On the basis of results of extensive comparisons with an authentic sample[9] using paper and starch chromatography, V has been identified as α,γ-diaminobutyric acid (VII), and it has apparently been derived from asparagine. The unknown and VII showed the same mobilities on paper in: 10:1:10 *n*-BuOH–HAc–H_2O, R_f 0.02; phenol, 0.28; 3:1 *n*-PrOH–0.2 *N* NH_3, 0.28; phenol–NH_3, 0.69; 4:1 pyridine–H_2O, 0.07, and on starch in 2:1 *n*-PrOH–0.5 *N* trichloroacetic acid[8] as well as in A. Chromatography of V in the presence of VII in several of these systems resulted in no separation. In addition, comparison showed the DNP derivative VI to be chromatographically identical with γ-DNP-α,γ-diaminobutyric acid.[10]

Preliminary experiments have traced this side reaction at least partly to initial changes in asparagine occurring during the preparation of the tetrapeptide II. Hydrolysis of the crude product yielded only aspartic acid and the other expected amino acids, while after reduction and hydrolysis VII also was detected. In contrast, reduction of purified II, as well as asparagine, carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteinamide, and other asparagine peptides led only to expected products. The asparagine residue appears to have been converted partially to one with reactivities reminiscent of, among other possibilities, a nitrile, being hydrolyzable to aspartic acid and reducible, at least in part, to a basic grouping, which yields after hydrolysis α,γ-diaminobutyric acid. This effect on asparagine has been noticed during the preparation of other similar asparagine peptides and may perhaps suggest a basis for a synthetic route to peptides of α,γ-diaminobutyric acid from the more readily available asparagine, if as seems possible, the α,γ-diaminobutyric acid residue is formed prior to hydrolysis.

Although the mechanism of this interesting reaction remains to be determined, it is stimulating to envision a comparable biosynthetic pathway which might account for the origin in nature from asparagine of α,γ-diaminobutyric acid, which occurs as an important constituent in a group of polypeptide antibiotic substances, the polymyxins.

(9) α,γ-L-Diaminobutyric acid dihydrochloride was obtained from Mann Chemical Company; m.p. 223–227°, $[\alpha]^{23}D$ +23.9° (2.5% in 5 *N* HCl); reported m.p. 195–196° (D. W. Adamson, *J. Chem. Soc.*, 1564 (1939)), m.p. 218–220° (A. C. Kurtz, *J. Biol. Chem.*, **180**, 1253 (1949)), $[\alpha]^{25}D$ +24.2° (2% monohydrochloride in 5 *N* HCl) (S. J. Fu, K. R. Rao, S. M. Birnbaum, and J. P. Greenstein, *J. Biol. Chem.*, **199**, 207 (1952)).

(10) Appreciation is expressed to Drs. L. C. Craig and W. Hausmann of the Rockefeller Institute for Medical Research for a sample of γ-DNP-α,γ-diaminobutyric acid which was derived from a hydrolysate of DNP-polymyxin B_1.

(11) An anhydro product has recently been isolated from a similar reaction (D. T. Gish, P. G. Katsoyannis, G. P. Hess, and R. J. Stedman, THIS JOURNAL, **78**, 5954 (1956)). Its possible relationship to the formation of α,γ-diaminobutyric acid from asparagine-containing peptides remains to be determined.

(12) The author wishes to thank Dr. Paula Zimmering and Mrs. Lorraine S. Abrash for assistance with the starch chromatography and Mrs. Sylvia Kirsimagi White for the bioassay. Appreciation is expressed to Dr. Vincent du Vigneaud for his encouragement and interest in the problem.

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ENTROPY CHANGE ASSOCIATED WITH HYDROTHERMAL SHRINKAGE OF COLLAGEN

Sir:

Native tendon fibers, when heated above 60° in water, undergo a dramatic, essentially irreversible shrinkage. Although the native fiber is nearly inextensible, the shrunken fiber (about one-quarter the original length) is quite rubbery, and can be stretched to nearly the original length. It is known that the shrink temperature, T_s, may be raised by external stress.[1] If as has been suggested,[2,3] shrinkage is a first-order phase transition, it should be possible, as suggested by Gee,[4] to apply an appropriately modified form of the Clausius–Clapeyron equation, *viz.*

$$df/dT = \Delta S/\Delta l \qquad (1)$$

In preliminary studies of this phenomenon, we first determined the increase in stress necessary to *prevent* hydrothermal shrinkage of strips of bovine foreleg tendon.[3,5] The resulting stress–temperature curves were entirely consistent with the force–length and length–temperature curves predicted by Flory.[6] Over the temperature range from 75–90°, df/dT = 630 g.cm.$^{-2}$ per °C. The small value of df/dT (*i.e.*, marked effect of stress on T_s) suggested strongly that ΔS must be quite small also. This is in contrast to the well-known small effect of pressure in lowering the m.p. of ice, where ΔS is large.

Several strips of tendon were then heated in water under various fixed loads, so that the rise of T_s with stress could be directly measured. From these results, df/dT was calculated to be about 330 g.cm.$^{-2}$ per °C. The calculated value of ΔS is given in Table I, where it is compared with ΔS for several more orthodox phase transitions.

TABLE I

ENTROPY OF MELTING OF SEVERAL SUBSTANCES

Substance	M.p., °C.	ΔS, cal.-g.$^{-1}$ – °C.$^{-1}$
Tendon	65 (T_s)	0.005
Ice	0	0.292
Polyethylene[7]	*ca.* 140	0.136
Poly-(N,N′-sebacoylpiperazine)[7]	180	0.054
Cellulose tributyrate[7]	207	0.017

If we assume[7,8] that each mole of chain bonds freed for rotation contributes 1.5–2.0 cal.-°C.$^{-1}$ to the entropy, we must conclude that the molecular weight of a freely-rotating unit of shrunken collagen is 300–400. Since the average weight per residue of the amino acids in collagen is 93,[9]

(1) E. Wohlisch, *Z. Biol.*, **85**, 406 (1927); *Biochim. Z.*, **247**, 329 (1932); F. G. Lennox, *Biochem. et Biophys. Acta* **3**, 170 (1949); C. E. Weir, *J. Research. Natl. Bur. of Standards*, **42**, 17 (1949).

(2) R. R. Garrett and P. J. Flory, *Nature*, **177**, 176 (1956).

(3) A. V. Tobolsky, *J. Applied Phys.*, **27**, 673 (1956).

(4) Geoffrey Gee, *Quarterly Rev.*, 1, 265 (1947).

(5) R. Haselkorn, B.A. Thesis, Princeton University, Princeton, N. J., 1956.

(6) P. J. Flory, *Science*, **124**, 53 (1956).

(7) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 573.

(8) H. Mark and A. V. Tobolsky, "The Physical Chemistry of High Polymers," Interscience Publication, N. Y., 1950, pp. 105–106.

(9) J. C. Kendrew, in "The Proteins," H. Neurath and K. Bailey (eds.), Academic Press, Inc., New York, N. Y., 1954, pp. 909–936.

there are 3 or 4 residues rigidly linked together in each segment. It is clear that even "melted" collagen is a rather rigid material on a molecular scale.[10]

Since this communication was first submitted, we have had the opportunity to discuss it with Professor P. J. Flory. He points out that the entropy change calculated by equation (1) is an over-all entropy change which included an integral entropy of mixing with solvent.

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ENZYMATIC SYNTHESIS OF S-METHYLCYSTEINE

Sir:

S-Methylcysteine, the lower homolog of methionine, has been found to be enzymatically synthesized in an extract of yeast. The synthesis utilizes methyl mercaptan and L-serine

$$CH_3SH + CH_2OHCHNH_2COOH \longrightarrow CH_3SCH_2CHNH_2COOH + H_2O$$

This reaction was discovered in the course of experiments designed to determine the metabolic role of another enzymatic process involving methyl mercaptan.[1]

Roberts, *et al.*, have shown that S-methylcysteine can be utilized by bacteria in isotope competition experiments and by an organic sulfur-requiring mutant of *E. coli* in a manner which suggests that it may be an intermediate in the microbial biosynthesis of cysteine.[2] It has also been considered as a possible precursor of S-methylcysteine sulfoxide, a major constituent of the nonprotein nitrogen fraction of certain plant tissues,[3] and its natural occurrence in plants was recently established.[4] Schlenk and Tillotson have reported[5] S-methyladenosine to be formed from CH_3SH in yeast, and it is possible that this occurs with the reaction reported here as an intermediate step.

