CORTICOTROPINS (ACTH)

I. ISOLATION OF $\alpha$-CORTICOTROPIN FROM SHEEP PITUITARY GLANDS

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Since the discovery (1) of an adrenal-stimulating activity in the anterior pituitary and the subsequent demonstration (2) of the therapeutic value in rheumatoid arthritis of adrenocorticotropic concentrates (corticotropins) from pituitary glands, many attempts have been made to isolate this active principle from hypophyseal extracts (3, 4). A preliminary report has been made from this laboratory (5) of the isolation from sheep pituitary glands of a polypeptide possessing adrenocorticotropic (ACTH) activity. Since the composition of this hormone and many of its physicochemical characteristics differ from those reported by White (6) for corticotropin A isolated from hog glands, it has been designated $\alpha$-corticotropin. In this and succeeding papers, we will report on its preparation, properties, and structure.

Isolation Procedure

Starting Material—The ACTH Fraction E obtained from the acid-ace tone extract (AAP) of whole sheep pituitaries after adsorption on oxy cellulose, as described previously (3, 7), was employed as the starting material for this work (Table I). This concentrate possesses approximately 25 U. S. P. units of adrenal-stimulating activity per mg.

Dioxane Fractionation—Eastman dioxane was refluxed over sodium and distilled before use. 200 mg. of Fraction E were dissolved in 200 ml. of 50 per cent aqueous dioxane, and the solution was adjusted to pH 9.3 to 9.4 with 0.1 M NaOH. A flocculent precipitate which formed was removed by centrifugation; the clear supernatant fluid was immediately frozen and lyophilized. The resulting white powdered material (Fraction F) weighed about 100 mg. and contained ACTH activity equivalent to 45 U. S. P. units per mg., whereas the precipitate from dioxane contained less than 1 unit per mg. Measurements of pH were made with a glass electrode in a Leeds and Northrup pH meter.

* Deceased, August 22, 1954.

1 All ACTH activities reported herein were estimated by comparing the effects of the unknown preparation in depleting ascorbic acid in the adrenals of hypophysectomized rats (8, 9) with those of the U. S. P. standard preparation.
Zone Electrophoretic Separation—Fraction F was next subjected to fractionation by means of zone electrophoresis on starch in a cold room at 3°. The technique employed was a modification of the method of Kunkel and Slater (10). Details, such as the type of trough used for the immobilized medium and the general procedure for packing the trough, were the same as those described previously (11). 100 mg. of Fraction F were dissolved in 1 ml. of 0.1 M Na₂CO₃ and placed on the trough about 6 cm. from the cathode end. The electrophoretic experiment was conducted at 200 volts for 16 hours. The starch in the trough was then cut into segments of 1 cm. and extracted three times with 5 ml. of water; a 0.1 ml. extract from each segment was analyzed for protein concentration by the method of Lowry et al. (12). A typical pattern may be seen in Fig. 1. Results of bio-assay revealed that the active material was concentrated in Segments 5 to 7. The extracts from these segments were combined, dialyzed against distilled water for 5 to 6 hours at 0°, and lyophilized. The yield was 50 mg. of material (Fraction G) having an activity of 75 units per mg.

Chromatographic Fractionation on Ion Exchange Resin—Further purification of Fraction G was carried out by means of column chromatography, with the carboxyl cation exchange resin, Amberlite IRC-50, as adsorbent. The resin, in 250 to 500 mesh range (XF-97), was washed three times with 3 volumes of water; 10 minutes were allowed for settling after each washing. The resin was then prepared by a procedure similar to that described by Hirs et al. (13). After the resin had become converted completely to the sodium form, it was washed thoroughly with water, dried in a Büchner funnel, and stored in this form.
For preparation of a column, 250 ml. of the moist resin were stirred with 0.05 M NaHCO₃ for 1 hour and allowed to settle. The supernatant fluid was decanted, and sufficient 0.05 M NaHCO₃ solution was added to make a slurry, which was then poured into a column (2.4 X 25 cm.) fitted at the bottom with a coarse sintered glass disk. An additional 250 ml. of 0.05 M NaHCO₃ were allowed to flow through the column before Fraction G was applied.¹

