$v_c(A)$, equation (13) for $\beta_e = 1$ gives the limiting mass for a cold star as $M = 5.8975\ \mu_e^{-2} M_\odot$. If we take $\mu_e = 2$,[10] then the limiting mass of rotating white dwarfs is as follows:

$$M = 1.474\ M_\odot \text{ (cold star)}$$

$$M = 1.704\ M_\odot \text{ (hot star)}.$$

The author is indebted to Professor William A. Fowler for valuable discussions and suggestions.

* Supported in part by the Office of Naval Research [Nonr-220(47)] and the National Aeronautics and Space Administration (NGR-05-002-028).

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*THE AMINO ACID COMPOSITION OF BONE AND TOOTH PROTEINS IN LATE PLEISTOCENE MAMMALS**

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Communicated by Ralph W. G. Wyckoff, May 6, 1965

In a recent paper from this laboratory,[1] results were given of preliminary determinations of the amino acid compositions of proteinaceous residues obtained from several Pleistocene fossil bones. These showed the presence of the same amino acids as could be obtained from similarly treated Recent bones. Electron microscopy[2, 3] has demonstrated that much of the collagen residue in such fossils has the characteristic morphology of collagen. The analytical results obtained in the preliminary study showed significant departures from those given by fresh collagen; and these could be attributed either to the presence of other proteins or to problems in the hydrolysis of the ancient collagen. The present investigation was undertaken to establish a satisfactory way of hydrolyzing the fossil residues and to provide further information on the composition of proteins of Pleistocene age. With this in view, controls were run on Recent material and procedures were used that led to analyses agreeing with determinations of collagen in fresh bone.

Methods and Results.—The fossil materials obtained for this study include: (*a*) the epiphysis of the femur of an unidentified species of ungulate (V63244); (*b*) the distal end of the horse limb bone of *Equus* sp. (V63245); (*c*) the scapula fragment of the extinct western horse *Equus occidentalis* (V642); (*d*) the diaphysis of the limb bone of the extinct dire wolf *Canis dirus* (V63247); (*e*) the canine of the extinct lion-

like cat *Felis atrox* (V63220); (*f*) the fragments of the cheek teeth of the extinct bison *Bison antiquus* (V63218); and (*g*) the upper molar of the extinct western horse *Equus occidentalis* (V63217). In addition to the fossil samples the diaphysis of a fresh ox femur and the premolars of a Recent cow were used as controls. These samples are different from those earlier analyzed, with the exception of V63245 which was from the same bone as that designated as B57 in the previous study.[1]

To obtain the results given below, chips of fossil materials were first freed from adhering foreign matter by brushing in petroleum ether and then washed in 1 *N* HCl for about 5 min to remove possible surface contaminants. The chips thus treated were powdered to pass through a 40-mesh sieve. The powdered materials were shaken in petroleum ether and acetone with several renewals of the extractants to remove tar and were then air-dried. Weighed amounts of dry powdered materials were demineralized by shaking in 1 *N* HCl for 3 hr and then centrifuged.

The free amino acids in the supernatant were recovered as follows: to remove the excess calcium ions that interfere with the ion-exchange column analysis of amino acids, a suitable amount of oxalic acid was added to the clear supernatant and the pH of the aliquot was adjusted to about 4–5 by 2 *N* NaOH. The precipitates of oxalates were removed by filtration. The filtrate, containing free amino acids, was evaporated to dryness at less than 50°C in a rotary evaporator.

The sediment, the protein fraction remaining after the centrifugation, was heated in 6 *N* HCl for 20 hr to hydrolyze the proteins. The hydrolysate was evaporated almost to dryness in a rotary evaporator and taken up in a suitable volume of pH 2.2 citrate buffer for amino acid analysis by the method of Moore, Spackman, and Stein.[4] The amino sugar identified in the column analysis was also checked by using hexosamine (Elson-Morgan) reagent in paper chromatography.

The sample of the amino acids in the proteins of the Recent bone and tooth was prepared as above with the exception that the length of time for hydrolysis was extended to 24 hr. For the preparation of bone collagen, as a control, noncollagen proteins in the ox bone were removed from the collagen by repeated extraction in $^1/_{10}$ *N* NaOH at room temperature.[5, 6]

The results of the analyses on the fossil bone and tooth proteins are summarized in Tables 1, 2, and 3; the results on Recent material agree with published analyses.[7, 8]

On the whole, there is a close agreement between the fossil and Recent analyses, but the following differences are to be noted: (*a*) a consistently lower content of the hydroxyproline in the fossil samples than that in the fresh collagens, and (*b*) a relatively large amount of glycine in most of the fossil samples. Furthermore, the molar ratios of the characteristic amino acids of collagen, glycine, alanine, and proline, to hydroxyproline are higher in the great majority of fossil samples. Particularly, the glycine/hydroxyproline ratios are higher in all fossil samples than those of the fresh collagen. Glucosamine was found in the fossil bones but not in the teeth, presumably because of the smaller original samples; nevertheless, a trace was observed in the free amino acid fraction of one tooth (V63217).

