Mechanism of Specificity in Radioimmunoassay for Human FSH after HCG Adsorption of Antisera (34289)

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A number of methods for the radioimmunoassay of human follicle-stimulating hormone (FSH) have been described in recent years (1–7). In all of these (1–4, 6, 7), except one (5), human chorionic gonadotropin (HCG) was added to the assay systems in order to confer complete specificity for FSH.

We have previously speculated that the HCG might be acting in one of two ways in these systems (3, 8). If the labeled FSH used as trace contained significant amounts of labeled luteinizing hormone (LH) and the antisera contained a population of antibodies directed against LH (or HCG since the immunological cross-reactivity of LH and HCG is well recognized (9)) in addition to a population of FSH-specific antibodies, then the addition of HCG would be acting by adsorbing out these anti-LH antibodies or diluting out the labeled LH so that little or none bound to the anti-LH antibodies. On the other hand, the initial nonspecificity of these various antisera might be due entirely to the antiserum alone—that is to the presence of a population of antibodies which fails to discriminate among the glycoprotein hormones [FSH, LH, HCG, and perhaps thyroid-stimulating hormone (TSH)], as well as a population of FSH-specific antibodies. The present communication presents three lines of evidence which indicate that this latter possibility is the case in our FSH radioimmunoassay system.

Methods. The radioimmunoassay procedure has been described in detail (3, 8). The first stage of this double-antibody procedure makes use of anti-FSH antisera prepared by immunizing guinea pigs with crude (potency 5–10 NIH-FSH-S1 U/mg) human pituitary FSH extracts; a highly purified human pituitary FSH (potency 100 NIH-FSH-S1 U/mg) for labeling and assay standard; and 0.7–1.4 IU of HCG/assay tube to confer specificity of the assay to FSH. The second stage consists of rabbit antiguinea pig serum and carrier guinea pig serum which results in precipitation of the antibody-bound labeled FSH. The antibody is used at a dilution which binds about 25–35% of the labeled FSH added, usually 0.1 mμg. In the results to be presented control tubes, containing no unlabeled FSH, are assigned a value of 100% bound.

In one experiment, described below, the FSH used for labeling, 11867C contained an equivalent FSH potency to that of our standard preparation 5765B both by bioassay and radioimmunoassay, but only about one-third the LH activity by radioimmunoassay, *i.e.*, approximately 5–6% on a weight basis (8).

In another experiment, the standard FSH label 5765B was adsorbed with an HCG antiserum, known to be specific for HCG and LH (10). One hundred and seventy mμg of ^{131}I-labeled FSH (320 μCi/μg) in a volume of 0.2 ml were incubated for 72 hr with 0.2 ml of HCG antiserum GP39B1 diluted 1:100 with 1% BSA–barbital buffer 0.07 *M*, pH8.6. Separation of antibody-bound label from free label was accomplished by layering the incubation mixture on a 45 × 1.2-cm column of Sephadex G-100 previously equilibrated with the BSA–barbital buffer. The material was eluted with barbital buffer and collected in 1-ml fractions. These were counted in a γ-well scintillation counter at a fixed distance from the detector. The retarded peak, presumably material not bound to

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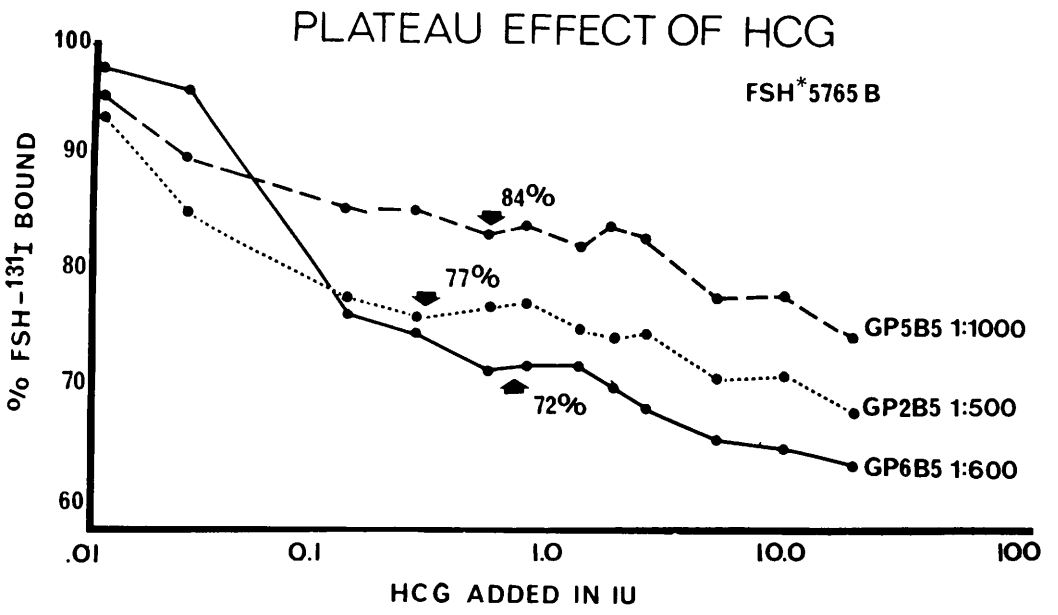

