THE CATABOLISM OF HOMOLOGOUS AND HETEROLOGOUS 7S GAMMA GLOBULIN FRAGMENTS*

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Digestion of 7S antibodies with papain and pepsin results in the formation of fragments which retain biological activity. It has been demonstrated by Porter that papain in the presence of cysteine splits the molecule into three fragments of about equal size with a sedimentation coefficient of 3.5S and a molecular weight of approximately 50,000 (1). Digestion of the 7S gamma globulin with pepsin has been shown by Nisonoff *et al.* to result in an active antibody fragment of a sedimentation coefficient of 5S and a molecular weight of 106,000 (2). The remaining part of the molecule is digested by pepsin into dialyzable polypeptides.

In the last few years a number of investigators have been able to localize certain biological features of the 7S gamma globulin in these subunits obtained by enzymatic digestion. Papain fragments I and II, also called the S (slow) fragments because of their slow electrophoretic mobility, each carry an antibody combining site (1). The pepsin fragment is composed of two antibody-combining sites linked by a disulfide bond which can be easily reduced to form two smaller fragments closely resembling the papain fragments I and II (2). The papain fragment III, or the F fragment (fast), does not interact with the antigen but is responsible for other biological functions of the antibody, such as complement fixation (3, 4), transmission to the fetal circulation (5), and fixation to the skin (6).

The antibody fragments are of great interest not only because they offer a means to study the submolecular structure of gamma globulin, but also because of their potential use as prophylactic and therapeutic agents in man. Pepsin-digested horse antitoxin has long been used for prophylactic treatment of tetanus and diphtheria (7). The prophylactic and therapeutic value of either the intact antibody or antibody fragments would depend on their persistence in the host. The present study has, therefore, been designed to determine the persistence in the circulation and the appearance in the urine of pepsin and papain

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fragments of homologous and heterologous 7S gamma globulin in rabbits, guinea pigs, and mice.

Materials and Methods

Animals.—New Zealand rabbits weighing 2.5 to 3.0 kg, Hartley strain guinea pigs weighing 250 to 350 gm, and Swiss Webster mice weighing 20 to 30 gm were used in the experiments. The animals were fed purina chow and water containing KI.

Isolation of 7S Gamma Globulin.—The globulin fractions of normal rabbit and guinea pig serum were precipitated and washed with 40 per cent saturated ammonium sulfate until the washing fluid was visibly free of hemoglobin. The precipitate was dissolved and dialyzed against 0.01 M potassium phosphate buffer pH 8.0. The globulin fraction was then applied to a DEAE cellulose column (selectacel standard, Brown Company, New Hampshire) previously equilibrated with 0.01 M phosphate buffer pH 8.0. The gamma globulin was eluted with the same buffer, concentrated by pressure dialysis and dialyzed against buffered saline pH 7.2 (0.14 M NaCl, 0.01 M sodium phosphate buffer pH 7.2). The rabbit gamma globulin (RGG) preparation formed a single precipitin line in the gamma 2 region when analyzed by immunoelectrophoresis (8) with a sheep anti-whole rabbit serum. The guinea pig gamma globulin (GpGG) formed a single precipitin line extending over the gamma 1 and gamma 2 regions when analyzed with a rabbit anti-whole guinea pig serum.

7S mouse gamma globulin (MGG) was prepared by zone electrophoresis on a Pevikon block (9) according to the method of Fahey and Askonas (10). The MGG formed a precipitin line extending over the gamma 1 and gamma 2 regions and the line separated into 2 lines (spur) at the cathodal end, indicating that the MGG preparation consisted of antigenically different types of 7S gamma globulin (11, 12). No contaminating gamma 1 M and gamma 1 A globulin could be detected.

Human gamma globulin (HGG) Cohn fraction II (E. R. Squibb and Sons, New York, lot 1895) was further purified by passage through a DEAE cellulose column equilibrated with 0.01 M phosphate buffer pH 8.0. In immunoelectrophoresis the HGG formed a single precipitin line in the gamma 2 region.

Horse gamma globulin (HoGG) Cohn fraction II (lot 9, Pentex, Inc., Kankakee, Illinois) was purified by DEAE cellulose chromatography using a 0.005 M phosphate buffer pH 8.0. As shown by immunoelectrophoresis, most of the fast migrating T globulin in the commercial preparation was removed by this procedure, however, a small amount remained.

Bovine gamma globulin (BGG) (lot W30512, Armour Pharmaceutical Co., Kankakee, Illinois) was purified by passage through a DEAE cellulose column equilibrated with 0.01 M phosphate buffer pH 8.0. The BGG formed a single precipitin line when analyzed by immunoelectrophoresis with a rabbit anti-whole bovine serum.

All gamma globulin preparations were stored in buffered saline at −20°C. Protein nitrogen determinations were performed according to a modified microKjeldahl technique using a Technicon autoanalyzer (13).

Preparation of Antisera.—Anti-bovine serum albumin (BSA) was obtained from hyperimmunized rabbits given several series of injections of soluble BSA, each series totaling 300 mg. The sera were obtained 7 days after the last injection. Sera from approximately 15 rabbits were pooled. Rabbit anti-*Shigella flexneri* sera were obtained from rabbits immunized with 10 mg lyophilized bacteria incorporated into incomplete Freund's adjuvant. The rabbits were bled 5 weeks after injection and their sera pooled. The 7S gamma globulin of these antisera was prepared as described above for the RGG preparation.

