EVIDENCE FOR SPECIES' DIFFERENCES IN THE EFFECT OF SERUM γ-GLOBULIN CONCENTRATION ON γ-GLOBULIN CATABOLISM

By STEWART SELL,* M.D.

(From the Laboratory of Germfree Animal Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda

(Received for publication, June 26, 1964)

The serum γ-globulin level depends on a dynamic equilibrium between synthesis and catabolism. The mechanisms which regulate this equilibrium are not well understood, but a balance must exist between the rate of production and the rate of breakdown in order to maintain a constant serum γ-globulin concentration. Injections of exogenous γ-globulin into mice increase the rate of catabolism of isotopically labeled homologous (mouse) γ-globulin (1). The rate of catabolism of homologous γ-globulin is also more rapid in hyperimmunized mice (1) or mice harboring γ-globulin-producing plasma cell tumors (2). On the other hand, germfree mice, which have subnormal serum γ-globulin concentrations, have slower rates of γ-globulin breakdown than normal mice (3). The rate of γ-globulin catabolism also appears to be related to the serum γ-globulin concentration in man (4–10).

In guinea pigs, however, the rates of homologous γ-globulin catabolism are the same in germfree and normal animals in spite of up to 6-fold differences in the serum γ-globulin levels (11), suggesting that the fractional catabolic rate of homologous γ-globulin is independent of the serum level in these animals.

Because of this apparent difference between the mouse and guinea pig and the relative ease with which their serum γ-globulin levels may be increased by administration of exogenous γ-globulins, the present study was planned to determine the relationship between serum γ-globulin levels and the fractional catabolic rate of γ-globulins in these two species. This was accomplished by raising the serum γ-globulin levels of mice and guinea pigs by hyperimmunization or by injections of exogenous γ-globulins and following the rate of catabolism of I^{131}–trace labeled serum proteins including: mouse, guinea pig, rabbit, bovine, and human γ-globulins, and human serum albumin. The results indicate a species difference in the relationship between the fractional rate of γ-globulin catabolism and the serum level of γ-globulin in the mouse and guinea pig. In addition, exogenous γ-globulins from man, mouse, guinea pig, and rabbit are all equally effective in increasing the fractional rate of γ-globulin catabolism in the mouse, but bovine γ-globulin is only partially active.

* Present address: Department of Experimental Pathology, University of Birmingham Medical School, Birmingham, England.

Materials and Methods

Mice.—5 groups of white Swiss-Webster mice were used. Group 1, germfree (GF), group 2, conventional low pathogen (CVN-LP), and group 3, conventional high pathogen (CVN-HP), were classified on the basis of potential environmental exposure to bacteria. The CVN-LP mice were raised in a clean animal room with no other strains or species and fed the same sterilized diet as the germfree, while the CVN-HP mice were transferred to another animal room which had a much higher density of animals of other species, placed in the same cages with C_{57}BL mice at a ratio of 4 CVN-HP's to 1 C_{57}BL and fed a non-sterilized stock diet (3). The GF and CVN-LP mice have subnormal serum γ-globulin levels, while the CVN-HP animals have above normal levels (*vide infra*). The 4th group of white Swiss-Webster mice was obtained from the general purpose supply colony of the National Institutes of Health (NIH-WS). Each mouse weighed from 25 to 35 gm. The 5th group consisted of 6 of these NIH-WS mice which were hyperimmunized (NIH-HI) with a total of 0.6 mg of alum-precipitated hemocyanin given in six doses as described by Fahey and Robinson (1).

Guinea Pigs.—Hartley guinea pigs weighing from 350 to 450 gm were obtained from the National Institutes of Health animal colony. 4 groups of 6 to 10 animals each were each immunized as follows: Group 1, bovine γ-globulin (lot T30103, Armour Laboratories, Chicago, Illinois) in a 20 mg/ml 0.85 per cent NaCl solution was emulsified with an equal volume of complete Freund's adjuvant (Boγ-CFA) (Bacto lot 0638, Difco Laboratories, Inc., Detroit, Michigan). Each guinea pig received 0.1 ml of this emulsion in each rear foot-pad. Group 2, *Escherichia coli* 0:127 in complete Freund's adjuvant (*E. coli*-CFA) was injected and boosters given as described by Bloch *et al.* (12). Group 3, 0.25 mg of keyhole limpet hemocyanin (KLH) (lot SP39, Pacific Bio-Marine Supply Co., Venice, California) diluted in 0.85 per cent NaCl was injected into each rear foot-pad and boosters of 0.1 mg given in each of 2 skin sites on the back every 3rd day thereafter for a total dose of 1.5 mg. Group 4, killed *E. coli* 0:127 in 0.85 per cent NaCl (6×10^{11}/ml) was injected in 0.2 ml of saline suspension (*E. coli*-SS) in each rear foot-pad and boosters of 0.1 ml in each of 2 skin sites were given on the back every 3rd day for a total dose of 1.4 ml. Turnover studies were initiated 3 weeks following immunization of group 1, and 3 weeks following the final booster in groups 2 to 4.

I^{131}-Labeled Proteins.—Mouse γ-globulin (Mγ) was prepared by zone (block) electrophoresis of pooled normal NIH-WS mouse sera (1). Guinea pig γ-globulin (GPγ) and bovine γ-globulin (Boγ) were obtained from the pooled sera of normal animals by the 0.005 M phosphate buffer eluate (pH 8.0) from a diethylaminoethyl cellulose (DEAE) column (13). Human γ-globulin (Huγ) was obtained from a normal serum by the 0.01 M phosphate buffer eluate (pH 8.0) from a DEAE column. Human serum albumin (HSA) was obtained commercially (lot 1916, Behring Werke Ag., Marburg-Laahn, Germany, kindly supplied by Dr. Thomas Waldmann, National Cancer Institute, Bethesda). These 5 proteins were individually tested by immunoelectrophoresis and double diffusion in agar using rabbit antisera against the respective whole serum, and found free of contaminating proteins. The preparations were individually trace labeled (<1 mole I per mole of protein) with I^{131} by the iodine monochloride method of McFarlane (14).

Unlabeled Exogenous Proteins.—0.85 per cent NaCl solutions of the following commercial proteins (Cohn fraction II) were prepared: bovine γ-globulin (Boγ, lot 16, Pentex, Inc., Kankakee, Illinois), human γ-globulin (Huγ, batches 2043 and 1801, E. R. Squibb and Sons, New York, kindly supplied by Dr. James H. Pert, American Red Cross), rabbit γ-globulin (Raγ, lot 33, Pentex, Inc.), guinea pig γ-globulin (GPγ, lot 664, Pentex, Inc.), bovine serum albumin (BSA, lot 3974, Nutritional Biochemicals Corp., Cleveland, Ohio), and human serum albumin (HSA, lot 952R, E. R. Squibb and Sons). In addition, unlabeled guinea pig γ-globulin (GPγP), bovine γ-globulin (BoγP), and mouse γ-globulin (MγP) were prepared in the laboratory from pooled normal sera as described for the I^{131}-labeled proteins. Pooled whole

normal NIH-WS mouse sera and pooled whole normal Hartley guinea pig sera were also given in some experiments.

