tion, by an excess of carbon dioxide acting at its own site, seems to us the most feasible of the possibilities which could produce the observed kinetic behaviour.

SUMMARY

1. Two different methods have been used to determine the effects of carbon dioxide on the rate of the reaction catalysed by phosphoenolpyruvic carboxylase.

2. The kinetics of the carbon dioxide effects have been studied with one of these methods in a reaction in which the carboxylase was linked with malic dehydrogenase.

3. The results have been discussed in relation to factors which are known to affect the rate of increase of acidity in the dark in Crassulacean plants.

The authors wish to express their indebtedness to Professor M. Thomas, F.R.S., at whose suggestion this work was undertaken, and to thank Miss J. Moyle, Dr R. Hill, F.R.S., and Dr S. L. Ranson for their invaluable advice and criticism. They are also grateful to Dr G. Maengwyn-Davies for her comments on specific points. Part of this work was carried out under grants from the D.S.I.R., and part during the tenure of an I.C.I. Fellowship held by one of us (D.A.W.).

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The Amino Acid Composition of Amphibian, Reptile and Avian Gelatins

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(Received 4 March 1957)

The present paper completes an investigation of the variation in amino acid composition found among the collagens and derived gelatins of the more important groups of vertebrates. Results are given for avian, reptilian and amphibian gelatins for comparison with the two previous sections of the work on mammalian (Eastoe, 1955) and fish (Eastoe, 1957) materials. Neuman (1949) included both chicken tendon and the subcutaneous membrane of the turtle in his survey, in which microbiological methods were used. No values for amphibian proteins of this type appear to have been published previously.

It has already been demonstrated (Eastoe, 1955) that, within the limits of experimental error, a carefully prepared gelatin adequately represents the composition of its collagen precursor, except in amide nitrogen. This is especially valuable when non-collagenous impurities, which cannot readily be removed, are present in a tissue and would intro-

duce errors if the whole tissue were analysed. The materials selected for analysis were gelatins prepared from toad, python and crocodile skins and from chicken-leg tendon. In this way representative values for the gelatins and collagens of three further classes of vertebrates were obtained by ion-exchange chromatography (Moore & Stein, 1951), a technique identical with that of the previous papers being used.

Following the suggestion of Gustavson (1953) that shrinkage temperatures are a measure of structural stability, these have been measured for each of the collagens studied.

EXPERIMENTAL

Materials

Python-skin gelatin. Python (probably *Python sebae*). The skin was limed at 0° for 2 months, washed, and gelatin extracted from it by heating with water for 2 hr. at 60° and pH 7.

Crocodile-skin gelatin. Crocodile skin from the back was soaked in 5% (w/v) HCl for 4 days at room temp. and then extracted with water by heating for 2 hr. at 80° and pH 3. The air-dried gelatin obtained was dissolved in water and adjusted to pH 7. The gelatin was precipitated at 40° by adding a suitable quantity of ethanol at the same temperature. After 2 hr. the supernatant liquor could be decanted off. The precipitated gelatin was swollen in 10 ml. of water, dissolved by warming, allowed to set to a gel and dried.

Chicken-leg-tendon gelatin. Chicken (*Gallus*) leg tendons were soaked overnight in SO₂-saturated water, and washed several times with water. Further water was added and saturated with SO₂, and the tendons were extracted at 80° for 3 hr. The liquor was filtered and the bulk of the SO₂ removed with N₂. The solution was neutralized, allowed to set to a gel and dried. Approximately half the ash content could be accounted for in terms of Na₂S and Na₂SO₄ resulting from the ashing of the Na₂SO₃ formed during neutralization.

Toad-skin gelatin. Skin from the back, belly and limbs of the South African clawed toad *Xenopus laevis* was washed with ether and water to remove mucus, soaked in 10% (w/v) NaCl (three changes for 4 days each) and the outer keratinous layer pulled off and discarded. The collagenous layer was washed successively with water, acetone and ether and finally air-dried. The skins were soaked in 0.02N-HCl for 24 hr., the pH being kept below 3, and then extracted for 3 hr. at 70° with the same liquor. The solution was adjusted to pH 7, filtered, and evaporated to dryness.

