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Research Article



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# Metabolism of Human Gamma Macroglobulins *

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The 18 S $\gamma_1$-macroglobulins ($\gamma_{1M}$- or $\beta_{2M}$-globulins) are antibody containing molecules which are present normally in all animal species that have been appropriately examined. In man, the $\gamma_1$-macroglobulins constitute about 7% of the total gamma globulin group and 1 to 2% of all serum protein. They differ from the more abundant 6.6 S $\gamma$-globulins in being larger (mol wt about 1,000,000), in being unable to pass the placental barrier, and in having distinctive antigenic characteristics (1, 2). Antibodies such as the isohemagglutinins, typhoid O antibodies, and cold agglutinins are predominantly in the $\gamma_1$-macroglobulin group. Infants respond principally with macroglobulin antibodies (3–5), and in adult animals and man the response to many (perhaps all) antigens normally passes through an early stage in which antibody activity is entirely limited to the $\gamma_1$-macroglobulins (6–9).

The amount of gamma macroglobulin (and macroglobulin antibody) in the serum depends on the rate and duration of macroglobulin synthesis and on the rate of $\gamma_1$-macroglobulin removal and catabolism.

Previous studies in animals (9, 10) and man (11–14) have indicated that $\gamma_1$-macroglobulins are catabolized more rapidly than 6.6 S $\gamma$-globulins. Half-times of 2 to 3 days have been reported for normal human gamma macroglobulin (11), and half-times of 6.5 to 13 days have been found with macroglobulins obtained from patients with Waldenström's macroglobulinemia (12, 13). In some of these studies (11), the methods used for isolating the $\gamma_1$-macroglobulin may have altered the protein and thus shortened the apparent survival. In other studies (12) where radioactive labeled amino acids were used *in vivo,* the apparent survival may have been spuriously prolonged due to persistent radioactivity in the precursor amino acid pool. In none of the previous studies was it possible to calculate the rate of normal $\gamma_1$-macroglobulin synthesis.

The present studies of normal $\gamma_1$-macroglobulin metabolism were undertaken when improved physicochemical methods made it possible to prepare purified and apparently unaltered normal 18 S $\gamma_1$-macroglobulin for turnover studies. The turnover of normal $\gamma_1$-macroglobulin was studied in normal subjects and in a variety of patients with diseases of the immunoglobulins. These included patients with congenital deficiency of 18 S $\gamma_1$-macroglobulin (agammaglobulinemia), patients with acquired deficiency (multiple myeloma, chronic lymphocytic leukemia), patients with protein-losing gastroenteropathy, and patients with excessive quantities of serum macroglobulin (Waldenström's macroglobulinemia). The turnover of the patient's own anomalous macroglobulin was compared with that of normal $\gamma_1$-macroglobulin in three cases. As a result of these observations, some of the factors affecting $\gamma_1$-macroglobulin synthesis and catabolism were defined and differences noted between the metabolic behavior of $\gamma_1$-macroglobulins and 6.6 S $\gamma$-globulins.

## Methods

*Patients.* Seven subjects without disease of the lymphocyte-plasma cell system, four with protein-losing enteropathy, four with severe hypogammaglobulinemia, five with multiple myeloma, eight with macroglobulinemia, and five with other diseases were studied. The pertinent clinical data are shown in Table I.

Of the four patients with protein-losing enteropathy, three were associated with intestinal lymphangiectasia (N.B., J.T., and L.H.) as shown by intestinal biopsies with a Crosby capsule. The fourth (M.R.) was an 8-year-old boy with an allergy to milk manifest by malabsorption and gastrointestinal protein loss. All four patients had been shown to have accelerated $I^{131}$ albumin turnover, and gastrointestinal protein loss was confirmed by $I^{131}$ polyvinylpyrrolidine and chromium$^{51}$-albumin studies.

The group with marked hypogammaglobulinemia in-

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TABLE I

*Clinical data on the 33 subjects studied with $I^{131}$-labeled gamma macroglobulin*

