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Amino Acid Compositions of Human and Rabbit γ -Globulins and of the Fragments Produced by Reduction

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Analyses of amino acid compositions have been reported for normal human γ -globulin (Brand, 1946; Heimer, Woods & Engle, 1962) and for several purified rabbit antibodies (Smith, McFadden Stockell & Buettner-Janusch, 1955; Askonas, Farthing & Humphrey, 1960; Fleischer *et al.* 1961). Similar values were obtained for rabbit antibodies to pneumococcal capsular polysaccharides, anti-(pneumococcal polysaccharide) antibodies present in different electrophoretic fractions of rabbit γ -globulin, and rabbit antibodies to bovine γ -globulin coupled either to a basic or an acidic hapten. Differences between analyses of human γ -globulin and between those of different rabbit antibodies are probably not significant and in all probability are due to variations in experimental technique. Waldschmidt-Leitz, Bretzel & Keller (1960) have reported that γ -globulins from rabbit and human sera contain appreciable amounts of hydroxylysine, 0.60 and 0.85 % of the total nitrogen respectively; Askonas *et al.* (1960) have also detected hydroxylysine (0.40 % of the total nitrogen) in a fraction of anti-(pneumococcal polysaccharide) antibodies produced in rabbits and separated by electrophoresis. In contrast with these reports, hydroxylysine has apparently not been detected in other Laboratories.

Human and rabbit γ -globulins have been shown (Cohen, 1963; Fleischman, Porter & Press, 1963) to

be made up of four peptide chains/molecule. Reduction of γ -globulin under mild conditions gives rise to two types of chains, called A and B, which can be separated in propionic acid. Similarly, reduction of piece II (Porter, 1959) formed by papain digestion of rabbit γ -globulin gives rise to two peptide chains, called A and B pieces.

The results of amino acid analyses of human and rabbit γ -globulins and their constituent peptide chains are presented below, together with the results of an examination of human and rabbit γ -globulins for the presence of hydroxylysine.

MATERIALS AND METHODS

Materials

Rabbit γ -globulin and fragments of rabbit γ -globulin were those described by Fleischman *et al.* (1963). Human γ -globulin and the human γ -globulin chains A and B were prepared by Cohen (1963); a sample of human γ -globulin (fraction G4; batch no. EG 137) that was kindly supplied by Dr R. A. Kekwick, The Lister Institute, London, S.W. 1, was also used.

Cysteic acid and DL- δ -hydroxylysine hydrochloride were purchased from L. Light and Co. Ltd., L-norleucine and L-2-amino-3-guanidinopropionic acid from California Corp. for Biochemical Research, Los Angeles, and DL-tryptophan and L-tyrosine from Roche Products Ltd. DL-S-Carboxymethylcysteine was synthesized by the method of Michaelis & Schubert (1934) and was recrystallized three times from

hot water; the product had m.p. 175–176° (decomp.) and was chromatographically pure when examined by the method of Spackman, Stein & Moore (1958). Amino acids were dried to constant weight *in vacuo* at 110° before being weighed. Aqueous solutions were stored at 2°.

Ox gelatin (Coignets Gold Label) was obtained from Hopkin and Williams Ltd. Indanetrione hydrate (AnalaR) was purchased from British Drug Houses Ltd., and 2-methoxyethanol (practical grade) from Kodak Ltd. The 2-methoxyethanol was tested for the absence of peroxides by mixing a sample with an equal volume of aq. 4% (w/v) KI. If no yellow colour formed the 2-methoxyethanol was used without any treatment.

Determination of protein concentration

Concentrations of protein solutions in either water or 0.01N-HCl were determined by relating the extinction at 280 m μ , measured with a Unicam SP. 500 spectrophotometer and a cell of 1 cm. light-path, to the specific extinction coefficient at the same wavelength.

Amino acid analysis

The procedure of Spackman *et al.* (1958) was followed, a Spinco model 120 Amino Acid Analyzer (serial no. 246) being used.

Calibration. The Amino Acid Analyzer was calibrated with the Standard Calibration Amino Acid Mixture, type 1 (Spinco part no. 120–220), to which were added measured amounts of cysteic acid, DL-S-carboxymethylcysteine, L-norleucine, L-2-amino-3-guanidinopropionic acid and DL- δ -hydroxylysine hydrochloride. The above mixture was diluted so that the concentration of each amino acid was 0.25 μ mole/ml.; 2.00 ml. samples were used for each determination.

