Biochem. J. (1969) **111**, 473
Printed in Great Britain

The Controlling Effect of Carbohydrate in Human, Rabbit and Bovine Immunoglobulin G on Proteolysis by Papain

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(*Received* 19 *July* 1968)

About two-thirds of the hexose of human and rabbit immunoglobulin G (IgG) was located in the Fc fragment and one-third in the 'hinge' region of the γ (heavy) polypeptide chain at the junction of the Fab and Fc fragments. In contrast, bovine IgG contained more hexose in the 'hinge' region than in the Fc fragment. The initial cleavage of susceptible IgG molecules into Fab and Fc fragments by papain under the conditions given by Porter (1959) had reached completion after digestion for 2hr., though bovine IgG was digested somewhat more slowly than human or rabbit IgG. The release of 'hinge' peptides from human and rabbit IgG had also reached completion by 2hr., but was slower from bovine IgG and continued for several hours longer. Since bovine IgG molecules contained on the average a greater amount of hexose in the 'hinge' region, carbohydrate on this part of the γ-chain may influence not only the initial rate of enzymic hydrolysis into Fab and Fc fragments, but also, and to a greater extent, the rate of further limited hydrolysis of the *N*-terminal regions of the Fc fragment. The presence of carbohydrate in the 'hinge' region does not appear to account for the resistance of some IgG molecules to papain digestion and of some Fc fragments to *N*-terminal degradation.

The carbohydrate content of normal human, rabbit and bovine IgG* is very similar, and the immunoglobulins contain similar proportions of hexose, hexosamine, fucose and sialic acid (reviewed by Press & Porter, 1966). In rabbit IgG about two-thirds of the carbohydrate is in the Fc fragment and one-third is in the region attacked by papain, the 'hinge' at the junction of the Fab and Fc fragments, and is released in glycopeptides during proteolysis (Fleischman, Porter & Press, 1963; Utsumi & Karush, 1965, 1967; Smyth & Utsumi, 1967). There is indirect evidence that hexose is distributed in a similar way in human IgG (Franklin, 1960).

Bovine IgG molecules have a four-polypeptide-chain structure that accords with the general model described by Porter (1962), since they are cleaved by papain primarily into Fab and Fc fragments (Payne, 1965; Kuchinskaya, Kul'berg & Tsvetkov, 1965; Murphy, Osebold & Aalund, 1965) and by mild reduction into heavy and light polypeptide chains (Payne, 1965; Milstein & Feinstein, 1968), the bovine fragments and chains having properties closely similar to those of rabbit, horse and human IgG. The distribution of carbohydrate in bovine IgG has not been reported, but the observation that polypeptides rich in carbohydrate are released during papain hydrolysis (Payne, 1966) suggested the possibility that bovine IgG may, like rabbit IgG, contain oligosaccharide units in two different locations.

In rabbit IgG, the presence of carbohydrate in the 'hinge' region of some molecules seems to limit the rate of initiation of papain hydrolysis of their γ-chains (Goodman, 1965). The purpose of the present work was to compare the distribution of hexose in human, rabbit and bovine IgG with the rate of digestion and of release of 'hinge' peptides during exposure to papain. Bovine IgG contained a greater proportion of hexose in the 'hinge' region. It was split into Fab and Fc fragments and it released 'hinge' peptides more slowly than either human or rabbit IgG.

*Abbreviation: IgG, immunoglobulin G; the nomenclature of immunoglobulin fragments and polypeptide chains used is that recommended by the World Health Organisation (1964).

MATERIALS AND METHODS

Human, rabbit and bovine IgG. Human, rabbit and bovine Cohn fraction II preparations were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.; human Cohn fraction II from Kabi AB, Stockholm,

Sweden; and bovine Cohn fraction II from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex. The solutions were freed of aggregated material and of traces of immunoglobulin M by gel filtration on Sephadex G-200. The purified preparations migrated as slow compact bands of comparable mobility on cellulose acetate electrophoresis, and gave the single precipitation line of IgG on immunoelectrophoresis against species-specific antisera to whole serum (Mann Research Laboratories, New York, N.Y., U.S.A.). The bovine IgG preparation was thus free of the fast-migrating serum immunoglobulin component designated IgG1 by Milstein & Feinstein (1968). The IgG solutions were stored at −15°. Repeated freezing and thawing was avoided.

