The Kinetics of Formation and Biological Activity of Native and Hybrid Molecules of Human Follicle-stimulating Hormone*

(Received for publication, February 26, 1974)

LEO E. REICHERT, JR.,† C. G. TROWBRIDGE, VINOD K. BHALLA, AND GEORGE M. LAWSON, JR.

From the Department of Biochemistry, Division of Basic Health Sciences, and Department of Chemistry, Emory University, Atlanta, Georgia 30322

SUMMARY

A rat testes tubule tissue receptor assay was utilized to study the kinetics of combination of the hormone-specific \( \beta \) subunit of human follicle-stimulating hormone (hFSH) with the \( \alpha \) subunits of hFSH, human luteinizing hormone (hLH), bovine luteinizing hormone (bLH), and human chorionic gonadotropin (hCG). In this competitive protein-binding assay, \(^{125}\text{I}\)-hFSH is the radioligand and FSH activity is measured by the ability of the test substance to inhibit uptake of the radioligand by the rat testes tubule receptors. Rate data for combination of subunits were analyzed for quality of fit to first or second order integrated rate equations by nonlinear regression analysis. The time course for the appearance of FSH activity upon incubation of the various subunit combinations was neither clearly first or second order, although each simple mechanism could be fitted to the experimental data. We conclude that at least one kinetically distinguishable state follows formation of the initial \( \alpha/\beta \) subunit complex and that this complex has a binding affinity for its receptors in rat testes tubules which is much less than that of subsequent states. Hybrid molecules of bLH-\( \alpha \)/hFSH-\( \beta \), hLH-\( \alpha \)/hFSH-\( \beta \), and hCG-\( \alpha \)/hFSH-\( \beta \) were formed at differing rates, but each was more rapid than the combination of the homologous hFSH-\( \alpha \)/hFSH-\( \beta \) pair. Maximum combination always occurred by 1440 min of incubation. Hybrid molecules formed after this time were tested for FSH activity by the in vivo rat testes tubule receptor assay and results compared with FSH activity as measured by a classical in vivo bioassay. The in vitro activity of hFSH-\( \alpha \)/hFSH-\( \beta \), hLH-\( \alpha \)/hFSH-\( \beta \), and hCG-\( \alpha \)/hFSH-\( \beta \) hybrid molecules was from 2 to 4 times greater than that of intact hFSH, but the activity of the hybrid molecules in vivo was less than that of intact hFSH. Of particular interest were the properties of the hybrid molecule formed between bLH-\( \alpha \)/hFSH-\( \beta \). This hybrid had an in vitro activity which was 2.8 times greater than that of purified hFSH. The activity of the bLH-\( \alpha \)/hFSH-\( \beta \) hybrid as measured by the in vivo bioassay, however, was only 0.14 times the activity of purified hFSH. The ratio of activity in vitro to in vivo for this artificial FSH molecule was 19.8. It is suggested that the bLH-\( \alpha \)/hFSH-\( \beta \) hybrid has a greater affinity than native hFSH for receptors in rat testes tubules, but that expression of in vivo biological activity is depressed probably because of factors related to its more rapid clearance from the circulation.

Fituitary and placental glycoprotein hormones of different types and from several sources have been shown to be composed of two dissimilar subunits, designated as the (common) and \( \beta \) (hormone-specific) subunits. It has been possible to form hybrid molecules from various combinations of these subunits, with the qualitative nature of the \( \alpha + \beta \) hybrid molecule being dependent upon the source of the \( \beta \) subunit. For example, a molecule formed from the \( \alpha \) subunit of LH and the \( \beta \) subunit of TSH will have TSH biological activity. We have previously reported initial studies on the formation and biological activity of native and hybrid molecules of LH and hCG. The rate of combination of the \( \alpha \) and \( \beta \) subunits of LH from various species was followed through use of a carefully validated rat testes Leydig cell receptor assay for LH (2), and the properties of the hormone-receptor interaction have also been studied in detail. We have demonstrated, on the basis of biological studies, that human FSH also consists of two dissimilar subunits (5, 6) and have succeeded in achieving a separation and purification of the hFSH \( \alpha \) and \( \beta \) subunits (7). The details of the interaction of hFSH with specific receptors in rat testes tubules have been described earlier in this journal (8), and application of the tissue receptor system to quantitation of human and animal pituitary FSH has also been reported.

