two antibodies selected on the basis of binding to different hCG epitope regions such as those shown in Figure 3. In an early study it was found that one such assay exhibited an affinity far beyond that expected on the basis of individual antibodies. A mathematical model was developed (*45,46*) that accounts for this observation by the prediction of the formation of a circular complex composed of one molecule of each an-

tibody and two molecules of antigen. Data illustrating these enhanced affinities are presented in Figure 4. Figure 5 depicts the concept of this high-affinity circular complex.

Development of a New Assay for hCG

Given access to the immunochemical reagents just described, it became possible to devise better assays for hCG. We chose urine specimens for measurement because they were easily collected in field studies; saving the first morning voided specimen seemed much less cumbersome than attempting to obtain a 24-hr collection (*47*). If this was to be the design, we next required an antibody to extract (i.e., concentrate) the hCG from urine by immunoaffinity adsorption. Monoclonal antibody B101 was our first β-specific antibody that became available, so we chose it as the capture antibody. B101 was coupled to Sepharose beads so that hCG could be bound to the immobilized antibody in a suspension of urine and then removed by centrifugation to form a pellet. This pellet, with the extracted hCG bound to the antibody, could then be washed and recentrifuged several times to remove any nonspecific, interfering substances, or proteolytic enzymes. Since antibodies to the β-CTP region were absolutely specific for hCG, we elected to purify the rabbit IgG to that region (R525) and radiolabeled it with ^{125}I as the measuring antibody. In other words, when this measuring antibody was incubated with the washed pellet, obtained from a Sepharose-B101-hCG suspension, the higher level of radioactivity bound to the solid phase, the greater the quantity of hCG attached to the capture antibody. The scheme for this immunoradiometric assay (IRMA) is shown in Figure 6*A*, and a standard curve for such an assay is illustrated in Figure 6*B*. It can be seen in Figure 6*B* that the hLH preparation (μg/mL) reacts less than one part in 10,000 with hCG (ng/mL). We do not believe that this is cross-reactivity between the two, rather it represents the minute quantities of hCG that have been reported in human pituitary preparations (*48–51*) copurified with hLH.

It can be seen from Figure 6*B* that this assay design permitted hCG detection at concentrations approaching 0.01 ng/mL, which was 50 to 100 times more sensitive than any other existing assay methodology.

Next we needed an indication that these low urinary concentrations actually reflected blood levels of the hormone (Fig. 7*A*) and also to apply the assay to a variety of normal individuals (Fig. 7*B*). Indeed, the decline of

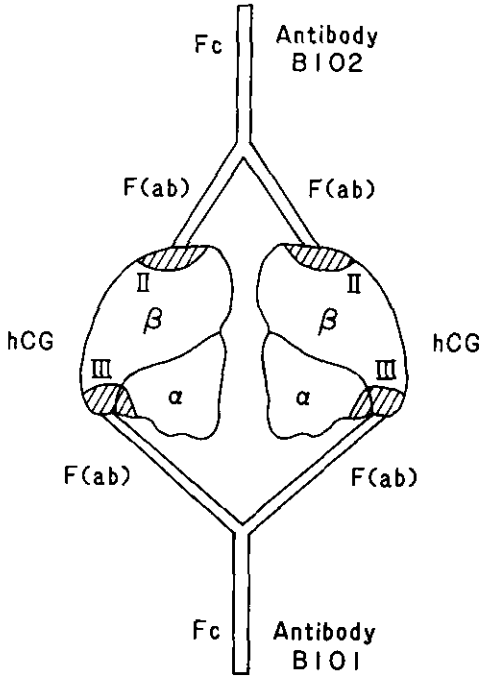

FIGURE 5. Circular complex formation. This diagram shows the circular molecular complex that is proposed as responsible for the affinity enhancement observed in some mixtures of monoclonal antibodies. Two monoclonal antibodies that bind to two distinct sites on hCG are capable of forming a complex as shown, which is composed of the two different antibodies molecules and two molecules of hCG (*46*).

