Abstract. The behavior and properties of ovine prolactin have been evaluated by measurements of fluorescence, polarization of fluorescence, absorption, optical rotation, and circular dichroism. The helical content of the native molecule at pH 8 is 60 per cent as determined by circular dichroism. Three molecular transitions have been followed. The one in acid affects only 20 per cent of the helical residues. More profound conformational changes occur in urea solutions (pH 5.2 and 8.0) where most of the helical residues are randomized. There is a close parallel between the behavior of ovine prolactin and bovine growth hormone both in aqueous solutions between pH 2 and 11.5 and in urea solutions at pH 5.2 and 8.0. Based on the similarities in behavior it is proposed that the conformations of these two horomones are homologous.

There is a growing list of proteins functioning by analogous mechanisms that appear to be structurally related.1 A high degree of sequence homology will result in conformational homology if the primary structure governs the folding of proteins. Sequence homology of about 50% as in the α- and β-chains of human hemoglobin, and much less in sperm whale myoglobin, produces similar three-dimensional structures.2-4 The probability of structural homology is increased, if, in addition, disulfide linkages are homologous.5,6 In the case of a group of enzymes, which are related by common sequences in their active sites, fewer sequence homologies may be sufficient to permit the inference of conformational homology.1,5,7,8 In some cases, a more limited extent of sequence homology, when combined with functional similarities, may be enough to predict structural interrelationships.9 A similar conclusion of conformational homology may be justifiable in the absence of definitive sequence homology if two proteins share some biological functions and, in addition, show similar physicochemical behavior. Since hormones generally show multiple activities, the significance of shared functions is probably of less importance in suggesting homology than with enzymes.

There is no unequivocal way of revealing structural homology between two proteins other than by x-ray analysis of their crystals. In the absence of the latter we have compared the solution properties of ovine prolactin with those of bovine growth hormone which have been previously published.10-13 The close resemblance in a number of properties which depend on structure suggests that the conformations of ovine prolactin and bovine growth hormone are homologous.
Materials and Methods. The ovine prolactin (PS-6-NIH Study Section) was a gift from Drs. R. W. Bates and P. G. Condliffe (National Institutes of Health). The material used for most studies was prepared by filtration on Sephadex G-100 in 0.01 M NaHCO₃, pH 8.1. About 15–20% of the protein was eluted ahead of the main protein peak. The latter was rerun on Sephadex G-100 and the center portion of the peak was collected and stored in small aliquots at −20°C. Polyacrylamide (7.5%) gel electrophoresis at pH 9.2 showed one prominent and two weaker bands. These results are very similar to those of Cheever and Lewis who showed that the smaller bands present in prolactin preparations represent deamidated and aggregated hormone. Velocity ultracentrifugation of a 0.6% solution at pH 8.8 of the Sephadex purified sample showed a single symmetrical boundary with an θ₂₀,₀ value of 2.3.

The circular dichroic spectra were measured on the Sephadex-purified material and on a more recent preparation of ovine prolactin without purification (PS-9-NIH Study Section). Both prolactins gave the same spectra in acid, alkali, and neutral pH.

Dimethylaminonaphthalene sulfonyl (DNS) conjugates of prolactin were synthesized by the same method as described for conjugates of bovine growth hormone, and their polarizations were determined in the same manner.

Fluorescence measurements were made with a Turner 210 spectrofluorometer. Circular dichroism and optical rotatory dispersion data were obtained with a Cary 60 spectropolarimeter and absorption spectra with a Cary 14 spectrophotometer.

Protein concentrations were calculated from the absorbance at 280 nm of their solutions. A value of 9.5 was found for the absorbance at 280 nm of a 1% solution in a 1 cm² cell of ovine prolactin.

Results. The number of peptide groups in α-helices can be assessed by circular dichroic or optical rotation spectra. No such direct method of analysis for tertiary structural interactions is possible. The latter, however, contribute significantly to the stability of a protein and can therefore be evaluated by examining the conformational changes produced by different environmental stresses. The behavior of prolactin and bovine growth hormone was therefore compared in (1) aqueous solutions in acid and alkali and in (2) urea solutions at pH 8.0 and 5.2.

