The Biological and Clinical Significance of Nicks in Human Chorionic Gonadotropin and Its Free β-Subunit

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Human chorionic gonadotropin (hCG) is a glycoprotein hormone composed of two dissimilar subunits, α and β. Nicks or missing peptide linkages have been found in the β44-52 region of the β-subunit of hCG, whether from pregnancy or trophoblast disease. This article reviews recent reports about the location of nicks in hCG, their origin and occurrence, their effects on the steroidogenic and receptor-binding activities of hCG, and on the immunological activities of hCG and its free β-subunit. Taken together, the reports show: (1) nicks occur primarily between β47 and β48, and to a lesser extent between β44 and β45; (2) the extent of nicking in hCG samples varies widely, from undetectable to 100 percent of molecules; (3) nicks greatly reduce the steroidogenic activity of hCG in vitro (nicked molecules have less than 20 percent of the activity of the intact hormone); (4) nicks may occur at the trophoblast-myometrial interface or in the circulation by the action of human leucocyte elastase or similar leucocytic protease; (5) hCG testing kits using dimer-specific antibodies may not detect nicked molecules and may give different results from those using other antibodies; (6) hCG international reference preparations and the CR series of hCG standards are variably nicked (10 percent to 20 percent), complicating the problem of discordant hCG results in nick-sensitive assays; (7) results from commonly used immunoassays for measurement of the hCG free β-subunit vary by as much as tenfold because some of the antibodies employed do not detect nick free β-subunit.

BACKGROUND

Human chorionic gonadotropin (hCG) is a glycoprotein hormone composed of two dissimilar subunits, α (92 residues) and β (145 residues), joined non-covalently. hCG is produced by the trophoblast in pregnancy and trophoblast disease. The α-subunit of hCG has the same peptide sequence as the α-subunit of the pituitary glycoprotein hormones. The β-subunit of hCG is similar to the β-subunit of human luteinizing hormone (hLH) (80 percent sequence homology). The β-subunits of hCG and hLH are responsible for directing binding of both hormones to their joint receptor.

hCG levels are used to examine the progress of pregnancy, to follow the evacuation of hydatidiform mole, and to follow the success of chemotherapy in women with persistent trophoblast disease (invasive mole and choriocarcinoma). In addition to hCG, a small amount of uncombined α-subunit (free α-subunit) and uncombined β-subunit (free β-subunit) are secreted by trophoblast tissue [1-7]. Levels of free

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Abbreviations: hCG: human chorionic gonadotropin  hLH: human luteinizing hormone  HLE: human leucocyte elastase  I.R.P.: international reference preparation

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β-subunit in pregnancy serum are one five-hundredth to one one-hundredth of those of hCG (from seven weeks to term). Higher levels of free β-subunit are present in serum from patients with hydatidiform mole (one one-hundredth to one-tenth of the hCG levels), and still higher levels in women with persistent trophoblast disease (one-twentieth to one-fourth of the hCG levels) [7–12]. Measurements of free β-subunit are now used in the diagnosis and management of hydatidiform mole and persistent trophoblast disease [8,9].

In 1973, Shome and Parlow [13] discovered nicks or missing peptide linkages at different sites between residues 44 and 49 on hLH β-subunit. More recently, the nicks have been located to between β-subunit residues 44 and 45, 47 and 48, or 48 and 49 on hLH [14,15]. These nicks diminish the biological activity of hLH. As shown by Ward and colleagues [14], highly nicked hLH preparations have 40 percent lower biological activity than less nicked samples. In 1988, Bousfield and Ward [16] found that hLH cleaved by endoproteinase Arg-C (nicks at an adjacent site, β43-44) also has diminished biological activity.

In 1988, Nishimura and colleagues used electrophoresis and immunoblotting procedures to examine a urine hCG sample from a woman with trophoblast disease [17]. On gels, a band migrating in the position of standard hCG β-subunit (Mr = 35,000) was detected with β-subunit C-terminal peptide antisera. After reduction of the disulfide bridges, however, a faster migrating band (Mr = 24,000) was detected with this antisera. This finding was the first indication of missing linkages in hCG. Since this time, ten reports have been published [4,18–26], originating in France, Japan, and the U.S.A. The results are reviewed here. Findings include the sites of nicks in hCG [4,18–20,21,23], the occurrence of nicks in individual hCG samples [4,22], the origin of nicks in hCG [24], the effect of nicks on the biological activity of hCG [19,22], the recognition of nicked hCG by different immunoassays [22,26], and nicks in hCG free β-subunit [20,25].