In this report, we describe application of the rat testes tubule receptor assay to the study of the kinetics of combination of the hormone specific \( \beta \) subunit of hFSH with the \( \alpha \) subunits of hFSH, hLH, bLH, and hCG. The newly formed FSH molecules are compared with respect to their FSH activity in the rat testes tubule receptor assay and in a hormone-specific in vivo bioassay.

* This is publication No. 1215 from the Division of Basic Health Sciences. This study was supported by United States Public Health Service Grants AM 3598 (L. E. R.), AM 4675 (C. G. T.), and HD 0828 (L. E. R.).
† To whom all correspondence should be addressed.

6472
Preparation of Rat Testes Tubule Homogenates—Mature rats (250 to 300 g) of the Sprague-Dawley strain were killed by Dry Ice asphyxiation immediately prior to use. Testes were taken and weighed to the nearest milligram. The tunica albuginea was then removed and the rat testes tubule homogenate prepared as described elsewhere (9).

Procedure for Iodination of hFSH—Highly purified human FSH, prepared as described previously (11, 12), was used as the radioligand in these studies. This preparation, LER-1575-C, had a FSH biological activity of 3608 i.u. per mg and an LH activity of 100 i.u. per mg, and showed a single component following acrylamide gel electrophoresis in a discontinuous buffer system (20) in 0.01 M phosphate buffer, pH 7.5, containing 5 mM MgCl₂, 0.1 M sucrose, and 0.1% egg albumin (twice crystallized), followed by aliquots of a standard or unknown to make a final volume of 500 μl. To this was added chilled receptor preparation (150 μl) and then 5 ng of 125I hFSH in 50 μl, thus making a total incubation volume of 1 ml. The tubes were then incubated in a reciprocal water bath shaker, 100 oscillations per min, at a temperature of 37°C for 3 hours. Following incubation, the tubes were centrifuged at 1500 × g for 10 min and the supernatant discarded. The tissue pellet was resuspended in the cold in 1 ml of chilled buffer using a Vortex mixer and centrifuged for an additional 10 min. One such wash was found adequate to remove interfering amounts of unbound radioactivity. The supernatant from the second centrifugation was decanted, the tips of the tubes wiped off with Kimwipes, and the tubes counted in an autogamma spectrometer.

Preparation of Subunits of hFSH—Highly purified hFSH, 3608 i.u. per mg, was treated with 8 M urea, pH 6.0, for 10 hours at 25°C. The dissociated subunit mixture was chromatographed on DEAE-cellulose in 0.007 M phosphate-0.003 M borate buffer, pH 8.0. The fraction unadsorbed on the exchanger represented the α subunit of hFSH (7). The activity at a minimum of three concentrations, 2 to 500 i.u./mg was treated with 8 M urea, pH 6.0, for 10 hours at 25°C. The dissociated subunit mixture was chromatographed on DEAE-cellulose in 0.007 M phosphate-0.003 M borate buffer, pH 8.0. The fraction unadsorbed on the exchanger represented the α subunit of hFSH (7). The adsorbed fraction was eluted with 0.5 M NaCl, recovered by dialysis and lyophilization, and then passed through a column of Sephadex G-100 to achieve a final purification of the hFSH-β subunit (7). The in vitro and in vivo biological activities of the hFSH α and β subunits are given in Table I.

Table I

| Combination             | Biological activity* | Per cent activity of pure FSH* | Ratio of per cent activity in vitro to in vivo |
|-------------------------|----------------------|-------------------------------|---------------------------------------------|
|                         | in vitro | in vivo | in vitro | in vivo | in vitro \(\frac{B_i}{B_r}\) to in vivo \(\frac{B_i}{B_r}\) |
| hFSH (monodissociated)  | 2538     | 2407   | 3608     | 3296   | \(57.6\)                               |
| hFSH-α + hFSH-β         | 802      | 810    | 510      | 530    | \(15.8\)                               |
| hLH-α + hLH-β           | 1200     | 1200   | 1200     | 1200   | \(1.0\)                                |
| hL-α + hL-β             | 3477     | 3363   | 2180     | 2180   | \(1.0\)                                |
| bLH-α + hFSH-β          | 7061     | 7061   | 510      | 530    | \(1.0\)                                |
| hFSH-α                  | 2.5 (2.6-116) | 10 | 0.1 (0.3) | 0.3 | \(2.7\)                                |
| hFSH-β                  | 78 (109-61) | 39 (35-43) | 3.1 (1.3) | 1.3 | \(2.7\)                                |

* LER-907, 20 i.u. per mg, run as standard in each type of assay.

