Clin. exp. Immunol. (1972) **10,** 599–607.

THE CATABOLISM OF HUMAN γG IMMUNOGLOBULINS OF DIFFERENT HEAVY CHAIN SUBCLASSES

III. THE CATABOLISM OF HEAVY CHAIN DISEASE PROTEINS AND OF Fc FRAGMENTS OF MYELOMA PROTEINS

H. L. SPIEGELBERG AND B. G. FISHKIN

Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California, and Laboratory Service, Wadsworth General Hospital, Veterans Administration Center, Los Angeles, California

(*Received* 13 *July* 1971)

SUMMARY

The catabolism of ^{131}I and ^{125}I paired labelled Fc fragments of myeloma proteins and of H chain disease proteins of different heavy chain subclasses was studied in men and monkeys. In contrast to the previously demonstrated catabolic heterogeneity of intact γG immunoglobulins, the Fc fragments and H chain disease proteins of all subclasses tested were catabolized at a similar rate. These data suggest that structures not present on the Fc fragments are responsible for the faster turnover rate of γG_3 immunoglobulins and for the differences in half-lives of myeloma proteins within a given subclass.

The catabolic features of the H chain disease proteins differed from those of intact γG. Although the whole body half-lives of the two proteins were similar, the fractional turnover rate of the H chain disease proteins was higher than that of γG, on the average 8% of the intravascular pool/day as compared to 4% for γG. One-half to 1% of the intravascular pool of the H chain disease protein and less than 0·1% of the γG was excreted into the urine. An average of 24% of the H chain disease proteins and 44% of the γG equilibrated into the intravascular compartment.

INTRODUCTION

Human γG myeloma proteins of different heavy chain subclasses differ in their *in vivo* catabolic rates (Spiegelberg, Fishkin & Grey, 1968; Morell, Terry & Waldmann, 1970). On the average, γG_1, γG_2 and γG_4 myeloma proteins are catabolized at a similar rate, whereas γG_3 myeloma proteins are catabolized more rapidly. In addition, the catabolic rates of

Correspondence: Dr H. L. Spiegelberg, Department of Experimental Pathology, Scripps Clinic and Research Foundation, 476 Prospect Street, La Jolla, California 92037, U.S.A.

individual myeloma proteins differ within each given subclass (Spiegelberg *et al.*, 1968). The reason for the catabolic heterogeneity of human γG myeloma proteins is unknown. Since Fc fragments are catabolized slowly at a rate similar to that of intact γG, whilst Fab fragments are eliminated from the circulation and rapidly catabolized, the submolecular structure related to the catabolic rate of γG is primarily localized in the Fc fragment (Spiegelberg & Weigle, 1965, 1966). In order to determine if the catabolic heterogeneity of γG immunoglobulins reflects differences in the Fc fragment, Fc fragments of myeloma proteins and heavy (H) chain disease proteins of the four γG subclasses were studied. In addition, the fractional turnover rate of H chain disease proteins and the excretion into the urine were compared with those of normal γG.

MATERIALS AND METHODS

Proteins. Normal human γG was isolated from a pool of normal sera and γG myeloma proteins from the sera of patients with multiple myeloma, either by DEAE-cellulose chromatography or Pevikon block electrophoresis (Spiegelberg *et al.*, 1968). The H chain disease proteins were isolated from the urine of patients Cra (Franklin *et al.*, 1964) and Zuc (Ossermann & Takatsuki, 1963) and from the serum of patient Gif by a combination of DEAE-cellulose chromatography and Sephadex G-100 gel filtration (Spiegelberg & Weigle, 1966). Two preparations of the protein from patient Zuc were available for study, but one had a much faster turnover rate than the other. It was assumed that the rapidly catabolized protein preparation had been altered either during shipping or isolation, and it was therefore not studied further. Fc fragments were isolated from γG_1, γG_3 and γG_4 myeloma proteins following digestion of the proteins with papain for 5 min as previously described (Spiegelberg & Weigle, 1965). γG_2 myeloma proteins were not fragmented by this method and were therefore isolated following digestion with papain for 1 hr in the presence of 0·1 M β-mercaptoethanol (Grey & Kunkel, 1967). The heavy chain subclass of the myeloma proteins and of the H chain disease proteins were determined by double diffusion in agar using rabbit antisera specific for heavy chain subclasses (Spiegelberg & Weigle, 1968). Protein nitrogen determinations were performed using a modification of the micro-Kjeldahl technique, using a Technicon AutoAnalyzer (Ferrari, 1960).

