The Relationship Between L-Chain Synthesis and γ-Globulin Production

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ABSTRACT The simultaneous administration of labeled human γG globulin and L-chains to subjects has allowed examination of the metabolic relationship between a component part and the whole immunoglobulin molecule. Studies were carried out in a series of control subjects and in a group of patients where substandard production of γ-globulin was anticipated, i.e., patients with the nephrotic syndrome on chronic Imuran therapy and patients with uremia. Full expression of the plasma decay curve was obtained for both substances, that for L-chain requiring only 4–5 days except in uremic subjects and that for γ-globulin requiring up to 30–40 days. Urinary excretion of inorganic iodide was also quantitated for 20–30 days. Equilibrium of the extravascular, vascular, and urinary radioactivity from the labeled γG globulin was usually not seen during this time interval suggesting more than one site of catabolism of the protein.

Excess L-chain poduction was about 45% of the total L-chain production and probably in no instance could account for the low γ-globulin production seen in certain patients with renal disease.

INTRODUCTION

Although the structure of the γG globulins is well defined (1, 2), factors governing the synthesis of the immunoglobulins are less well understood (3). It seems likely that L-chains are produced in excess of their capability for use in synthesis of γ-globulins in normal individuals, since they are found in measurable concentration in the blood and are known to undergo rapid degradation (4). Heavy-chains are not normally found in blood and the possibility that these components are rate limiting in normal synthesis seems reasonable.

In states of suboptimal γ-globulin synthesis several mechanisms for impaired production suggest themselves. First, less than normal amounts of either L-chains or heavy-chains or both might be produced. Second, production of these chains might be normal, but fabrication of the total molecule might be faulty. Insight into this problem was sought by the simultaneous study of L-chain and γ-globulin production in a series of normal individuals and in two conditions where γ-globulin synthesis is usually subnormal, i.e., the nephrotic syndrome and uremia. Although definitive answers to the above questions concerning impaired synthesis were not obtained, it seems likely that failure of L-chain production per se was not responsible for the reduced rate of γ-globulin turnover in our abnormal subjects.

METHODS

Patient material and procedure. Five persons with renal disease and nine control subjects, three of whom were without disease, were chosen for study. As seen in Table I, minor chronic disease was present in six of the control subjects. Pertinent data concerning the patients with renal disease are also included in Table I. It is perhaps significant that despite proteinuria, neither patient with the nephrotic syndrome had measurable L-chain or γG globulin excretion. All patients with uremia excreted abnormal amounts of L-chain and one, J. Ga., had γG globulin proteinuria.

All were hospitalized on the Clinical Research Center and given 10 gtt Lugol solution daily, and on one occasion scanning over the thyroid gland was carried out which showed no uptake of radioactive iodine. Each was given approximately 25 μCi of ^{125}I-labeled L-chains and 25 μCi of ^{125}I-labeled γ-globulin by single intravenous injection. Blood samples were taken at 2, 3, 5, 10, 30, and 60 min and then at 4, 8, 12, 16, and 24 h and usually daily thereafter for at least 20 days. Spot samples were collected thereafter for the time interval up to 40 days. Urines were collected continuously during the time of hospitalization and at intervals thereafter usually coinciding with the plasma samples.

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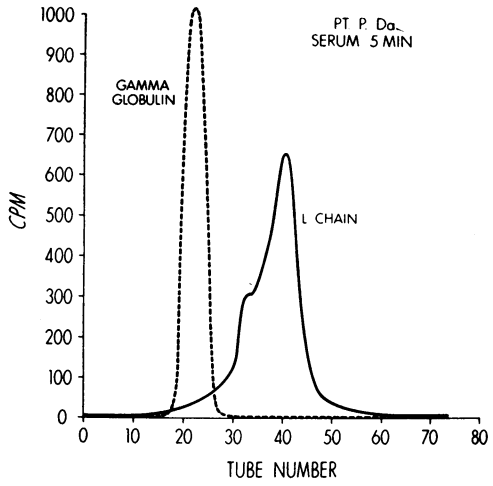

FIGURE 1 Results of fractionation of a serum taken 5 min after injection of ^{131}I-labeled γ-globulin and ^{125}I-labeled L-chain on a Sephadex G-100 column. γG globulins are eluted first, with the L-chains appearing later. Inorganic iodide, if present, comes off the column in the last tubes. Slight overlap of the γ-globulin on L-chains will be seen. For L-chain determinations, pools were made which excluded any γG globulin, and the values were subsequently corrected by a factor of the total integral divided by the integral of the analyzed fraction.

