TABLE I

THE COMPARATIVE ACTIVITIES OF PANTOTHENIC ACID AND BIS-[N-(PANTOTHENYL)]-L-CYSTINE FOR *Acetobacter suboxydans*

Compound tested	Amount required for half maximum growth, μM. × 10³ per 10 ml.
Calcium pantothenate	11.2
Calcium pantothenate + sulfhydryl compound[a]	11.2
Bis-[N-(pantothenyl)]-L-cystine (III)	2.0
Bis-[N-(pantothenyl)]- + sulfhydryl compound	0.7

[a] Cysteine, β-mercaptoethylamine, thiomalic acid and sodium thioglycolate were equally effective. Each compound was added to III at a 200 to 1 molar ratio.

bis-[N-(pantothenyl)]-cystine (III),[3] corresponded closely in biological properties to I. Thus, III was less than 2% as active as pantothenate for *L. arabinosus*, *L. casei* and *S. carlsbergensis*, and less than 2% as active as pantethine for *L. helveticus*. For *A. suboxydans*, however, III was 2 to 3 times as active as pantothenate, and following treatment with any of several sulfhydryl compounds, was about 8 times as active (Table I). The latter did not alter the activity of pantothenate, and hence presumably acted by reducing the disulfide III to the more active thiol, II. Finally, dried cells of *A. suboxydans* convert II to pantetheine (Table II).

TABLE II

THE ENZYMATIC CONVERSION OF N-PANTOTHENYLCYSTEINE TO PANTETHEINE

The complete system contained per 4 ml.: 0.021 μmole of bis-[N-(pantothenyl)]-L-cystine, 35 μmoles of –SH compound (cysteine or β-mercaptoethylamine), 80 mg. of vacuum-dried cells of *A. suboxydans*, and 1 ml. of 0.25 *M* phosphate buffer, *p*H 6.5; incubation was for 3 hours at 30°.

System	Pantetheine formed, μM. × 10³
Complete	2.9
Complete, heated[a]	0.0
Minus bis-[N-(pantothenyl)]-L-cystine	.0
Minus –SH compound	.0
Minus dried cells	.0

[a] Heated at 100° for 5 min. immediately after the addition of the dried cells.

These experiments indicate that pantothenylcysteine is an intermediate in the formation of pantetheine (and hence CoA) from pantothenic acid. The fact that both II and CoA have high activities for *A. suboxydans*, whereas pantethine is no more active than pantothenic acid, may indicate that pantothenylcysteine is phosphorylated before decarboxylation, thus yielding phosphopantetheine (which has high activity for *A. suboxydans*[4]) directly.

Under conditions that liberate the theoretical amount of pantothenate from pantethine, an extract of pigeon liver liberated only 7% of the bound pantothenate from III. If animals can utilize III, its natural occurrence could explain the observations of Lih, *et al.*,[5] that the pantothenate content of some natural materials as indicated by assay with rats was higher than when the material was subjected to enzyme treatment and pantothenate was measured microbiologically.

(3) We are indebted to Drs. J. F. Cavella and R. E. Bowman, Parke, Davis and Co., Ltd., Hounslow, England, for a sample of this compound.

(4) G. D. Novelli, private communication.

(5) H. Lih, T. E. King, H. Higgins, C. A. Baumann and F. M. Strong, *J. Nutrition*, **44**, 361 (1951).

THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH
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GENE M. BROWN
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RECEIVED APRIL 30, 1953

HELICAL POLYPEPTIDE CHAIN CONFIGURATION IN COLLAGEN[1]

Sir:

Methods for testing X-ray diffraction data for evidence of helical molecular chain configuration have recently been provided.[2] Applications have shown that helical structures hitherto proposed do not account for wide-angle diffraction by collagen.[3] We wish to describe specifications for a helix which promises to be satisfactory.

The table lists the commonly observed near-meridional diffractions of kangaroo tail tendon, as well as new ones photographed in a cylindrical camera with specimens tilted at appropriate angles to the incident Cu Kα radiation. Assignment of layer indices (k) further supports the 20 Å. pseudo-period previously suggested.[4]

Observed spacings, d, Å.	k	$b_0 = kd$, Å.	n
∞	0	...	0
9.55	2	19.1	1
5.0	4	20.0	2
3.97	5	19.9	1
2.86	7	20.0	0
2.27	9	20.4	1
1.83	11	20.1	2
1.64	12	19.7	1
1.45	14	20.3	0

A discontinuous helix with 2 turns and 7 roughly equivalent scattering groups per 20 Å. of axial projection should contribute to near-meridional diffraction according to Bessel functions whose orders, n, are shown. The observed layer lines are those for which n is 2 or less (indeed often one or zero). For missing layer lines n is 2 or greater. The higher layers observed with shorter wavelength radiation[5] would have zero n's, and k indices which are multiples of 7.

The approximately seven-fold screw axis does not permit exact hexagonal packing of these chains in fibrils, but there is no evidence requiring this. Pseudo-hexagonal packing, with about 12 Å. between chain centers (dry fibers), accounts for the

(1) Supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council. One of us (C. C.) gratefully acknowledges fellowship support by the National Science Foundation.

(2) W. Cochran, F. H. C. Crick and V. Vand, *Acta Cryst.*, **5**, 581 (1952).

(3) L. Pauling and R. B. Corey, *Proc. Roy. Soc.* (*London*), **B141**, 31 (1953).

(4) R. S. Bear, *Advances in Protein Chem.*, **7**, 115, 130 (1952).

(5) M. F. Perutz, *Ann. Repts., Chem. Soc., London*, **48**, 379 (1952).

principal equatorial reflection. Density requires about 21 amino-acid residues along 20 Å. of helix axis.[4] The 7 nearly equivalent groups should, therefore, comprise 3 residues each.