SITES OF NICKS IN HCG

In July 1988, Nishimura and colleagues published electrophoresis results indicating nicks in an individual trophoblast disease hCG sample. Four weeks later, Bidart and colleagues published their findings [18], which demonstrated nicks in hCG purified from a crude pregnancy urine preparation (“Pregnyl” from Organon, crude preparation from large number of pregnancy urines). Using sequence analysis, Bidart and colleagues demonstrated a single nick between β-subunit residues 47 and 48, in this preparation. In the following year, Lustbader, Birken, and colleagues performed similar experiments, purifying hCG from Organon crude and examining the N-terminal sequences [21]. They found nicks at two of the three hLH cleavage sites, between β-subunit residues 44 and 45, and between β-subunit residues 47 and 48 (Fig. 1). Recently, Birken and colleagues examined the sequence of eight international hCG standards [24]. These included the first I.R.P. (international reference preparation) for immunoassay, and the CR series (CR117, 119, 121, 123, 125, and 127) distributed by the National Institutes of Health. To determine accurately the sites and extents of nicking, Birken and colleagues isolated the nicked β-subunit of hCG by reverse-phase chromatography before doing N-terminal sequence analysis. Preparation CR119 (same as first I.R.P.) was shown to be 10 percent nicked (at β47-48 or β44-45), preparation CR127 to be 20 percent nicked, and the
of the 13 individual urine nicks, primarily at one site. 

other standards to be nicked within this 10 to 20 percent range. These findings show hCG isolated from large pools of pregnancy urine (i.e., Organon crude) is, on average, 10 to 20 percent nicked at one of two sites, β44-45 or β47-48. 

The studies with hCG standards or hCG purified from Organon crude gave a general idea about the sites and extent of nicking. They said nothing, however, about individual samples. Are individual samples all 10 to 20 percent nicked or are some 0 percent and others 100 percent nicked? Are individual samples nicked at both β44-45 and 47-48, or are some nicked at one site and others at the second site? To address this issue, Kardana and colleagues [23] examined the hCG in urines from five individuals with first trimester (P1, P2, P3, P5, P6) and from one individual with second trimester pregnancy (P4), from three individuals with complete hydatidiform mole (M1, M2, M4), and from four women with advanced choriocarcinoma. They used classical solvent extraction and chromatographic methods to purify hCG. Using N-terminal sequence analysis (Table 1) Kardana and colleagues [23] showed that 11 of the 13 individual urine samples were nicked. Most samples were nicked at just one site, β47-48 (three of the 11 were also nicked at β44-45 or β46-47), and that the extent of nicking in individual urine samples varied widely (0 to 100 percent). In conclusion, hCG standards purified from pooled urines, and clinical hCG preparations like “Pregnyl,” represent an average mixture (10 to 20 percent nicking at β44-45 or β47-48), and so, in extent and site of nicking, are not necessarily representative of individual samples, which display a wide variation in the extent of nicking (0 to 100 percent nicked, primarily at one site).

