

studies of the kinetics of histidine biosynthesis by the mutant and by the parent strain will permit us to determine which of these mechanisms is responsible for the bacteriostatic effect of 2-TA (10).

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References and Notes

1. H. E. Umbarger and B. Brown, *J. Biol. Chem.* 233, 415 (1958).
2. H. J. Strecher, *ibid.* 225, 825 (1957).
3. R. A. Yates and A. B. Pardee, *ibid.* 221, 757 (1956).
4. DL-6-fluorotryptophan was a gift of Dr. Jacob Mager of the Hebrew University, and DL-2-thiazole alanine was kindly supplied by Dr. R. G. Jones of the Lilly Research Laboratories.
5. C. Yanofsky, *J. Biol. Chem.* 223, 171 (1956).
6. H. S. Moyed and B. Magasanik, *J. Am. Chem. Soc.* 79, 4812 (1957).
7. A similar basis for resistance to the tryptophan analog 6-FT has not yet been observed in mutants selected for ability to grow in the presence of that compound. Instead, resistance to 6-FT in these organisms appears to be due to the loss of the ability to concentrate the aromatic amino acids and their analogs.
8. E. A. Adelberg, *J. Bacteriol.* 76, 326 (1958).
9. G. N. Cohen and E. A. Adelberg, *ibid.* 76, 328 (1958).
10. This work has been supported by a grant from the William F. Milton fund.

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Isolation of Gelatin from Ancient Bones

Abstract. The isolation and characterization of gelatin from 12,000-year-old deer antlers is described. Use of gelatin from ancient bones for carbon-14 dating may improve the accuracy of the dating procedure because gelatin is not likely to be contaminated by extraneous carbon.

Estimates of the age of buried objects based on their carbon-14 content has been of great value in anthropology. We believe that gelatin from ancient bones might be used for such dating with advantage. Charcoal has been the material most extensively used by anthropologists for dating. It is assumed that carbon produced by pyrolysis of ancient materials could not subsequently be contaminated with contemporary carbon. Charcoal, however, is not always pure carbon (1) but is, rather, a complex organic material. It presents a large surface area and may absorb other organic substances.

It is necessary to separate the organic and inorganic carbon fractions of bone before the count is made, because calcium carbonate in the mineral matrix may exchange with contemporary carbon dioxide of the air or ground water (2). Furthermore, ancient bones are porous and are capable of absorbing organic material from the soil.

Approximately 80 percent of the carbon of bone is collagen carbon, consti-

tuting 8.7 percent of air-dried bone. Collagen is soluble in hot water, in which it dissociates into a soluble protein gelatin. With time, the amount of this collagen decreases. However, appreciable amounts of collagen may be found in bone up to 100,000 years old (3). Gelatin is 18 percent nitrogen. The alpha-amino nitrogen of gelatin is 14 percent. In humic acids the alpha-amino nitrogen is between 0.2 and 1.5 percent (4, 5). The hydroxyproline content of gelatin is 13.5 percent; of humic acids, less than 0.05 percent.

We have attempted to prepare gelatin from approximately 10 g of deer antler (6). The deer antler was the remainder of radiocarbon sample Y-158, approximately 12,000 years old (5). Ten grams of pulverized antler were extracted with three 50-ml portions of 10 percent Versene, pH 7.05, in a boiling water bath for 1-hour periods with occasional shaking. The pooled extractions were dialyzed on a rocking dialyzer against distilled water at 2°C for 72 hours with eight water changes. After the dialysate had been concentrated in a vacuum to 50 ml, the solution was made 5 percent in trichloroacetic acid and allowed to stand overnight at 2°C. The precipitate was removed by centrifugation, and the supernatant was dialyzed as before. The gelatinous precipitate which formed was filtered and dried. It weighed 249 mg.

The material collected was dissolved in H₂O at room temperature with the addition of a small amount of NaOH which brought the pH of the solution to between 4.0 and 4.5. A 1-percent solution of this material gelled at 2°C.

The molecular weight of this gelatin was estimated by the viscosity procedure of Pouradier and Venet (7). A molecular weight of 41,000 was obtained. Such a molecular weight is not significantly different from that of commercial gelatins isolated from fresh bone.

Hydroxyproline determinations (8) on material dried in a vacuum oven at 100°C for 24 hours indicated that this material contained 12.9 percent hydroxyproline (the usual literature value is 13.5 percent) and 17.4 percent nitrogen (the usual literature value is 13 percent). The carbon content was 96.0 percent of theoretical. The material did not contain detectable amounts of tyrosine (9) or uronic acid (10).

This material was apparently gelatin of a purity of at least 96 percent. We feel that carbon-14 dating of purified gelatin would be as reliable as charcoal dating, or possibly more so, since analytical evidence may be obtained that the organic material is what it appears to be, namely bone protein (11).

