The Presence of a Common Type of Subunit in Bovine Thyroid-stimulating and Luteinizing Hormones*

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SUMMARY

Bovine thyroid-stimulating hormone (thyrotropin, TSH) has been separated into two different subunits by gel filtration in ammonium bicarbonate solution after prior treatment with 1 M propionic acid. The two subunits, which have been designated TSH-α and TSH-β, resemble each other more closely in amino acid composition than do the two subunits of luteinizing hormone (LH, interstitial cell-stimulating hormone), CI and CII, but there are differences in the contents of aspartic acid, isoleucine, leucine, and tyrosine. Maps of the tryptic peptides and the biological properties of TSH-α and -β differ markedly. TSH-α and the CI chain of LH are closely related both in amino acid and carbohydrate composition; their peptide maps are also very similar. By themselves each subunit of TSH has little or no TSH activity, but activity is restored to about 50% of that of undissociated TSH by combining the two in equal amounts. The CI chain of LH can substitute fully for TSH-α in reconstitution of TSH activity, and other experiments indicate that TSH-α and the CII chain of LH will combine to form a hybrid active LH. TSH-β and CII differ markedly in amino acid composition and immunological properties, and do not appear to interact with each other.

The data obtained thus far show that the TSH-α chain and the LH-CI chain have very similar or nearly identical primary structures. Thus, the different hormonal activities of TSH and LH have originated from the combination of this type of chain with one of two other peptide chains, TSH-β and LH-CII, respectively, whose amino acid sequences most probably differ considerably.

Although recent studies in this laboratory, especially on the glycopeptides obtained after tryptic hydrolysis, have shown striking similarities between parts of the primary structure of bovine thyrotropin and one of the two subunits of luteinizing hormone (2), several attempts to produce dissociation of TSH yielded no evidence for subunits (4, 5). Countercurrent distribution experiments (4) were of particular interest because, although this technique has enabled complete separation of the two subunits of LH, its use gave no indication of TSH dissociation. In fact, the combined use of countercurrent distribution and gel filtration (4) has permitted preparation of fully active TSH which is free of LH or its subunits (3, 6). One indication, however, that TSH might consist of more than one peptide chain was the observation of Condliffe (7) that in gel filtration experiments with reduced, S-carboxymethylated TSH, the elution volume of the derivative was greater than that of the untreated hormone.

We now wish to report that TSH can be dissociated and separated into two distinctly different subunits, which we have designated TSH-α and TSH-β. Details of their separation, composition, and electrophoretic and other properties and of experiments in which TSH activity is restored by recombination of TSH-β with either TSH-α or the CI chain of LH are given.

EXPERIMENTAL PROCEDURE

Hormone Preparations and Bioassay—The TSH and LH are preparations whose properties are those recently described (4). As our countercurrent distribution system removes all material previously separated from TSH by chromatography on carboxymethylcellulose, i.e. the fraction containing the heterothyrotropic factor (8), the preparation of TSH has been modified as follows.

Subsequent to initial chromatography of TSH on DEAE-cellulose, which separates the bulk of the LH plus many other unidentified glycoproteins from the fraction containing TSH and the heterothyrotropic material (see References 2 and 4 for an outline of the procedure and references to details of the chromatography), the TSH-heterothyrotropic fraction, 200 to 400 mg, was subjected to a 30 transfer countercurrent distribution in an all-glass apparatus with 10 ml in each phase. The heterothyrotropic material and other impurities strongly favored the upper phase and were well separated from TSH peak. Gel filtration on Sephadex G-100 was then used to separate any CI chain of LH from the TSH. The experimental details of the distribution and the gel filtration are given in Reference 4. TSH obtained by the above procedure, which is much simpler than the earlier procedure of rechromatography on both DEAE- and carboxymethylcellulose, had the same electrophoretic properties, amino acid composition, and biological activity as our previously described TSH.

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1 The abbreviations used are: TSH, thyroid-stimulating hormone (thyrotropin); LH, luteinizing hormone (interstitial cell-stimulating hormone); TSH-α, the first of two subunits of TSH to emerge from Sephadex columns; TSH-β, the second of the two subunits; CI, the subunit of LH whose distribution coefficient favors the lower phase of a countercurrent distribution system; CII, the subunit favoring the upper phase (3, 4).
composition, immunochemical properties, and biological activity as those previously described (4). All TSH preparations were shown to be free of LH by immunoelectrophoresis (4).