| Patient | Sex | Age | Hgb. | Renal damage* | Total serum protein | Albumin | γ-Globulin | $\gamma_1$-Macroglobulin | Clinical status and therapy |
|---|---|---|---|---|---|---|---|---|---|
| | | | *g/100 ml* | | *g/100 ml* | *g/100 ml* | *g/100 ml* | *mg/100 ml* | |
| Controls | | | | | | | | | |
| L.C. | M | 49 | 14.4 | | 6.3 | 3.57 | 1.03 | 57 | Acute intermittent porphyria |
| C.G. | M | 21 | 14.5 | | 6.3 | 3.53 | 0.97 | 59 | Normal subject |
| B.H. | M | 44 | 14.4 | | 7.2 | 3.92 | 1.27 | 70 | Normal subject |
| R.T. | M | 44 | 13.6 | | 6.3 | 3.44 | 1.33 | 77 | Cerebral aneurysm |
| S.W. | M | 28 | 13.1 | | 6.4 | 3.33 | 1.50 | 91 | Idiopathic epilepsy |
| J.T. | M | 31 | 14.0 | | 6.8 | 3.79 | 1.04 | 98 | Chronic adhesive pachymeningitis |
| E.C. | F | 72 | 13.3 | | 6.8 | 3.65 | 1.63 | 198 | Polyps in colon; iron deficiency anemia |
| Protein-losing enteropathy | | | | | | | | | |
| L.H. | F | 25 | 14.0 | | 3.4 | 1.53 | 0.38 | 27 | Intestinal lymphangiectasia |
| M.R. | M | 8 | 13.5 | | 4.9 | 2.02 | 0.99 | 31 | Food allergy (milk) |
| N.B. | M | 32 | 15.3 | | 4.0 | 1.82 | 0.54 | 32 | Intestinal lymphangiectasia |
| J.T. | M | 39 | 16.9 | | 3.5 | 1.65 | 0.51 | 62 | Intestinal lymphangiectasia |
| Agammaglobulinemia | | | | | | | | | |
| C.G. | F | 63 | 9.2 | | 4.9 | 3.31 | 0.26 | 6 | Chronic lymphocytic leukemia, *Salmonella enteritis* |
| T.C. | M | 23 | 14.0 | | 4.9 | 2.98 | 0.15 | 8 | Chronic otitis media, recurrent pneumonia |
| L.S. | F | 14 | 13.5 | | 5.4 | 3.28 | 0.21 | 10 | Chronic otitis media, recurrent pneumonia bronchiectasis |
| P.B. | M | 18 | 16.6 | | 6.1 | 4.25 | 0.23 | 12 | Chronic otitis media, rheumatoid arthritis, sinusitis |
| Multiple myeloma | | | | | | | | | |
| L.C. | F | 60 | 12.2 | +‡ | 10.0 | 2.7 | 6.2 | 9 | γ-Myeloma, Bence Jones proteinuria, 1.0 g/day |
| C.M. | M | 43 | 9.6 | +‡ | 8.9 | 2.4 | 5.1 | 24 | γ-Myeloma, nephrotic syndrome, proteinuria, 6.0 g/day |
| W.M. | M | 38 | 12.2 | | 8.3 | 2.6 | 5.2 | 25 | $\beta_{2A}$-Myeloma, prednisone, 20 mg/day, cytoxan, 200 mg/day |
| A.P. | M | 38 | 8.0 | | 12.0 | 1.6 | 9.4 | 25 | γ-Myeloma, amyloidosis, no treatment in past 3 months |
| A.T. | M | 54 | 12.5 | | 8.8 | 2.8 | 4.4 | 28 | γ-Myeloma, no treatment in past 4 months |
| Macroglobulinemia | | | | | | | | | |
| E.C. | M | 52 | 14.0 | | 7.7 | 2.9 | | 2,640 | Partial remission, no treatment |
| J.G. | F | 55 | 10.4 | +§ | 6.6 | 2.5 | | 2,760 | Hypertension, renal disease, chlorambucil, 4 mg/day for past year |
| F.C. | M | 62 | 10.7 | | 7.4 | 2.6 | | 3,850 | Chlorambucil, 4 mg/day for past year, plasmapheresis† |
| J.W. | M | 48 | 13.2 | | 9.1 | 2.5 | | 5,380 | Chlorambucil, 4 mg/day for past year |
| H.M. | F | 73 | 11.2 | | 8.7 | 1.8 | | 5,390 | Hemolytic anemia, prednisone, 20 mg/day for past year |
| R.W. | M | 65 | 8.0 | | 9.4 | 2.3 | | 6,460 | Osteolytic bone lesions, no treatment |
| K.I. | M | 56 | 8.0 | | 10.3 | 2.1 | | 7,000 | Lymphoma right testis, plasmapheresis† |
| W.F. | M | 38 | 9.3 | | 10.5 | 2.0 | | 7,290 | Generalized lymphadenopathy, no treatment |
| Others | | | | | | | | | |
| S.L. | F | 23 | 15.3 | +‡ | 3.1 | 1.76 | 0.45 | 96 | Idiopathic nephrosis, relapse, proteinuria, 4.4 g/day |
| B.G. | F | 25 | 12.2 | +‡ | 4.5 | 1.85 | 0.76 | 224 | Idiopathic nephrosis, relapse, proteinuria, 1.1 g/day |
| A.L. | M | 72 | 10.0 | +§ | 7.5 | 1.65 | 4.7 | 16 | Diabetes mellitus, hypergammaglobulinemia |
| H.D. | M | 62 | 12.2 | | 5.8 | 3.31 | 0.82 | 30 | Malignant lymphoma, ascites, pleural effusions |
| G.R. | M | 61 | 10.4 | | 7.0 | 4.05 | 1.04 | 61 | Chronic lymphocytic leukemia, recurrent pulmonary infections |

* +‡ = proteinuria; +§ = normal blood urea nitrogen, no proteinuria, creatinine clearances, 60 ml per minute.
† Plasmapheresis, see text.

cluded two males and two females. The two males had congenital agammaglobulinemia of the sex-linked recessive type, and one of the females had acquired idiopathic agammaglobulinemia. The other female (C.G.) with hypogammaglobulinemia secondary to chronic lymphocytic leukemia ran a low grade fever during the study and was found to have *Salmonella enteritis* on stool culture. These patients all had had recurrent infections in the past. The serum gamma globulin levels were markedly reduced, and all patients had received parenteral gamma globulin therapy. No gamma globulin was given, however, during the study or during the month before study.

Four patients with γ-myeloma proteins and one patient with $\beta_{2A}$-myeloma protein were studied. The myeloma proteins were characterized by immunoelectrophoresis using specific antisera (15). The group included one patient (L.C.) with Bence Jones proteinuria (1.0 g per day) and another (C.M.) with a nephrotic syndrome (6.0 g proteinuria per day), but no Bence Jones proteinuria. One patient (W.M.) was receiving cytoxan (200 mg per day) and prednisone (20 mg per day) before and during the study period.

Eight patients with macroglobulinemia were studied. In each case, the diagnosis was confirmed by analytic ultracentrifugation. Three patients (J.G., F.C., and J.W.) received chlorambucil (4 mg per day) during the study, all having been on the drug for more than 1 year.

Two patients (F.C. and K.I.) had received plasmapheresis therapy of 12 and 2 months duration, respectively, and required removal of 4 U of plasma per week, but plasmapheresis was discontinued during the study period.

The five other patients studied included two with idiopathic nephrosis, one with chronic lymphocytic leukemia, one with a malignant lymphoma, and one with hypergammaglobulinemia due to an anomalous protein with a sedimentation value of 14 S.

All patients studied were considered suitable for turnover studies. None of the patients was critically ill during the study period. All patients maintained body weight and their normal dietary intake during the study. Adequate urinary and fecal collections were possible in all cases.

*Preparation of labeled $\gamma_1$-macroglobulin.* Two separate studies were performed with radioiodine-labeled normal $\gamma_1$-macroglobulin. For each study normal $\gamma_1$-macroglobulin was prepared from freshly obtained serum of a healthy normal donor. In addition, three patients with macroglobulinemia received radioiodine-labeled preparations of their own macroglobulin. All reagents were prepared with sterile pyrogen-free water, and all equipment was autoclaved. The fractionation procedure has been described in detail previously (15). The modification employed here will be described briefly.

Twenty ml of fresh serum was concentrated twice by ultrafiltration and applied to a block composed of 240 g of polyvinyl chloride particles [1] and 240 g of polyvinyl chloride-polyvinyl acetate copolymer [2] in sodium barbiturate buffer pH 8.6. After electrophoresis at + 6° C for 18 hours the section of the block containing the gamma globulin was removed, the proteins were eluted with saline, concentrated to 2 ml by ultrafiltration, and dialyzed against 1.0 M NaCl, 0.1 M Tris buffer pH 8. The electrophoretically prepared gamma globulin fraction was then applied to a column of Sephadex G-200 [3] equilibrated with the pH 8 Tris NaCl buffer. Two major protein peaks were recovered in the effluent. The first fraction contained the 18 S gamma macroglobulin, and the second contained 6.6 S $\gamma$-globulin and a small amount of $\beta_{2A}$-globulin. Only the first fraction was used for $I^{131}$-labeling. Before iodination, samples of the two preparations of normal gamma macroglobulin were tested by immunoelectrophoresis, and one by ultracentrifugation and found to contain only 18 S $\gamma_1$-macroglobulin. The anomalous gamma macroglobulins of three patients with Waldenström's macroglobulinemia were isolated and prepared in the same manner as described above.