Patients. Seven volunteers, hospitalized because of a neoplasm other than multiple myeloma, were injected with the trace labelled proteins. None of the patients had kidney disease when laboratory tested. One patient (Buttl) suffered from diabetes insipidus resulting from a metastasis of an oat cell carcinoma in the pituitary gland. Lugols solution was given daily to the patients beginning 3 days before injection of the iodinated proteins. The patients were stationed in a metabolic ward where complete 24-hr urine and stool collections were made.

Animals. Squirrel monkeys were obtained from Tarpon Zoo, Tarpon Springs, Florida. The monkeys were kept in metabolic cages and urine and stool samples were collected daily. Potassium iodide was added to the drinking water. As demonstrated previously, squirrel monkeys do not show an immune elimination of intravenously injected, aggregate-free human γG globulin, as indicated by the absence of a more rapid immune elimination of the labelled protein 8–14 days following injection (Spiegelberg & Grey, 1968).

Iodination of proteins. Either 2·5 or 5 mg aliquots of protein dissolved in 2 or 5 ml of phosphate buffer pH 7·0 were labelled with ^{131}I or ^{125}I, according to a procedure previously

described (Weigle & Dixon, 1959), or according to a modified chloramine T method (McConahey & Dixon, 1966), using 20 μg of chloramine T per mg of protein. No significant difference in half-lives of two aliquots of the same Fc fragment preparation (γG_1 Fc Mag, γG_3 Fc Nix) of two subclasses was observed when paired labelled with ^{131}I and ^{125}I by these two methods and injected into monkeys. 99% of the radioactivity of the γG and 95–97% of the Fc fragments and the H chain disease proteins were precipitable by addition of an equal volume of 20% trichloracetic acid (TCA). The radioactivity of both the ^{131}I and ^{125}I, as well as mixtures of the two isotopes, was determined using a dual channel scintillation counter (Baird Atomic, Inc., Cambridge, Mass.). The labelled preparations were centrifuged and sterilized by millipore filtration as previously described (Spiegelberg *et al.*, 1968).

Determination of turnover rates. The patients were injected intravenously with about 1 mg aliquots of two proteins, one labelled with 50–75 μCi of ^{131}I and the other with 25–40 μCi of ^{125}I. The first blood sample was drawn 10 min after injection from the arm opposite to the injected arm and subsequent samples were obtained three times per week. Blood was collected into tubes containing dried EDTA. Urine was collected in plastic containers, over a 24-hr period the volume measured and aliquots frozen at $-20°C$ before being analysed. The stools were collected over a 3-day period, diluted with water, homogenized and stored in the frozen state. 2 ml of plasma and 10 ml of urine were counted. In order to determine the protein bound radioactivity excreted into the urine, 1 ml of normal serum was added to 10 ml of urine as a carrier, and the protein was precipitated and washed at a concentration of 10% TCA. Plasma half-lives were determined from the slope of the semilogarithmic plot of the TCA precipitable radioactivity remaining in the plasma. The intra- and extravascular distribution of the proteins were extrapolated from the straight plasma elimination curve to the day of injection. The total body half-lives of the protein were obtained from the slope of the semilogarithmic plot of the radioactivity retained in the body (total injected radioactivity minus radioactivity recovered in the urine and stool per day). The fractional turnover rate (Waldmann & Strober, 1969) was calculated from the radioactivity excreted daily into the urine, expressed as per cent of the average intravascular pool in the same 24-hr period.

The difference in the plasma half-lives of the paired labelled proteins in monkeys was calculated as previously described: per cent difference $=(t_{\frac{1}{2}}$ long$-t_{\frac{1}{2}}$ short$)/t_{\frac{1}{2}}$ long $\times 100$ (Spiegelberg *et al.*, 1968). Only a single protein labelled with ^{131}I was injected into monkeys when the whole body half-lives were to be determined with a whole body counter.