TSH was assayed by measurement of the uptake of $^{32}$P into the thyroids of chicks (9) with 0.1% bovine serum albumin in 0.9% NaCl as a diluent. LH activity was assayed by the method of Parlow (10).

Analytical Methods—The techniques employed for amino acid and carbohydrate analyses, electrophoresis in gels containing 7% polyacrylamide, immunoelectrophoresis, and peptide mapping have been described in previous publications (2, 4). The amino acid analyses were for the purposes of comparison and only 24-hour hydrolyses were performed. Amino-terminal analyses were carried out both by the Edman degradation (11) with the terminal amino acid determined after its regeneration from the anilinothiazolinone as described by Africa and Carpenter (12) and by thin layer chromatography (18) of the 1-dimethylaminonaphthalene 5-sulfonl-derivative (14).

The amino acids released by carboxypeptidase A were determined on the amino acid analyzer after enzymic hydrolysis had been stopped by freeze-drying. The digest was dissolved in 0.1 N NaOH before adjustment of pH to 2.2. Any precipitate formed was removed by centrifugation. The enzyme ( Worthington) was treated with diisopropyl phosphofluoridate at 40° for 1 hour before use.

Preparation of Subunits of TSH by Gel Filtration—TSH preparations, after their passage through Sephadex G-100 (4), were dissolved in 1 M propionic acid (1 mg of protein per ml) and allowed to stand at room temperature overnight. The solution was then freeze-dried and the material dissolved in 0.126 M ammonium bicarbonate and subjected to gel filtration on a column (200 × 2.5 cm) of Sephadex G-100 in the same bicarbonate solution. Quantities of 50 to 80 mg of TSH were used in each run; other details are given in the legends to Figs. 1 and 2. The two subunits and a fraction which is undissociated TSH were recovered by freeze-drying until all the ammonium bicarbonate sublimed.

Reconstitution of TSH Activity—One hundred micrograms of each fraction were dissolved in 100 μl of 0.012 M sodium glycinate, pH 9.5, in a 3-ml centrifuge tube. The tube was flushed with nitrogen, covered, and kept at 37° overnight. Samples were then diluted appropriately for TSH bioassay on the assumption that the material had a specific TSH activity of 20 units per mg of glycoprotein. The same conditions were used to prepare reconstituted material for electrophoretic examination and to reconstitute LH activity.

RESULTS

The observations which led to the recognition of the dissociation of TSH are shown in Fig. 1. TSH, purified by countercurrent distribution, was subjected to gel filtration on Sephadex G-100 (Fig. 1a) to remove the CI chain of any LH which had not previously been removed by ion exchange chromatography. The column was eluted with 0.126 M ammonium bicarbonate (pH 8.3). TSH, as before (4), emerged in a single sharp peak, with the material from the trailing peak having the composition and immunological properties of CI of LH (4). In the next experiment (Fig. 1b), 54 mg of the recovered TSH were dissolved in 1 M propionic acid and passed through the identical column equilibrated in 1 M propionic acid. The elution volume of the peak was greater than when gel filtration was in the presence of ammonium bicarbonate, but no distinct separation occurred. However, as shown in Fig. 1c, when this material, recovered by freeze-drying, was rerun under the identical conditions used for the experiment shown in Fig. 1a, it separated into three peaks. The amino acid compositions of the fractions isolated from the last two peaks to emerge are given in Table I and although the mole fractions, in each, of many amino acids are nearly the same and are close to those of native TSH (4), there are obvious differences in the contents of aspartic acid, tyrosine, isoleucine, and leucine. It thus appeared that TSH had dissociated into two dissimilar fractions. The resolution of the two peaks was improved by increasing the length of the column (Fig. 2). The combined yields of the two fractions were 50 to 60% of the starting material in many runs. These yields were approximately equal and the two fractions were designated TSH-α and -β. The data in Table I are expressed on the assumption that TSH-α contains 2 leucyl residues per chain and TSH-β 4 residues. Clearly TSH-α and CI of LH are very similar but pos.
is in agreement with our studies on cyanogen bromide cleavage of TSH have quite different amino acid sequences. The map of TSH-a very closely resembles the map.2 The map of TSH-a! and -p are not extensive, peptide maps of tryptic digests to be the major NHz-terminal amino acid of bovine TSH with some aspartic acid also present (4, 5). The molecular weight of the CI chain which is given for comparison (2) (Fig. 4) and shows that the near identity in composition between these two materials is not fortuitous while a comparison of the maps of TSH-β (Fig. 3) and CII of LH (2) shows them to be clearly dissimilar.