Methods

Hydrolysis. About 0.2 g. of a gelatin was hydrolysed with 20 ml. of 20% (w/w) HCl at 100° in a sealed tube for 24 hr.

Amino acid analysis. The analyses were carried out by the method of Moore & Stein (1951) as described by Eastoe (1955); the corrections applied were the same. Hydroxyproline was determined by the method of Neuman & Logan (1950).

Moisture. The loss of weight on drying a thin film of the gelatin at 105° for 24 hr. was used to calculate the moisture content.

Ash. The gelatin was ignited in a platinum dish, then heated at 550° to constant weight, and the residue weighed as ash.

Shrinkage temperature. Observations were made on water-saturated samples, freely suspended in toluene. Preliminary determinations were carried out at a temperature rise of 2°/min. The final determination was carried out at a 1° rise/min., when the temperature was within 10° of the preliminary value. A maximum variation between readings of 1.5° was obtained, and the true value taken as the average of three determinations.

RESULTS

A summary of the analytical data is given in Table 1. The weight recovery is the weight of amino acids recovered from 100 g. of protein multiplied by the average residue weight and divided by the average amino acid weight. The average residue weight is equal to the average amino acid weight less the weight of one molecule of water. The nitrogen recoveries of these materials were slightly lower than average.

As with collagens and gelatins from other vertebrates, traces of cysteic acid were found in the hydrolysates. This presumably originated from small amounts of cystine.

DISCUSSION

Throughout this section the figures refer to g./100 g. of dry, ash-free protein.

Analysis of chicken-leg tendon by microbiological and ion-exchange techniques

The results in Table 1 for chicken-leg tendon show reasonable agreement with those of Neuman (1949) for the same material analysed by microbiological methods. Large differences (greater than 1 in 20) between the two methods occur for tyrosine, methionine and histidine, where the absolute values are very small, and for lysine where Neuman's figures may include hydroxylysine. Alanine and serine show discrepancies of about 1 in 6, and isoleucine 1 in 4. Similar differences between the techniques have been reported for other samples (Eastoe, 1955).

In view of the totally different techniques used, and the considerable difficulty of securing reliable results by microbiological assay, the general agreement must be regarded as very satisfactory.

Characteristics of the amino acid composition of chicken-leg tendon

The composition is very similar to mammalian collagen and gelatins, with the exception of a few amino acids (cf. Eastoe, 1955, for the mammalian figures). Although the hydroxyproline is the same as for mammalian material, the hydroxylysine content of 1.68 is extremely high, and is only approached by the figure of 1.5 for human tendon. The increase in hydroxyl groups due to hydroxylysine is offset by the decrease in serine. The serine content is lower than for either mammalian or fish materials (cf. Eastoe, 1955, 1957). This adjustment is not shown in the composition of human tendon. The value for lysine is the lowest so far encountered for mammalian collagens and gelatins, and is only partially compensated by the high hydroxylysine value. Other differences are not large enough to be significant.

Amino acid composition of amphibian- and reptile-skin gelatins

The proportions of certain amino acids in the amphibian-skin gelatin suggest that this material occupies an intermediate position between mammalian and fish materials. The comparison is complicated by the wide range of values for certain amino acids, given by the different groups of fish materials. The toad-skin gelatin shows considerable

Table 1. *Amino acid composition of gelatins and related analytical data*

G, g. of amino acid/100 g. of dry ash-free protein. *N*, Amino acid N as % of total N. *M*, Moles of amino acid/10⁶ g. of dry ash-free protein. *Z*, weight of amino acids given on hydrolysis of 100 g. of protein (Chibnall, 1942).