Helical Content. The circular dichroic spectrum of prolactin at pH 7.8 contains the twin, negative extrema at 207 and 222 nm, characteristic of the peptide chromophore in an α-helical conformation. The molecular ellipticity at 222 nm gives a helical content of about 60% ([θ]₂₂₂ = −18,700) if we use the limits suggested for the α-helical and random polypeptide configurations (i.e., [θ]₂₂₂ = −35,000 and +4,000 respectively). Bovine growth hormone shows a similar circular dichroic spectrum from which a helical content of 52% was calculated. Bewley et al. have recently found 55% α-helix for human growth hormone.

At pH 2.5 the number of helical peptide groups of prolactin decreased by 20% from its value near neutrality ([θ]₂₂₂ = −14,300). A similar percentage of decrease has been observed recently by circular dichroic measurements with bovine growth hormone. A smaller decrease has been reported from analysis of optical rotatory dispersion data on this hormone.

Increasing the pH of prolactin to 11.5 produced only a marginal change in dichroic activity of the peptide group whereas at pH 11.8 no change had occurred in the circular dichroic spectrum of bovine growth hormone. Optical rotatory dispersion data on this hormone also showed no change in the helical parameter b₀ of the Moffitt equation.
Conformational Transitions. Aqueous solutions: (1) Acid—A reversible, configurational transition in prolactin between pH 5 and 3 has been followed by the increase in tryptophanyl fluorescence, the decline in polarization of DNS-prolactin and by the "denaturation blue shifts" in the absorption of both tryptophanyl and tyrosyl chromophores (Fig. 1). A similar transition has been shown with bovine growth hormone by the same procedures. Although the pH dependence is similar in the two proteins the prolactin curve is displaced to more neutral pH values by 0.4 pH units (Fig. 1). A complete difference absorption curve could not be obtained because of the greater concentration needed for this type of measurement and the limited solubility of prolactin near pH 4-5. The enormous increase in quantum yield observed with bovine growth hormone (ninefold) was not encountered with prolactin. This difference is not very surprising, since the quantum yield of the indole chromophore is especially sensitive to its environment and minor changes could produce very large effects.

(2) Alkali—The hydrodynamic properties of bovine growth hormone in alkali not only reflect its dissociation into subunits but also show that the subunits
are as compact as their precursor. In addition, the helical content of this hormone is invariant to pH between 9 and 11.8. Prolactin is however, slightly less stable in alkali than bovine hormone. Figure 2 shows the effect of pH on the molecular ellipticity at 222 nm, the polarization and fluorescence of DNS-labeled prolactin. Between pH 9 and 11.4 there is only a marginal change in all three parameters. They continue to change more rapidly at higher pH values, although relatively little disorganization has occurred by pH 12.0, as indicated by either the ellipticity or polarization values.

**Urea solutions.** The urea dependence of the optical activity of the peptide groups, tryptophanyl fluorescence, and tryptophanyl difference absorption of prolactin at pH 8.0 is shown in Figure 3. It is evident that the three sets of data represent the same structural transition. The magnitude of the optical rotatory change indicates that nearly all the helical peptide groups are eliminated in 10 M urea. The transition curve of prolactin at pH 8.0 is parallel to that of bovine growth hormone, but displaced by 1.5 units to lower urea concentrations. The prolactin curve at pH 5.2 (Fig. 4), as measured by optical activity at 233 nm or tryptophanyl difference absorption, is similarly shifted from that of bovine growth hormone at the same pH.

**Discussion.** It is first necessary to justify the comparison of prolactin with bovine growth hormone rather than with human growth hormone since hormonal activities are shared mainly between prolactin and the human hormone. Although native bovine hormone neither cross reacts with antisera against human hormone nor is hormonally active in humans, it acquires both activities when it is partially digested with proteolytic enzymes. In addition, common
antigenic determinants are found in the fully reduced forms of bovine and human hormones.\textsuperscript{23}

The sequence of amino acids of bovine growth hormone is still incomplete, although partial sequences have been established which show significant identities and similarities to many of the sequences in human growth hormone in two-thirds of the chain from the C-terminal end.\textsuperscript{24,25} Dellacha et al. have summarized their results by the statement, "All this information suggests the existence of great analogy in the secondary and tertiary structures of human and bovine growth hormones."