Calculations.—The total amount of γ-globulin in an average (25 gm) mouse was calculated from the following formula:

$$T\gamma = W \times SV \times R \times S\gamma$$

in which $T\gamma$ = total body γ-globulin content
W = body weight (25 gm)
SV = serum volume (4 per cent of W)
R = ratio of total body γ-globulin to the intravascular γ-globulin (2.2), and
$S\gamma$ = serum γ-globulin concentration (5 mg/ml):

Or, $T\gamma = (25)(.04)(2.2)(5) = 11$ mg γ-globulin/animal.

The figure of 11 mg γ-globulin/animal was used as a basis for calculations of the amounts of exogenous γ-globulin necessary to elevate the total body γ-globulin of each animal a given number of times. The values for SV, R, and $S\gamma$ are based on previous studies (1, 2). The total body albumin was calculated to be 66 mg/25 gm mouse based on a serum concentration of 30 mg/ml. The amount of exogenous protein given was calculated to raise the total body level from 4 to 25 times.

Similar calculations indicated 120 mg of γ-globulin/450 gm guinea pig using the following values: W = 450 gm, SV = 4 per cent of W, R = 2.2, and $S\gamma$ = 3.0 mg/ml (11, 15). Calculations of the total body albumin of a 450 gm guinea pig based on a serum concentration of 25 mg/ml gave a value of approximately 1 gm albumin/animal. The amount of exogenous unlabeled proteins given was calculated to raise the serum levels of each guinea pig injected 2 to 16 times.

Experimental Protocols.—All animals were housed either in plastic cages containing wood shavings that were frequently changed, or in cages with wire floors which allowed urine and fecal droppings to pass through to a tray below. All animals were given drinking water containing 0.45 per cent NaCl and 0.01 per cent KI. Mice were injected intraperitoneally with from 0.3 to 0.5 μc of I^{131}-labeled protein in 0.1 ml 0.85 per cent NaCl. The whole body radioactivity of mice was measured in a gamma ray bulk spectrometer (Sharpe Laboratories, La Jolla, California) for 1 minute. The radioactivity in each mouse was determined within 30 minutes of injection, then once every 1 to 2 days. A radioactive standard was prepared by injecting 1 dose of the given labeled protein in 20 ml of 0.85 per cent NaCl in a 30 ml plastic bottle. This standard was counted daily with the experimental animals and the results used for correction of physical decay.

The whole blood radioactivity decay was followed in some experiments using mice which were injected with 2 to 3 μc of I^{131} protein. Every 1 to 2 days following injection of the I^{131} protein approximately 0.3 ml of whole blood was collected from the retroorbital venous sinus of each animal into citrated test tubes. 0.25 ml of whole unclotted blood was then pipetted into 0.75 ml of 0.85 per cent NaCl and the amount of radioactivity determined on a gamma well scintillation counter (auto-gamma spectrometer, series 410A, Packard Instrument Co., La Grange, Illinois) as described previously for the guinea pig (11). The amount of radioactivity in the whole blood was converted to the proportion of the I^{131} protein remaining in the total blood volume using a blood volume of 7 per cent of the body weight (2). Although immune elimination of soluble proteins in mice does not take place unless the mice are given a prior injection of the protein in adjuvant (16, 17), all half-lives were determined using elimination data obtained prior to day 6; *i.e.*, before immune elimination would take place.

Guinea pigs received from 5 to 12 μc of I^{131} protein intraperitoneally in 0.5 to 1.0 ml of 0.85 per cent NaCl. Every 1 to 2 days following injection, approximately 0.75 ml of whole

blood was obtained from the retroorbital venous sinus of each animal, and the radioactivity present in 0.5 ml of whole unclotted blood determined as described above for mouse blood. The amount of radioactivity was converted to the proportion of the injected I^{131} protein remaining in the total blood volume using an estimated blood volume of 8 per cent of the body weight (15).

Whole body counts were also made on some guinea pigs. After injection with 10 to 15 μc of I^{131} protein, the animals were counted every 1 to 3 days by placing them a given distance from a 2 inch NaI crystal attached to a scintillation counter. The distance used depended upon the amount of radioactivity present in each animal at the time of counting. In order to avoid immune elimination (18) all half-lives for heterologous labeled proteins in the guinea pig were determined from the radioactivity decay curves prior to day 7.

The fractional rate of catabolism (T ½) of the I^{131} proteins was determined from the graphic plots of the decay curves corrected for physical decay of the isotope. The total serum proteins were quantitated by the microbiuret technique (19), and the serum protein fractions by paper electrophoresis (20).

RESULTS

Effect of Exogenous Proteins on γ-Globulin Catabolism in Mice.—

Radioiodine-labeled normal 7S human (Huγ-I^{131}), mouse (Mγ-I^{131}), guinea pig (GPγ-I^{131}), and bovine (Boγ-I^{131}) γ-globulins and human serum albumin (HSA-I^{131}) were each separately injected intraperitoneally into groups of 25 to 40 NIH-WS mice. After following the rate of catabolism for 3 to 4 days, each group was subdivided into 3 to 5 mice each and injected intraperitoneally with a loading dose of unlabeled protein 5 times the maintenance dose shown in Table I, calculated to raise the given serum protein level by the factor given. The maintenance dose was then given the subsequent 3 days. 1 day following the last injection each animal was weighed and bled and the serum was saved for protein determinations.

The whole body radioactivity decay curves for a typical γ-globulin experiment is shown in Fig. 1, and for the albumin experiment in Fig. 2. The data from all experiments is collected in Table I.

Saline, HSA, and BSA had no effect on the fractional rate of catabolism of any of the labeled proteins tested. Raγ, Huγ, GPγ, and Mγ markedly increased the fractional rate of catabolism of Huγ-I^{131}, Mγ-I^{131}, GPγ-I^{131}, and Boγ-I^{131}, but had no effect on HSA-I^{131}. Boγ had a moderate effect on the fractional rates of the γ-globulins tested, and no effect on HSA-I^{131}. To determine if the decreased effect of the commercial Boγ used might be due to partial denaturation, 2 control experiments were carried out: (*a*) 5 times the usual doses of unlabeled commercial bovine γ-globulin (BoγC) and (*b*) the usual doses of a bovine γ-globulin (BoγP) isolated by DEAE chromatography were given to mice that had been injected with Boγ-I^{131} or Mγ-I^{131} 3 days previously. The higher doses of BoγC increased the rate of Mγ-I^{131} and Boγ-I^{131} catabolism only slightly. Injection of BoγP resulted in essentially the same T ½'s for Mγ-I^{131}, and Boγ-I^{131}, as had been found with injection of the commercial product.