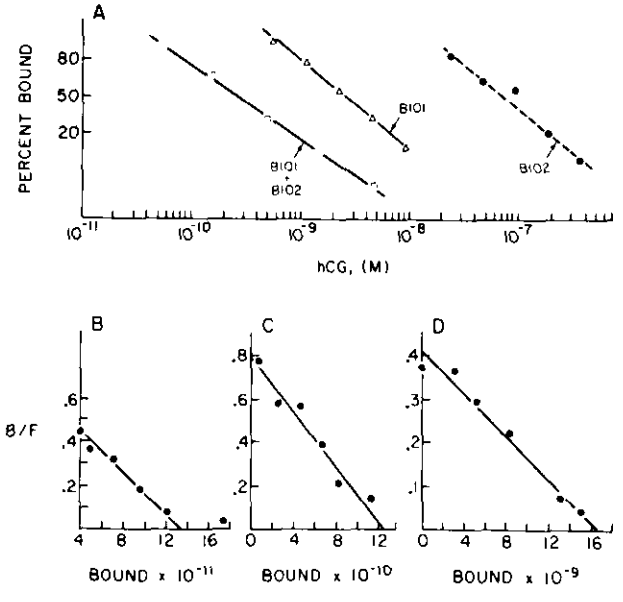

FIGURE 4. Antibody affinity enhancement due to circular complex formation. This figure shows cooperative effect that occurs when two antibodies that bind to different sites form a circular complex resulting in an affinity to hCG higher than that of either antibody alone. (*A*) the increase in sensitivity for measurement of hCG when two antibodies are mixed; (*B*) the estimated affinities of the antibodies alone or in the mixture. The data in (*A*) were obtained by radioimmunoassay for hCG using the double-antibody liquid-phase assay with antibodies B101, B102, and a mixture of B101 and B102. The amounts of labeled hCG bound in the absence of unlabeled inhibitor was approximately the same for each antibody or antibody mixture. Rabbit anti-mouse IgG was the second antibody. The Scatchard analysis of the binding of hCG to a mixture of antibodies B101 and B102 (*B*), antibody B101 alone (*C*), and antibody B102 (*D*) yielded values for the equilibrium binding constant of 5.4×10^9 for (*B*); 5.1×10^8 for (*C*); and 1.9×10^7 for (*D*). Reproduced from (*61*).

