FACTORS CONTROLLING SERUM γ-GLOBULIN CONCENTRATION

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The mechanisms which regulate the serum level of γ-globulin (or other serum proteins) are poorly understood, although a balance between the rate of production (synthesis) and rate of removal (catabolism) must exist for the maintenance of a constant serum level. γ-Globulin synthesis has been demonstrated in plasmacytes and related cells of the spleen, lymph node, bone marrow, and other tissues following antigenic stimulation (1–3), and the turnover of γ-globulin has been determined by means of radioisotopically labeled protein. The anatomic and cellular sites of degradation are still unknown, however, and the mechanisms regulating the rate of γ-globulin catabolism are poorly understood.

Several observations indicate that the rate of γ-globulin catabolism may be related to the serum γ-globulin level. γ-Globulin catabolism is generally accelerated in patients with increased γ-globulin levels due to inflammatory diseases (4–9) or plasma cell malignancy (7–11). Similarly in mice with plasma cell tumors and large amounts of γ myeloma proteins, γ-globulin catabolism is accelerated (12). Conversely, prolonged γ-globulin survival has been found when the serum γ-globulin level is low (9, 11, 13, 14). Two variable factors, however, were present in these studies; *i.e.*, the amount of serum gamma globulin and the number of plasma cells. Patients with low γ-globulin levels had reduced numbers of plasma cells, and patients with large amounts of γ-globulin had increased numbers of plasma cells. Soons and Westenbrink (15), on the basis of isotopically labeled protein studies, obtained some evidence that plasma cells might be the site of γ-globulin destruction. If this is the case, then γ-globulin catabolism would be increased when the number of plasma cells is increased and decreased with plasma cell reduction. Because of the uncertainty about the roles of the plasma cells and the serum gamma globulin level, the present studies were undertaken to clarify the factors determining the rate of γ-globulin catabolism. Inbred BALB/c mice were used (*a*) to reduce genetic variability to a minimum, (*b*) because β_{2A}-globulins as well as γ-globulins from BALB/c mice were available in large quantities, (*c*) because transplantable

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plasma cell tumors are available in these mice, and the relative amount of plasma cells can be estimated in mice bearing subcutaneous transplants of plasma cell tumors, data which cannot be obtained in man, and (*d*) because parenteral administration of large quantities of gamma globulin fractions is feasible in the mouse, whereas quantitative and qualitative factors make this impractical in man. Tracer amounts of radioiodine-labeled normal mouse γ-globulin ($NM\gamma\text{-}I^{131}$) were used to measure γ-globulin catabolic rates in normal mice, in hyperimmune hypergammaglobulinemic mice, in hypergammaglobulinemic mice bearing transplantable plasma cell tumors, and in normal mice receiving parenterally administered γ-globulins and other serum proteins.

Methods and Materials

Mice.—BALB/c mice were obtained from the animal production section, National Institutes of Health. Female mice, 3 months or more of age and weighing 20 to 25 gm, were used in these studies.

Ten mice were immunized by injecting a total of 0.6 mg hemocyanin in six progressively enlarging parenteral injections of alum-precipitated hemocyanin to each mouse. A second group of ten mice was immunized by intraperitoneal injection of 1 mg hemocyanin and Freund's adjuvant and after 6 months given injections of 1 mg of alum-precipitated hemocyanin 1 and 2 weeks prior to the gamma globulin turnover studies.

Plasma Cell Tumors.—Plasma cell tumor lines MPC-1 (16) and MPC-11 were obtained from Dr. Ruth Merwin, and plasma cell tumor line Adj.PC-5 (17) was obtained from Dr. Michael Potter of the National Cancer Institute. The tumors were maintained by subcutaneous implantation of tumor pieces with a trocar. Tumor mice appear to be in good general health until the tumor exceeds 5 gm. These mice show no morphologic abnormalities in the kidney. The proteins produced by the MPC-11 and Adj.PC-5 tumors are 6.6S γ-type globulins by immunochemical and physicochemical characteristics (17–19), and they migrate as gamma globulins on zone electrophoresis. The MPC-1 myeloma protein is more rapidly migrating and has immunochemical and physicochemical features of β_{2A}-globulin (18, 19). The quantity of myeloma protein and of normal gamma globulin was determined by quantitative paper electrophoresis (19) of serum obtained at the end of the turnover study.

Proteins for Injection.—Mouse γ myeloma protein was isolated by diethylaminoethyl (DEAE) cellulose chromatography of serum obtained from mice bearing the MPC-11 plasma cell tumor. Normal human gamma globulin fractions obtained from commercial sources had been prepared by ethanol fractionation (Lederle Laboratories, Pearl River, New York) and by salting out procedures (Courtland Laboratories, Los Angeles) and stored in sterile solution until used. The normal human γ-globulin preparation obtained by salt fractionation procedures induced a more rapid catabolism than the ethanol-prepared human γ-globulin or the other γ-globulin preparations. For example, (as discussed below) a 20 mg/day dose of the ethanol preparation produced a T½ (half-time) of 1.9 days and the salt preparation caused a 1.4 day half-time for mouse γ-globulin. The basis for this difference is uncertain, but a second ethanol preparation of normal human γ-globulin at a dose of 20 mg/day also induced a T½ of 1.9 days for $NM\gamma\text{-}I^{131}$. Human γ myeloma protein (type I) was prepared by DEAE cellulose chromatography from the serum of a patient (E.C.) with multiple myeloma. Human β_{2A} myeloma protein (type II) was prepared by a combination of zone electrophoresis and DEAE cellulose chromatography from the serum of a patient (G.F.) with multiple myeloma. Human gamma macroglobulin (type I) was obtained from the serum of a patient with primary (Waldenström's) macroglobulinemia by precipitating the macroglobulin from serum by

addition of ammonium sulfate to 33 per cent of saturation, reprecipitating twice with ammonium sulfate and fractionating the macroglobulin concentrate by DEAE cellulose chromatography to obtain γ_1-macroglobulin essentially free of 6.6S γ-globulin. The procedures used to obtain these purified proteins are described in detail elsewhere (20).

The protein preparations were characterized by paper electrophoresis, immunoelectrophoresis, starch gel electrophoresis, and, in some cases, by ultracentrifugation (18–20). The mouse and human myeloma proteins and macroglobulinemic macroglobulins were essentially free of contaminating serum proteins. The normal human gamma globulin preparations were composed largely of 6.6S γ-globulin but contained up to 10 per cent of the total protein as other serum protein components. The albumin preparation was at least 80 per cent albumin and contained no detectable gamma globulin.

Preparation of S and F Pieces of γ-Globulin.—Normal human 6.6S γ-globulin preparations and mouse γ myeloma protein were treated with papain and cysteine as described by Porter (21) except that human γ-globulin was treated for only 6 hours and the mouse γ myeloma protein for only 1 hour. The S (slow) and F (fast) pieces were separated by DEAE cellulose chromatography (22) and shown by immunoelectrophoresis and Ouchterlony tests to be free of detectable intact 6.6S γ-globulin.