Hydrolysis. Suitable volumes of solutions of known concentration containing about 4 mg. of protein were quantitatively transferred to 16 mm. $\times$ 150 mm. thick-walled rimless Pyrex tubes. Known amounts (2 μ moles) of L-norleucine and of L-2-amino-3-guanidinopropionic acid were added as internal standards (Walsh & Brown, 1962), and the mixture was dried *in vacuo* over P₂O₅. In certain cases, 2 μ moles of DL- δ -hydroxylysine were also added. A portion (1.2 ml.) of constant-boiling HCl (three-times redistilled) was added to the dried material. The mixture was frozen, the tube was evacuated to a pressure of 0.05 mm. Hg, which was maintained while the solution was allowed to thaw, and the tube was sealed. Samples were hydrolysed for periods of 24 and 72 hr. in a boiling-toluene bath (110°). Hydrolysates were dried at 40° *in vacuo* in a rotary evaporator. The dried material was dissolved in 8.5 ml. of 0.20N-sodium citrate-HCl buffer, pH 2.2; analysis was carried out in duplicate on 2.00 ml. samples.

Determination of hydroxylysine. Hydroxylysine was determined on a 50 cm. column (column no. 3) of Amberlite IR-120 resin (25–30 μ) eluted at 30° with 0.38N-sodium citrate-HCl buffer, pH 4.26.

Determination of tryptophan. Tryptophan was determined by a modification (Melamed & Green, 1963) of the method of Beaven & Holiday (1952). Absorption spectra were measured with a Unicam SP. 700 recording spectrophotometer and a cell of 1 cm. light-path. Weighed dried samples of γ -globulin, fragments of γ -globulin, DL-tryptophan and L-tyrosine were dissolved in 0.01N-HCl. Molecular

extinction coefficients (ϵ) for tryptophan and tyrosine were determined at the wavelength of the inflexion (approx. 286 m μ) in the tryptophan spectrum. The wavelength of the inflexion due to tryptophan present in protein samples was measured (approx. 290 m μ). The extinction at this wavelength was corrected for background absorption which was determined by linear extrapolation of the spectrum between 340 and 380 m μ , and for the absorption due to tyrosine which was calculated from the tyrosine content determined with the Amino Acid Analyzer, the ϵ of tyrosine and the concentration of protein. The amount of tryptophan was then calculated by relating the corrected extinction to the ϵ of tryptophan.

Determination of cystine as cysteic acid. This was carried out by using a modification (Moore, 1963) of the method of Schram, Moore & Bigwood (1954). Performic acid was used as the oxidizing agent, and excess of performic acid was destroyed by the addition of HBr. The procedure was checked with a mixture of L-cystine and L-alanine, and with a sample of insulin.

Protein samples (about 4 mg.) together with added L-norleucine (2 μ moles) contained in a thick-walled Pyrex test tube were treated with 2 ml. of performic acid at 0° overnight. Excess of performic acid was destroyed with HBr and the mixture was evaporated to dryness. Constant-boiling HCl (1.2 ml.) was added and the oxidized protein was hydrolysed for 24 hr. as described above. The hydrolysate was dried and redissolved in 5.00 ml. of citrate-HCl buffer, pH 2.2; 2.00 ml. samples were used for duplicate analyses. The amount of cysteic acid relative to the amount of protein oxidized was calculated by reference to the amount of alanine determined in the same analysis, the exact amount of alanine being known from previous analyses of unoxidized samples of the protein.

RESULTS

Specific extinction coefficients

Specific extinction coefficients at 280 m μ were determined for human and rabbit γ -globulins, rabbit- γ -globulin chains A and B, and A piece obtained from piece II formed by papain digestion of rabbit γ -globulin. The results are shown in Table 1. Solutions (about 10 mg./ml.) of rabbit pseudo- γ -globulin in water, and of the rabbit γ -globulin chains A and B and A piece in 0.01N-hydrochloric acid, were dialysed exhaustively against the respective solvent and then clarified by centrifuging. Duplicate dilutions (1:20 in 0.01N-hydrochloric acid) were used for measurement of absorption spectra between 220 and 380 m μ . No evidence of light-scattering due to incomplete solution was obtained. The concentrations of solutions other than human γ -globulin were determined in duplicate by weighing suitable volumes (1.00 ml.) that had been dried to constant weight *in vacuo*. For human γ -globulin an accurately known amount (47.5 mg.) contained in a sealed ampoule was dissolved in 90 ml. of 0.9% sodium chloride and made up to 100 ml. with 0.1N-hydrochloric acid.

