Catabolism of Human γG-Immunoglobulins

of Different Heavy Chain Subclasses

I. CATABOLISM OF γG–MYELOMA PROTEINS IN MAN

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A B S T R A C T   The rates of catabolism of human γG-immunoglobulins of subclasses γG$_1$, γG$_2$, γG$_3$, and γG$_4$ were studied by determining the rates of elimination from the circulation of pairs of ^{131}I- and ^{125}I-labeled γG-myeloma proteins in 57 patients suffering from cancer other than multiple myeloma. On the average, γG$_1$-, γG$_2$-, and γG$_4$-myeloma proteins were catabolized at a rate similar to that of normal γG-immunoglobulin, whereas γG$_3$-myeloma proteins were catabolized more rapidly than normal γG-immunoglobulin. The average half-lives for the myeloma proteins were 12.3 days for normal γG, 11.6 days for γG$_1$, 12.4 days for γG$_2$, 8.2 days for γG$_3$, and 11.3 days for γG$_4$. However, significant differences in catabolic rates were observed when individual myeloma proteins of a single subclass were compared. These individual variations were present within all four heavy chain subclasses. The extent of differences ranged from 10 to 47%. The catabolic rate of normal γG was in an intermediate range when compared with myeloma proteins of relatively long and short half-lives. The rate of catabolism of an individual myeloma protein did not correlate with its light chain type, Gm factor, carbohydrate content, or electrophoretic mobility.

These findings indicate that the structure(s) related to the catabolism of γG-immunoglobulins are complex and differ from one immunoglobulin molecule to another.

INTRODUCTION

It has been shown by a number of investigators working with different species that immunoglobulins of different classes differ in their rates of in vivo catabolism. In man, γG-immunoglobulins are relatively slowly catabolized with a half-life of about 20 days (1, 2), whereas γA-, γM-, and γD-immunoglobulins are more rapidly catabolized with average half-lives of 6 (3), 5 (4), and 2.8 (5) days, respectively. Recently it has been demonstrated that human γG-immunoglobulins can be divided into four subclasses, γG$_1$, γG$_2$, γG$_3$, and γG$_4$, on the basis of antigenic differences in the heavy chain (6, 7).[1] These four subclasses differ also in certain biological properties. Terry reported that γG$_1$-, γG$_3$-, and γG$_4$-, but not γG$_2$-immunoglobulins induce a reverse passive cutaneous anaphylaxis in the guinea pig (8). In addition, the subclasses differ in their abilities to fix complement (C'). γG$_1$- and γG$_3$-immunoglobulins fix C' very well, whereas γG$_2$-immunoglobulins fix

[1] Nomenclature according to the Communication in the *J. Immunol.* **99:** 465. 1967. The γG–heavy chain subclasses have previously been called γG$_1$ = We = γ2B ; γG$_2$ = Ne = γ2a ; γG$_3$ = Vi = γ2c ; and γG$_4$= Ge = γ2d (6, 7).

less C' and γG_4-immunoglobulins probably do not fix C' at all (9, 10). The present experiments were undertaken to study the catabolic rate of human γG-immunoglobulins of different heavy chain subclasses. It has previously been demonstrated that the submolecular structure responsible for the persistence in the circulation and the slow rate of catabolism of γG-immunoglobulins is also located in the heavy chain (11–13). The catabolism of γG-myeloma proteins was studied since myeloma proteins are at the present time the only available source of relatively pure immunoglobulins of a particular heavy chain subclass. Because of the considerable variations in the rate of catabolism of γG-globulin in man, pairs of [131]I- and ^{125}I-labeled myeloma proteins were injected to determine differences in the rate of catabolism in the same individual.

METHODS

Myeloma proteins. Eight γG_1-, five γG_2-, four γG_3-, and three γG_4-myeloma proteins were isolated from the sera of patients with multiple myeloma either by Pevikon block electrophoresis (14) or by DEAE-cellulose chromatography (12). The sera were stored at $-20°C$ before the isolation of the myeloma proteins. Normal γG-immunoglobulin was isolated from the sera obtained from seven healthy donors by DEAE-cellulose chromatography using 0.01 M potassium phosphate buffer, pH 8.0 (12). The heavy chain subclass of the isolated myeloma proteins was determined by double diffusion in agar as previously described (6), using either monkey or rabbit antisera specific for the heavy chain subclasses. The light chain type of the myeloma proteins was similarly determined using rabbit antisera specific for human κ and λ light chains (15). The Gm factors of most of the myeloma proteins were kindly determined by Dr. S. Litwin and a few myeloma proteins according to the method described by Steinberg (16) using Gm reagents obtained from Hoechst, Inc., Cincinnati, Ohio. Starch gel electrophoresis was performed in 0.05 M glycine buffer, pH 8.8, using a discontinuous buffer system (17).