RESULTS

Catabolism of Fc fragments of myeloma proteins in monkeys

The plasma half-lives of sixteen pairs of Fc fragments obtained from six γG_1, two γG_2, three γG_3 and two γG_4 myeloma proteins were studied in monkeys. The Fc fragments were isolated from myeloma proteins which had been shown previously to have either a relatively long or short half-life. The shape of the body elimination curve indicated that all preparations of Fc fragments of γG_1, γG_3 and γG_4 myeloma proteins consisted of at least two populations of molecules, one being relatively rapidly eliminated within the first few days following injection and the other persisting in the body over a longer period of time (Fig. 1). As previously reported, the quantity of the rapidly eliminated fraction increased after prolonged digestion with papain (Spiegelberg & Weigle, 1965). Extrapolation of the whole body elimination curves to the day of injection indicated that 15–25% of the Fc fragments obtained

from γG_1, γG_3 and γG_4 myeloma proteins were rapidly eliminated. The Fc fragments of the γG_2 myeloma proteins were all rapidly eliminated within the first 3 days following injection. As described under Methods, γG_2 myeloma proteins could only be fragmented by papain in the presence of 0·1 M β-mercaptoethanol for a relatively long period of 1 hr, which probably resulted in destruction of the sites responsible for maintaining the Fc fragments in the circulation. Because of the biphasic elimination curve of the γG_1, γG_2 and γG_4 Fc fragments, only the plasma half-life determined between days 4 and 14 following injection was studied and given in the tables. The differences in plasma half-lives of representative pairs of intact γG myeloma proteins and Fc fragments are shown in Table 1. In contrast to the intact

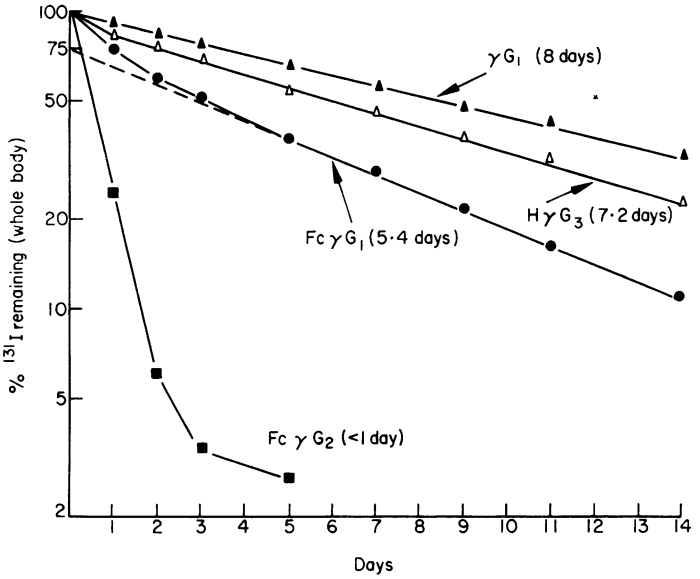

FIG. 1. Whole body elimination curves in monkeys of ^{131}I labelled myeloma protein γG_1 Mag (γG_1), γG_3 H chain disease protein Zuc (HγG_3), Fc fragment of γC_1 myeloma protein Mag (Fc γG_1) and of Fc fragment of γG_2 myeloma protein Sheff (Fc γG_2).

γG myeloma proteins, which had significantly different half-lives, the Fc fragments of the subclasses γG_1, γG_3 and γG_4 were all eliminated at very similar rates. The average plasma half-lives of the γG_1 and γG_4 myeloma proteins were 7·2 and 7·1 days, respectively, and those of the γG_3 myeloma proteins 5·1 days. The average half-life of all Fc fragments tested was 4·3 days. It is interesting to note that the half-life of the Fc fragment of the γG_3 protein Nix was longer (3·9 days) than that of the intact γG_3 protein Nix (2·9 days). When measured 6–12 days following injection, 0·1–0·6% of the intravascular pool of the intact γG and 2–3% of the intravascular pool of the Fc fragments were recovered in the urine as protein bound radioactivity. Only small and variable amounts of radioactivity were found in the stools, which were probably contaminated with urine.

Catabolism of H chain disease proteins in men and monkeys

The catabolism of three H chain disease proteins of subclasses γG_1 (Cra), γG_2 (Gif) and γG_3 (Zuc), which were paired labelled with ^{131}I and ^{125}I, was studied. As can be seen in

Table 2, the plasma half-lives, fractional turnover rates, amounts of protein bound radioactivity excreted into the urine, and the extent of intra- and extravascular equilibrium did not differ significantly in any of the three pairs of H chain disease proteins. In different patients the fractional turnover rates of the H chain disease proteins varied from 4·0 to 8·8%. Three-tenths to 0·7% of the intravascular pool of the H chain disease proteins was recovered in the urine as protein bound radioactivity. Less than 1% of the radioactivity excreted in the urine was recovered in the stool. The elimination from the circulation of the three pairs of H chain disease proteins was also studied in monkeys. In monkeys, as in man, no significant

TABLE 1. Plasma half-lives in monkeys of ^{131}I and ^{125}I paired labelled γG myeloma proteins and myeloma Fc fragments