Human- γ -globulin chains A and B gave absorption spectra that were closely similar to those of the rabbit- γ -globulin chains A and B. As a result, the human- γ -globulin chains were assumed to have the same specific extinction coefficients at 280 $m\mu$ as the respective rabbit- γ -globulin chains.

Amino acid analysis

Calibration of the Amino Acid Analyzer. The standard values used for the determination of amino acid compositions represent average values calculated from 16 determinations of the calibration mixture carried out over a period of 6 months. During this period any two determinations did not differ by more than $\pm 4\%$; usually the agreement between different determinations was much better than this. This variation was apparently independent of the column of resin used, the age of the ninhydrin reagent, the batch of ninhydrin reagent and the stock solution of the standard amino acid mixture.

The molar colour yield for norleucine (19.9×10^6) was slightly greater than that for leucine (19.7×10^6). The colour yields for cysteic acid and *S*-carboxymethylcysteine were 96 and 88% re-

spectively of the 'average standard HW constant' compared with values of 97 and 88% quoted by Spackman (1960).

Analysis of γ -globulin and fragments of γ -globulin.

The results of a typical analysis obtained with the Amino Acid Analyzer are shown in Table 2; proteins were hydrolysed for 24 and 72 hr. and each hydrolysate was analysed in duplicate. Norleucine was completely stable during 72 hr. in constant-boiling hydrochloric acid at 110° and recoveries of 98–100% were consistently obtained. Values for other amino acids were corrected for a 100% recovery of norleucine. In some cases 2-amino-3-guanidinopropionic acid was also added to the protein sample before hydrolysis. However, the recovery of this amino acid was significantly less than that of norleucine; further, recoveries were not reproducible and its use was discontinued. As shown by Table 2, duplicate analyses gave values that were in close agreement. Hydrolysis for 72 hr. gave smaller amounts of serine, threonine and tyrosine, and greater amounts of histidine and valine, than did hydrolysis for 24 hr. As a result, amounts of serine, threonine and tyrosine were determined by extrapolation to zero hydrolysis time and only those values for histidine and valine determined after 72 hr. were used to calculate the amino acid composition.

Amounts of cystine determined after hydrolysis for 24 and 72 hr. were much less than those expected. As a result, cystine was determined as cysteic acid after oxidation of the protein. An unknown peak that was eluted 20–24 ml. before glycine was detected in both hydrolysates. This peak was not, however, seen when samples of oxidized proteins were analysed. It may therefore be related to the poor recovery of cystine from the unoxidized protein.

Human γ -globulin and the fragments of human and rabbit γ -globulins behaved on hydrolysis in an identical manner with that described above for rabbit γ -globulin.

The amino acid compositions of rabbit γ -globulin, rabbit- γ -globulin chains A and B, and the A and B pieces obtained from piece II formed by papain digestion of rabbit γ -globulin, are shown in Table 3; those of human γ -globulin and its chains A and B are shown in Table 4. Chains A and B and the A and B pieces contain *S*-carboxymethylcysteine as well as cystine. This was expected since they had been produced by reduction of some of the disulphide bonds of the protein and subsequent treatment with iodoacetamide (Fleischman *et al.* 1963; Cohen, 1963). Amounts of *S*-carboxymethylcysteine determined after hydrolysis for 24 and 72 hr. were not significantly different, and it would appear that *S*-carboxymethylcysteine is stable to the conditions of hydrolysis employed.

Table 1. *Specific extinction coefficients at 280 $m\mu$ for rabbit and human γ -globulins, the rabbit- γ -globulin chains A and B and the rabbit- γ -globulin A piece*

Experimental details are given in the text.