Patients. 57 patients, 55 men and 2 women (M. Y. and V. H.), hospitalized because of a neoplasm other than multiple myeloma and volunteering for this study, were injected with trace-labeled myeloma proteins. No clinical evidence of renal disease was evident in these patients, as revealed by laboratory tests, except for one patient who had a hypernephroma, but no proteinuria. The patients were given Lugol's solution 3 days before and during the study to inhibit incorporation of liberated radioactive iodine into the thyroid. The γG-serum concentration of the patients was determined by the radial immunodiffusion method (18) on three samples obtained on days 0, 12, and 21 using commercially available antibody-agar plates (Immunoplates, Hyland Laboratories,

Los Angeles, Calif.). The γG-serum concentrations given in the tables represent the average of three determinations.

Radioiodination. 2.5- or 5.0-mg aliquots of myeloma or normal γG-immunoglobulins dissolved in 2 or 5 ml 0.05 M phosphate buffer, pH 7.0, were labeled with either ^{131}I or ^{125}I according to a modification of the chloramine T method described by McConahey and Dixon (19), using 20 μg chloramine T/mg protein and an incubation period of 5 min before addition of the sodium metabisulfite. Some myeloma proteins were labeled using a different iodination procedure previously described (20). The specific radioactivity was usually about 50 μc ^{131}I and 25 μc ^{125}I/mg protein. 97–99% of the radioactivity was 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 the mixture of the two isotopes, was determined using a dual channel scintillation counter (Baird-Atomic, Inc., Cambridge, Mass.). The labeled myeloma proteins were kept at 4°C and were injected between 24 and 48 hr after labeling. The radioactively labeled myeloma proteins were routinely examined by radioautography. A small amount of the labeled myeloma proteins was added to the respective unlabeled myeloma proteins serving as carrier, and the mixtures were analyzed by radioimmunoelectrophoresis using a goat anti–whole human serum obtained from Hyland Laboratories, Los Angeles, Calif. The radioactivity was always localized in the precipitin arc characteristic of the myeloma protein. Neither fragmentation nor contamination of the myeloma proteins with serum proteins other than a trace amount of normal γG-immunoglobulin was demonstrated by this method. After the labeling procedure, we prepared mixtures of two myeloma proteins containing 50–75 μc ^{131}I and 25–40 μc ^{125}I/2 ml phosphate-buffered 0.15 M NaCl, pH 7.2. The mixtures were subsequently centrifuged in a Spinco model L ultracentrifuge at 40,000 rpm for 60 min using a Titanium 50 rotor equipped with 2-ml tube adapters. Ultracentrifugation was performed to remove any aggregates that might be present. After ultracentrifugation, the upper 1.5 ml was carefully aspirated into disposable plastic syringes. The myeloma proteins injected into patients were sterilized by Millipore filtration using 0.45 mμ filters.

Determination of differences in half-lives. The patients were injected intravenously with a mixture containing two myeloma proteins, one labeled with 50–75 μc ^{131}I and the other with 25–40 μc [125]I. 10 min after injection, the first blood sample was drawn from the opposite arm used for the injection. The blood was collected in tubes containing dried EDTA. Subsequent blood samples were usually obtained 5, 8, 12, 15, and 21 days after injection. Blood samples of the first six patients and of all patients injected with γG_3-myeloma proteins were obtained three times per week during the first 2 wk after injection. A single urine specimen was collected from all patients between 5 and 12 days after injection of the labeled myeloma proteins. Both plasma and urine samples were kept frozen at $-20°C$ for a maximum of 2 wk before being analyzed. The TCA-precipitable radio-

activity of 2 ml plasma was counted. The plasma sample obtained 10 min after injection was taken as the 100% value in order to calculate the per cent radioactivity remaining in the plasma after subsequent time intervals. The per cent ^{131}I and ^{125}I radioactivity in the plasma was plotted against time on semilogarithmic paper and the half-lives of both ^{131}I- and ^{125}I-labeled myeloma proteins were determined from the slope of the elimination curves following intra- and extravascular equilibration. The difference in the half-lives of two myeloma proteins in a patient was expressed as the per cent difference calculated by dividing the difference in half-lives by the longer half-life according to the formula: per cent difference = (t½ long − t½ short)/t½ long × 100. A typical protocol is shown in Fig. 1.

