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THE METABOLISM OF GAMMA GLOBULINS IN MYELOMA AND ALLIED CONDITIONS *

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Previous investigations of the turnover of normal and "pathological" gamma globulins using radioisotopically *in vitro* trace-labeled proteins in patients with multiple myeloma, macroglobulinemia, and benign M-type hypergammaglobulinemia (1–10) have yielded different results. The present study was undertaken to delineate and compare simultaneously the metabolism of normal gamma globulin and M-components in nine patients with M-component disease. This has been made possible by the use of *a*) two radioactive isotopes of iodine to study two proteins at the same time in a single patient and *b*) a recently developed method of analysis particularly suited to this sort of investigation.

MATERIALS AND METHODS

Patients. A summary of the clinical and routine laboratory findings of the nine patients investigated is presented in Table I.

Patient AA died of an Adams-Stokes attack after a short period of paraproteinemic coma about 2 months after the completion of this investigation. Post-mortem examination confirmed the diagnosis of multiple myeloma as well as myeloma renal disease. Patient JS also died several months after being studied, but no post-mortem examination was performed.

Exact classification of the M-component disease was clear-cut in most of the patients, but was somewhat uncertain in three patients. Patient ILL is considered to have multiple myeloma rather than benign M-type hypergammaglobulinemia because of the abnormal appearance of her plasma cells and the progressive increase in the serum concentration of M-component during the 9 months she has been observed. Nevertheless, unequivocal osteolytic lesions have not yet been demonstrated. The period of observation of patient GP is thought to be too short to allow one to exclude completely the diagnosis of myeloma, although the evidence to date supports the diagnosis of benign M-type hypergammaglobulinemia. Because of pathological vertebral body fractures and an increased number of plasma cells in the sternal marrow, patient NM is considered to have multiple myeloma even though the complete clinical syndrome is lacking.

Protein preparations. Normal γ-globulin [1] was prepared from the sera of four healthy blood donors fractionated separately. Four of the preparations were produced by ammonium sulfate fractionation followed by chromatography on diethylaminoethyl cellulose (DEAE) with gradient elution (11) from 0.005 M Tris-phosphate, pH 8.0, to 0.3 M Tris-phosphate, pH 7.4. The protein peak eluted with the starting buffer was reprecipitated with ammonium sulfate, dissolved in isotonic saline, and dialyzed against two changes of isotonic saline. One normal γ-globulin preparation was prepared by first precipitating non-γ-globulin material with Rivanol (6,9-diamino-2-ethoxyacridine) (12) and then carrying out further purification as described above.

M-components were purified by the methods outlined by Laurell (13), which included the techniques already described plus chromatography on carboxymethyl cellulose (CM) using gradient elution from 0.01 M acetate buffer, pH 5.4, to 0.3 M acetate buffer, pH 5.4 (14). All operations were carried out at 4 to 10° C.

All protein preparations used in this study were characterized by paper electrophoresis (15), starch gel electrophoresis (16), cellulose acetate electrophoresis (17), and microimmunoelectrophoresis (18). Antisera for the latter included commercial rabbit antisera against whole human serum and rabbit antisera prepared in this laboratory against purified normal γ-globulin and various γ_{1A} and γ_{1M} M-components. By these criteria all preparations were between 90 and 98% pure. Immunoelectrophoresis revealed one or two non-γ-contaminants in six of the preparations, but these were not generally detectable by the other techniques. On starch gel electrophoresis, the two γ_{1A}-proteins characteristically resolved into multiple bands, and Mγ_{1A}ILL showed a small amount of α_2

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[1] The following are synonyms for the nomenclature used here: γ-globulin = γ_2-globulin, γ_{ss}-globulin, 7Sγ-globulin; γ_{1A}-globulin = γ_1-globulin, β_{2A}-globulin; γ_{1M}-globulin = 19Sγ-globulin, gamma macroglobulin, β_{2M}-globulin; γ_μ = micromolecular γ-globulin, γ_L (including Bence Jones protein). The term M-component refers to proteins related immunochemically to the above globulins occurring as greatly increased serum or urine concentrations of one or at most a few molecular species.

TABLE I

A summary of the clinical and laboratory findings

Patient	Sex	Age	Weight	Bone lesions	Marrow	Hemoglobin	M-component: Type	M-component: Serum concentration	M-component: Paper electrophoretic mobility	Normal γ-globulin concentration	Proteinuria*	Non-protein nitrogen	Diagnosis†	Known duration	Symptoms	Treatment
		yrs	*kg*			*g/100 ml*		*g/100 ml*		*g/100 ml*		*mg/100 ml*				
GP	F	58	50	0	Normal	11.5	γ	2.04	γ_3	0.28	0	30	H	3 yrs	Psychosis	l-Phenyl-alanine mustard
OE	M	73	84	0	Normal	13.1	γ	2.46	γ_2	0.23	0	40	H	4 yrs	Dyspnea, headache, palpitations, infections	
EH	F	57	68	+	Plasma cells, 25%	10.4	γ	4.81	β_2	0.34	0	28	A	3 yrs	Back pain, hip pain, fatigue	
JP	M	59	82	+	Increase in plasma cells		γ	1.90	γ_1	0.80	0	38	A	3 mos	Dyspnea	
JS	F	54	59	+	Increase in plasma cells	10.8	γ	3.20	γ_1	0.40	+	46	A	1 yr	None	
ILL	F	47	68	±	Plasma cells, 5%	12.6	γ_{1A}	2.00‡	β_2	0.30	0	27	A	9 mos	Back pain, fatigue	
NM	F	76	53	+	Plasma cells, 11%	12.8	γ_{1A}	1.06‡	β_2	0.27	0	19	A	3 mos	Back pain, anorexia, fatigue	
AA	M	77	67	+	Plasma cells, 19%	12.8	γ_μ	0	γ_2	0.35	+	55	A	4 mos	Back pain	Prednisone
SH	M	52	85	0	Normal	14.4	γ_{1M}	2.96	γ_1	0.83	0	30	C	4 yrs	Fatigue, oral bleeding, coagulation defect	

