tirely consistent with the results found in the present investigation, which was carried out at slightly higher temperature, where the mid-point of the curve is found in the range 9.92–9.98.

Electrostatic Interactions.—Values for the electrostatic parameter w (see equation 1) are presented in Table III; limits of reliability, accounting for randomness of the data, are also included. In the carboxylic region, however, a definite curvature appears in the plot of (pH *vs.* log $r/(n - r)$) *versus* $(-Z)$ (*cf.* Fig. 5). Deviations from the linearity implicit in equation 1 could arise from any one of the following reasons, or from a combination.

TABLE III

VALUES OF THE ELECTROSTATIC PARAMETER (w) IN CORTICOTROPINS

Nature of the group	Sheep α-corticotropin trichloroacetate	Sheep and beef corticotropins, desalted samples
Carboxylic	0.086(0.076–0.096)[a]	0.090(0.080–0.099)
Phenolic	0.034(0.026–0.042)	0.025(0.010–0.040)
ε-Amino	0.027(0.013–0.041)	0.039(0.025–0.053)

[a] The 95% confidence interval is given in parentheses.

First, it might be an oversimplification to assume a single pK_i value for all carboxylic groups. Indeed, four of the carboxyl groups appear in the very unusual sequence –Glu.Asp.Asp.Glu– in corticotropin.[5] Alternately, the value for Z obtained from the titration curve might be inadequate, if pH-dependent ion binding occurs. In view of the significant difference between isoionic and isoelectric pH, this alternative possibility must be considered. Finally, it might be that the value of w is not constant throughout the pH range of from 3 to 6. Since the w factor is directly related to the size and shape of the molecule,[26] this would mean that α-corticotropin is endowed with appreciable deformability or flexibility.

The very large difference in w observed between the acidic and the basic portions of the titration curve also favors this latter view. That this difference is real is indicated by a significant departure of the data from a titration curve calculated with a constant value of w throughout, the same value as that of the carboxylic groups (dotted curve in Fig. 2); in order to obtain a reasonably close fit between the data and the calculated curve around pH 12, the very unlikely value of $pK_i \leqslant 11$ would have to be used for the guanidino groups. However, agreement between calculated and observed values is, as expected, extremely close if the set of values for w given in Table III are used (solid curves in Figs. 2 and 3). The change in w might in part reflect the variation between values for the sedimentation constant measured in acid and in alkaline solutions.[27] By means of equations discussed by Tanford,[26] it is calculated that a change of w from 0.09 to 0.035 (or 0.025) would correspond to an increase in molecular weight by a factor of at least 5 (or 10) if the molecule is assumed to retain spherical symmetry and to undergo no change in hydration. Actually, a spherical, unhydrated molecule of the size of corticotropin would have a value for w quite different (namely, 0.17) from those recorded in Table III. Figure 5 clearly indicates that the model of a rigid sphere becomes a reasonable approximation only in the neighborhood of the isoelectric pH. Accordingly, it seems justified to assume that the molecule has a compact configuration in the pH range of limited solubility but undergoes some expansion outside of this range.

(26) C. Tanford, *J. Phys. Chem.*, **59**, 788 (1955).

(27) C. H. Li and H. Papkoff, unpublished experiments.

BERKELEY, CALIF.

[CONTRIBUTION FROM THE HORMONE RESEARCH LABORATORY AND THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Corticotropins (ACTH). XV. The Action of Chymotrypsin on α-Corticotropin[1]

BY JOSÉ LÉONIS,[2] CHOH HAO LI AND DAVID CHUNG

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The following peptide fragments have been isolated from chymotryptic digests of α-corticotropin and identified: Ser.Tyr; Ser(Tyr,Ser,Met,Glu)His.Phe; Arg.Try; Arg(Try,Gly,Lys,Pro,Val); Gly(Lys,Pro)Val.Gly.Lys; Lys(Arg$_2$,Pro)Val; Lys(Glu$_2$,Asp$_2$,Ala$_2$,Val,Tyr,Pro,Gly,Ser)GluNH$_2$.Ala.Phe; Ala(Phe,Pro)Leu; and Glu.Phe. These sequences are consistent with the proposed structure for α-corticotropin. One of the two amide groups in the peptide hormone has been located as glutamine at amino acid position 33. The specificity of chymotryptic hydrolysis of peptide bonds has been discussed.