### TABLE 1
N-Terminal Sequence Analysis of β-Subunit of Purified hCG

| Sample Code | N-Terminal Sequence Detected Starting at | Proportion of Nicked hCG Molecules (%) |
|-------------|------------------------------------------|---------------------------------------|
|             | β45                                      | β47                                   | β48                                   |                            |
|             | hCG Purified from Individual Pregnancy Urines |                                       |                                       |                            |
| P1          | 1.0                                      | 0                                     | 0                                     | 0                         |
| P2          | 1.0                                      | 0                                     | 0                                     | 0.35                      |
| P3          | 1.0                                      | 0                                     | 0                                     | 0.59                      |
| P4          | 1.0                                      | 0                                     | 0                                     | 0.86                      |
| P5          | 1.0                                      | 0                                     | 0                                     | 0.58                      |
| P6          | 1.0                                      | 0                                     | 0.55                                  | 1.00                      |
|             | hCG Purified from Individual Trophoblast Disease Patient Urines |                                       |                                       |                            |
| M1          | 1.0                                      | 0                                     | 0                                     | 0.15                      |
| M2          | 1.0                                      | 0                                     | 0                                     | 0                         |
| M4          | 1.0                                      | 0                                     | 0                                     | 1.0                       |
| C1          | 1.0                                      | 0.23                                 | 0                                     | 0.39                      |
| C2          | 1.0                                      | 0.51                                 | 0                                     | 0.45                      |
| C3          | 1.0                                      | 0                                     | 0                                     | 0.24                      |
| C5          | 1.0                                      | 0                                     | 0                                     | 1.28                      |

N-terminal sequence determined for 15 consecutive amino acid residues, and compared with the known peptide structures of the α- and β-subunits of hCG. The amount of each sequence was estimated from the concentration of PTH-amino acids (pmol), and was normalized to the sequence starting at β1. Proportion of molecules nicked is the percentage of peptides starting at β45 or 48 compared with β1 [23].
OCCURRENCE OF NICKS IN INDIVIDUAL hCG SAMPLES

Electrophoresis of reduced hCG molecules and immunoblotting with β-subunit C-terminal peptide antisera was used in 1988 to identify nicked hCG in individual and pooled urine samples [17,18]. In 1989, Cole and colleagues used the same methods to identify nicked hCG in individual pregnancy and trophoblast disease serum samples [4]. Shortly thereafter, Puisieux and colleagues confirmed this finding, by identifying nicked hCG in the serum from four individuals with choriocarcinoma [20]. Recently, electrophoresis and immunoblotting were used to examine nicks in 34 urine and serum samples from patients with pregnancy and trophoblast disease [22]. A fast-migrating band (Mr = 24,000), indicative of nicked hCG, was observed in 71 percent of pregnancy urine samples and 80 percent of the matching sera, and in 82 percent of trophoblast disease urine samples and in seven of seven matching sera. These findings show that hCG molecules are, to some extent, nicked in most pregnancy and trophoblast disease serum and urine samples. The extent of nicking in serum is not significantly different to that in urine based on these semi-quantitative electrophoresis and immunoblotting techniques. This was confirmed by immunoassays for nicked hCG (described below). While realizing that individual hCG samples vary widely, the 10 to 20 percent range, as indicated by standard or pooled preparations, may reflect the average extent of nicking in individual pregnancy hCG serum and urine samples.

THE ORIGIN OF NICKS IN hCG

Since nicks are present in both serum and urine, it is likely that nicking occurs inside the trophoblast cell, at the time of secretion, or in the circulation. Human leucocyte elastase (HLE) is a protease that cleaves after glycine, valine, or leucine residues within exposed hydrophobic regions of proteins [27]. Nicks in hCG occur at β44-45 (Val-Leu) and β47-48 (Gly-Val) (Fig. 1). The nicks occur within an amphipathic intercysteine loop on β-subunit. An amphipathic loop is hydrophilic on one side and hydrophobic on the other, making it a good substrate for HLE with an exposed hydrophobic face. Leucocytes and HLE are known to be present in high concentrations at the trophoblast-myometrial interface and at the choriocarcinoma-normal tissue interface [28]. To test the hypothesis that HLE may play a role in nicking of the loop region, Birken and colleagues [24] used sequence analysis to investigate the cleavage of CR127 standard hCG by HLE. As found, after a two-hour period, HLE cleaved 30 percent of hCG molecules at β44-45, and at no other site on the α- or β-subunit. With longer incubations, further cleavages occurred at β44-45, β47-48, and β48-49. We conclude that HLE may be responsible for nicks at β44-45 in hCG preparations. The identity of the enzyme that nicks at β47-48 is less certain. While HLE may be involved, it appears more likely that other enzymes with specificities similar to HLE, possibly also located at the trophoblast-myometrial and choriocarcinoma-normal tissue interfaces, nick at this site.

