Plasma

terone is 90.9 ± 2.8 in the follicular phase and 90.0 ± 4.8 in the luteal phase, and thus the binding does not vary.

How then may the apparent change in ratio be explained? One obvious explanation is by the heteroscedastic nature of the error function in radioimmunoassays. If the assay for plasma progesterone is set up for the optimal determination of all samples likely to be received, without dilution, the upper standard will correspond with 60 nmol/L or thereabouts and the lower standard (assuming a dynamic range of 20) with 3 nmol/L. Thus, most follicular-phase samples will fall between 0 and 3 nmol/L and would be reported as <3 nmol/L in many laboratories. A similar situation will occur in the saliva progesterone assay. Thus, the ratio in the luteal range will be determined with satisfactory precision, but in the follicular phase the plasma:saliva ratio will consist of the quotient of two poorly defined numbers, e.g., (<3 nmol/L)/(<60 nmol/L).

The author's estimate of 47.1 ± 38.5 illustrates the uncertainty.

Apart from precision, another crucial factor is the element of accuracy or bias. The specificity of Evans' assay will not be assisted by extracting progesterone with diethyl ether. It is surely a truism that the least-polar solvent giving adequate extraction efficiency should be used. Because, in general, cross-reaction curves are non-parallel, with the cross-reactant having a lesser slope, the cross reaction of any hydroxylated steroid extracted with ether will be greatest at low dosage. In brief, the bias will be relatively greater in the follicular phase.

Can bias explain Evans' data? If we assume that the true plasma:saliva ratio is 100, and consider samples from the follicular and luteal phase assayed by (a) an accurate assay and (b) an assay with a constant bias of 50 pmol/L, the results will be as summarized in Figure 1.

The results in the biased assay—plasma:saliva ratio in the luteal phase (91), follicular phase (50)—may be compared with Evans' data—luteal phase (115), follicular phase (47).

Interestingly, the follicular-phase values for saliva shown in Evans' figure are almost all at 100 pmol/L or more, whereas we would expect the majority of follicular-phase samples in our assay (1, 2) to be ≤56 pmol/L. This, again, could be interpreted as consistent with a bias of about 50 pmol/L. Similarly, his cutoff value of 15 nmol/L in plasma is higher than many would consider appropriate for the follicular:

![Figure 1. Effect of a bias of 50 pmol/L in a salivary progesterone assay on the apparent plasma:saliva concentration ratio (1984).](image)

The ratio is assumed to be actually constant. • • •, accurate assay; • • •, assay with consistent 50 pmol/L bias.

luteal transition.

These arguments do not, of course, prove that the assay of Evans is biased. They do, however, prove that a relatively small bias of 50 pmol/L, which is similar to the assay sensitivity, would account for the apparent variation of the plasma:saliva ratio. In our opinion, the case for lack of parallelism remains unproven.

References

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Argument In Favor of Using Mass Units to Calibrate and Report Concentrations of Human Chorionic Gonadotropin

To the Editor:

In recent years, treatment of patients with germ-cell tumors and gestational trophoblastic disease has been guided by information on the concentration of human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) in serum (1). Ordinarily these substances are measured with commercially available kits. Each manufacturer supplies its own polyclonal or monoclonal antibodies, calibrator, tracer, and controls. Almost without exception, the methods for hCG are calibrated in terms of International Units (int. units) and related either to the World Health Organization 2nd International Standard (2nd IS) or the 1st International Reference Preparation (1st IRP).

The decision to assign values in terms of international units was made by the National Committee for Clinical Laboratory Standards (NCCLS) in 1981 after it conducted a collaborative “round robin” study to determine which standard(s) was (were) suitable as National Reference Preparations for hCG. The 2nd IS and the 1st IRP from which were included in the survey, as were a pooled specimen of urine from first-trimester pregnancies, a “medium-pure” hCG preparation, a highly purified hCG preparation, and the USP standard. At a London meeting of the National Institute for Biological Standards and Control in the same year, it also was decided that potency assigned to standards should be expressed in international units.

Unfortunately, the values in such units obtained by the various methods cannot be related to each other: different kits give different values for the normal reference interval and indeed for the same samples. For example, the values ascribed to Lypchohek Immunoassay controls I, II, and III (Bio-Rad EOC Division, Anaheim, CA) for 24 different kits, in one from 7.7 to 24.9 for I, from 16.1 to 45.2 for II, and from 40.2 to 112.5 for III. Thus, there is no necessary relationship between values obtained for patients in one institution and those obtained in another, and institutional guidelines for treatment based upon these values cannot be compared easily.

An international unit of hCG can be any of the following:

- The relationship between the amount of hCG administered to a male frog (Rana pipiens) by intraperitoneal injection and the time required for gametokinesis to occur. This time varies with the season, 50 int. units being equal to 18 min in the spring, 42 min in the summer, and 88 min in the winter (2).
- Immature rat or mouse uterine weight or hyperemia units. One unit is
the amount of urine injected intraperitoneally that will cause a positive response. Thus, 4, 2, 1, or 0.2 mL injected corresponds to 250, 500, 1000, or 5000 hyperemia units/L (3).