Cystine (or cystein) is absent, and tyrosine and methionine are present in reduced amounts in fossil samples. Since these amino acids are minor constituents and are subject to some destruction during hydrolysis, little meaning can, however, be attached to these results.

Free amino acids were found in all fossil samples, but they were usually present in

TABLE 1

AMINO ACID AND AMINO SUGAR COMPOSITION OF PROTEINS FROM LATE PLEISTOCENE BONES (VALUES ARE GIVEN AS MICROMOLES OF AMINO ACIDS PER GRAM OF BONE)

	Fossil Bones				Recent Femur of Ox		
	Horse *Equus* sp., V63245	Ungulate, V63244	Dire wolf *C. dirus*, V63247	Horse *E. occidentalis*, V642	Whole bone	Bone collagen	Bone collagen as reported*
Hydroxylysine	1.530	6.308	14.700	9.600	17.327	16.450	12.862
Lysine	9.540	27.218	108.000	49.300	63.838	60.850	52.378
Histidine	1.680	7.216	23.400	9.300	17.525	12.150	11.557
Arginine	18.000	45.815	176.500	79.920	110.790	106.100	97.800
Hydroxyproline	27.264	59.184	127.250	142.462	200.880	205.938	201.312
Aspartic acid	26.040	35.040	50.700	96.253	144.960	85.375	99.538
Threonine†	5.736	21.384	12.400	32.206	55.720	37.375	39.330
Serine†	11.592	26.640	19.750	65.780	85.560	69.813	75.492
Proline	31.392	80.304	164.950	230.546	249.878	237.188	237.101
Glutamic acid	26.688	49.056	112.150	128.859	164.800	129.500	151.357
Glycine	138.936	276.228	567.350	624.992	753.800	636.438	628.169
Alanine	41.448	75.216	95.000	208.275	244.680	216.750	219.207
Valine	3.216	11.220	8.000	46.609	36.720	49.000	42.313
Cystine (half)	—	—	—	—	13.120	—	—
Methionine	7.968	5.880	9.050	24.372	27.280	10.000	10.252
Isoleucine	3.504	6.300	8.000	19.871	16.280	26.938	24.605
Leucine	8.928	23.040	26.000	47.910	60.920	60.313	55.734
Tyrosine	0.452	0.576	1.150	0.600	14.840	14.438	5.778
Phenylalanine	3.528	12.252	22.300	26.639	36.120	38.813	32.620
Glucosamine	6.552	Trace	2.000	0.733	2.240	—	—
Total μM/gm	373.998	768.901	1548.650	1844.227	2317.278	2008.429	1997.470
Total mg/gm	40.680	83.944	177.082	199.975	255.961	223.243	220.552
Ammonia	20.040	66.692	31.190	33.600	145.500	77.300	83.507

* Converted from the original data[7] on the basis that an ox femur diaphysis contains 18.64% of collagen.[9]
† No corrections for hydrolytic loss were made for these amino acids analyzed.

TABLE 2

AMINO ACID AND AMINO SUGAR COMPOSITION OF PROTEINS FROM LATE PLEISTOCENE BONES (VALUES ARE GIVEN AS MOLE PER CENT OF AMINO ACIDS FOUND)