EFFECT OF NICKS ON THE BIOLOGICAL ACTIVITY OF hCG

Sakakibara and colleagues [19] purified hCG β-subunit from a pool of pregnancy urines and separated nicked from non-nicked hCG β-subunit molecules by reverse phase chromatography. Nicked hCG β-subunit was then recombined with α-subunit. The product (α:nicked β) had less than one percent of the steroidogenic activity of
natural hCG. Cole and colleagues [22] used HLE to generate nicked CR127 standard hCG. The product had approximately 20 percent of the steroidogenic activity of non-treated CR127 standard hCG. The discrepancy in these results may come from the use of different hCG preparations, or it may be due to abnormal combination of Sakakibara and colleagues’ nicked β-subunit preparation, or to damage to this preparation by the trifluoroacetic acid used in the reverse phase procedure. All told, a single nick at β44-45 or β47-48 dramatically reduces the steroidogenic activity of hCG.

Cole and colleagues [22] also looked at the steroidogenic and receptor binding activities of eight individual hCG preparations (Table 2). No correlation was found between the proportion of intact or non-nicked hCG molecules in the individual preparations and their ability to displace ^125^I-hCG in binding the hCG/LH receptor. A close correlation was found, however, between the proportion of intact (non-nicked) molecules and their steroidogenic activity in vitro (correlation coefficient
TABLE 2
The Biological and Receptor Binding Activities of Individual Pure Preparations of hCG

| Sample | Proportion of Non-Nicked hCG Molecules (%) | Concentration by Amino Acid Analysis, μg/ml | Steroidogenic Activity μg/μg hCG | Radioreceptor Assay EC50 |
|--------|---------------------------------|---------------------------------|---------------------------------|-----------------------|
| P1     | 100                             | 620                             | 1.0‡                            | 1.0‡                  |
| P3     | 41                              | 530                             | 0.48                            | 1.6                   |
| P4     | 14                              | 2,450                           | 0.51                            | 1.7                   |
| P5     | 42                              | 1,460                           | 0.50                            | 1.1                   |
| M1     | 85                              | 1,090                           | 0.87                            | 1.3                   |
| M2     | 100                             | 1,490                           | 1.2                             | 0.82                  |
| C1     | 38                              | 4,300                           | 0.58                            | 2.2                   |
| C5     | 0                               | 2,100                           | 0.20                            | 1.6                   |

Steroidogenic activity was determined in the isolated rat luteal cell progesterone test, and radioreceptor assay activity in the competitive binding assay using homogenated luteinized rat ovaries.

*The proportion of non-nicked hCG molecules (percentage) is calculated as 100 minus percentage of nicked molecules (from Table 1).

*Values are the concentration (μg/ml by amino acid analysis) necessary to displace 50 percent of 125I-hCG in binding receptor; values are normalized to result for P1 hCG.

*In a regression analysis, steroidogenic activity (μg/μg hCG) versus percentage non-nicked hCG, correlation coefficient was greater than 95 percent.

*In a regression analysis, receptor binding activity (EC50 in radioreceptor assay) versus percentage non-nicked hCG, significant correlation not observed (correlation coefficient = 68 percent)

> 95 percent). It was estimated (intercept of regression line) that the nicked components of the individual preparations had approximately 24 percent of the steroidogenic activity of non-nicked molecules. This estimate supported the studies with HLE-cleaved standard hCG, which suggested that nicked hCG had about 20 percent of the activity of non-cleaved molecules. These findings indicated that nicks, which do not have a major effect on hCG receptor binding, were an important cause of discordance in steroidogenic activity between individual hCG preparations. It is concluded that nicked molecules, a component of most hCG preparations, have low steroidogenic activity and could antagonize hCG action.