* Nitric acid ring test.
† H = benign M-type hypergammaglobulinemia; A = multiple myeloma; C = macroglobulinemia.
‡ The M-components in these patients' sera migrated as double bands in paper electrophoresis.

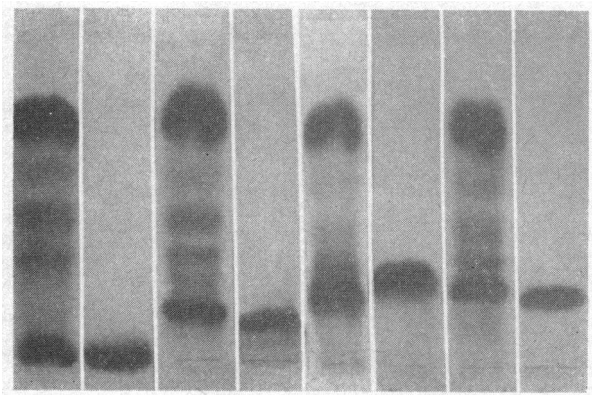

FIG. 1. PAPER ELECTROPHORETIC PATTERNS OF FOUR WHOLE SERA AND THE CORRESPONDING PURIFIED M-COMPONENTS. From left to right the strips are GP serum, MγGP, SH serum, Mγ_{1M}SH, MM serum, Mγ_{1A}MM, EH serum, and MγEH. Fresh normal human serum shows two distinct bands in the beta region when studied by this technique (15), owing to the addition of calcium to the standard barbiturate buffer.

contaminant. The γ_{1M}-proteins, as expected, did not enter the gel. The relative electrophoretic mobility on paper and the immunologic type of each M-component are given in Table I. Figure 1 shows the paper electrophoretic patterns of four whole sera and their corresponding purified M-components.

The fraction designation scheme is as follows: Pathological proteins are preceded by the letter "M." The immunoelectrophoretic type is followed by the designation for the donor, either I through IV for the normal γ-globulin preparations or code letters for patients. Finally, the isotope of iodine used for labeling is appended where that is pertinent. If two separate preparations of M-components have been used, these are designated I and II.

Protein concentrations were determined by the biuret method (19) and related to a human serum albumin standard. Sera and protein fractions were stored at $-20°$ C until used.

Trace-labeling. I^{131} and I^{125}, free of reducing agents and carrier iodide,[2] were used to label the protein preparations by the iodine monochloride technique of McFarlane (20). Labeling efficiency varied from 20 to 80%. No more than a mean of 1 atom of iodine per molecule of protein was introduced. Free iodide was removed after labeling by chloride-saturated Deacidite FF[3] resin. Human serum albumin added at this point reduced self-irradiation of the protein. Sterilization was accomplished by passing the protein solution through a membrane filter. All preparations were injected within 24 to 48 hours of labeling. Less than 3% of the radioactivity of these preparations was not precipitated in 10% trichloroacetic acid.

[2] Obtained from the Radiochemical Centre, Amersham, England.

[3] Permutit Co., Ltd., London, England.

A small amount of each labeled protein was added to the donor patient's serum, and the latter was then subjected to paper electrophoresis. The paper electrophoretic strip was then scanned in a strip radioactivity counter to assure that the radioiodinated protein's mobility was the same as that of the native M-component. The γ_{1M} M-components were tested further for absence of depolymerization after labeling by subjecting them to starch gel electrophoresis and radioautographing the gel. All the material remained at the application zone and hence was presumably intact.

Administration of labeled proteins. Each patient received two proteins, one labeled with I^{125} and the other with I^{131}, as outlined in Table II. The dose of each protein consisted of 5 to 40 mg containing 5 to 60 μc of radioactivity. The two proteins selected for each patient were administered as a single mixed dose intravenously. No untoward reactions were observed. Each patient in the study received 50 mg inactive sodium iodide 4 times daily by mouth for 1 day before, during, and for 1 week after the labeled protein study. Those patients whose radioactive iodide renal excretion was investigated also received sodium iodide before and during these studies that usually lasted only 24 to 30 hours.

Plasma and urine samples. Heparinized blood samples of about 5 ml each were collected at frequent intervals during the first 2 days and then every 24 hours for a total of 7 to 25 days. Two ml of plasma from each sample was pipetted into polyethylene counting tubes. All urine was collected in 24-hour portions for from 7 to 14 days, the total volumes were measured, and 2.0-ml samples were pipetted into counting tubes.