Papain Digestion.—Digestion with papain of 7S gamma globulin of all species was performed according to the method of Porter (1). Gamma globulin was digested in the presence of 0.01 M cysteine and 0.002 M EDTA-tetrasodium salt at a concentration of 10 mg protein

per ml buffered saline pH 7.0 (equal parts of 0.15 M NaCl and 0.15 M sodium phosphate buffer pH 7.0). One mg of two times crystallized papain (Worthington Biochemical Corp., Freehold, New Jersey) was added per 100 mg protein and the digestion permitted to proceed for 18 hours at 37°C. When shorter incubation periods with papain were used, the gamma globulin was first incubated for 2 hours a 37°C with 0.01 M cysteine and 0.002 M EDTA (14). The papain was inactivated after shorter digestion periods by cooling the reaction mixture in a 0°C waterbath immediately followed by removal of the cysteine by passage through a sephadex G-25 column equilibrated with buffered saline pH 7.0.

Isolation of the Papain Fragments.—Crystalline fragment III of RGG was obtained by dialyzing papain digests of RGG against buffered saline pH 7.2. The crystals were removed by centrifugation and washed twice with cold buffered saline. The supernatant containing fragments I and II and some soluble fragment III was dialyzed against 0.01 M acetate buffer pH 5.5 and the fragments isolated by gradient chromatography on carboxymethyl cellulose (CM) according to Porter (1). Fragment I was eluted with the solvent front. The second protein peak eluted from the column representing fragment II was slightly contaminated with the immediately following eluted fragment III. To avoid this contamination, only the fractions representing the ascending portion of the protein peak II were used. Both fragments I and II were concentrated by pressure dialysis and dialyzed against buffered saline. The fragments were analyzed by immunoelectrophoresis with a sheep anti-rabbit globulin serum. In contrast to all the other gamma globulins studied, RGG fragments I and II had a faster electrophoretic mobility and fragment III had a slower mobility than RGG.[1]

The fragments obtained by papain digestion of HGG were separated according to Franklin by DEAE cellulose and CM cellulose gradient column chromatography (15). No crystallization of fragment III was observed after dialysis against 0.01 M phosphate buffer pH 8.0. However, crystallization of fragment III could be achieved by increasing the protein concentration. One hundred mg HGG per ml buffered saline pH 7.0 containing 0.01 M cysteine and 0.002 M EDTA were incubated at 37°C for 2 hours. One mg papain was added per 100 mg protein and the mixture incubated at 37°C. After 5 minutes the mixture was cooled to 0°C in a water bath and dialyzed against cold 0.01 M phosphate buffer pH 8.0 at 4°C. Crystals which formed after dialysis overnight were centrifuged and washed twice with 0.01 M phosphate buffer pH 8.0. Analysis by immunoelectrophoresis of the dissolved crystals in buffered saline showed a single precipitin line with a faster electrophoretic mobility than HGG.

Fragments obtained following digestion of MGG with papain were separated as described by Fahey and Askonas by gradient column chromatography on DEAE cellulose using 0.01 M phosphate buffer as the starting buffer and 0.3 M phosphate buffer pH 8.0 as the end buffer (10). The S papain fragment was eluted with the solvent front and two protein peaks eluted later contained F fragments of different fast electrophoretic mobilities corresponding to the fast and slow moving MGG. The F papain fragment with the slower mobility representing the major protein peak was used in the present study. MGG was only partly digested after incubation for 5 minutes with papain, the undigested MGG was eluted with the S fragment and no contamination of the F fragment with undigested MGG could be demonstrated.

The fragments obtained after papain digestion of GpGG were isolated by DEAE cellulose column chromatography using the same gradient elution procedure as described for the MGG. The S papain fragment was eluted with the solvent front and two protein peaks were eluted later, representing F fragments of different fast electrophoretic mobilities corresponding to the fast and slow moving 7S GpGG (16). The F fragment with the slower electrophoretic mobility representing the major protein peak was used in the present study. As with MGG, GpGG was only partly digested after incubation for 5 minutes with papain. The undigested

[1] Papain fragments I and II have also been designated A and C or the S (slow) fragments. Papain fragment III has also been designated fragment B or F (fast) fragment.

GpGG was eluted with the S fragment and no contamination of the F fragment with GpGG could be demonstrated.

All fragments were concentrated by pressure dialysis, dialyzed against buffered saline pH 7.2, and stored at 4°C.

Pepsin Digestion.—The 7S gamma globulin of all species examined was digested with pepsin according to the method of Nisonoff *et al.* (2). The gamma globulin was dialyzed against 0.1 M acetate buffer pH 4.0 in a concentration of 10 mg protein per ml. Three mg of two times crystallized pepsin (Worthington Biochemical Corp., Freehold, New Jersey) were added per 100 mg protein and the digestion proceeded for 18 hours at 37°C. The pH was then raised to 8.0 with 1.0 N NaOH and the pepsin fragment precipitated by adding slowly 25 per cent Na_2SO_4 to a final concentration of 18 per cent Na_2SO_4. The precipitate formed was centrifuged at room temperature, dissolved in distilled water, and dialyzed against buffered saline. Examination of the pepsin fragment in the Spinco model E analytical ultracentrifuge revealed a single peak with a sedimentation coefficient of approximately 5S.

