myeloma.[3] Results on the II-1,2 and II-3 fractions have been confirmed by Putnam,[4] who has analyzed other γ-globulin fractions and performed extensive studies on a series of myeloma proteins. His results, privately communicated to us, show an even more striking range of variations in the N-terminal amino acids of human γ-globulins.

Studies on a highly purified bovine γ-globulin (γ-globulin A[5]) show that all the N-terminal residues are present in less than molar quantities. This suggests that bovine γ-globulin, like the human, is a mixture of closely related proteins which differ in the nature of their N-terminal residues. A preparation of bovine γ-globulin (B)[6] from animals hyperimmunized to mixed antigens (vaccinia, *H. pertussis*, and diphtheria toxin) yields the same end groups as A but contains greater amounts of N-terminal valine and only traces of the other DNP amino acids. An equine γ-globulin[6] gives glutamic acid, aspartic acid, serine, threonine and leucine (or isoleucine) as N-terminal residues, each present in less than a molar quantity.

The DNP proteins were prepared and hydrolyzed by the methods of Sanger (see Porter[7]). The quantity of protein in the DNP protein was estimated from the amide NH_3 of the untreated and the DNP proteins. The ether-soluble DNP amino acids were separated on a series of buffered Celite columns[8] at *p*H 4.0, 5.6, 6.5 and 7.1, with water-saturated ethyl acetate, chloroform and various chloroform–ether and chloroform–butanol mixtures as developing solvents. Positive identification of the separated DNP amino acids was made on Whatman No. 1 and No. 4 papers, buffered with phthalate at *p*H 6.0. ε-DNP-lysine was separated from the acid fraction of the hydrolysate on 1 *M* HCl–Celite columns. Quantitative measurements were made according to the method described by Sanger. Because breakdown of the protein may occur during treatment with dinitrofluorobenzene, *e.g.*,[9] the mother liquors were examined for DNP amino acids. No liberation of DNP amino acids or peptides has been found during the preparation of DNP γ-globulins. Further study of these and other γ-globulins and of specific antibodies is in progress.[10]

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(3) The "cryoglobulin" spontaneously precipitated from the cooled serum of this patient. It was washed several times with cold water and further purified by separation in the electrophoresis cell. The electrophoretic mobility of this γ-globulin is -1.4×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ at *p*H 8.5 in veronal buffer. This protein precipitates completely with rabbit antisera to human γ-globulin. We are indebted to Dr. B. V. Jager and Mr. D. M. Brown for their coöperation in these studies.

(4) F. W. Putnam, THIS JOURNAL, **75**, 2785 (1953).
(5) E. L. Smith, *J. Biol. Chem.*, **164**, 345 (1946).
(6) E. L. Smith and T. D. Gerlough, *ibid.*, **167**, 679 (1947).
(7) R. R. Porter, "Methods in Medical Research," Year Book Publishers, Inc., Chicago, Ill., **3**, 256 (1950).
(8) J. C. Perrone, *Nature*, **167**, 513 (1951).
(9) E. O. P. Thompson, *Biochim. et Biophys. Acta*, **10**, 633 (1953).

(10) Supported by grants from the National Institutes of Health, United States Public Health Service. We are indebted to Dr E. O. P. Thompson for his advice and help in these studies.

N-TERMINAL GROUPS OF NORMAL HUMAN GAMMA GLOBULIN AND OF MYELOMA PROTEINS

Sir:

Although proteins of different isoelectric point and antibodies of unlike specificity have been demonstrated in normal human γ-globulin, there is yet no firm evidence for forms differing in molecular weight or chemical structure. To be sure, the electrophoretic inhomogeneity of γ-globulin is well known, variation in the amino acid content of subfractions has been reported, and a faster sedimenting component is observed after ethanol fractionation of pooled sera.[1] On the other hand, in support of the theory of the chemical identity of normal and antibody globulins, Porter[2] has found in the rabbit that both the normal γ-globulin and the antibody to egg albumin end in the same pentapeptide sequence with a single N-terminal group of alanine per molecule. The aberration in protein synthesis occurring in patients with multiple myeloma has prompted a similar study of the N-terminal groups of normal human γ-globulins and of the myeloma proteins produced by different individuals. This has led to the finding that normal human γ-globulins contain aspartic and glutamic acids as the major N-terminal groups, whereas the pathological γ-globulins we have so far investigated contain neither amino acid nor only aspartici n this position.[3]