Determination of protein. The concentrations of solutions of human, rabbit and bovine IgG were determined by using the appropriate specific extinction coefficients at 280 mμ (Crumpton & Wilkinson, 1963; Payne, 1965). The specific extinction coefficients of $F(ab')_2$ fragments and of mixtures of Fab and Fc fragments were assumed to be the same as those of the IgG preparations from which they were derived.

Determination of protein-bound hexose. Hexose was determined by the modification by François, Marshall & Neuberger (1962) of the Winzler orcinol–H_2SO_4 method. When the volume of protein solution containing 50–500 μg. of hexose was greater than 1·0 ml., the protein was precipitated by the addition of an equal volume of 10% (w/v) trichloroacetic acid. In some experiments, trichloroacetic acid was also used to terminate enzyme digestions. In either case, the precipitate was washed several times with ethanol and dissolved in 1·0 ml. of 0·1 N-NaOH before the addition of the orcinol–H_2SO_4 reagent. Mannose was used as the standard.

Gel filtration. The Cohn fraction II preparations were purified by chromatography on a column (2·5 cm. × 35 cm.) of Sephadex G-200 (Pharmacia, Uppsala, Sweden) in 0·1 M-sodium phosphate buffer, pH 7·0, and the products of digestion with papain and with pepsin were separated on a column (2·5 cm. × 35 cm.) of Sephadex G-200 in 0·1 M-tris–HCl buffer, pH 7·5, containing 0·5 M-NaCl and 0·01% (w/v) of NaN_3. Molecular weights were determined from elution volumes by the principle described by Andrews (1964), but by upward-flow chromatography on a column (2·5 cm. × 35 cm.) of Sephadex G-200 column in the tris buffer, pH 7·5. Samples and buffer were applied with a peristaltic pump. The column was calibrated with bovine IgG (from Armour Pharmaceutical Co. Cohn fraction II), assumed molecular weight 160000, crystalline bovine albumin (Armour Pharmaceutical Co.), assumed molecular weight 67000, and twice-recrystallized ovalbumin (Koch–Light Laboratories Ltd., Colnbrook, Bucks.), assumed molecular weight 45000. Chromatography on Sephadex G-200 was carried out at room temperature at flow rates of 10–12 ml./hr.

Electrophoresis. Electrophoresis and immunoelectrophoresis were carried out as described by Payne (1965).

Two-dimensional immunodiffusion. The supporting medium for this was 1% (w/v) Ionagar no. 2 (Oxoid Ltd., London, E.C. 4) in 0·1 M-tris–HCl buffer, pH 7·5, either in Petri dishes or on microscope slides.

Pepsin digestion. Human, rabbit and bovine IgG (20 mg./ml.) were incubated at 37° with 3% (w/v) three-times recrystallized pepsin (Calbiochem, Spring Valley, N.Y., U.S.A., or Mann Research Laboratories, New York, N.Y., U.S.A.) in a 0·1 M-sodium acetate buffer, pH 4·5, for 18 hr. Digestion was stopped by adjusting the mixture to pH 7·0 or by precipitation with an equal volume of 10% (w/v) trichloroacetic acid.

Papain digestion. The IgG preparations were digested with twice-recrystallized papain (Worthington Biochemical Corp., Freehold, N.J., U.S.A.) by using the conditions described by Porter (1959). Digestion was stopped by the addition of an equal volume of 0·04 M-*N*-ethylmaleimide, or by precipitation with an equal volume of 10% (w/v) trichloroacetic acid.

Reduction and alkylation. Pepsin-digested bovine IgG, prepared by gel filtration in the 0·1 M-tris–HCl buffer, pH 7·5, was exposed to 0·2 M-mercaptoethanol for 1 hr. at room temperature and then cooled to 2–4°. The reduced protein was alkylated by the addition of an equal volume of ice-cold 0·2 M-iodoacetamide. The reaction mixture was placed immediately in a Radiometer type TTT1 automatic titrator and the pH maintained at pH 7·5 with 1 N-NaOH. After the reaction was complete, the molecular weight of the reduced alkylated protein was determined by chromatography on Sephadex G-200.