The pepsin fragment of RGG was reduced by incubation with 0.01 M cysteine for 2 hours at 37°C, followed by dialysis against 0.01 M iodoacetate in buffered saline and buffered saline alone.

Iodination of the Gamma Globulin and the Gamma Globulin Fragments.—Five mg portions of gamma globulin or gamma globulin fragments were labeled at pH 7.0 with I^{131} (I*) according to a modified method (17) of Hunter and Greenwood (18). Only 1.5 mg of the RGG papain fragment III were labeled because of its low solubility at neutral pH. Ninety-eight to 99 per cent of the radioactivity of all preparations was protein bound as determined by trichloracetic acid (TCA) precipitation. All of the gamma globulin fragments were labeled within 3 to 5 days after isolation.

Determination of the Half-Lives of Gamma Globulin and Gamma Globulin Fragments.—Rabbits were injected with 1.0 ml buffered saline containing approximately 1 mg I*-labeled protein. Three ml of blood were obtained by bleeding from the ear vein or by cardiac puncture and the blood collected in tubes containing dried oxalate and heparin. The TCA precipitate from 0.5 ml plasma was counted in a well-type scintillation counter containing an NaI crystal. The counts were converted to per cent injected protein remaining in the plasma using an arbitrary plasma volume of 39.1 ml per kg bodyweight. Guinea pigs were injected by cardiac puncture with 0.5 ml buffered saline containing approximately 0.5 mg I*-labeled protein. Twenty-five hundredths ml blood was obtained from the retroorbital venous plexus with a calibrated heparinized pipette. The cells were lysed in 1 ml water and the TCA-precipitable fraction counted. Mice were injected intravenously with 0.2 ml saline containing approximately 0.2 mg I*-labeled protein. Five hundredths ml blood was obtained with a calibrated pipette from the retroorbital venous plexus, the cells lysed in 0.5 ml water and the TCA-precipitable fraction counted. The counts were converted to per cent injected radioactivity (protein-bound) remaining in the plasma using an arbitrary blood volume of 8 per cent and a hematocrit of 40 per cent for both guinea pigs and mice. All counts were corrected for decay.

The remaining radioactivity in the plasma was plotted against time and the half-life determined graphically from the slope of the semilogarithmic curve after initial equilibration between intra- and extravascular spaces. The values given in the tables represent average values from 3 to 11 animals.

Measurement of the I Activity in the Urine.*—The animals were kept for 3 days following injection of the I*-labeled proteins in metabolic cages and the urine was collected daily. Urine samples were centrifuged and the radioactivity of 1 ml aliquots counted and the total excreted I* activity calculated. In order to determine the protein-bound activity excreted, 0.1 ml of normal rabbit serum was added to 1 ml urine and the protein precipitated with 1 ml of 20 per cent TCA. The precipitate was centrifuged and washed with 2 ml of 20 per cent TCA. The washed precipitate was counted and the counts converted to per cent of total in-

jected radioactivity. The I* activity of the excreted gamma globulin and gamma globulin fragments precipitable with TCA will be referred to as protein-bound radioactivity, although TCA will precipitate relatively large polypeptides as well as intact proteins.

Analysis of Antisera.—The rabbit anti-BSA gamma globulin and its pepsin and papain digests were analyzed by the ammonium sulfate technique for antibody activity (19). The pepsin-digested anti-BSA was dialyzed against saline and not purified by Na_2SO_4 precipitation. Comparing equal volumes of undigested and digested anti-BSA gamma globulin, 54 per cent of the anti-BSA activity was recovered in the pepsin digest. No activity was recovered in either the papain digest or a preparation of cysteine reduced pepsin digest. Agglutinating antibodies to *Shigella* were measured according to Harris *et al.* (20). Pepsin digestion of the anti-*Shigella* gamma globulin resulted in a twofold dilution drop in titer.

RESULTS

Elimination of RGG and RGG Fragments in Rabbits.—The elimination curves of RGG and RGG fragments obtained after digestion for 18 hours with pepsin and papain are shown in Fig. 1, and the respective half-lives are summarized in