The differences in the factor (calculated times serum or total body protein

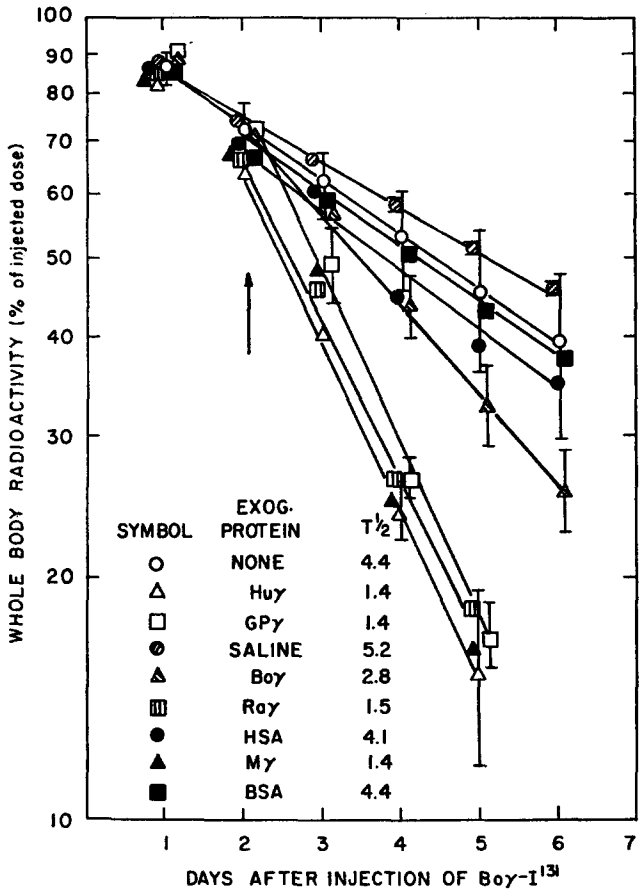

FIG. 1. Elimination of Boγ-I^{131} in mice receiving exogenous serum proteins. Total body radioactivity counts were measured daily, corrected for physical decay, and recorded on the ordinate as the per cent of the injected dose. Each symbol represents the mean value of 3 to 4 mice and the brackets, when given, the range within each group; the arrow indicates the 1st day of injection of the unlabeled proteins and saline.

elevated by exogenous injection) and the actual serum value as determined by paper electrophoresis may be explained by 3 factors: (*a*) The serum samples were collected 1 day following the last injection of exogenous protein and thus represent a minimum value. (*b*) The maintenance dose is based on the normal half-life of mouse γ-globulin (4 to 5 days). As the rate of catabolism was markedly increased (1 to 2 days) by the exogenous injections, the maintenance dose given may not have been capable of maintaining the calculated level. (*c*) The serum levels of the mice used were higher (7.6 mg/ml) than had been previously

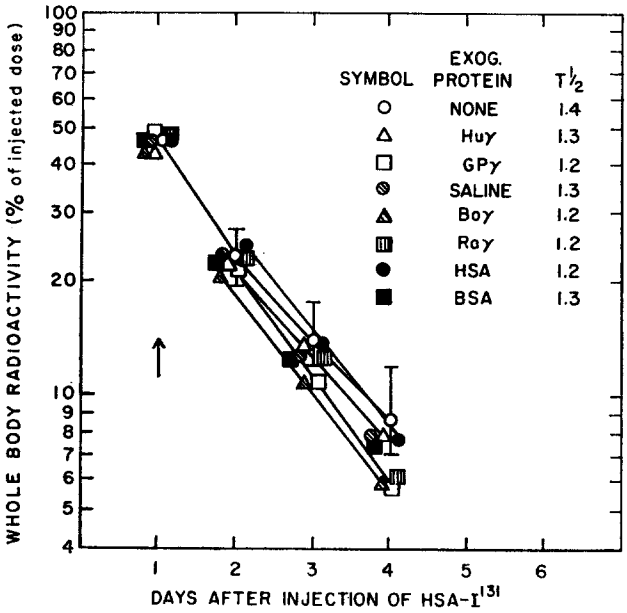

FIG. 2. Elimination of HSA-I^{131} in mice receiving exogenous serum proteins. Each symbol represents the mean value of 3 to 4 animals. The range as indicated by the brackets is given for the control group only.

TABLE I

Effect of Exogenous Proteins on I^{131} Protein Catabolism in NIH-WS Mice

Exogenous injection	Maintenance* dose	Factor‡	Serum proteins		T ½ of I^{131} proteins				
			γ-Globulin	Albumin	Huγ	Mγ	GPγ	Boγ	HSA
			mg/ml	*mg/ml*	*days*	*days*	*days*	*days*	*days*
None	0	0	7.6	34.7	5.1	3.2	7.7	4.4	1.4
Saline	2	0	6.6	34.8	5.2	3.2	7.7	5.2	1.3
HSA	50	4	6.1	50.1	5.1	3.3	7.5	4.4	1.2
BSA	50	4	8.0	40.7	—	—	—	4.4	1.3
BoγC	50	25	27.7	28.8	—	2.4	—	2.1	—
BoγC	10	5	18.7	31.3	3.5	2.8	6.7	2.8	1.2
Raγ	10	5	13.5	30.0	1.4	1.8	1.8	1.5	1.2
Huγ	10	5	12.4	29.6	1.8	1.8	2.1	1.4	1.3
GPγP	10	5	14.0	28.9	1.8	1.0	1.6	1.4	1.2
MγP	10	5	14.9	29.0	—	1.8	—	1.4	—

* Milligrams protein/day except for saline, which is given in milliliters. Initial loading dose is 5 times maintenance dose.

‡ Calculated times given total body protein elevated.

found for normal NIH-WS mice (4 to 5 mg/ml). This latter finding may also explain the more rapid catabolic rate for normal mice (3.2 days) than the 4 to 5 days previously found (2, 3).

I^{131} Protein Catabolism in Hyperimmunized Mice or Mice with Different Bacterial Exposures.—

The 5 labeled serum proteins were each individually injected intraperitoneally into groups of 4 to 5 germfree (GF), 3 to 5 conventional low pathogen (CVN-LP), 4 to 5 conventional high pathogen (CVN-HP), 3 to 5 hyperimmunized (NIH-HI), and 6 normal (NIH-WS) mice, and the half-times determined for each protein in each group.

An example of the whole body radioactivity decay curves for γ-globulin in these animals is shown in Fig. 3. The serum γ-globulin and albumin levels, and half-times are given in Table II. The mean serum γ-globulin levels of the GF (2.9 mg/ml) and CVN-LP (3.4 mg/ml) mice are lower than those of the NIH-WS mice (7.6 mg/ml), while the levels of the NIH-HI (12.9 mg/ml) and CVN-HP (15.5 mg/ml) are higher than normal as determined by electrophoresis. These differences are not as great as those determined previously by an immunochemical technique (precipitation inhibition) (3). The half-lives of each of the 4 labeled γ-globulins are slower than normal in the GF and CVN-LP mice and faster than normal in the NIH-HI and CVN-LP mice, while there is little or no difference between the half-life of HSA-I^{131} in any of the groups of mice. An unexplained finding is the consistently longer T ½ of all γ-globulins in CVN-LP mice when contrasted to the half-lives of these proteins in GF mice, in light of the higher serum γ-globulin levels in the CVN-LP mice.

Effect of Exogenous Proteins on I^{131} Protein Catabolism in Guinea Pigs.—

The 5 labeled serum proteins (Huγ-I^{131}, Mγ-I^{131}, GPγ-I^{131}, Boγ-I^{131}, and HSA-I^{131}) were each individually injected into groups of approximately 20 guinea pigs. After 3 to 5 days each group was subdivided into groups of 2 to 4 each and each subgroup injected intraperitoneally with unlabeled HSA, Huγ, or GPγ in amounts calculated to elevate the given serum protein from 2 to 16 times.