The macroglobulins were labeled with $I^{131}$ by the iodine monochloride method of McFarlane (16). After dialysis against saline to remove unbound $I^{131}$, normal human albumin was added to prevent damage of the macroglobulin by self-irradiation, and the mixture was sterilized by filtration. The products were calculated to have less than 1 mole of $I^{131}$ per mole of macroglobulin, and 96 to 100% of the radioactivity was precipitable with trichloroacetic acid. Throughout the preparation, labeling, and storage, the $\gamma_1$-macroglobulin remained in the soluble state and the protein solutions were not frozen.

The radioiodinated protein was characterized by paper electrophoresis and Sephadex G-200 filtration (Figure 1). A sample of the $I^{131}$ gamma macroglobulin preparation was added to normal serum, and the mixture was examined by paper electrophoresis. Analysis of the paper strip for radioactivity with a continuously recording counting rate meter, equipped with an end-window Geiger-Mueller tube, showed that radioactivity was present in the faster half of the gamma globulin region (i.e., the normal $\gamma$-macroglobulin region). When the $I^{131}$ $\gamma_1$-macroglobulin containing serum was applied to a G-200 Sephadex column, the radioactivity was limited to the initial effluent peak, i.e., the fraction containing the 18 S macroglobulins (Figure 1).

*Study protocol and calculation of data.* Each patient received 0.5 ml of Lugol's solution three times daily before and during the entire study period to prevent thyroidal uptake of the released $I^{131}$. Approximately 20 to 25 $\mu$c (0.2 to 0.3 mg) of $I^{131}$ $\gamma_1$-macroglobulin was injected intravenously from a calibrated syringe, and serum samples were obtained at 10 minutes, 20 minutes, 4 hours, 8 hours, 16 hours, 24 hours, and daily thereafter. Urine was collected in 24-hour pools throughout the entire study, and feces were examined for radioactivity in 24-, 48-, or 72-hour lots. Serum and urine samples were counted with an appropriate standard in an automatic gamma-ray well-type scintillation counter (employing a thallium-activated sodium iodide crystal). Stools were collected in paint cans, brought to a constant volume, and shaken in a paint can shaker. The homogenized samples were counted in a gamma-ray scintillation counter.

The data were analyzed according to the method of Berson, Yalow, Schreiber, and Post (17). Plots were constructed on semilogarithmic paper of the serum radioactivity and of the remaining whole body activity as plotted against time. Whole body activity was calculated by cumulative subtraction of radioactivity excreted in urine and stool from that originally administered. After initial equilibration, the two curves each declined as a single exponential function, each having a comparable slope. The biological half-life ($t_{\frac{1}{2}}$) was calculated from these curves. With the $t_{\frac{1}{2}}$, the fraction of the total body $\gamma_1$-macroglobulin catabolized per day could be determined. Fraction of the total body $\gamma_1$-macroglobulin catabolized per day = 0.693/survival $t_{\frac{1}{2}}$ (days).

The fractional catabolic rate was also expressed as the fraction of the intravascular pool catabolized per day. Fraction of the intravascular pool catabolized per day = daily urinary radioactivity/mean intravascular radioactivity during that day. This value was determined

[1] Geon resin, B. F. Goodrich Chemical Co., Niagara Falls, N. Y.

[2] Pevikon, Superfosfat Fabrika, Aktiebolog, Stockholm, Sweden.

[3] Pharmacia Fine Chemicals, New York, N. Y.

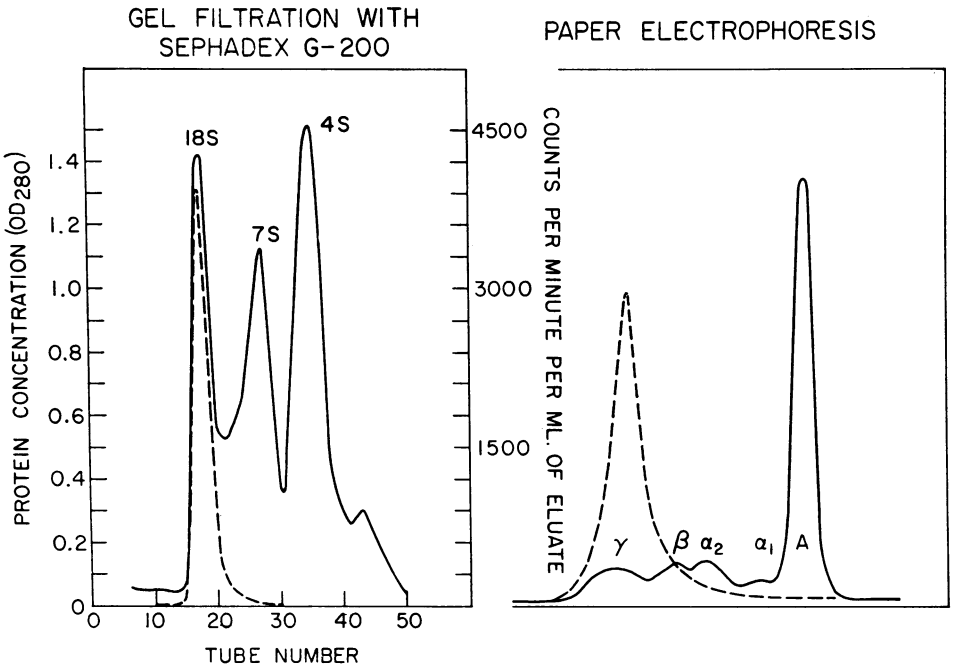


FIG. 1. DISTRIBUTION OF LABELED GAMMA MACROGLOBULIN ADDED TO NORMAL SERUM. The dashed lines depict the radioactivity.

for each daily urinary period, and the mean of the different periods for a given patient was recorded.

Plasma volume was calculated on the basis of initial distribution of the administered radioactivity, using the specific activity of the 10-minute serum sample. Plasma volume (milliliters) = counts per minute administered/10-minute serum activity (counts per minute per milliliter). The proportion of administered $I^{131}$ gamma macroglobulin which remained intravascular, after equilibration with the extravascular space was complete, was calculated from the serum and whole body activity curves. Fraction intravascular after equilibration = counts per minute per milliliter serum × plasma volume/counts per minute remaining in whole body.

The total body content of gamma macroglobulin was calculated from the serum concentration, fraction intravascular, and plasma volume. Total body $\gamma_1$-macroglobulin = serum concentration (milligrams per milliliter) × plasma volume/fraction intravascular.

The total quantity of $\gamma_1$-macroglobulin catabolized each day was calculated from the total body $\gamma_1$-macroglobulin and fraction of the total body $\gamma_1$-macroglobulin catabolized per day. With the assumption of a steady state, this is equivalent to the synthetic rate for the protein. Turnover rate (milligrams per kilogram per day) = total body $\gamma_1$-macroglobulin (milligrams) × fraction of the total body $\gamma_1$-macroglobulin catabolized per day/body weight (kilograms).