	Crocodile-skin gelatin			Python-skin gelatin		
	<i>G</i>	<i>N</i>	<i>M</i>	<i>G</i>	<i>N</i>	<i>M</i>
Alanine	11.1	9.5	124.5	10.2	9.86	114.0
Glycine	26.5	27.6	354	21.6	24.9	288
Valine	1.98	1.29	16.9	2.15	1.59	18.4
Leucine	2.87	1.68	22.0	3.07	2.02	23.4
Isoleucine	1.63	0.95	12.4	1.40	0.92	10.6
Proline	16.1	10.7	139.7	12.5	9.41	108.8
Phenylalanine	3.20	1.48	19.4	2.14	1.12	13.0
Tyrosine	0.64	0.27	3.6	0.30	0.14	1.7
Serine*	4.83	3.51	46.0	4.18	3.43	39.7
Threonine*	2.87	1.84	24.1	1.94	1.41	16.3
Cystine	Trace	Trace	Trace	Trace	Trace	Trace
Methionine†	1.02	0.54	7.1	0.82	0.48	5.5
Arginine	9.42	16.6	54.2	7.92	15.7	45.5
Histidine	0.79	1.17	5.1	0.67	1.12	4.3
Lysine	4.04	4.23	27.7	3.67	4.35	25.1
Aspartic acid	6.62	3.79	49.7	5.83	3.78	43.8
Glutamic acid‡	11.69	6.07	79.5	8.35	4.91	56.9
Hydroxyproline	13.3	7.74	101.4	12.2	8.04	93.0
Hydroxylysine	0.87	0.82	5.4	0.59	0.63	3.7
Total	119.44	—	—	99.51	—	—
Amide N§	0.39	2.13	27.9	0.28	1.74	20.1
Total N	—	18.35	—	—	16.19	—
Mean residue wt.	—	91.40	—	—	91.18	—
<i>Z</i>	—	119.7	—	—	119.8	—
Recovery by wt. (%)	—	99.8	—	—	83.1	—
Recovery of N (%)	—	101.8	—	—	95.5	—
Moisture	—	14.4	—	—	19.7	—
Ash	—	2.1	—	—	6.5	—
Shrinkage temperature	—	59°	—	—	57°	—

	Toad-skin gelatin			Chicken-leg-tendon gelatin		
	<i>G</i>	<i>N</i>	<i>M</i>	<i>G</i>	<i>N</i>	<i>M</i>
Alanine	8.7	7.6	97.3	11.1	9.8	124.4
Glycine	24.4	23.2	298	27.0	28.2	359
Valine	2.55	1.69	21.8	2.52	1.69	21.5
Leucine	3.75	2.22	28.6	3.38	2.03	25.8
Isoleucine	1.83	1.08	13.9	1.55	0.93	11.8
Proline	12.5	8.48	108.9	16.2	11.05	140.5
Phenylalanine	3.17	1.49	19.2	2.56	1.22	15.5
Tyrosine	1.11	0.47	6.1	0.67	0.29	3.7
Serine*	6.92	5.12	65.8	3.26	2.44	31.0
Threonine*	3.12	2.04	26.2	2.46	1.62	20.7
Cystine	Trace	Trace	Trace	Trace	Trace	Trace
Methionine†	1.29	0.67	8.61	1.00	0.52	6.7
Arginine	8.42	15.2	48.9	8.46	15.3	48.6
Histidine	1.01	1.52	6.5	0.75	1.14	4.8
Lysine	4.23	4.51	28.9	3.01	3.24	20.6
Aspartic acid	7.25	4.25	54.5	6.94	4.10	52.1
Glutamic acid‡	11.36	6.02	77.3	11.82	6.32	80.3
Hydroxyproline	10.1	5.96	77.0	14.0	8.41	106.8
Hydroxylysine	0.70	1.09	4.31	1.68	1.63	10.4
Total	110.42	—	—	118.31	—	—
Amide N§	0.75	4.17	53.5	0.61	3.42	43.5
Total N	—	17.99	—	—	17.80	—
Mean residue wt.	—	93.33	—	—	91.13	—
<i>Z</i>	—	119.4	—	—	119.8	—
Recovery by wt. (%)	—	92.5	—	—	98.8	—
Recovery of N (%)	—	96.8	—	—	103.3	—
Moisture	—	4.2	—	—	14.4	—
Ash	—	4.9	—	—	4.6	—
Shrinkage temperature	—	54°	—	—	64°	—

* Corrected for decomposition during hydrolysis (Eastoe, 1955).