* Obtained by dividing the activity of each combinator by 2538 (for in vitro assay) and 3608 (for in vivo assay) (9).

* The in vitro assay refers to the tissue receptor assay as described in Ref. 9. The in vivo assay refers to the Steelman-Pohley assay (14) as modified in (15). For additional description, see text.
FIG. 1. A, combination of hFSH-α + hFSH-β subunits as a function of time. The subunits of hFSH were prepared as described elsewhere (7). One milligram of each subunit was dissolved in 1 ml of 0.01 M phosphate buffer, pH 7.0 (final protein concentration 2 mg per ml), and allowed to incubate for various periods of time (m, minutes of incubation). After each time interval, an aliquot was withdrawn and immediately diluted and frozen to stop the process of combination, which is temperature and concentration dependent. Following removal of the final aliquot (1440 min of incubation), the various samples were analyzed for FSH activity by the tissue receptor assay (9). The time-dependent increase in the ability of the various incubates to inhibit the uptake of 125I-hFSH (5 ng) by the receptor preparation (45 mg, wet weight) is taken to reflect gradual combination of the hFSH-α + hFSH-β subunits. The FSH specific activity at each time of incubation was calculated in terms of LER-1575-C (3608 i.u. per mg), a highly purified hFSH preparation which was always run as the reference preparation. B, combination of bLH-α + hFSH-β as a function of time. This and the other combinations discussed in the text were carried out as described for A. Note that in this instance, maximum combination again occurred after 1440 min of incubation, but the specific activity of the newly formed hybrid was greater than that of LER-1575-C. In this assay, the response curve at 0 min was not parallel to the other curves shown.

All three equations are in forms suitable for direct application of nonlinear fitting procedures. Equations 1 and 2 have two adjustable parameters whose values are to be found; Equation 3 has three adjustable parameters, p being the ratio of $a_0$:$\beta_0$. The program used in the analysis provides for fixing selected coefficients at predetermined values, and in the present work p was treated as both free and fixed in the model fitting experiments. By assuming 100% subunit content of the preparation on a mass basis, it was possible to calculate the second order rate constant, $k_2$, in the familiar units of $h^{-1}$ (Table II). Because of the assumptions involved, however, the values of $k_2$ in the last column of Table II must be regarded as estimates.

RESULTS

Kinetic Analysis—Seven data sets were obtained, four of them for the combination of the α and β subunits of hFSH and one each for the combination of the hFSH-β subunit with the α subunits of hLH, bLH, and hCG. Each data set was tested for fit
trials, small systematic deviations between fits of similar quality. In all cases, whether first or second order fitting trials show that the first and second order equations gave results depicted in the upper panel can be related to Fig. 1A. The curve depicted in the lower panel can be related to Fig. 1B.

The ratio of activity in vitro to in vivo ranged from 2 to 4 for all comparisons, with the exception of that for the bLH-α + hFSH-β hybrid, where the ratio was 19.8. The significance of this discrepancy was even more marked with the combination bLH-α + hFSH-β. Here the in vitro activity of the hybrid was 7081 i.u. per mg or 27.8% that of undissociated human FSH. In contrast, the in vivo activity of the hybrid was 510 i.u. per mg.

This discrepancy was even more marked with the combination bLH-α + hFSH-β. Here the in vitro activity of the hybrid was 7081 i.u. per mg or 27.8% that of undissociated human FSH. In contrast, the in vivo activity of the hybrid was 510 i.u. per mg.