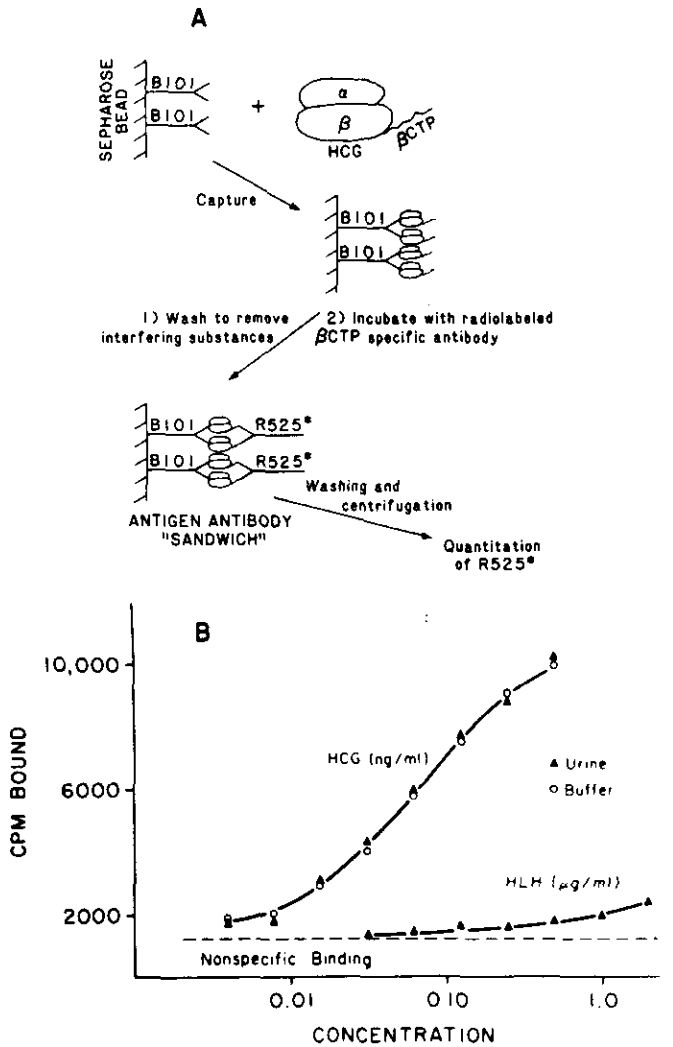

FIGURE 6. Design of the immunoradiometric assay (IRMA) for hCG and standard curve illustrations. (*A*) The hormone is first extracted from urine with immobilized monoclonal antibody B101, specific for region III. The immobilized antibody-antigen complex is washed free of interfering substances and then incubated with the radiolabeled β-COOH-terminal specific antibody R525. After washing and centrifugation to remove unbound trace, the "sandwich" is counted in a gamma counter. The quantity of radiolabel present is directly proportional to the amount of antigen (hCG) present in the sample. (*B*) Measurement of hCG and hLH in urine by the IRMA. Urine is shown not to interfere with measurement of hCG. hLH is shown to cross-react less than one part in 10,000. IRMA of hCG added to buffer (○) and to urine (▲) of a normal man; hLH added at high concentration to urine (▲) of a normal man. The units of concentration for hCG and hLH are ng/mL and μg/mL, respectively. Reproduced from (*3*).

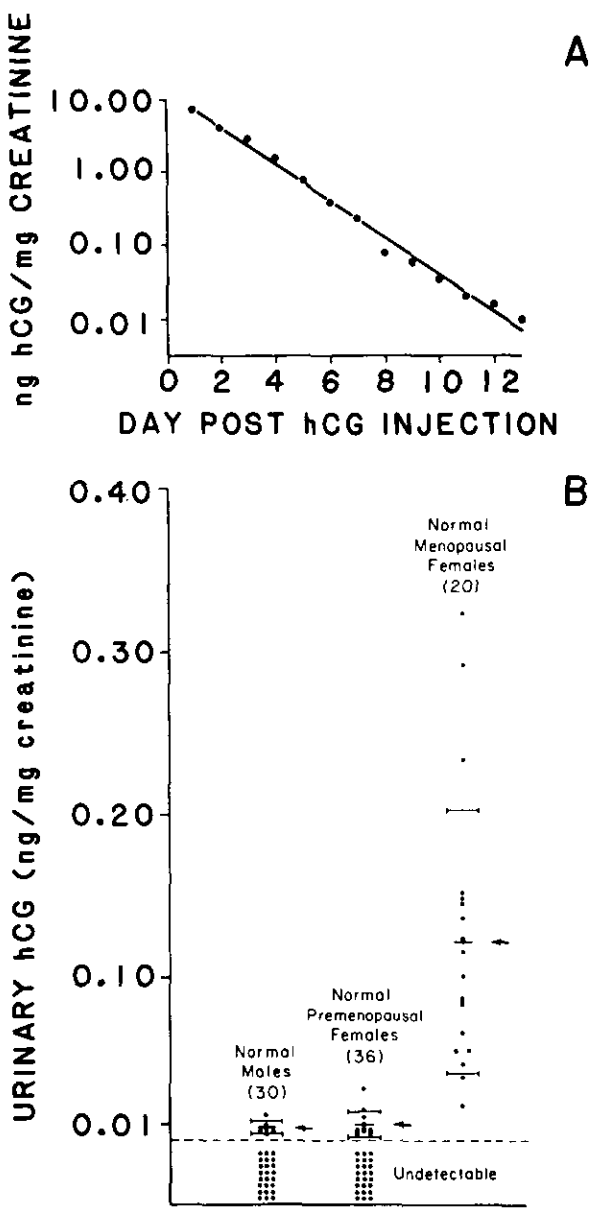

FIGURE 7. Urinary hCG assay results from normal human subjects. (*A*) hCG excretion rate for a man injected with 3000 IU of hCG for treatment of infertility. Daily first morning voided urine specimens were collected, and the concentration of hCG in ng/mg creatinine were determined by IRMA. The half-life of hCG was 29 hr. (*B*) Values for ng hCG/mg creatinine obtained by IRMA of urine specimens from normal men and normal premenopausal and menopausal women. The arrows indicate the mean values and the brackets represent ± 1 SD of the means for detectable levels in each group. Reproduced from (*3*).

hormone in urine in men who had received a single injection of hCG follows the expected decline in blood levels. While men and women of reproductive age generally had undetectable or extremely low levels of urinary hCG, we found that menopausal females did have detectable quantities covering a rather wide range. This has also been noted by others (*48,49*) and probably represents the effect of ovarian failure in the menopause leading to a profound stimulus for excess gonadotropin secretion that includes a small fraction of hCG.