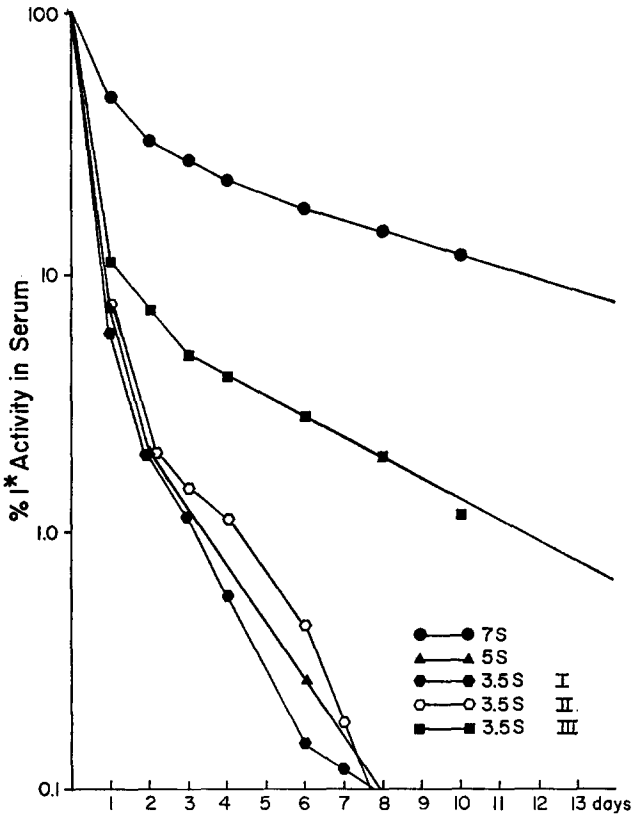

FIG. 1. Elimination of RGG and RGG fragments (isolated after digestion for 18 hours) from the circulation of rabbits.

Table I. Approximately 90 per cent of all fragments were eliminated from the circulation within 24 hours after injection. Rapid elimination of the pepsin fragment and the papain fragments I and II continued until day 3, then the elimination became slower when the plasma concentration fell below 1 per cent of the injected radioactivity. In contrast, the portion of fragment III remaining

TABLE I

*Half-Lives of Different Gamma Globulins and Gamma Globulin Fragments**

Animal	Source of gamma globulin	7S gamma globulin average (range)	Gamma globulin fragments		
			Pepsin 5S	Papain 3.5S	
				I (S)	III (F)‡ average (range)
Rabbit	Rabbit	6.0 (4.5–7.5)	<0.5	<0.5	4.7 (4.1–5.6)
Guinea pig	Rabbit	4.3 (4.0–4.7)	<0.5	<0.5	4.0 (3.9–4.1)
Mouse	Rabbit	5.7 (5.3–6.4)	<0.5	<0.5	4.4 (3.6–5.9)
Guinea pig	Guinea pig	4.2 (3.4–5.0)	<0.5	<0.5	2.2 (1.7–2.5)
Rabbit	Guinea pig	3.3 (2.9–3.7)	<0.5	<0.5	2.1 (1.6–2.5)
Mouse	Guinea pig	4.9 (4.6–5.2)	<0.5	<0.5	2.2 (2.0–2.4)
Mouse	Mouse	4.0 (3.0–5.2)	<0.5	<0.5	1.0 (0.8–1.1)
Rabbit	Mouse	1.5 (1.2–1.7)	<0.5	<0.5	1.4 (1.3–1.5)
Guinea pig	Mouse	2.3 (2.2–2.5)	<0.5	<0.5	1.5 (1.4–1.7)
Rabbit	Human	5.0 (3.7–5.8)	<0.5	<0.5	3.8 (2.6–4.9)
Guinea pig	Human	3.8 (3.7–3.9)	<0.5	<0.5	3.1 (3.0–3.4)
Mouse	Human	4.5 (4.2–4.9)	<0.5	<0.5	2.9 (2.6–3.6)
Rabbit	Horse	1.7 (1.6–1.8)	<0.5	—	—
Rabbit	Bovine	1.6 (1.4–1.8)	<0.5	—	—

* Half-life given in days.

‡ Half-life of F papain fragment (III) obtained after 5 minutes digestion with papain.

in circulation after 24 hours showed a rate of elimination comparable to that of the intact RGG. Exact half-lives for the pepsin fragment and the papain fragment I could not be determined since no definite equilibration was observed. Persistence of a significant amount of the pepsin fragment and the papain fragment I in the extravascular space was excluded by whole body counting of some rabbits and by the recovery in the urine of most of the injected radioactivity within 3 days after injection.

Pepsin and papain fragments obtained from RGG which were labeled with I* before digestion were removed from the circulation in the same manner as the fragments labeled with I* after digestion. Univalent fragments obtained by

reduction of the pepsin fragment with cysteine were eliminated in the same manner as the papain fragments I and II and the divalent pepsin fragment.

The RGG papain fragment I, isolated after digestion for 5 minutes, was eliminated from the circulation identical with fragment I obtained after 18 hours of

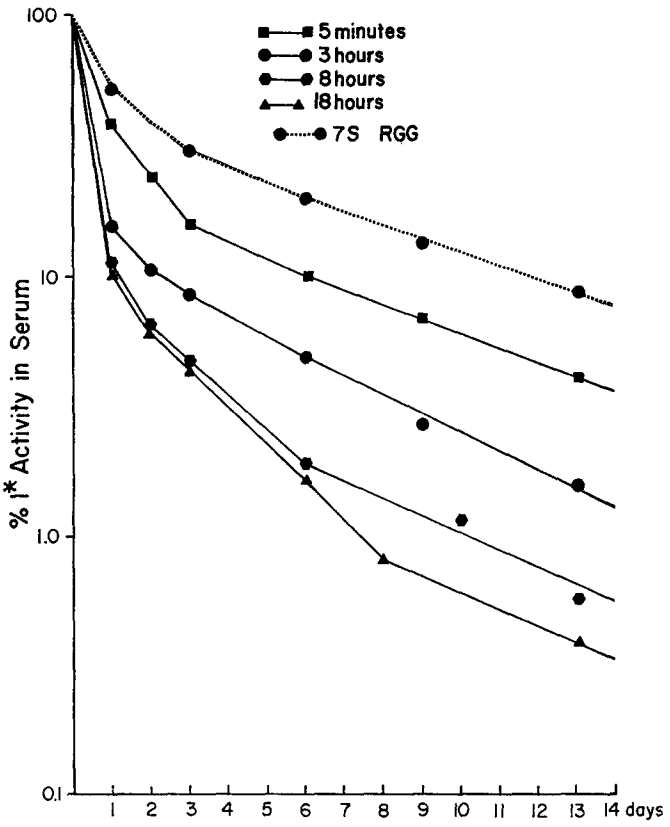

FIG. 2. Elimination of RGG and RGG papain fragment III (isolated after digestion for 5 minutes, 3, 8, and 18 hours) from the circulation of rabbits.