Proteins for Radioiodine Labeling.—Normal mouse gamma globulin was prepared from pooled serum of normal adult BALB/c mice. Zone electrophoresis was carried out on polyvinyl particle blocks (composed of a mixture of polyvinyl chloride and polyvinyl chloride–polyvinyl acetate copolymer particles (20, 23). Serial fractions from the gamma globulin region were individually tested for the presence of gamma globulin and other serum proteins by the Ouchterlony technique (agar double diffusion employing potent rabbit antisera against normal mouse serum and against normal mouse gamma globulin). Those gamma globulin fractions which were free from contaminating protein were pooled and concentrated by ultrafiltration and tested for purity by starch gel electrophoresis and immunoelectrophoresis employing rabbit antinormal mouse serum. The characteristics of the normal mouse gamma globulin preparation (NMγ) are seen in Fig. 1. Ultracentrifugation revealed that most of the protein sedimented ($s^{\circ}_{20,w}$) at about 6.6S. A small amount of a beta globulin component was detected by immunoelectrophoresis, but the bulk of the protein had the features of 6.6S γ-globulin. Gamma myeloma proteins from mice bearing the MPC-11 or Adj.PC-5 plasma cell tumor were isolated by DEAE cellulose chromatography and characterized by starch gel electrophoresis, immunoelectrophoresis, and ultracentrifugation, and shown to be 6.6 γ myeloma proteins.

Iodination with I^{131}.—Iodination was performed by the iodine monochloride method of McFarlane (24). The amount of iodine introduced corresponded to 0.5 to 1.5 atom per molecule of protein, taken as 160,000 molecular weight for normal mouse γ-globulin and for γ myeloma protein. Approximately 60 per cent of the added iodine became attached to the protein. After iodination, free iodine was removed by dialysis against sterile saline, and normal mouse serum was added so that the total radioactivity was less than 10 μc per mg of protein. The iodinated protein was tested by starch gel electrophoresis, the starch gel being cut into 5 mm segments which were counted individually in a gamma scintillation counter, and the relative radioactivity was related to the distribution of protein as determined by amido-black staining of the opposing half of the starch gel. As seen in Fig. 1, the distribution of radioactivity corresponded to the γ-globulin region of mouse serum. Three normal mouse gamma globulin preparations were used in these studies, and each was shown to have the characteristic properties of the gamma globulins in normal serum.

Experimental Protocols.—Mice were housed in cages with wire floors which allowed urine and fecal droppings to pass through to a tray below. Three or four mice were in each cage and were provided with drinking water containing 0.45 per cent NaCl and 0.01 per cent KI in

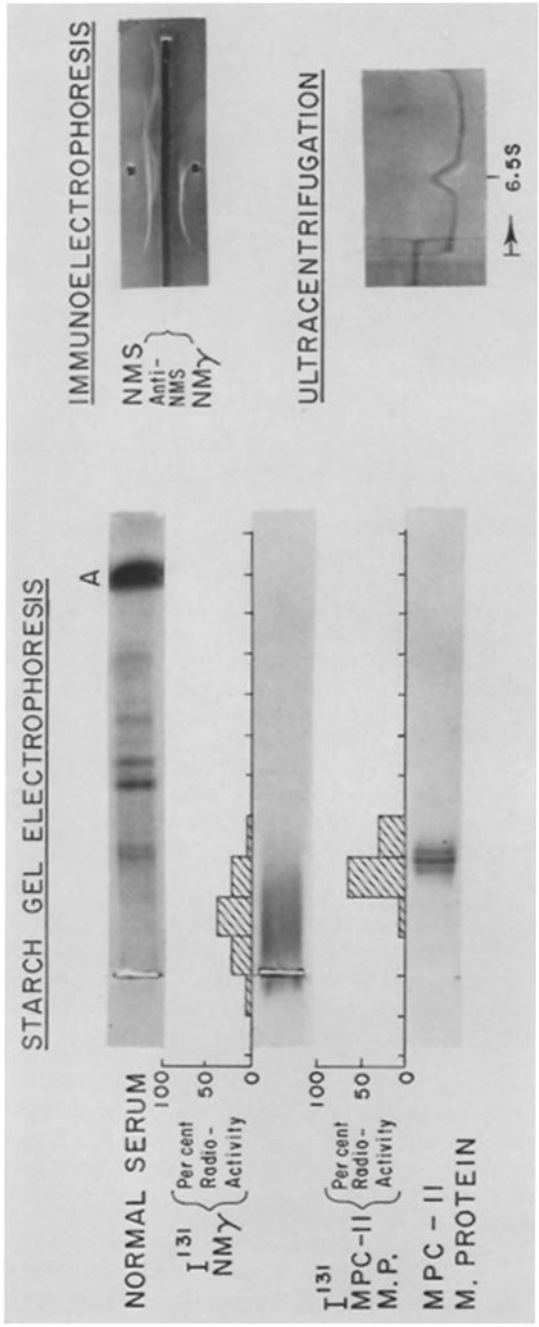

FIG. 1. Physicochemical and immunochemical characteristics of mouse γ-globulins labeled for metabolic studies. Characteristics of normal 6.5S γ-globulin-I^{131}. Normal γ-globulin (NMγ) prepared by zone electrophoresis on pevikon® blocks was characterized by paper electrophoresis and starch gel electrophoresis. The ultracentrifugation photo was obtained 42 minutes after reaching 59,780 RPM with a cell containing 3 mg protein/ml in 0.14 M NaCl. The radioactivity distribution after starch gel electrophoresis and counting 5 mm wide gel sections is graphically illustrated. Preparations of γ myeloma proteon (MPC-11) were similarly characterized but only the data for starch gel electrophoresis and radioiodine label distribution are illustrated.

order to accelerate renal excretion of free iodine and to reduce uptake of I^{131} by the thyroid gland. Whole body radioactivity was measured daily or more frequently in a gamma ray bulk spectrometer (Sharpe Laboratories, LaJolla). Counting was continued long enough to give an accuracy of at least ±5 per cent. All figures were corrected for radioactive decay.

Mice were injected intravenously with 0.3 μc of I^{131} labeled mouse γ-globulin (NMγ-I^{131}) in 0.1 ml saline. Each animal was counted within 60 minutes of injection, then at daily intervals. Data for each animal were calculated separately. Counting was continued until at least one biological half-time survival of the radioiodinated protein was complete.

Each plasma cell tumor line (γ-tumors MPC-11 and Adj.PC-5, and β_{2A} tumor MPC-1) was tested in two separate experiments. In the first, three or four mice bearing tumors weighing 2 to 5 gm were studied. Subsequently, tumors were transplanted to a large number of mice. From this group were selected mice with tumor sizes ranging from none or barely detectable to approximately 3 gm tumor weight. This second group of turnover studies included mice with small tumors and serum myeloma protein levels in a range which also could be attained by parenteral protein injection.

Mice receiving exogenous protein were given NMγ-I^{131} 1 or 2 days prior to starting the injections of exogenous protein. Most unlabeled protein injections were given intraperitoneally although in the case of human macroglobulins and human β_{1A}-globulins the initial loading dose was given intravenously. An initial loading dose five times greater than the maintenance dose was given on the 1st day of protein injection. Subsequently a daily maintenance dose of protein was given, and it is this maintenance dose which is recorded in the graphic presentation of results. For example, an animal listed as receiving 1 mg of normal human gamma globulin per day had been given 5 mg on the 1st day and subsequently received 1 mg of protein per day. A maintenance dose was given for 3 successive days, and the following day the experiment was terminated when the animal was weighed, bled, and the serum saved for protein determinations.