Total serum proteins were determined by the biuret method, and serum paper electrophoresis was performed by methods previously described (18). The serum gamma macroglobulin level in all subjects except those with macroglobulinemia was determined by a quantitative immune inhibition method (19). Gamma macroglobulin levels in patients with macroglobulinemia were determined by paper electrophoresis. The data were analyzed and compared using the significance of the difference of the means.

## Results

Two preparations of $I^{131}$-labeled normal 18 S $\gamma_1$-macroglobulin were injected into three and four control subjects in separate studies. The metabolic behavior of these two preparations was similar in the control subjects and in four patients who received both $\gamma_1$-macroglobulin preparations. Therefore, the data obtained in the two studies have been combined. A total of 37 observations was made in 33 patients (Table II).

*Control subjects.* Intravenously injected $I^{131}$-labeled normal $\gamma_1$-macroglobulin rapidly equilibrated between the intravascular and extravascular compartments. Approximately 76% of the $\gamma_1$-macroglobulin was located in the intravascular compartment.

The biological half-time in seven control subjects averaged 5.1 days with a range of 3.8 to

6.5 days (Table II). The percentage of circulating $\gamma_1$-macroglobulin catabolized per day averaged 18.7%, the observed values ranging from 14.1 to 25.1%.

The serum $\gamma_1$-macroglobulin level was found to average 98 mg per 100 ml, with a range of 57 to 198 mg per 100 ml. The total exchangeable $\gamma_1$-macroglobulin averaged 49 mg per kg body weight. The mean turnover rate was 6.9 mg per kg per day in the control group. The absolute turnover (synthetic) rate varied from 0.2 to 0.7 g per day, the mean value being 0.41 g per day. The serum $\gamma_1$-macroglobulin level showed a gross correlation with the synthetic rate. The serum level was highest in the subject who had the greatest rate of $\gamma_1$-macroglobulin synthesis and was correspondingly lower in those with lesser rates of macroglobulin synthesis. Comparison of the serum $\gamma_1$-macroglobulin level with the biological half-time in the control group, however, showed no correlation between serum level and fractional catabolic rate. Further studies of the factors influencing

TABLE II

*$I^{131}$-labeled gamma macroglobulin turnover in 33 patients*

| Patient | Serum $\gamma_{1M}$-globulin | Plasma volume | % intravascular $\gamma_{1M}$-globulin | Total intravascular $\gamma_{1M}$-globulin | Total body $\gamma_1$-macroglobulin | $t_{\frac{1}{2}}$ | % of circulating $\gamma_1$-macroglobulin catabolized | $\gamma_{1M}$-Globulin turnover |
|---|---|---|---|---|---|---|---|---|
| | *mg/100 ml* | *ml/kg* | *%* | *mg/kg* | *mg/kg* | *days* | *%/day* | *mg/kg/day* |
| Controls | | | | | | | | |
| L.C. | 57 | 26.9 | 69 | 15.4 | 22.3 | 4.5 | 22.3 | 3.42 |
| C.G. | 59 | 33.3 | 88 | 20 | 22.8 | 3.8 | 19.0 | 4.13 |
| B.H. | 70 | 33.1 | 78 | 23.2 | 29.7 | 6.5 | 15.5 | 3.16 |
| R.T. | 77 | 35.3 | 100 | 27.2 | 27.2 | 4.9 | 14.1 | 3.83 |
| S.W. | 91 | 59 | 70 | 53.8 | 76.9 | 5.9 | 17.9 | 9.09 |
| J.T. | 98 | 40.7 | 65 | 39.8 | 61.3 | 5.5 | 17.3 | 7.65 |
| E.C. | 198 | 34.4 | 65 | 68.2 | 105 | 4.3 | 25.1 | 16.9 |
| Protein-losing enteropathy | | | | | | | | |
| L.H. | 27 | 36.4 | 95 | 9.84 | 10.35 | 1.0 | 73.3 | 7.18 |
| M.R. | 31 | 37.4 | 80 | 11.6 | 14.5 | 1.7 | 51.0 | 5.9 |
| N.B. | 32 | 24.0 | 78 | 7.69 | 9.86 | 1.5 | 66.0 | 4.56 |
| J.T. | 62 | 28.6 | 68.5 | 17.7 | 25.9 | 1.5 | 60.0 | 12 |
| Agammaglobulinemia | | | | | | | | |
| C.G. | 6 | 47.3 | 77 | 2.83 | 3.69 | 4.5 | 18.3 | 0.57 |
| T.C. | 8 | 30.1 | 76 | 2.4 | 3.15 | 2.2 | 36.4 | 0.99 |
| L.S. | 10 | 49.4 | 70 | 4.93 | 7.04 | 3.7 | 26.4 | 1.31 |
| P.B. | 12 | 35 | 87 | 4.2 | 4.83 | 6.5 | 12.2 | 0.52 |
| Multiple myeloma | | | | | | | | |
| L.C. | 9 | 30.8 | 80 | 2.8 | 3.47 | 13.2 | 6.1 | 0.18 |
| C.M. | 24 | 31.1 | 92 | 7.5 | 8.11 | 9.1 | 9.3 | 0.62 |
| W.M. | 25 | 48.6 | 90 | 12.2 | 13.5 | 7.0 (6.8)* | 18.4 | 1.34 |
| A.P. | 25 | 46.9 | 72 | 11.7 | 16.3 | 8.6 (6.1)* | 12.2 | 1.31 |
| A.T. | 28 | 30.4 | 80 | 8.5 | 10.6 | 5.8 | 15.4 | 1.27 |
| Macroglobulinemia | | | | | | | | |
| E.C. | 2,640 | 31.7 | 94 | 838 | 891 | 5.2 | 14.1 | 119 |
| J.G. | 2,760 | 41.6 | 88 | 1,149 | 1,306 | 6.8 | 11.6 | 133 |
| F.C. | 3,850 | 40.6 | 95 | 1,564 | 1,647 | 5.8(4.7)* | 13.5 | 196 |
| J.W. | 5,380 | 41.4 | 82 | 2,230 | 2,719 | 6.4 | 12.4 | 295 |
| H.M. | 5,390 | 43 | 90 | 2,317 | 2,573 | 4.7 | 16.2 | 381 |
| R.W. | 6,460 | 51.4 | 86 | 3,319 | 3,859 | 5.5 | 13.5 | 486 |
| K.I. | 7,000 | 65 | 90 | 4,550 | 5,050 | 7.1 | 10.9 | 495 |
| W.F. | 7,290 | 64.2 | 83 | 4,677 | 5,634 | 6.2 | 14.1 | 630 |
| Others | | | | | | | | |
| S.L. | 96 | 28.9 | 92 | 27.8 | 30.2 | 4.9 | 15.2 | 4.25 |
| B.G. | 224 | 31.9 | 66 | 71.4 | 108.2 | 4.0 | 22.9 | 18.7 |
| A.L. | 16 | 46.3 | 85 | 9.7 | 11.4 | 11.2(13.3)* | 8.3 | 0.82 |
| H.D. | 30 | 45.6 | 71 | 13.7 | 19.3 | 4.8 | 22.7 | 2.77 |
| G.R. | 61 | 37.1 | 81 | 22.6 | 27.91 | 4.7 | 19.6 | 4.12 |

* Number in parentheses = results of second study.