Chemical analysis. Hexoses were determined by the orcinol reaction as described by Svennerhölm (21) reducing the sample and reagent volumes to 1 and 2 ml, respectively. An equimolar mixture of galactose and mannose was used as standard in concentrations of 0.058–0.233 μmole/ml. Spectrophotometric readings were taken at 505 mμ. The sialic acid was determined by the thiobarbituric acid assay described by Warren (22). The protein preparations were first hydrolyzed in a final concentration of 0.1 N H_2SO_4 for 1 hr at 80°C. Protein nitrogen determinations were performed by a modification of the micro-Kjeldahl technique using an AutoAnalyzer (Technicon Co., Chauncey, N. Y.) (23).

RESULTS

In all, 57 patients were injected either with different pairs of ^{131}I- and ^{125}I-labeled myeloma proteins or with normal γG-immunoglobulin paired with myeloma proteins. Each patient received approximately 1 mg of each trace-labeled myeloma protein or normal γG-immunoglobulin. As can be seen in Table I, the average half-lives of γG₁-, γG₂-, and γG₄-myeloma proteins were similar with 11.6, 12.4, and 11.3 days, respectively, and did not differ significantly from the average half-life of
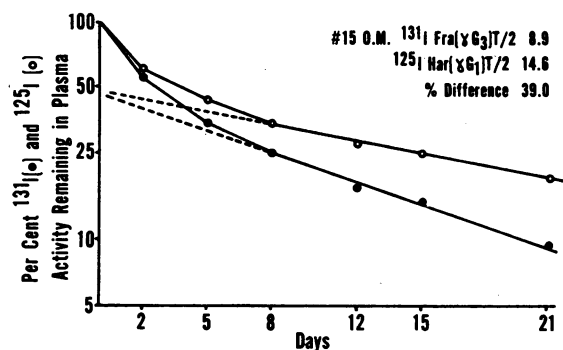

FIGURE 1 Plasma elimination curves of the ^{131}I trace-labeled γG₃-myeloma protein Fra and the ^{125}I trace-labeled myeloma protein Har in patient O. M.

TABLE I
*Average Half-Lives of Normal and Myeloma
γG-Immunoglobulins in Humans*

	Number of proteins	Number of patients	Average half-life
Normal γG		11	12.3 ± 3.4*
Myeloma γG₁	8	44	11.6
Myeloma γG₂	5	16	12.4
Myeloma γG₃	4	15	8.2 ± 3.2
Myeloma γG₄	3	10	11.3

* SD.

12.3 days for normal γG-immunoglobulin. In contrast, γG₃-myeloma proteins were more rapidly eliminated, with an average half-life of 8.2 days. As shown in Table II, individual myeloma proteins within a given subclass were heterogeneous with respect to their catabolic rates. Certain myeloma proteins were eliminated at a similar rate, whereas others differed significantly in their half-lives. The heterogeneity in the rate of catabolism of different myeloma proteins existed within all four heavy chain subclasses. In different patients, a particular myeloma protein consistently had either a relatively long, medium, or short half-life. Myeloma proteins having comparable rates of catabolism were present in the γG₁-, γG₂-, and γG₄- subclass, whereas all γG₃-myeloma proteins were eliminated faster than any of the γG₁-myeloma proteins examined. The differences in half-lives were expressed in per cent in order to better compare the result from one patient to that of another, since the absolute half-lives of a myeloma protein varied greatly among individual patients. Of 33 different pairs of myeloma proteins injected, 24 pairs differed significantly with differences varying between 10.4 and 49.5%, and 9 pairs differed only slightly or not at all. No difference in the elimination from the plasma of the ^{131}I and ^{125}I protein-bound radioactivity was observed in the control patient injected with the same doubly labeled myeloma protein. To study the consistency with which a particular pair of myeloma proteins was catabolized in different individuals, we injected patients with certain pairs of myeloma proteins using different labeled preparations and two different iodination procedures (Table III). Of the γG₁-myeloma protein pair Har and Cov, the myeloma protein Cov was always eliminated more rapidly than the myeloma protein Har. The per