RECOGNITION OF NICKED hCG BY DIFFERENT IMMUNOASSAYS

Our group showed that monoclonal antibodies specific for dimer hCG have much lower affinity for nicked hCG [22,24,26]. This finding was an important observation since this type of antibody is commonly used in kits for the clinical measurement of hCG and could result in errors when nicked molecules were a major component of the hCG sample. Table 3 shows the results of recent tests of eight different immunoassays [26]. Each was used to measure the hCG level at six different concentrations in three hCG standards. These were hCG standard batch CR127 (20 percent nicked), hCG batch CR127 incubated for 21 hours with HLE (90 percent nicked), and C5 individual hCG preparation (100 percent nicked). Results were plotted and variation in values was examined at the level corresponding to 50 percent of maximum response. As shown, nicks only minimally (±29 percent) affect the
TABLE 3
Recognition of Nicked and Non-Nicked hCG by Different Immunoassay

| Immunoassay                                      | CR127 hCG (20% Nicked) (%) | CR127 hCG 21-hour HLE (90% nicked) (%) | C5 hCG (100% nicked) (%) |
|-------------------------------------------------|-----------------------------|----------------------------------------|--------------------------|
| 2119-12/BP052 anti-α:anti-β-peroxidase sandwich assay | 100                         | 107                                    | 71                       |
| Hybritech Tandem-R anti-α:125I-anti-β sandwich assay | 100                         | 114                                    | 129                      |
| Organon NML anti-β C-terminus:125I-anti-β sandwich assay | 100                         | 108                                    | 116                      |
| Biomerica hCG anti-β:125I-anti-β sandwich assay   | 100                         | 117                                    | 117                      |
| Diagnostic Products anti-β radioimmunoassay      | 100                         | 118                                    | 67                       |
| Amersham Amerlex-M anti-β radioimmunoassay       | 100                         | 95                                     | 98                       |
| B109/BP052 anti-hCG: anti-β-peroxidase sandwich assay | 100                         | 522                                    | 1,580                    |
| Serono MAIA clone anti-hCG:125I-anti-β sandwich assay | 100                         | 465                                    | 1,370                    |

hCG standards were prepared 20 to 100 percent nicked. These were hCG standard CR127 hCG, standard CR127 further nicked by incubation 21 hours with HLE, and individual hCG preparation C5. Each standard was tested in each immunoassay kit at six different dilutions (between 0.25 and 200 mg/ml). Values are relative concentrations (percentage of hCG standard CR127 level) needed for 50 percent maximum response.

potency of hCG in kits using anti-α:anti-β, anti-β C-terminus:anti-β and anti-β: anti-β sandwich protocols, and in hCGβ RIAs. Nicking, however, have a very big effect on the potency of hCG in kits using antibodies to hCG dimer (anti-hCG:anti-β sandwich assays). As much as 16 times more nicked hCG was needed to achieve a similar response as non-nicked hCG (Table 3). The assays using an hCG dimer antibody include the widely used Serono MAIA clone kit and the B109 anti-hCG assay from Columbia University. This latter assay has been distributed by Drs. Canfield and O’Connor at Columbia University to a large number of test centers in North America for the ultrasensitive detection of early pregnancy hCG levels [29]. A survey of different quantitative hCG kits available in North America revealed that approximately one-quarter utilize an hCG dimer antibody. Nicking ablates the biological activity of hCG [19,22]. Thus, levels of non-nicked hCG (determined by Serono MAIA clone, B109 anti-hCG or other immunoassays with similar specificities) could be more representative of biologically active hormone than levels of total hCG (measured with the other immunoassays) and may be preferable in situations where knowing the potency of hCG is critical.

Using the B109 anti-hCG dimer assay to measure the level of non-nicked, and the Hybritech Tandem assay to measure total hCG, attempts were made to assess the extent of nicking in pregnancy and trophoblast disease hCG samples [22]. By these methods, approximately 25 percent nicking was indicated in 38 pairs of serum and urine samples. Consistent with sequence analysis studies with individual urine hCG
samples (Table 1), very wide variation in the extent of nicking (0 to 94 percent of molecules) was indicated.

We conclude that hCG kits might be categorized as either immunoassays for total hCG, which measure nicked plus non-nicked molecules, or as immunoassays for intact hCG, which only detect non-nicked molecules. The existence of these two categories of hCG kits may be a major cause of discordance in results between different immunoassays and the cause of confusion in interpreting assay results from different test centers. The two categories of kits could have separate applications. Total hCG kits might be optimal for general pregnancy, cancer, and trophoblast disease applications. Intact hCG kits might be optimal for assessing the level of biologically active hCG; thus they may be preferable for monitoring women with threatened abortion or other situations requiring assessment of hCG potency.