digestion. No detectable undigested RGG was present in these preparations of fragment I as shown by immunoelectrophoresis and elimination from the circulation. In contrast to fragment I, the elimination of fragment III depended on the duration of digestion (Fig. 2). The portion eliminated within the first 24 hours after injection was significantly less after shorter digestion periods. The elimination curve of fragment III isolated after digestion for 5 minutes was close to the elimination of the intact RGG. It was apparent that a progressive alteration of fragment III occurred as the period of digestion was extended.

In order to determine if the cysteine was responsible for the alteration of fragment III during prolonged digestion, a portion of fragment III obtained after digestion for 5 minutes was further incubated for 18 hours at 37°C with 0.01 M cysteine at pH 7.0 and another portion of the same preparation was similarly incubated with buffered saline pH 7.0. After incubation the cysteine was removed by dialysis and both preparations were labeled with I*. Both preparations were injected intravenously into rabbits and their elimination from the blood followed. The fragment III incubated in buffered saline was eliminated at the same rate as the preparation of fragment III only digested for 5 minutes, whereas the portion incubated with cysteine was eliminated in the same manner as fragment III obtained after digestion for 18 hours. The I* uptake of the preparation incubated with cysteine was approximately twice as high as that of the preparation incubated without cysteine. In contrast to papain fragment III, no significant difference was found between either the elimination from the circulation or the I* uptake of native RGG and native RGG treated with 0.01 M cysteine.

The recovery of the radioactivity in the urine during the first 3 days following injection of the RGG and RGG fragments is summarized in Table II. Most of the radioactivity of RGG and RGG fragment III excreted in the urine was recovered in the form of non–protein-bound I* activity. In contrast, 25 per cent of the pepsin fragment and 21.7 per cent of the papain fragment I were excreted as protein-bound radioactivity. With all preparations, approximately 90 per cent of the protein-bound I* activity was excreted within 24 hours following injection.

Elimination of 7S and 5S Anti-BSA and Anti-Shigella Antibodies in Rabbits.— The elimination of 7S and 5S anti-BSA and anti-*Shigella* antibodies was compared with the elimination of the respective I*-labeled proteins to test for possible *in vivo* reduction of the 5S pepsin fragment. The ammonium sulfate technique was chosen to determine the anti-BSA activity because the divalent pepsin fragment but not the univalent fragment gave a positive result and because of the great sensitivity of the test system. The elimination of the 7S and 5S anti-BSA activities are shown in Fig. 3. The 7S and 5S anti-BSA activities were eliminated at the same rate as the corresponding I*-labeled proteins. The rate of elimination of the 7S and 5S anti-*Shigella* activities from the circulation of rabbits was also similar to that of the corresponding I*-labeled proteins.

The recovery of the 5S antibody activity in the urine 24 hours after injection was significantly less than the recovery of protein-bound I* activity. Only 2 per cent (average of 3 animals) of the injected anti-BSA activity was detected in the urine, whereas 20 per cent of the injected I* activity was recovered as protein-bound radioactivity (Fig. 3). The concentrated protein of none of the urines of 5 rabbits injected with the pepsin fragment of the anti-*Shigella* gamma globulin contained agglutinins.

TABLE II

Per Cent Radioactivity Excreted in Urine during the First 3 Days following Injections of Gamma Globulin and Gamma Globulin Fragments‡

Animals	Source of gamma globulin	7S gamma globulin		Gamma globulin fragments						Duration of digestion
				Pepsin 5S		Papain 3.5S				
						I (S)		III (F)		
		Total I*	Protein I*	Total I*	Protein I*	Total I*	Protein I*	Total I*	Protein I*	
Rabbit	Rabbit	43.3	0.6	89.5	25.0	80.4	21.7	68.1	1.9	18 hrs.
						77.3	13.0	52.5	2.1	5 min.
Guinea pig	Rabbit	31.3	2.3	86.5	35.2	77.5	46.2	74.3	11.9	18 hrs.
						87.8	41.6	56.9	10.7	5 min.
Mouse	Rabbit	26.0	0.7	79.2	15.1	90.8	33.3	57.6	6.2	18 hrs.
						64.0	17.2	58.7	3.8	5 min.
Guinea pig	Guinea pig	50.2	2.8	85.7	9.3	91.7	28.0	85.9	14.8	18 hrs.
								61.0	12.0	5 min.
Rabbit	Guinea pig	47.8	1.8	82.3	4.3	95.0	13.9	84.3	6.7	18 hrs.
								69.9	4.3	5 min.
Mouse	Guinea pig	37.1	1.1	80.5	4.2	80.9	3.7	79.2	3.6	18 hrs.
								65.1	2.2	5 min.
Mouse	Mouse	45.5	1.9	77.7	2.9	85.6	9.7	67.9	2.2	18 hrs.
Rabbit	Mouse	69.9	1.6	78.5	4.3	72.8	18.2	66.5	3.5	18 hrs.
Guinea pig	Mouse	66.3	2.0	89.7	3.9	83.2	25.9	81.3	5.5	18 hrs.
Rabbit	Human	42.2	0.5	83.9	2.5	96.9	3.6	72.7	8.9	18 hrs.
								66.5	6.6	5 min.
Guinea pig	Human	29.9	0.8	91.9	5.4	94.9	35.3	96.8	18.8	18 hrs.
								39.7	6.0	5 min.
Mouse	Human	40.9	0.9	97.1	2.8	84.8	6.7	86.5	10.3	18 hrs.
								52.6	9.9	5 min.