Field Studies of Early Fetal Loss: Testing the Utility of the hCG Assay

Thus far we have described how biochemical and immunochemical information has been used to devise a

new, sensitive assay for hCG in urine specimens, but its applicability as a biomarker for early fetal loss in epidemiologic studies required evaluation. To demonstrate the applicability of hCG as a biomarker, a relatively small trial was conducted to ascertain whether the design of the study was feasible and whether new assays for hCG in urine, with enhanced sensitivity, would yield new data. Specimens were also analyzed by the method employed by an earlier investigator (*53*).

The field studies enrolled women as paid volunteers. Each woman collected approximately 1 ounce of urine each morning and stored the containers frozen until they were picked up for assay. Details of the study design have been described elsewhere (*47*). To date, we have completed three studies testing the utility of these assays. In the first, 30 women collected daily urine specimens from the time they stopped contraception until they became pregnant, or for six months if no pregnancy occurred (*47*). This study not only showed the feasibility of the epidemiologic design, but it also permitted the detection of several cycles with early fetal loss that would not have been detected by any other available methodologies. This preliminary study is the basis for a larger study currently in progress, which will more accurately define the incidence of such clinically unrecognized early fetal loss.

A second study group was composed of women who had undergone tubal ligation; no patterns suggestive of pregnancy have been seen so far. In the third group, women who were using intrauterine devices as a means of contraception were evaluated to determine whether this contraceptive technique prevented implantation of the fertilized ovum. Urinary hCG was detected in only 1 of 107 menstrual cycles in this study (*54*), indicating that implantation is an infrequent event among women who use IUDs for contraceptive purposes. The assay technique could not rule out the possibility that the single pregnancy loss detected might have been a tubal implantation.

Figure 8 has been reproduced (*55*) to illustrate the type of findings that may be obtained in field studies.

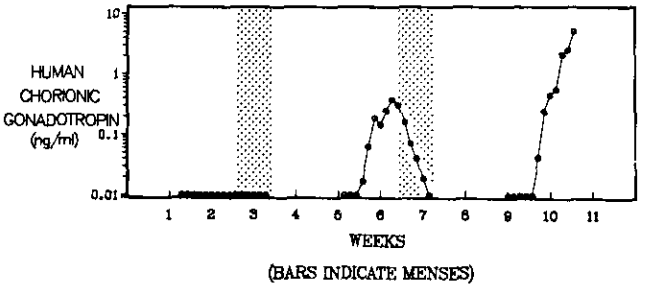

FIGURE 8. Menstrual cycle hCG values obtained by IRMA technique. Three consecutive menstrual cycles in which daily urines were collected during the second half of the cycle. Assay of hCG indicates that no conception occurred during the first cycle (hCG < 0.01 ng/mL). During the second cycle, hCG values rose for 6 consecutive days (peak value of 0.38 ng/mL hCG) and then fell, indicating a preclinical fetal loss. The third cycle indicates the rising level of hCG of a normal pregnancy. Reproduced from (*55*).

Three consecutive menstrual cycles of one individual are illustrated in which urine specimens were collected daily. During the first cycle no conception occurred. All hCG values were less than 0.01 ng/mL. During the second cycle, 3 weeks after the onset of the prior menstrual period, urinary hCG rose for 6 days to 0.38 ng/mL. Human chorionic gonadotropin steadily declined over the next 6 days, returning to undetectable levels. With the decline of hCG came the onset of menses, 27 days after the onset of the previous menses. This individual was unaware of her pregnancy or the episode of early fetal loss. However, the finding of 11 consecutive elevations of urinary hCG above the normal background for this individual, in a pattern of rise and then fall, leave little doubt that a loss has occurred. Three weeks after the apparent fetal loss, this woman again exhibited a rising level of urinary hCG. This time she remained pregnant and delivered a normal, full-term infant.

One additional point should be added concerning the advantage of an immunoradiometric assay. When a conventional radioimmunoassay is performed on a urine specimen, contaminating proteolytic enzymes in the urine may degrade the radiolabeled tracer during its incubation. This proteolytic artifact may lead to falsely elevated measurements of hCG. In addition, other interfering urinary substances may add to background baseline variations and decrease the signal-to-noise ratio for detection of pregnancy. In the immunoradiometric assay, the radioactive tracer is introduced after proteases and interfering substances have been washed away. For example, the SB-6 radioimmunoassay in urine displays a poor signal-to-noise ratio with a wide variation between 1 to 10 ng/mL (*47*). This contrasts with the data from the immunoradiometric assay performed on the same specimens which display greater sensitivity for hCG without the widely fluctuating baseline (*47*). These factors may have contributed to the differing results (8–57%) obtained in prior studies of early fetal loss (*56–58*).