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References and Notes

1. *Thorpe's Dictionary of Applied Chemistry* (Longmans, Green, New York, ed. 4, 1938), vol. 2.
2. I. May, *Science* 121, 508 (1955).
3. R. F. Heizer and S. F. Cook, *Am. J. Phys. Anthropol.* 10, 289 (1952).
4. W. Flaig, F. Sheffer, B. Klamroth, *Pflanzenenernah. Dung. Bodenk.* 71, 33 (1955); J. M. Bremner, *ibid.* 71, 63 (1955).
5. G. W. Barendsen, E. S. Deevey, L. J. Gralenski, *Science* 126, 908 (1957).
6. The antlers were obtained from E. S. Deevey, Geochronometric Laboratory, Yale University.
7. J. Pouradier and A. M. Venet, *J. chim. phys.* 49, 238 (1952).
8. D. S. Mayada and M. A. Tappel, *Anal. Chem.* 28, 909 (1956).
9. S. Udenfriend and J. R. Cooper, *J. Biol. Chem.* 196, 227 (1952).
10. Z. Dische, *ibid.* 167, 189 (1947).
11. This work was generously supported by the Massachusetts Heart Association as part of a study of the stability of collagen with time. We are indebted to Robert Braidwood of the Oriental Institute of the University of Chicago, C. A. Reed of the University of Illinois, and H. E. Wright of the University of Minnesota for advice, encouragement, and bones from Jarmo, Iraq, used in preliminary studies.

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Proposed System of Terminology for Preparations of Adrenocorticotrophic Hormone

Abstract. During the past few years there has been a considerable amount of confusion with respect to the terminology used for adrenocorticotropins (ACTH) isolated from the pituitary glands of various species. In this paper (1) a unified system of nomenclature for these hormones is proposed. It is hoped that this system will furnish readily and at a glance, from the terminology itself, pertinent information about the source of the particular preparation as well as the chemical formula of any active degraded product derived from the natural hormone.

Some confusion among investigators is usually unavoidable with respect to the term or name used to designate a biologically active substance derived from natural products before it is isolated in pure form and before its structure is known. The confusion is compounded when preparations of the same substance isolated from tissues of different species differ chemically but have similar biological effects. The terminological problem becomes even more complicated when the pure substances can be modified chemically without loss of biological activity. The present state of the terminology used to designate the adrenal-stimulating substance from the adeno-hypophysis is a case in point.

Since the discovery of the existence of adrenal-stimulating activity in pituitary glands by Smith in 1927 (2), many names have been proposed for the hormone: adrenotropic hormone, adrenocorticotrophic hormone, corticotrophic hormone, adrenotropin, corticotropin, adrenocorticotropin, and ACTH. In the past few years, adrenal-stimulating pep-

Table 1. Structural differences among adrenocorticotropins isolated from pig, sheep, and beef pituitary glands.

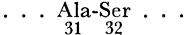
Structure of bovine ACTH:																					
Ser	Tyr	Ser	Met	Glu	His	Phe	Arg	Try	Gly	Lys	Pro	Val	Gly	Lys	Lys	Arg	Arg	Pro	Val		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
													NH ₂								
Lys	Val	Tyr	Pro	Asp	Gly	Glu	Ala	Glu	Asp	Ser	Ala	Glu	Ala	Phe	Pro	Leu	Glu	Phe			
21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39			
Species		Laboratory		Amino acid residue in position																	
				25	26	27	28	29	30	31	32	33									
Pig	Armour Company (12)		Gly-Ala-Glu-Asp-Asp-Glu-Leu-Ala-Glu																		
Pig	American Cyanamid Company (7)		Asp-Gly-Ala-Glu-Asp-Glu-Leu-Ala-Glu																		
Sheep	University of California (10)		Ala-Gly-Glu-Asp-Asp-Glu-Ala-Ser-Glu																		
Beef	University of California (13)		Asp-Gly-Glu-Ala-Glu-Asp-Ser-Ala-Glu																		

tides have been prepared in highly purified form from sheep (3, 4), pig (5-8), and beef (9) pituitaries, and their amino acid sequences (10-13) are known. Three different names have been employed by the investigators to designate their products: corticotropin A (5, 8) and β -corticotropin (6) for the porcine hormone, and α -corticotropin (3) for the ovine hormone. In addition, Brink *et al.* (14) named an ACTH-active product obtained from peptic digests of porcine ACTH concentrates corticotropin-B. It is the purpose of this note to propose a system of terminology for the adrenal-stimulating hormone and its degraded products possessing biological activity. As far as I am aware, no discussion of this sort has appeared in print, and I hope that these remarks will serve to stimulate further discussion among investigators who are actively interested in the field of pituitary hormones.

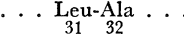
At the outset, the hormone, regardless of the species, should be called *adrenocorticotrophic hormone* (ACTH) or *adrenocorticotropin*. The terms "adrenotropic hormone" and "adrenotropin" should not be employed, for they imply that the hormone stimulates the whole adrenal gland. It is established now beyond any doubt that the hormone controls only the development and function of the cortical part of the adrenal gland. Similarly, the designation of the hormone as "corticotrophic hormone" or "corticotropin" may lead to confusion, for many other glands, such as the cere-brum, kidney, lymph gland, thymus, and ovary, all have cortices.