Five well-characterized preparations of human γ-globulin obtained by ethanol fractionation of pooled plasma were received from various sources.[4,5] All contained 15 to 25% of a second component with a sedimentation constant of 9S and migrated with a diffuse boundary in electrophoresis. These were compared with myeloma globulins prepared by salt fractionation of the serum of five patients.[6] Electrophoretically four of the myeloma proteins were of the gamma type, one with a mobility of $-0.7\ u$ at *p*H 8.6 in Veronal buffer, and three with a mobility of $-1.1\ u$. These proteins migrated with a sharp single boundary in electrophoresis; they had an s_{20} of 6.6*S* and exhibited only 0 to 5% of a heavy component in the ultracentrifuge. A fifth myeloma protein was of the "beta" type; it had a mobility of $-3.4\ u$ at *p*H 8.6, was 90% homogeneous in electrophoresis, but contained two major components on ultracentrifugation (s_{20} = 6.2 and 8.8*S*). The N-terminal amino acids were determined by Sanger's method with use of a buffered silica gel column for separation of the dinitrophenyl-(DNP) amino acids and paper chromatography for their identification.[7,8]

(1) E. L. Smith and B. V. Jager, *Ann. Rev. Microbiol.*, **6**, 207 (1952).
(2) R. R. Porter, *Biochem. J.*, **46**, 479 (1950).

(3) McFadden and Smith[4] report that a "cryoglobulin" from a patient with multiple myeloma contained 1.2 and 1.3 moles, respectively, of N-terminal glutamic and aspartic acids. One of our proteins of lower mobility ($-1.1\ u$ at *p*H 8.6) was also a cryoglobulin; it yielded 1.8 moles of N-terminal aspartic but only 0.14 mole of N-terminal glutamic acid per mole protein. The other myeloma globulins of the same mobility were devoid of detectable N-terminal seilne and glutamic acid.

(4) M. L. McFadden and E. L. Smith, THIS JOURNAL, **75**, 2784 (1953).

(5) Two lots of Fraction II kindly supplied by Dr. John T. Edsall, γ_2 globulin by Dr. R. A. Alberty, and Fractions II-1,2 and II-3 by Dr. E. L. Smith.

(6) F. W. Putnam and B. Udin, *J. Biol. Chem.*, in press.
(7) F. Sanger, *Advances in Protein Chem.*, **7**, 1 (1952).
(8) S. Blackburn and A. G. Lowther, *Biochem. J.*, **48**, 126 (1951).

Unlike rabbit γ-globulin, normal human γ-globulin (Fraction II or γ_2) contains approximately one mole each of N-terminal aspartic and glutamic acids per 160,000 g. It also yields 0.1 to 0.2 mole of N-terminal serine and small amounts of an unidentified DNP-derivative. In contrast, the three myeloma globulins of mobility -1.1 *u* had two moles of N-terminal aspartic acid per 160,000 g., and in accord with their greater physical homogeneity, were entirely free or nearly so of other N-terminal groups. The myeloma globulin of mobility -0.7 *u* was essentially devoid of N-terminal glutamic or aspartic acids, whereas these occurred in an almost equimolar ratio in the heterogeneous β-globulin.

This appears to be the first demonstration of a difference from normal in the chemical structure of serum proteins elaborated in any disease. It may, however, be interpreted in terms of the physiological occurrence of three (or more) gamma globulins only one of which is synthesized profusely by a given patient with multiple myeloma. Of these proteins one may contain at least two peptide chains both terminating in glutamic acid, another two chains with only aspartic acid as the amino end-group, and the third has neither amino acid in the N-terminal position. This hypothesis is supported by results communicated to us by McFadden and Smith[4] and confirmed in this laboratory, in which it was found that human γ-globulin subfraction II-1,2 has 1.7 times as many moles of N-terminal glutamic acid as of aspartic, whereas subfraction II-3 has one mole of each. There is no correlation of N-terminal groups with the isoelectric point, for in three proteins with a *p*I of 7.3 to 7.5, the molar ratio of N-terminal glutamic to aspartic ranged over twenty-fold. Since physicochemical analysis has indicated great variation in the nature of myeloma globulins,[8] further terminal group analysis may reveal new end-groups and other information of interest to the study of normal serum proteins and of antibody globulins.[9]

(9) Supported by grants of the Lasdon Foundation and The National Cancer Institute, National Institutes of Health, United States Public Health Service. The aid and encouragement of Dr. Fred Sanger is gratefully acknowledged.