Determination of radioactivity. Plasma and urine samples were counted in a well-type scintillation counter coupled to a pulse-height analyzer as in the method of Cohen and Freeman (23). This technique gave good discrimination between I^{125} and I^{131} radioactivity. The same material used for labeling was used to prepare I^{125} and I^{131} standard solutions, which were counted at the same time as the samples. The error of counting was 1% or less at the beginning and 3% or less toward the end of each study.

The amount of radioactivity given to each patient (dose) was determined as I^{131} by counting the bottle containing the dose on a lead plate over the well of the counter and recounting the bottle after administration with the washings from the syringe used to inject the labeled proteins. Care was taken to ensure constant geometrical relationships. Counts per minute measured by this arrangement were related empirically to counts measured in the well directly. The I^{125} dose was easily calculated from the I^{125} radioactivity in the 10-minute plasma sample and the plasma volume calculated from the I^{131} radioactivity in the same sample and the known I^{131} dose. It is assumed that at this time mixing in the plasma compartment was complete and that passage from this compartment owing to either distribution or catabolism was negligible.

Treatment of data. All plasma radioactivities as counts per minute were corrected for background, radioactive

decay, and fluctuations in plasma volume. The last was accomplished by dividing the counts per minute by the total protein concentration of the sample, thus obtaining the "total protein-specific activity." I^{125} counts per minute were also corrected for contribution from I^{131} as described elsewhere (23). Finally, the plasma "protein-specific activity" was expressed as a percentage of the 10-minute plasma sample radioactivity and hence as percentage of dose.

Excreted radioactivity per milliliter of urine, corrected for background, decay, and contribution of I^{131} radioactivity to the I^{125} counting channel, was multiplied by the 24-hour urine volume to obtain total radioactivity excreted per period. This was finally divided by the dose and thus expressed as the percentage of dose excreted per 24-hour period. The urine of all patients with proteinuria was tested for protein-bound radioactivity. In no case was this of significant amount.

Iodide excretion. This was measured by the method of Zizza, Campbell, and Reeve (24) and expressed as parts per day removed from the body. The latter is termed k_5.

RESULTS

A. *Evidence for extravascular catabolism.* Although it is well established that serum albumin is catabolized in a pool in very rapid exchange with the plasma (25–28), there is no evidence that this is the case for the proteins considered here in the present patients. In this report the term "intravascular catabolism" will be used to include catabolism in an apparent pool in rapid equilibrium with the intravascular space.

Some information with regard to site of catabolism may be obtained from the daily urinary radioactivities. If catabolism takes place in a compartment in slow exchange with the plasma, one would expect rising excretion over the first few days, reflecting the relatively slow influx of labeled protein molecules into this pool. Conversely, if catabolism occurs in a compartment in very rapid exchange with the plasma, excreted radioactivity would be maximal on the first day. The bottom portions of Figures 2 and 3 show examples of both of these types of patterns. Thus, there is evidence that both proteins studied in GP are catabolized partly extravascularly and that in SH normal γ-globulin is catabolized intravascularly.

TABLE II

Kinetic tracer constants, extravascular/intravascular pool ratios, and mass turnovers as calculated by Nosslin's method