† Sum of methionine and methionine sulphoxide peaks.

‡ Corrected for decomposition on column.

§ Corrected for ammonia formed by decomposition of serine and threonine.

Table 2. *Shrinkage temperatures of collagens and their hydroxyproline and serine plus threonine contents*

Shrinkage temperature ranges for cold-water and warm-water fishes and mammals are taken from Gustavson (1953). For other references to sources of data see Discussion.

Collagen from		Shrinkage temp.	Range of shrinkage temp.	Content of hydroxyproline (g./100 g.)	Content of serine + threonine (g./100 g.)
Cold-water fishes	Halibut	40°	36-43°	9.1	9.3
	Cod	40		8.3	11.1
Warm-water fishes	Pike	55	53-57	7.9	—
	Shark	53		10.9	8.2
Amphibia	Toad	54	57-59	10.1	10.0
Reptiles	Python	57		12.2	6.1
	Crocodile	59		13.3	7.7
Birds	Chicken	64	59-67	14.0	5.7
Mammals	Ox	65		14.1	6.8
	Pig	62		13.5	6.3
	Whale	63		12.8	7.8
Hot-water fish	Lung fish	63		10.8	7.9

similarities to the main group of fish materials (cf. Eastoe, 1957) with respect to its content of hydroxyproline, proline, serine, threonine and, to some degree, methionine. The increased serine and threonine contents offset the loss of hydroxyl groups due to decreased hydroxyproline and hydroxylysine contents. The remaining amino acid contents are not sufficiently different from those for fish or mammalian materials to enable any distinctions to be drawn. The impurity level, indicated by the low weight recoveries, is rather higher than for many of the mammalian materials where nearer 100 % weight recoveries are obtained.

Crocodile-skin and python-skin gelatins are broadly similar to one another in composition, but appear to have much closer affinities to mammalian materials than the toad-skin gelatin. The serine contents are high, resembling fish materials, and this is the principal departure from a mammalian composition, since the methionine, threonine and hydroxyproline contents are not very different from those of mammalian materials.

Thermal stability of collagens and their hydroxyproline content

Gustavson's views (1953, 1955) on the connexion between the hydroxyproline content and thermal stability of collagen, as indicated by the shrinkage temperature, have been discussed by Eastoe (1957). Gustavson (1955) suggested that the stabilizing feature of collagen is the interchain-hydrogen bonding between the hydroxy groups of the hydroxyproline molecules and carbonyl groups of the adjacent chain or chains. The results of the present paper may be usefully incorporated with those already published to give a more complete picture. Table 2 includes data from Neuman (1949), Gustavson (1953, 1955), Eastoe (1955, 1957) and Dr A. Courts (private communication).

From Table 2 it can be seen that the increase in shrinkage temperature is very approximately associated with an increase in hydroxyproline content, but the exceptions are sufficient to throw doubt on the hypothesis. If the toad and python materials are corrected for low weight recoveries, the amounts of the amino acids under discussion become very close to those of shark and crocodile gelatin respectively. The shrinkage temperature appears also to be in roughly the same order as maximum environmental temperature, and here lung fish fits well into the scheme. The low hydroxyproline content of lung-fish skin, and the somewhat low values for whale skin and pike skin compared with members of the same shrinkage temperature group, suggests an adaptation to life in a different medium. It is not clear whether the physical environment or the available diet has produced, in the course of time, the changes observed.