The first peak to emerge from the gel filtration column after incubation of TSH with propionic acid (Fig. 1c) is undissociated TSH as judged by bioassay (the material was nearly as active as the starting material), immunoelectrophoresis and gel electrophoresis (Fig. 3, top left, Sample e). Upon reincubation with propionic acid and fractionation exactly as before, further dissociation occurs and more TSH-α and -β are obtained. These results are shown in Fig. 2a. Fig. 2b shows that bovine LH can be dissociated under the same conditions, but with an interesting difference. The last peak to emerge, which is at the same relative position as TSH-α, is CI, as identified by its amino acid composition, while the middle peak has the composition of CII (2).

Although the differences in amino acid composition between TSH-α and TSH-β are not extensive, peptide maps of tryptic digests of their reduced S-carboxymethyl derivatives, as shown in the lower part of Fig. 3, are completely different. This observation is in agreement with our studies on cyanogen bromide cleavage products.2 The map of TSH-α very closely resembles the map sequences of fragments obtained from TSH by cleavage with cyanogen bromide. These studies have shown that the two halves of TSH have quite different amino acid sequences.

### Table I

| Residue       | TSH-α CI from LH | TSH-β CI from LH | TSH-α + β CI from LH | Native TSH |
|---------------|-----------------|-----------------|---------------------|------------|
| Lysine        | 11.0            | 8.8             | 9.0                 | 2.0        | 20.0 | 19.8 |
| Histidine     | 3.1             | 2.6             | 2.7                 | 2.9        | 5.8  | 6.0  |
| Arginine      | 3.4             | 2.7             | 4.5                 | 7.6        | 7.9  | 7.4  |
| Aspartic acid | 5.8             | 5.1             | 8.0                 | 4.4        | 13.8 | 14.1 |
| Threonine     | 8.6             | 7.6             | 9.4                 | 6.4        | 18.0 | 18.0 |
| Serine        | 5.6             | 5.0             | 4.7                 | 6.9        | 10.3 | 9.8  |
| Glutamic acid | 7.8             | 6.9             | 6.3                 | 5.8        | 14.1 | 14.2 |
| Proline       | 6.1             | 6.2             | 5.9                 | 18.9       | 12.0 | 12.1 |
| Glycine       | 3.7             | 3.5             | 3.9                 | 6.7        | 7.6  | 7.4  |
| Alanine       | 6.6             | 6.9             | 5.4                 | 7.6        | 12.0 | 11.9 |
| Half-cystine  | 8.1             | 8.5             | 9.2                 | 13.1       | 17.3 | 17.6 |
| Valine        | 4.4             | 4.1             | 4.6                 | 6.0        | 9.0  | 9.2  |
| Methionine    | 3.8             | 3.4             | 3.7                 | 2.8        | 7.5  | 7.7  |
| Isoleucine    | 2.0             | 1.8             | 5.3                 | 3.8        | 7.3  | 7.5  |
| Leucine       | 2.0             | 2.0             | 4.0                 | 11.1       | 6.0  | 6.0  |
| Tyrosine      | 5.1             | 4.0             | 9.3                 | 1.9        | 14.4 | 14.5 |
| Phenylalanine | 4.7             | 3.5             | 4.1                 | 2.8        | 8.8  | 8.4  |
| Pheoe         | 0.3             | 0.4             | 0.9                 | 0.7        | 1.2  | 1.3  |
| Mannose       | 5.8             | 6.8             | 2.5                 | 2.5        | 8.3  | 8.7  |
| Galactosec    | 0.2             | 0.1             | 0.1                 | 0.1        | 0.2  | 0.3  |
| Glucosamine   | 6.4             | 5.3             | 3.1                 | 3.6        | 9.5  | 9.9  |
| Galactosamine | 2.5             | 1.6             | 1.5                 | 1.4        | 4.0  | 4.1  |