The above analysis does not determine uniquely the chemical connection of the residues. Primitive helical connection has the merit of allowing several-fold chain extensibility from the average 0.95 Å. of axial projection per residue.[4] Intensity relationships and stereochemical considerations are being used to derive detailed models.

DEPARTMENT OF BIOLOGY CAROLYN COHEN
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RECEIVED MAY 16, 1953

REACTIONS OF ALIPHATIC AMINES WITH SUGARS*

Sir:

We have found that reactions of long-chain primary aliphatic amines with sugars can go far past the amine glycoside stage. In our work as many as five or six moles of amine have reacted with one mole of hexose sugar. In effect, these reactions are replacement of hydroxyl groups by alkylamino groups. The actual mechanism of the reaction, however, is probably a series of Amadori rearrangements, each followed by reaction of the carbonyl group so formed with another mole of amine.

Ketoses appear to be more reactive than aldoses toward amines. In general, formation of amine glycosides takes place very readily at room temperature. A solution of equimolar amounts of fructose and octadecylamine in aqueous isopropyl alcohol, however, reacts in a day or two to give a good yield of a white solid, m.p. 105.5–106.2° (dec.). Elementary analysis shows that this compound is formed from two moles of amine and one of fructose by loss of two moles of water. From this fact it is clear that the product is not an aldehyde-ammonia type of compound or a mixture of amine and amine glycoside.

Anal. Calcd. for $C_{42}H_{86}N_2O_4$: C, 73.81; H, 12.69; N, 4.10. Calcd. for $C_{42}H_{88}N_2O_5$: C, 71.92; H, 12.65; N, 4.00. Found: C, 74.36; H, 12.52; N, 4.29.

To obtain a similar product with glucose it is necessary to use an excess of amine and it is desirable to heat the mixture. By increasing the severity of the reaction conditions, one may introduce still more amino groups. A solution of six moles of octadecylamine and one mole of glucose in aqueous isopropyl alcohol, heated several hours at 60–70°, yields a yellow solid, m.p. 66.5–68°, derived from four moles of amine and one of sugar.

Anal. Calcd. for $C_{78}H_{160}N_4O_2$: C, 78.96; H, 13.60; N, 4.72. Found: C, 78.66; H, 13.37; N, 4.41.

This octadecylamine-glucose product forms a monopicrate of uncertain m.p. (*ca.* 50–95°).

Anal. Calcd. for $C_{84}H_{163}N_7O_9$: C, 71.29; H, 11.61; N, 6.93. Found: C, 71.83; H, 11.77; N, 6.70.

* Paper No. 151, Journal Series, Research Laboratories, General Mills, Inc.

Five moles of octadecylamine react with one mole of sorbose on extended heating in alcohol to give a tan solid, m.p. 67–69.5°.

Anal. Calcd. for $C_{96}H_{197}N_5O$: C, 80.19; H, 13.81; N, 4.87. Found: C, 80.24; H, 13.25; N, 4.79, 4.53.

This sorbose-octadecylamine product forms a yellow monopicrate, m.p. 45–47°.

Anal. Calcd. for $C_{102}H_{200}N_8O_8$: C, 73.50; H, 12.10; N, 6.72. Found: C, 72.95; H, 11.43; N, 6.73.

These findings suggest new approaches to the study of the browning reaction. A more detailed report on this work will be published later.

CHEMICAL LABORATORIES
GENERAL MILLS, INC. JOHN G. ERICKSON
MINNEAPOLIS, MINN.

RECEIVED APRIL 20, 1953

THE FREE AMINO GROUPS OF γ-GLOBULINS OF DIFFERENT SPECIES

Sir:

Porter[1] has found that normal rabbit γ-globulin and the specific rabbit antibody to hen's ovalbumin possess the identical N-terminal peptide sequence (Ala.Leu.Val.Asp.Glu-). It has been suggested that this is in agreement with theories of antibody formation which ascribe specificity to specific surface configuration rather than to differences in amino acid sequence or composition. In contrast are earlier findings by many investigators[2] which have shown that human, bovine and equine γ-globulins are heterogeneous by a variety of criteria. We now wish to report a study of the free amino groups of these globulins by reaction with dinitrofluorobenzene to form the dinitrophenyl (DNP) derivatives by the procedure of Sanger. The present results indicate differences among preparations of human γ-globulins and considerable species variation (Table I).

TABLE I

NUMBER OF FREE AMINO GROUPS IN VARIOUS γ-GLOBULINS

	Human II-1,2	Human II-3	Human cryoglobulin	Bovine A
Asp (60%)[a]	1.06	1.01	1.3	0.13
Ser (81%)	.10	.17	[c]	.09
Glu (56%)	1.82	1.06	1.2	.15
Ala (55%)				.09
Val (57%)				.11
Lys (90%)	75	74	70	73
Lys[b]	79	69		74

[a] Parentheses give recovery values for DNP amino acids after hydrolysis for 24 hours in a sealed tube at 105°. The tabulated values are based on these recoveries, and on an assumed molecular weight of 160,000 for all these proteins. Data on II-1,2 and II-3 globulins are the averages of five determinations each. The cryoglobulin values are averages of three determinations and the bovine, of four independent measurements. [b] Values calculated from microbiological assays.[2] [c] None detectable.

The results on the II-1,2 and II-3 fractions suggest the presence of two or more distinct molecules with different N-terminal residues. The "cryoglobulin" is a γ-globulin from a patient with multiple

(1) R. R. Porter, *Biochem. J.*, **46**, 473 (1950).

(2) E. L. Smith and B. V. Jager, *Ann. Rev. Microbiol.*, **6**, 207 (1952).