- Storring et al. (4) report that the unit value of the IRP was measured by seminal vesicle weight gain, but fail to mention what animal was used.

- Bell et al. (5) assayed preparations by mouse uterine weight, finding differences in potency from 13.7 milli-int. units/ng to 18.6 milli-int. units/ng, depending upon the carbohydrate content of the preparations. The relationship between Canfield's preparation CR115 and the biological activity of the international standards is defined as follows: 1.0 int. unit = 1.28 μg of 2nd IS; thus the potency of the 2nd IS is 0.781 int. units/μg and of the 1st IRP is 9.3 int. units/μg (6).

- 1 int. unit = 100 μg of IS (League of Nations), the bioassay being the rat or mouse uterine weight gain in response to intraperitoneal injection of urine (7).

- Hyperemia of the rat testes in response to intraperitoneal injection of urine, no unit being defined (8).

The NCCLS in their prepared guidelines for Immunodiagnostic Procedures and Reagents (9) state: "The terms standard, control, and calibrator do not directly apply to immunodiagnostic procedures. This is due to the nature of most immunological reagents. It is difficult, and in fact, almost impossible to determine absolute mass units with most of these reagents." If this statement was ever true, it is not true today.

The London-based National Institute for Biological Standards and Control stated (personal communication, February 1986): "The rationale of assigning international units (instead of mass) to international standards consisting of mixtures of complex incompletely characterized substances is a fundamental principle of biological standardization adhered to over the years by the Expert Committee on Biological Standardization of WHO." This is an antiquated and illogical stance. Suitably pure preparations of hCG and of its subunits are available that can be and are being used to establish mass calibration units of hCG (5). In addition, because most of the methods that are being used for immunoaassay of hCG are based on antibodies to the β subunit, both the whole molecule and the free β subunit are being measured simultaneously. In cases of gestational trophoblastic disease and certain germ-cell tumors, free β subunit can constitute a sizable proportion of the total hormone being measured. Because these subunits have no biological activity, it is inappropriate to express values in international units that are based on biological activity.

Table 1 illustrates the extent of the confusion that has been engendered by the use of international units to calibrate the hCG kits available from different manufacturers. The values in column 3 (milli-int. units/ng) are rarely included in the kit inserts. The values listed were obtained by personal communication with the technical services division of each of the vendors. Where the value is not listed, it is because the information could not be supplied by the vendor. Of particular interest is the variation in biological potency of the preparations provided by the laboratory of Dr. Canfield. Each of these is a highly purified preparation of the whole molecule of hCG, which could be used as a standard for mass. The variations in biological potency are probably caused by variations in the degrees of sialylation, a consequence of the purification methods (4).

Thus, the use of international units to calibrate immunoassays for hCG is an anachronism that should be abandoned in favor of mass units. In fact, mass units should be used in every instance where there is sufficiently pure material to construct an accurate calibration.

### Table 1. Concentration of hCG, in Mass Units, in Various Commercial Kits

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Standard</th>
<th>milli-Int. units/ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>WHO 2nd Is 61/6</td>
<td>2.5</td>
</tr>
<tr>
<td>Amersham</td>
<td>WHO 2nd Is 61/6</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>1st IRP 75/537</td>
<td>9.2</td>
</tr>
<tr>
<td>Bio-Rad</td>
<td>1st IRP</td>
<td>9.8</td>
</tr>
<tr>
<td>Boehringer Mannheim</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Cinecisco</td>
<td>2nd Is 61/6</td>
<td>5.0–5.5</td>
</tr>
<tr>
<td>Clinical Assays</td>
<td>1st IRP</td>
<td>9.3</td>
</tr>
<tr>
<td>Corning</td>
<td>2nd IS</td>
<td>6.6</td>
</tr>
<tr>
<td>Diagnostic Products</td>
<td>1st IRP</td>
<td>14.3</td>
</tr>
<tr>
<td>Endotech (RSL)</td>
<td>1st IRP</td>
<td>9.8</td>
</tr>
<tr>
<td>Hybritech</td>
<td>1st IRP</td>
<td>12.0</td>
</tr>
<tr>
<td>Leeco</td>
<td>1st IRP</td>
<td>10.0/9.3</td>
</tr>
<tr>
<td></td>
<td>2nd IS</td>
<td>5.0</td>
</tr>
<tr>
<td>Mallinckrodt</td>
<td>2nd IS</td>
<td>11.0 ± 1.0</td>
</tr>
<tr>
<td>Micromedic</td>
<td>2nd IS</td>
<td>4.0</td>
</tr>
<tr>
<td>NML</td>
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<td>NA</td>
</tr>
<tr>
<td>NMS</td>
<td>2nd IS</td>
<td>10.0</td>
</tr>
<tr>
<td>Sorono</td>
<td>2nd IS</td>
<td>NA</td>
</tr>
<tr>
<td>Wampole</td>
<td>2nd IS</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>CR115</td>
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<tr>
<td></td>
<td>CR117</td>
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<tr>
<td></td>
<td>CR119</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>CR121</td>
<td>13.45</td>
</tr>
</tbody>
</table>

