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.

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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.

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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).

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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RECEIVED MAY 6, 1953

(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 aspartic in 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 serine 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).