FIG. 1. The effect of HCG on the displacement of labeled FSH from binding to three different FSH antisera.

antibody and thus representing ^{131}I-FSH now freed of LH contamination was subsequently used as trace in the radioimmunoassay.

Results and Discussion. Plateau effect of added HCG in FSH assay. Figure 1 shows the effect of added commercial HCG[2] on the binding of FSH label to three different FSH antisera. It should be noted that a plateau is observed with all three antisera but at different points with each, 84, 77, and 72%. It was previously reasoned, based on the observation that our two original FSH antisera (3, 8) both showed a plateau near 80% bound, and that the LH contamination of the FSH used for iodination amounted to 15–20%[3] on a weight basis by radioimmunoassay for LH, that the explanation for the conferring of specificity in the FSH assay by adding a plateau amount of HCG could well be due to adsorbing out LH-specific antibodies which were reacting with the LH-^{131}I known to contaminate our FSH label. (We assumed that the LH present in our FSH preparation iodinated equally well. We now have evidence to suggest that LH, in fact, iodinates less well.) However, this explanation does not now appear tenable since if this were true the level of our plateau should never be less than 80%, *i.e.*, the difference from 100% should never be more than the maximum percentage of LH-^{131}I present in our FSH label. Thus, the possibility of a second species of antibodies which does not distinguish between FSH and HCG is suggested.

Effect of added HCG in FSH assay using FSH label relatively free of LH activity. It was reasoned that if part of the problem were due to the FSH label's containing significant quantities of labeled LH, then using an FSH preparation relatively devoid of LH activity should minimize the effect of added HCG on displacing the label from antibody-binding sites. Thus, the FSH preparation 11867C, relatively LH-free was used as trace. The effects of added HCG, in this case a more purified preparation,[4] are shown in Fig. 2. It should be Noted that a plateau is seen at "80% bound" which is the same as that noted with this particular antiserum and the

[2] A. P. L. Ayerst, Montreal, Canada; containing approx 3500 IU/mg.

[3] Assuming homogenous LH to have a potency of 4 NIH-LH-S1 U/mg.

[4] Gift of Dr. A. Albert, Mayo Clinic, containing 8700 IU/mg.