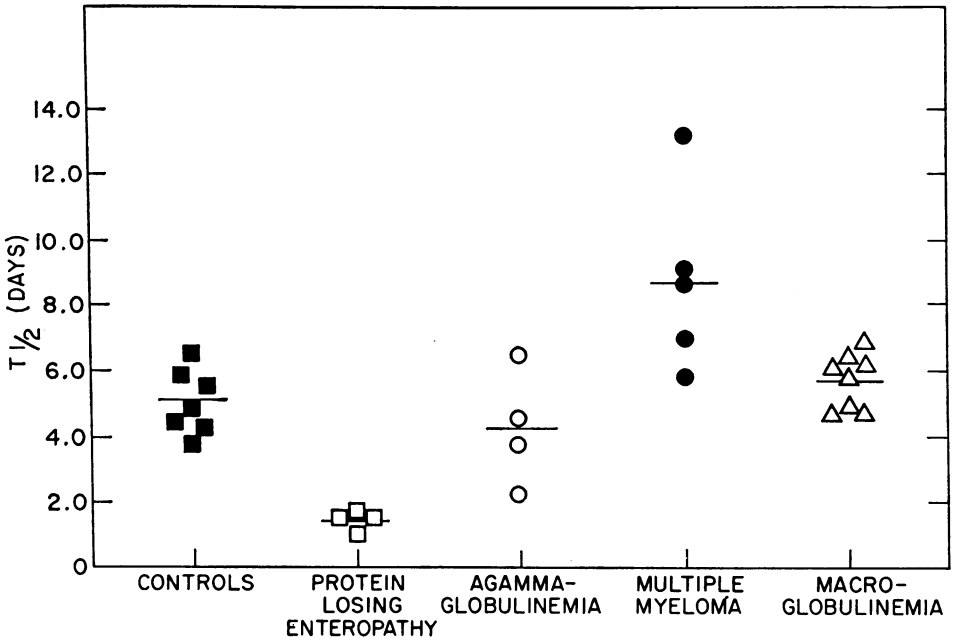


FIG. 2. SURVIVAL TIME ($t_{\frac{1}{2}}$) OF $I^{131}$-LABELED NORMAL GAMMA MACROGLOBULIN.

the serum $\gamma_1$-macroglobulin level and the rates of synthesis and catabolism were undertaken in a variety of diseases.

*Protein-losing gastroenteropathy.* Four patients, previously shown to have marked gastrointestinal protein loss with $I^{131}$ albumin and $Cr^{51}$ albumin (20) were studied to determine the fate of the $\gamma_1$-macroglobulins in the presence of protein-losing gastroenteropathy. The serum $\gamma_1$-macroglobulin levels were subnormal in three patients (27 to 32 mg per 100 ml) and at the lower range in the fourth.

The survival of $I^{131}$-labeled $\gamma_1$-macroglobulin was shorter in this group of patients than in any other group studied (Figure 2). The mean $t_{\frac{1}{2}}$ was 1.4 days with a range of 1.0 to 1.7 days. A typical study is shown in Figure 3. Calculation of the $\gamma_1$-macroglobulin turnover rates (Table II), however, revealed that the $\gamma_1$-macroglobulin synthetic rate was in the normal range (i.e., 4 to 12 mg per kg per day). Thus, there was no evidence that these patients had any compensatory increase in $\gamma_1$-macroglobulin synthesis to counterbalance the rapid loss. These observations suggest that $\gamma_1$-macroglobulins are among the proteins lost into the gastrointestinal lumen in this disease, where digestion presumably occurs.

In the nephrotic syndrome, the smaller serum proteins are lost preferentially, and macroglobulins are lost into the urine only with severe renal damage (21). The fate of labeled $\gamma_1$-macroglobulin was studied in two patients with the nephrotic syndrome (patients S.L. and B.G. under *Others* in Tables I and II). Although urinary protein losses were 4 and 1 g per day, in neither case was trichloroacetic acid-precipitable radioactivity found in the urine, evidence that $\gamma_1$-macroglobulin was not being lost via the kidney. In one case the serum $\gamma_1$-macroglobulin level was normal and in the other it was high, and a corresponding normal or increased turnover (synthetic) rate was found (Table II). The $\gamma_1$-macroglobulin half-times were normal ($t_{\frac{1}{2}} = 4.9$ and 4.0 days, respectively). In these two nephrotic patients, renal loss of other proteins and hypoproteinemia did not alter $\gamma_1$-macroglobulin catabolism.

*Agammaglobulinemia.* Four patients with severe hypogammaglobulinemia all had markedly depressed serum $\gamma_1$-macroglobulin levels (6 to 12 mg per 100 ml). Despite the low serum levels, there was no evidence of prolonged $\gamma_1$-macroglobulin survival. The average $t_{\frac{1}{2}}$ for the group was 4.3 days with a range of 2.2 to 6.5 days (Figure 2). The basis for the rapid catabolism of $\gamma_1$-