TABLE II

Differences in Half-Lives of Human
γG-Myeloma Proteins in Humans

Patient	γG		t½		t½	Per cent difference
	g/100 ml	γG₁	*vs.*	γG₁		
S. J.	2.1	Ing	13.1	Den	13.1	0.0
R. L.	0.8	Car	7.2	Jac	7.6	5.3
W. W.	0.7	Cut	13.0	Cov	12.1	6.9
M. F.	0.5	Car	11.5	Den	10.3	10.4
C. G.	0.6	Car	10.0	Ing	8.6	14.0
W. S.	1.5	Car	9.8	Har	12.0	18.3
F. K.	1.8	Cov	7.2	Har	9.2	21.6
C. M.	0.6	Jon	18.4	Jac	14.0	24.0
		γG₂	*vs.*	γG₂		
M. H.	1.1	Tsc	23.0	Dah	23.0	0.0
M. Y.	0.8	Tsc	10.7	Lig	13.4	20.1
J. B.	1.2	Spa	10.2	Dah	7.7	24.6
L. B.	1.2	Lig	12.0	Dom	7.2	40.0
L. M.	0.3	Spa	12.8	Dom	6.8	46.9
		γG₃	*vs.*	γG₃		
T. C.	1.1	Fra	7.3	Fel	7.5	2.7
E. C.	0.7	Fra	10.0	Dep	9.7	3.0
V. H.	1.1	Fra	6.4	She	9.1	29.6
		γG₄	*vs.*	γG₄		
F. J.	1.1	Ger	9.2	Fer	9.7	5.2
M. P.	1.4	Ger	11.5	Heb	13.4	14.2
T. J.	2.5	Heb	6.7	Fer	5.3	20.9
		γG₁	*vs.*	γG₂		
J. R.	1.0	Car	9.8	Dah	10.2	3.9
H. H.	0.7	Har	19.5	Dah	17.0	12.8
L. E.	0.8	Cov	8.3	Dah	10.0	17.0
W. B.	3.5	Car	7.4	Lig	10.8	31.5
B. H.	1.1	Car	7.1	Spa	11.6	38.8
		γG₁	*vs.*	γG₃		
R. E.	0.7	Cov	9.2	Dep	7.8	15.2
J. A.	1.7	Cov	7.9	She	5.8	26.6
V. A.	1.0	Car	6.6	Dep	4.8	33.4
A. A.	1.2	Car	7.3	Fel	4.7	35.6
O. M.	1.7	Har	14.6	Fra	8.9	39.0
A. S.	1.1	Har	10.1	She	5.0	49.5
		γG₁	*vs.*	γG₄		
C. W.	1.3	Car	16.0	Heb	17.1	6.4
C. H.	1.5	Har	10.3	Heb	11.5	10.4
H. J.	1.5	Cov	9.2	Heb	11.4	19.3
		Control				
W. O.	1.3	Ing	15.8	Ing	15.8	0.0

cent difference was relatively constant, on the average 20.2% with a range of 15.9–27.5%, despite the fact that in this group of patients the absolute half-life of the two proteins varied greatly from one patient to the next. Similar differences in half-lives between γG_1- and/or γG_3-myeloma proteins existed when the proteins were labeled by a different iodination procedure.

To determine the relative rate of catabolism of normal γG-immunoglobulin, we compared the rate of elimination of normal γG-immunoglobulin with the elimination of a few selected γG-myeloma proteins. Furthermore, the elimination of γG-immunoglobulin obtained from one individual was compared with pooled γG-immunoglobulin obtained from six donors. As can be seen in Table IV, the γG-immunoglobulin obtained from a single individual was eliminated in two patients either at the same rate or at a very similar rate to that of pooled γG-immunoglobulin. In contrast, the half-lives of certain myeloma proteins differed significantly from the half-life of normal γG-immunoglobulin. A γG_1 (Har)-, a γG_2 (Spa)-, and a γG_4 (Heb)-myeloma protein, each having a relatively long half-life when compared with other myeloma proteins, were all more slowly eliminated than normal γG-immunoglobulin. One γG_1 (Car)-myeloma protein with a medium half-life was eliminated at the same rate as normal γG-immunoglobulin and one γG_1 (Cov)-myeloma protein with a relatively short half-life compared to other myeloma proteins was more rapidly eliminated than normal γG-immunoglobulin. The two γG_3-myeloma proteins examined were both eliminated more rapidly than normal γG-immunoglobulin.

The myeloma proteins were arbitrarily divided into three groups having either a relatively long, medium, or short half-life, in order to determine a possible correlation between half-life and known structural features of the myeloma proteins. As can be seen in Fig. 2, no correlation existed between rate of catabolism and light chain type, Gm factor, hexose content, sialic acid content, and electrophoretic mobility of the γG_1-myeloma proteins studied. Similar data were obtained for the γG_2-, γG_3-, and γG_4-myeloma proteins (Table V).