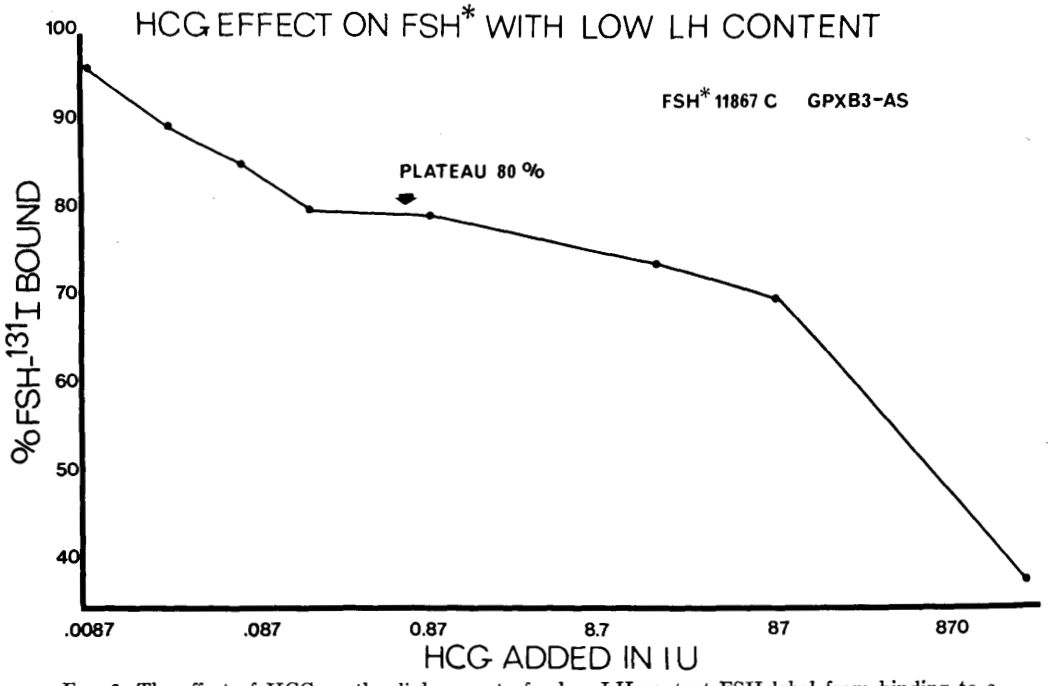

FIG. 2. The effect of HCG on the diplacement of a low-LH-content FSH label from binding to a FSH antiserum.

5765B label which contains far more LH (8).

Were the plateau effect due to LH contamination of the label alone then the plateau would be expected to be no less than 94–95%. The fact that it was significantly less again points to a partially cross-reacting population of antibodies which are being saturated by HCG. The explanation for the further displacement of label from antibody-binding sites at very high HCG concentrations is probably due to the small amounts of immunologically-reactive FSH previously shown to be present in HCG (10).

Effect of added HCG in FSH assay using FSH label with LH removed by adsorption with HCG antiserum. The elution pattern from the FSH label 5765B incubated with HCG antiserum on a Sephadex G-100 column is shown in Fig. 3. Two peaks are shown. The unretarded peak coincident with the blue dextran void volume presumably represents labeled LH which is antibody bound (mol wt γ-globulin approximately 160,000). The retarded peak presumably represents the FSH label which does not bind to the HCG antibody. It should be noted that 18.4% of the label bound to antibody which agrees very well with the approximate 15–20% contamination by LH known to be present in this preparation by radioimmunoassay for LH (8).

A pool of the back half of the second peak, shown in Fig. 3, was used as trace in the FSH radioimmunoassay and the results are shown in Fig. 4. Note that despite the fact that the trace is now theoretically devoid of LH, the same plateau phenomenon is shown with the addition of HCG (compare with Fig. 2), so that one must conclude that the initial cross-reactivity observed with HCG is due to the presence of a species of antibodies in the FSH antiserum which does not discriminate between labeled FSH and HCG, *i.e.*, a cross-reacting species of antibody.

It is well recognized that certain antisera, perhaps most, prepared by immunization with semipurified FSH material, contain solely cross-reacting antibodies which appear to be directed against a portion of the molecule which is common to the four glycoprotein hormones—FSH, LH, HCG, and also TSH