The whole blood decay curve for a typical experiment is shown in Fig. 4. The mean half-lives of the labeled proteins, the mean serum protein levels, and the doses of exogenous unlabeled proteins in all experiments are summarized in Table III. None of the unlabeled proteins had any effect on the fractional rate of catabolism of any of the labeled proteins, even though the unlabeled γ-globulins increased the serum γ-globulin levels up to 4½ times the preinjection levels. The sharp drop in the per cent of GPγ-I^{131} remaining in the total blood volume in guinea pigs on the day of injection of the heterologous unlabeled γ-globulin (Fig. 4) is most likely caused by dilution or a small change in distribution of the GPγ-I^{131} *in vivo*. This is supported by the observation that the fraction of

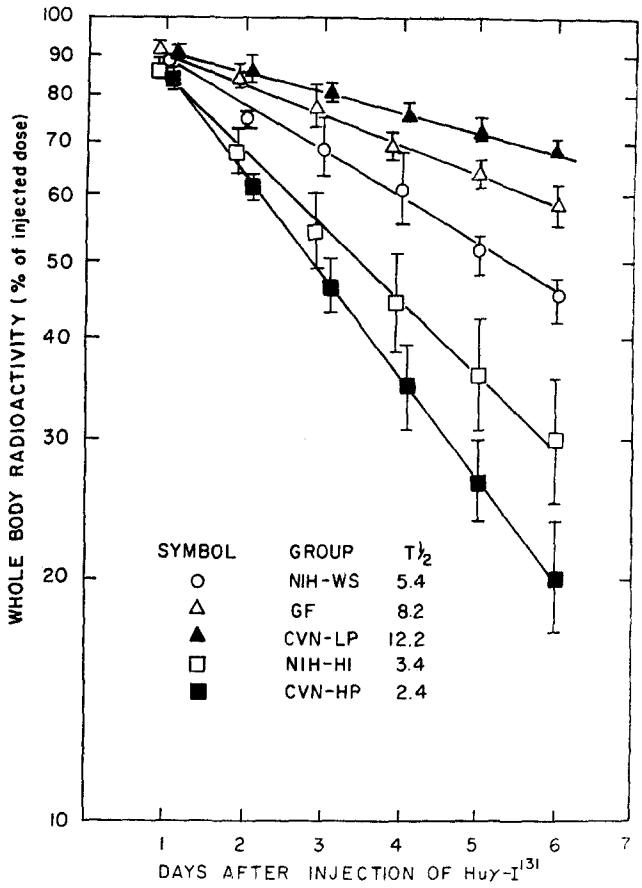

FIG. 3. Elimination of Huγ-I^{131} in mice with different serum γ-globulin levels due to different bacterial exposures or hyperimmunization. Each symbol represents the mean value of 3 to 5 animals.

TABLE II

I^{131} Protein Catabolism in GF, CVN-LP, NIH-WS, NIH-HI, and CVN-HP Mice

Group	Serum proteins		T ½ of I^{131} proteins				
	γ-Globulin	Albumin	Huγ	Mγ	GPγ	Boγ	HSA
	mg/ml	*mg/ml*	*days*	*days*	*days*	*days*	*days*
GF	2.9	30.4	8.2	4.5	8.3	4.9	1.3
CVN-LP	3.4	34.2	12.2	6.2	9.5	10.3	1.3
NIH-WS	7.6	34.7	5.4	3.3	7.7	4.4	1.4
NIH-HI	12.9	33.8	3.4	2.8	6.0	3.0	1.6
CVN-HP	15.5	32.2	2.4	2.5	3.6	2.4	1.6

the GPγ-I^{131} remaining on day 10 in the animals receiving heterologous γ-globulin is higher than would be expected from the slope of the decay curves from days 5 to 8. Injections of the heterologous γ-globulin were discontinued on day 7 and any remaining heterologous γ-globulin may have been subject to removal by day 10 through immune elimination (18). Again, since the serum pro-

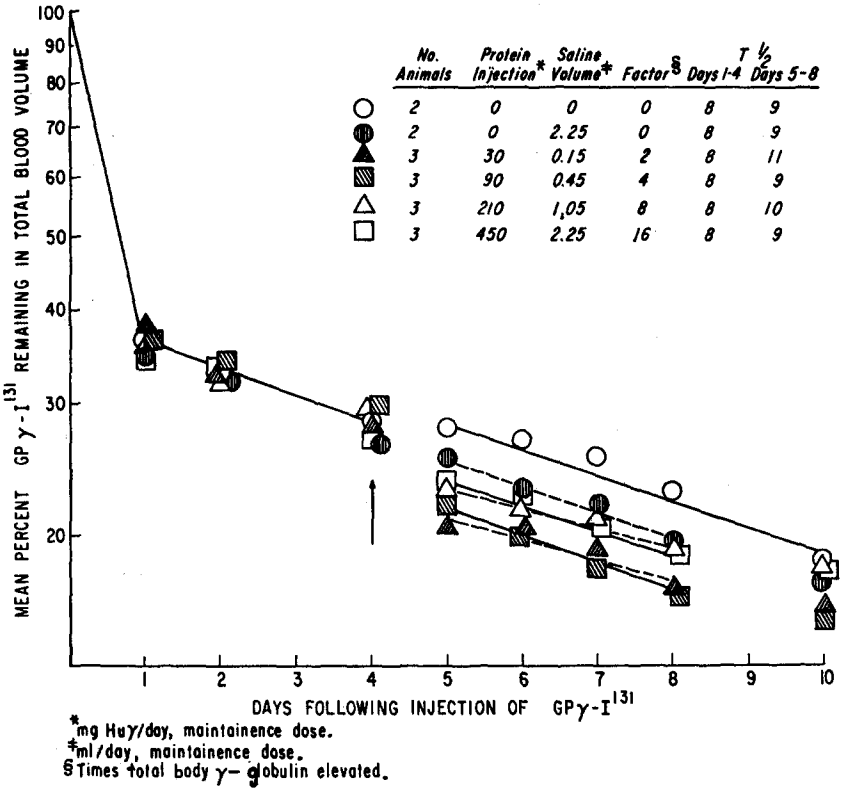

FIG. 4. Elimination of GPγ-I^{131} in guinea pigs receiving exogenous γ-globulin (Huγ). The radioactivity in a sample of whole blood was determined every 1 or 2 days, converted to the per cent of the injected dose remaining in the total blood volume of each animal, and the mean value for each group recorded on the ordinate.

tein determinations were done on serum samples taken day following the last injection of unlabeled protein, these determinations represent minimum values.

Two separate preparations of labeled guinea pig γ-globulin (GPγ-I^{131}) were used. One had a control T ½ of 8 days, the other a control T ½ of 6.6 days. This, plus the fact that the half-lives were determined later following injection

in the experiment in which exogenous unlabeled Huγ was given, explains the difference in the half-lives given in Table III for GPγ-I^{131}.