The structure of α-corticotropin has been deduced from peptide fragments isolated after cleavage of the hormone by means of peptic, tryptic and acid hydrolysis.[3] The peptide pattern obtained when α-corticotropin was digested with chymotrypsin, however, was so complex that only relatively limited information could be derived from it during the early stages of the structural investigation; and, indeed, the data presented here indicate that the number of peptide bonds susceptible to the enzyme is unexpectedly high. Furthermore, since the rate of splitting of susceptible bonds seems to differ and also to vary with the average extent of digestion, it was found that isolation of the resultant peptides in nearly stoichiometrical amount could not be expected. It is the purpose of this paper to demon-

(1) This work was aided in part by grants from the National Institutes of Health of the United States Public Health Service (G 2907) and the Albert and Mary Lasker Foundation. For Paper XIII of this series see C. H. Li, R. D. Cole, D. Chung and J. Leonis, *J. Biol. Chem.*, **227**, 207 (1957); and for Paper XIV see C. H. Li, J. S. Dixon and D. Chung, THIS JOURNAL, **80**, 2587 (1958).

(2) Fulbright Grantee 1955–1957, on leave of absence from the University of Brussels, Belgium.

(3) C. H. Li, I. I. Geschwind, R. D. Cole, I. D. Raacke, J. I. Harris and J. S. Dixon, *Nature*, **176**, 687 (1955).

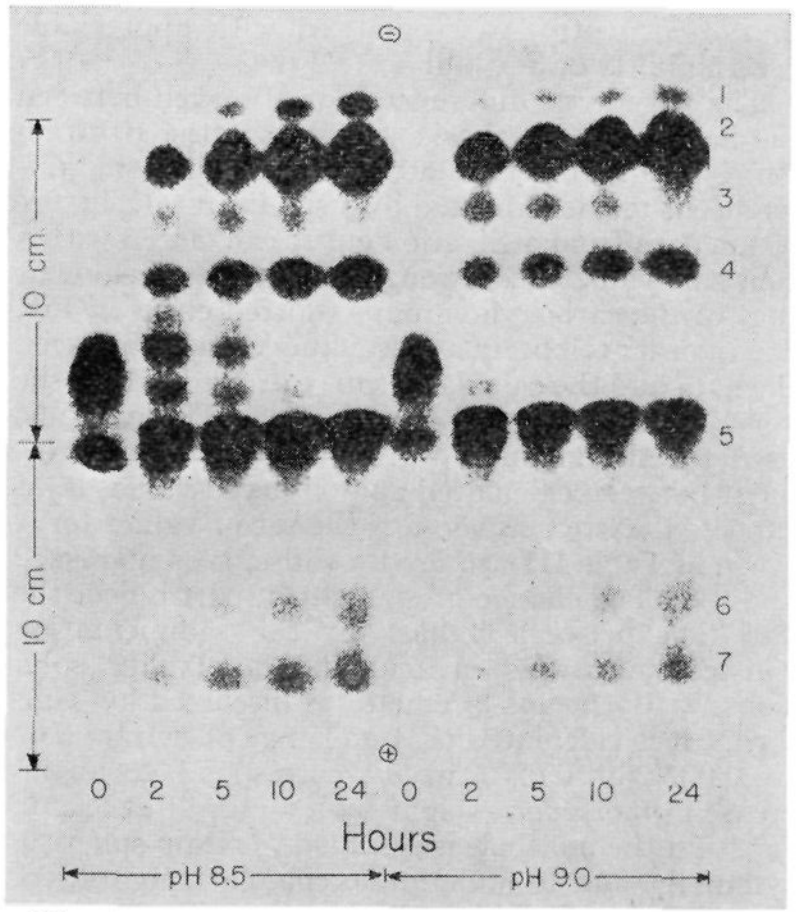

Fig. 1.—Separation by electrophoresis on paper of the peptide mixture after digestion of α-corticotropin with chymotrypsin at *p*H 8.5 and 9.0 for different time intervals.

times crystallized α-chymotrypsin (Armour, Lot 381–092) in 0.25 ml. of a 0.2 *M* ammonium acetate–ammonia buffer, at a temperature of 40°, but at two different *p*H's (8.5 and 9.0) and for differing time intervals (0, 2, 5, 10, 24 hours). After the designated intervals had elapsed, 0.05–ml. aliquots were removed, immersed in a boiling water-bath for 5–10 minutes and applied to Whatman 3 MM filter paper strips for fractionation by electrophoresis. Zone electrophoresis on paper was carried out in a Spinco apparatus[5] for 7 hr. at 200 volts, with a collidine–acetic acid buffer[6] of *p*H 6.5. Figure 1 shows the peptide pattern obtained after development with ninhydrin.