100 mg. of Fraction G in 2.5 ml. of 0.05 M NaHCO₃ were put onto the column; the column was then washed with 200 ml. of 0.05 M NaHCO₃, and the emergent solution was collected in 10 ml. fractions in a drop-counting fraction collector. After the 0.05 M NaHCO₃ solution had passed through the column, a gradual increase in the strength of the eluent was effected by allowing 350 ml. of 0.15 M NaHCO₃ to drop into 250 ml. of 0.05 M NaHCO₃ in the magnetically stirred reservoir in which mixing took place, as described previously ((14), cf. (15, 16)). 10 ml. fractions were collected in test-tubes, each of which contained 0.1 ml. of concentrated HCl. Finally, 300 ml. of 0.1 M Na₂CO₃ were passed through the column to elute the remainder of the adsorbed material. The concentration of material in each effluent fraction was estimated by measuring its absorption at 275 nm in a model DU Beckman spectrophotometer; the result of a typical experiment may be seen in Fig. 2. Bioassay revealed that Peak II, containing 50 to 60 per cent of the original material, possessed the major part of the ACTH activity.

In order to accomplish the recovery of the active material, the contents of tubes 34 to 49 were combined, adjusted to about pH 4 with 1.0 M HCl, and lyophilized. In order to remove the salt, the dry solid (2 gm.) was dissolved in 50 ml. of water, and an equal volume of 50 per cent trichloroacetic acid (TCA) was added. The solution was then centrifuged, and the resulting supernatant fluid was discarded. The precipitate was dissolved in about 10 ml. of water, to which a few drops of 1.0 M HCl were added, and then extracted six times with an equal volume of peroxide-free ether. After careful removal under vacuum of the last traces of ether, the solution was lyophilized. The product (Fraction H) had an activity of 100 units per mg.; the yield was 50 mg.

Counter-Current Distribution—The final purification of α-corticotropin was achieved by counter-current distribution in a 2-butanol-aqueous trichloroacetic acid system. An automatic all-glass apparatus (17) consisting of 240 cells was employed, each cell in the train having been constructed to contain 5 ml. of lower, or stationary, phase. The counter-current dis-

¹ By altering the diameter of the column, the scale of the experiment has been increased (3.6 X 25 cm.) in order to prepare larger amounts of material or decreased (0.9 X 35 cm.) for analytical studies.
distribution experiments were carried out in a room maintained at 19° ± 1°. The following description of the steps involved in carrying out a specific purification of Fraction H may be taken as representative of the general procedure.

Distilled water and dry 2-butanol (Shell Chemical Corporation, distilled from SnCl₂) were shaken together in a volume ratio of 1.155:1 for equilibration, and the two layers were separated. The lower phase was made up to 0.2 per cent trichloroacetic acid by the addition of a calculated quantity of 50 per cent (weight per volume) TCA solution, shaken with an equal volume of upper phase, and allowed to equilibrate, with occasional shaking, for at least 24 hours before use.

The lower phase was then pipetted into each cell in the counter-current apparatus, and the device for the automatic addition of upper phase was put into operation. Preliminary experiments had shown that shaking the two layers for 70 seconds accomplished adequate mixing, while a settling time of 2.5 minutes was generally sufficient for good separation. The total time required for one complete transfer was 4.5 minutes.

After a forerun of twenty-four transfers had been completed, 236 mg. of Fraction H were dissolved in lower phase solvent, which had been removed from the 0 cell of the counter-current train, and returned to the cell along with 5 ml. of upper phase. This material was then submitted to a total

![Image](http://www.jbc.org/)

**Fig. 2.** Chromatography on the Na form of Amberlite IRC-50 resin (column 2.4 x 25 cm.) of material (Fraction G, 100 mg.) obtained from zone electrophoresis, 10 ml. per tube. The hormonal activity is located in tubes 34 to 49.
of 500 transfers, and the upper phase material issuing from the 239th (last) tube in the train was collected in an automatic fraction collector. After completion of the distribution, lower phase solution was removed for analysis. The optical density of each of these lower phase samples was measured at 275 μm, with fresh lower phase as the reference blank, and the optical density of those upper phase samples which had been collected in the fraction collector was also measured, in this case against a water blank. No material was found in these upper phases. The experimental distribution of material as determined from the optical density measurements and the corresponding theoretical distribution are illustrated in Fig. 3.