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REVERSIBLE CATALYTIC CLEAVAGE OF HYDROXY-AMINO ACIDS BY PYRIDOXAL AND METAL SALTS

Sir:

Pyridoxal and salts of copper, iron and aluminum catalyze the deamination of serine to pyruvate.[1] We have now found that while threonine and allothreonine undergo similar reactions they are much more rapidly cleaved to glycine and acetaldehyde throughout the *p*H range 3–12 in the presence of pyridoxal and copper, iron or aluminum salts. The reactions are readily reversible. Some analyses on reaction mixtures heated at 100° are given in Tables I and II. Similar results were obtained by allowing the reaction to proceed at room temperature for a few days. The amino acid concentrations were determined microbiologically or by the ninhydrin color reaction after separation on a column of cation exchange resin.

(1) D. E. Metzler and E. E. Snell, *J. Biol. Chem.*, **198**, 353 (1952).

The cleavage of serine to glycine and formaldehyde can be demonstrated readily at *p*H 7 where its deamination is slow as can the conversion of formaldehyde and glycine to serine and pyruvate.

Other aldehydes such as propionaldehyde, glyoxylic acid, and pyridoxal can be condensed with glycine under the same conditions to yield a variety of β-hydroxy amino acids.

The presence in animal livers of an enzyme which splits threonine, allothreonine, β-hydroxyvaline, β-hydroxynorvaline and β-phenylserine to glycine and carbonyl compounds has been reported.[2] The reactions are inhibited by carbonyl reagents and despite the lack of decrease in activity in livers of vitamin B_6-deficient rats we predict that this will be found to be a pyridoxal-containing enzyme. A similar enzyme which splits serine is stated to require a cofactor related to folic acid and not to be inhibited by carbonyl reagents. However, vitamin B_6 is also required by both bacteria[3] and chicks[4] for synthesis of serine from glycine.

The combination of the following three vitamin B_6-catalyzed reactions provides an attractive mechanism of amino acid biosynthesis.

TABLE I

CLEAVAGE OF THREONINE TO GLYCINE AND ACETALDEHYDE

Reaction mixtures 20 mM. in threonine, *p*H 5, were heated 30 min. at 100°.

Additions, millimoles per l. Pyridoxal	Alum[a]	Products, millimoles per l. Threonine	Glycine	Acetaldehyde	Ammonia
0	2	19.2	0.0		
10	0	17.5	1.2		
10	2	3.7[b]	12.5	14.0	1.3

[a] $KAl(SO_4)_2 \cdot 12H_2O$. [b] A small amount of allothreonine was also produced.

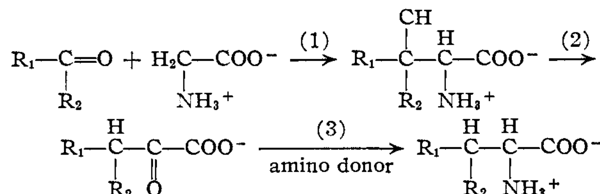
TABLE II

THE REVERSIBILITY OF THREONINE CLEAVAGE

Reaction mixtures containing 10 mM. pyridoxal and 2 mM. alum at *p*H 5 were heated at 100°.

Reactants, millimoles per l. Threonine	Glycine	Acetaldehyde	Heating time, hr.	Threonine, found millimoles per l.
20	20	80	0.25	11.7
20	20	80	2.0	4.4
0	40	100	0.25	2.6[a]
0	40	100	2.0	3.7[a]

[a] Allothreonine was also produced in similar amounts.



The non-enzymatic reactions catalyzed by pyridoxal and metal salts have been carried out for

(2) G. Ya. Vilenkina, *Doklady Akad. Nauk S.S.S.R.*, **84**, 559 (1952), from *C. A.*, **46**, 10227 (1952), and preceding papers.

(3) J. Lascelles and D. D. Woods, *Nature*, **166**, 649 (1950).

(4) S. Deodhar and W. Sakami, *Fed. Proc.*, **12**, 195 (1953).