γ-Globulin Catabolism in Hyperimmunized Guinea Pigs.—The rate of catabolism of the 5 labeled serum proteins was followed in guinea pigs immunized

TABLE III

Effect of Exogenous Proteins on I^{131} Protein Catabolism in Hartley Guinea Pigs

Exogenous protein	Maintenance* dose	Factor‡	Serum proteins		T ½ of I^{131} proteins				
			γ-Globulin	Albumin	Huγ	Mγ	GPγ	Boγ	HSA
			mg/ml	*mg/ml*	*days*	*days*	*days*	*days*	*days*
None	0	0	6.6	25.8	4.2	2.9	9§	2.4	3.4
Saline	2.25	0	6.2	24.0	3.3	3.0	9	—	3.3
Huγ	30	2	10.6	23.8	3.4	—	11	—	—
Huγ	90	4	13.0	23.1	3.3	2.6	9	—	2.9
Huγ	210	8	17.2	21.7	3.0	2.8	10	2.3	3.2
Huγ	450	16	26.0	21.5	3.3	3.6	9	2.2	—
HSA	800	4	4.4	70.0	—	—	6.5‖	—	3.0
GPγ	30	2	9.1	26.5	—	—	6.6‖	—	—
GPγ	90	4	12.2	25.2	—	—	6.8‖	—	—

* Milligrams protein/day except for saline, which is given in milliliters.

‡ Calculated times total body protein elevated.

§ Determined days 5 to 9.

‖ These determinations were done on a different GPγ-I^{131} preparation for which the control T ½ was 6.6 days.

TABLE IV

I^{131} Protein Catabolism in Hyperimmunized Guinea Pigs

Group	Serum proteins		T ½ of I^{131} proteins					γ-Globulin intravascular, mean
	γ-Globulin	Albumin	Huγ	Mγ	GPγ	Boγ	HSA	
	mg/ml	*mg/ml*	*days*	*days*	*days*	*days*	*days*	*per cent*
Control	6.6	25.8	4.2	2.9	6.6*	2.4	3.4	43
Boγ-CFA	17.0	27.0	2.4	1.5	3.8	—	2.7	41
E. coli-CFA	16.5	23.5	2.4	1.8	4.1	2.0	2.4	40
KLH	13.5	26.4	—	—	6.9	—	3.3	39
E. coli-SS	13.6	26.2	—	—	6.2	—	3.1	40

* Determined days 1 to 7.

with Boγ in complete Freund's adjuvant (Boγ-CFA), killed *E. coli* in complete Freund's adjuvant (*E. coli*-CFA), killed *E. coli* in saline suspension (*E. coli*-SS), and keyhole limpet hemocyanin in saline (KLH). The serum γ-globulin levels, the per cent intravascular I^{131} γ-globulin determined by extrapolating the decay

curves back to 0 time, and the half-lives are given in Table IV. The fractional rate of GPγ-I^{131} and HSA-I^{131} catabolism is the same in normal and in KLH animals in spite of greater than 2-fold differences in serum γ-globulin levels. In contrast, the fractional rate of catabolism is markedly increased over normal

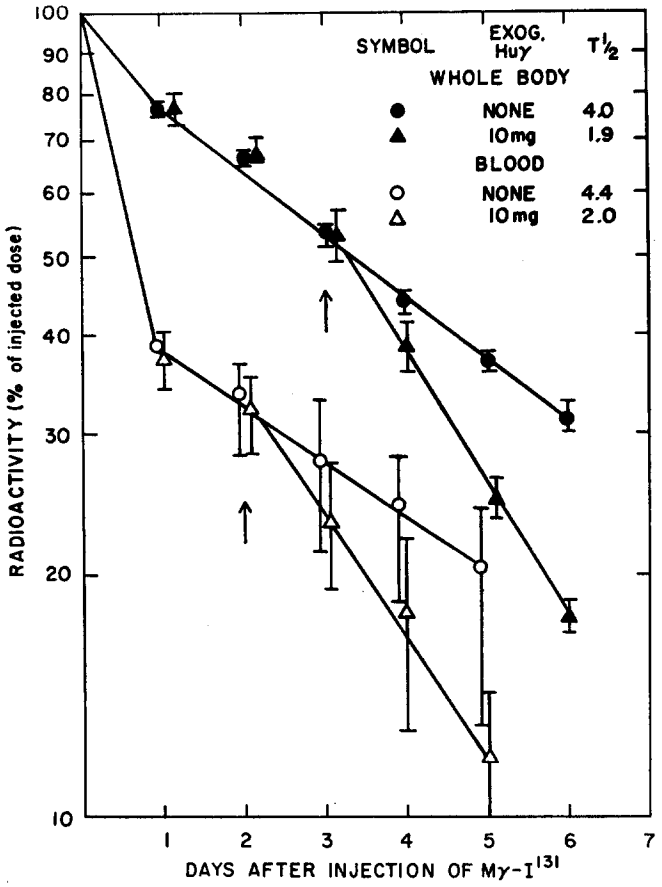

FIG. 5. Elimination of Mγ-I^{131} in mice receiving exogenous Huγ as determined separately by whole body and blood radioactivity sampling. Each solid symbol is the mean per cent of the injected dose remaining in the total body in 3 to 4 animals as determined by whole body counting, and each open symbol is the mean per cent of the injected dose remaining in the total blood volume of 6 animals as determined by whole blood counting. The arrows indicate the 1st day of injection of the exogenous unlabeled Huγ.

for all I^{131} proteins in Boγ-CFA and *E. coli*-CFA animals. The rates of catabolism of GPγ-I^{131} and HSA-I^{131} in the *E. coli*-SS animals are only slightly increased over normal, not nearly as much as in the adjuvant immunized animals. The per cent of γ-globulin (homologous) in the intravascular space is essentially the same in all groups.

Whole Body Radioactivity Determinations Compared to Whole Blood Determinations.—Mathematical analyses of the protein decay curves of *in vivo* tracer experiments indicate that labeled proteins are distributed in a dynamic equilibrium between a central compartment (intravascular) and outer compart-

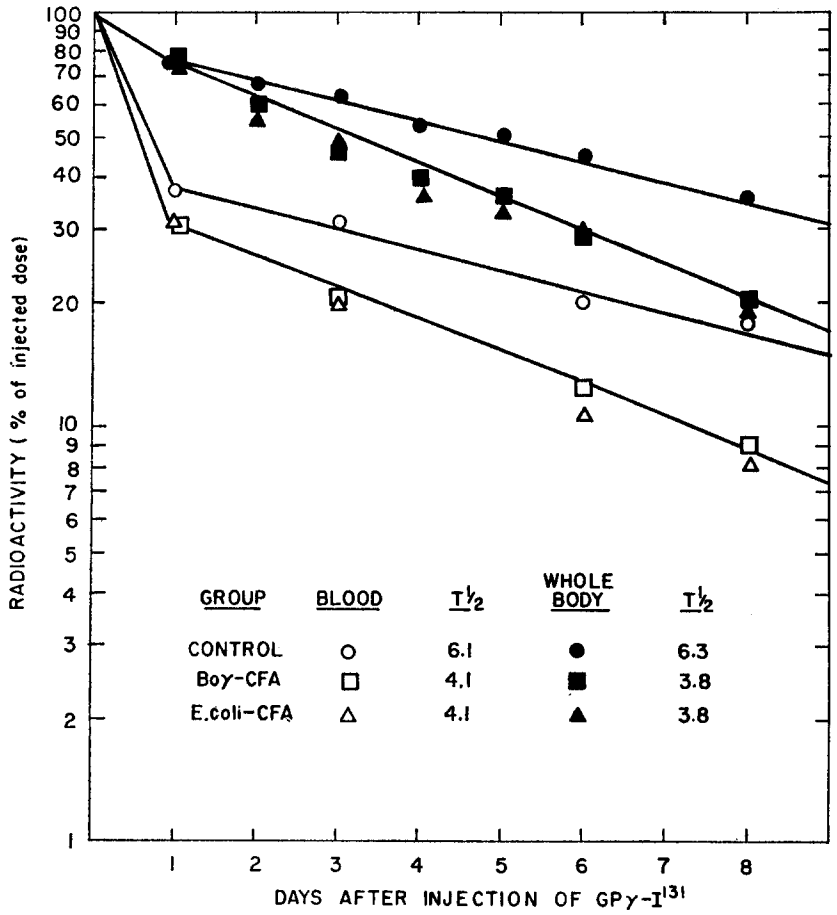

FIG. 6. Elimination of GPγ-I^{131} in guinea pigs immunized with antigens in complete Freund's adjuvant (CFA). Each symbol represents the mean value of 4 to 6 animals. Whole body counts and blood counts were done on the same animals.