Protein	Patient	k_1*	k_2	k_3	k_4	k_5†	Pool ratio‡ E/P 1	Pool ratio‡ E/P 2	Mass turnover‡ 1	Mass turnover‡ 2
									g/day	
γI-125	GP	0.236 ± 0.008	0.446 ± 0.018	0.064 ± 0.002	0.076 ± 0.005	1.66	0.45	0.67	0.53	0.62
MγGP-131	GP	0.135 ± 0.002	0.077 ± 0.002	0.054 ± 0.002	0.028 ± 0.002	1.66	1.29	2.45	4.24	5.76
γI-125	EH	0.155 ± 0.018	0.169 ± 0.032	0.057 ± 0.003	0.054 ± 0.006	1.73	0.70	1.26	0.79	1.04
MγEH-131	EH	0.177 ± 0.017	0.152 ± 0.026	0.023 ± 0.006	0.060 ± 0.009	1.73	0.84	1.32	8.59	11.96
γIV-125	JP	0.357 ± 0.003	0.284 ± 0.003	0.037 ± 0.003	0.028 ± 0.003	[1.00]	1.15	1.39	1.92	2.11
		0.335 ± 0.003	0.283 ± 0.003	0.060 ± 0.013	0.010 ± 0.011§	[3.00]	1.14	1.40	1.98	2.06
MγJP-131	JP	0.335 ± 0.005	0.201 ± 0.003	0.038 ± 0.008	0.012 ± 0.005§	[1.00]	1.66	1.95	3.94	4.17
		0.390 ± 0.004	0.198 ± 0.003	0.024 ± 0.002	0.021 ± 0.005	[3.00]	1.78	2.09	4.11	4.54
γIV-131	JS	0.341 ± 0.005	0.331 ± 0.006	0.014 ± 0.003	0.016 ± 0.004	[1.00]	0.94	1.07	0.60	0.66
		0.344 ± 0.002	0.324 ± 0.006	0.013 ± 0.001	0.032 ± 0.004	[3.00]	0.97	1.10	0.59	0.65
MγJS-125	JS	0.336 ± 0.014	0.296 ± 0.035	0.014 ± 0.001	0.037 ± 0.003	[1.00]	1.01	1.18	5.50	6.17
		0.340 ± 0.034	0.290 ± 0.033	0.013 ± 0.003	0.036 ± 0.003	[3.00]	1.04	1.22	5.50	6.20
γII-125	ILL	0.109 ± 0.003	0.080 ± 0.009	0.052 ± 0.001	−0.002 ± 0.001§	1.89	1.40	2.02	0.41	0.41
$M\gamma_{1A}$ILL-I-131	ILL	0.045 ± 0.003	0.089 ± 0.016	0.086 ± 0.006	0.007 ± 0.011§	1.89	0.47	1.48	5.22	5.22
$M\gamma_{1A}$MM-125	NM	0.214 ± 0.024	0.345 ± 0.043	0.134 ± 0.008	−0.029 ± 0.014§	2.08	0.68	1.01	(3.13)‖	(3.13)‖
$M\gamma_{1A}$ILL-II-131	NM	0.191 ± 0.019	0.235 ± 0.027	0.130 ± 0.004	−0.013 ± 0.006§	2.08	0.86	1.37	(3.27)‖	(3.27)‖
γI-131¶	AA	0.104 ± 0.021	0.191 ± 0.053	0.032 ± 0.002	−0.001 ± 0.006§	[1.00]	0.55	0.71	0.36	0.36
		0.153 ± 0.011	0.295 ± 0.027	0.021 ± 0.004	−0.019 ± 0.009§	[3.00]	0.55	0.66	0.38	0.38
γI-125	AA	0.103 ± 0.014	0.255 ± 0.050	0.032 ± 0.003	−0.004 ± 0.005§	[1.00]	0.41	0.53	0.38	0.38
		0.107 ± 0.015	0.254 ± 0.047	0.035 ± 0.002	−0.009 ± 0.005§	[3.00]	0.44	0.56	0.41	0.41
γI-125	SH	0.302 ± 0.026	0.369 ± 0.036	0.047 ± 0.006	0.013 ± 0.008§	2.77	0.79	0.95	1.74	1.74
$M\gamma_{1M}$SH-131	SH	0.283 ± 0.031	0.556 ± 0.088	−0.003 ± 0.017§	0.265 ± 0.033	2.77	0.34	0.50	12.97	19.07

* All values for k_{1-4} are given ± standard error.
† Where k_5 was not measured directly, the assumed limits of the normal variation of this constant, 1.00 and 3.00 (21, 22), have been used separately in the calculation of the other constants.
‡ 1 =entirely intravascular and 2 =entirely extravascular synthesis.
§ Not significantly different from zero ($p > 0.05$ in a "one-tailed" test).
‖ Assuming that the patient's M-component behaved as did the labeled homologous M-component.
¶ Rivanol was used in purification.

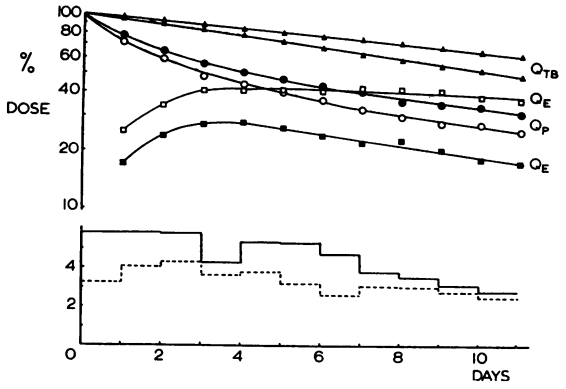

FIG. 2. PLASMA (Q_P, CIRCLES), EXTRAVASCULAR (Q_E, SQUARES), AND RETAINED OR TOTAL BODY (Q_{TB}, TRIANGLES) RADIOACTIVITY AS PERCENTAGE OF THE DOSE IN PATIENT GP AS PLOTTED ON A SEMILOGARITHMIC SCALE. Open symbols represent the M-component (MγGP-131) and closed symbols represent normal γ-globulin (γI-125) radioactivity. Daily excreted radioactivity of the normal γ-globulin is shown as a solid line and that of the M-component as an interrupted line on a linear scale in the bottom portion of the figure.

whereas the M-component is largely, if not exclusively, degraded in an extravascular pool. A low iodide-excretion rate would tend to produce an extravascular pattern of excretion, but a difference in pattern between two proteins studied in a given patient cannot be ascribed to this cause. Furthermore, iodide excretion has been studied in these patients and was found to be normal. Denatured protein or free label in the injected material would tend to mask the extravascular type of pattern.

