TABLE IV

THE TERMINAL AMINO GROUPS OF CASEIN FRACTIONS

	Alpha	Beta
Terminal arginine[a]	10.7	5.3
Terminal lysine[a]	1.5	2.4
Non-terminal lysine[a]	23.8	24.6
Unassigned amino groups[a,b] (detn.)	12	13
Unassigned amino groups[a,c] (theory)	33	16
Average weight per terminal lysine	67,000	42,000
Av. wt. per terminal amino group	8,200	13,000
Average chain weight[12]	10,000	14,300

[a] Moles per 100,000 g. of protein. [b] Van Slyke amino nitrogen on a swollen solid sample of derivative. [c] From Van Slyke amino nitrogen on pure protein sample.

α-Casein gives a slightly higher number of terminal amino groups than is expected from the difference between the Van Slyke amino nitrogen analysis and the lysine content, but the values are still within experimental error. The average chain weight approaches the 10,000 figure calculated by Hoover, *et al.*,[12] and is remarkably close to the 9,000 value which Hoover, *et al.*, find for unfractionated casein. The minimum molecular weight for the α-casein determined by the number of terminal lysines is about twice the value reported for unfractionated casein by Burk and Greenberg.[13] The large correction for decomposition of the didinitrophenyllysine derivative during the analysis and the possible non-homogeneity of the α-casein may account for this discrepancy. The terminal lysine was not determined on the retreated sample of α-casein and an upward revision of the value may be necessary to account for incomplete reactivity of the lysineamino groups. The change required in this value to reach a reasonable molecular weight would be small compared with the total amount of substitution in the α-casein.

In α-casein there now appears to be seven terminal arginyl residues for each terminal lysyl residue and an average weight per terminal group of 8,200. In β-casein there appears to be only two terminal arginyl residues for each terminal lysyl residue and an average weight per terminal group of 13,000.

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PHILADELPHIA, PENNA.

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Amino Acid Composition of γ-Casein

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γ-Casein has been analyzed for its constituent amino acids. Such physical properties of the protein as solubility, electrophoretic mobility and specific volume have been related to its amino acid composition.

Methods for the separation of γ-casein, a minor component of casein which is soluble in 50% ethanol, have been developed by Hipp, *et al.*[3] The availability of this purified protein has enabled us to supplement our investigation of the amino acid composition of α- and β-casein[4] with an analysis of γ-casein. We have previously reported analyses of γ-casein for alanine, glycine and proline.[5] Additional amino acid analyses, which make it possible to account for essentially all of the nitrogen of this protein, are recorded in the present paper.

Experimental

The γ-casein used in the analyses was provided by N. J. Hipp and M. L. Groves of this Laboratory. The protein was free of α- and β-casein as shown by electrophoresis. It contained 15.40% N, 0.11% P, 1.03% S and 0.15% true ash.[3a]

Methods of Analysis.—Analyses for moisture, amino nitrogen and tyrosine, and microbiological determinations of valine, leucine, isoleucine, phenylalanine and aspartic acid were run by the same procedures used in our earlier work.[4] Methionine, arginine, histidine and lysine were also determined by bioassay using the same general technique. *S. faecalis R.* and Stokes' basal medium with citrate were used for the methionine and arginine analyses, *L. Mesenteroides* P-60 and Steele's medium VI[6] for histidine and lysine.

Additional analyses for methionine were carried out spectrophotometrically by the method of Bakay and Toennies.[7] Cystine could not be detected in γ-casein by the Sullivan–Lugg method applied to HI hydrolyzates.[8] Tryptophan was determined in the unhydrolyzed protein by the procedure of Spies and Chambers.[9] Glutamic acid analyses were made by the enzymatic decarboxylation procedure of Meister, *et al.*[10] Amide nitrogen, serine and threonine were determined by the methods described by Rees.[11]

The preceding methods were applied not only to γ-casein, but to α- and β-casein under identical conditions in order that the results would be strictly comparable. This was especially important in the analyses for valine, leucine, isoleucine, phenylalanine and aspartic acid since it was possible to carry out only a single bioassay of each of these amino acids.