For preparative experiments, the time interval chosen was 24 hr. and the *p*H, 9.0. Seven and one half micromoles of α-corticotropin trichloroacetate and 0.046 micromole of α-chymotrypsin, dissolved in 5.1 ml. of ammonium acetate–ammonia buffer of *p*H 9.0 were incubated at 40° for 24 hr. The whole digest was applied as a band to the paper and then submitted to electrophoresis as described above, for 10 hours. Peptide bands, numbered as in Fig. 1, were located by means of narrow guide strips developed with ninhydrin; bands 1 to 7 were eluted separatedly with 3% aqueous ammonia and were then submitted twice successively to purification by chromatography on Whatman No. 1 filter paper. The solvents used were *n*-butanol/pyridine/acetic acid/water[7] (for basic and neutral peptides) or *n*-butanol/acetic acid/water[8] (for acidic peptides), followed in either case by *n*-butanol/formic acid/water.[9] Both paper electrophoresis and chromatography were carried out at room temperature. Figure 2, a facsimile of the pilot chromatograms, shows the separation that was achieved.

After the final chromatography in *n*-butanol/formic acid/water, the purified peptides were eluted and submitted to analysis by means of the paper-DNP method[10] of Levy,[11] to identify the amino acid residue at the N-terminus and to assertain the complete amino acid composition. Analysis at