More information regarding this question can be

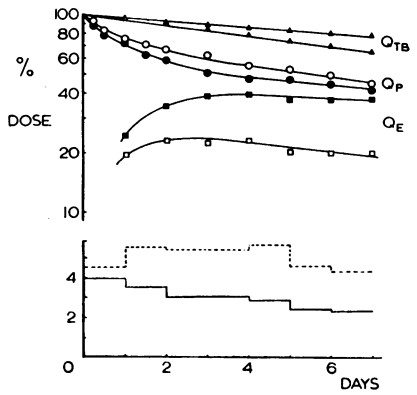

FIG. 3. PARAMETERS DEPICTED IN FIGURE 2 SHOWN HERE FOR PATIENT SH. The symbols are as in Figure 2. The M-component is Mγ_{1M}SH.

obtained by plotting daily excreted radioactivity divided by the corresponding mean daily radioactivity in the plasma, extravascular, and total body compartments (26). For the compartment where catabolism occurs, the resulting line should be straight and horizontal, especially during the first few days. After equilibration the test no longer applies, since all the quotients will then be constant (i.e., curves for the individual compartments become parallel). This test as applied to the two proteins of GP is seen in Figure 4. Since the ini-

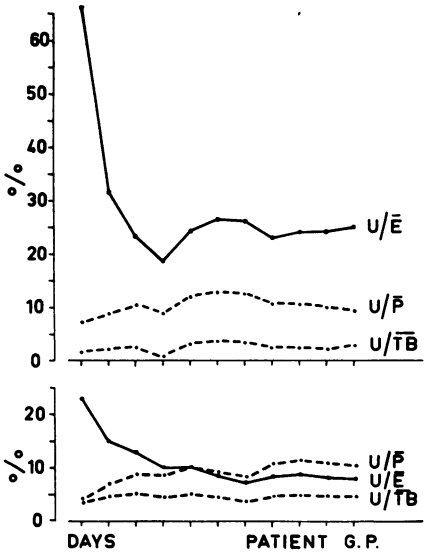

FIG. 4. QUOTIENTS OF DAILY EXCRETED RADIOACTIVITY DIVIDED BY MEAN PLASMA RADIOACTIVITY ($U/\bar{P}$), MEAN EXTRAVASCULAR RADIOACTIVTY ($U/\bar{E}$), AND MEAN TOTAL BODY RADIOACTIVITY ($U/\overline{TB}$) PLOTTED ON A LINEAR SCALE VERSUS TIME. The upper portion of the figure represents γI-125, and the lower part shows MγGP-131 studied in patient GP. After day 6 all quotients form more or less horizontal lines (are constant), whereas before this time the lines they form are most different and only $U/\overline{TB}$ for both proteins and $U/\bar{P}$ for γI-125 form horizontal lines.

tial portions of the $U/\overline{TB}$ plot and the $U/\bar{P}$ plot of normal γ-globulin are most horizontal, we can conclude that both proteins are catabolized intra- and extravascularly. The results of this test applied to the remainder of the proteins are presented in Table IV. Differences in the results of the two proteins studied simultaneously in the same patient are probably most significant, since experimental errors (particularly those of urine collection) would affect the behavior of both

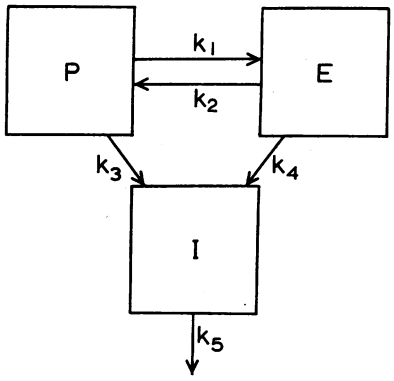

FIG. 5. MODEL USED FOR THE NOSSLIN ANALYSIS. It is the same as model C of Reeve and Roberts (33). The labeled protein is introduced into the intravascular compartment (P) where equilibrium takes place with the extravascular space (E) at rates defined by the tracer kinetic constants k_1 and k_2. Catabolism occurs potentially from the intravascular compartment at the rate k_3 and from the extravascular compartment at the rate k_4. The label released from catabolism of the labeled protein then enters the degradation products distribution space (I) from which it is excreted at the rate k_5.

labels in the same way. Hence, there is qualitative evidence that some of the proteins are catabolized extravascularly, at least in part.

B. *The application of Nosslin's method.* Nosslin's analysis (29) was applied to the data of the present investigation in a form based on the model shown in Figure 5. The data are plotted on a linear scale, and first derivatives of the plasma (P) and first and second derivatives of the cumulated urinary (U) curves are determined mechanically (30). Radioactivity in the degradation products distribution space (I) is determined from k_5 and the first derivative of U by the relationship

$$\frac{dU}{dt} = k_5 I. \qquad [1]$$

The extravascular radioactivity (E) is $100 - P - U - I$. Then, since the total rate of label release from catabolism equals the total rate of appearance of nonprotein-bound label,

$$\frac{dU}{dt} + \frac{1}{k_5}\frac{d^2U}{dt^2} = k_3P + k_4E \qquad [2]$$

or (rearranged)

$$\frac{\frac{dU}{dt} + \frac{1}{k_5}\frac{d^2U}{dt^2}}{P} = k_3 + k_4\frac{E}{P}. \qquad [3]$$

If the data can be described by the chosen model, then the line obtained by plotting values for the left-hand expression of Equation 3 versus E/P is straight, its intercept on the ordinate is k_3, and its slope is k_4. The values of k_3 (the intravascular catabolic constant) and k_4 (the extravascular catabolic constant) may thus be determined by regression analysis, and errors may be calculated. These latter, of course, are measures of "fit" and not estimates of experimental error.