The fraction of the myeloma proteins remaining in the intravascular compartment was determined in all patients by extrapolating the plasma elimination curves after equilibration between intra- and extravascular spaces back to day 0 (Fig. 1). But for a single exception, the degree of intra- and extravascular equilibration of any pair of myeloma proteins injected never differed by more than 3%. On the average 44% of the injected myeloma proteins or of the normal γG-immunoglobulin remained in the intravascular compartment. The exception to this was a γG_3-myeloma protein (Fel). This protein was unique, in that it contained bound lipoprotein and it is possible that some globulin-lipoprotein complexes

TABLE III

Differences in Half-Lives of Pairs of Labeled Myeloma Proteins with Differently Labeled
Preparations and Different Iodination Procedures

Patient	γG	Expt. No.	[131]I protein	$t_{\frac{1}{2}}$	[125]I protein	$t_{\frac{1}{2}}$	Per cent difference
	g/100ml		γG$_1$	vs.	γG$_1$		
			Iodination method 1 (19)				
F. K.	1.8	1	Cov	7.2	Har	9.2	21.6
R. O.	1.2	2	Har	15.6	Cov	11.3	27.5
R. G.	0.4	3	Har	13.2	Cov	11.2	15.2
K. T.	1.9	3	Har	7.9	Cov	6.3	20.2
J. D.	0.7	3	Har	11.0	Cov	9.0	18.2
W. A.	1.2	3	Har	10.0	Cov	8.1	19.0
						Average:	20.2
			Iodination method 2 (20)				
			γG$_1$	vs.	γG$_1$		
R. F.	1.2	10	Har	17.8	Cov	13.8	22.5
W. B.	1.3	10	Har	21.7	Cov	14.9	31.4
			γG$_1$	vs.	γG$_3$		
C. H.	0.7	10	Cov	13.3	She	11.2	15.8
W. M.	1.5	10	Cov	17.7	She	15.6	11.9
A. G.	1.3	10	Cov	8.7	Fra	5.0	42.5
W. S.	1.2	10	Cov	10.0	Fra	6.3	37.0
T. B.	1.1	10	Har	10.6	Fra	5.5	48.1
C. Ha.	1.3	10	Har	19.8	She	15.6	21.2

might have been more rapidly eliminated from the circulation during the first few days after injection.

A single urine specimen obtained between 5 and 12 days after injection was analyzed from every patient for its content of both total and protein-bound radioactivity. No significant amount of protein-bound radioactivity was detected in the urine of any patient. A single stool specimen of two patients (T. B. and C. Ha., Table III) obtained 1 wk after injection of γG$_1$- and γG$_3$-myeloma proteins contained about 0.2% of the total injected radioactivity which was not precipitable with TCA.

The γG-serum concentration was determined in every patient in order to study the relationship between γG-serum concentration and half-life of

TABLE IV

Differences in Half-Lives between Normal and Myeloma γG-Proteins in Humans

Patient	γG		$t_{\frac{1}{2}}$		$t_{\frac{1}{2}}$	Per cent difference
	g/100 ml					
		γG Pool	vs.	*Individual γG*		
J. R.	1.1	γG Pool	12.2	Spi (γG)	12.2	0.0
R. B.	1.0	γG Pool	13.4	Spi (γG)	13.0	3.0
		Normal γG	vs.	*Myeloma proteins*		
W. M.	1.3	γG	10.7	Car (γG$_1$)	10.7	0.0
F. K.	1.8	γG	9.7	Spa (γG$_2$)	11.3	14.1
J. B.	0.5	γG	15.0	Heb (γG$_4$)	17.5	14.3
W. D.	0.6	γG	18.0	Cov (γG$_1$)	15.3	15.0
A. B.	0.7	γG	11.7	Har (γG$_1$)	15.0	22.0
J. B.	1.3	γG	12.9	She (γG$_3$)	9.5	26.4
C. W.	1.4	γG	11.7	Fra (γG$_3$)	7.7	34.2