Methods of Calculation.—Plasma volumes were calculated from the radioactivity of blood samples taken 2 to 3 minutes after injection of known amounts of radioactive protein into a tail vein and from a knowledge of the hematocrit. The intravascular γ-globulin was calculated from the plasma volume and serum concentration data. The fraction of γ-globulin present in the intravascular plasma pool in relation to the total body γ-globulin content was calculated from a determination of the plasma radioactivity present 24 hours after injection, and compared with the total body radioactivity at the same time. The catabolism of γ-globulin was determined by daily measurement of the total body radioactivity. In most experiments the radioactivity fell as a straight line function for 1 week when plotted on semilogarithmic graph except in two experiments using γ-globulins which had been stored at 5°C for more than 1 week. In these cases, an additional 10 to 20 per cent of the radioactivity was removed during the first 24 hours, presumably due to removal of altered protein, but within 24 hours of injection, the decay curves became straight lines with normal T½ values. The per cent I^{131} protein degraded per day was obtained by dividing the observed half-time into 0.693. The quantity of γ-globulin in the circulation, the total amount of exchangeable γ-globulin, and the quantity of γ-globulin degraded per day were calculated as previously described (12). The quantity of γ-globulin degraded per day was determined from the calculation of the total body γ-globulin and the fraction catabolized per day.

RESULTS

Turnover of γ-Globulin in Normal BALB/c Mice.—Normal 6.6S γ-globulin prepared from BALB/c mice and labeled with radioiodine (NMγ-I^{131}) was administered intravenously on eight occasions to a total of twenty-two normal

BALB/c mice. The whole body radioactivity decay curve for a typical experiment is shown in Fig. 2. The observed T½ ranged from 3.7 to 4.8 days with an average value of 4.1 days. The fractional rate of γ-globulin catabolism per day was 0.169.

The plasma volume was found to be 5 per cent of the body weight, and 49 per cent of the total body γ-globulin-I^{131} was calculated to be in the intra-

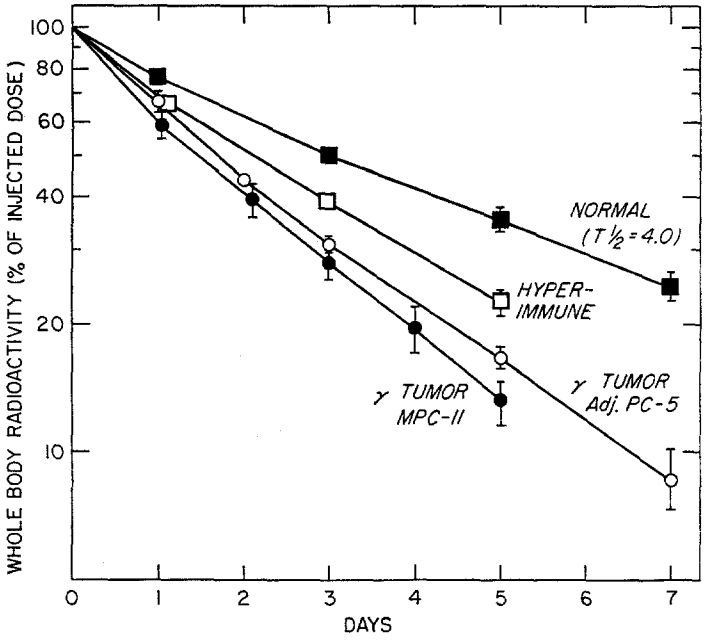

FIG. 2. Turnover of normal mouse γ-globulin-I^{131}. Catabolic Decay Curves of radioiodine-labeled normal mouse γ-globulin (NMγ-I^{131}). Median and range of observed data for three or more mice in each group are indicated. The hyperimmune mice represent a group of three with a mean γ-globulin level of 1.56 gm per cent. The plasma cell tumors MPC-11 and Adj. PC-5 produce γ myeloma proteins. The MPC-11 tumors weighed 2.5 to 5.0 gm with a mean value of 3.8 gm. The Adj.PC-5 tumors weighed 1.0 to 4.0 (mean 2.5) gm.

vascular pool. The normal serum γ-globulin level was 0.5 gm per cent and the mean total body γ-globulin was 12.5 mg/25 gm mouse.

On the basis of this data and the rate of γ-globulin catabolism determined above, normal BALB/c mice were calculated to degrade (and, presumably, synthesize) approximately 2.1 mg γ-globulin per day per 25 gm mouse; *i.e.*, 0.084 gm/kg/day.

Hyperglobulinemia in Hyperimmunized Mice.—Sixteen mice, previously immunized with hemocyanin, were injected intravenously with NMγ-I^{131} and the half-time determined for each mouse. The observed half-times for NMγ-I^{131}

ranged from 4.3 to 1.9 days in these mice. The mice were then grouped according to their observed T½ values, with two to four mice in each of the five groups. The mice were sacrificed, the sera pooled for each group, and the serum gamma globulin levels determined.

The serum gamma globulin levels and T½ values are compared in Table I. The γ-globulin half-time is seen to become progressively shorter as the serum gamma globulin levels rise. Accelerated γ-globulin catabolism is readily evident in Fig. 2 where the whole body radioactivity curves for the three hypergammaglobulinemic mice included in group D are plotted.

These observations indicated that the γ-globulin catabolic rate might be related to the level of serum γ-globulin or to the number of plasma cells which

TABLE I

Comparison of NMγ-I^{131} Half-Life with Serum γ-Globulin Levels in Immunized Mice

Group	No. of mice	T½	Serum γ-globulin	γ-Globulin catabolized (synthesized)
		days	*gm per cent*	*mg/25 gm body weight/day*
A	4	4.0–4.3	0.50	2.1
B	2	3.5–3.6	0.72	3.6
C	3	3.3	0.68	3.6
D	3	2.5–2.7	1.56	10.4
E	4	1.9–2.2	2.80	24.3

had increased in response to antigenic stimulation. In normal and hyperimmune mice, it was not feasible to measure the number of plasma cells, but in mice bearing transplantable plasma cell tumors the relative quantity of malignant plasma cells can be estimated by tumor measurements.

Hyperglobulinemia (γ Myeloma Protein) in Mice with Plasma Cell Tumors.—Catabolism of NMγ-I^{131} was accelerated in BALB/c mice bearing plasma cell tumors producing γ myeloma proteins. Half-times of 4.4 to 1.5 days were observed in eighteen mice bearing MPC-11 tumors and in twenty-one mice with Adj.PC-5 tumors (Fig. 3). The observed half-times could be correlated roughly both with the size of the tumor and with the quantity of γ myeloma protein (Fig. 3); the shortest half-times were found in mice with the largest tumors and the greatest amounts of serum γ myeloma protein.

The tumor studies, however, did not indicate whether the increased number of γ-globulin producing plasma cells or the increased serum γ-globulin level was responsible for the accelerated catabolism of γ-globulin. Therefore, the effects of individual proteins on γ-globulin metabolism were investigated.

Effect of Exogenous γ-Globulin.—Purified mouse γ myeloma protein MPC-11 was prepared in large quantity for parenteral administration. This protein was

injected intravenously or intraperitoneally 1 or 2 days after the intravenous injection of NMγ-I^{131}; *i.e.*, after equilibration of the labeled γ-globulin between intravascular and extravascular γ-globulin pools was complete. The initial loading dose was five times larger than the daily maintenance dose.