The serine plus threonine contents appear to decrease in a rather irregular manner with increase in shrinkage temperature. The sum of threonine, serine and hydroxyproline throughout the series examined is approximately constant.

A start has been made on the study of the amino acid composition of invertebrate materials possessing collagen-like X-ray-diffraction patterns. Watson & Smith (1956) have shown that earthworm cuticle contains amino acids with non-polar side chains in amounts similar to collagens. The most striking features of the composition of this material are the extremely high hydroxyproline and the very low proline values.

SUMMARY

1. The amino acid composition of gelatins from two reptiles, an amphibian and a bird, has been determined by ion-exchange chromatography.

2. The agreement in values for gelatin from chicken-leg tendon by both microbiological and ion-exchange methods is very good except for alanine, serine and isoleucine.

3. Chicken-leg-tendon gelatin is similar in composition to mammalian materials. The composition of toad-skin gelatin resembles certain fish gelatins more closely than mammalian materials, and the reverse is true for the two reptile gelatins.

4. The relationship between shrinkage temperature and hydroxyproline and serine plus threonine contents was studied. A very approximate relationship appears to exist between hydroxyproline content and shrinkage temperature.

The author is very grateful to Dr G. R. Tristram, Biochemistry Department, St Andrews, for the amphibian and avian materials, and to Dr A. Courts, of the British Gelatine and Glue Research Association, for the determination of the shrinkage temperature values of the crocodile and python skins.

The author is indebted to Dr J. E. Eastoe of the British Gelatine and Glue Research Association for an insight into the Moore & Stein (1951) technique and much valuable discussion.

This paper is published by permission of the Director and Council of the British Gelatine and Glue Research Association.

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The Metabolism *in vitro* of Tissue Slices from Rats given Triethyltin Compounds

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(Received 14 February 1957)

A detailed account of the signs of the acute and chronic toxicity of alkyltin compounds in animals has been given by Stoner, Barnes & Duff (1955), who concluded that the main site of action of trialkyltin compounds appears to be in the central nervous system. Histological studies have shown that rats fed on a diet containing triethyltin hydroxide develop a specific lesion of the white matter of the brain and spinal cord (Magee, Stoner & Barnes, 1957). The lesion is characterized by an increase in the fluid content of the white matter without any apparent damage to the cells. A second characteristic is the reversibility of the lesion and recovery of animals from chronic poisoning when triethyltin is no longer administered.

The present work is an attempt to elucidate the reason for the selective action of triethyltin on the central nervous system *in vivo*, since previous biochemical studies showed that rat-liver mitochondrial preparations were highly sensitive to triethyltin (Aldridge & Cremer, 1955) and that the site of action was on the oxidative-phosphorylation mechanism.

This paper describes the respiratory activity of rat-tissue slices in the presence of triethyltin. Of the

tissues studied, only brain slices are sensitive to triethyltin and the altered metabolic activity is paralleled by the concentration of triethyltin in the slice whether it is added *in vitro* or administered to the rat before removal of the brain.

A preliminary account of the work has already appeared (Cremer, 1956).

MATERIALS AND METHODS

Tissues. The tissues were from adult albino rats, of 170–200 g. body wt., which had been fed on M.R.C. diet no. 41 (Bruce & Parkes, 1949) except where otherwise stated. In experiments where triethyltin was added to the diet triethyltin hydroxide in arachis oil was mixed with powdered M.R.C. diet no. 41. Food consumption was recorded and paired feeding carried out on control rats.

Slices of brain cortex, liver, kidney and heart were cut with a Stadie-Riggs type of tissue slicer (Stadie & Riggs, 1944). Brain subcortical white matter was prepared according to McIlwain (personal communication). Diaphragms and pieces of washed small intestine were cut into segments. Tissue slices of 0.5–0.7 mm. thickness were quickly prepared at room temperature and placed directly in Warburg flasks. Dry weights were determined by removing the slices from the flasks at the end of the incubation period and heating at 110° for 24 hr.