^{14}C-labelling of 'hinge' inter-γ-chain disulphides. Human, rabbit and bovine IgG (200 mg.) in 4·0 ml. of 0·1 M-sodium phosphate buffer, pH 8·0, were exposed to 0·001 M-mercaptoethanol at 30° for 5 hr., conditions known to reduce inter-γ-chain disulphide bonds in rabbit IgG with minimal reduction of the disulphide bonds between heavy and light chains (Hong & Nisonoff, 1965). Incubations were continued for a further 1 hr. after the addition of 1·0 ml. of 0·007 M-iodo-[1-^{14}C]acetic acid (7·03 mc/m-mole; New England Nuclear Corp., Boston, Mass., U.S.A.). Material of low molecular weight, including unbound iodo[^{14}C]acetate, was removed by gel filtration on a column of Sephadex G-75 equilibrated with 0·1 M-sodium phosphate buffer, pH 7·0. The immunoglobulins alkylated with iodo[^{14}C]acetate were eluted in the void volume and were stored at 2–4° in the presence of toluene. The specific radioactivity of the preparations was determined by digesting portions (0·1 ml.) each with 0·5 ml. of 1 M-Hyamine hydroxide in methanol (Packard Instrument Co. Inc., Downers Grover, Ill., U.S.A.) for 2 hr., adding 20 ml. of scintillation fluid (containing 38·5 ml. of xylene, 38·5 ml. of dioxan, 23 ml. of ethanol, 8·09 g. of naphthalene, 0·504 g. of 2,5-diphenyloxazole and 5·05 mg. of α-naphthylphenyloxazole/100 ml.) and counting in a Packard Tri-Carb liquid-scintillation spectrometer at an efficiency of 84%. A minimum of 1000 counts above background were recorded. Quenching of the radioactivity of [^{14}C]toluene in this system was negligible. The supernatants after precipitation of the labelled immunoglobulins with an equal volume of 10% (w/v) trichloroacetic acid contained only background amounts of radioactivity. The immunoglobulins were labelled with about 0·3 mole of [^{14}C]carboxymethyl groups/mole of IgG.

Release of labelled peptides during papain digestion. The ^{14}C-labelled IgG preparations were digested with papain under the conditions described by Porter (1959), but with 0·02 M- in place of 0·01 M-cysteine, in closed tubes in the presence of toluene. Digestion was terminated after 30 and 69 hr. by the addition of an equal volume of 10% (w/v) trichloroacetic acid, and the radioactivity of 0·5 ml. of supernatant in 20 ml. of scintillation fluid was measured to determine the maximum release of radioactivity. Quenching of the radioactivity of [^{14}C]toluene in this system was negligible. The rates of release of radioactivity were

Table 1. *Hexose content of IgG before and after enzyme digestion*

Values are given as g. of hexose/100g. of undigested IgG. 'Pepsin-digested' represents $F(ab')_2$ fragments freed of peptides, and 'papain-digested' represents Fab and Fc fragments freed of non-covalently bound glycopeptide by chromatography on Sephadex G-200 in 0·5M-NaCl–0·1M-sodium phosphate buffer, pH7·5. Details are given in the text.

Sample	Fragment	Hexose content (g./100g. of IgG)		
		Human	Rabbit	Bovine
(*a*) Undigested	IgG	1·01	0·95	0·98
(*b*) Papain-digested	Fab+Fc	0·66	0·65	0·38
(*a*)−(*b*)	'Hinge'	0·35	0·30	0·60
(*c*) Pepsin-digested	$F(ab')_2$	0·28	0·26	0·53

determined by pipetting samples (0·1ml.) of the digestion mixtures into 1·9ml. of 10% (w/v) trichloroacetic acid and measuring the radioactivity of 0·5ml. of the supernatants in 20ml. of scintillation fluid.

RESULTS

Hexose content of papain-digested human, rabbit and bovine IgG. The undigested human, rabbit and bovine pooled IgG preparations all contained about 1% of hexose by weight (Table 1), close to the published values reviewed by Press & Porter (1966). Fleischman *et al.* (1963) reported that papain digestion of rabbit IgG released a glycopeptide containing one-third of the total carbohydrate, which became bound to Fab prepared by CM-cellulose chromatography and which could be dissociated from it by gel filtration in 1M-propionic acid. Rabbit IgG digested with papain under the conditions described by Porter (1959) was applied to a Sephadex G-200 column and eluted with 0·1M-sodium phosphate buffer, pH7·4, containing 0·5M-sodium chloride. The hexose content of the main protein peak, shown by immunoelectrophoresis to consist of Fab and Fc fragments, was only two-thirds of that of the intact immunoglobulin. The remaining hexose was eluted later from the column with low-molecular-weight material. Thus the non-covalently bound glycopeptide was dissociated under these chromatographic conditions. Human Fab and Fc fragments were also found to contain about two-thirds of the hexose of the starting material. In contrast, bovine Fab and Fc fragments, prepared in a similar way, contained only just over one-third of the hexose of the starting material (Table 1).