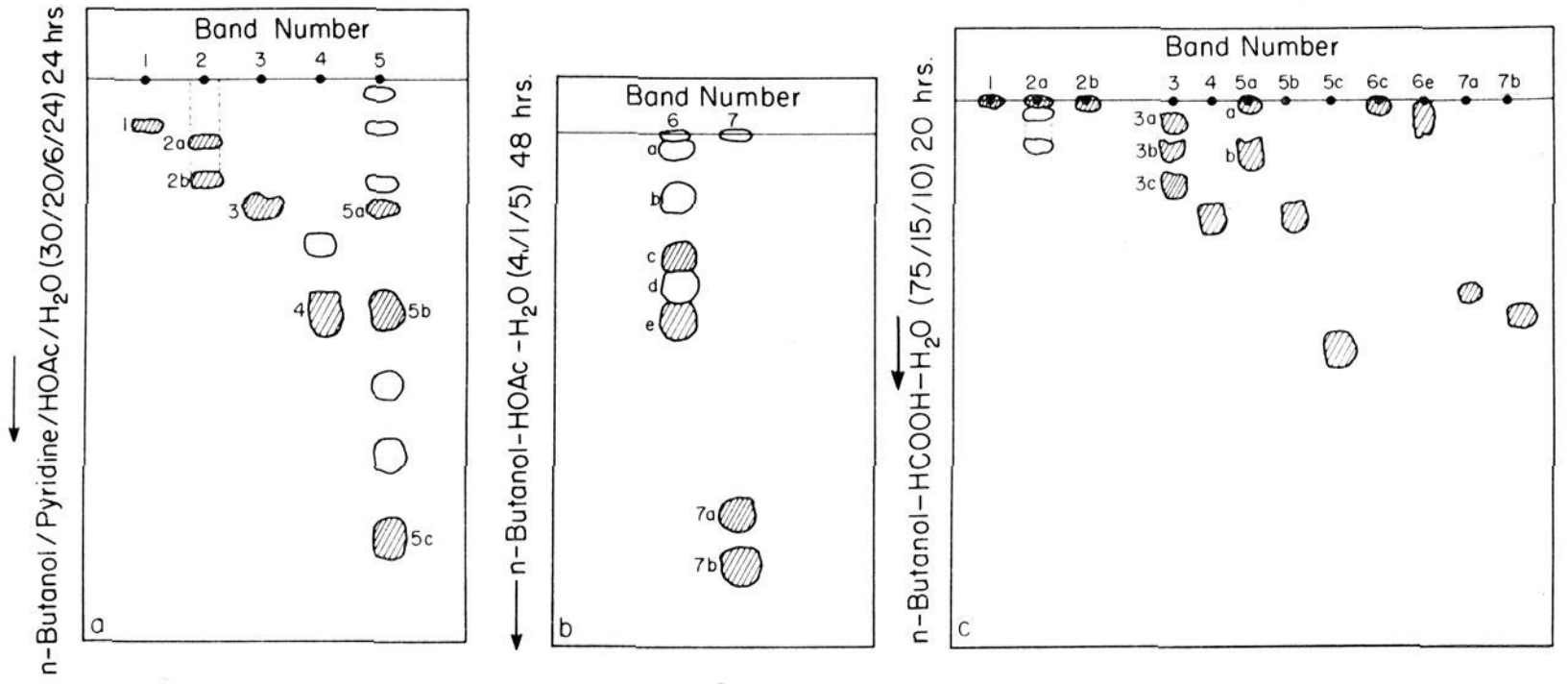

Fig. 2.—Paper chromatography of fractions obtained from paper electrophoresis (bands are numbered as in Fig. 1): (a) neutral and basic peptides, first solvent; (b) acidic peptides, first solvent; (c) all peptides, second solvent. In the preparative experiment, in which the first solvent was used, bands 1, 2 and 6 were chromatographed for 3 days and band 3 for 2 days. Bands 1, 2a, 2b, 3 and 5a only were rechromatographed in the second solvent (for 3 days). First solvent, *n*-butanol/pyridine/HOAc/H_2O (30/20/6/24); second solvent, *n*-butanol/HCOOH/H_2O (75/15/10).

strate that those peptides that have been characterized in the present study are nonetheless entirely consistent with the whole amino acid sequence postulated for α-corticotropin.[3]

Experimental

Isolation and Analysis of Peptides.—During the exploratory phase of this study, comparative enzymic digestions were performed. *Ca.* 2 mg. of ovine α-corticotropin trichloroacetate[4] was allowed to react with 0.05 mg. of three

(4) C. H. Li, I. I. Geschwind, J. S. Dixon, A. L. Levy and J. I. Harris, *J. Biol. Chem.*, **213**, 171 (1955).

(5) F. G. Williams, Jr., E. G. Pickels and E. L. Durrum, *Science*, **121**, 829 (1955).

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(8) S. M. Partridge, *ibid.*, **42**, 238 (1948).

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(10) The quantitative estimation of amino acid content by means of 2-dimensional chromatography on paper of the dinitrophenylated derivatives of the amino acids liberated after acid hydrolysis of the peptide or protein under investigation.

(11) A. L. Levy, *Nature*, **174**, 126 (1954).

TABLE I

COMPOSITION AND STRUCTURE OF NEUTRAL AND BASIC PEPTIDES

Peptide	N-terminus[a,b]	Other constituent amino acids[a]	C-terminus[a,g]	Amino acids as contaminants[c]	Recovery,[d] %	Proposed structure
5b	Ser(0.8)	Tyr[e]	.....	.....	35	Ser.Tyr
5ab	Ser(0.4)	Ser(1.0),Met(0.9), Glu(1.1),His(0.9), Phe(1.0),Tyr[e]	*C* (semi-quantitatively) ...His.Phe	Gly(0.2)	60	Ser(Tyr,Ser,Met,Glu)His.Phe
5c	Ala(1.0)	Phe(1.0),Pro(1.0). Leu(1.0)	*C:* Leu(1.0),Pro(0.02)	.....	10	Ala(Phe,Pro)Leu
4	Ar(1.0)	Try[f]	.....	.....	25	Arg.Try
2a	Gly(0.8)	Lys(1.8),Pro(0.6), Val(1.3),Gly(1.3)	*C:* Lys(0.14),Gly(0.11), Val(0.05)	.....	15	Gly(Lys,Pro)Val.Gly.Lys
2aH_2O	Ag(0.7)	Gly(1.1),Lys(0.6), Pro(1.2),Val(1.1), Try[f]	.....	Asp + Glu(0.1)	10	Arg(Try,Gly,Lys,Pro,Val)
2b	Lys(0.5)	Arg(1.9),Pro(0.9), Val(0.9)	*H:* Val(0.27)	Gly(0.1), Lys(0.2)	40	Lys(Arg_2,Pro)Val