The similarity in helical content of prolactin and bovine growth hormone is an impressive criterion of structural similarity since, with the exception of hemoglobinlike molecules, most other proteins have much smaller values.\textsuperscript{16} Perhaps of even more significance is the trivial change in the number of helical peptide groups produced in the acid transitions, compared to the important changes in other parameters representative of tertiary structure.\textsuperscript{11-13} Moreover, the helical organization of both proteins is indifferent to alkali, i.e., 11.8 with bovine hormone and almost 11.5 with prolactin. It is evident that the structure determined by the helical peptide groups of these two proteins is very stable in aqueous media between very wide pH limits.

As criteria of tertiary structural interactions, three molecular transitions have been compared, one in acid and two in urea. The acid transition of prolactin has the same pH dependence as bovine growth hormone except that the transition is shifted to higher pH values by 0.4 pH units. Both hormones also share an important difference in stability to urea denaturation between pH 8.0 and 5.2.
This difference is quite striking and unexpected since the pH values are still in the neutral region and most molecular parameters are unaffected by this pH change.

In aqueous solution, in both acid and alkali, and in urea solutions at pH 5.2 and 8.0 there are minor but significant differences in stability between the two hormones. Nevertheless, the various curves parallel each other and the transitions consistently show that prolactin is slightly less stable than bovine growth hormone.

There are no identical sequences of more than three residues between prolactin and human growth hormone.26 27 If one allows the usual type of conservative substitutions,3 and a few nonconservative, there are several sequences which may be considered homologous.

Of interest are the homologous C-terminal sequences which include two half-cystine residues which form disulfide bonds to half-cystine residues in other parts of the molecule.

It is not clear whether the homologies indicated above, representing about 30% of all the residues, with much less than half being identical, are enough to determine the conformations of the two proteins. In the absence of extensive homology or similar functional groups, it is useful to see if the distribution of nonpolar and charged groups is similar in the two proteins. One of the important principles of protein structure which has emerged from the x-ray studies on crystalline proteins is that the charged groups are essentially confined to the exterior whereas the interior consists mostly of nonpolar groups.2 4

If we compare the ratios of nonpolar2 (i.e., Trp, Ileu, Tyr, Phe, Pro, Leu, Val, Cys, Met), charged groups (i.e., Lys, Arg, His, Asp, Glu), and neutral residues (Ala, Gly, Thr, Ser, Asn, Gln) in the two hormones they come out to be very similar, as seen in Table 1. The distribution of each type is clearly compatible with conservative replacements, although there are significant differences (>20%) in composition in about half the amino acids.26 27

If prolactin and growth hormone were derived from a common ancestor, then as new structural patterns emerged with evolutionary changes, some activities may have been modified or eliminated and new ones introduced. Depending on the extent of evolutionary change, which may vary with each species, the prolactin may be identical to the growth hormone of the same species, as is con-

<table>
<thead>
<tr>
<th></th>
<th>Prolactin</th>
<th>Percentage</th>
<th>Human growth hormone</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpolar</td>
<td>82</td>
<td>41.4</td>
<td>77</td>
<td>41.0</td>
</tr>
<tr>
<td>Polar</td>
<td>54</td>
<td>27.3</td>
<td>51</td>
<td>27.1</td>
</tr>
<tr>
<td>Neutral</td>
<td>62</td>
<td>31.3</td>
<td>60</td>
<td>31.9</td>
</tr>
<tr>
<td>Total</td>
<td>198</td>
<td></td>
<td>188</td>
<td></td>
</tr>
</tbody>
</table>
considered to be the case in humans, or it may be distantly related or unrelated, as may hold with the bovine or ovine hormones.

We would like to thank Drs. R. W. Bates and P. G. Condliffe for suggesting this problem to us.

† Requests for reprints may be addressed to Dr. H. Edelhoch, Room 8N-313, Bldg. 10, Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. 20014.
17 Edelhoch, H., and R. E. Lippoldt, accepted for publication, J. Biol. Chem.