Rationale for Future Assay Developments

As noted at the outset, tests for hCG have evolved over the past 5 decades from crude bioassays to highly sensitive and specific immunoassays. The hCG molecule is well-characterized and a relatively specific product of pregnancy. Its ectopic secretion by some tumors and its slight elevation in postmenopausal women (*49–52*) do not lessen its epidemiologic value as a biomarker for pregnancy in healthy women of reproductive age. Other placental proteins that have been described as pregnancy-specific have proven to be of less utility to detect early pregnancy. Furthermore, these products are typically assayed in blood, and their fate in urine is less well known (*59*).

The careful description of early pregnancy loss requires not only a reliable assay but also frequent mea-

surement. The collection of daily urine specimens from women attempting pregnancy has proven to be feasible. Data from such studies show that hCG can be detected within a few days of the expected date of implantation and, on average, at least 2 days before the onset of the expected next menses. Early fetal losses are detectable with this approach, although an assay needs to be sensitive and reliable to at least 0.05 ng/mL of hCG in order to detect most of these losses clearly.

Many research groups have contributed to advances in the techniques for studying early pregnancy. Still more remains to be done if epidemiologic studies are to be carried out efficiently. The best available methods for measuring hCG are complex, time-consuming, and expensive. We suggest here some specific and feasible ways in which hCG assays might be refined.

New Monoclonal Antibodies for hCG Detection

Although urinary hCG is predominantly in its intact form, there are several forms of fragmentary hCG as well, each with distinct immunochemical properties. In order to measure the complete urinary products of hCG, new antibodies with high affinities for the various products are needed. Ideally, these should be monoclonal antibodies, in order to assure a continuous supply of well-characterized reagents. The complete detection of hCG in its various forms would maximize sensitivity and assure that unusual events such as a decrease in the secretion of one of the subunits could be described.

Nonradioactive Methods for hCG Detection

The use of radioactive iodine in immunoassays has been highly successful, but not without disadvantages. First, radiolabeled reagents must be freshly prepared and characterized every few weeks and then shipped to users. Second, the use of radioactive materials in the laboratory requires special training and surveillance. Third, there is an increasing problem with radioactive waste disposal. The application of newer technologies such as a fluorescent immunoassay would allow greater ease and flexibility of laboratory support for field studies.

Systematic Comparisons of hCG Assays

The suggestions above are aimed toward making hCG assays as accurate, rapid, and inexpensive as possible. Such improvements would greatly increase the applicability of such assays in epidemiologic studies. However, with every change in assay method, there is the problem of potential noncomparability of results among different assays. Every effort should be made to characterize the properties of new assays in a way that will allow the results of epidemiologic studies to be meaningfully compared.

Related Research Opportunities

Urinary hCG is a useful marker for detecting the fertilized ovum around the time of implantation. However, this marker cannot detect earlier loss of the ovum. Possible methods for earlier detection of pregnancy should be pursued (*60*).

In the broader context of reproductive studies, there is a need for continued progress in the development of markers of ovarian function, including ovulation. As with hCG, the most useful markers for epidemiologic research are those detectable in easily collected biological specimens such as urine or saliva. The pituitary gonadotropins (hLH and hFSH) can be useful for determining the timing of ovulation (*1*). The various estrogen and progesterone derivatives can be used to identify ovulation as well as to measure the follicular and luteal functions of the ovary. The same improvements that were discussed for hCG assays would be useful for assays of these hormones.

The measurement of reproductive dysfunction (with early pregnancy loss as a specific example) may be useful in studies of environmental toxins. For such studies of early loss to be reliable, more information about major factors that affect such risk are needed. Does the risk of early loss increase with the mother's age? Is risk affected by the type of birth control previously used? Is the risk associated with other types of reproductive failure, such as spontaneous abortion? Is the risk of loss a function of other events of the cycle, such as length of the luteal phase or the timing of intercourse in relation to ovulation?

There are clinical questions as well that can be addressed by studies of early fetal loss. These focus mainly on the possible contribution of unrecognized loss to infertility. Syndromes involving very early loss may be so rare as to be unimportant to epidemiologic studies of environmental hazards, but the syndromes are important nonetheless for diagnosis and treatment of infertility. All these basic questions, whether coming from the epidemiologist or the clinician, are now answerable with tools that were unavailable until recently. Work will be further enhanced when more convenient assays for hCG are available.

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