The remaining constants, k_1 and k_2, are determined analogously from the equation

$$\frac{k_3P + \frac{dP}{dt}}{P} = -k_1 + k_2\frac{E}{P}. \qquad [4]$$

In practice, only the values from days 2 to 5 are used to determine the constants. Thus, the first 48 hours, when the effects of denatured protein and free label in the dose are most likely to be maximal, are eliminated (this, then, is a form of self-screening of the labeled protein). Avoiding later values minimizes cumulative errors, and since these values tend to cluster, they give little information on slope or intercept.

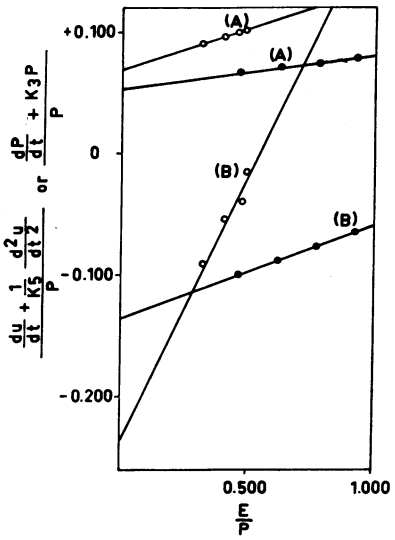

FIG. 6. FINAL PLOT OF THE ANALYZED DATA OF γI-125 (OPEN CIRCLES) AND MγGP-131 (CLOSED CIRCLES) IN PATIENT GP ACCORDING TO NOSSLIN'S METHOD. The lines (A) represent $[(dU/dt)+(1/k_5)(d^2U/dt^2)]/P$ plotted versus E/P, and the lines (B) represent $[(dP/dt)+(k_3P)]/P$ plotted versus E/P.

In this method, pool ratios are calculated by the following equations:

$$\frac{E}{P} = \frac{k_1}{k_2 + k_4} \quad [5]$$

and

$$\frac{E}{P} = \frac{k_1 + k_3}{k_2}. \quad [6]$$

Equation 5 is based on the assumption of intravascular synthesis, whereas 6 is based on the assumption of extravascular synthesis. Therefore, since it is not known which of these assumptions is correct, E/P is obtained as a range.

When this method was applied to the present data, the plots for determining the kinetic constants were all straight lines (Figure 6) except those of both proteins of OE. Since the plasma curves in this patient required three exponentials for resolution in conventional analysis, it is not surprising that the simple model chosen did not fit the data. In some cases, the early (up to 36 hours) points in the plot for k_1 and k_2 did not fall on a straight line, probably reflecting the fact that there are many extravascular pools which become "lumped" by 48 hours and thereafter behave as a single pool.

Since statistical fitting errors are obtained for the kinetic constants, it is possible to *a*) determine whether the constants are statistically different from zero and *b*) compare corresponding constants for both proteins studied in single patients.

The values for kinetic constants with their standard errors, mass turnover data, and pool ratios as calculated by Nosslin's method are given in Table II. Table III gives the statistical comparisons of the paired constants in each patient.

C. *Comparison with conventional analyses.* To compare these results with those in the literature, the data have also been analyzed by several conventional techniques. For these, plasma radioactivity (Q_P), total body or retained radioactivity (Q_{TB}, calculated by subtracting the cumulated urinary radioactivity from 100), and extravascular radioactivity (Q_E, retained minus plasma radioactivity) are plotted on a semilogarithmic scale. Figures 2 and 3 show the plots for two patients, GP and SH.

The results according to the Sterling (31), Matthews (32), Campbell, Cuthbertson, Matthews, and McFarlane (26), and Reeve and Roberts (33) methods are given in Table IV. The fractional catabolic rates as calculated by these methods, in general, differ from each other and from those of Nosslin's analysis, whereas all the mass turnover data are similar no matter which method is used. The Sterling method catabolic rate is based on the final slope of the plasma curve and is thus an apparent degradation rate of the total exchangeable pool after equilibration. Therefore, there is little basis of comparison with Nosslin's k_3 and k_4. The same reasoning applies to the $t_{\frac{1}{2}}$ of the final slope of the plasma curve, since this is simply ln 2/degradation rate in the Sterling analysis. The $t_{\frac{1}{2}}$'s of the final exponentials of each of the plasma curves are given in Table IV. These varied between 7.4 and 28.2 days for all proteins studied, and no consistent differences were noted between those of normal γ-globulin and M-components or between those of proteins of different immunoelectrophoretic types.