**NICKS IN hCG FREE β-SUBUNIT**

Studies at Yale University [4] and at other centers throughout the world [3,5,7,8–12] show a higher free β-subunit to hCG ratio in sera from patients with persistent trophoblast disease than in that from patients with hydatidiform mole, and a higher free β-subunit to hCG ratio in the latter than in normal seven- to 40-week pregnancies. An outcome of these reports is the availability of monoclonal antibodies and kits using these antibodies for specifically measuring free β-subunit, and the testing for free β-subunit at trophoblast disease centers. Reports from different centers on the free β-subunit to hCG ratio in serum, however, vary widely. Reports on the average free β-subunit to hCG ratio for persistent trophoblast disease, for instance, range from 2.6 percent [11] to 37 percent [10]. The discrepancy in reported levels has deterred physicians from using these tests and has limited the interpretation of results [25,30].

Kardana and Cole [25] investigated the cause of the wide variation in free β-subunit to hCG ratios. They determined levels in serum using two different free β-subunit immunoassays. The first assay used the monoclonal antibody 1E5. An immunoassay using antibody 1E5 was employed in several reports on free β-subunit levels, by Khazaeli and colleagues ([8], and four other articles), by Thomas and colleagues ([11], and one other article), and by our laboratory [2,4,6]. The second assay used monoclonal antibody FBT11. This assay has also been used in several reports on free β-subunit levels, by Ozturk and colleagues ([3,9], and four other articles). The two assays were carried out by similar procedures, using the free β-subunit specific monoclonal as capture antibody, and anti-β antisera peroxidase to detect bound molecules. As found (Table 4), qualitatively the two immunoassays gave similar results (higher percentage free β-subunit in trophoblast disease), but, quantitatively, the values differed up to tenfold. As such, results are assay-specific and not interchangeable.

Puisieux and colleagues [20] examined the free β-subunit produced in trophoblast disease and found that it was more extensively nicked than hCG. Kardana and colleagues [25] prepared 0 and 100 percent nicked β-subunit standards and tested the recognition of nicked molecules in the 1E5 and FBT11 free β-subunit immunoassays. The assay using antibody 1E5 did not recognize nicked β-subunit (level for nicked β-subunit was 4 percent of non-nicked level), detecting only intact β-subunit molecules. The immunoassay using antibody FBT11, however, gave similar results with nicked and non-nicked standards (nicked β-subunit level was 96 percent of
TABLE 4
Testing of Individual Serum Samples for hCG and Percentage of Free β-Subunit