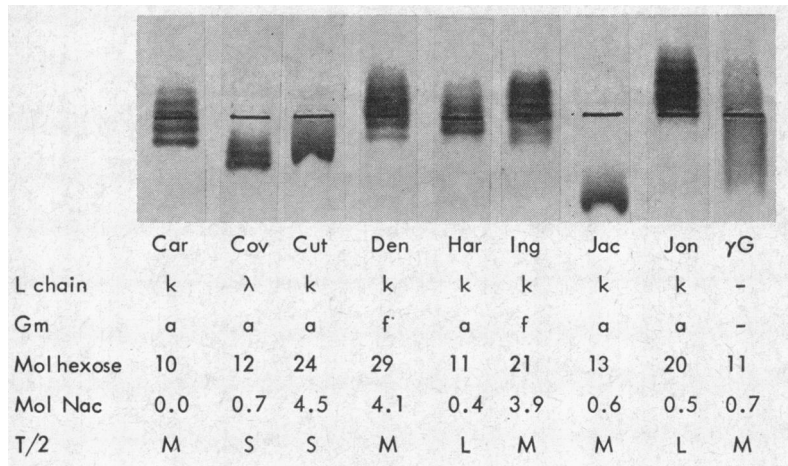|	Car	Cov	Cut	Den	Har	Ing	Jac	Jon	γG
L chain	k	λ	k	k	k	k	k	k	–
Gm	a	a	a	f	a	f	a	a	–
Mol hexose	10	12	24	29	11	21	13	20	11
Mol Nac	0.0	0.7	4.5	4.1	0.4	3.9	0.6	0.5	0.7
T/2	M	S	S	M	L	M	M	L	M

FIGURE 2 Electrophoretic mobility of a starch gel of pH 8.8, light chain type, Gm factor, hexose and sialic acid contents, and relative rates of catabolism of eight γG$_1$-myeloma proteins and normal γG-immunoglobulin. *L, M, or S:* relatively long medium, or short half-life of a myeloma protein.

either the myeloma proteins or normal γG-immunoglobulin. The γG concentration remained constant during the 21 days of the study in 45 patients, whereas it either increased or decreased significantly in four patients. This change, however, had no apparent effect on the plasma elimination curve of the injected proteins. The γG-serum concentrations varied in the 49 patients between 0.4 and 3.5 g/100 ml (Tables II, III, and IV). Comparison of the γG-concentration and half-life revealed a considerable variation and no good correlation between γG-concentration and half-lives.

DISCUSSION

The present data demonstrate that human γG-myeloma proteins are heterogeneous with respect to their catabolic rates. On the average, γG$_3$-

TABLE V
Summary of 12 γG-Myeloma Proteins of Subclasses
γG$_2$, γG$_3$, and γG$_4$ Studied

Myeloma protein	Heavy chain subclass	Light chain type	Mol/mol hexose	Half-life
Dah	γG$_2$	λ	28	Medium
Dom	γG$_2$	κ	33	Short
Lig	γG$_2$	λ	13	Long
Spa	γG$_2$	κ	9	Long
Tsc	γG$_2$	λ	12	Medium
Dep	γG$_3$	λ	Not tested	Short
Fel	γG$_3$	κ	28	Short
Fra	γG$_3$	κ	19	Short
She	γG$_3$	κ	13	Short
Fer	γG$_4$	κ	18	Short
Ger	γG$_4$	κ	25	Medium
Heb	γG$_4$ ·	λ	13	Long

myeloma proteins were more rapidly eliminated than γG$_1$-, γG$_2$-, and γG$_4$-myeloma proteins, which were catabolized at a rate similar to that of normal γG-immunoglobulin. In addition, when individual myeloma proteins within a single subclass were compared, significant differences in catabolic rates were observed. This heterogeneity was observed within all four heavy chain subclasses and the rate of catabolism also did not correlate with the light chain type, Gm factor, carbohydrate content, or electrophoretic mobility of the myeloma proteins.

That heterogeneity in the catabolic rate of human γG-myeloma proteins is significant and reflects a heterogeneity in the molecular structure rather than a nonspecific alteration of the myeloma proteins occurring during the isolation and labeling procedures is indicated by the following findings. (*a*) The differences in half-lives of a particular pair of myeloma proteins were repeatedly and similarly observed both in different patients and with different preparations of the same myeloma protein. (*b*) A particular myeloma protein when injected into different patients behaved consistently in that it always had either a relatively long, medium, or short half-life. (*c*) The differences in half-lives that existed between myeloma proteins in man were not observed when the same proteins were injected into certain heterologous species (24).

It has previously been demonstrated that the submolecular structure related to the rate of catabolism of γG-immunoglobulin is located in the Fc fragment (11, 12). The Fc fragment is eliminated from the circulation in a manner similar to

the intact γG-molecule, whereas the Fab and F(ab')$_2$ fragments are rapidly eliminated and catabolized (12). Peptide maps of Fc fragments of myeloma proteins belonging to the same heavy chain subclass are, however, very similar except for allotypic differences (25, 26), and do not suggest a heterogeneity in the amino acid sequence that could be responsible for the heterogeneity in the catabolism of myeloma proteins of the same heavy chain subclass. When Fc fragments of γG-myeloma proteins that differed from one another in their catabolic rates were injected into mice and monkeys, no significant differences in the rate of elimination of the Fc fragments were observed.[2] These findings suggest that a structure not present in the Fc fragment in some as yet undefined manner influences the rate of catabolism of γG-immunoglobulins.