[a] Recoveries in residues per mole of peptide are given in parentheses. [b] Corrected also for breakdown during hydrolysis.[29] [c] Considered significant if in the amount of at least 0.1 residue/mole of peptide.* [d] Computed from amino acid analysis, and corrected for obvious operational losses (guide strips, pilot runs, etc.). [e] Detected with α-nitroso-β-naphthol.[14] [f] Detected with *p*-dimethylaminobenzaldehyde.[15] [g] *H:* Hydrazinolysis; *C:* Carboxypeptidase.

TABLE II

COMPOSITION AND STRUCTURE OF ACIDIC PEPTIDES

Peptide		7b	6b	6c	6d	6e
N-terminus[a,b]	Lys	..	0.3	0.3	0.2	0.3
	Glu	1.0	..	..	..	..
Other constituent amino acids[a]	Ala	..	2.3	3.0	3.0	3.0
	Glu	..	2.6	2.7	2.9	2.8
	Asp	..	1.8	2.2	1.9	1.9
	Pro	..	1.0	0.9	1.3	2.0
	Ser	..	0.9	1.0	1.0	0.9
	Gly	..	1.2	1.2	1.0	1.1
	Val	..	0.8	1.0	0.9	0.7
	Phe	0.8	..	0.6	1.1	0.8
	Leu	..	..	..	0.4	0.7
	Tyr	..	[c]	[c]	[c]	[c]
Amino acids as contaminants[a]	Lys	..	..	0.2	0.4	..
	Leu	..	0.2	0.2	..	..
	Phe	..	0.1	..	..	..
C-terminus (carboxypeptidase)		..	..	Phe(0.46) Ala(0.39) $GluNH_2$(0.23)	..	..
Recovery, %[d]		60%	5%	5%	5%	20%
Proposed structure	7b	Glu.Phe				
	6b	Lys(Glu_3,Asp_2,Ala_2,Val,Tyr,Pro,Gly,Ser)				
	6c	Lys(Glu_2,Asp_2,Ala_2,Val,Tyr,Pro,Gly,Ser).$GluNH_2$.Ala.Phe				
	6d	Approximately $^2/_3$ of 6c + $^1/_3$ of 6e				
	6e	Lys(Glu_3,Asp_2,Ala_3,Val,Tyr,Pro_2,Gly,Ser,Phe,Leu)				

[a] In residues per mole of peptide. [b] Corrected only for breakdown during hydrolysis.[29] [c] Tyrosine was detected with α-nitroso-β-naphthol.[14] [d] Computed from amino acid analysis and corrected for losses during purification.

the C-terminus was performed on those peptides isolated in high enough yield, either by digestion with carboxypeptidase[12] or by hydrazinolysis.[13] Color reactions were also used to confirm the presence in some peptides of tyrosine[14] or tryptophan.[15]

Proposed Structure of Peptide Fragments.—Of all the peptides obtainable in purified form from digests after successive fractionation by paper electrophoresis and paper chromatography, only those which showed a fairly intense reactivity with ninhydrin[16] were submitted to complete analysis. The combined results of the analysis of these peptides for amino acid composition, and for N- and C-terminal residues, are presented in Tables I and II, together with their proposed structure. R_f values of these peptides in three solvent systems are also given (Table III).

Certain peptides appear in Fig. 1 and 2 but are not included in Tables I and II, since some difficulties were encountered in their analysis. However, there are some pertinent comments that should be made here about them. The peptide in electrophoretic band No. 1 (Fig. 1) became insoluble upon dinitrophenylation and was subsequently difficult to handle for analysis; its properties, including a high positive charge and low R_f values (see Fig. 2a and C) would point to a fragment derived from the basic sequence... Lys. Lys. Arg. Arg. . . .in α-corticotropin. Peptides 2a and 2a$_{H_2O}$ could not be resolved by the *n*-butanol/formic acid/water system but were separated after dinitrophenylation of the mixture when it was discovered that DNP-2a$_{H_2O}$ is water soluble whereas DNP-2a is soluble in ether. Peptides in band No. 2 seem to contain a variable number of basic

(12) J. I. Harris and C. H. Li, *J. Biol. Chem.*, **213**, 499 (1955).