| Approximate number of residues |
|-------------------------------|
| 108                           |
| 98                            |
| 109                           |
| 122                           |
| 217                           |

| Approximate molecular weight calculated from nearest whole number of residues |
|-------------------------------|
| 13,300                        |
| 12,000                       |
| 13,600                       |
| 13,700                       |
| 26,600                       |

a Taken from Reference 4.
b Residues were calculated after setting leucine as 6.0, 4.0, or 2.0 per molecule of TSH, TSH-β, and TSH-α, respectively. These values were assigned on the basis of the composition of cyanogen bromide fragments.3

sibly not identical in composition. TSH-β, however, does not resemble CI of LH in its amino acid composition. The sum of the compositions of TSH-α and TSH-β is in excellent agreement with that of the native hormone. The carbohydrate compositions of TSH-α and -β are in agreement with the compositions of the oligosaccharides of the three tryptic glycopeptides obtainable from TSH, two of which must be derived from TSH-α (2).

The terminal groups—Previous studies have shown phenylalanine to be the major NH2-terminal amino acid of bovine TSH with some aspartic acid also present (4, 5). The molecular weight found for TSH, as determined by low speed sedimentation equilibrium in the presence of 6 M guanidine hydrochloride at pH 4.5 (either with or without reduction of the disulfide bonds), was approximately 25,000 (5). A value of 28,000 was found by sedimentation velocity-diffusion which is in better agreement with our preliminary sequence studies.3 On the basis of a
FIG. 3. Top left, electrophoresis in polyacrylamide gels of TSH and its separated subunits. Sample a is native TSH, specific activity 30 to 40 units per mg. Sample b is TSH dissolved in 1 M propionic acid and immediately freeze-dried. Sample c is TSH incubated in the acid for 1 hour at 40°. Sample d is TSH incubated in the acid for 16 hours at room temperature. Sample e is undissociated TSH (the first peak to emerge from Sephadex G-100, Fig. 1c). Sample f is TSH-α; Sample g is TSH-β, sample size, 100 μg. Top middle, reconstitution experiments with TSH-α and TSH-β. Each sample was incubated overnight in 50 μl of 0.012 M sodium glycinate, pH 9.5. Samples a and b are incubated controls of TSH-α and -β, 50 μg, respectively. Samples c, d, and e are incubated mixtures of 50 μg of TSH-β with 10, 30, and 50 μg of TSH-α, respectively. (Sample e was run in a separate electrophoretic experiment than the others.) Top right, reconstitution experiments with CI of LH plus TSH-β and CI of LH plus TSH-α. a, an incubated control of 50 μg of a CI preparation. The arrow indicates the position of the weakly staining band. It was shown by starch gel electrophoresis that no other components migrated in the opposite direction. b, a mixture of 50 μg of CI with 50 μg of TSH-β incubated overnight. c, an incubated control of 50 μg of a CI preparation which was run in a separate electrophoretic experiment than the others. The staining at the top of the gel indicates some aggregation. d, a mixture of 50 μg of TSH-α with 50 μg of CI incubated overnight. The control TSH-α and -β samples are given in the top middle, a and b. Electrophoresis was for 2 hours at 2 ma per tube. Bottom, peptide maps of tryptic digests of reduced, S-carboxymethylated TSH-α and TSH-β, sample size, 0.5 mg, and of the CI chain of LH, sample size, 2.0 mg.
molecular weight of 28,000, 0.8 to 0.85 residue of NH₂-terminal phenylalanine and 0.13 to 0.2 residue of NH₂-terminal aspartic acid were found by the method of Stark and Smyth (16). Although these data are in agreement with a single chain of molecular weight 25,000 to 28,000 with some NH₂-terminal heterogeneity, they are also compatible with a two chain structure if one chain were blocked at its NH₂ terminus. After treatment with 1-dimethylamino-naphthalene 5-sulfonyl chloride and hydrolysis of intact TSH, TSH-α, and TSH-β, surprisingly, phenylalanine and 0.15 to 0.2 residue of NH₂-terminal aspartic acid were found by this procedure.