Monkey	^{131}I protein	^{131}I $T_{\frac{1}{2}}$ (days)	^{125}I protein	^{125}I $T_{\frac{1}{2}}$ (days)	% difference
1	γG_1 (Mag)	6·1	γG_3 (Nix)	3·6	41·0
2	Fc γG_1 (Mag)	4·0	Fc γG_3 (Nix)	4·2	4·8
3	γG_1 (Ing)	4·5	γG_1 (Har)	8·9	49·5
4	Fc γG_1 (Ing)	3·6	Fc γG_1 (Har)	3·8	5·3
5	γG_4 (Heb)	8·2	γG_3 (She)	4·8	41·5
6	Fc γG_4 (Heb)	4·1	Fc γG_3 (She)	4·0	2·4
7	γG_3 (Nix)	2·9	Fc γG_3 (Nix)	3·9	25·6

TABLE 2. Turnover studies in man of ^{131}I and ^{125}I paired labelled H chain disease proteins of subclasses γG_1 (Gra), γG_2 (Gif) and γG_3 (Zuc)

Recipient	Injected protein	Plasma $T_{\frac{1}{2}}$	% of plasma pool		% intravascular
			Catabolized/day	Excreted/day	
Burg.	Cra	16·5	6·2	0·65	24
	Gif	16·0	6·4	0·7	24
Page.	Cra	13·2	8·8	0·8	24
	Zuc	13·2	8·5	0·8	24
Bare.	Cra	19·6	4·0	0·3	22
	Zuc	21·6	4·1	0·4	22

differences in the catabolic rates of these proteins was observed. In contrast to the Fc fragments, the H chain disease proteins were homogeneous, as shown by a straight, semi-logarithmic, whole body elimination curve (Fig. 1). The average fractional turnover rate of the H chain disease proteins in monkeys was 16%. One to 3% of the intravascular pool was recovered in the urine as protein bound radioactivity.

The catabolism of the H chain disease proteins was also studied paired with normal human γG. As can be seen in Table 3, the whole body half-lives of the γG and the H chain disease proteins were similar in four patients. In contrast, the fractional turnover rate, the excretion into the urine and the intra- and extravascular distribution differed for the two types of

proteins. The fractional turnover rate was on the average 8% for the H chain disease proteins and 4% for γG. 24 %of the H chain disease proteins remained in the intravascular compartment, as compared to 44% of the γG. One patient (Buttl) suffered from diabetes insipidus and excreted 6–7 litres of urine per day. Despite this fact, no more than 1·5% of the intravascular pool of the H chain disease proteins were recovered in the urine as protein bound radioactivity.

TABLE 3. Turnover studies in man of ^{131}I and ^{125}I paired labelled normal γG and H chain disease proteins of subclasses γG_1 (Cra), γG_2 (Gif) and γG_3 (Zuc)

Recipient	Injected protein	Whole body $T_{\frac{1}{2}}$	% of plasma pool		% intravascular
			Catabolized/day	Excreted/day	
Nuttl.	γG	26·5	5·0	<0·1	40
	Cra	26·0	9·0	0·4	22
Cart.	γG	28·0	5·3	<0·1	46
	Gif	27·0	7·0	0·75	28
Par.	γG	33·0	4·5	<0·1	42
	Zuc	28·0	10·5	0·35	28
Buttl.*	γG	35·0	6·0	0·2	37
	Gif	22·0	9·0	1·5	24

* Diabetes insipidus.

DISCUSSION

The present data demonstrate that Fc fragments and H chain disease proteins of different heavy chain subclasses are catabolized at a similar rate. This is in contrast to intact γG immunoglobulins. γG_3 myeloma proteins are catabolized more rapidly than myeloma proteins of the other three subclasses, and in addition, γG myeloma proteins differ in this parameter within each given subclass (Spiegelberg *et al.*, 1968). Previously the Fc fragment was shown to be responsible for persistence in the circulation and slow rate of metabolism of γG. In those studies normal γG and Fc fragments, which represent a mixture of the different subclasses, were analysed, and therefore the differences between the subclasses and individual myeloma patients could not be detected. The present data indicate that, though the Fc fragment is of major importance for determining the catabolic rate of γG immunoglobulins, the more subtle differences in turnover rates between the different subclasses and individual myeloma proteins are not the result of structural differences in the Fc fragment. These differences must be caused by additional sites within the molecule, probably on the Fab fragment. It is possible that a structure in the constant region of the Fd fragment is responsible for the more rapid catabolism of γG_3 immunoglobulins and that differences in the variable region are causing the catabolic heterogeneity of myeloma proteins within a given subclass. However, another structural characteristic, such as susceptibility to proteolysis, which has been shown to differ among subclasses (Gergely, Medgyesi & Stanworth, 1967), might also be responsible for the catabolic heterogeneity of γG immunoglobulins. In any event, apparently the submolecular structure related to the rate of catabolism of γG is complex and is not restricted to a particular site on the Fc fragment. This finding is