	$E_{1\text{cm}}^{1\%}$ at 280 $m\mu$
Rabbit γ -globulin	13.5
Rabbit- γ -globulin chain A	13.7
Rabbit- γ -globulin chain B	11.8
Rabbit- γ -globulin piece A	14.4
Human γ -globulin	13.6

Table 2. *Amino acid analyses of rabbit γ -globulin hydrolysed for 24 and 72 hr.*

Experimental details are given in the text. Values are given as g. of amino acid residue/100 g. of γ -globulin.

Amino acid	After hydrolysis for 24 hr.		After hydrolysis for 72 hr.	
Lys	5.74	5.72	5.69	5.90
His	1.60	1.57	1.70	1.77
Arg	4.42	4.39	4.46	—
Asp	8.04	8.12	8.14	8.04
Thr	9.85	9.91	8.96	8.82
Ser	7.46	7.69	6.04	5.95
Glu	10.97	11.05	11.11	11.08
Pro	6.71	6.64	6.99	6.83
Gly	3.96	3.99	4.06	3.95
Ala	3.71	3.71	3.74	3.69
Val	8.11	7.99	8.35	8.37
Met	1.14	1.11	1.14	1.13
Ileu	3.50	3.48	3.55	3.45
Leu	6.71	6.73	6.81	6.69
Tyr	5.94	5.97	5.47	5.54
Phe	4.29	4.12	4.09	4.12

Table 3. *Amino acid composition of rabbit γ -globulin, the chains A and B, and the A and B pieces*

Experimental details are given in the text. Values are given as g. of amino acid residue/100 g. of protein.

Amino acid	Rabbit γ -globulin	Chain A	Chain B	A Piece	B Piece
Lys	5.76	5.79	4.99	4.22	4.78
His*	1.73	1.79	0.89	1.06	0.98
Arg	4.42	4.90	2.04	3.85	2.13
Asp	8.08	7.53	8.91	6.18	9.18
Thr†	10.37	9.79	12.20	11.98	12.24
Ser†	8.32	8.52	8.36	9.93	8.08
Glu	11.05	10.39	11.80	7.35	11.31
Pro	6.79	7.03	4.85	6.47	4.83
Gly	3.98	3.71	4.47	4.76	4.40
Ala	3.71	3.37	4.75	3.87	4.51
Val*	8.36	8.07	8.69	8.68	9.02
Met	1.13	1.21	0.33	0.77	0.57
Ileu	3.49	3.39	3.49	2.36	3.30
Leu	6.73	6.79	5.56	7.21	6.07
Tyr†	6.17	5.52	7.79	5.68	7.66
Phe	4.15	4.07	3.88	3.56	3.92
Cys‡	2.63	1.91	2.68	2.00	—
Cys·CH ₂ ·CO ₂ H	0	1.17	0.80	1.54	0.73
Try	2.90	2.91	1.71	2.98	—
Total	99.2	97.9	98.2	94.5	—

* Amounts determined after 72 hr. of hydrolysis only.

† Corrected to zero time for decomposition during hydrolysis.

‡ Calculated as cysteic acid after oxidation and hydrolysis of the protein.

Table 4. *Amino acid composition of human γ -globulin and the chains A and B*

Experimental details are given in the text. Values are given as g. of amino acid residue/100 g. of protein.

Amino acid	Human γ -globulin	Chain A	Chain B
Lys	7.06	7.35	6.23
His*	2.44	2.62	1.75
Arg	4.02	3.81	4.57
Asp	7.77	7.64	7.33
Thr†	7.04	6.83	7.42
Ser†	9.13	8.60	10.28
Glu	11.18	10.05	13.04
Pro	6.40	6.41	5.10
Gly	3.37	3.23	3.33
Ala	3.29	2.73	4.19
Val*	7.92	8.15	6.27
Met	0.93	1.02	0.42
Ileu	2.16	1.86	2.74
Leu	7.40	7.11	7.29
Tyr†	5.76	5.52	6.16
Phe	4.07	3.77	4.31
Cys‡	2.07	1.41	1.51
Cys·CH ₂ ·CO ₂ H	0	1.15	0.69
Try	2.63	2.79	2.15
Total	94.6	92.1	94.8

* Amounts determined after 72 hr. of hydrolysis only.

† Corrected to zero time for decomposition during hydrolysis.

‡ Calculated as cysteic acid after oxidation and hydrolysis of the protein.

insulin gave 90 % of the cysteic acid expected from analyses of the unoxidized protein.