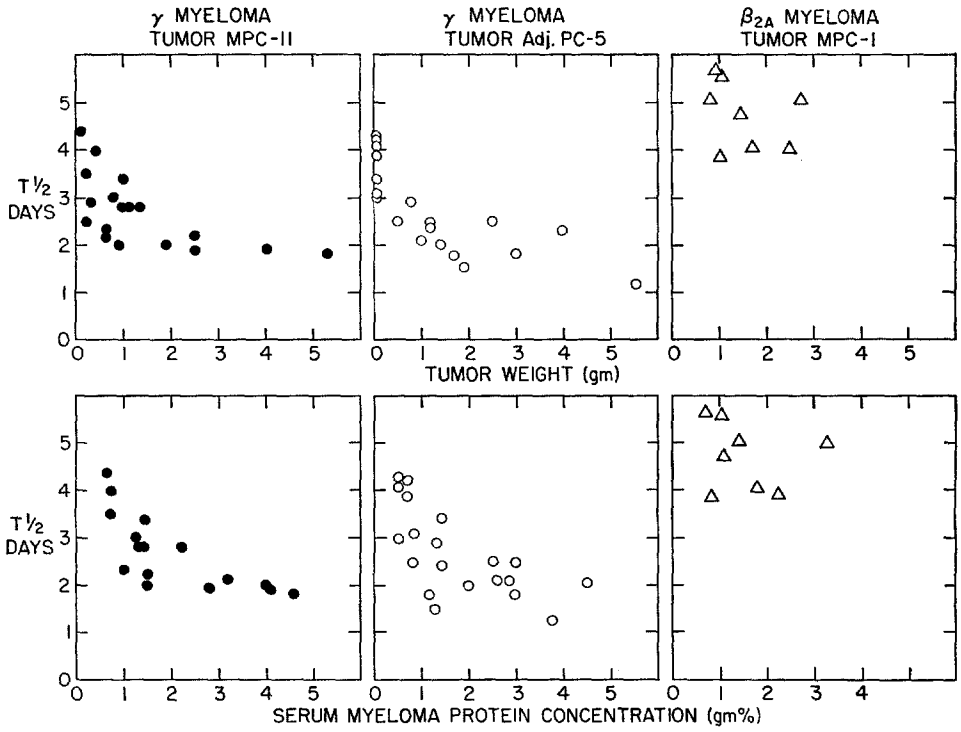

FIG. 3. Relation of NMγ-I^{131} Half-time (T½) to tumor weight and serum myeloma protein concentration. After determining the T½ values, individual mice were sacrificed, tumors weighed, and the serum analyzed by paper electrophoresis. With the γ myeloma protein tumors, MPC-11 and Adj.PC-5, the serum myeloma protein value includes the normal serum γ-globulin. In several mice that had received subcutaneous implants of the Adj.PC-5 tumor, no tumor or serum myeloma protein was evident at the time of sacrifice, thus accounting for the normal serum γ-globulin levels in this experiment. In the mice with the MPC-1 plasma cell tumor, which produces a β_{2A} myeloma protein, the data for myeloma protein concentration does not include the normal serum γ-globulins.

The effect of injected mouse γ-globulin (γ myeloma protein MPC-11) on the catabolism of normal mouse γ-globulin is recorded in Table II. As little as 1 mg of γ myeloma protein reduced the half-time of NMγ-I^{131} from 3.8 to 3.3 days. With increasing amounts of γ myeloma protein, the half-time of normal γ-globulin became progressively shorter, until values of 1.8 to 2.2 days were consistently observed.

TABLE II

Effect of Injected Mouse γ-Globulin (γ-MP MPC-11) on Catabolism of Normal Mouse γ-Globulin

Amount of γ myeloma protein given i.p.* daily	T½‡
mg	*days*
Control§	3.8
1	3.3
2	2.7
4	2.6
10	1.8
20	1.6

* Initial dose of γ myeloma protein given intraperitoneally (i.p.) was five times greater than the maintenance dose listed.

‡ Average values for 3 mice in each group, except the last, which included 2 mice.

§ 1 ml isotonic saline injected daily.

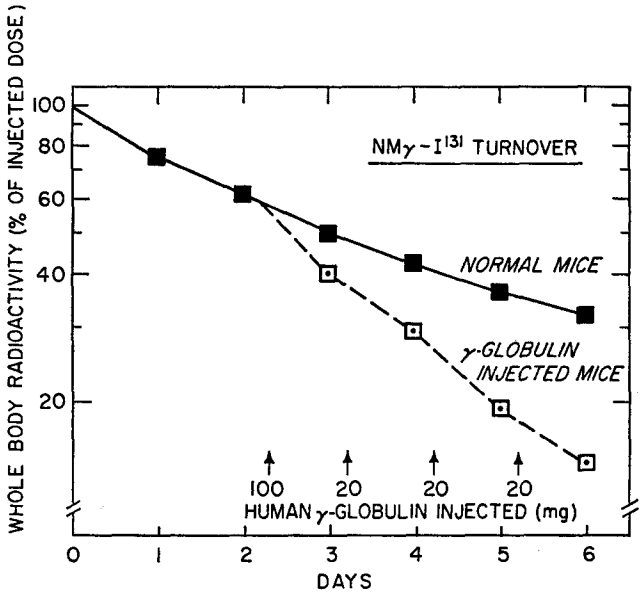

FIG. 4. Effect of γ-globulin injections on the half-times (T½) of NMγ-I^{131}. Normal mice were injected intravenously with 0.1 mg of NMγ-I^{131}. Two days later 100 mg of normal human γ-globulin (0.65 ml) was injected intraperitoneally and then 20 mg daily thereafter. Several mice from the original group that received I^{131}-labeled protein were not injected with human γ-globulin and served as controls.

Injection of human γ-globulins also reduced the biological half-time of mouse γ-globulin. The effect of normal human γ-globulin on the survival of NMγ-I^{131} is graphically illustrated in Fig. 4. Following intraperitoneal injection of human γ-globulin, a marked acceleration of NMγ breakdown is evident

within 24 hours. Mouse γ-globulin catabolism continues at a rapid rate with continued human γ-globulin injections. The effect of human γ-globulin in increasing the catabolism of mouse γ-globulin was confirmed in six separate studies using normal human γ-globulins prepared by ethanol fractionation or by salting-out fractionation procedures or using γ myeloma protein prepared by DEAE cellulose chromatography.

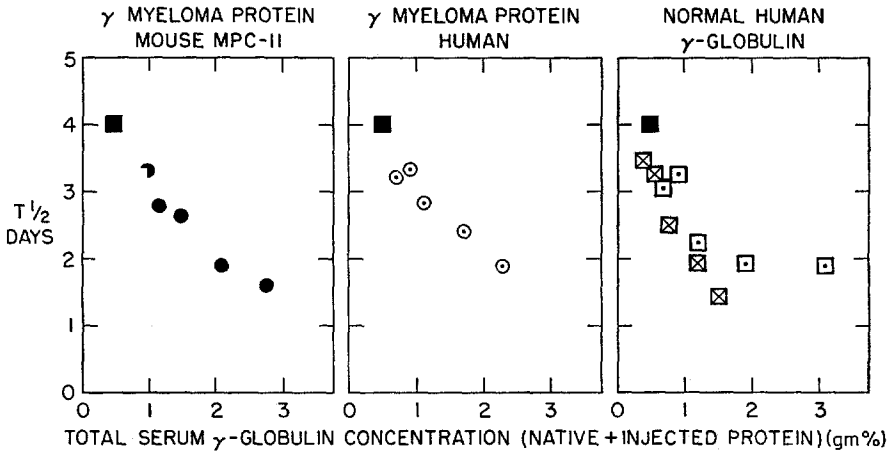

FIG. 5. Effect of γ-globulin injection on the half-time (T½) of NMγ-I^{131}. Labeled normal mouse γ-globulin (NMγ-I^{131}) was injected intravenously into normal BALB/c mice. Two days later injections of the proteins listed at the top of each box were begun and continued for 3 or 4 days with maintenance doses of 1, 2, 4, 10, or 20 mg per day (the initial, loading dose was five times greater in each instance). The T½ of the NMγ-I^{131} was determined and compared with the total serum γ-globulin concentration (including normal plus injected protein) as determined by paper electrophoresis. Two studies were made with normal human γ-globulin preparations:

⊠, Prepared by salt fractionation; ⊡, prepared by ethanol fractionation of human plasma... ■, mean control value; ●, MPC-11 mouse γ myeloma protein injected; ⊙, human γ myeloma protein injected.