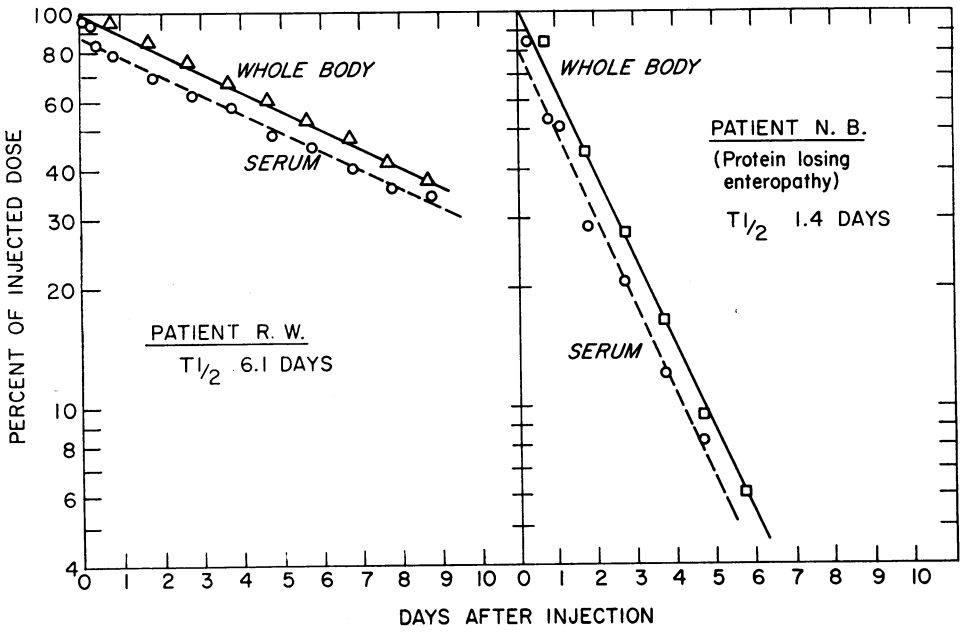


FIG. 3. WHOLE BODY AND SERUM RADIOACTIVITY CURVES AFTER ADMINISTRATION OF LABELED GAMMA MACROGLOBULIN. On the left, R.W. with macroglobulinemia (△); on the right, N.B. with protein-losing enteropathy (□).

macroglobulin in T. C. ($t_{\frac{1}{2}} = 2.2$ days) was unexplained. Subsequent studies with intravenously administered $I^{131}$ albumin showed normal catabolism ($t_{\frac{1}{2}} = 16$ days) and, therefore, failed to show any evidence for gastrointestinal protein loss. The synthetic rates of $\gamma_1$-macroglobulin were uniformly reduced in the agammaglobulinemic patients, being 10 to 15% of the control values. The agammaglobulinemic patients, as well as those with protein-losing gastroenteropathy, had reduced serum gamma macroglobulin levels but based on different pathologic processes, i.e., deficient synthesis in the former group (Figure 4) and accelerated catabolism in the latter (Figure 2).

*Multiple myeloma.* Low serum $\gamma_1$-macroglobulin levels were found in five patients with multiple myeloma (the levels being 10 to 28% of the mean control value). The finding of reduced $\gamma_1$-macroglobulin levels in one patient with a $\beta_{2A}$-myeloma protein, as well as in four with $\gamma$-myeloma proteins, is representative of the experience in a larger group of patients with multiple myeloma (22).

Labeled $\gamma_1$-macroglobulin had half-times of 5.8 to 13.2 days with an average $t_{\frac{1}{2}}$ of 8.5 days in the five patients with multiple myeloma. The $t_{\frac{1}{2}}$ values for $\gamma_1$-macroglobulin were longer in this group than in any other (Figure 2), differing significantly from the control group ($t = 2.95$; $p = 0.01$ to $0.02$).

The calculated turnover (synthetic) rates for $\gamma_1$-macroglobulin in the patients with multiple myeloma were lower than normal, ranging from 3 to 20% of the mean control value. In multiple myeloma, as in agammaglobulinemia, the low serum $\gamma_1$-macroglobulin levels are due to depressed synthetic rates. In multiple myeloma, however, the effects of depressed synthesis are partially offset by a prolongation of $\gamma_1$-macroglobulin survival.

*Macroglobulinemia.* Radioiodine-labeled normal $\gamma_1$-macroglobulin metabolism was studied in eight patients with Waldenström's macroglobulinemia. The serum macroglobulin concentration was 27 to 73 times greater than the average normal level. This was associated with an increased plasma volume which averaged 47 ml per kg for the group (control group average = 37 ml per kg). The total exchangeable $\gamma_1$-macroglobulin for this group was calculated to be 18 to 115 times greater than normal.

The biological half-time of $I^{131}$ $\gamma_1$-macroglobulin

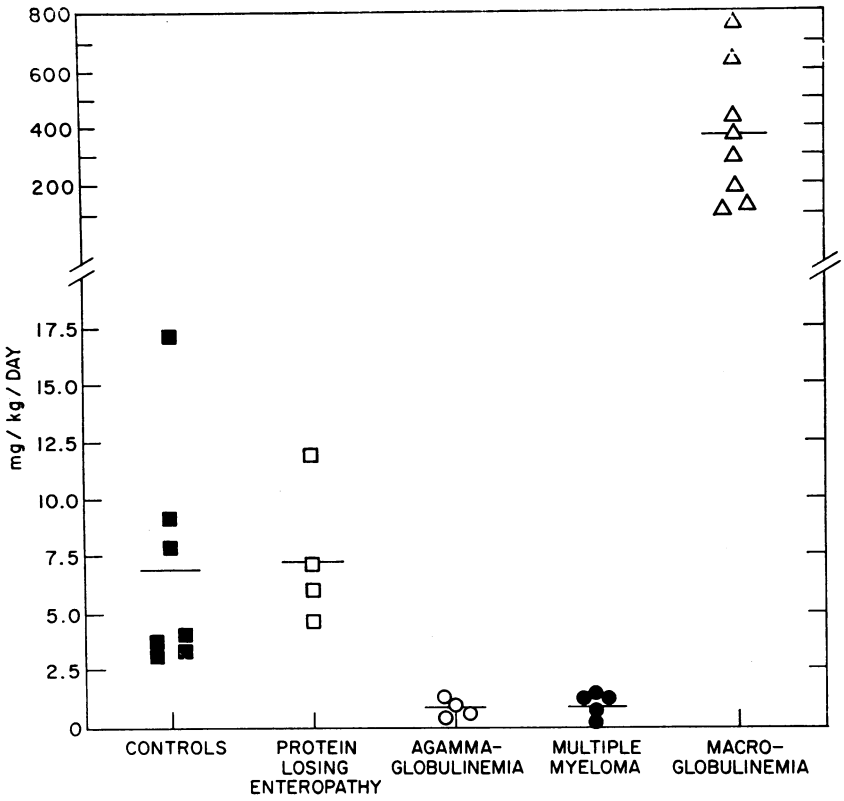


FIG. 4. CALCULATED SYNTHETIC RATES OF GAMMA MACROGLOBULIN.

in the macroglobulinemic patients ranged from 4.6 to 8.6 days (average, 5.9 days). This does not differ significantly from the control group data ($t = 1.69$; $p = 0.10$ to $0.20$).

The turnover of $\gamma_1$-macroglobulin calculated in Table II is based on the assumption that the anomalous macroglobulin is catabolized at the same fractional rate as normal $\gamma_1$-macroglobulin (see below). In the absence of altered catabolism, it is evident that increased macroglobulin synthesis is responsible for the elevated serum macroglobulin levels.

*Macroglobulinemic macroglobulins.* The normal $\gamma_1$-macroglobulins are a heterogeneous group of proteins differing in electrophoretic mobility, antigenic type, and antibody activity. This heterogeneity contrasts with discrete physicochemical and immunochemical properties of the anomalous macroglobulins found in Waldenström's macroglobulinemia. Therefore, the metabolic behavior of anomalous and normal macroglobulin was compared in three patients. The anomalous macroglobulins were isolated from the serum of each patient, labeled with $I^{131}$, and reinjected into the donor. The two studies in each patient were performed in the same manner.

