References and Notes

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Isolation and Properties of Corticotropin from Bovine Pituitary Glands

The structural formulas that have been elucidated for ACTH preparations isolated from ovine (*1*) and porcine (*2, 3*) pituitary glands (α-corticotropin and corticotropin-A, respectively) have revealed that these preparations are not identical. A recent note by White and Peters (*4*) describes the results of preliminary physical and chemical studies on a bovine ACTH preparation; similarities in amino acid composition between the bovine preparation and porcine corticotropin-A, as well as an identity in the patterns of amino acids that were released when these two preparations were treated with enzymes, were noted. We would like to report (*5*) that in this laboratory an identical amino acid composition has been found for bovine ACTH as for the ovine (*6, 7*) product (α-corticotropin), and in addition, that the hormones of these latter two species manifested identical behavior in resin column chromatography and in countercurrent distribution. Hence, the properties of bovine ACTH would seem to be closer to those of the ovine than to those of the porcine hormone.

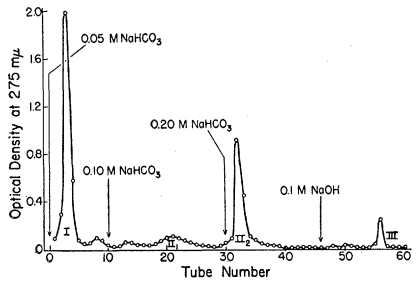

Fig. 1. Chromatography on the Na form of Amberlite XE-97 resin (dimensions of column, 0.9 by 24 cm) of an ACTH concentrate (20 mg) obtained from beef pituitary glands; 3 ml per tube. The hormonal activity is located in peaks II_1 and II_2.

The bovine corticotropin was isolated from whole beef pituitaries by the same procedure previously described for the hormone from sheep glands (*6, 7*), except for omission of the step involving zone electrophoresis on starch. The chromatographic pattern of the concentrate obtained at the dioxane step on the Amberlite IRC-50 (XE-97) column may be seen in Fig. 1; the activity was found in peaks II_2 and II_1. It may be noted that the positions of these two active peaks are identical with those obtained (*7*) with the ovine ACTH concentrate. The material in peak II_2 was desalted and submitted to 200 transfers in an all-glass countercurrent distribution apparatus (*8*) in a 2-butanol/0.2-percent trichloroacetic acid system (Fig. 2). The material in those tubes falling within the theoretical distribution curve for a partition coefficient (K) of 1.06 was found to be active (*9*), and it behaved as a single substance when it was submitted to terminal amino acid analyses. It may be recalled that corticotropin-A (of porcine origin) distributes in the 2-butanol/0.2-percent trichloroacetic acid system with a K value of 1.82 (*10*), whereas α-corticotropin in the same solvent system distributes with a K value of 1.0 (*11*).

The molar ratios of amino acids in the bovine hormone are as follows: alanine, 3; arginine, 3; aspartic acid, 2; glutamic acid, 5; glycine, 3; histidine, 1; leucine, 1; lysine, 4; methionine, 1; phenylalanine, 3; proline, 4; serine, 3; tryptophan, 1; tyrosine, 2; and valine, 3. Tyrosine and tryptophan were estimated spectrophotometrically (*12*), while the other amino acids, including tyrosine, were estimated by quantitative paper chromatography of their dinitrophenyl derivatives (*13*). It can be noted that these values are identical with those found for α-corticotropin (*14*). Earlier investigations (*2, 6, 14*) showed that there is a difference in amino acid composition between the peptide hormones isolated from sheep and from pig glands —namely, one more serine and one less leucine in the former.

N-terminal amino acid anaylsis of the bovine hormone by means of both the fluorodinitrobenzene and phenyl*iso*thiocyanate procedures (*15*) disclosed serine as the sole terminal residue. The paper-strip modification (*16*) of the phenyl*iso*-thiocyanate method yielded the following N-terminal sequence for bovine corticotropin: serine, tyrosine, serine, methionine. . . . The amino acid released from the carboxyl end of the peptide hormone obtained by the carboxypeptidase procedure (*17*) was phenylalanine. Thus, with respect to N-terminal amino acid sequence and the C-terminal residue, the hormones of all three species are identical (*1–3, 18*).

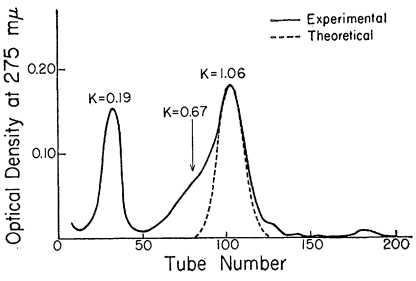

Fig. 2. Countercurrent distribution (200 transfers) of material (77 mg) obtained from chromatography on an XE-97 resin column (see Fig. 1); system, 2-butanol/0.2-percent aqueous trichloroacetic acid. The component with $K = 0.6$ is the bovine corticotropin.

The findings reported here indicate an identity between bovine ACTH and α-corticotropin, but the final proof for this conclusion must await the elucidation of the structural formula of the bovine hormone. Such structural studies are now being carried out and will be reported in a subsequent paper.

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5. This report is paper XI of the corticotropins (ACTH) series. This work was supported in part by grants from the Albert and Mary Lasker Foundation and from the National Institutes of Health of the U.S. Public Health Service (grant No. G 2907).
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9. From 1 kg of whole beef pituitaries, the yield of the hormone averaged about 8 mg. When the material was assayed in hypophysectomized rats, it possessed an ACTH potency similar to that of α-corticotropin. Like the sheep hormone, the corticotropin from beef pituitaries has a me'anocyte-stimulating activity.
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