(13) C. I. Niu and H. Fraenkel-Conrat, THIS JOURNAL, **77**, 5882 (1955).

(14) R. Acher and C. Crocker, *Biochim. et Biophys. Acta*, **9**, 704 (1952).

(15) I. Smith, *Nature*, **171**, 43 (1953).

(16) These are indicated by shaded areas in Fig. 2.

TABLE III

CHROMATOGRAPHIC BEHAVIOR ON PAPER OF CERTAIN PEPTIDES (R_f VALUES)

Peptide no.[a]	Proposed sequence	Solvent[b] 1	Solvent[b] 2	Solvent[a,c] 3 This paper	Solvent[a,c] 3 White[28]
7b	Glu.Phe	..	0.53	Arg	Arg
4	Arg.Try	0.44	.26	Val	Val
5b	Ser.Tyr	.43	.27	Pro	Pro
5c	Ala.Phe.Pro.Leu	.80	.78	..	1.4 × Phe
2a	Gly.Lys.Pro.Val.Gly.Lys	.07	.01	Glu	...
2b	Lys.Arg.Arg.Pro.Val	.12	.01	Glu	...
$2a_{H_2O}$	Arg.Try.Gly.Lys.Pro.Val	.07	.01	Glu	...
5a 6	Ser.Tyr.Ser.Met.Glu.His.Phe	.23	.06	..	...

[a] See Fig. 2. [b] Solvents: (1) *n*-butanol/pyridine/acetic acid/water[8]; (2) *n*-butanol/formic acid/water[9]; (3) *s*-butyl alcohol/ammonia.[30] [c] Since this system is used in such a way that the position of the solvent front cannot be determined, R_f values are given as in reference 28 in terms of the nearest amino acid.

residues (compare 2a with 2b, for instance); this is presumably related to the fact that there are two overlapping zones in band No. 2, easily recognized by visual inspection of the ninhydrin spot, although impossible to resolve with the present techniques. Although judging from ninhydrin color the peptides in band No. 3 seem to be present in reasonable amounts, their component amino acids could not be clearly identified. That these peptides (in band No. 3) appear in the early stages of digestion and subsequently decrease in amount, seems to indicate fragments of rather large size. Among the peptides in band No. 5, spot 5aa gave analytical values that suggest a long peptide extending from position 16 to 37 (Table IV); however, because of the low recovery and high complexity of this material, it was not possible to ascertain its complete structure. Band No. 6 contains a family of related peptides, decreasing in length toward the C-terminus.

Considering the large variety of C-terminal amino acids encountered in the products of digestion, one must conclude that the action of chymotrypsin was not restricted here to hydrolysis of peptide bonds involving the carboxyl groups of aromatic amino acids.[17] However, the yields of all peptides were not comparable; there was a series of peptides liberated in relatively low yield, and with a good deal of overlapping in structure. This overlapping does serve to provide helpful clues to the position of peptides relative to one another, thus facilitating elucidation of larger segments of the molecule.

Partial Structure of α-Corticotropin Derived from Chymotryptic Digestion.—The one methionine residue and one histidine residue that are present in α-corticotropin[18] are found in peptide 5ab; the partial sequence Ser (Tyr, Ser, Met, Glu) His.Phe (which will be designated *1*) accounts as well for one of the two tyrosine and two of the three serine residues in the molecule. The third serine is common to peptides of family No. 6 (Table II), where it is always bracketed by other amino acids; if the serine at the N-terminus of the hormone[19] is taken into account, sequence (*1*) must then occur at the amino end of α-corticotropin.

Peptides 6b, 6c, 6e and 5c can be seen to derive from another single sequence of the molecule, as follows: Lys(Ala_2,-Glu_2Asp_2,Val,Tyr,Pro,Gly,Ser)$GluNH_2$.Ala.Phe.Pro.Leu(*2*). This sequence accounts for the single leucine and the three alanine residues present in α-corticotropin, as well as for the second tyrosine and the third serine not accounted for by sequence (*1*).