Dische & Franklin (1964) noted that, when human Fab fragment prepared by CM-cellulose chromatography was rechromatographed on DEAE-cellulose, a glycopeptide soluble in 5% (w/v) trichloroacetic acid was obtained. It has been suggested that this glycopeptide is analogous to that isolated by Fleischman *et al.* (1963) from rabbit Fab (Press & Porter, 1966). The material of low molecular weight that was released during papain digestion for 2 or 24hr. from human, rabbit and bovine IgG, which was eluted from Sephadex G-200 after Fab and Fc fragments, was examined; the peptides from all three species of IgG were found to be soluble in 5% and 10% (w/v) trichloroacetic acid. It was thus possible to re-examine the carbohydrate contents of rabbit and bovine IgG after papain digestion by precipitating undigested IgG, Fab fragment and Fc fragment from the digestion mixtures with trichloroacetic acid (see the Materials and Methods section). It was confirmed by this technique that rabbit IgG lost one-third of its hexose during digestion. The trichloroacetic acid-insoluble material in the bovine IgG papain digests, which is shown below to have included about 11% of the original undegraded IgG, accounted for less than half of the hexose of the starting material, thus confirming that bovine IgG lost a greater proportion of its hexose during papain digestion than human or rabbit IgG.

The hexose-rich peptide material (obtained by chromatography on Sephadex G-200 of papain digests of all three species of IgG without alkylation with *N*-ethylmaleimide) was fractionated by rechromatography on Sephadex G-25. In each case peptides that contained hexose were excluded from the bed material, indicating minimum molecular weights of about 5000, and suggesting the possibility of dimerization due to disulphide formation.

Pepsin digestion of bovine IgG. Digestion of rabbit and human IgG with pepsin results in degradation of most of the Fc portion of the molecule, but leaves intact a bivalent unit designated fragment $F(ab')_2$, consisting of two Fab fragments and two 'hinge' regions linked by at least one inter-γ-chain disulphide bridge (Nisonoff, Wissler, Lipman & Woernley, 1960; Mandy, Rivers, & Nisonoff, 1961).

The effect of pepsin digestion on bovine IgG has not been reported hitherto. When bovine IgG was digested with pepsin in 0·1M-acetate buffer, pH4·5, an immediate precipitate appeared, which redissolved when digestion was stopped after 18hr. by adjusting the mixture to pH7·0. Immunoelectrophoresis and double-diffusion studies in agar

of the washed redissolved precipitate against an antiserum to bovine IgG produced in rabbits (Payne, 1965) showed that it contained at least two peptides that were antigenically distinct from peptides present in the supernatant. Chromatography of the whole digestion mixture, including the redissolved precipitate, on Sephadex G-200 separated two peaks, the first containing two-thirds and the second one-third of the material absorbing at 280mμ. Immunoelectrophoresis of the protein of the first peak against the antiserum to bovine IgG gave a single precipitin arc with a mobility slightly slower than that of the starting material. The second peak contained a mixture of peptides. Ouchterlony-plate studies showed that the digested protein was antigenically deficient with respect to bovine IgG, but gave a reaction of identity with bovine Fab. The elution volumes of the first peak of pepsin-digested bovine IgG and of human $F(ab')_2$ fragment from Sephadex G-200 were identical, and reduction and alkylation of the bovine material halved the apparent molecular weight, determined by gel filtration. It was therefore concluded that the material was bovine $F(ab')_2$ fragment.

Distribution of hexose in human, rabbit and bovine $F(ab')_2$ fragments. Human and rabbit $F(ab')_2$ separated by gel filtration of pepsin digests contained just over one-quarter of the hexose of intact IgG. In contrast, over one-half of the original hexose was retained in bovine $F(ab')_2$ (Table 1). None of the pepsin digests contained detectable amounts of IgG of unchanged molecular weight.

IgG resistant to papain hydrolysis. The proportion of the human, rabbit and bovine IgG preparations resistant to papain digestion for various periods under the conditions described by Porter (1959) was determined by chromatography of the digestion mixture on Sephadex G-200 after the reaction had been stopped with *N*-ethylmaleimide. The protein content of the peak appearing in the same elution volume as the starting material was measured (Table 2).

Chromatography on Sephadex G-200 of rabbit IgG digested with papain for 3min. showed a poorly resolved peak between the peak of intact IgG and that of the mixture of the Fab and Fc fragments, probably representing the 5·0s intermediate described by Goodman (1965). The protein of this peak was included with intact IgG in calculating the proportion of rabbit IgG that had not been degraded in this period. It was not seen after the longer periods of digestion.