The effects of three γ-globulins on the catabolism of NMγ-I^{131} are illustrated in Fig. 5. The T½ of NMγ-I^{131} is compared to the total serum γ-globulin concentration at the end of each experiment, a value which includes the endogenous normal γ-globulin and the exogenously administered protein (Fig. 6). In each case the T½ values fall as the serum γ-globulin level rises, and the different γ-globulin preparations are seen to produce similar responses in the catabolism of NMγ-I^{131}.

The γ myeloma proteins MPC-11 and Adj.PC-5 were isolated and labeled with I^{131}. Their survival in normal mice was found to be similar to that of normal γ-globulins. In mice with γ-type plasma cell tumors or in mice given exog-

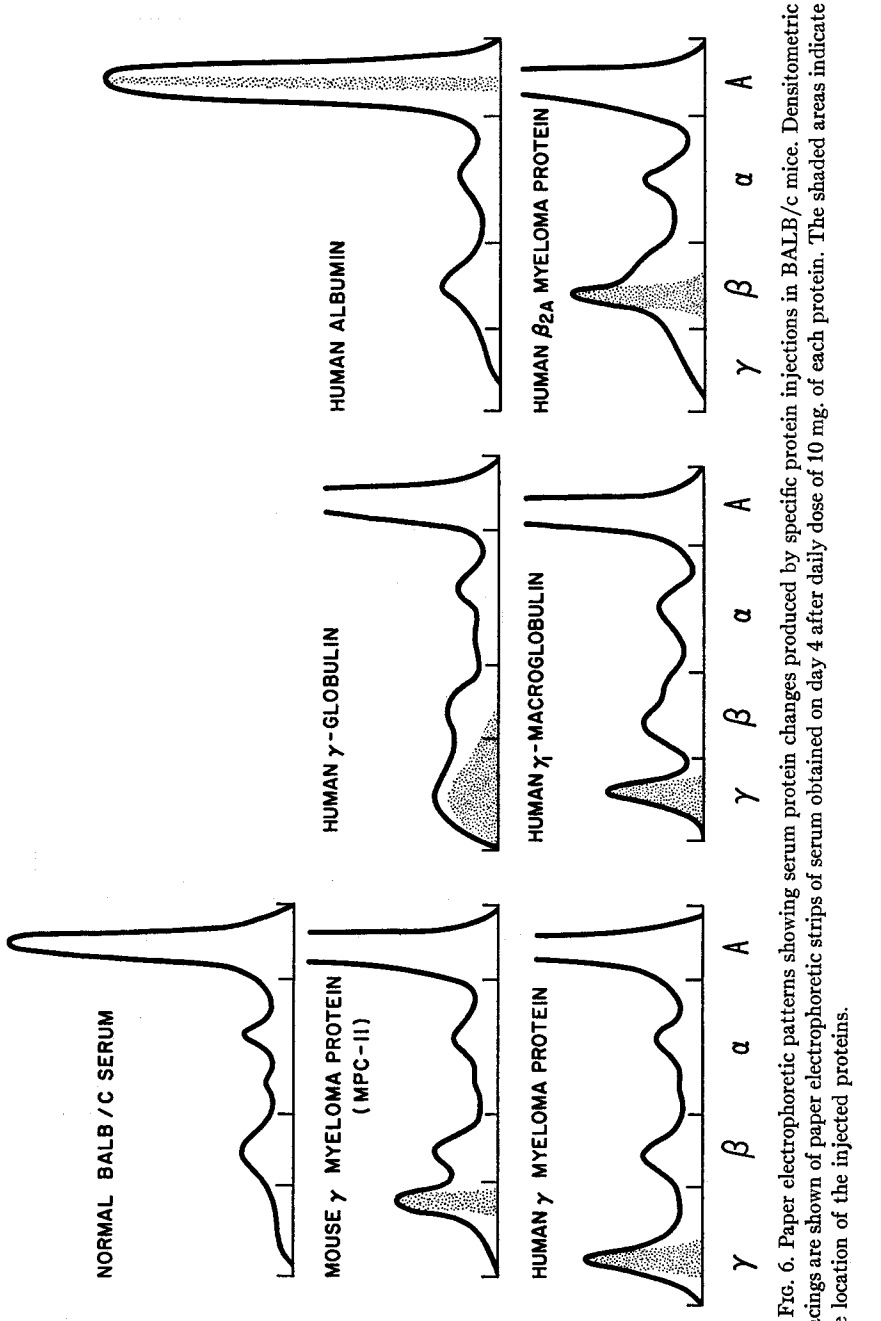

FIG. 6. Paper electrophoretic patterns showing serum protein changes produced by specific protein injections in BALB/c mice. Densitometric tracings are shown of paper electrophoretic strips of serum obtained on day 4 after daily dose of 10 mg. of each protein. The shaded areas indicate the location of the injected proteins.