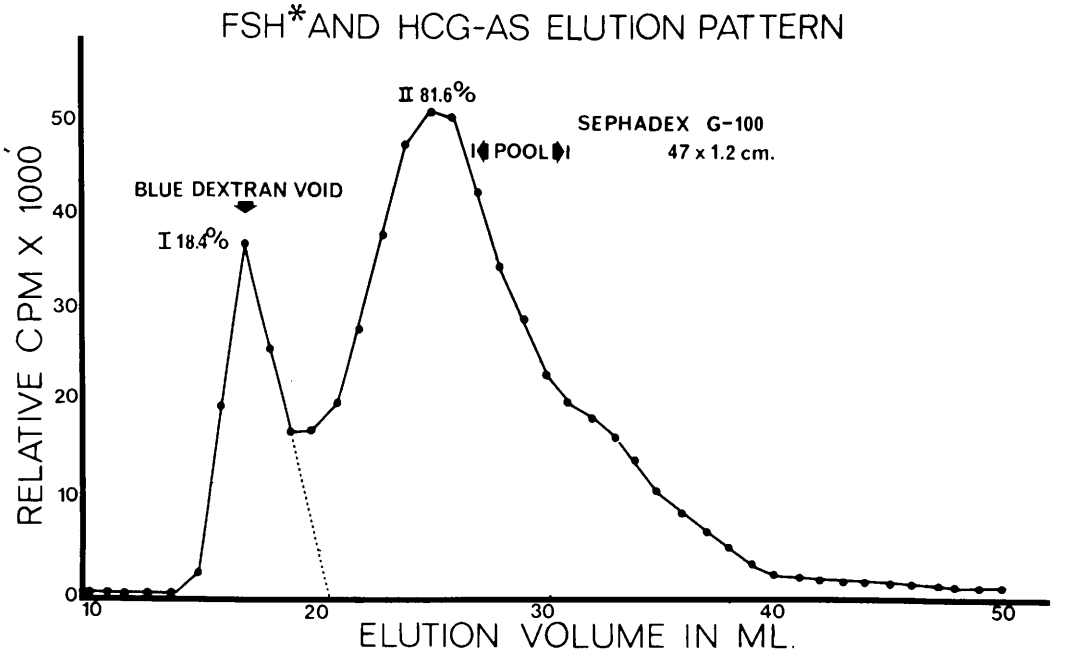

FIG. 3. Elution pattern of FSH label preincubated with HCG antiserum on a Sephadex G-100 column.

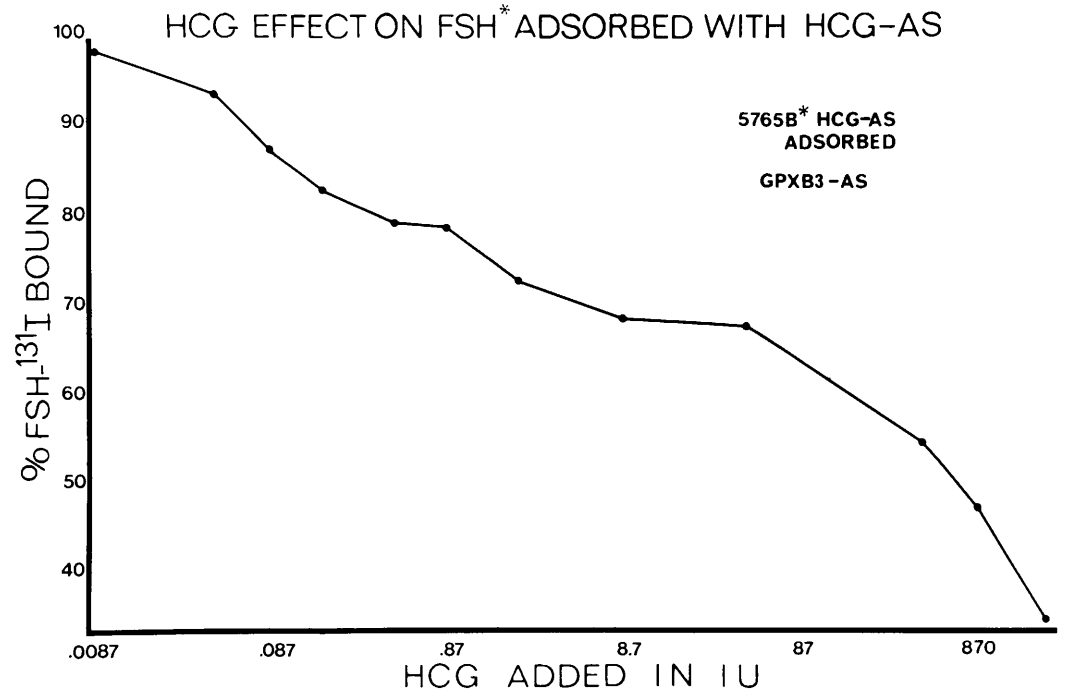

FIG. 4. The effect of HCG on the displacement of LH-adsorbed FSH label from binding to a FSH antiserum.

and fail to even partially distinguish among these (8, 9, 11–13). Thus, it is not difficult to imagine that certain antisera prepared against FSH will contain antibodies directed solely against FSH, and others will contain a cross-reacting species of antibodies in addition to the FSH-specific antibodies, as has been demonstrated in the present study. These cross-reacting antibodies can be adsorbed out by HCG, leaving the FSH-specific antibodies to react with the FSH label and not be further interfered with by the other glycoprotein hormones.

Summary. Three lines of evidence are presented which show that the mechanism whereby the addition of HCG confers FSH specificity on the FSH radioimmunoassay is by adsorbing out a species of antibodies which fails to distinguish among the glycoprotein hormones, FSH, LH, and HCG, leaving a second species of FSH-specific antibodies to react with the FSH label in the system.

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