in contrast to other biological properties of γG. It has been shown that myeloma proteins of the various subclasses differ in their capacity to fix complement (Ishizaka *et al.*, 1967) and to induce passive cutaneous anaphylaxis in guinea-pigs (Terry, 1965). However, unlike the differences in the rate of catabolism, these differences were also found in the Fc fragment.

Although the whole body half-life of the H chain disease proteins was similar to that of γG, the catabolic features of the two proteins were quite different. The fractional turnover rate of the H chain disease proteins was almost twice as high as that of γG, and more of the H chain disease protein was excreted into urine. The comparable whole body half-lives, despite differences in fractional turnover rates, is probably explained by the unequal distribution between intra- and extravascular spaces of the two proteins. A larger portion of the H chain disease protein than of the γG equilibrated into the extravascular space. For normal γG, catabolism took place at a site having rapid equilibrium with the intravascular pool and little, if any, of the γG present in the extravascular space was catabolized (Anderson, 1964). The H chain disease proteins were probably catabolized at the same site as was γG, and since more of the H chain disease protein was in the extravascular compartment, the higher fractional turnover rate of the H chain disease protein did not result in a significantly faster whole body half-life. The quantity of H chain disease protein excreted into the urine as protein bound radioactivity was about 5% of the fractional turnover rate, indicating that catabolism rather than excretion into the urine was the major route of elimination of this protein. However, more H chain disease protein was excreted into the urine than intact γG. It is likely that greater quantities of the small H chain disease protein was filtered in the glomeruli and not all of it was reabsorbed into the serum. Some patients with H chain disease having apparently normal kidney function excreted a large amount of H chain disease protein into the urine: 5% of the intravascular pool in patient Cra (Franklin *et al.*, 1964). The high concentration of H chain disease protein in such patients may result in filtration of large quantities of H chain protein which no longer can be reabsorbed because of saturation of the reabsorption mechanism.

Recently, metabolic studies of a γG_3 H chain disease protein (JM) were reported by Ein & Waldmann (1969). These authors found a higher fractional turnover rate than the one observed for the γG_3 proteins Zuc in this study, and they attributed the high fractional turnover rate to the fact that γG_3 myeloma proteins were catabolized more rapidly than myeloma proteins of the other subclasses. Although the reason for the different fractional turnover rates of proteins JM and Zuc is not known, amino acid sequence and peptide analyses demonstrate that the two H chain disease proteins differ in the amino (N)-terminal structure. The amino acid sequence of the N-terminus of the protein Zuc is identical to that of normal γG_3 heavy chains (Prahl, 1967; Frangione & Milstein, 1969), whereas the protein JM lacks this structure (Ein, Buell & Fahey, 1969). The studies of the half-lives in monkeys showed that the Fc fragments were always more rapidly eliminated than the H chain disease proteins. The Fc fragments, like the γG_3 H chains disease protein JM, did not contain the N-terminal structure of the γ chains. It is likely, therefore, that the difference in the N-terminal structure was responsible for the disparate turnover rates of the proteins JM and Zuc. Whether the N-terminal structure of the γ chain is involved in the sites related to the catabolism of γG or merely protects the H chain disease proteins from a more rapidly occurring *in vivo* proteolysis is presently unknown.

Because of the remote possibility of transferring ontogenic viruses by injecting myeloma proteins and heavy chain disease proteins, the catabolic studies were made in patients

suffering from terminal neoplasias. Turnover studies of γG immunoglobulins in normal volunteers and in cancer patients suggest that chronically ill patients have a more rapid turnover than normal (Watkins & Tee, 1970). Therefore, the half-lives of the myeloma proteins and heavy chain disease proteins might be longer in normal individuals; the differences in catabolic rates, however, are most likely similar to those found in cancer patients.

ACKNOWLEDGMENTS

The authors thank Dr D. Feinstein, Dr E. C. Franklin and Dr E. F. Osserman for supplying the H chain disease proteins Gif, Cra and Zuc, and Mrs Solbritt Singer and Mr W. James Mills for excellent technical assistance.