Bovine IgG was digested with papain under the conditions described by Porter (1959) for 24hr., and the digestion mixture was fractionated by chromatography on Sephadex G-200. The hexose content of the IgG of unchanged molecular weight was found to be 0·81g./100g. of IgG, lower than the hexose content of bovine IgG before digestion, which was 0·98g./100g. Papain-resistant human IgG prepared

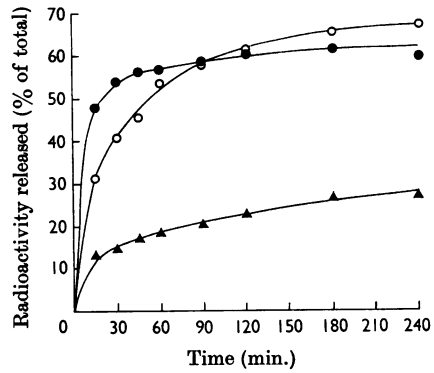

Fig. 1. Rate of release of ^{14}C-labelled peptides during papain digestion under the conditions described by Porter (1959) of IgG radioactively labelled at the 'hinge' disulphide bond by alkylation of mildly reduced molecules with iodo[^{14}C]acetate. Values are the percentages of the initial trichloroacetic acid-insoluble radioactivity that had become trichloroacetic acid-soluble in samples taken from the digestion mixtures at intervals. Details are given in the text. ●, Human IgG; ○, rabbit IgG; ▲, bovine IgG.

Table 2. *Undigested IgG remaining during digestion with papain*

Values are the weight of IgG of unchanged molecular weight as a percentage of that present at the start of incubation. Details are given in the text.

Incubation time (min.)	Wt. of undigested IgG (% of original wt.)		
	Human	Rabbit	Bovine
3	33·7	27·8	77·2
120	23·7	0·0	11·5
1440	18·7	0·0	10·5

Table 3. *Release of ^{14}C-labelled 'hinge' peptides during prolonged digestion of IgG with papain*

Values are the radioactivity soluble in 5% (w/v) trichloroacetic acid as a percentage of the total radioactivity present at the start of incubation. Details are given in the text.

Incubation time (hr.)	IgG ...	Acid-soluble radioactivity (% of original total)		
		Human	Rabbit	Bovine
30		70·6	65·7	72·3
69		69·5	66·4	73·7

in the same way contained 0·75g. of hexose/100g. of IgG compared with 1·01g./100g. in the starting material.

Rate of release of 'hinge' peptides during papain digestion. The rate of liberation of 'hinge' peptides was lowest from bovine IgG (Fig. 1). The three species of IgG released a maximum of 66–74% of the total radioactivity as trichloroacetic acid-soluble peptides during 30hr. incubation, and this proportion remained unchanged when the incubation was continued for a further 39hr. (Table 3).

DISCUSSION

The content and distribution of carbohydrate in normal pooled IgG represents the average of molecules of widely different composition; for example, the greater the electrophoretic mobility of IgG, the higher the hexose content, whereas hexosamine and fucose remain constant (see review by Press & Porter, 1966). To distinguish variations between species of IgG from differences due to heterogeneity within species the present work was carried out with IgG preparations that had limited and similar slow electrophoretic mobilities.

Carbohydrate is present in the rabbit IgG molecule in two locations. Fleischman *et al.* (1963) found that one-third was released in a glycopeptide during pepsin digestion, the rest being retained in the Fc fragment. Utsumi & Karush (1965) showed that the carbohydrate moiety of papain-digested rabbit IgG [fragment $F(ab')_2$] closely resembled the glycopeptide released during papain digestion, indicating that this glycopeptide was located at the junction of the Fab and Fc fragments, the flexible 'hinge' region of the intact molecule (Valentine & Green, 1967). The carbohydrate content of the fragments produced by papain digestion of human IgG (Franklin, 1960) suggests that it too contains carbohydrate in two locations. The present work confirmed the presence of hexose at two loci in both human and rabbit IgG and showed that hexose is present in the same two sites in bovine IgG: at the 'hinge' and in the Fc fragment. Table 1 shows that the amount of hexose in the $F(ab')_2$ fragment, which includes both the Fab fragment and the 'hinge' region, is approximately the same as the amount lost as 'hinge' peptides during the papain digestion. Thus significant amounts of carbohydrate could not have been present in the Fab part of most molecules, though some mouse and human myeloma proteins (and therefore presumably some normal IgG molecules) contain large amounts in this region (Melchers, Lennox & Facon, 1966; Abel, Spiegelberg & Grey, 1968). In agreement with earlier observations, one-third of the hexose of human and rabbit IgG was present in the 'hinge' region. An unexpected finding was that over one-half of the hexose of bovine IgG was present in this part of the molecule.