The hybrid molecules resulting from combination of bLH-α + hFSH-β and hCG-α + hFSH-β had in vivo activities (receptor assay) at least as great as that of undissociated hFSH, while in each instance, the in vitro activity of the hybrid molecules was significantly less than that of undissociated hFSH (Table I). This discrepancy was even more marked with the combination bLH-α + hFSH-β. Here the in vitro activity of the hybrid was 7081 i.u. per mg or 27.8% that of undissociated human FSH. In contrast, the in vivo activity of the hybrid was 510 i.u. per mg.

| Subunit source | Rate constants |
|----------------|----------------|
|               | First order    | Second order   |
| α             | β              | Per cent⁻¹ hr⁻¹ | M⁻¹ hr⁻¹ |
| hFSH          | hFSH           | hFSH           | hFSH     |
| hCG           | hFSH           | hFSH           | hFSH     |
| hLH           | hFSH           | hFSH           | hFSH     |
| hLH           | hLH            | hLH            | hLH     |
| hLH-α + hFSH-β| 0.190 ± 0.0143 | 0.0032 ± 0.0000 | 2.490 |
| hCG-α + hFSH-β| 0.110 ± 0.0114 | 0.0045 ± 0.0004 | 6.360 |
| bLH-α + hFSH-β| 0.238 ± 0.0159 | 0.0019 ± 0.0007 | 3.430 |
| bLH-α + hFSH-β| 0.267 ± 0.0230 | 0.0028 ± 0.0007 | 4.500 |
| hLH-α + hFSF-β| 2.60 ± 0.310  | 0.0870 ± 0.0008 | 56.000 |

* Range of the 95% confidence limits.
* Estimated from per cent⁻¹ hr⁻¹ by assuming an average molecular weight for each subunit of 15,000.
* Rate constant for combination of hLH subunits calculated as described in (2).

**Table II**

Rate constants for combination of gonadotropin subunits

| Subunit source | Rate constants |
|----------------|----------------|
|               | First order    | Second order   |
| α             | β              | Per cent⁻¹ hr⁻¹ | M⁻¹ hr⁻¹ |
| hFSH          | hFSH           | hFSH           | hFSH     |
| hCG           | hFSH           | hFSH           | hFSH     |
| hLH           | hFSH           | hFSH           | hFSH     |
| hLH           | hLH            | hLH            | hLH     |
| hLH-α + hFSH-β| 0.190 ± 0.0143 | 0.0032 ± 0.0000 | 2.490 |
| hCG-α + hFSH-β| 0.110 ± 0.0114 | 0.0045 ± 0.0004 | 6.360 |
| bLH-α + hFSH-β| 0.238 ± 0.0159 | 0.0019 ± 0.0007 | 3.430 |
| bLH-α + hFSH-β| 0.267 ± 0.0230 | 0.0028 ± 0.0007 | 4.500 |
| hLH-α + hFSF-β| 2.60 ± 0.310  | 0.0870 ± 0.0008 | 56.000 |

* Range of the 95% confidence limits.
* Estimated from per cent⁻¹ hr⁻¹ by assuming an average molecular weight for each subunit of 15,000.
* Rate constant for combination of hLH subunits calculated as described in (2).

**Table II**

Rate constants for combination of gonadotropin subunits

| Subunit source | Rate constants |
|----------------|----------------|
|               | First order    | Second order   |
| α             | β              | Per cent⁻¹ hr⁻¹ | M⁻¹ hr⁻¹ |
| hFSH          | hFSH           | hFSH           | hFSH     |
| hCG           | hFSH           | hFSH           | hFSH     |
| hLH           | hFSH           | hFSH           | hFSH     |
| hLH           | hLH            | hLH            | hLH     |
| hLH-α + hFSH-β| 0.190 ± 0.0143 | 0.0032 ± 0.0000 | 2.490 |
| hCG-α + hFSH-β| 0.110 ± 0.0114 | 0.0045 ± 0.0004 | 6.360 |
| bLH-α + hFSH-β| 0.238 ± 0.0159 | 0.0019 ± 0.0007 | 3.430 |
| bLH-α + hFSH-β| 0.267 ± 0.0230 | 0.0028 ± 0.0007 | 4.500 |
| hLH-α + hFSF-β| 2.60 ± 0.310  | 0.0870 ± 0.0008 | 56.000 |

* Range of the 95% confidence limits.
* Estimated from per cent⁻¹ hr⁻¹ by assuming an average molecular weight for each subunit of 15,000.
* Rate constant for combination of hLH subunits calculated as described in (2).
ble I). Assuming no increase in FSH activity resulting from combination of the subunits to form active $\alpha \beta$ dimers, the in vivo biological activity of a 1:1 mixture of the subunits would be the mean value of the activity of the individual subunits, or 26 i.u. per mg. The observed potency of 510 i.u. per mg, therefore, represented a highly significant 21-fold augmentation of FSH activity. By similar reasoning, the activity of hFSH-$\alpha + hFSH-\beta$ as measured by the in vitro assay, 882 i.u. per mg, represents a highly significant 25-fold increase in activity over what would be expected if no combination of subunits occurred. It should be noted that the in vitro activity of the hCG-$\alpha + hFSH-\beta$ hybrid relative to intact hFSH, was relatively high, about 60%. Therefore, the low recovery of activity in the hFSH-$\alpha + hFSH-\beta$ combinator would seem to reside in a defect in the hFSH-$\alpha$, rather than with the hFSH-$\beta$ subunit.