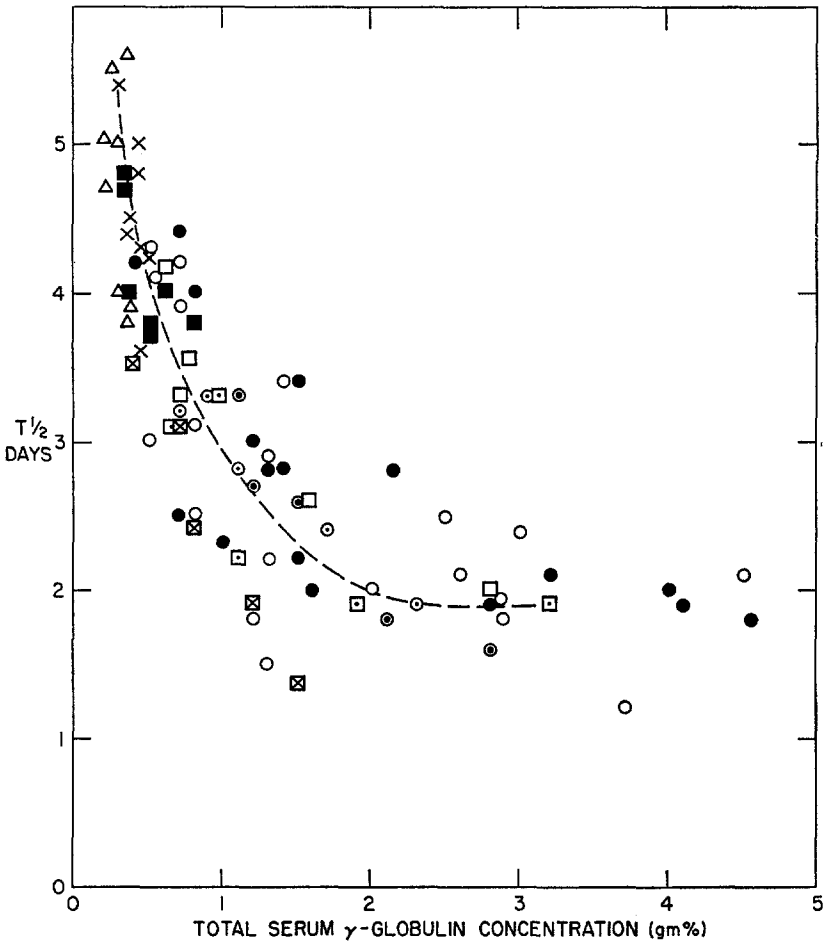

FIG. 7. Comparison of NMγ-I^{131} half-time (T½) with total serum γ-globulin level. Observation on 85 groups of mice or individual mice are recorded. The total γ-globulin concentration includes the normal γ-globulin plus γ myeloma protein or injected γ-globulin if present. The conditions of the study are indicated by the code: ■, normal BALB/c mice; □, immunized; ●, MPC-11 γ plasma cell tumor; ○, Adj.Pc-5 γ-plasma cell tumor; △, MPC-1 β_{2A} plasma cell tumor; ⊠ ⊡, injected normal human γ-globulin (two experiments); ⊙, injected human γ myeloma protein; ×, injected human albumin; ◉, injected MPC-11 myeloma protein.

enous γ-globulin, the catabolism of both myeloma proteins was increased, and the dimensions of the changes in labeled myeloma protein catabolism were similar to those seen with labeled normal γ-globulin. These observations indicate that γ myeloma proteins and normal γ-globulins are susceptible to the same processes controlling the rate of γ-globulin catabolism.

Comparison of γ-Globulin Survival (T½) and Serum γ-Globulin Levels.—The half-time of NMγ-I^{131} is compared to the serum gamma globulin level (normal gamma globulin or normal gamma globulin plus γ myeloma protein) in Fig. 7. The impression that γ-globulin catabolism is progressively accelerated with increasing γ-globulin levels (noted above in individual experiments) is amply confirmed in the composite data.

In the lower range of serum γ-globulin levels, *i.e.*, at γ-globulin levels below 0.4 gm per cent, T½ of 5 days or more is seen (Fig. 7). Survival half-time of

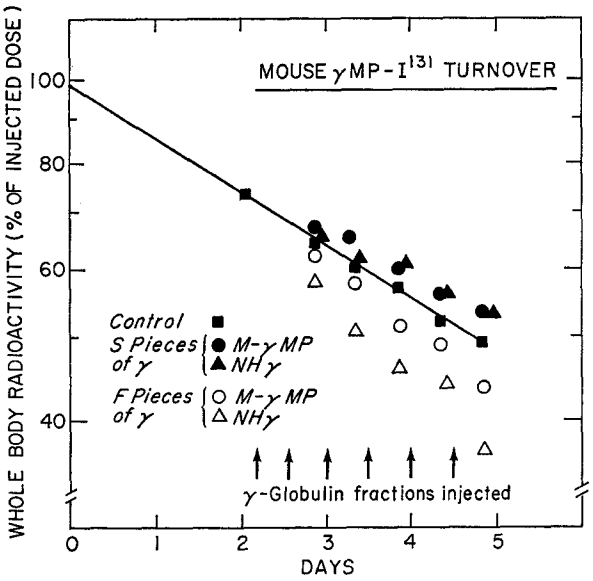

FIG. 8. Effect of S and F pieces of γ-globulins on the catabolism of I^{131}-labeled γ-globulin. Alterations in γ-globulin turnover produced by injection of S and F pieces obtained by papain treatment of γ-globulin. Mouse γ myeloma protein Adj.PC-5 labeled with I^{131} was injected intravenously into normal BALB/c mice and 2 days later intraperitoneal injections of purified S or F pieces were started and repeated at 12 hour intervals.

normal γ-globulin shortened as γ-globulin levels rose, reaching T½ values of 1.8 to 2.0 days at serum γ-globulin levels of 2 gm per cent. Further increases in serum γ-globulin concentration above 2 gm per cent had progressively smaller effects on γ-globulin half-time. These observations indicate that the fractional rate of γ-globulin catabolism approaches a maximum; *i.e.*, the greatest rate of γ-globulin catabolism is approximately 40 per cent of total body γ-globulin per day in BALB/c mice.

Molecular Location of Catabolic Configuration.—γ-Globulin preparations were split into S (slow) and F (fast) pieces by treatment with papain and cysteine. Normal human γ-globulin and mouse γ myeloma protein Adj.PC-5 were treated

in this manner and the S and F pieces separated by DEAE cellulose chromatography. The effect of injection of these materials on labeled γ-globulin (I^{131}-Adj.PC-5 γMP) turnover in normal mice is seen graphically in Fig. 8.

F (fast) pieces from mouse γ myeloma protein or from normal human γ-globulin accelerated the catabolism of the mouse γ myeloma protein in the three mice given F piece injections. F pieces from human γ-globulin were given in two dose levels, and the larger amount caused a greater increase in γ-globulin catabolism (Table III). The F piece represents about one-third of the intact γ-globulin molecule (21, 25, 26). Therefore, the effect of F piece administration

TABLE III

*Effect of F and S Pieces (Obtained by Papain Digestion of γ-Globulin) on the Turnover of Intact γ-Globulin**

	Control	F pieces			S pieces		
		γMP (mouse)	Normal γ-globulin (man)		γMP (mouse)	Normal γ-globulin (man)	
Dose, *mg/day*‡	—	2, 1, 1	2, 1, 1	3, 2, 2	4, 2, 2	4, 2, 2	6, 4, 4
Equivalent amount of intact γ-globulin	—	3	3	6	3	3	6
T½, *days*	4.7	3.6	3.0	2.4	5.7	6.2	6.3

* I^{131}-labeled Adj.PC-5 mouse γ myeloma protein was injected intravenously into normal BALB/c mice 2 days prior to starting intraperitoneal injections of F or S pieces.

‡ The daily dose of S and F pieces was divided into two equal amounts which were injected intraperitoneally at 12 hour intervals. The total daily dose given on each of 3 days is recorded.

at a dose of 1 and 2 mg per day may be compared to the effect of 3 and 6 mg of intact γ-globulin per day (Table III). On this basis, the effect of F piece injection appears to be approximately equivalent to the effect of intact γ-globulin in accelerating γ-globulin catabolism.

S pieces from human and mouse γ-globulins failed to increase γ-globulin catabolism (Fig. 8). Indeed, γ-globulin survival appeared to be prolonged by injection of S pieces (Table III). The mechanism by which this is achieved is not clear. At the conclusion of the experiment, the mice receiving these injections were sacrificed and the kidneys examined by hemotoxylin and eosin staining of paraffin blocks and by immunofluorescent examination of frozen sections (27). No renal tubular or glomerular damage was evident in the mice receiving either S or F pieces. The renal tubules were free of detectable quantities of protein, although appropriate proteins and protein pieces were found intravascularly, both by immunofluorescent and immunoelectrophoretic studies. Thus there was no morphologic evidence of renal lesions which might impair iodide excretion or promote proteinuria.

Effects of β_{2A}-Globulin, γ_1-Macroglobulin, and Albumin.—Human β_{2A} myeloma protein, γ_1-macroglobulin, and albumin were injected in parallel experiments. As seen in Table IV, these proteins failed to accelerate the catabolism of NMγ-I^{131}, although appreciable amounts of these proteins were detected by paper electrophoresis (Fig. 6) and immunoelectrophoresis in the sera of injected mice.