Matthews' analysis is based on the resolution of

TABLE III

*P values for the statistical comparisons between the tracer kinetic rate constants of the two proteins studied in each patient as determined by Nosslin's method**

Patient	k_1	k_2	k_3	k_4
GP	0.20–0.10	$<0.01^{++}$	$<0.05^{+}$	$<0.01^{++}$
EH	0.50–0.40	0.70–0.60	0.70–0.60	0.70–0.60
JP ($k_5 = 1$)	0.70–0.60	$<0.01^{++}$	0.80–0.70	$<0.05^{+}$
($k_5 = 3$)	$<0.01^{++}$	$<0.01^{++}$	0.10–0.05	0.40–0.30
JS ($k_5 = 1$)	0.90–0.80	0.50–0.40	0.60–0.50	0.60–0.50
($k_5 = 3$)	0.95	0.50–0.40	0.20–0.10	0.50–0.40
ILL	$<0.05^{+}$	0.70–0.60	0.80–0.70	0.50–0.40
NM	0.50–0.40	0.20–0.10	0.80–0.70	0.40–0.30
AA ($k_5 = 1$)	0.90–0.80	0.10–0.05	0.95–0.90	0.50–0.40
($k_5 = 3$)	0.20–0.10	0.60–0.50	0.20–0.10	0.50–0.40
SH	0.80–0.70	0.30–0.20	$<0.05^{+}$	$<0.01^{++}$

* Symbols used: $^{+}$ = probably significant difference; $^{++}$ = significant difference.

TABLE IV

The results of analyzing the data of the present investigation by several of the usual methods

Protein and label	Patient	Duration of plasma sampling	Duration of urine sampling	$t_{\frac{1}{2}}$	Fractional catabolic rate					Mass turnover			Result of qualitative test for site of catabolism‖
					1*	2†	3‡	4§ k_3	4§ k_4	1*	2†	3‡	
		days	*days*	*days*	*%/day*					*g/day*			
γI-125	GP	11	11	10.8	6.4	9.5	10.3	10.7	−0.7	0.5	0.5	0.6	TB, P
MγGP-131	GP			11.4	6.1	8.4	9.2	6.7	2.6	6.7	4.0	4.3	TB
γIII-125	OE	28	9	12.6	5.5	9.3	9.2	12.7	−4.6	1.2	1.0	1.0	TB
MγOE-131	OE			16.7	4.1	6.4	7.4	5.3	2.9	8.9	7.2	8.4	E
γI-125	EH	7	7	8.3	8.3	8.1	14.2	10.4	15.9	0.9	0.7	1.2	TB
MγEH-131	EH			9.1	7.6	5.3	10.7	2.5	16.9	13.1	6.2	12.5	TB
γIV-125	JP	24	10	20.0	3.5	7.2	7.8			2.2	2.0	2.2	TB, P
MγJP-131	JP			21.7	3.2	5.7	8.5			5.9	3.8	5.7	TB
γIV-131	JS	25	14	26.7	2.6	5.0	5.1			0.7	0.7	0.7	TB, P
MγJS-125	JS			28.2	2.5	4.4	5.1			5.6	4.7	5.5	TB
γII-125	ILL	8	8	7.8	8.9	10.2	7.7	10.8	−4.2	1.4	0.8	0.6	P
$M_{\gamma 1A}$ILL-I-131	ILL			7.4	9.4	10.4	11.3	13.9	−20.4	7.3	6.2	6.8	P
$M_{\gamma 1A}$MM-125	NM	28	12	8.5	8.2	12.7	13.9	14.5	−1.2	3.8	3.4¶	3.7¶	TB, P
$M_{\gamma 1A}$ILL-II-131	NM			9.7	7.1	11.8	13.4	10.0	4.8	4.0	3.2¶	3.6¶	TB
γI-125	AA	8	8	26.3	2.6	3.3	3.7			0.4	0.4	0.4	P
γI-131**	AA			19.2	3.6	3.5	4.1			0.7	0.4	0.5	P
γI-125	SH	7	7	16.9	4.1	5.5	6.1	10.0	4.8	2.3	1.7	1.9	TB
$M_{\gamma 1M}$SH-131	SH			10.6	6.5	8.5	9.5	6.7	−0.8	13.3	13.3	14.9	TB, E

* Sterling method.
† Method of Campbell and others.
‡ Matthews' method.
§ Reeve's and Roberts' method.
‖ See text. TB = total body; P = plasma; E = extravascular.
¶ Assuming that the patient's M-component behaved as did the labeled homologous M-component.
** Rivanol was used in purification.

the plasma curve into exponentials. All the curves of the present investigation could be analyzed into two such exponentials except for the curves of both proteins of OE, which required three. Fractional catabolic rates as calculated by this technique and that of Campbell and co-workers would be expected to agree with the k_3 values of Nosslin's analysis only in those cases where $k_4 = 0$, since the former methods assume intravascular catabolism only. This was approximately true, although these methods gave slightly higher intravascular catabolic rates than did Nosslin's analysis where k_4 was zero.

The Reeve and Roberts method, as applied to their model C (the same as that in Figure 5), allows for extravascular catabolism. The results of applying this method to cases where k_5 was measured are given in Table IV. In a few cases, there was agreement between k_3 and k_4 determined by this method and that of Nosslin, but many results were very discrepant. This may have been because some of our experiments were too short for accurate assessment of all slopes and intercepts. In our hands, however, slight changes in the exponential analysis resulted in markedly different kinetic constants in the Reeve and Roberts analysis. Finally, there are no criteria for deciding when a small value for k_3 or k_4 is actually zero. In two experiments large negative values for k_4 were obtained by this method (ILL and OE). These are very difficult to interpret.