Peptide No. 7b, Glu.Phe, represents a third sequence, which, together with the other two, accounts completely for the three phenylalanine residues in α-corticotropin.[18] One of these phenylalanines is C-terminal in the hormone[20]; it cannot belong to sequence (*1*), which was just shown to be at the amino end of the molecule, nor to sequence (*2*), since here phenylalanine is bracketed by other residues. The

(17) α-Corticotropin contains 1 residue of tryptophan, 2 of tyrosine and 3 of phenylalanine.[18]

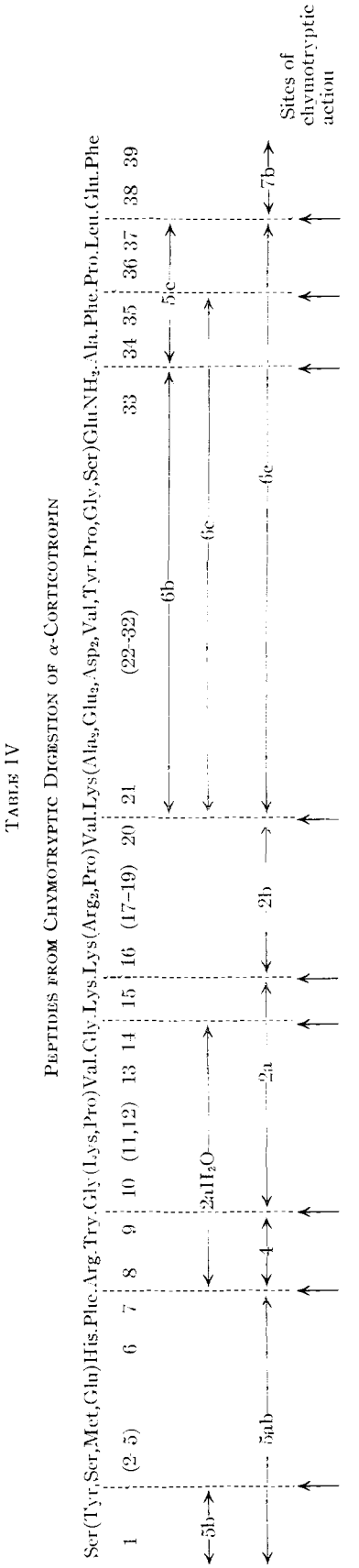
(18) A. L. Levy, I. I. Geschwind and C. H. Li, *J. Biol. Chem.*, **213** 187 (1955).

(19) A. L. Levy and C. H. Li, *ibid.*, **213**, 487 (1955).

(20) J. I. Harris and C. H. Li, *ibid.*, **213**, 499 (1955).

TABLE IV

PEPTIDES FROM CHYMOTRYPTIC DIGESTION OF α-CORTICOTROPIN



sequence Glu.Phe(*3*) must consequently be placed at the carboxylic end of α-corticotropin.

The single tryptophan residue of the hormone is found in peptide 4 and in peptide $2a_{H_2O}$ (Table I), both of which must derive from a common sequence (Arg.Try(Gly,Lys, Pro,Val)). This together with sequence (*2*) locates two of the three glycine residues in the molecule. Peptide 2a also has the glycine residues and consequently must possess a section in common with either peptide 4 or peptide $2a_{H_2O}$; its structure Gly. . . .Gly.Lys, however, rules out any connection with sequence (*2*). It must be concluded that peptides 2a, $2a_{H_2O}$ and 4 all arise from the single sequence Arg. Try.Gly(Lys, Pro)Val.Gly.Lys(*4*).

Peptide 2b affords the remaining sequence, Lys(Arg_2,Pro) Val(*5*), necessary to account for the complete amino acid composition of the whole molecule.[18] From the presence of peptide 5aa (see discussion of this peptide), it may be concluded that sequence (*5*) and (*2*) were originally linked. For the following discussion, it will be helpful to arrange sequences (*1*) to (*5*) in the order indicated by previous work on α-corticotropin with other enzymes[3,21] (Table IV).