Results and Discussion

The averaged analytical results, corrected for moisture, are summarized in Table I. They may be compared directly with our analyses of α- and

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TABLE I

COMPOSITION OF γ-CASEIN

	G. per 100 g. protein	G. amino acid N per 100 g. protein N
Total N	15.40[a]	
Total P	0.11[a]	
Amino N	0.67 (0.66–0.69)[3][b]	
Glycine	1.5[c] (1.43–1.58)[3]	1.8
Alanine	2.3[c] (2.27–2.32)[3]	2.3
Valine	10.5[1]	8.2
Leucine	12.0[1]	8.3
Isoleucine	4.4[1]	3.1
Proline	17.0[c] (16.8–17.3)[3]	13.4
Phenylalanine	5.8[1]	3.2
Cystine	0.0	0.0
Methionine	4.1[d]	2.5
Tryptophan	1.2 (1.16–1.17)[2]	1.1
Arginine	1.9 (1.7–2.1)[2]	4.0
Histidine	3.7 (3.6–3.7)[2]	6.5
Lysine	6.2 (6.0–6.3)[2]	7.7
Aspartic acid	4.0[1]	2.7
Glutamic acid	22.9 (22.4–23.7)[4]	14.2
Amide N	1.6 (1.57–1.60)[2]	10.4
Serine	5.5[e] (5.44–5.50)[2]	4.8
Threonine	4.4[e] (4.39–4.45)[2]	3.4
Tyrosine	3.7 (3.67–3.75)[3]	1.9
Total	113.3[f]	99.5

[a] Values reported by Hipp, *et al.*[3a] [b] Figures in parentheses show the range of the individual determinations from which the final average value was calculated; each determination was run in duplicate, whenever possible, and each microbioassay was run at 5 levels of unknown concentration; the number of individual determinations is shown in brackets. [c] Analyses previously reported,[5] obtained by the radioisotope derivative (pipsyl) technique. [d] Averaged results of 2 methods; found, by bioassay, 4.2(4.0–4.5)[3] and by spectrophotometric method, 3.99 (3.80–4.12)[4]. [e] Corrected for decomposition during hydrolysis by factors of Rees.[11] [f] Total includes amino acids, amide N calculated as ammonia, and P calculated as phosphoric acid.

β-casein[4,5] except in the case of tryptophan.[12] In general the distribution of amino acids in γ-casein resembles that in β-casein. If the data are calculated in terms of side chain groups in the manner previously described,[4] the differences between β-casein and γ-casein become more apparent, the most pronounced being the difference in phosphoserine groups. The calculations show that γ-casein contains 444 non-polar groups and 421 polar groups per 10^5 g. protein. The low phosphorus content of γ-casein is primarily responsible for the preponderance of non-polar groups which, in turn, is reflected in the relatively high solubility of the protein in 50% ethanol.[3a] Also, whereas γ-casein contains 168 anionic and cationic groups, β-casein has 219 per 10^5 g. protein and this difference in composition can be related to the lower electrophoretic mobility of γ-casein.[3a]

It will be noted that the summation of nitrogen in Table I is close to 100%. Calculation of amino acid residue weights gives a summation of 96.1. It may be that the disparity in these figures is an indication of the presence of an undetected non-nitrogenous component in γ-casein but a number of the analyses cannot be considered sufficiently accurate for this interpretation to be unequivocal. The proximity of the summation of nitrogen to 100% could well be the result of compensating errors. From the amino acid residue weights the specific volume of γ-casein has been computed.[13] The result, 0.749, is in excellent agreement with the observed value of 0.750 from density measurements.[14] The agreement may be regarded as evidence for the general accuracy of both the amino acid analyses and the specific volume determinations.[15]

Hipp, *et al.*,[3a] have pointed out the similarity in composition and properties between γ-casein and the alcohol-soluble, low-phosphorus casein prepared by Osborne and Wakeman.[16] Osborne and Wakeman found that their protein contained 2.9% arginine, 2.3% histidine, 4.0% lysine, 2.5% tyrosine and 1.6% amide nitrogen. A direct comparison of these values with the present analyses of γ-casein cannot be significant because it is unlikely that Osborne and Wakeman's alcohol-soluble casein was a single component of casein and because the analytical methods used were quite different. However, Osborne and Wakeman observed that casein contained more of each of the above amino acids than did their alcohol-soluble casein and that both proteins contained the same amount of amide nitrogen. Similarly, we have found casein to contain more arginine, lysine and tyrosine than γ-casein, the same amount of amide nitrogen, but less histidine. In this very limited sense Osborne and Wakeman's alcohol-soluble casein and γ-casein may be considered similar in amino acid composition.

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PHILADELPHIA 18, PENNA.

(12) Analysis for tryptophan by the Spies and Chambers method gave the following results: whole casein, 1.7%; α-casein, 2.2%; β-casein, 0.83%; γ-casein, 1.2%.

(13) For the method of calculation, see T. L. McMeekin, M. L. Groves and N. J. Hipp, THIS JOURNAL, **71**, 3298 (1949).

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