NA = not available. *Values supplied by vendors. Milli-int. units are used here to conform to common usage. *Preparations made by the lab. of Dr. Robert Canfield, Columbia University; distributed by NIMDD.
Triclonal Gammopathy in a Patient with AIDS

To the Editor:

Two recent reports (1,2) have documented an increased incidence of monoclonal gammopathies in patients with acquired immunodeficiency syndrome (AIDS). In these studies the para-proteins were uniformly of the IgG kappa type. In light of these findings we now report a case of triclonal gammopathy in a patient with AIDS.

In a previous case report (3) we described a triclonal gammopathy with cryoglobulinemia in a 50-year-old man who presented with symptoms of hyperviscosity syndrome and has subsequently developed AIDS. The gammopathy was composed of IgM kappa, IgG kappa, and IgG lambda components. The patient had a prior history of bisexual activity two years before and had an inverted T helper cell to T suppressor cell ratio at the time of his presentation. At that time there was no history of lymphadenopathy, opportunistic infections, or Kaposis sarcoma. Bone-marrow biopsy revealed a mildly increased proportion of plasma cells (10–20%) with a "polyclonal" staining pattern on immunohistochemical staining for kappa and lambda light chains.

The patient was treated with plasmapheresis and a course of chlorambucil and prednisone for three months, with control of his symptoms but with persistent immunoglobulin and T-cell abnormalities. Two months after stopping therapy he developed severe watery diarrhea; *I. belli* was isolated. This organism has been associated with diarrhea in some AIDS patients (4). One month later he developed cutaneous Kaposis sarcoma and a diagnosis of AIDS was made. His health deteriorated progressively and he died six months later.

Many B-cell abnormalities have been reported in association with AIDS, including increased bone-marrow lymphoplasma cytosis (5); plasmacytoma (6); B-cell lymphomas (7); and polyclonal (8), oligoclonal (9), and monoclonal gammopathies (1,2). The etiology of these abnormalities is unknown; they are variably attributed to the loss of T-cell regulation of B-cells, the stimulation of numerous infectious agents, or direct infection of B-cells by HTLV-III virus. In our patient a triclonal gammopathy developed in association with immune system abnormalities (altered T-cell subsets) but prior to the development of frank AIDS. This case expands the range of gammopathies described in association with AIDS to include IgM heavy chains and lambda light chains, which had not been described in other reports.

References


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Higher Values for Digitalis-Like Factors with TDx Digoxin II

To the Editor:

The importance of digitalis-like factors (DLF) in relation to the assay of serum digoxin has been recently reviewed (7). Clinically important values by radioimmunoassay are most often found in neonates, late pregnancy, and in patients with hepatic and (or) renal failure.

The TDx automated system for digoxin (Abbott Laboratories, Abbott Park, IL 60064) was used by more than 40% of the laboratories in recent CAP and AAC surveys (2). We have previously reported that values for DLF were often of little clinical consequence when measured by the Abbott "TDx Digoxin II" fluorescence polarization method in patients with combined hepatic and renal failure (3) and in renal failure (4), in contrast to clinically important values found in some of these patients by RIA. Our results confirmed the observations of others using the TDx Digoxin I method in patients with renal failure (5,6). Also, we found that values for DLF by that TDx Digoxin I method were generally lower for cord bloods than those obtained by various RIAs.

In late 1985, Abbott introduced "TDx Digoxin II" to eliminate the potential variation in results due to abnormal concentrations of serum proteins seen with TDx Digoxin I. In the new method a 30 g/L solution of sulfosalicylic acid in water/methanol (1:1) is used for protein precipitation, instead of the trichloroacetic acid used in TDx Digoxin I. The only other change was apparently in the "pretreatment solution," where there has been a change from phosphate to Tris buffer.

We have found that the TDx Digoxin II assay gave falsely high values for digoxin in three patients with combined hepatic and renal failure, and clinically important values in one patient with hepatic failure and for sera or plasma from 11 cord bloods (Table I).

Detectable values for DLF in plasma were found in four of five patients with dialysis-dependent renal failure and in four of 10 healthy subjects. None of these patients and none of the mothers of the infants had ever received digoxin. Higher DLF values were found with TDx Digoxin II in 19 of 20 sera or plasmas as compared with values found with use of two RIA kits for the patients with hepatic and (or) renal failure, and with four RIA kits for all 11 cord bloods. TDx Digoxin II values ranged from 1.2 to 1.5 mmol/L for the