The β_{2A} myeloma protein tumor (MPC-1) of the mouse, also, failed to accelerate the catabolism of NMγ-I^{131} (Fig. 3). Half-times of 3.8 to 6.7 days (average = 4.8 days) indicate that γ-globulin survival may be prolonged in the presence of large quantities of mouse β_{2A}-globulin.

TABLE IV

Effect of Injected Proteins on Catabolism of Normal Mouse γ-Globulin

Protein injected*	T½
	days
Control	4.1
Mouse γ myeloma protein	1.8
Human normal γ-globulin	1.8
Human γ myeloma protein	2.4
Human β_{2A} myeloma protein	4.1
Human γ_1-macroglobulin	4.4
Human serum albumin	4.6

* 10 mg per day given intraperitoneally (except human γ_1-macroglobulin, which was given intravenously) as maintenance dose. Initial dose was 50 mg in each case.

Effects of Plasma Cells.—The data in Fig. 3 indicate that the large numbers of plasma cells in the β_{2A}-type tumor (MPC-1) did not increase γ-globulin catabolism. Further, as seen in Fig. 7, γ-globulin catabolism is about the same in mice with normal plasma cell numbers and elevated γ-globulin levels (due to exogenously supplied γ-globulin) as in mice with increased plasma cells and increased γ-globulins (hyperimmunized mice and mice with γ-type plasma cell tumor). These observations indicate that the numbers of plasma cells probably have little direct effect on γ-globulin catabolism.

DISCUSSION

The present observations indicate that the rate of γ-globulin synthesis is the primary factor determining the serum γ-globulin level. As synthetic rates increase, the serum γ-globulin levels rise and catabolic processes accelerate. The mechanisms controlling catabolic rates, however, lag sufficiently that the serum γ globulin level rises to a new level before catabolic rates match synthetic rates.

Synthesis of gamma globulin appears to depend largely on antigenic expe-

rience. In germ-free animals with very little exposure to antigenic substances, the serum gamma globulin levels are low, and may be as low as 10 per cent of normal (28, 29). With exposure to infection, serum gamma globulin levels rise in germ-free animals (28, 29), presumably due to increased synthesis of γ-globulins. Evidence that immunization causes an increase of γ-globulin synthesis in normal mice is seen in Table I. γ-Globulin synthetic rates were found to be increased to as much as twenty times normal in the immunized mice. Associated with the increase of synthetic rate was an increase in the serum γ-globulin level. γ-Globulins from a variety of sources, such as those (*a*) synthesized endogenously in normal response to antigenic stimulation, or (*b*) synthesized in plasma cell tumors, or (*c*) provided from exogenous sources, will all raise the total serum γ-globulin level. Synthetic rate appears to be the primary factor determining the serum γ-globulin level, except in a few pathologic states as noted below. Whether factors other than antigenic experience influence serum γ-globulin synthesis, however, is uncertain.

The importance of factors other than synthetic rate in determining serum γ-globulin levels is illustrated by a comparison of mice, rabbits, and man. Normal mice synthesize about 85 mg of γ-globulin per kg of body weight each day as shown in the present study. Rabbits synthesize about 80 mg/kg (calculated from data in references 30–32) and man about 36 mg/kg each day (11, 33). Yet the serum γ-globulin levels in these species do not reflect the synthetic rates, for the serum levels in mouse, rabbit, and man are 0.5, 0.75, and 1.2 gm per cent, respectively. The differences between the relative synthetic rate and the serum γ-globulin levels, however, are accounted for by the differences in catabolic rates. The per cent of the total body γ-globulin catabolized daily is about 17, 12, and 3 per cent respectively in mouse, rabbit, and man. Although the mouse has a high rate of γ-globulin synthesis, it has the highest relative rate of γ-globulin catabolism and, as a result, has the lowest serum γ-globulin level of the three species.

Species differences in γ-globulin catabolism are related, at least in part, to differences in metabolic rate. Observations on γ-globulin catabolism in various species (30, 34, 35) indicate that the species with the highest metabolic rates had the most rapid rates of γ-globulin catabolism. This view is supported by the increase of γ-globulin catabolism during thyroxin administration (30) and fever (11).

Factors, in addition to variations in metabolic rate, also operate to determine the rate of γ-globulin catabolism within a species. The serum γ-globulin level is one factor as shown in the present studies. Disease may increase γ-globulin catabolism by a febrile increase of total body metabolism and by antigenic stimulation and inflammatory processes which increase the serum γ-globulin level. Certain diseases of the gastrointestinal tract (36) and kidney (37), which cause excessive γ-globulin losses may markedly reduced serum γ-globulin levels.

Catabolic rates apparently have a limit, in the sense that a maximal fractional rate of γ-globulin catabolism is approached. As γ-globulin levels increase in the mouse, the fraction of the total body γ-globulin pool which is catabolized daily may increase to 40 per cent per day. This fractional catabolic rate is approached when the serum γ-globulin level in the mouse reaches 2 gm per cent. Further increases in serum level have little, if any, effect on increasing the fractional catabolic rate.

These findings in the mouse are in accord with the demonstration of a similar relationship between serum γ-globulin concentration and catabolic rate in man (7, 11). The fractional catabolic rate in man, usually about 3 per cent of the total body pool per day, may be increased to about 6 to 7 per cent at very high γ-globulin levels (5 gm per 100 ml or more). Higher fractional catabolic rates have not been observed, however, in afebrile, euthyroid subjects even though turnover measurements have been made at γ-globulin levels as high as 9 gm per 100 ml. In man and in the mouse, the maximal fractional rate of γ-globulin catabolism appears to be between two and three times greater than the normal fractional catabolic rate.

In contrast to the apparent limit, or approach to a limit, in the fractional rate of γ-globulin catabolism there is no evidence of a quantitative limit on γ-globulin catabolism. A mouse with 6 gm per cent serum myeloma protein concentration apparently catabolized about twice as much γ-globulin per day as does a mouse with 3 gm per cent γ myeloma protein.

Under circumstances where the maximal fractional rate of γ-globulin catabolism is closely approached, the fractional rate of γ-globulin removal for practical purposes is independent of the rate of γ-globulin synthesis. This was reported by Humphrey and McFarlane (31) who compared normal γ-globulin and antibody catabolism in normal rabbits, in a rabbit given a large amount of γ-globulin by passive transfer, and in a rabbit actively hyperimmunized to a high level of serum antipneumococcal antibody. They observed a constant fractional rate of γ-globulin catabolism (about 12.5 per cent per day) but did not detect any relationship between antibody level and fractional rate of catabolism. Although serum γ-globulin levels were not reported, these observations need not be at variance with those in the present study if the rabbit studies were done at or above the serum γ-globulin level which produces the maximal fractional rate of γ-globulin catabolism in the rabbit. It is also possible that the rabbit differs from mouse and man in this aspect of γ-globulin control.

Albumin metabolism has been extensively investigated, but the relative contribution of catabolic processes in determining serum albumin levels is not clear. The findings of Reeve and Roberts (38) in normal rabbits indicated that albumin catabolic rates were not related to serum level or synthetic rate. Studies in hyperimmunized rabbits (32), however, indicated that the fractional rate of albumin catabolism was depressed after serum albumin levels fell. Similarly,

patients with analbuminemia have low albumin catabolic rates which are increased toward normal by raising the serum level (39). Where high serum albumin levels were produced in normal subjects by albumin infusions, the fractional rate of albumin catabolism was increased (40). These latter observations on albumin metabolism indicate that the albumin catabolic rate may be influenced by serum albumin level in the same manner that γ-globulin catabolism is influenced by the serum γ-globulin concentration.