Preliminary studies concerning the COOH termini by hydrolysis with carboxypeptidase B, which had some contamination with carboxypeptidase A, showed release of the same amino acids from intact TSH as previously reported after hydrolysis with both carboxypeptidases (5), namely serine, tyrosine, and lysine with lesser amounts of methionine and histidine. The amino acids released by carboxypeptidase A from each subunit and from intact TSH are also given in Table II. Those from the intact TSH agree with previous data (5). Serine was released in greatest amounts from TSH-α; hydrazinolysis (17) showed it to be COOH-terminal in this subunit. However, the data are insufficient to permit unequivocal assignment of the COOH terminus of TSH-β.² Most important is that these experiments show that no hydrolysis of peptide bonds took place during dissociation and separation of the subunits.

² A probable COOH terminal sequence of His-Tyr-Lys-Ser-Tyr-COOH for bovine TSH had previously been proposed on the basis of the amino acids released from reduced, S-carboxymethyl TSH by a combination of carboxypeptidases A and B and the isolation of the dipeptide Ser-Tyr from tryptic digests (5). This peptide has not yet been obtained from cyanogen bromide fragments and may have resulted from residual chymotryptic action of the trypsin.

Table I

| Residue       | TSH-α | TSH-β | TSH* |
|---------------|-------|-------|------|
| NH₂ terminus  | 0.18  | 0.37  | 0.59 |
| COOH terminus |       |       |      |
| Lysine        | 0.00  | 0     | 0.07 |
| Histidine     | 0.14  | 0     | 0.11 |
| Serine        | 0.78  | 0.08  | 0.90 |
| Methionine    | 0     | 0.18  | 0.19 |
| Tyrosine      | 0.37  | 0.60  | 1.06 |

* The starting material for preparation of the two subunits. Corrected for 10% moisture. The molecular weight of TSH was taken as 28,000; that of each subunit as 14,000.

Electrophoretic Properties and Reconstitution of Biological Activity—The electrophoretic characteristics in polyacrylamide gels at pH 9.5 of TSH, before and after its incubation in 1 M propionic acid, and of the separated subunits are shown in Fig. 3 (top left). Incubation of TSH, which exhibited the usual polymorphism (18), either for 1 hour at 40° or 16 hours at room temperature (about 22°) gave a complete change in pattern (Fig. 3, c and d); the new components correspond to a combination of those in the patterns of TSH-α and -β (Samples f and g). The pattern of the first peak from the gel filtration column (Sample e) is almost identical with that of the starting material. The results of mixing varying proportions of TSH-α with a fixed amount (50 μg) of TSH-β in glycine buffer, pH 9.5, and incubation at 37° overnight before beginning electrophoresis are shown in Fig. 3 (top middle). Samples a and b are controls; the patterns of e, d, and c show that the mixture of equal amounts of TSH-α and TSH-β results in a marked but not complete restoration to a pattern similar to that of undissociated TSH. The restoration is best demonstrated by the reappearance of the middle band of the three bands seen in the starting sample (a, left).

Recombinations of CI of LH with TSH-β and of TSH-α with CI are shown in Fig. 3 (top right). Our preparations of CI chain have not stained well (4) most probably because the material is not fixed strongly in the gels during staining and destaining. Sample a, right, shows that the control CI after incubation gave a faint, diffuse band at the position indicated by the arrow while b, right, shows the striking change resulting from the incubation of 50 μg of the CI with 50 μg of TSH-β (whose control pattern is shown by Sample b, middle). While the mobility of the hybrid product is different from that resulting from recombination with TSH-α and TSH-β, bioassays showed that TSH activity was restored in both instances. Typical results of bioassays for TSH, with intact TSHI as a standard, are summarized in Fig. 4. The dilutions were made as if each sample was 20 units per mg (actual potency of the intact TSH was 30 to 40 units per mg). Maximum response was obtained with 2.4...
of either TSH-cr plus TSH-3 or CI plus TSH-fi gave a maximal response (not shown). The responses in a second assay at lower dose levels are shown in Fig. 4a; the data show that at least 50% of the activity of the starting material was recovered by the reconstitution of TSH-3 with either TSH-cr or CI. The same results have been found with a second preparation of the TSH subunits and two other preparations of CI.