The heterogeneity in the catabolic rates of individual myeloma proteins may be partially explained by the theory proposed by Brambell for the mechanism of γG-catabolism (27). This theory assumes that γG-immunoglobulins are pinocytosed at a constant rate and that a fraction of the pinocytosed γG attaches via a specific site to receptors which protect this fraction from proteolysis and subsequently return it to the circulation. The present data demonstrating different rates of catabolism of two γG-myeloma proteins in the same patient could be explained in at least two ways. (a) A heterogeneity of the site on the γG-molecule attaching to the protective receptor could allow certain molecules to compete better for the receptor and therefore they would be catabolized more slowly. (b) The site reacting with the receptor could be the same on each γG-molecule but differences in susceptibility to proteolysis from one species of molecules to another could result in different rates of catabolism, since an equilibrium between attached and unattached molecules would exist and the equilibrium would shift in favor of unattached molecules for those molecules being more rapidly digested.

The reason for the more rapid catabolism of γG$_3$-myeloma proteins is not known. It probably

does not reflect structural differences in the Fc fragment, since Fc fragments of γG$_3$-myeloma proteins are catabolized at a rate similar to that of γG$_1$-Fc fragments.[2] Other structural differences between γG$_3$ and those of the other subclasses might be responsible for the increased rate of catabolism of γG$_3$-myeloma proteins. It has been shown in vitro that γG$_3$-myeloma proteins are more susceptible to papain digestion than myeloma proteins of the other subclasses (28). As mentioned above, an increased susceptibility to proteolysis might result in a more rapid rate of catabolism of γG$_3$-immunoglobulins. Although the present experiments offer no evidence for it, an alteration of the γG$_3$-myeloma proteins could not definitely be excluded as a cause of the more rapid catabolism of γG$_3$-myeloma proteins.

A heterogeneity in the catabolic rate of mouse myeloma proteins similar to that observed in the present study for human myeloma proteins has been described by Fahey and Sell (29). Although these authors did not study the catabolism of paired labeled myeloma proteins, the average half-lives reported for individual mouse myeloma proteins suggest a catabolic heterogeneity within a given heavy chain subclass. In mice, γ$_{2b}$-myeloma proteins which share certain biological and chemical features with human γG$_3$-myeloma proteins [3] were, as were human γG$_3$-myeloma proteins, catabolized more rapidly than the other myeloma proteins.

The average half-lives of normal γG-immunoglobulin in the present study were shorter than those reported by other investigators for normal γG-immunoglobulin (1, 2). The explanation of this difference might lie in different catabolic rates of healthy individuals and chronically ill cancer patients and in technical differences. The longer half-lives have usually been obtained by measuring the retention of radioactivity in the body (1, 2). The serum concentration of most of the injected patients was either within normal limits or slightly decreased. No good correlation between the γG-serum concentration and the half-lives of both normal and myeloma γG-immunoglobulins could be demonstrated. These findings are in agreement with those of Solomon, Waldmann, and Fahey, who also observed a considerable variation in healthy subjects whose γG-concen-

[2] Spiegelberg, H. L., and B. G. Fishkin. The catabolism of human γG-immunoglobulins of different heavy chain subclasses. III. The catabolism of Fc fragments of γG-myeloma proteins and of heavy chain disease proteins. Manuscript in preparation.

[3] Grey, H. M. Unpublished observations.

tration was within normal limits, and only in patients with extremely high or low γG-serum concentrations could a definite correlation between γG-serum concentration and rate of γG-catabolism be demonstrated (2).