ments (extravascular) (21). To check if fixation of the labeled proteins to tissues or pooling in another kind of non-exchangeable compartment might affect the rate of catabolism, comparisons of the radioactivity decay curves obtained by whole body counting and by whole blood counting in a group of mice and guinea pigs receiving homologous labeled γ-globulin were made and are shown in

Figs. 5 and 6. There was no difference in the fractional rate of decay as determined by whole body or whole blood counting in either mice or guinea pigs. This agrees with previous findings reported in man (7).

Effect of Infusions of Whole Blood.—Because an imbalance of γ-globulin over the other serum proteins, rather than the quantity of γ-globulin present, might be the factor causing increased γ-globulin catabolism in hyper-γ-globulinemic mice, the effect of infusions of whole normal mouse serum (WMS, 3 ml intraperitoneally daily) on Mγ-I^{131} catabolism, and the effect of whole normal guinea pig serum (WGPS, 5 ml intraperitoneally daily) on GPγ-I^{131} catabolism in mice was determined and the results tabulated in Table V. WMS increased Mγ-I^{131} catabolism and WGPS increased GPγ-I^{131} catabolism.

Proportion of Intravascular γ-Globulin in Mice.—Because of differences in the intravascular:extravascular distribution between normal and hyperim-

TABLE V

Effect of Injections of Whole Sera on I^{131} γ-Globulin Catabolism in NIH-WS Mice

Exogenous injection	Dose	I^{131} γ-globulin	Serum proteins		T ½
			γ-Globulin	Albumin	
	ml/day		*mg/ml*	*mg/ml*	*days*
Saline	3	Mγ-I^{131}	5.6	34.0	3.3
WMS	3	Mγ-I^{131}	8.6	38.4	2.3
Saline	5	GPγ-I^{131}	7.1	35.7	7.7
WGPS	5	GPγ-I^{131}	9.4	39.4	1.8

munized rabbits recently reported (22), whole blood radioactivity decay of GPγ-I^{131} was determined in GF, CVN-LP, NIH-WS, and CVN-HP mice, in addition to mice receiving the initial injections of exogenous unlabeled proteins 1 day prior to the injection of GPγ-I^{131}. Extrapolation of the radioactivity decay curves to 0 time revealed little or no difference in the per cent of the labeled protein in the intravascular space (47 to 50 per cent in uninjected animals; 43 to 50 per cent in mice receiving exogenous proteins).

DISCUSSION

A summary of the fractional rates of γ-globulin catabolism in 5 different species including data from the literature (1–11, 22–28) is given in Table VI. In mice (1–3) and man (4–10) subnormal serum γ-globulin levels are associated with slower than normal rates of catabolism while above normal serum γ-globulin levels are associated with faster than normal rates of catabolism. In the rabbit the newborn has a significantly slower γ-globulin fractional catabolic rate than the adult (23, 24), and increased fractional catabolic rates have been

reported in rabbits having elevated serum γ-globulin levels due to hyperimmunization (22, 26). However, Humphrey and McFarlane (25) report no alteration in the fractional rate of γ-globulin catabolism in rabbits with up to 4 times normal serum γ-globulin levels following hyperimmunization or in a rabbit given large amounts of γ-globulin by passive transfer. In the guinea pig the same rate of γ-globulin catabolism is found regardless of low, normal, or elevated serum γ-globulin levels (11, 27). Germfree and normal chickens also appear to have the same fractional catabolic rate (28). Thus a feedback mechanism for the control of γ-globulin catabolism may be active in some species, and not in others.

TABLE VI

The Relationship between the Half-Life of Homologous γ-Globulin and Conditions Affecting Serum γ-Globulin Levels in 5 Species

Condition	Serum γ-globulin	Species				
		Man (4–10)*	Mouse (1–3)	Rabbit (22–26)	Guinea pig (11, 27)	Chicken (28)
		days	*days*	*days*	*days*	*days*
Newborn	< Normal	30–35‡	—	13–33	7–8§	—
Germfree or hypo-γ-globulinemia (man)	< Normal	34	5.5	—	7–8	4.2
Normal	Normal	20	4	5.6	6–9	4.3
Hyperimmune	> Normal	—	2	3.5–5.4	6–7	—
Exogenous transfusion	> Normal	—	1.8	5	6.5–10	—
γ-Globulin–producing tumors	> Normal	5.7–22	1.8	—	—	—

* References.

‡ Half-life.

§ Heterologous γ-globulin (Huγ) used in newborn guinea pigs.

One explanation of the species difference might be a difference in the serum level above which γ-globulin catabolism is no longer affected. Serum γ-globulin limits have been found in both mice (1) and man (7) above which no increase in the rate of γ-globulin catabolism is observed. However, it appears likely that at least for the guinea pig, no level exists at which alterations in the serum γ-globulin level will affect the fractional rate of γ-globulin catabolism as a range of serum γ-globulin values from 50 (germfree) to 2500 (exogenous injections) mg per cent has no effect on γ-globulin catabolism.

There appears to be a site (or sites) on the γ-globulin molecule that affects γ-globulin catabolism in the mouse. These sites or configurations must exist on γ-globulin molecules from other species (guinea pig, rabbit, and human) as well as homologous γ-globulin. This effect appears to be specific for γ-globu-

lin as bovine serum albumin and human serum albumin fail to increase the rate of γ-globulin catabolism, and the exogenous γ-globulins listed above fail to increase the rate of labeled albumin catabolism. However, bovine γ-globulin injected into the mouse is only partially effective in increasing the fractional catabolic rate of the labeled γ-globulins studied.

The decreased ability of exogenous bovine γ-globulin to affect the metabolism of γ-globulin in the mouse as compared to the γ-globulins of the other species tested does not appear to be related to antigenic cross-reactivity as antisera produced in chickens to rabbit γ-globulin cross-react equally with bovine and human γ-globulins and antisera produced in rabbits to human and bovine γ-globulins cross-react equally with bovine and human γ-globulins (29). This does not rule out the possibility that antisera raised in mice to human and bovine γ-globulins might recognize differences in antigenic cross-reactivity between these γ-globulins not detectable with antisera produced in chickens and rabbits.

The sites or configurations that affect the fractional rate of γ-globulin catabolism in mice are not present in all of the immunoglobulin groups of man. Injections of human 7S γ-globulin has a marked effect on 7S γ-globulin catabolism in mice, but human β_2A-myeloma proteins and human γ_1-macroglobulins have no effect (1). Recent observations also show that mouse β_2A-myeloma proteins do not affect mouse 7S γ-globulin catabolism (30).