The electrophoretic pattern of an incubation mixture of the reverse situation, i.e. the reconstitution of LH by mixture of TSH-cr and CI1 of LH, indicates that a hybrid LH resulted as in Fig. 3 (top right, Sample 4). Patterns of TSH-3 plus CII showed that no interaction occurred between these two chains. Sample c, right, shown a control of CII. In one bioassay for LH activity, mixtures of 100 µg each of CI plus CI1 of LH, TSH-cr plus CI1 of LH, and a control of TSH-3 plus CI1 of LH gave the following results: CI plus CI1, 2.6 times the National Institutes of Health LH reference standard, S-1; TSH-cr plus CI1, 0.9 times the standard; the control TSH-3 plus CI1, 0.06 times the standard. In a second experiment, two samples of TSH-cr plus CI1 gave responses of 0.5 and 0.7 times the standard while the responses of control samples, CI, TSH-cr, and CI1 were 0.04, < 0.02, and 0.08 times the standard, respectively. Thus, the combination of TSH-cr and the CI1 chain of LH gave a partial restoration of LH activity. The LH sample, before dissociation, had previously been assayed twice at 2.2 and 2.9 times the standard.

**Immunological Cross-reactivity**—When examined by immunoelectrophoresis, by techniques previously described (4, 19), against anti-CI, anti-CI1, anti-TSH, and anti-LH, TSH-cr gave a strong line of precipitation against anti-CI but reacted weakly against anti-TSH, while TSH-fi gave a strong reaction against both anti-TSH and anti-CI. The latter cross-reactivity indicates that the immunochemical determinants of TSH-cr are more similar to those of the TSH-cr-CI type of subunit than are those of CI1, as no cross-reactivity has been found between CI and CI1 (4). TSH-cr showed traces of cross-reactivity against anti-LH; TSH-fi showed none. It is noteworthy that the immunological characteristics of TSH-cr are the same as previously assigned to “denatured TSH” (20).

**DISCUSSION**

The present results demonstrate that TSH can be dissociated into two subunits without breaking peptide bonds because study of the amino and carboxyl termini gave no evidence for the appearance of new end groups. Thus, the hormonal functions of TSH and LH must originate by a combination of a common type of subunit, the TSH-cr-CI type with one of two other subunits, TSH-3 or CI1. Close similarity in structure between TSH-cr and CI is demonstrated by studies on tryptic glycopeptides (2) and by the data presented above including the peptide maps and the fact that TSH activity can be regenerated by a combination of either CI or TSH-cr with TSH-3 and LH activity by TSH-cr plus CI1. However, TSH-cr and CI are probably not completely identical. Extensive studies on composition remain to be done but the data indicate that CI contains a different number of residues of lysine, phenylalanine, and perhaps of some other amino acids than does TSH-cr. The electrophoretic mobilities of TSH-cr and CI and those of their recombination products with TSH-3 also differ. The COOH terminus of CI from bovine LH has been reported by Reichert et al. (15) to be serine as is found for TSH-cr, but they did not detect any free NH-terminal groups. Any differences between TSH-cr and CI would presumably reflect changes occurring during evolution of the separate hormonal functions of TSH and LH.

The composition, immunological properties, peptide maps, and thus presumably the amino acid sequences of TSH-3 and CI of LH differ markedly, and it should be noted that these two subunits seem to carry, respectively, the major immunological determinants of intact TSH or LH. Thus, TSH-3 reacted strongly with anti-TSH as does CI1 with anti-LH (4, 15) while TSH-cr or CI gave a much weaker or no precipitation reaction with antisera against the intact hormones.