	Fossil Bones				Recent Femur of Ox		
	Horse *Equus* sp., V63245	Ungulate, V63244	Dire wolf *C. dirus*, V63247	Horse *E. occidentalis*, V642	Whole bone	Bone collagen	Bone collagen as reported*
Hydroxylysine	0.41	0.82	1.02	0.52	0.75	0.82	0.64
Lysine	2.55	3.54	6.93	2.67	2.75	3.03	2.62
Histidine	0.45	0.94	1.61	0.50	0.76	0.61	0.58
Arginine	4.81	5.96	10.34	4.33	4.78	5.03	4.90
Hydroxyproline	7.29	7.70	8.31	7.72	8.67	10.25	10.08
Aspartic acid	6.96	4.56	3.31	5.22	6.26	4.25	4.98
Threonine†	1.53	2.78	0.81	1.74	2.40	1.86	1.97
Serine†	3.10	3.46	1.29	3.57	3.69	3.48	3.78
Proline	8.39	10.44	10.76	12.50	10.78	11.81	11.87
Glutamic acid	7.14	6.38	7.33	6.99	7.11	6.45	7.58
Glycine	37.15	35.93	37.07	33.89	32.53	31.69	31.45
Alanine	11.08	9.78	6.21	11.29	10.56	10.79	10.97
Valine	0.86	1.46	0.52	2.53	1.58	2.44	2.12
Cystine (half)	—	—	—	—	0.57	—	—
Methionine	2.13	0.76	0.60	1.32	1.18	0.50	0.51
Isoleucine	0.94	0.82	0.52	1.08	0.70	1.34	1.23
Leucine	2.34	3.00	1.70	2.60	2.63	3.01	2.79
Tyrosine	0.12	0.07	0.08	0.03	0.64	0.72	0.29
Phenylalanine	0.94	1.59	1.46	1.44	1.56	1.93	1.63
Glucosamine	1.75	Trace	0.13	0.04	0.10	—	—
Total	99.95	99.99	100.00	99.98	100.00	100.02	99.99
Molar ratio Gly/Hypro	5.09	4.67	4.46	4.39	3.75	3.09	3.12
Molar ratio Ala/Hypro	1.52	1.14	0.75	1.46	1.22	1.05	1.09
Molar ratio Pro/Hypro	1.15	1.35	1.30	1.62	1.24	1.15	1.15

* Converted from the original data of Eastoe.[7]
† No corrections for hydrolytic loss were made for these amino acids analyzed.

TABLE 3

AMINO ACID COMPOSITION OF PROTEINS FROM LATE PLEISTOCENE TEETH

	μmoles Amino Acids per Gm of Tooth				Mole % Amino Acids Found				
	Lion-like cat *F. atrox*, V63220	Horse *E. occidentalis*, V63217	Bison *B. antiquus*, V63218	Recent cow, whole tooth	Lion-like cat *F. atrox*, V63220	Horse *E. occidentalis*, V63217	Bison *B. antiquus*, V63218	Recent cow, whole tooth	Recent dentine collagen of steer*
Hydroxylysine	0.716	1.020	18.710	18.312	0.73	0.84	1.82	1.14	1.30
Lysine	1.237	2.900	26.502	37.932	1.26	2.38	2.58	2.37	1.90
Histidine	0.605	0.300	8.792	11.936	0.62	0.25	0.85	0.75	0.37
Arginine	2.808	4.220	58.588	83.549	2.87	3.47	5.69	5.22	4.70
Hydroxyproline	7.582	9.090	84.375	122.625	7.74	7.48	8.20	7.66	10.30
Aspartic acid	5.320	6.200	52.225	92.378	5.43	5.10	5.06	5.77	5.20
Threonine†	2.422	2.180	18.700	27.223	2.47	1.79	1.82	1.70	1.60
Serine†	3.810	3.860	34.250	57.961	3.25	3.17	3.33	3.62	3.90
Proline	9.059	14.020	105.238	178.044	9.25	11.53	10.23	11.12	11.60
Glutamic acid	7.516	8.210	76.050	116.249	7.67	6.75	7.39	7.26	7.20
Glycine	36.856	46.100	324.125	528.514	37.63	37.91	31.50	33.00	32.70
Alanine	11.388	13.460	128.250	162.110	11.63	11.07	12.46	10.12	11.20
Valine	2.770	2.550	27.000	31.147	2.83	2.10	2.62	1.94	2.20
Cystine (half)	—	—	—	—	—	—	—	—	—
Methionine	0.636	0.080	8.463	24.116	0.65	0.07	0.82	1.51	0.41
Isoleucine	1.136	1.410	10.675	22.890	1.16	1.16	1.04	1.43	1.10
Leucine	2.860	3.720	29.913	50.930	2.92	3.06	2.91	3.18	2.50
Tyrosine	0.202	0.150	0.313	7.439	0.21	0.12	0.03	0.46	0.32
Phenylalanine	1.658	2.130	16.788	28.367	1.69	1.75	1.63	1.77	1.60
Total μM/gm	97.951	121.600	1028.957	1601.722	100.01	100.00	99.98	100.02	100.10
Total mg/gm	10.455	12.898	107.690	177.315					
Ammonia	7.652	8.320	20.290	92.705					
Molar ratio Gly/Hypro					4.86	5.07	3.84	4.31	3.17
Molar ratio Ala/Hypro					1.50	1.48	1.52	1.32	1.09
Molar ratio Pro/Hypro					1.19	1.54	1.25	1.45	1.13

* Converted from the data of Piez and Likins.[8]
† No corrections for hydrolytic loss were made for these amino acids analyzed.