**DISCUSSION**

We have recently reported studies on the kinetics of formation and biological activity of native and hybrid molecules of LH and hCG, utilizing as a tool for assessment of recombination a rat testes Leydig cell receptor assay for LH (2, 3). The rates of dissociation and combination of subunits of hLH and hCG have also been studied by application of a fluorescence probe, anilinonaphthalene sulfonate, to follow the course of the reaction (22). There is no information, however, on similar studies with FSH.

We have reported a procedure for separation and isolation of subunits of hFSH (7) and have developed a rat testes tubule receptor assay suitable for quantitation of FSH biological activity (9). It has been previously reported that the fluorescent probe anilinonaphthalene sulfonic acid does not bind to hFSH (26) but even if binding were observed, it would remain to be demonstrated that enhanced anilinonaphthalenesulfonic acid fluorescence is correlated with biological activity. Thus, the receptor assay for FSH (9) seems ideally suited for studies of kinetics of formation of native and hybrid molecules of FSH, as well as for comparisons of receptor binding activity with in vivo biological activity.

The general mechanism suggested in our previous communication (9) and represented by Equation 4 provides a basis for discussion of the FSH subunit combination reaction.

$$\alpha + \beta \rightarrow X_1 \rightarrow X_2 \rightarrow \cdots \rightarrow X_n$$  \hspace{1cm} (4)

The model states that a bimolecular reaction between the subunits leads to formation of an initial complex, $X_1$, which is formed from 1 molecule each of $\alpha$ and $\beta$ subunit. Proceeding to the right, the formation of $X_1$ is followed by formation of $X_2$, $X_3$, and so forth, until the "final" species, $X_n$, results. The species $X_1, \ldots, X_n$ are shown as reversibly related, with the implication that equilibrium among them exists. The degree to which this can occur depends upon the relative magnitudes of the various rate constants. The apparent kinetics to be observed in such a model will also depend upon the relative magnitudes of the rate constants, but at a sufficiently low subunit concentration, the reaction would necessarily appear as second order because of the first step in the sequence.

In the present study, the analysis for extent of reaction is based upon the ability of FSH formed in the reaction mixture after various periods of incubation to compete with $^{34}P$-labeled hFSH for FSH-specific binding sites in the rat testes tubule homogenate. In terms of the general mechanism (Equation 4), it is not known at the outset whether any of the species beyond $X_1$ are able to bind to the hormone receptor sites, or how many such species there may be. If second order kinetics were clearly established, it could indicate that (a) there is no species beyond $X_1$, (b) limiting low concentration conditions for $\alpha$ and $\beta$ existed during the rate measurements, or (c) active species may exist beyond $X_1$ but the bimolecular step is rate-limiting. If first order kinetics were seen, then the existence of at least one species beyond $X_1$ would be established.

Our kinetic measurements show that although the combination of $\alpha$ and $\beta$ subunits of hFSH gives a satisfactory fit to the second order integrated rate equation (Equation 2), a fit of similar quality is obtained with the first order equation (Table II). To provide a more stringent test, the ratio of $\alpha$ to $\beta$ subunit was varied; the rate data however proved insensitive to these variations in reactant concentration ratios ($\rho$ in Equation 3). The quality of fit to the first order law was also substantially unaffected, keeping in mind that small systematic deviations are seen in our fits to both kinetic laws. It does not seem likely that more data would alter either the systematic deviations in fit or the insensitivity to initial subunit concentration ratios. The evidence then, appears to rule out a single step bimolecular formation of active hormone, that is, only species $X_1$ being formed. The lack of response to variations in the $\alpha: \beta$ ratio supports the view that at least one species beyond $X_1$ does exist, and that $X_1$ itself does not compete for receptor sites. The last conclusion follows since if $X_1$ were active in the assay, second order kinetics for its appearance would be expected.