The metabolic behavior of anomalous and normal macroglobulin in these three patients is compared in Table III. The intravascular : extravascular distributions of the protein were comparable. The rate of $\gamma_1$-macroglobulin synthesis calculated from the autologous $\gamma$-macroglobulin studies in these patients was 31.7, 12.3, and 3.6 g per day (i.e., 10 to 100 times greater than normal). These observations extend the evidence that increased synthesis of a discrete $\gamma_1$-macroglobulin is responsible for the elevated macroglobulin levels in macroglobulinemia.

The studies in patient K.I. show the survival, distribution, and synthetic rate of the anomalous $\gamma_1$-macroglobulin and the normal gamma macroglobulin to be approximately the same. The fractional catabolic rate for the anomalous $\gamma_1$-macroglobulins in patients R.W. and E.C. was lower

TABLE III

*Comparison of the metabolism of normal and anomalous gamma macroglobulin in three patients with macroglobulinemia*

| Patient | Protein | Serum $\gamma_{1M}$-globulin concentration | Per cent intravascular $\gamma_{1M}$-globulin | $t_{\frac{1}{2}}$ | % of circulating $\gamma_1$-macroglobulin catabolized | Turnover rate |
|---|---|---|---|---|---|---|
| | | *mg/100 ml* | % | *days* | %/day | *mg/kg/day* |
| K.I. | Normal $\gamma_{1M}$ | 7,000 | 90 | 7.1 | 10.9 | 495 |
| | KI $\gamma_{1M}$ | 6,820 | 100 | 6.8 | 10.2 | 528 |
| R.W. | Normal $\gamma_{1M}$ | 6,460 | 86 | 5.5 | 13.5 | 486 |
| | RW $\gamma_{1M}$ | 3,980 | 84 | 9.6 | 8.1 | 158 |
| E.C. | Normal $\gamma_{1M}$ | 2,640 | 94 | 5.2 | 14.1 | 119 |
| | EC $\gamma_{1M}$ | 1,820 | 80 | 7.0 | 12.4 | 54 |

than that found for the normal $\gamma_1$-macroglobulin. The $t_{\frac{1}{2}}$ of 9.6 days observed in patient R.W. using his own gamma macroglobulin was appreciably longer than that found for any other macroglobulin preparation in the macroglobulinemic patients (Tables II and III). Whether these differences noted in the catabolism of normal and anomalous gamma macroglobulins in R.W. and E.C. represent an intrinsic feature of their macroglobulins or were due to a difference occurring during the preparation or labeling of the proteins remains unexplained. Although these differences were noted, the data relating the normal and anomalous macroglobulins were sufficiently comparable to warrant the assumption that the estimated synthetic rates of $\gamma_1$-macroglobulins in these patients (Tables II and IV) are close to the actual values.

Reduction of serum $\gamma_1$-macroglobulin levels has been reported with penicillamine therapy in some patients with high levels of gamma macroglobulins (23). To determine if penicillamine would increase the rate of gamma macroglobulin catabolism, two patients, R.W. and E.C., were given *d*-penicillamine orally (2.0 g per day) from days 8 to 14 of their turnover study. No difference was noted in the rate of catabolism (or serum $\gamma_1$-macroglobulin level) in either patient during this short period of observation.

## Discussion

The 18 S $\gamma_1$-macroglobulins were shown in the present study to be predominantly intravascular proteins whose serum concentration depends upon the rate of synthesis. Observations in subjects with $\gamma_1$-macroglobulin levels ranging from 5 to 5,000% of normal demonstrated that low rates of synthesis were associated with low serum $\gamma_1$-macroglobulin levels, and high synthetic rates with elevated serum $\gamma_1$-macroglobulin levels. No correlation was observed between the serum concentration of the proteins and their biological half-time.

The metabolic characteristics of 18 S $\gamma_1$-macroglobulin differ in several respects from those of 6.6 S $\gamma$-globulin. Only about 20% of the total body pool of $\gamma_1$-macroglobulin lies in the extravascular space, the rest remaining intravascular. In contrast, about 60% of 6.6 S $\gamma$-globulin is ex-

TABLE IV

*Summary of data on the metabolism of normal human gamma macroglobulin**

| | Number of subjects | Serum $\gamma_{1M}$-globulin concentration | % intravascular $\gamma_{1M}$-globulin | $t_{\frac{1}{2}}$ | Turnover rate (Synthetic rate) | |
|---|---|---|---|---|---|---|
| | | *mg/100 ml* | % | *days* | *mg/kg/day* | *g/day* |
| Control | 7 | 93 | 76.4 | 5.1 | 6.88 | 0.2 – 0.7 |
| Protein-losing gastroenteropathy | 4 | 38 | 80.7 | 1.4 | 7.41 | 0.1 – 0.7 |
| Agammaglobulinemia | 4 | 9 | 77.5 | 4.2 | 0.85 | 0.02– 0.06 |
| Multiple myeloma | 5 | 22 | 82.8 | 8.5 | 0.94 | 0.01– 0.1 |
| Macroglobulinemia | 8 | 5,100 | 88.5 | 5.9 | 342 | 8.0 –40.3 |

travascular. The observation that the larger molecules remain principally intravascular is compatible with a hypothesis that normal passage of proteins out of the intravascular compartment involves transit through pores or other structures of a limiting size. Evidence for this has been obtained from studies of other plasma proteins.

Fibrinogen, with a molecular weight of 340,000, was found to be predominantly intravascular (80%) in recent studies (24). In contrast, the smaller serum proteins such as albumin (mol wt, 69,000), 6.6 S γ-globulin (160,000), and transferrin (90,000) are approximately 40 to 50% intravascular (11, 17, 25–26).

$I^{131}$-labeled normal γ-macroglobulin has a survival $t_{\frac{1}{2}}$ of about 5 days in contrast to 23 days for normal 6.6 S γ-globulin (11, 25). This observation means that about 14% of the total body macroglobulin is catabolized and replaced each day, while only 3% of the 6.6 S γ-globulin is replaced per day. Because of this and because of the difference in distribution, the serum levels of $\gamma_1$-macroglobulin (0.1 g per 100 ml) and 6.6 S γ-globulin (1.2 g per 100 ml) do not accurately reflect their relative rates of synthesis. The serum levels of 18 S γ-macroglobulin are only 5 to 10% of the 6.6 S γ-globulin level, but the quantity of macroglobulin synthesized is 15 to 20% of the amount of 6.6 S γ-globulin.