Hydrolysis for 24 and 72 hr. of samples of rabbit γ -globulin that had been oxidized with performic acid at 0° for 16 and 40 hr. gave closely similar amounts of cysteic acid. As a routine, therefore, cysteic acid was determined after oxidation for 16 hr. and hydrolysis for 24 hr. Oxidation resulted in a 100 % conversion of the methionine into methionine sulphone, which was eluted from the 150 cm. column of the Amino Acid Analyzer between aspartic acid and threonine, almost complete destruction of tyrosine and a small decrease in the phenylalanine. Oxidation of proteins containing *S*-carboxymethylcysteine would be expected to give rise to *S*-carboxymethylcysteine sulphone and, indeed, chromatograms of the oxidized chains A and B had no peak in the position of *S*-carboxymethylcysteine. However, a new peak due to the sulphone was not seen. It is possible that *S*-carboxymethylcysteine sulphone is either eluted together with another amino acid or that it is not stable to acid hydrolysis. To determine which of these possibilities is correct, samples of *S*-carboxymethylcysteine were analysed after oxidation and hydrolysis.

Weighed amounts (about 20 mg.) of *S*-carboxymethylcysteine and alanine were dissolved in 2 ml. of *N*-sodium hydroxide and diluted with water to 10 ml. The concentrations of the amino acids were determined by using 0.100 ml. of the solution diluted to 5.00 ml. with citrate-HCl buffer, pH 2.2.

Analysis of oxidized proteins. Oxidation of standard mixtures of cystine and alanine gave 99 % recoveries of cysteic acid, whereas oxidation of

Two samples (each 0.100 ml.) were oxidized for 16 hr. as described above; one of these was re-dissolved in 5.00 ml. of citrate-HCl buffer, pH 2.2, and the other was hydrolysed for 24 hr. before being redissolved in the same volume of buffer. Analysis revealed that identical amounts of alanine were recovered after oxidation and hydrolysis, but no *S*-carboxymethylcysteine. Chromatograms of oxidized *S*-carboxymethylcysteine showed three peaks; the first of these occupied a position very close to that of cysteic acid, the next gave a shoulder on the left-hand side of the aspartic acid peak and the last a shoulder on the right-hand side of the glutamic acid peak (Spackman *et al.* 1958). When cysteic acid was added to the oxidized *S*-carboxymethylcysteine before analysis a single slightly asymmetrical peak was eluted in the cysteic acid position. Oxidation of closely similar amounts of *S*-carboxymethylcysteine on different occasions always gave rise to the above three peaks but the ratio of their areas varied. Hydrolysis of oxidized *S*-carboxymethylcysteine resulted in the complete destruction of the substance responsible for the peak eluted close to that of glutamic acid, a decrease of about 66% in the area of the peak in the cysteic acid position and a small decrease in the peak close to that of aspartic acid.

Examination for the presence of hydroxylysine. Hydroxylysine is eluted before lysine from the 15 cm. column of the Analyzer. Hexosamines and artifacts produced during the hydrolysis of proteins by the degradation of tryptophan and chlorination of tyrosine are eluted in the same region. Consequently, if hydroxylysine were present in hydrolysates of γ -globulin it would not have been possible to detect it with certainty. For this reason, the 50 cm. column of the Analyzer was used since with this column a much better resolution of hydroxylysine, hexosamine and the above artifacts is achieved (Spackman, 1960). The procedure was checked by the analysis of samples of δ -hydroxylysine, of ox gelatin that had been hydrolysed with constant-boiling hydrochloric acid for 24 hr. *in vacuo* at 110° and of ox gelatin to which a known amount of δ -hydroxylysine had been added before hydrolysis. Hydroxylysine was eluted as two overlapping peaks; the first represented hydroxylysine and the second alhydroxylysine. Hydrolysed gelatin gave a double peak in an identical position. This peak was equivalent to 1.10 g. of hydroxylysine/100 g. of gelatin; according to Eastoe (1955) 100 g. of ox-hide gelatin contains 0.97 g. of hydroxylysine. Gelatin to which hydroxylysine was added before hydrolysis gave a 100% recovery of the calculated amount of hydroxylysine.