Total I*, total recovered I^{131} activity

Protein I*, total TCA precipitable recovered I^{131} activity.

‡ Average of 3 to 6 animals.

Elimination of RGG and RGG Fragments in Guinea Pigs and Mice.—The half-lives of RGG and RGG fragments in guinea pigs and mice are summarized in Table I. The pepsin fragment and the papain fragment I were rapidly eliminated. The papain fragment III obtained after digestion for 5 minutes showed an elimination similar to that of untreated RGG.

The recovery of the radioactivity in the urine is summarized in Table II. As observed with rabbits, a significant amount of the pepsin fragment and the papain fragment I was excreted as protein-bound I* activity.

Elimination of GpGG and GpGG Fragments in Guinea Pigs, Rabbits, and Mice.—The half-lives of the GpGG and the GpGG fragments in guinea pigs, rabbits, and mice are summarized in Table I. The F fragment isolated after digestion for 18 hours was eliminated as fast as the S papain fragment. In con-

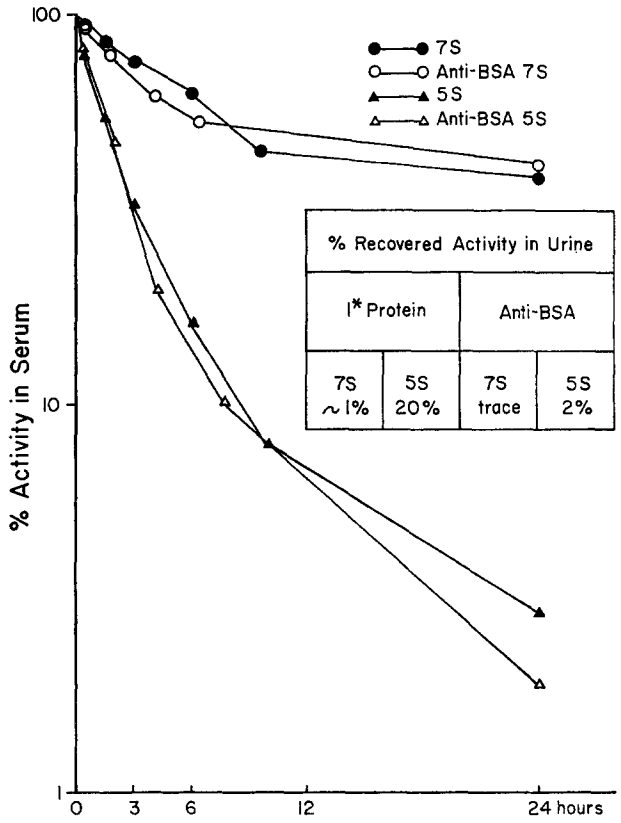

FIG. 3. Elimination from the circulation of rabbits and recovery in the urine of homologous anti-BSA (7S) and pepsin-digested BSA (5S) activity compared with the elimination and urinary excretion of the respective I*-labeled proteins.

trast, a portion of the F fragment isolated after digestion for 5 minutes remained in circulation and was eliminated at a rate similar to that of the GpGG. The recovery of the radioactivity in the urine is summarized in Table II. Most of the injected radioactivity was excreted by all three species as non-protein-bound I* activity.

Elimination of MGG and MGG Fragments in Mice, Rabbits, and Guinea Pigs.—The half-lives of MGG and MGG fragments in mice, rabbits, and guinea pigs are shown in Table I. The F papain fragment obtained after digestion for 4 or 18

hours was eliminated from the circulation similar to the S papain fragment. In contrast, a portion of the F papain fragment isolated after digestion for 5 minutes remained in circulation.

The recovery in the urine of the MGG and MGG fragments is summarized in Table II. Only small amounts of the MGG fragments were excreted as protein-bound radioactivity and most of the I* activity recovered was non-protein-bound.