Fig. 3. Counter-current distribution (500 transfers) of material (236 mg. of Fraction H) obtained from chromatography on IRC-50 resin. System, 2-butanol-0.2 per cent aqueous trichloroacetic acid. The component with K = 0.719 has been designated α-corticotropin.

The material which had been distributed throughout the apparatus was combined into five fractions, designated Components a, b, c, d, and e, as follows: tubes 30 to 60, 80 to 135, 136 to 170, 171 to 203, and 204 to 239. The volume of each fraction was reduced to about 50 ml. at room temperature under reduced pressure; trichloroacetic acid was removed by extraction with ether, six successive extractions usually being sufficient to reduce the acidity of the water solution to pH 4.5 to 5. After extraction, each sample was taken to dryness in vacuo at room temperature, redissolved, and lyophilized for isolation of the material as the trichloroacetate salt. The yields were as follows: Component a, 46 mg.; Component b, 20 mg.; Component c, 32 mg.; Component d, 48 mg.; and Component e, 66 mg. Components a to c had an activity of less than 10 units per mg., whereas
Components d and e possessed equal potency equivalent to 150 units per mg.; the latter two were combined. The active product isolated in this manner was designated α-corticotropin (5). Table I summarizes the yield and estimated ACTH potency of fractions obtained from each step of the isolation procedure. It may be noted that 0.012 gm. of α-corticotropin was isolated from 1 kilo of whole sheep pituitaries. The over-all yield with respect to activity was about 15 per cent.

Homogeneity Studies

Counter-Current Distribution—Approximately 25 mg. of α-corticotropin were submitted to 50 transfers in a 2-butanol-0.1 per cent aqueous tri-chloroacetic acid system. The distribution patterns may be seen in Fig. 4; the experimental pattern appears to follow very closely the theoretical distribution curve for a partition coefficient (K) of 0.41. Moreover, the K values were found to be constant throughout the curve when distribution coefficients were calculated from the contents of adjacent pairs of tubes (18).

Partition Chromatography—The success which Porter achieved with the technique of partition chromatography in his studies on insulin (19), led us to explore the utility of this method for investigating the homogeneity of α-corticotropin. Hyflo Super-Cel (Johns-Manville) treated with silane (General Electric Company, Dri-Film), as described by Howard and Martin (20), was employed as the supporting medium. The solvent system was that used by Porter (19) for insulin and had the following composition: 9 ml. of 5 M sodium dihydrogen phosphate adjusted to pH 3.0 by the addition of phosphoric acid; 6.67 ml. of ethyl Cellosolve, 3.33 ml. of butyl Cellosolve.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Procedure</th>
<th>Yield from 1 kilo fresh sheep glands</th>
<th>Estimated ACTH potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAP</td>
<td>Acid-acetone extract</td>
<td>25</td>
<td>0.5</td>
</tr>
<tr>
<td>D</td>
<td>NaCl precipitation</td>
<td>6.5</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>Oxy cellulose adsorption</td>
<td>0.2</td>
<td>25</td>
</tr>
<tr>
<td>F</td>
<td>Dioxane fractionation</td>
<td>0.1</td>
<td>45</td>
</tr>
<tr>
<td>G</td>
<td>Zone electrophoresis on starch</td>
<td>0.05</td>
<td>75</td>
</tr>
<tr>
<td>H</td>
<td>IRC-50 resin column</td>
<td>0.025</td>
<td>100</td>
</tr>
<tr>
<td>α-Corticotropin</td>
<td>Counter-current distribution</td>
<td>0.012</td>
<td>150</td>
</tr>
</tbody>
</table>
solve, and 15 ml. of water; the glycol ethers were redistilled in the presence of SnCl₂. The preparation and operation of the column followed exactly the details described by Porter (19).³

The distribution of α-corticotropin in this system is in favor of the organic phase, with a partition coefficient of 7.3. Fig. 5 presents the chromatographic pattern obtained when 3 mg. of the hormone were run on a 6 gm. column. The peptide concentration in the effluent was estimated by ultra-

![Graph showing counter-current distribution](attachment:image.png)

Fig. 4. Counter-current distribution (50 transfers) of α-corticotropin (see Fig. 3). System, 2-butanol-0.1 per cent aqueous trichloroacetic acid.

violet absorption at 275 μm. α-Corticotropin behaved as a chromatographically homogeneous component (R = 0.5). When Fraction F was similarly chromatographed, its heterogeneity was indicated by at least five peaks on the effluent curve.