| Diagnosis                        | hCG Assay | Free b-Subunit Assays |
|----------------------------------|-----------|----------------------|
|                                  | 2119-12/BP052 | 1E5/BP052 | FBT11/BP052 |
|                                  | Anti-α-Anti-β | Anti-Free β-Anti-β | Anti-Free β:Anti-β |
|                                  | Sandwich Assay | Sandwich Assay | Sandwich Assay |
|                                  | (μg/ml (mIU/ml)) | (% of hCG level) | (% of hCG level) |
| First trimester pregnancy, 5 weeks | 7.3 (68,000) | 1.0 | 0.55 |
| First trimester pregnancy, 6 weeks | 2.0 (18,600) | 1.0 | 0.45 |
| First trimester pregnancy, 6 weeks | 4.1 (38,100) | 0.25 | 0.37 |
| First trimester pregnancy, 7 weeks | 8.9 (83,000) | 0.17 | 0.52 |
| First trimester pregnancy, 7 weeks | 2.5 (23,000) | 0.53 | 0.57 |
| First trimester pregnancy, 7 weeks | 2.4 (22,000) | 1.7 | 0.66 |
| First trimester pregnancy, 7 weeks | 5.8 (54,000) | 0.97 | 0.67 |
| First trimester pregnancy, 7 weeks | 2.5 (23,000) | 0.28 | 0.79 |
| First trimester pregnancy, 8 weeks | 15.5 (144,000) | 0.43 | 0.58 |
| First trimester pregnancy, 8 weeks | 3.9 (36,000) | 0.26 | 0.13 |
| **Mean ± S.D.**                  | **5.5 ± 4.2** | **0.66 ± 0.49** | **0.53 ± 0.18** |
| Partial mole, pre-evacuation     | 60 (558,000) | 0.43 | 2.12 |
| Partial mole, pre-evacuation     | 30 (279,000) | 0.71 | 18.6 |
| Partial mole, pre-evacuation     | 9.6 (89,000) | 0.20 | 1.95 |
| Partial mole, pre-evacuation     | 19.6 (182,000) | 1.2 | 13.7 |
| Partial mole, pre-evacuation     | 3.1 (28,000) | 0.16 | 1.6 |
| Partial mole, pre-evacuation     | 195 (1,810,000) | 2.7 | 5.6 |
| Complete mole, pre-evacuation    | 9.0 (84,000) | 0.29 | 1.3 |
| Complete mole, pre-evacuation    | 61 (567,000) | 0.44 | 4.4 |
| Complete mole, pre-evacuation    | 146 (1,360,000) | 0.06 | 0.89 |
| Complete mole, pre-evacuation    | 116 (1,080,000) | 0.09 | 1.3 |
| Complete mole, pre-evacuation    | 49 (456,000) | 0.83 | 7.3 |
| Complete mole, pre-evacuation    | 49 (456,000) | 0.11 | 3.3 |
| **Mean ± S.D.**                  | **62 ± 60** | **0.61 ± 0.76** | **5.2 ± 5.6** |
| Invasive mole, pre-therapy       | 11.3 (105,000) | 0.91 | 11.6 |
| Invasive mole, pre-therapy       | 0.65 (6,050) | 2.2 | 7.4 |
| Invasive mole, pre-therapy       | 19 (177,000) | 0.89 | 14.7 |
| Choriocarcinoma, pre-therapy     | 121 (1,125,000) | 2.3 | 7.2 |
| Choriocarcinoma, pre-therapy     | 8.7 (81,000) | 0.18 | 2.7 |
| Choriocarcinoma, pre-therapy     | 8.8 (82,000) | 0.54 | 25.7 |
| **Mean ± S.D.**                  | **28 ± 46** | **1.2 ± 0.86** | **11.6 ± 8.0** |

*aCalculated assuming 70 μg = 650 IU (or 1μg = 9.3 IU), as established for preparing the first I.R.P. for immunoassay [32]

*bFree β-subunit level (μg/ml) presented as a percentage of hCG level (μg/ml)

non-nicked value), measuring total β-subunit. Kardana and colleagues [25] attributed the discordance in values seen with 1E5 and FBT11 assay results, and possibly the discordance with other free β-subunit immunoassay results, to differences in recognition of nicked free β-subunit molecules. Using the 1E5 assay, which only detects non-nicked molecules, a relatively small difference was observed in values for pregnancy and persistent trophoblast disease (twofold). Using the FBT11 assay, which detects total free β-subunit, a much bigger difference is observed (twentyfold). We infer that the FBT11 or total free β-subunit type of assay is preferable for the differential diagnosis of pregnancy, hydatidiform mole, and persistent trophoblast disease.
GENERAL CONCLUSIONS

The finding of a wide variation in the extent of peptide bond nicks in hCG isolated from both pregnancy and trophoblast disease urines was surprising. The additional discovery that nicks not only diminished the biological activity of hCG but also reduced the immunoreactivity of the hormone as measured by common monoclonal antibodies gave this finding greater importance. The potential diagnostic values of measurements of nicking are as yet unknown, but the necessity to evaluate measuring systems for sensitivity to nicked molecules is a serious and urgent consideration.

The finding of nicking in hCG may help to explain the variable biological activity of different hCG samples and standards, the discordant results obtained for hCG between different immunoassays, and the wide variation in free β-subunit immunoreactivity results. The origin of the nicking is still uncertain, and the possibility that nicked molecules have other, non-hCG, activities, such as immunosuppressive or growth factor activities should also be investigated.

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