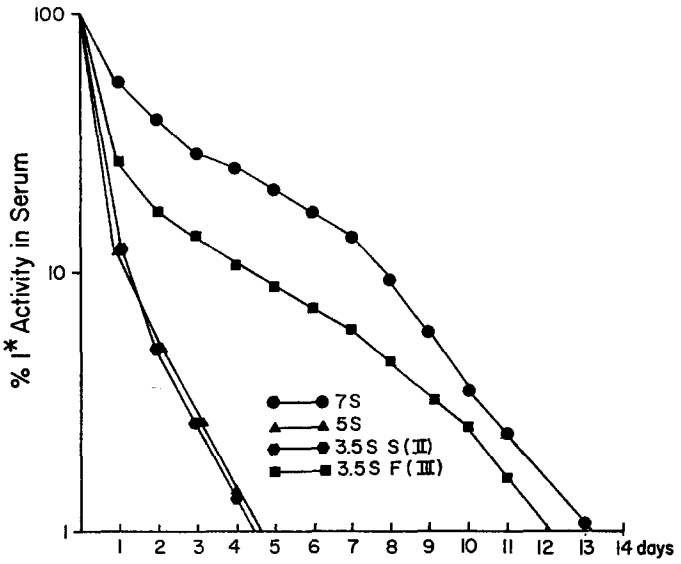

FIG. 4. Elimination of HGG and HGG fragments from the circulation of rabbits. The F papain fragment III was isolated after digestion for 5 minutes.

Elimination of HGG and HGG Fragments in Rabbits, Guinea Pigs, and Mice.— The elimination curves of HGG and HGG fragments in rabbits are shown in Fig. 4 and the half-lives summarized in Table I. Seven and one-tenth per cent of the papain fragment III isolated after digestion for 18 hours and 28 per cent of fragment III isolated after digestion for 5 minutes remained in the circulation 24 hours after injection. All 6 rabbits injected with HGG and all 6 rabbits injected with HGG papain fragment III showed a terminal immune elimination of these proteins.

The elimination of HGG and HGG fragments in mice and guinea pigs was similar to that observed in rabbits. The half-lives of the HGG and HGG fragments in guinea pigs and mice are summarized in Table I.

The recovery of the radioactivity in the urine of rabbits, guinea pigs, and mice is summarized in Table II. All three species excreted only small amounts of the pepsin fragment as protein-bound radioactivity. In contrast to rabbits and

mice, guinea pigs excreted a large amount of papain fragment I (35.3 per cent) as protein-bound radioactivity.

Elimination of HoGG, BGG, and Pepsin Fragments of HoGG and BGG in Rabbits.—The half-life of HoGG in rabbits was 1.7 days and that of BGG was 1.6 days (Table I). The pepsin fragments of HoGG and BGG were eliminated with half-lives of less than 12 hours. Three and two-tenths per cent of the injected HoGG and 3.6 per cent of the BGG pepsin fragments were excreted as protein-bound I* activity and 80 to 90 per cent as non–protein-bound radioactivity.

DISCUSSION

The present data demonstrate that papain fragment III is largely responsible for the rate of elimination and catabolism of 7S gamma globulin. The rate of elimination from the circulation of papain fragment III was slow and closely related to that of the intact gamma globulin. In contrast, papain fragments I or II and the pepsin 5S fragment were rapidly catabolized. These observations were made with both homologous and heterologous gamma globulin fragments in rabbits, guinea pigs, and mice. The present findings are in agreement with those recently reported by Fahey and Robinson who studied the influence of the gamma globulin concentration on its catabolism in mice (21). These authors found that passive administration of gamma globulin or papain fragment III, but not papain fragment I, resulted in an increased turnover rate of the 7S gamma globulin.

The behavior of papain fragment III *in vivo* depended, in part, on the duration of digestion. Fragment III digested for short periods of time was more slowly eliminated. The change in the *in vivo* behavior of fragment III during prolonged digestion was shown to be the result of the presence of cysteine in the digestion mixture. Fragment III, isolated after digestion for 5 minutes and incubated for 18 hours with cysteine alone, was eliminated in the same manner as fragment III obtained after incubation for 18 hours with both papain and cysteine. It may be that the incubation of fragment III in the presence of cysteine results in a reduction of disulfide bonds, however, other changes may occur which are responsible for the rapid elimination and the higher I* uptake. In any event, incubation of RGG with cysteine for 18 hours had no effect on its elimination which indicates that fragment III is not affected by cysteine in the intact molecule. Cysteine also appears to have no effect on the elimination of papain fragment I since fragment I, isolated after digestion for either 5 minutes or 18 hours, was eliminated identically.

The structure, rather than the molecular weight, is apparently responsible for the rate of elimination of the fragments. Pepsin fragments and papain fragment I of RGG, which have molecular weights of 106,000 and 50,000, respectively, are eliminated at a very fast rate, while papain fragment III, which has

a molecular weight of 50,000, is eliminated considerably slower. Other than the difference in molecular weight, papain fragment I and the pepsin fragment are similar in their structure. The pepsin fragment is composed of two univalent fragments closely resembling papain fragment I. The possibility of a reduction of the RGG pepsin fragment in the circulation is unlikely since the elimination of the antibody activity of rabbit anti-BSA and anti-*Shigella* gamma globulin digested with pepsin paralleled that of the I*-labeled pepsin fragment. If a major portion of the pepsin anti-BSA or anit-*Shigella* fragments would have been reduced in the circulation to univalent fragments, the elimination of the activity should have been much faster than that of the I*-labeled pepsin fragment, since even reduced and alkylated pepsin fragments were not immediately eliminated. As mentioned previously, the divalent, but not the univalent fragment, gave a positive reaction in the serological techniques employed.