DISCUSSION

When we compare the results of the various methods of analysis of data (Tables II and IV), we find that the mass turnover data of a given protein are in good agreement no matter which method of analysis or model is used. The present results indicate that in patients with myeloma and allied conditions, the M-components are synthesized at a higher rate than normal γ-globulin in normal subjects [approximately 1.0 to 2.0 g per day (23, 34)]. In some patients rates as much as 6 times the normal were observed. This is in agreement with earlier findings of other workers (2, 7). The high serum concentrations of M-components that are usually seen are thus attributable to these high synthesis rates.

Normal γ-globulin concentrations are often re-

duced in patients with the diseases under consideration here (35, 36). The mass turnover of γ-globulin in all patients where this was studied was less than that in normal subjects with two exceptions. These were JP with myeloma and SH with macroglobulinemia, both of whom had normal serum concentrations and plasma pools of this protein. Part of the reason for the low levels of normal γ-globulin in patients with diseases associated with M-components can thus be ascribed to a production deficit. Our findings are consistent with those reported briefly by Solomon, Fahey, and Waldmann (9).

The fractional catabolic rates of M-components and normal γ-globulin in M-component disease have previously been reported in two forms: as $t_{\frac{1}{2}}$'s of the final slopes of the plasma or total body curves (1–6, 8, 10) or as fractions of the plasma pool per day (7). The $t_{\frac{1}{2}}$ alone is, in our view and that of others (37, 38), not a meaningful parameter for comparison without other information, such as is provided by the intercept of the final slope and the slopes and intercepts of the other exponentials that make up the observed and derived curves. Nevertheless, the $t_{\frac{1}{2}}$'s of I^{131}-labeled M-components have been reported as varying between about 7 and 18 days, which is the same range reported for I^{131}-labeled γ-globulin in normal subjects (39–42). In the present study, this same range was observed, although a few $t_{\frac{1}{2}}$'s were longer than 20 days. The $t_{\frac{1}{2}}$'s of the two proteins studied in each patient were more similar to each other than were those of similar proteins in different individuals. We did not observe, as has one group (3–6), a longer $t_{\frac{1}{2}}$ of normal γ-globulin and γ-mobility M-components in patients with a β-mobility M-component compared with those in patients with a γ-mobility M-component. In fact, the opposite was the case in our study: in four γ patients, normal γ-globulin and γ-mobility M-components had a mean $t_{\frac{1}{2}}$ of 19.8 days as compared with a mean $t_{\frac{1}{2}}$ of 8.0 days for normal γ-globulin and 8.7 days for β-mobility M-components in three patients with β-mobility serum M-components.

Since normal γ-globulin in normal subjects is catabolized at the rate of 3 to 8% of the plasma pool per day without extravascular catabolism (23, 43), the results of the qualitative tests and Nosslin's analysis indicate that the presence of an M-component may in some cases be associated with extravascular catabolism as well. In general, the fractional catabolic rates were the same for both proteins studied in each patient, as seen from Table III, so that the abnormal site and possibly the mode of catabolism affects both proteins. The two exceptions to this were in the case of macroglobulinemia (SH) and one patient with benign M-type hypergammaglobulinemia (GP). The findings in the former patient where γ-globulin was catabolized normally but the macroglobulin was catabolized only extravascularly are most interesting. This finding implies a different mode of catabolism for these two proteins. In the two patients with γ_{1A} myeloma all proteins were catabolized intravascularly.

Since the intravascular catabolic rates were normal or slightly subnormal in patients where extravascular catabolism occurred, it may be that the superimposition of extravascular catabolism contributes to the observed decrease in normal γ-globulin concentration in these patients.

It is tempting to speculate that the extravascular component of catabolism reflects breakdown of γ-globulins by malignant plasma cells that may represent a compartment in relatively slow equilibrium with the plasma. This hypothesis was first put forth several years ago (1). Since different cell types are usually involved in macroglobulinemia than in myeloma, it may be for this reason that normal γ-globulin levels are less often disturbed in this condition than in myeloma.

The curious but general finding that γ M-components had a higher *E/P* ratio than normal γ-globulin may possibly reflect complex-formation between the pathological proteins and cellular elements of the blood or vascular endothelium not observed with normal γ-globulin.

The finding of others (7, 8) that γ_{1M}-proteins are more confined to the plasma compartment than are γ- or γ_{1A}-globulins was confirmed.

SUMMARY

Normal γ-globulin and M-component metabolism is studied in nine patients with myeloma and allied diseases. By two isotopic labels, I^{125} and I^{131}, two proteins are studied simultaneously in each patient. Because of evidence for extravascular catabolism of immune globulins in some

of these patients, a new method of analysis is applied to the data. Intravascular and extravascular catabolic rates, extravascular/intravascular pool ratios, and mass turnover are calculated for 16 proteins in eight patients. The catabolic rates of the pair of proteins studied in each patient are compared statistically and are shown to be the same in most instances. A notable exception is a patient with macroglobulinemia in whom normal γ-globulin is catabolized intravascularly at a normal rate, whereas the M-component is catabolized only extravascularly.

The present data support the contention that the high serum concentrations of M-components and the low serum concentrations of normal γ-globulin observed commonly in these patients are secondary to high and low synthetic rates, respectively. Extravascular catabolism superimposed on essentially normal intravascular catabolism may also contribute to the low serum concentrations of normal γ-globulin.

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