Zone Electrophoresis—The behavior of α-corticotropin in zone electrophoresis on starch at pH 5.1, 7.0, 8.2, 9.1, 10.2, and 11.2 has been examined, and in every case the hormone has been found to migrate as a single zone. The electrophoretic pattern obtained when 4 mg. of α-corticotropin were subjected to electrophoresis in an acetate buffer of pH 5.1 and 0.1 ionic

³The authors wish to thank Dr. R. R. Porter for the advice on these chromatographic experiments, which he so generously gave during his visit to this laboratory.
strength, at 170 volts for 16 hours, may be seen in Fig. 6. The experimental
details were the same as those described above. Each segment along the
curve appeared to possess equal activity according to biological assay.

![Image](Fig. 5. Chromatography of α-corticotropin (3 mg.). System, 15 ml. of water,
6.67 ml. of ethyl Cellosolve, 3.33 ml. of butyl Cellosolve, 9 ml. of 5 M NaH₂PO₄ (ad-
justed to pH 3 with H₃PO₄). Column, 6 gm. of silane-treated Hyflo Super-Cel.)

![Image](Fig. 6. Zone electrophoresis of α-corticotropin (4 mg.) on starch in acetate buffer
of pH 5.1 and 0.1 ionic strength at 170 volts for 16 hours. The arrow indicates the
site of application of the hormone.)

**Terminal Group and Amino Acid Analyses**—Evidence for the purity of
α-corticotropin has also been adduced on the basis of chemical studies.
Briefly, reactions of α-corticotropin with phenyl isothiocyanate (21) re-
vealed that serine occurs as the only N-terminal amino acid, in an amount
close to 1 mole per mole (4541 gm.) of the peptide hormone (22). When
the hormone is treated with carboxypeptidase, 1 mole of phenylalanine is released per mole of α-corticotropin (22). The results of amino acid analyses of various preparations of α-corticotropin are in close agreement, and the molar ratios of the constituent amino acids very closely approximate whole numbers (23). These results suggest that the hormone peptide is a single chemical species.

Biological Tests—As has been mentioned above, α-corticotropin possesses an activity of 150 i.u. per mg. as estimated by the extent of ascorbic acid depletion in the adrenals of hypophysectomized rats. A daily dose of 0.005 mg. of α-corticotropin in beeswax-peanut oil suspension, injected into hypophysectomized rats on the day of operation and subsequently for 15 days, maintained normal adrenal weight at 23 mg., compared with 8 mg. for the controls. In experiments for measuring adrenal stimulation, a daily dose of 0.01 mg. in beeswax-peanut oil suspension given for 4 days to hypophysectomized male rats (operated on at 40 days of age and injected 4 days postoperatively) causes an increase of adrenal size from 9 to 24 mg. Moreover, a total dose of 1 mg. to hypophysectomized female rats (operated on at 27 days of age and injected for 4 days beginning on the 14th postoperative day) produces no histological evidence of the presence of thyrotropic and gonadotropic hormonal contaminations. Furthermore, a dose of 1 mg. injected locally into month-old squabs gives no indication of prolactational response. Bioassay for melanophore-expanding activity in hypophysectomized Rana pipiens indicates that α-corticotropin possesses an intermedin contamination of less than 0.1 per cent. A complete account of the biological behavior of the hormone will be reported elsewhere.

DISCUSSION

The successful concentration of ACTH activity has been achieved by a number of workers by means of ion exchange chromatography on oxy-cellulose (4, 24, 25) and on the polycarboxylic acid resin Amberlite IRC-50 (25–27). In this study, the use of gradually increasing cation concentration and pH on Amberlite IRC-50 (XE-97) has afforded an effective purification of α-corticotropin.