The hormones of the pituitary gland have already provided several interesting examples of the utilization of similar sequences for different functions, such as oxytocin and vasopressin in which two amino acid substitutions in the same cyclic octapeptide give rise to the two different hormones. The corticotropins, melanotropins, and β-lipotropins, single chain molecules containing from 13 to 90 amino acid residues, also share common sequences (see Reference 22 for a recent review) and some sequential homology between pituitary prolactin and growth hormone has also been indicated (23). The utilization, seen here, of a single type of peptide chain in combination with one of two others to obtain different hormonal functions appears to be a type of conservation of structure during evolution which has not previously been recognized, at least in the pituitary hormones. The conservation of the TSH-cr-CI type of structure appears to be very extensive and the possibility that this type of structure may have a biological function additional to that of forming LH and TSH with CI1 and TSH-3-CI should not be overlooked. Partial analogies can be drawn between the present case and the occurrence of α-lactalbumin either as a milk protein apparently devoid of specific physiological functions or as one chain of the enzyme lactose synthetase (24), and with the different catalytic properties of the tryptophan synthetase of Escherichia coli and its subunits (25). The α chain of hemoglobin can also combine with β or γ chains with resulting differences in the properties of the hemoglobins (26).

The observations in this paper raise both questions concerning details of the structures of TSH-cr and CI and questions of more general biological significance. For example, while polymorphism of both TSH-cr and CI is not unexpected in view of the polymorphism of undissociated TSH, it will be of interest to see if more discrete differences can be found between the two major electrophoretic components of either TSH-cr or TSH-fi (see Fig. 3) than have been found with intact TSH or its S-carboxamidomethyl derivative (4). The TSH subunits each also manifest additional polymorphism after reduction and S-carboxamidomethylation, with the components from each showing partially overlapping electrophoretic mobilities. The nature of any heterogeneity in both the amino and carboxyl
termini must also be determined as well as differences in sequence between CI of LH and TSH-α.

Particularly interesting general questions are those concerning the biosynthesis of TSH and LH, possible control mechanisms which could involve the production of the common type of chain, and whether the different cell types in the pituitary which have been implicated in the synthesis of TSH and LH each produce the TSH-α-CI type. Whether any of the subunits circulate in the blood either under normal or pathological conditions must be investigated as well as the effects that any circulating subunits would have on radioimmunoassays of either TSH or LH. The exophthalmic activity (27) of the two subunits also should be studied.

It is interesting that countercurrent distribution and reisolation in an acid system (40% ammonium sulfate-0.15 M p-toluene sulfonic acid-1 propanol-ethanol, 60:60:27:33, by volume) did not lead to separation of the subunits (4). The distributions yielded TSH of higher specific activity than the starting material because of removal of inactive material. Thus, if dissociation had occurred, the over-all compositions of the TSH-α and -β must be sufficiently alike so that a 120 transfer distribution gave no evidence of even partial separation, and recombination and restoration of hormonal activity must have been essentially quantitative during reisolation of the TSH. The probable explanation for the success with propionic acid is that nonpolar interactions were overcome and this together with electrosstatic repulsion at low pH caused nearly complete dissociation even though at a slow rate. The changed electrophoretic pattern of the material recovered after incubation supports this concept (Fig. 3, top left, c and d). Separation of the subunits during passage through Sephadex in 1 M propionic acid does not occur because both subunits have nearly the same hydrodynamic volume and interactions between either subunit and the Sephadex would be minimized in this solvent. At alkaline pH, reassociation clearly occurs but at a rate which also must be slow. Separation at alkaline pH on the Sephadex column probably occurs, not altogether because of differences in hydrodynamic volume, but because of greater interaction between TSH-β (the last peak to emerge) and the Sephadex, and it may be that these interactions also reduce markedly the rate of recombination. TSH-α does, however, contain two oligosaccharide units and TSH-β only one; any resulting differences in hydrodynamic volume at alkaline pH thus may also contribute to separation as may any differences in the state of aggregation of the individual subunits.

Finally, the structural similarity between TSH-α and CI of LH and the ease of recombination and formation of hybrid molecules raises the possibility that exchange of subunits between TSH and LH could take place during their isolation. Such recombinations might be the explanation of some of the polymorphism observed with both TSH and LH (4) and could introduce difficulties in the unequivocal determination of the amino acid sequence of each hormone.

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*Previously published gel electrophoresis patterns and immunological data after countercurrent distribution suggest that some TSH-β was in a non-rechromatographed fraction subjected to countercurrent distribution (Fraction 7 in Fig. 5a in Reference 4).
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