Examination of the elution curves given by human γ -globulin (5.10 mg.) and by rabbit γ -globulin (4.68 mg.) after hydrolysis with constant-

Table 5. *Analysis of human and rabbit γ -globulins hydrolysed for 24 hr. in the absence and presence of measured amounts of hydroxylysine*

Experimental details are given in the text. The amount of hydroxylysine added was equivalent to 1.27 and 1.38 g./100 g. of human and rabbit γ -globulins respectively. Values for amino acids other than hydroxylysine are given as g. of amino acid residue/100 g. of γ -globulin.

Amino acid	Human γ -globulin		Rabbit γ -globulin	
	Without Hylys	With Hylys	Without Hylys	With Hylys
Hylys	0	1.21	0	1.34
Lys	7.20	7.15	5.73	—
His	2.20	2.20	1.53	1.44

Fig. 1. Elution curves of the hydrolysate from 5.10 mg. of human γ -globulin, chromatographed on the 50 cm. column of the Spinco model 120 Amino Acid Analyzer. (a) With 0.40 μ mole of hydroxylysine added before hydrolysis; (b) without added hydroxylysine. Experimental details are given in the text. Amino acids are eluted in the order: hydroxylysine plus alhydroxylysine; ammonia; lysine; histidine.

boiling hydrochloric acid for 24 hr. *in vacuo* at 110° revealed no trace of a peak in the position of hydroxylysine. Further, analysis of human and rabbit γ -globulins that had been hydrolysed in the presence of measured amounts of δ -hydroxylysine (0.40 μ mole) showed that the amount of hydroxylysine was equal to that added (Table 5). The elution curves given by human γ -globulin hydrolysed in the absence and presence of added hydroxylysine and chromatographed on the 50 cm. column of the Analyzer are shown in Fig. 1.

DISCUSSION

As serine, threonine and tyrosine were progressively destroyed and valine and histidine were slowly liberated during hydrolysis, amino acid compositions were determined from analyses

carried out on materials hydrolysed for 24 and 72 hr. Unfortunately, cystine could not be determined from these analyses since poor recoveries were obtained even after hydrolysis for 72 hr. The reason for this is not known but, as satisfactory recoveries of cystine were obtained for insulin hydrolysed under the same conditions, it is probable that the low recovery is related in some way to the structure of γ -globulin. Cystine was therefore determined as cysteic acid. The measured amount of cysteic acid was not corrected as suggested by Moore (1963) since the recovery of cysteic acid may not be the same for all proteins; the results quoted therefore represent minimum values. Oxidation of proteins also resulted in the complete conversion of methionine into methionine sulphone, almost complete destruction of tyrosine and a small decrease in phenylalanine. If the protein contained *S*-carboxymethylcysteine then this was completely destroyed. Examination of oxidized *S*-carboxymethylcysteine in the Amino Acid Analyzer revealed two major and one minor peaks. In all probability the peak in the position of cysteic acid represents *S*-carboxymethylcysteine sulphone which is unstable to acid hydrolysis. Recoveries of the sulphone were not reproducible. As a result it was not possible to correct the area of the cysteic acid peak given by *S*-carboxymethylcysteine-containing proteins after oxidation and hydrolysis for the presence of the sulphone. However, the fragments of γ -globulin contain less *S*-carboxymethylcysteine than cystine. Also, as 1 g. of *S*-carboxymethylcysteine gave after oxidation and 24 hr. of hydrolysis an amount of sulphone equivalent to about 0.06 g. of cysteic acid, the sulphone contributed a small amount only to the areas of the cysteic acid peaks of the γ -globulin fragments, in all probability not more than 3%. Therefore measurements of cysteic acid uncorrected for the presence of the sulphone are within the experimental error of the method. A more satisfactory method would, however, have been to hydrolyse oxidized proteins for 72 hr., since under these conditions cysteic acid is stable whereas approximately 90% of the *S*-carboxymethylcysteine sulphone would have been destroyed.