Turnover data for the proteins presumably apply to specific antibody as well. The rapid turnover of $\gamma_1$-macroglobulin indicates that newly formed macroglobulin antibodies would be available for a shorter time than 6.6 S γ-globulin antibodies. At the same time, the 18 S antibodies would be predominantly located in the intravascular space while the smaller 6.6 S antibodies distribute themselves more widely. Since macroglobulin antibodies are formed first in response to antigen administration (7, 9), intravascular retention of antibodies may have some significance in the early phase of the immune response. Later, with formation of 6.6 S antibodies, the large antibodies may no longer be needed or may even be deleterious, so that their rapid removal would be of value. Further work is needed, however, to determine the roles of macroglobulin and 6.6 S γ-globulin antibodies in the immune response.

The survival of $I^{131}$ γ-macroglobulin in the present study appeared to be independent of the serum concentration or total body pool of γ-macroglobulin. The $\gamma_1$-macroglobulin survival ($t_{\frac{1}{2}}$) was essentially the same in the normal controls as in the patients with markedly reduced $\gamma_1$-macroglobulin levels (agammaglobulinemia) and markedly increased levels (macroglobulinemia). Gamma macroglobulin degradation took place by a first order process with a constant fraction of the body $\gamma_1$-macroglobulin pool being degraded over a wide range of serum concentrations. The survival of ceruloplasmin (27) and fibrinogen (28, 29) has also been shown to be independent of the serum concentration. On the other hand, a direct relationship between the fractional catabolic rate and the serum concentration has been found for 6.6 S γ-globulin (25, 30) and possibly for albumin (31, 32). The fractional catabolic rate of 6.6 S γ-globulin is increased in most subjects with an elevated 6.6 S γ-globulin concentration due to chronic infection (33), hyperimmunization (30), or multiple myeloma with γ-myeloma proteins (25, 34, 35). In contrast, 6.6 S γ-globulin catabolic rate is normal or decreased in patients with agammaglobulinemia or macroglobulinemia where the levels of 6.6 S γ-globulin are reduced (25, 36). The present observations confirm and extend previous observations (25, 30) that 6.6 S γ-globulins and 18 S $\gamma_1$-macroglobulins are catabolized by separate processes.

Evidence for a role of bacterial environment in stimulating total gamma globulin synthesis has been found in studies with germ-free animals (37). The factors controlling the synthesis of the separate classes of immune globulins, however, have not been defined. The observations presented here indicate that the synthetic rate of $\gamma_1$-macroglobulin in patients with gastrointestinal protein loss falls within the normal range. No compensatory increase in synthetic rate was found, despite the low serum gamma macroglobulin levels and the rapid catabolism found in this disorder. Similarly, little or no increase in the synthesis of 6.6 S γ-globulin has been found in protein-losing gastroenteropathy (38) or in nephrosis (39), disorders characterized by low serum γ-globulin levels and rapid catabolism. These considerations are in accord with the concept that the rate of synthesis of the immune globulins is not controlled by their serum concentration or pool size, but by other factors such as antigenic exposure.

The rapid catabolism of $\gamma_1$-macroglobulin in patients with protein-losing enteropathy suggests that in this syndrome, substantial quantities of the protein are lost into the intestinal tract where breakdown takes place. If all of the increase, beyond the normal endogenous catabolism of 18.7% per day, is assumed to be due to loss into the gastrointestinal tract, it can be calculated that an average protein equivalent of about 40% of the intravascular pool of $\gamma_1$-macroglobulin was lost in this manner each day. All four of the patients with protein-losing enteropathy had previously been studied with $I^{131}$ albumin, and two of these had also had $I^{131}$ 6.6 S gamma globulin turnover studies. In these patients the increase in catabolism (above the normal) for albumin, 6.6 S $\gamma$-globulin, and 18 S $\gamma_1$-macroglobulin was similar (35 to 40% of the intravascular pool). The finding that plasma proteins of different size are lost at similar rates in gastroenteropathy contrasts with the urinary protein losses in nephrotic syndrome where size determines the relative amount lost as shown by Blainey, Brewer, Hardwicke, and Soothill (21). The findings in patients with gastrointestinal protein loss are compatible with transmission of plasma itself, or a substance closely related to plasma, into the gastrointestinal tract in quantities that may be equivalent to more than one-third of the total plasma volume per day.

## Summary

The metabolic behavior of normal human $\gamma_1$-macroglobulin was studied in seven normal subjects and in 26 patients with a variety of diseases. $\gamma_1$-Macroglobulins were isolated from normal human serum, labeled with $I^{131}$, and utilized in 37 turnover studies in 33 subjects. The relative roles of synthesis and catabolism in determining serum $\gamma_1$-macroglobulin levels were assessed.

The $\gamma_1$-macroglobulins remained predominantly in the intravascular compartment with an average value of 82% for the whole group. This is similar to observations on the distribution of fibrinogen (80% intravascular) but differs from 6.6 S $\gamma$-globulins, albumin, and transferrin where only about 40% of the body content is intravascular.

The biological half-time ($t_{\frac{1}{2}}$) of $\gamma_1$-macroglobulin was found to average 5.1 days in the control subjects. Approximately 18% of the circulating $\gamma_1$-macroglobulin was catabolized per day. In the control subjects the turnover (synthetic) rate of $\gamma_1$-macroglobulin was found to be 0.2 to 0.7 g per day, averaging 6.9 mg per kg per day.

$\gamma_1$-Macroglobulin metabolism differs from 6.6 S gamma globulin metabolism in the shorter survival of $\gamma_1$-macroglobulin (5.1 vs. 23 days) and in the lower net synthetic rate (15 to 20% of the amount of 6.6 S gamma globulin synthesized per day).

Low serum $\gamma_1$-macroglobulin levels were found in agammaglobulinemia, multiple myeloma, and protein-losing gastroenteropathy. In agammaglobulinemia and multiple myeloma, deficient $\gamma_1$-macroglobulin synthesis was responsible for low serum levels.

In protein-losing gastroenteropathy, increased catabolism ($t_{\frac{1}{2}}$ averaged 1.5 days) was responsible for the low serum $\gamma_1$-macroglobulin levels. The rate of $\gamma_1$-macroglobulin synthesis was normal, however, indicating that no compensatory increase in $\gamma_1$-macroglobulin synthesis had occurred in the patients with protein-losing gastroenteropathy.

In macroglobulinemia the high serum macroglobulin level was due to markedly increased synthesis of the anomalous macroglobulin. *d*-Penicillamine had no effect on the rate of $\gamma_1$-macroglobulin catabolism in two patients.

Observations were made in patients with serum $\gamma_1$-macroglobulin levels ranging from 5 to 5,000% of normal. These indicate that the serum $\gamma_1$-macroglobulin level is directly related to the rate of $\gamma_1$-macroglobulin synthesis (in the absence of abnormal gastrointestinal protein loss) and that there is no relationship between the fractional catabolic rate and the serum concentration.

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