It seems clear, however, that the catabolic processes of some serum proteins are not directly influenced by the serum level. In studies of γ_1-macroglobulin metabolism, subjects with serum γ_1-macroglobulin levels ranging from 5 to 5000 per cent of the normal level were found to have similar fractional catabolic rates (41). In studies of transferrin metabolism, Awai and Brown (42) found that the fractional rate of transferrin catabolism was often less when serum transferrin levels were increased in disease, and in one subject whose transferrin level was raised to twice the normal level *via* transferrin infusions, the fractional catabolic rate was markedly reduced,—the opposite effect from that observed here with γ-globulin.

The relative discrepancy between albumin and γ-globulin survival in normal mice, (half-times of 2.1 and 4.1 days respectively) can be narrowed if the serum γ-globulin concentration is raised to a level similar to that of albumin (Table V). Gamma globulin levels increased to approximately 3 gm per cent by hyperimmunization (Table I, group E) or by other means (Fig. 6), reduce γ-globulin half-time to approximately 2.0 days, similar to the 2.1 day half-time observed for albumin. As seen in Table V, the absolute rate of degradation is similar for both proteins at this serum level. It would be of interest to know if this holds true for other species as well. In man, normal γ-globulin survival is somewhat longer, $T\frac{1}{2}$ = 23 days (11, 33), than albumin, $T\frac{1}{2}$ = 17 days (43, 44). Extrapolation from the data of Solomon *et al.* (11) indicates that raising the γ-globulin level to 4 gm per cent in man (*i.e.* equivalent to the normal serum albumin level), reduces γ-globulin half-time to about 14 days (*i.e.* somewhat shorter than for albumin). More data on human albumin and γ-globulin turnover at several serum levels are needed, however, before a definite comparison is possible.

Albumin and γ-globulin catabolic processes are similar in being susceptible to differences in metabolic rate (34, 35). In other respects, however, processes of albumin and γ-globulin catabolism appear to be under separate control. Albumin catabolism is normal when γ-globulin catabolism is reduced (13) or increased (12). In the present study, changes in serum albumin level were found to have no effect on the rate of γ-globulin catabolism. Also, γ-globulin metabolism may be normal in patients with analbuminemia (45).

The selectivity of the mechanisms determining 6.6S γ-globulin catabolism is demonstrated by their failure to respond to marked increases of the closely

related β_{2A}-globulin and γ_{1M} macroglobulin groups. Processes such as pinocytosis, which have been considered as possible mechanisms for removal of serum proteins, would not account for the selectivity exhibited by γ-globulin catabolic processes.

Two processes may contribute to the present observations on γ-globulin metabolism, one mechanism concerned with actual removal and catabolism of γ-globulin and a second mechanism regulating the rate of γ-globulin removal. If γ-globulin were catabolized by a non-selective mechanism which was dependent upon the concentration of γ-globulin, one would expect a constant rate of catabolism. In this study, however, the rate of catabolism was increased as

TABLE V

Comparison of γ-Globulin and Albumin Catabolism in BALB/c Mice

	Albumin normal	γ-Globulin	
		Normal	Raised to 3.0 gm per cent
Serum concentration, *gm per cent*	3.0	0.5	3.0
Total body content, *mg*	75	12.5	75
T½, *days*	2.1	4.1	2.0*
Fractional rate of degradation (per day)	0.333	0.16	0.347
Absolute turnover rate, *mg/25 gm mouse/day*	25	2.1	26

* Obtained from data in Table I and Fig. 6.

concentration increased. One biologic mechanism which would explain this observation is that more than one molecule of γ-globulin on the catabolic receptor might be required before catabolism occurs. The simultaneous presence of two molecules is attractive because of its simplicity. Algebraic solution of our graphic data does not support a hypothesis that two simultaneous molecular hits on the receptor are necessary for catabolism, but the simultaneous presence of two molecules would be plausible if some factor allowed a delay between the two hits. Whatever the method of catabolism, there appears to be a homeostatic mechanism sensitive to the serum level and responding to increased levels by acceleration of γ-globulin catabolism.

The nature of the sensing mechanism responding to changes in γ-globulin levels is unknown. Indeed, it is not certain whether the serum concentration or the amount of γ-globulin in the intravascular pool, in another body pool, or in the total body γ-globulin content determines the activity of catabolic processes. In the present work, the observed catabolic rates were expressed in terms of the serum γ-globulin concentration because this γ-globulin parameter could be most accurately measured and a correlation was readily evident.

Specificity of control of γ-globulin catabolism implies that γ-globulin molecules have a specific configuration which is recognized by the sensing mechanism of the process regulating γ-globulin catabolic rate. The finding that injection of F pieces from γ-globulin accelerates γ-globulin catabolism indicates that the catabolic control site is on the F piece. This is supported by evidence that β_{2A}-globulins and γ_1-macroglobulins do not accelerate γ-globulin catabolism. Current hypotheses (46–49) view the 6.6S γ-globulin molecules as being composed of several types of polypeptide chains. One type of polypeptide chain is present only in 6.6S γ-globulin molecules and is not present in β_{2A}-globulins or γ_{1M}-macroglobulins. At least a part of the polypeptide chain unique to 6.6S γ-globulin (*i.e.* not on β_{2A}-globulin or γ_{1M}-globulin), is represented in the F piece obtained after papain digestion. The finding of the catabolic control site on the F piece is in accord with evidence that other specific properties of 6.6S γ-globulin are on the F pieces.

The control of γ-globulin catabolism probably is similar in many species. The turnover rate of heterologous γ-globulin is largely determined by the metabolic rate of the recipient (12, 30, 50). Also, large amounts of exogenous γ-globulin of heterologous and isologous origin similarly accelerate the catabolism of isologous normal γ-globulin.

The observations reviewed above indicate that several factors determine the metabolic fate of γ-globulin. These include metabolic rate of the host; specific features of the protein molecule; the serum γ-globulin level and, indirectly, the γ-globulin synthetic rate. Further studies are needed to elucidate the mechanisms controlling γ-globulin catabolism, the anatomic location of the homeostatic and catabolic sites, and the means of communication between these sites.

SUMMARY

Both synthetic and catabolic processes determine the serum γ-globulin level. The rate of γ-globulin synthesis appears to be the primary factor determining the amount of serum γ-globulin. Increase of γ-globulin synthesis (as may occur following immunization or development of plasma cell tumor) elevates the serum γ-globulin level. This, in turn, accelerates the fractional rate of γ-globulin catabolism. The change in catabolic rate reduces the dimensions of the serum change from that which would occur if synthesis alone determined the serum γ-globulin level. The present studies indicate the existence of a homeostatic mechanism controlling the rate of γ-globulin catabolism.

The mechanisms of γ-globulin catabolism are specific and selective. Marked serum increase of other immunoglobulin components (β_{2A}-globulins and γ_1-macroglobulins) do not accelerate γ-globulin catabolism. Similarly, serum albumin increases do not influence γ-globulin catabolism.

The site determining γ-globulin catabolism is restricted to a part of the

γ-globulin molecule; *i.e.*, on the F piece obtained by papain digestion and, by inference, on the H chains obtained by reduction and alkylation of γ-globulin molecules.

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