small quantities (with free amino-nitrogen ranging from 0.3% to 21% that of the bound amino acids). For this reason and because of insufficient starting material only two analyses were made. Their free amino acids and amino sugar were present in the following order of decreasing abundance: (*a*) Bone (V63244): glycine, alanine, aspartic acid, glutamic acid, arginine, proline, lysine, hydroxyproline, leucine, histidine, isoleucine, methionine, glucosamine, hydroxylysine, and phenylalanine; (*b*) Tooth (V63217): glycine, alanine, arginine, aspartic acid, lysine, serine, methionine, glucosamine, hydroxylysine, leucine, glutamic acid, histidine, phenylalanine, isoleucine, and tyrosine. Among these amino acids, 50 and 40 per cent of the total free amino acids in the bone and the tooth, respectively, are represented by glycine.

Discussion.—The similarity in the amino acid composition of the fossils and of the Recent bone and teeth suggests that collagen is the predominant protein in the fossils, as was suggested by electron microscopic studies.[2, 3] The amino sugar in the protein fraction of the fossil samples points to the presence of mucoprotein, probably the remains of the ground substance in bone and tooth. No attempt was made to recover the amino sugar in the proteins by means of more moderate hydrolysis, and it is therefore probable that more glucosamine is present in the sample than the tables suggest.

The existing data are insufficient to explain adequately the relatively high percentage of the glycine in the fossils analyzed, but it could be due to a greater stability of glycine-rich polypeptides resulting from the collagen degradation that has already occurred. This is consistent with the observations that the free amino acids resulted from the further degradation of the polypeptides, consist chiefly of glycine, and that the fossils having rather low contents of bound amino acids contain a relatively large amount of glycine.

Summary.—Four samples of the bones and three samples of the teeth of the Late Pleistocene mammals from the deposits in the Rancho La Brea tar pits were analyzed for the amino acid composition of their proteins, using Recent bone and tooth as controls. The total amount of the amino acids in the proteins of the fossils ranges from about $^1/_6$ to $^4/_5$ that of the Recent bone, and $^1/_{16}$ to $^2/_3$ that of the Recent tooth. The amino acid composition of fossil samples is similar to that of the Recent bone and tooth with the exception of the rather high percentage of glycine in the fossil samples. In addition to the collagen, which is a predominant constituent of the proteins in the fossil bones and teeth, the presence of mucoprotein and glycine-rich polypeptides in the fossil specimens was suggested.

We are indebted to Dr. J. Reid Macdonald of the Los Angeles County Museum for the Rancho La Brea specimens, and to Dr. Theodore Eaton of the Department of Zoology, University of Kansas, for the premolars of the Recent cow.

* This work was carried out in the laboratory of Dr. Ralph W. G. Wyckoff under grants to him from the National Institutes of Health (DE-01919) and the National Science Foundation (GB-1322).

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A PROCEDURE FOR MOLECULAR WEIGHT MEASUREMENTS: APPLICATION TO CHYMOTRYPSINOGEN A

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Communicated by J. W. Williams, April 19, 1965

The use of short columns[1] has proved advantageous for sedimentation equilibrium studies of protein solutions. However, such studies require a second experiment on the sample in the form of a boundary-forming experiment for determining the initial concentration of the solution. With this second experiment, one has, in addition to the possibility that a proper boundary may not be formed, the problem of partial loss of the protein solute by adsorption onto the walls of the ultracentrifuge cell. Indeed, this latter problem has already been discussed in detail by Yphantis.[2] Other disadvantages of methods requiring synthetic boundary-forming experiments are the need for sample in addition to that required for the equilibrium experiment, and the introduction of error into measurements of initial concentration by evaporation of solvent from the solution on which the sedimentation equilibrium experiment was performed.

Wales *et al.*[3] introduced a technique which circumvents the need for the boundary-forming experiment; they accomplished this by carrying out an experiment on the sample at a speed high enough that the concentration at the meniscus is negligibly small. The method outlined here extends the spirit of this analysis to use with Rayleigh interference optics and thereby requires measurements at high speed after the sample has been brought to equilibrium at low speed. This procedure differs from that of Yphantis[2] which requires data only from a single high-speed experiment. However, the present method permits measurement at the base of the cell and allows evaluation of the weight-average molecular weight over the entire cell volume,[4] as well as equilibrium constants for associating systems;[5, 6] the single high-speed experiment, on the other hand, does not provide such information.

Procedure.—For the study of dilute solutions the following is a good approximation:[6]

$$\frac{d \ln c_r}{dr^2} = AM_{wr\ \mathrm{app}} \tag{1}$$

$$A = \frac{(1 - \bar{v}\rho)\omega^2}{2RT} \tag{1a}$$