With respect to location of amide groups, these experiments show that one appears at position 33 and that none occurs at position 5 or 38. The second amide group known to be present in the molecule[18] consequently should be sought in the sequence Glu.Asp.Asp.Glu (positions 27 to 30).[3]

Aspects of the Specificity of Chymotrypsin.—Primarily intended as a structural investigation, this study also yielded some observations concerning the specificity of chymotrypsin. When subjected to proteolysis, α-corticotropin does not evince some of the usual complications associated with more typical proteins, since it lacks a secondary structure[22] and contains no –S–S bridges.[18] In other words, digestion of the peptide hormone could neither involve denaturation steps nor lead to an enzyme-resistant core, which makes the situation considerably simpler.

In agreement with early work on small synthetic substrates, the peptide bonds in α-corticotropin that involve aromatic amino acids usually were hydrolyzed by chymotrypsin. Two expected breaks, at the –Tyr.Pro– and –Met.-Glu–bonds, did not occur, but in both instances, conditions unfavorable to the action of the enzyme are persumably introduced by the other partner in the bond (*cf.* 23). Recent work with proteins of small size, insulin,[24] ribonuclease[25] and lysozyme,[26] further indicated the possibility of splitting after a leucine, a valine or an amide of dicarboxylic acid; examples of such breaks were indeed observed here.

It is of interest to note that the liberation of peptide 4 (or opening of the bond $\overset{7}{\text{Phe}}$–$\overset{8}{\text{Arg}}$) occurs earlier than the liberation of peptide 6c (or opening of the bond $\overset{35}{\text{Phe}}$–$\overset{36}{\text{Pro}}$) (Figs. 1 and 2b). Furthermore, the Val–Gly bond is resistant to chymotrypsin whereas Val–Lys (peptide 2b) is susceptible to the action of the enzyme. These results would seem to indicate that a neighboring positive charge increases the susceptibility of the bond to the enzyme. This might possibly be related to the opposite effect, namely, the well-known inhibition by a negative charge of substrate activity in small molecules.[23] Besides, it becomes tempting to speculate whether the splitting of a –Lys.Lys–bond in α-corticotropins by chymotrypsin, which has also been observed by others[27] and usually has been attributed to some contaminating trypsin, could not arise from this charge effect and be inherently due to the chymotrypsin itself.

Even the very short time required to inactivate the enzyme immediately after it is mixed with the α-corticotropin is sufficient to produce some breakdown of the hormone. Indeed, preliminary studies indicate that more than half of the –Phe.Arg– bond (see Table IV) are cleaved within that period, a reaction which is seen to liberate two peptides, one short and neutral (positions 1 to 7), the other very long and somewhat basic (positions 8 to 39). This is evidenced by two spots recognizable after paper electrophoresis of the digest at time "zero" (Fig. 1). The peptide pattern is also seen to depend somewhat on the *p*H at which proteolysis by chymotrypsin is conducted (Fig. 1); the temperature might be a further variable of the system.

With these effects in mind, it becomes less puzzling to observe significant differences in the results of investigations on related corticotropins. Using chymotrypsin at a *p*H of 7.8 and 25°, with a rather low enzyme/substrate ratio, Shepherd, *et al.*,[27] obtained evidence of splitting at only five bonds in the polypeptide chain (the first five in Table IV). The very high recovery of peptides, quantitative in nearly all cases, points to an extremely sharp specificity on the part of chymotrypsin under these conditions. White, *et al.*[28] using a higher temperature and a lower *p*H, found evidence for a further cleavage at the –Val.Lys– bond. The substrates in these studies, namely, β-corticotropin and corticotropin-A, were both obtained from hog glands but by different isolation procedures. They both differ from sheep α-corticotropin at positions 33–34, where a susceptible–GluNH_2.Ala– bond is present instead of the chymotrypsin-resistant linkage –Glu.-Ala–, which is encountered in the porcine hormones.[29] On the other hand, the porcine corticotropins possess large segments that are identical with their counterparts in α-corticotropin, from positions 1 to 24 and also from 33 to 39; yet, only in the ovine hormone do the –Val.Gly– and –Phe.Pro– bonds that are located in these areas of identity evince some susceptibility to chymotrypsin.[30] This again might well arise from differences in the conditions of reaction, or, alternately, it might be taken to indicate that the influence of structure upon rate of digestion extends significantly beyond the two partners of the bond being broken.

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