Although adrenocorticotropins isolated from unhydrolyzed extracts of pig, sheep, and beef pituitaries possess biological properties similar to one another, there are slight differences among them in

amino acid composition as well as structural sequence. Adrenocorticotropins from these three species are single-chain polypeptides composed of 39 amino acids with serine and phenylalanine as N- and C-terminal residues, respectively (9, 15). The only difference in amino acid composition between the porcine and ovine hormones appears to be one more leucine in the former and one more serine in the latter; this difference occurs in the sequence



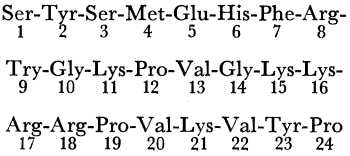
which appears in the ovine peptide in place of the



sequence in the porcine peptide. Although there are no differences in amino acid composition between the ovine and bovine hormones, their sequential isomerism seems to support the conclusion that the ovine and bovine adrenocorticotropins are distinct chemical entities. Table 1 summarizes the structural differences that exist among these adrenocorticotropins. Because of the differences, it is necessary to distinguish among adrenocorticotropins isolated from glands of the various species, and a suggestion might be made that the polypeptides possessing ACTH activity obtained from unhydrolyzed extracts of sheep, pig, and beef pituitaries be designated α_s -, α_p - and α_b -adrenocorticotropin, respectively. If more than one active component can be derived from the unhydrolyzed extract, as in the case of the sheep (3, 4) and pig (6) hormones, these could be called β_s -, γ_s -, or β_p -, γ_p -, and so on down the alphabet.

It is now well established that re-

moval of certain portions from the C-terminus of the hormone does not cause loss of activity (15). Such active fragments that have been obtained by *enzymic* action could be distinguished by the species letter in subscript and the remaining amino acid sequence in superscript; for example, α_s^{1-28} -ACTH would represent the active fragment obtained by the action of pepsin, which cleaves 11 amino acids from the C-terminus of the ovine α_s -ACTH (15, 16). There is some evidence (15) that as many as 15 amino acids can be removed from this terminus without damage to the activity. Since the amino acid sequence of the first 24 residues from the N-terminus is identical for the three species (Table 1), then in this case, particularly if the synthetic peptide with this amino acid sequence, namely,



is biologically active, the subscripts s, p, and b could be omitted and the active peptide could be designated as α^{1-24} -adrenocorticotropin or α^{1-24} -ACTH.

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References and Notes

1. This report is paper XVI of the adrenocorticotropin (ACTH) series; for paper XV, see J. Léonis, C. H. Li, D. Chung, *J. Am. Chem. Soc.* 81, 419 (1959). I wish to thank J. S. Dixon, W. R. Lyons, H. Papkoff, R. Schwyzler, P. G. Squire, and others for valuable discussions during the preparation of this paper.
2. P. E. Smith, *J. Am. Med. Assoc.* 88, 158 (1927); *Am. J. Anat.* 45, 205 (1930).
3. C. H. Li, I. I. Geschwind, A. L. Levy, J. I. Harris, J. S. Dixon, N. G. Pon, J. O. Porath, *Nature* 173, 251 (1954).
4. C. H. Li, I. I. Geschwind, J. S. Dixon, A. L. Levy, J. I. Harris, *J. Biol. Chem.* 213, 171 (1955).
5. W. F. White, *J. Am. Chem. Soc.* 75, 503 (1953).
6. P. H. Bell, *ibid.* 76, 5565 (1954).
7. R. G. Shepherd, K. S. Howard, P. H. Bell, A. R. Cacciola, R. G. Child, M. C. Davies, J. P. English, B. M. Finn, J. H. Meisenhelder, A. W. Moyer, J. van der Scheer, *ibid.* 78, 5051 (1956).
8. H. B. F. Dixon and M. P. Stack-Dunne, *Biochem. J.* 61, 483 (1955).
9. C. H. Li and J. S. Dixon, *Science* 124, 934 (1956).
10. C. H. Li, I. I. Geschwind, R. D. Cole, I. D. Raacke, J. I. Harris, J. S. Dixon, *Nature* 176, 687 (1955).
11. K. S. Howard, R. G. Shepherd, E. A. Eigner, D. S. Davies, P. H. Bell, *J. Am. Chem. Soc.* 77, 3419 (1955).
12. W. F. White and W. A. Landman, *ibid.* 77, 1711 (1955).
13. C. H. Li, J. S. Dixon, D. Chung, *ibid.* 80, 2587 (1958).
14. N. G. Brink, F. A. Kuehl, Jr., J. W. Richter, A. W. Bazemore, M. A. P. Meisinger, D. E. Ayer, K. Folkers, *ibid.* 74, 2120 (1952).
15. C. H. Li, *Advances in Protein Chem.* 11, 101 (1956).
16. R. D. Cole, C. H. Li, J. I. Harris, N. G. Pon, *J. Biol. Chem.* 219, 903 (1956).

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