# A Quantitative Amino Acid Analysis of Sheep Adrenocorticotropic (ACTH) Protein[1]

Richard M. Mendenhall[2]

*Laboratory Services, Veterans Administration Hospital, Oakland, California*

The amino acid analysis of sheep adrenocorticotropic (ACTH) protein, prepared by the method of Li, Evans, and Simpson (*1*) is presented. Three investigators working independently and in separate laboratories have produced these results. The starting material in each case was taken originally from the same bottle, thus eliminating any question as to differences in results being caused by variations in preparative technique. The analyses may, therefore, be directly compared.

passage through the column. Thus, known quantities (1.2 μM) of each amino acid were added to the column and the total optical density of the developed ninhydrin color was recorded for each peak, being determined with the Beckman DU spectrophotometer. The standardization was performed 3 or 4 times for each amino acid and the average value used in the determination of the unknown. In so doing, the ratio of optical densities of unknown to standard multiplied by 1.2 equaled the number of micromoles of the particular amino acid under consideration in a known quantity of the chromatographed protein hydrolysate.

Table 1, method 1, presents the data obtained by Shankman Laboratories (*6, 7*) using microbiological techniques, except for cystine which was determined by Li (*8*). Methods 2 and 3 present the data obtained by Hier (*9*), using microbiological techniques, and those of the author, respectively.

TABLE 1

AMINO ACID ANALYSIS OF ADRENOCORTICOTROPIC (ACTH) PROTEIN

| Amino acid | Amino acid content of protein (g/100 g) | | | | Estimated number of residues | | |
|---|---|---|---|---|---|---|---|
| Method* | 1 | 2 | 3 | Av | 1 | 2 | 3 |
| Alanine | — | — | 7.04 | 7.04 | — | — | 18 |
| Arginine | 8.7 | 9.0 | 10.28 | 9.3 | 11 | 12 | 13 |
| Aspartic acid | 6.7 | 6.3 | 6.83 | 6.6 | 11 | 12 | 12 |
| Cystine | 7.2 | 8.6 | 8.21 | 8.0 | 7 | 8 | 8 |
| Glutamic acid | 15.6 | 16.1 | 14.99 | 15.6 | 24 | 25 | 23 |
| Glycine | 8.0 | — | 8.66 | 8.3 | 24 | — | 26 |
| Histidine | 1.3 | 1.3 | 1.34 | 1.3 | 2 | 2 | 2 |
| Isoleucine | 3.1 | 3.4 | 2.97 | 3.2 | 5 | 6 | 5 |
| Leucine | 7.8 | 7.2 | 7.42 | 7.5 | 13 | 12 | 13 |
| Lysine | 5.0 | 4.6 | 5.32 | 5.0 | 8 | 7 | 8 |
| Methionine | 1.9 | 1.2 | 0.91 | 1.5† | 3 | 2 | — |
| Phenylalanine | 4.0 | 4.2 | 4.13 | 4.1 | 5 | 6 | 6 |
| Proline | 8.2 | 7.7 | 9.61 | 8.5 | 16 | 15 | 19 |
| Serine | 6.0 | — | 6.69 | 6.2 | 13 | — | 14 |
| Threonine | 3.2 | 2.1 | 3.63 | 3.0 | 6 | 4 | 7 |
| Tyrosine | 2.4 | 2.4 | 2.99 | 2.6 | 3 | 3 | 4 |
| Valine | 3.4 | 3.3 | 3.49 | 3.4 | 7 | 6 | 7 |

| Method | Mean Molecular Weight | ± Standard Deviation |
|---|---|---|
| 1 | 22,600 | 950 |
| 2 | 22,900 | 800 |
| 3 | 22,900 | 700 |

* See text.
† Chromatographic value not included in the average.

The method used by the author was essentially the starch-column chromatographic technique of Moore and Stein (*2–5*). The only major departure from their procedure was the standardization of the amino acids. Although they used color recovery, the method used in these determinations was based on amount of color developed from the eluted amino acid without concern for that which might be lost in some manner during

[1] This work was supported in part by grants from the U. S. Public Health Service, the Armour Laboratories, and the Merck Co., and was taken, in part, from a section of the author's thesis submitted in partial satisfaction of the requirements for the Ph.D. degree in Biochemistry at the University of California, Berkeley.

[2] Cutter Laboratories Fellow in Biochemistry, 1950–51.

Three sets of data are included in the table for comparative purposes. The calculated molecular weights of the protein by the 3 analyses are not significantly different, nor is the spread of the 3 sets of values significantly different. For the molecular weight estimation from the chromatographic data, the value for methionine was omitted, apparently being too low. The sum, however, of the methionine plus cystine sulfur is 2.38%, while the data from method 1 yields a sum of 2.33%.

The chromatographic values for serine and threonine are corrected according to Rees (*10*). If these correction factors for loss during hydrolysis are ne-

glected, the values become 5.96 for serine and 3.41 for threonine as g per 100 g protein.

The estimated number of amino acid residues per molecule of protein appears in the second set of figures for each method. A comparison of these numbers reveals the 3 determinations to agree on histidine only; although aspartic acid, cystine, isoleucine, leucine, lysine, phenylalanine, tyrosine, and valine show agreement in 2 out of 3 of the analyses. In the latter 8 instances, 7 of the identical sets of numbers include the chromatographic data, whereas one set includes the 2 microbiological data. If the uncorrected chromatographic data for serine were used, it would agree with one of the microbiological figures for this amino acid. The remaining numbers do not show agreement although the differences are not large, except for the chromatographic values for methionine and proline.