Conclusions of a related nature were reached by Ingham et al. (22) for the recombination of hLH $\alpha$ and $\beta$ subunits, as followed by use of fluorescent probe techniques.

We have included in Table II data on the kinetics of combination of the isolated subunits of hLH as determined using a radioligand receptor assay for LH (3). As can be seen, the rate constant for the combination of the hLH subunits is an order of magnitude higher than observed for any of the combinations involving the $\beta$ subunit of hFSH.

The rate equations (Equations 1, 2, and 3) were utilized because they represent mechanisms easily accommodated within the framework of the general reaction model (Equation 4). The failure of the hFSH-$\alpha + hFSH-\beta$ combination data to respond as expected to variations in the $\alpha: \beta$ ratio, together with the systematic deviations observed at times early in the course of the reaction, suggest that neither simple expression will suffice, and that further analysis will be needed to permit a more precise description of the kinetic events. Nevertheless, it is clearly shown in the present study that the subunit combination reactions do lead to formation of $\alpha \beta$ dimer molecules capable of competing with labeled hFSH for specific receptor sites in the testes tubule homogenate, as well as stimulating ovarian growth in vivo (Table I). Together with the in vivo biological activity of the hybrid molecules in the specific Steelman-Pohley bioassay this would seem to confirm the hormone-specific character of the hFSH-$\beta$ subunit fraction, as well as the efficacy of the subunit dissociation and isolation procedure previously reported (7).

The high activity of the hLH-$\alpha + hFSH-\beta$ hybrid molecule in vivo was somewhat surprising. However, there is a precedent for hybrid molecules having receptor binding activity greater than the most active intact hormone. A similar situation was encountered in our earlier studies with LH and hCG (2) where it was observed that a hybrid molecule formed between the $\alpha$ subunit of porcine LH and the $\beta$ subunit of hCG had a receptor binding activity somewhat greater than that of intact hCG, which was the most active LH molecule examined in that study. Presumably, the configuration of the hybrid dimer is such as to confer a greater affinity of the molecule for the receptor than that of the native hormone.
It is of interest that the bovine LH-α/hFSH-β fraction discussed above had such a low activity in vivo. A possible explanation could be related to the sialic acid content of hFSH (27), which is considerably greater than that of bovine LH (28). Morell et al. (29) have shown that sialic acid content is related to sustained levels of glycoproteins in plasma, and Vaitukaitis and Ross (30) have shown a relationship between sialic acid content of urinary FSH and biological activity. It is possible that the discrepancy between in vitro and in vivo activity of the bLH-α/hFSH-β dimer is due to differences in the sialic acid content of the bovine LH-α compared to that of hFSH-α. This might result in an accelerated clearance of the hybrid molecule from the circulation. Such an explanation has been invoked to explain certain pharmacological differences in the biological properties of bovine, porcine, and human FSH (15, 31). The greater in vitro activity of bLH-α/hFSH-β hybrid compared to that of the homologous human subunit pair could also be due to damage to the hFSH-α subunit during its dissociation and separation. bLH-α subunit is prepared by acid dissociation and separated from bLH β subunit by counter current distribution, while hFSH-α was dissociated from hFSH-β by incubation with urea. The latter dissociating agent may be responsible for a structural perturbation in the hFSH-α subunit which prevents efficient recombination with the hFSH-β subunit. For example, variations in the degree of denaturation of the α subunits could explain the differences in measured activity of the respective hybrid FSH molecules. Also, the possible presence of impurities in the subunit preparations could account for failure to recover greater biological activity after incubation under conditions favoring formation of α/β dimers.

To our knowledge, this is the first report dealing with the kinetics of formation of subunits of hFSH, as well as kinetics of formation and biological activity, in vitro and in vivo, of hybrid FSH molecules. We are currently extending these studies to probe the structural requirements for FSH subunit combination and receptor binding affinity.