This is publication No. 527 from the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California. The investigation was supported by Grants 70 710 from the American Heart Association, No. 531 Special Grant from the American Cancer Society, California Division, A1-07007 United States Public Health Service Grant and Veterans Administration. Dr Spiegelberg is a Faculty Research Associate of the American Cancer Society (PRA-38).

REFERENCES

ANDERSON, S.B. (1964) *Metabolism of Human Gamma Globulin*, p. 97. Davis, Philadelphia, Pa.

EIN, D., BUELL, D.N. & FAHEY, J.L. (1969) Biosynthetic and structural studies of a heavy chain disease protein. *J. clin. Invest.* **48,** 785.

EIN, D. & WALDMANN, T.A. (1969) Metabolic studies of a heavy chain disease protein. *J. Immunol.* **103,** 345.

FERRARI, A. (1960) Nitrogen determination by a continuous digestion and analysis system. *Ann. N.Y. Acad. Sci.* **87,** 782.

FRANGIONE, B. & MILSTEIN, C. (1969) Partial deletion in the heavy chain disease protein Zuc. *Nature (Lond.)*, **224,** 597.

FRANKLIN, E.C., LOWENSTEIN, J., BIGELOW, B. & MELTZER, M. (1964) Heavy chain disease—a new disorder of serum γ-globulins. *Amer. J. Med.* **37,** 332.

GERGELY, J., MEDGYESI, G.A. & STANWORTH, D.R. (1967) Structural studies of immunoglobulins. II. The varying susceptibility to papain-digestion of a group of human myeloma γ-globulins. *Immunochemistry*, **4,** 369.

GREY, H.M. & KUNKEL, H.G. (1967) Heavy chain subclasses of human γG-globulin. Peptide and immunochemical relationships. *Biochemistry*, **6,** 2326.

ISHIZAKA, T., ISHIZAKA, K., SALMON, S. & FUDENBERG, H. (1967) Biologic activities of aggregated γ-globulin. VIII. Aggregated immunoglobulins of different classes. *J. Immunol.* **99,** 82.

MCCONAHEY, P.J. & DIXON, F.J. (1966) A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy*, **29,** 185.

MORELL, A., TERRY, W.D. & WALDMANN, T.A. (1970) Metabolic properties of IgG subclasses in man. *J. clin. Invest.* **49,** 673.

OSSERMAN, E.F. & TAKATSUKI, K. (1963) Plasma cell myeloma: Gamma globulin synthesis and structure. *Medicine*, **42,** 357.

PRAHL, J.W. (1967) N- and C-terminal sequences of a heavy chain disease protein and its genetic implications. *Nature (Lond.)*, **215,** 1386.

SPIEGELBERG, H.L., FISHKIN, B.G. & GREY, H.M. (1968) Catabolism of human γG-immunoglobulins of different heavy chain subclasses. I. Catabolism of γG-myeloma proteins in man. *J. clin. Invest.* **47,** 2323.

SPIEGELBERG, H.L. & GREY, H.M. (1968) Catabolism of human γG immunoglobulins of different heavy chain subclasses. II. Catabolism of γG myeloma proteins in heterologous species. *J. Immunol.* **101,** 711.

SPIEGELBERG, H.L. & WEIGLE, W.O. (1965) The catabolism of homologous and heterologous 7S gamma globulin fragments. *J. exp. Med.* **121,** 323.

SPIEGELBERG, H.L. & WEIGLE, W.O. (1966) Studies on the catabolism of γG subunits and chains. *J. Immunol.* **95,** 1034.

SPIEGELBERG, H.L. & WEIGLE, W.O. (1968) The production of antisera to human γG subclasses in rabbits using immunologic unresponsiveness. *J. Immunol.* **101,** 377.

TERRY, W.D. (1965) Skin-sensitizing activity related to γ-polypeptide chain characteristics of human IgG. *J. Immunol.* **95,** 1041.

WALDMANN, T.A. & STROBER, W. (1969) Metabolism of immunoglobulins. *Progr. Allergy*, **13,** 1.

WATKINS, J.R. & TEE, D.E.H. (1970) Catabolism of γG-globulin and myeloma proteins of the subclasses γG_1 and γG_2 in a healthy volunteer. *Immunology*, **18,** 537.

WEIGLE, W.O. & DIXON, F.J. (1959) The antibody response of lymph node cells transferred to tolerant recipients. *J. Immunol.* **82,** 516.