γ-Globulin molecules are believed to be composed of 2 pairs of polypeptide chains (31). Fahey and Robinson (1) have demonstrated that the portion of the molecule that is capable of increasing the fractional catabolic rate resides in the F piece of the H chain of γ-globulin. Other biologic properties such as skin-binding capacity, placental transfer, complement fixation (32), and the major activity for antigenic cross-reactivity between the γ-globulins of different species also resides in the F piece (33). Studies on the metabolism of isolated chains or pieces from the γ-globulins of different species or the different immunoglobulins of the same species might clarify the presence and location of an active site or configuration.

Although γ-globulin synthesis has been shown in plasma cells and their precursors (34), the site of γ-globulin catabolism is unknown. Evidence suggests that I^{131} γ-globulin catabolism most likely takes place in the intravascular compartment or in a compartment in rapid equilibrium with the intravascular compartment (35) and that the freed I^{131} is excreted in the kidney (26). Denatured serum proteins are disposed of by the action of the reticuloendothelial (RE) system (36), and it has been reported that the rate of disappearance of homologous native proteins from the circulation of the rat is slowed by blocking of the RE system with thorotrast (37). This suggests that the catabolism of homologous proteins is at least partially effected by the RE system. However, Freeman *et al.* (38) reported that while blocking of the RE system of the

rat with carbon (India ink) greatly decreased the clearance rate of heat-denatured HSA-I^{131}, the rate of catabolism of I^{131}-labeled native rat albumin was increased by 40 per cent, and the T ½ of native I^{131} rat γ-globulin changed from 130 to 96 hours. They conclude that, if the RE system is important in the catabolism of normal serum proteins, the process by which this is accomplished must be different from that of denatured proteins.

An increased fractional rate of γ-globulin and albumin catabolism in guinea pigs immunized with antigens in complete Freund's adjuvant (CFA) and killed *E. coli* saline suspension (*E. coli*-SS) was found in the present study. These increases in catabolic rate do not appear to be the result of the elevation in serum γ-globulin induced by immunization as guinea pigs hyperimmunized with keyhole limpet hemocyanin had comparable increases in serum γ-globulin level with no increases in the fractional catabolic rate of guinea pig γ-globulin or human serum albumin. Animals immunized with *E. coli*-SS gave only slight if any increased catabolic rates of both guinea pig γ-globulin and human serum albumin. This minimal increase may have been the result of the increased clearance rate in the RE system found following injections of endotoxins (39). However, a much greater increase in the rates of γ-globulin and albumin catabolism were found following immunization with CFA. Marked proliferation and stimulation of the RE system is seen following injections of CFA (40), but the clearance of latex particles is not affected (41). It is likely that the increased catabolic rate following CFA immunization is not the result of the same process as the increased rate following *E. coli*-SS immunization, but is related to the marked increased breakdown of natural albumin and γ-globulin found by Freeman *et al.* (38) after administration of carbon.

The site of γ-globulin catabolism and the anatomical location of its control (or recognition) may not be the same (1). Whatever the location, the γ-globulin catabolic recognition system of the mouse appears to depend on the absolute quantity of γ-globulin present and not a relative increase in γ-globulin over the other serum proteins as injections of whole serum are effective in increasing the fractional catabolic rate of γ-globulin. Dixon and Maurer (42) were able to demonstrate an increase in the fractional catabolic rates of both γ-globulin and albumin in rabbits infused with large volumes of normal human and normal bovine serum.

Recent studies in hyperimmunized rabbits led to different conclusions in spite of similar increases in serum γ-globulin levels and decreases in labeled γ-globulin plasma survival times (22, 26). Catsoulis *et al.* (26) concluded that the fractional rate of homologous γ-globulin catabolism was increased over normal in hyperimmunized rabbits while Anderson and Bjørneboe (22) state that the rate is the same in normal and hyperimmunized rabbits. This difference may be explained by the fact that Anderson and Bjørneboe (22) found a marked difference in the distribution of γ-globulin in the intravascular and

extravascular pools (50 per cent intravascularly in the normal *vs.* 67 per cent intravascularly in the hyperimmunized), while Catsoulis *et al.* (26) found essentially the same distribution in normal (46 per cent intravascularly) and hyperimmunized rabbits (48 per cent intravascularly). Both workers used a metabolic breakdown method for calculating fractional catabolic rates which reflects the size of the intravascular pool (21). No differences in the per cent of intravascular γ-globulin were found in the present study on analysis of whole blood radioactivity from germfree, normal, or hyperimmunized mice or guinea pigs. In addition, the I^{131} γ-globulin distribution is also the same in mice receiving exogenous γ-globulin, in mice bearing plasma cell tumors (2), and in normal mice.

SUMMARY

The fractional rates of catabolism of isotopically labeled mouse, human, bovine, and guinea pig γ-globulins and human serum albumin were determined in mice and in guinea pigs whose serum γ-globulin and serum albumin levels were elevated by immunization or by injections of exogenous serum proteins. These serum proteins were also followed in mice with different serum γ-globulin levels due to different bacterial environments. The fractional rates of catabolism of the labeled γ-globulins from all species tested were markedly increased in mice with elevated γ-globulins due to immunization; to injections of human, mouse, guinea pig, or rabbit γ-globulins; to exposure to supra normal numbers of bacteria in the environment. Injections of bovine γ-globulin were only partially effective, and injections of human serum albumin had no effect. The γ-globulin catabolic rates were decreased in mice with subnormal serum γ-globulin levels (germfree mice). The catabolic rate of human serum albumin was essentially the same in all mice in spite of differences in serum γ-globulin levels. In contrast, elevation of the serum γ-globulin levels by injections of exogenous γ-globulins or by hyperimmunization with keyhole limpet hemocyanin produced no change in the fractional catabolic rates of the isotopically labeled γ-globulins and labeled albumin in guinea pigs. Thus, a feedback mechanism for the control of the serum γ-globulin concentration appears to be operative in the mouse, but not in the guinea pig.

Guinea pigs immunized with antigens in complete Freund's adjuvant or a saline suspension of killed *E. coli* had an increase in the catabolic rates of all labeled proteins tested including human serum albumin. Evidence is presented that the mechanism of this increase in catabolism is not the same as that seen in mice with elevated serum γ-globulin levels.

I would like to express my gratitude to Dr. John L. Fahey and Dr. Thomas Waldmann for their advice and stimulation and for the use of their laboratories. The technical assistance of Miss Martha B. Hylton, Miss Betty J. Sanders, and Miss Barbara G. Mance helped make this work possible.