Similar to the elimination from the circulation, the excretion or lack of excretion in the urine of RGG and RGG fragments depends on a submolecular structure. A large portion of the I*-labeled pepsin fragment and papain fragment I was recovered in the urine in the form of protein-bound I* activity. In contrast, only very small amounts of papain fragment III and intact RGG were excreted as protein. It has been shown that some gamma globulin is filtered in the glomeruli and mostly reabsorbed in the proximal convoluted tubuli (22). Filtration may occur with all fragments, but because of their molecular structure, only fragment III and intact RGG might be reabsorbed. The pepsin fragment of RGG was recovered as protein in the urine, but apparently had been altered. Approximately 20 per cent of the injected I* activity of the pepsin fragments was recovered as protein-bound radioactivity, whereas only 2 per cent of the anti-BSA and no anti-*Shigella* activity were recovered. The alteration could have occurred prior to filtration, during filtration or even after excretion. Additional degradation of the pepsin fragment may have occurred since precipitation with TCA does not distinguish between relatively large polypeptides and intact fragments.

The excretion of protein-bound I* activity of fragments obtained from several other gamma globulins differed markedly from that of RGG fragments. Only a very small portion of the pepsin fragment of several gamma globulins other than RGG was excreted in the urine. The amount of papain fragment I excreted in the urine varied considerably and depended on the source of gamma globulin and on the animal species in which fragment I was injected. The portion of papain fragment III of other gamma globulins excreted in the urine was generally low but always higher than that observed with RGG fragment III. A difference in the biological properties of gamma globulin fragments of different species which do not appear between the intact gamma globulins is not a unique observation. Franklin and Ovary have reported that fragment III of HGG neither fixes to the skin nor crosses the placental barrier (6) and differs in these two

biological activities from RGG fragment III which has been shown to do both (5, 6).

The catabolism of HGG papain fragment I in rabbits and mice is similar to the catabolism of Bence Jones protein in the human and the rabbit. As mentioned above, papain fragment I of HGG was rapidly catabolized in rabbits and mice and only small amounts were excreted as protein-bound I* activity in the urine. Similarly, Bence Jones protein is rapidly catabolized in the human (23) and in the rabbit (24) and little, if any, Bence Jones protein is excreted in the urine of patients with a normal kidney function (23). Like their *in vivo* behavior, the structures of papain fragment I and Bence Jones protein are similar. Bence Jones protein is composed of two light chains (L or B chains) and has a molecular weight of approximately 40,000 (25). Papain fragment I is composed of one L chain and the so called A piece of the heavy chain (H or A chain) and has a molecular weight of approximately 50,000 (26). Both papain fragment I and Bence Jones protein lack the structure of papain fragment III which has been shown to be responsible for the persistence in the circulation. A different observation was made in guinea pigs which excreted a large portion of the human papain fragment I in the urine. The reason for the different behavior of fragment I in guinea pigs than in rabbits and mice in unknown, however, the difference may be related to the ability of the guinea pigs to excrete larger amounts of the gamma globulin fragments and a unique catabolic mechanism in this species (27).

The fact that antibody fragments which contain the antigen-combining site are rapidly catabolized suggests that their use as prophylactic agents is limited. A protective effect of pepsin and papain I antibody fragments can only be expected to last a short period of time. Hartley, who compared the elimination of native and pepsin-treated homologous diphtheria antitoxin in guinea pigs, found that the *in vivo* protective effect of pepsin-treated diphtheria antitoxin was brief (28). Guinea pigs injected with pepsin-treated homologous diphtheria antitoxin were Schick-negative for only 3 to 6 days after injection, whereas, guinea pigs injected with the same amount of native antitoxin were Schick-negative for over 21 days.

SUMMARY

The catabolism of homologous and heterologous 7S gamma globulin fragments obtained by pepsin and papain digestion was studied in rabbits, guinea pigs, and mice. The elimination from the circulation of I* labeled gamma globulin fragments was followed and the urinary excretion of the total and protein-bound I* activity determined.

Evidence is presented that the molecular structure responsible for the catabolism of 7S gamma globulin is located in papain fragment III. The elimination of papain fragment III was slow and closely related to the intact gamma

globulin, whereas the pepsin fragment and papain fragments I and II were rapidly eliminated and catabolized in all species examined.

Prolonged incubation with cysteine altered papain fragment III as shown by a rapid catabolism of a large portion of incubated fragment III within 24 hours after injection.

Small amounts of intact RGG and RGG papain fragment III were excreted as protein-bound I* activity in the urine. On the other hand, large amounts of the pepsin fragment and papain fragments I and II of RGG were excreted as protein-bound I* activity in the urine. The possibility of a molecular structure present in papain fragment III, which may be responsible for tubular reabsorption in the kidney, is discussed. The rate of urinary excretion of fragments obtained from RGG was different from that of fragments obtained from gamma globulin of several other species. In general, small amounts of the pepsin fragment and papain fragment III obtained from gamma globulin other than RGG were excreted as protein-bound I* activity. The amounts of fragment I* excreted as protein-bound I* activity depended on the species in which it was injected, as well as the source of the gamma globulin.

The rapid catabolism of the pepsin fragment and papain fragments I or II which bear antibody-combining sites suggest that their use for the prophylactic treatment of tetanus and diphtheria in man is limited.

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