One of the carbohydrate moieties of human, rabbit and bovine IgG is linked to an aspartyl residue (Rosevear & Smith, 1961; Nolan & Smith, 1962*a*,*b*) in a sequence in the Fc fragment (Hill, Delaney, Lebovitz & Fellows, 1966) that is very similar in ten different mammalian species (Howell, Hood & Sanders, 1967). The glycopeptide isolated from bovine IgG by Nolan & Smith (1962*b*) contained 5 moles of hexose and 4 moles of hexosamine to 1 mole of aspartic acid, whereas the whole IgG contained about 10 moles of hexose and 15 moles of hexosamine. The presence of such an oligosaccharide unit on both γ-chains of all bovine IgG molecules would account for all the hexose but less than two-thirds of the hexosamine (Press & Porter, 1966). These results, taken in conjunction with the present finding of rather less hexose in the bovine Fc fragment than in the 'hinge' region, suggest either that less than half of the bovine IgG molecules carry two symmetrical aspartyl-linked oligosaccharide units in the Fc fragment or that most of them carry a single asymmetrical unit. The possibility of an asymmetrical distribution of the oligosaccharide in the second site, the 'hinge' region, in rabbit IgG was discussed by Smyth & Utsumi (1967).

Rabbit IgG was wholly susceptible to papain digestion in the presence of 0·01M-cysteine, but appreciable proportions of human IgG (19%) and bovine IgG (11%) were resistant even to prolonged digestion (Table 2), as observed by other authors (Franklin, 1960; Hsiao & Putnam, 1961; Payne, 1965; Murphy *et al.* 1965). Since the papain-resistant IgG contained somewhat less hexose than the starting material, it is unlikely that the lack of digestibility is directly related to carbohydrate content.

Comparison of Table 2 with Fig. 1 shows that the splitting by papain of susceptible IgG molecules of all three species, but particularly of bovine IgG, into Fab and Fc fragments proceeds considerably faster than the release of labelled 'hinge' peptides. This is in agreement with the conclusion of Inman & Nisonoff (1966) that the first locus of cleavage of the γ-chain of rabbit IgG is on the *N*-terminal side of the inter-γ-chain disulphide bridge, and the demonstration by Smyth & Utsumi (1967) of the production of 'hinge' peptides by further digestion of Fc fragments prepared by brief papain digestion.

A maximum of about 70% of the radioactive label was released in peptides during prolonged papain digestion of the 'hinge'-labelled IgG preparations (Table 3). Smyth & Utsumi (1967) found similarly that only 80% of the label was released from rabbit IgG. The data of Hong &

Nisonoff (1965) and Steiner & Porter (1967) suggest that significant labelling of disulphides between heavy and light chains and of intrachain disulphides is unlikely to have taken place under the conditions used here. The easily reduced cysteine that is not released during prolonged papain digestion is therefore presumably retained in resistant whole IgG molecules (except in the case of rabbit IgG) and in the *N*-termini of resistant Fc fragments.

Goodman (1965) found that rabbit IgG that had not been degraded after exposure to papain for 10min. contained almost twice as much hexose as the starting material, whereas the carbohydrate contents of Fc fragments derived from resistant molecules and the starting material were the same. The more slowly digested IgG molecules therefore contained more hexose in the 'hinge' region. The present work has shown that bovine IgG carries more 'hinge' carbohydrate than human and rabbit IgG and is degraded into Fab and Fc fragments more slowly. 'Hinge' peptides are also released more slowly from molecules of bovine IgG that have been cleaved into Fab and Fc fragments. Thus it may be concluded that carbohydrate in the 'hinge' region of IgG controls the rate, but not the occurrence, of the initial cleavage of γ-chains by papain to produce Fab and Fc fragments, and also the rate of release of hinge peptides from the *N*-termini of Fc fragments.

This work was supported by grants from the Arthritis and Rheumatism Council for Research and the U.S. Public Health Service. Part of the work was carried out in the Division of Biological and Medical Sciences, Brown University, Providence, R.I., U.S.A. I am grateful to Dr Julien Van Lancker for his generous provision of facilities and apparatus at Brown University.

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