α-Corticotropin is adsorbed on IRC-50 at pH 8.3 if the sodium ion concentration is less than 0.10 M; at concentrations equal to or greater than 0.10 M the activity is no longer adsorbed. In actual practice it has been found expedient to introduce a sample, which has been purified by zone electrophoresis on starch, onto a column of resin equilibrated with 0.05 M NaHCO₃. Some inactive material passes through the column with the hold-up volume under these conditions, whereas the activity is strongly adsorbed. No further elution of material results even when quantities of buffer equal to as much as 20 hold-up volumes are passed through the
column. If a solution of 0.15 M NaHCO₃ is then put through the column, very rapid elution of activity occurs in the form of a single peak. All the remaining strongly adsorbed material can be removed by a final washing with a 0.1 M NaOH or 0.1 M Na₂CO₃ solution. The fraction obtained with strongly alkaline solutions emerges with approximately 6 hold-up volumes of eluent and has an activity of less than 10 units per mg.

Since no special precautions had been taken to prevent contact of the bicarbonate solutions with air, with possible subsequent loss of CO₂ and change in pH, experiments were undertaken to demonstrate that elution of activity actually resulted from an increasing concentration gradient of sodium ions at constant pH. Mineral oil was layered on the bicarbonate solutions in order to prevent loss of CO₂. The pH of the effluent solutions was determined and found to be constant, and the distribution of ultraviolet-absorbing material was identical to that found in earlier experiments. Additional proof of the importance of the gradient of the sodium ion concentration was obtained by employing as eluent of the activity a solution which is 0.05 M with respect to NaHCO₃ and 0.10 M with respect to NaCl. The results showed that the distribution pattern of material was the same as that obtained with a 0.15 M NaHCO₃ solution. Thus, by simply increasing the sodium ion concentration at constant pH, elution of activity may be effected.

However, when a fraction derived from steps in the isolation procedure which precede the zone electrophoresis, such as Fraction F, is introduced onto the resin, chromatography on IRC-50 yields two incompletely resolved peaks which appear to be equally active (Fig. 7). Two minor components were also revealed; one of these appears just before the first active peak and is inactive, whereas the other follows the second major peak and possesses activity.

The results of a series of counter-current distributions in the system 2-BuOH-0.2 per cent TCA, performed on the active material (combined material of Peaks II₁ and II₂ in Fig. 7) may be seen in Fig. 8, A; the activity was confined to the peak having \( K = 0.71 \). The active peak was then cut and redistributed in the same system. The material giving the distribution pattern shown in Fig. 8, B was also cut and isolated. When each of these fractions was submitted to counter-current distribution, \( C₁, C₂, C₃, \) and \( C₄ \) were obtained. Chromatographic analyses indicate that \( C₄ \) contained both Peaks II₁ and II₂, whereas \( C₁ \) consisted almost exclusively of Peak II₁ when corrected for conversion taking place on the column (see below). Thus it appears that Peak II₁ has a slightly greater \( K \) value than Peak II₂ in this system, and that these two active components were not separated to a practical degree by the counter-current procedure.

\[ \text{This fraction binds copper and travels down the column as a distinct blue band under the experimental conditions required for its displacement.} \]
In order to clarify the relationship of these two active peaks and to ascertain why material obtained by zone electrophoresis on starch shows only Peak III, each of the two active peaks was rechromatographed on IRC-50 in the continuous gradient system. While Peak II_1 behaved as a single component, chromatography of the second peak (II_2) revealed the presence of Peak II_1 as well as the expected Peak II_2; in fact, repeated chromatography of isolated Peak II_2 invariably led to the appearance of material in the Peak II_1 region.

It is apparent that Fraction F manifests two active peaks when chromatographed on a resin column; during the chromatography a gradual conversion of Peak II_2 → Peak II_1 also occurs. This may mean that Peak II_1 represents at all times an artifact, created from Peak II_2 under the general conditions of isolation.\(^5\)

Since complete conversion of Peak II_2 to Peak II_1 appears to take place during zone electrophoresis, it was thought probable that this conversion, like that which takes place on the column, was due to the alkaline conditions maintained during the experimental procedure.\(^6\) In fact, the extent

\(^6\) It should be pointed out that there is no evidence of such a conversion occurring during the counter-current procedure, for, when Peak II_2 is submitted to counter-current analysis, followed by rechromatography, only as much Peak II_1 is found as is to be expected from the chromatography itself. It would follow, therefore, that the appearance of the two active peaks in the chromatographic experiments is not due to impurities present in Fraction F, since conversion on the column occurs even with the pure material isolated from the counter-current procedure.