The amino acid compositions of rabbit and human γ -globulins are in fairly good agreement with previously published results. The majority of the differences are of an order which would be expected to be due to experimental errors and different techniques. The major exception is the apparent absence of hydroxylysine. Waldschmidt-Leitz *et al.* (1960) have reported that human and rabbit γ -globulins contain appreciable amounts of hydroxylysine. Hydroxylysine could not, however, be detected in the present investigation. The reason for this discrepancy is not clear. It is possible, however, that it is due to differences in experi-

mental technique; for instance, Waldschmidt-Leitz *et al.* (1960) determined hydroxylysine by using a column of Amberlite IRC-50 resin eluted with 0.2M-citrate buffer, pH 7.0 (Hannig, 1959). Poorer resolutions are achieved with this method than with the 50 cm. column of the Amino Acid Analyzer. On the other hand, Waldschmidt-Leitz *et al.* (1960) also detected hydroxylysine by paper chromatography. Greater amounts of tryptophan were found in the present investigation than were reported by Smith *et al.* (1955). Anderson & Gill (1962) have, however, obtained values for tryptophan that are in closer agreement with the present results.

Human and rabbit γ -globulins possess similar amino acid compositions. Each contains large amounts of serine, threonine, valine, proline and lysine, and relatively small amounts of methionine, histidine and isoleucine. The compositions of chains A and B are generally similar. There are, however, some noteworthy differences. For instance, the rabbit- γ -globulin chain B contains significantly more tyrosine and much less arginine, methionine and tryptophan than chain A; the human- γ -globulin chain B also contains less methionine and tryptophan than chain A. The B piece isolated from the papain-digestion piece II of rabbit γ -globulin has an amino acid composition that is identical with that of chain B obtained from undigested γ -globulin. Thus piece II must contain all of chain B. The distribution of amino acids in the A piece separated from the papain-digestion piece II is similar to that for γ -globulin and the entire chain A. It appears therefore that the presence of an antibody site cannot be correlated with a characteristic amino acid composition of A piece (Fleischman *et al.* 1963), such as a high content of either cystine or serine plus threonine.

The total weight of amino acids recovered was, in each case, close to 100% when allowances were made for the presence of amide groups and for carbohydrate in γ -globulin and chain A.

Values for the specific extinction coefficients at 280 m μ agreed, in general, with the tyrosine and tryptophan contents. However, although rabbit γ -globulin A piece and chain A contained similar amounts of tyrosine and tryptophan, the A piece had a significantly greater specific extinction coefficient. No reason can be given for this discrepancy.

SUMMARY

1. Amino acid compositions have been determined, by using the Spinco model 120 Amino Acid Analyzer, for human and rabbit γ -globulins, for the chains A and B separated after partial reduction of human and rabbit γ -globulins, and for the A and B pieces obtained from piece II formed by papain digestion of rabbit γ -globulin. The compositions of

the rabbit- γ -globulin chain B and B piece were not significantly different.

2. Tryptophan was determined spectrophotometrically on the intact protein and cystine was determined as cysteic acid after oxidation of the protein. Oxidation of *S*-carboxymethylcysteine gave three products; one of these was eluted from the 150 cm. column of the Analyzer very close to the position of cysteic acid and was unstable to hydrolysis with acid.

3. In contrast with a previous report, hydroxyllysine could not be detected in either human γ -globulin or rabbit γ -globulin.

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The Molecular Weights of the Peptide Chains of γ -Globulin

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Mild reduction of γ -globulin with mercaptoethanol in aqueous solution breaks five interchain disulphide bonds, allowing the separation of two fractions, termed chains A and B (Fleischman, Pain & Porter, 1962). The molecular weights of the peptide chains A and B of horse γ -globulin were reported as 105 000 and 40 000 respectively. It became apparent that the persistence of aggregate in the solutions examined had given rise to high values with the Archibald technique. More accurate results have now been determined for chains A and B by using short-column equilibrium sedimentation (Van Holde & Baldwin, 1958). Determinations of the molecular weights of the

products of reduction of papain-digestion piece I (Fleischman, Porter & Press, 1963) and of whole horse γ -globulin have also been carried out.

MATERIALS

γ -Globulin. Horse and rabbit γ -globulins were separated from sera on a diethylaminoethylcellulose column equilibrated with 17.5 mM-sodium phosphate buffer, pH 6.5. Solutions were concentrated to 4 g.dl.⁻¹, sterilized by filtration and stored at 3°.

Peptide fragments of γ -globulin. These were prepared as described by Fleischman *et al.* (1963).

Other materials. Crystalline ribonuclease and crystalline bovine plasma albumin were purchased from Armour