Incubation of CH_3SH and serine with a partially purified enzyme yielded a product which, upon purification on a cation exchange column, moved on paper chromatograms in four solvent systems just as authentic S-methylcysteine does (Table I). After treatment with H_2O_2 the product, when chromatographed on paper, behaved as the mixture of diastereo-isomers of S-methylcysteine sulfoxide which is obtained by treating S-methylcysteine with H_2O_2[3] (Table I). The enzymatic product develops a color in the nitroprusside test for methionine[6] which has a somewhat different absorption spectrum from that developed by methionine but which is identical to that found with S-methylcysteine.

TABLE I

The 8-fold purified enzyme was prepared from a yeast extract[7] by precipitating it as a protamine complex from dilute neutral solution after removing an inactive precipitate at *p*H 5.0. The incubation mixture contained the enzyme preparation (600 mg. of protein), 50.0 millimoles of triethanolamine chloride buffer (*p*H 7.5), 40.0 millimoles of DL-serine, and 3.0 millimoles of CH_3SH in a final volume of 1.0 liter. The mixture was incubated at 24° for 2.5 hours. After deproteinization the product, 1.1 millimoles, was purified by chromatographing on a column of Dowex 50 (H^+ form) and precipitating from water by addition of ethanol. A portion of the reaction product was oxidized with H_2O_2 as indicated by Morris and Thompson for the preparation of S-methylcysteine sulfoxide from S-methylcysteine.[8] Oxidized and unoxidized samples were then chromatographed on Whatman paper No. 1 in the following solvents: A, *n*-butanol–acetic acid–H_2O, 200:30:75; B, 2,4-lutidine–2,4,6-collidine–H_2O–diethylamine, 100:100:100; C, methanol–H_2O–pyridine, 80:20:4; and D, phenol–H_2O, 80:20. The compounds were located on the chromatograms with ninhydrin.

	R_f			
Solvent	SMC[a]	Product	Oxidized SMC	Oxidized Product
A	0.12	0.12	0.025	0.025
B	.41	.40	.23	.23
C	.56	.57	.43, .35	.47, .38
D	.72	.70	.65	.63

[a] SMC = S-methylcysteine.

The extent of the enzymatic reaction may be determined by use of the nitroprusside test, or by measuring the incorporation of $CH_3S^{35}H$ into a heat-stable, non-volatile product. Using these tests the enzyme was found to be specific for L-serine and completely inactive with D-serine, L-threonine, and L-alanine. It was also inactive with DL-homoserine, which suggests that methionine is not formed in an analogous reaction. C_2H_5SH reacts in the system at about 60% of the CH_3SH rate.[8] No cysteine or cystine was found when H_2S was substituted for CH_3SH in the incubation mixture.

The enzyme was completely inactivated by incubation at 60° for 10 minutes. The ability of a crude yeast extract to form S-methylcysteine deteriorated 70% when stored at 0° for several days, and was completely restored by addition of pyridoxal phosphate. Pyridoxal phosphate-requiring preparations were rapidly inactivated by dialysis and other purification procedures in contrast to fresh preparations which were more stable.

Though this reaction is not measurably reversible it is inhibited considerably by the product, S-methylcysteine. The inhibition was partially relieved by a relatively high concentration of serine which

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(2) R. B. Roberts, P. H. Abelson, D. B. Cowie, E. T. Bolton and R. J. Britten, "Studies of Biosynthesis in Escherichia Coli," Carnegie Institution of Washington, Washington, D. C., 1955, p. 318.

(3) C. J. Morris and J. F. Thompson, THIS JOURNAL, **78**, 1605 (1956).

(4) J. F. Thompson, C. J. Morris and R. M. Zacharius, *Nature*, **178**, 593 (1956).

(5) F. Schlenk and J. A. Tillotson, *Federation Proc.*, **13**, 290 (1954).

(6) E. A. Csonka and C. A. Denton, *J. Biol. Chem.*, **163**, 329 (1946).

(7) S. Black and N. G. Wright, *J. Biol. Chem.*, **213**, 27 (1955).

(8) S-Ethylcysteine was used as an analytical standard in this test.