REFERENCES

1. Pierce, J. G., Liao, T. H., Howard, S. M., Shome, B., and Connell, J. S. (1971) Recent Progr. Hormone Res. 27, 165-200
2. Reichert, L. E., Jr., Lawson, G. M., Jr., Leidenberger, F. L., and Trowbridge, C. G. (1973) Endocrinology 93, 938-946
3. Reichert, L. E., Jr., Leidenberger, F. L., and Trowbridge, C. G. (1973) Recent Progr. Hormone Res. 29, 497-526
4. Leidenberger, F., and Reichert, L. E., Jr. (1972) Endocrinology 91, 901-900
5. Reichert, L. E., Jr. (1971) Endocrinology 80, 925-928
6. Reichert, L. E., Jr. (1972) Endocrinology 90, 1119-1124
7. Reichert, L. E., Jr., and Ward, D. N. (1974) Endocrinology 94, 653-664
8. Bhalla, V. K., and Reichert, L. E., Jr. (1974) J. Biol. Chem. 249, 45-51
9. Reichert, L. E., Jr., and Bhalla, V. K. (1974) Endocrinology 94, 485-491
10. Reichert, L. E., Jr., and Bhalla, V. K. (1974) Gen. Comp. Endocrinol. 23, 111-117
11. Reichert, L. E., Jr., Kathan, R. H., and Ryan, R. J. (1968) Endocrinology 90, 109-114
12. Reichert, L. E., Jr. (1973) in Methods in Investigative and Diagnostic Endocrinology (Berson, S., and Yalow, R. S., eds) Vol. 2A, pp. 509-514, American Elsevier Publishing Co., New York
13. Greenwood, F. C., Hunter, W. M., and Glover, J. S. (1963) Biochem. J. 89, 114-123
14. Steelman, S. L., and Fohley, F. M. (1953) Endocrinology 53, 604-616
15. Reichert, L. E., Jr. (1967) Endocrinology 80, 1180-1181
16. Reichert, L. E., Jr., and Parlow, A. F. (1963) Endocrinology 73, 285-293
17. Reichert, L. E., Jr. (1970) J. Clin. Endocrinol. Metab. 31, 331-335
18. Finney, D. J. (1946) Statistical Methods in Biological Assay, Hafner Publishing Co., New York
19. Borth, R. (1957) Ergebnisse 13, 115-120
20. Ward, D. N., Reichert, L. E., Jr., Liu, W. K., Nahm, H. E., Hsi, J., Lamkin, W. M., and Jones, N. S. (1973) Recent Progr. Hormone Res. 29, 497-526
21. Reichert, L. E., Jr., Rasco, M. A., Ward, D. N., Niswender, G. D., and Midgley, A. R., Jr. (1969) J. Biol. Chem. 244, 510-5117
22. Ingham, K. C., Aloi, M., and Edelhoch, E. (1973) Arch. Biochem. 159, 566-505
23. Draper, N. R., and Smith, H. (1968) Applied Regression Analysis, p. 263, Wiley, New York
24. Arihood, S. A., and Trowbridge, C. G. (1970) Arch. Biochem. 141, 131-140
25. Albert, A., Rosemberg, E., Ross, G. T., Paulsen, C. A., and Ryan, R. J. (1968) Clin. Endocrinol. 28, 1214-1219
26. Aloi, S. M., Ingham, K. C., and Edelhoch, E. (1973) Arch. Biochem. 158, 478-485
27. Reichert, L. E., Jr., Kathan, R. H., and Ryan, R. J. (1968) Endocrinology 83, 169-114
28. Kathan, R. H., Reichert, L. E., Jr., and Ryan, R. J. (1967) Endocrinology 81, 45-48
29. Morell, A. G., Gregoriades, G., Scheinberg, I. H., Hickman, J., and Ashwell, G. (1971) J. Biol. Chem. 246, 1461-1467
30. Vaitukaitis, J. L., and Ross, G. T. (1971) J. Clin. Endocrinol. Metab. 33, 308-311
31. Parlow, A. F., and Reichert, L. E., Jr. (1963) Endocrinology 73, 740-744
The Kinetics of Formation and Biological Activity of Native and Hybrid Molecules of Human Follicle-stimulating Hormone
Leo E. Reichert, Jr., C. G. Trowbridge, Vinod K. Bhalla and George M. Lawson, Jr.

*J. Biol. Chem.* 1974, 249:6472-6477.

Access the most updated version of this article at [http://www.jbc.org/content/249/20/6472](http://www.jbc.org/content/249/20/6472)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/249/20/6472.full.html#ref-list-1](http://www.jbc.org/content/249/20/6472.full.html#ref-list-1)