The preponderance of acidic over basic amino acids is in keeping with the isoelectric point of the protein (*11*) and also points to a site of action for the cationic exchange properties which this protein quite possibly displays (*12*).

### References


1. Li, C. H., Evans, H. M., and Simpson, M. E. *J. Biol. Chem.*, **149**, 413 (1943).
2. Stein, W. H., and Moore, S. *Ibid.*, **176**, 337 (1948).
3. Moore, S., and Stein, W. H. *Ibid.*, 367.
4. Stein, W. H., and Moore, S. *Ibid.*, **178**, 53 (1949).
5. Moore, S., and Stein, W. H. *Ibid.*, 79.
6. Shankman Laboratories, Los Angeles, California.
7. Li, C. H. *J. Am. Chem. Soc.*, **73**, 4146 (1951).
8. ———. *Federation Proc.*, **5**, 144 (1946).
9. Hier, S. W. The Wilson Laboratories, Chicago, Illinois. Western Avenue Boulevard, Chicago 9, Illinois.
10. Rees, M. W. *Biochem. J.*, **40**, 632 (1946).
11. Li, C. H., Evans, H. M., and Simpson, M. E. *Science*, **96**, 450 (1942).
12. Astwood, E. B. *Adrenal Cortex*. New York: Josiah Macy Jr. Fdn. (1950).


Manuscript received December 1, 1952.

## $P^{32}$ Distribution in the Serum Proteins of the Chicken[1]


R. E. Clegg and R. E. Hein

*Kansas State College, Manhattan*


The source of the phosphorus found in the egg has been the subject of numerous investigations and, in general, these studies have shown that extra phosphorus appears in the blood of birds during egg formation. In this respect, the relationship between the phospholipids and the site and rate of formation of these substances has been discussed previously (*1, 2*). Recently, differences in the number and amount of the electrophoretic components of the serum proteins of laying and nonlaying birds have been observed (*3, 4*); however, except for the possible lipoprotein nature of certain of these components (*4*), no other characteristics have been reported. According to Chargaff (*5*), phosphorus plays an important role in binding the lipid to the protein, and the amount of phosphorus found in various lipoproteins tends to confirm this statement. A method for measuring the $P^{32}$ distribution in the various components of protein mixtures is now available (*6*), and by using this technique the $P^{32}$ in the electrophoretic components of the sera of birds has been under investigation. Certain of the results, and the relationship of these results to the distribution of the lipoprotein fractions reported by Moore (*4*), are discussed in this communication.

The sera were prepared from the blood of 8-week-old chickens, from 8-week-old chickens injected with diethylstilbestrol, and from laying hens. The 8-week-

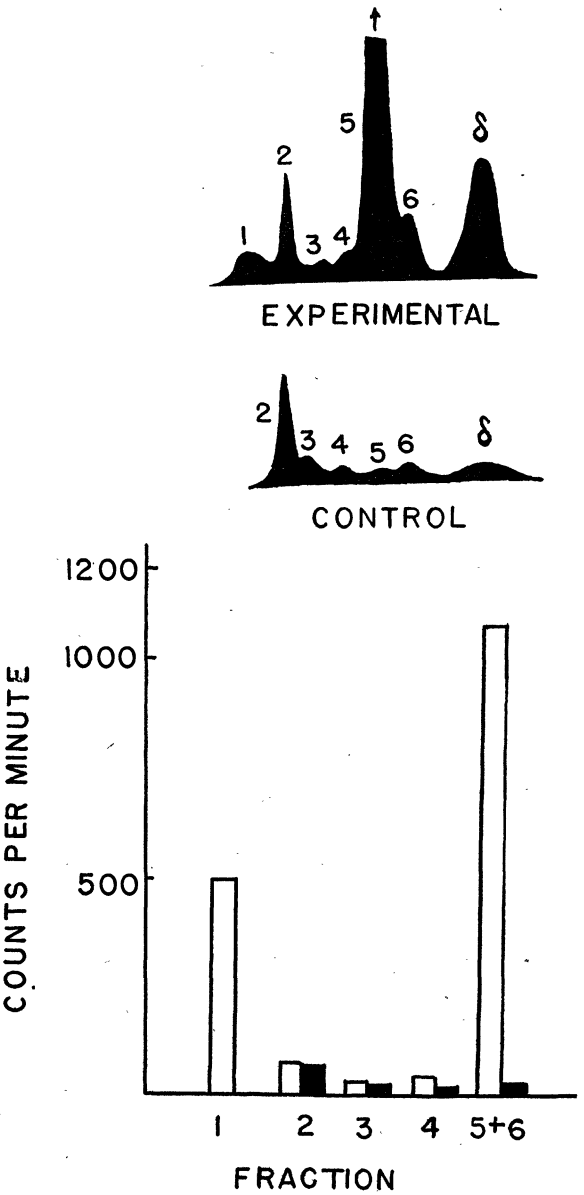


FIG. 1. $P^{32}$ activity of the electrophoretic components of the blood serums of normal and diethylstilbsetrol-treated chickens. The solid bars represent the activity of the components of the control; the open bars represent the activity of the hormone-treated birds.

[1] Contribution No. 480, Department of Chemistry.