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REFERENCES

1. Cohen, S., and T. Freeman. 1960. Metabolic heterogeneity of human γ-globulin. *Biochem. J.* **76**: 475.
2. Solomon, A., T. A. Waldmann, and J. L. Fahey. 1963. Metabolism of normal 6.6S γ-globulin in normal subjects and in patients with macroglobulinemia and multiple myeloma. *J. Lab. Clin. Med.* **62**: 1.
3. Solomon, A., and T. B. Tomasi, Jr. 1964. Metabolism of IgA (β_2A) globulin. *Clin. Res.* **12**: 452. (Abstr.)
4. Barth, W. F., R. D. Wochner, T. A. Waldmann, and J. L. Fahey. 1964. Metabolism of human gamma macroglobulins. *J. Clin. Invest.* **43**: 1036.
5. Rogentine, G. N., Jr., D. S. Rowe, J. Bradley, T. A. Waldmann, and J. L. Fahey. 1966. Metabolism of human immunoglobulin D (IgD). *J. Clin. Invest.* **45**: 1467.
6. Grey, H. M., and H. G. Kunkel. 1964. H chain subgroups of myeloma proteins and normal 7S γ-globulin. *J. Exptl. Med.* **120**: 253.
7. Terry, W. D., and J. L. Fahey. 1964. Subclasses of human γ₂-globulin based on differences in the heavy polypeptide chains. *Science.* **146**: 400.
8. Terry, W. D. 1965. Skin-sensitizing activity related to γ-polypeptide chain characteristics of human IgG. *J. Immunol.* **95**: 1041.
9. Ishizaka, T., K. Ishizaka, S. Salmon, and H. Fudenberg. 1967. Biologic activities of aggregated γ-globulin. VIII. Aggregated immunoglobulins of different classes. *J. Immunol.* **99**: 82.
10. Müller-Eberhard, H. J., M. A. Calcott, and H. M. Grey. 1968. Interaction between C'1q and γG-globulins of different heavy chain subtypes. *J. Immunol.* In press.
11. Fahey, J. L., and A. G. Robinson. 1963. Factors con-trolling serum γ-globulin concentration. *J. Exptl. Med.* **118**: 845.
12. Spiegelberg, H. L., and W. O. Weigle. 1965. The catabolism of homologous and heterologous 7S gamma globulin fragments. *J. Exptl. Med.* **121**: 323.
13. Spiegelberg, H. L., and W. O. Weigle. 1965. Studies on the catabolism of γG subunits and chains. *J. Immunol.* **95**: 1034.
14. Müller-Eberhard, H. J. 1960. A new supporting medium for preparative electrophoresis. *Scand. J. Clin. Lab. Invest.* **12**: 33.
15. Fahey, J. L., and C. McLaughlin. 1963. Preparation of antisera specific for 6.6S γ-globulins, β₂A-globulins, γ₁-macroglobulins and for type I and II common γ-globulin determinants. *J. Immunol.* **91**: 484.
16. Steinberg, A. G., J. Wilson, and S. Ranset. 1962. A new human gamma globulin factor determined by an allele at the inv locus. *Vox Sanguinis.* **7**: 151.
17. Fahey, J. L. 1963. Heterogeneity of myeloma proteins. *J. Clin Invest.* **42**: 111.
18. Mancini, G., A. O. Carbonara, and J. F. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry.* **2**: 235.
19. McConahey, P. J., and F. J. Dixon. 1966. A method of trace iodination of proteins for immunologic studies. *Intern. Arch. Allergy Appl. Immunol.* **29**: 185.
20. Weigle, W. O., and Dixon, F. J. 1959. The antibody response of lymph node cells transferred to tolerant recipients. *J. Immunol.* **82**: 516.
21. Svennerholm, L. 1956. The quantitative estimation of cerebrosides in nervous tissue. *J. Neurochem.* **1**: 42.
22. Warren, L. 1959. The thiobarbituric acid assay of sialic acids. *J. Biol. Chem.* **234**: 1971.
23. Ferrari, A. 1960. Nitrogen determination by a continuous digestion and analysis system. *Ann. N. Y. Acad. Sci.* **87**: 792.
24. Spiegelberg, H. L., and Grey, H. M. The catabolism of human γG immunoglobulins of different H chain subclasses. II. The catabolism of γG myeloma proteins in heterologous species. *J. Immunol.* In press.
25. Frangione, B., E. C. Franklin, H. H. Fudenberg, and M. E. Koshland. 1966. Structural studies of human γG-myeloma proteins of different antigenic subgroups and genetic specificities. *J. Exptl. Med.* **124**: 715.
26. Grey, H. M., and H. G. Kunkel. 1967. Heavy-chain subclasses of human γG-globulins. Peptide and immunochemical relationships. *Biochemistry.* **6**: 2326.
27. Brambell, F. W. R. 1966. The transmission of immunity from mother to young and the catabolism of immunoglobulins. *Lancet.* **2**: 1087.
28. Takatsuki, K., and E. F. Osserman. 1964. Structural differences between two types of "heavy chain" disease proteins and myeloma globulins of corresponding types. *Science.* **145**: 499.
29. Fahey, J. L., and S. Sell. 1965. The immunoglobulins of mice. V. The metabolic (catabolic) properties of five immunoglobulin classes. *J. Exptl. Med.* **122**: 41.