\(^6\) In most of the previous studies with IRC-50 phosphate buffers in the region of
of conversion was found to be dependent on pH as well as on time and temperature. Thus, more rapid and extensive conversion took place in 0.1 M Na₂CO₃ than in 0.2 M NaHCO₃, and complete conversion occurred if the starting material (Fraction F) was allowed to stand at room tempera-

![Experimental vs Theoretical](http://www.jbc.org/)

**Fig. 8.** Counter-current distribution of combined material of Peaks II₁ and II₂ (see Fig. 7). System, 2-butanol-0.2 per cent aqueous trichloroacetic acid.

It may be recalled, however, that the original application (28) of IRC-50 for the chromatography of a protein, cytochrome c, made use of buffers of pH 10.8. Similar alkaline conditions have been employed more recently for the chromatography of cytochrome c (29) and corticotropin A (27).
the second peak (II₂). After as short a period as 6 hours in 0.1 M Na₃CO₃ at room temperature, 90 per cent of the activity was found in Peak II₁.

On the basis of these results, it appears that conversion is undoubtedly due to the alkaline conditions under which zone electrophoresis on starch is performed. When the step involving electrophoresis is omitted, any conversion which occurs results not only from the alkaline conditions per se of the column, but also from the basicity of the solutions in the collecting tubes. To prevent conversion due to this latter cause, effluent was collected in tubes containing a small volume of concentrated HCl. Rechromatography of an active region which had been collected in this manner demonstrated that about one-half of the usual conversion had been prevented. Preliminary chemical investigations indicate that the hormone derived from Peak II₂ (α₂-corticotropin) possesses four amide groups per mole, whereas only two amide groups are found per mole of α-corticotropin. Since no value intermediate between two and four amide groups has been found, it would appear that two amide groups in α₂-corticotropin are probably alkali-labile. Moreover, qualitative carboxyl and amino terminal group analyses of these two fractions are the same. The finding of a greater K value for α-corticotropin than for α₂-corticotropin is consistent with similar findings with respect to insulin. Insulins A and B differ in their distribution characteristics in that Insulin B has the greater K value in the system 2-BuOH-0.1 per cent dichloroacetic acid (30), whereas they differ chemically only in that Insulin B possesses one amide group less than Insulin A (31).

It was pointed out earlier that counter-current distribution in a 2-butanol-aqueous TCA system is incapable of differentiating Peak II₂ from Peak II₁. On the other hand, the Peak II₁ derived from chromatography on the Amberlite IRC-50 (XE-97) column, can be easily resolved into at least three components, one of which possesses the partition coefficient of 0.72 in a 2-butanol-0.2 per cent TCA system (Fig. 3), and represents the active component (α-corticotropin). The K value for α-corticotropin in 2-butanol-aqueous TCA is sensitive to the concentration of TCA, whereas that of the inactive Component α varies very little. Fig. 9 presents the partition coefficient of α-corticotropin as a function of the concentration of TCA; a change in this concentration from 0.10 to 0.5 per cent causes an increase in K value from 0.42 to 3.2.

Although the results of this investigation demonstrate that counter-current distribution is indeed an effective tool for separation and isolation, it should be pointed out that caution must be employed in comparing experimental distribution curves with those plotted according to theory. The anomaly which appears in the experimental distribution curves presented in this investigation, namely, broadening of the distribution patterns, may
probably be explained in terms of a deviation from ideality of the partition isotherm which obtains for the particular peptide-solvent system under investigation (32). Experimentally, even a single pure component when fractionated in a non-ideal system may give a distribution curve which differs from what would be theoretically expected. In such cases the experimental distribution curve cannot be employed as the ultimate criterion for purity or lack thereof.

![Graph](image)

**Fig. 9.** Partition coefficient of α-corticotropin in 2-butanol-aqueous trichloroacetic acid system as a function of TCA concentration.

**SUMMARY**

A procedure involving dioxane fractionation, zone electrophoresis on starch, chromatography on Amberlite IRC-50 (XE-97) resin column, and counter-current distribution has been described for the isolation from sheep pituitary glands of a peptide which possesses ACTH activity equivalent to 150 i.u. per mg. The purity of this peptide, designated α-corticotropin, has been examined by means of counter-current distribution, partition chromatography, zone electrophoresis, terminal group and amino acid analysis, and biological tests.

Evidence is presented for the existence of an alkali-labile active precursor of α-corticotropin, whose behavior during the isolation procedure is discussed.

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