BIBLIOGRAPHY

1. Fahey, J. L., and Robinson, A. G., Factors controlling serum γ-globulin concentration, *J. Exp. Med.*, 1963, **118,** 845.
2. Humphrey, J. H., and Fahey, J. L., The metabolism of normal plasma proteins and gamma-myeloma protein in mice bearing plasma-cell tumors, *J. Clin. Inv.*, 1961, **40,** 1696.
3. Sell, S., and Fahey, J. L., Relationship between γ-globulin metabolism and low serum γ-globulin in germfree mice, *J. Immunol.*, 1964, **93,** 81.
4. Havens, W. P., Dickensheets, J., Bierly, J. N., and Eberhard, T. P., The half-life of I^{131}-labeled normal human gamma globulin in patients with hepatic cirrhosis, *J. Immunol.*, 1954, **73,** 256.
5. Cohen, S., and Hansen, J. D. L., Metabolism of albumin and γ-globulin in kwashiorkor, *Clin. Sc.*, 1962, **23,** 351.
6. Lippincott, S. W., Korman, S., Fong, C., Stickley, E., Wolins, W., and Hughes, W. L., Turnover of labeled normal gamma globulin in multiple myeloma, *J. Clin. Inv.*, 1960, **39,** 565.
7. Solomon, A., Waldmann, T. A., and Fahey, J. L., Metabolism of normal 6.6S γ-globulin in normal subjects and in patients with macroglobulinemia and multiple myeloma, *J. Lab. and Clin. Med.*, 1963, **62,** 1.
8. Martin, C. H., Gordon, R. S., Felts, W. R., and McCullough, N. B., Studies on gamma globulin. I. Distribution and metabolism of antibodies and gamma globulin in hypogammaglobulinemic patients, *J. Lab. and Clin. Med.*, 1957, **49,** 607.
9. Barr, M., Glenny, A. T., and Randall, J. K., Concentration of diphtheria antitoxin in cord blood and rate of loss in babies, *Lancet*, 1949, **2,** 324.
10. Wiener, A. S., The half-life of passively acquired antibody globulin molecules in infants, *J. Exp. Med.*, 1951, **94,** 213.
11. Sell, S., γ-Globulin metabolism in germfree guinea pigs, *J. Immunol.*, 1964, **92,** 559.
12. Bloch, K. J., Kourilsky, F. M., Ovary, Z., and Benacerraf, B., Properties of guinea pig 7S antibodies. IV. Antibody response to *E. coli* bacteria, *Proc. Soc. Exp. Biol. and Med.*, 1963, **114,** 52.
13. Peterson, E. A., Wyckoff, M. M., and Sober, H. A., Gradient chromatography of human serum proteins and its application to the examination of "albumin" and "globulin" obtained by ammonium sulfate fractionation, *Arch. Biochem. and Biophysics*, 1961, **93,** 428.
14. McFarlane, A. S., Efficient trace-labelling of proteins with iodine, *Nature*, 1958, **182,** 53.
15. Edmondson, P. W., and Wyburn, J. R., The erythrocyte life-span, red cell mass and plasma volume of normal guinea pigs as determined by the use of ^{51}chromium, ^{32}phosphorus labelled di-isopropyl fluorophosphonate and ^{131}iodine labelled human serum albumin, *Brit. J. Exp. Path.*, 1963, **44,** 72.
16. Dresser, D. W., Specific inhibition of antibody production. II. Paralysis induced in adult mice by small quantities of protein antigen, *Immunology*, 1962, **5,** 378.
17. Dietrich, F. M., and Weigle, W. O., Induction of tolerance to heterologous proteins and their catabolism in C_{57}Bl/6 mice, *J. Exp. Med.*, 1963, **117,** 621.

18. Weigle, W. O., and Dixon, F. J., The elimination of heterologous serum proteins and associated antibody responses in guinea pigs and rats, *J. Immunol.*, 1957, **79,** 24.
19. Caraway, W. T., and Fanger, H., Ultramicro procedures in clinical chemistry, *Am. J. Clin. Path.*, 1955, **25,** 317.
20. Technical Bulletin No. TB6050A, Spinco Division, Beckman Instruments, Inc., April, 1958.
21. Matthews, C. M. E., The theory of tracer experiments with ^{131}I-labelled plasma proteins, *Physics Med. and Biol.*, 1957, **2,** 36.
22. Anderson, S. B., and Bjørneboe, M., Gamma globulin turnover in rabbits before and during hyperimmunization, *J. Exp. Med.*, 1964, **119,** 537.
23. Humphrey, J. H., The metabolism of homologous and heterologous serum proteins in baby rabbits, *Immunology*, 1961, **4,** 380.
24. Deichmiller, M. P., and Dixon, F. J., The metabolism of serum proteins in neonatal rabbits, *J. Gen. Physiol.*, 1960, **43,** 1047.
25. Humphrey, J. H., and McFarlane, A. S., Rate of elimination of homologous globulins (including antibody) from the circulation, *Biochem. J.*, 1954, **57,** 6.
26. Catsoulis, E. A., Franklin, E. C., Oratz, M., and Rothschild, M. A., Gamma globulin metabolism in rabbits during the anamnestic response, *J. Exp. Med.*, 1964, **119,** 615.
27. Humphrey, J. H., and Turk, J. L., Immunological unresponsiveness in guinea pigs. I. Immunological unresponsiveness to heterologous serum proteins, *Immunology*, 1961, **4,** 301.
28. Wostmann, B. S., and Olson, G. B., Precipitating antibody production in germfree chickens, *J. Immunol.*, 1964, **92,** 41.
29. Maurer, P., The cross reaction between albumins of different species and gamma globulins of different species, *J. Immunol.*, 1954, **72,** 119.
30. Fahey, J. L., and Sell, S., data in preparation.
31. Porter, R. R., The structure of gamma globulin and antibodies, *in* Symposium on Basic Problems in Neoplastic Diseases, (A. Gellhorn and E. Hirschberg, editors), New York, Columbia University Press, 1962, 177.
32. Fahey, J. L., Heterogeneity of γ-globulins, *Advances Immunol.*, 1962, **2,** 42.
33. Stanworth, D. R., personal communication.
34. Nossal, G. J. V., Genetic control of lymphopoiesis, plasma cell formation, and antibody production, *Internat. Rev. Exp. Path.*, 1962, **1,** 1.
35. Campbell, R. M., Cuthbertson, D. P., Matthews, C. M., and McFarlane, A. S., Behavior of ^{14}C- and ^{131}I-labelled plasma proteins in the rat, *Internat. J. Appl. Radiation Isotopes*, 1956, **1,** 66.
36. Benacerraf, B., Halpern, B. N., Stiffel, C., and Mouton, D., Phagocytosis of heat-denatured human serum albumin labelled with ^{131}I and its use as a means of investigating liver blood flow, *Brit. J. Exp. Path.*, 1957, **38,** 35.
37. Hyman, C., and Paldino, R. L., Possible role of the reticuloendothelial system in protein transport, *Ann. New York Acad. Sc.*, 1960, **88,** 232.
38. Freeman, T., Gordon, A. H., and Humphrey, J. H., Distinction between catabolism of native and denatured protein by the isolated liver after carbon loading, *Brit. J. Exp. Path.*, 1958, **39,** 459.

39. Thorbecke, G. J., and Benacerraf, B., The reticulo-endothelial system and immunologic phenomena, *Progr. Allergy*, 1962, **6,** 559.
40. Moore, R. D., Lamm, M. E., Lockman, L. A., and Schoenberg, M. D., Cellular aspects of the action of Freund's adjuvant in the spleen and lymph nodes, *Brit. J. Exp. Path.*, 1963, **44,** 300.
41. Schoenberg, M. D., Gilman, P. A., Mumaw, V., and Moore, R. D., Proliferation of the reticuloendothelial system and phagocytosis, *Exp. Mol. Path.*, 1963, **2,** 126.
42. Dixon, F. J., and Maurer, P. H., Effects of large infusions of heterologous serum proteins on the serum protein metabolism of rabbits, *J. Exp. Med.*, 1955, **101,** 233.