Preparation of the labeled proteins. Human IgG prepared with 25% cold ethanol was commercially obtained as Cohn Fraction II from E. R. Squibb & Sons, Princeton, N. J. This preparation was further purified by passage over a DEAE column equilibrated with 0.15 M phosphate buffer, pH 7.2. The preparation thus obtained contained only IgG when tested by immunodiffusion and immunoelectrophoresis with rabbit antisera specific for the human IgG, IgA, and IgM immunoglobulin class determinants, and a horse polyvalent antiwhole human serum. The same preparation was used for all experiments reported in this study.

An additional study was done to test the integrity of the starting material, namely the γ-globulin obtained from Cohn Fraction II. Pooled fresh sera from normal individuals were precipitated at 15% sodium sulfate saturation. The precipitate was dissolved in neutral phosphate-buffered saline (0.154 M). After dialysis against 0.015 M phosphate buffer, the sample was placed on a DEAE column which had been equilibrated with the same buffer. The preparation was labeled with ^{125}I and given simultaneously with the ^{131}I-labeled Cohn Fraction to a single patient. Plasma disappearance was identical with the two preparations as was urinary excretion through the first 2 wk.

Light-chains were obtained from the purified IgG by reduction and alkylation. IgG was reduced for 60 min at room temperature in 0.2 M Tris-HCl buffer pH 8.3, 0.05 M in dithiothreitol. No attempt was made to exclude air. A 20% molar excess of dry iodoacetamide over reducing agent was added and alkylation was allowed to proceed for 90 min at 0°C. The reduced and alkylated IgG was dialyzed against phosphate-buffered 0.154 M NaCl, pH 7.4, for 12 h, and 1 M acetic acid at 4°C. It was then placed on a Sephadex G-100 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) column equilibrated with 1 M acetic acid. The L-chains obtained were immediately dialyzed against cold distilled water for 12 h and then two changes of borate-buffered saline pH 8.0 at 4°C. The L-chain preparation contained both κ and λ L-chain determinants and was shown to be free of heavy-chain contamination when tested in immunodiffusion with a rabbit antiserum specific for human γ-chain. All L-chains were in the monomeric form as demonstrated by Sephadex G-100 column chromatography and by analytical ultracentrifugation.

Again this preparation of L-chains was tested against an isolated Bence Jones protein, a monomer of the κ type, in a single patient. The plasma disappearance curves and the urinary excretion patterns were similar.

The IgG and L-chains were labeled as described by Bale, et al. (5). Various preparations of the labeled proteins contained from 0.2 to 0.6 mol of radioiodine per mol of IgG or L-chain. 1 mg of protein contained a maximum of 50 μCi of specific activity. In order to remove any possibility of administering aggregated protein, all radiolabeled preparations were centrifuged at 35,000 rpm for 1 h in a Spinco model L preparative ultracentrifuge before injection. The top two-thirds of the centrifuged material was removed and utilized for injection. A sample from these preparations was precipitated in 5% trichloroacetic acid. In all cases greater than 99% of the labeled protein was acid precipitable. Samples of the injected material were also placed on Sephadex G-100 columns to ensure that no acid-precipitable protein subunits were present. All preparations demonstrated

TABLE I
Patient Data

Control subjects				Patients with renal disease						
Patient	Sex	Age	Diagnosis	Patient	Sex	Age	Diagnosis	Creatinine clearance	Proteinuria	Imuran
		yr				*yr*		*cm³/min*	*g/day*	*mg/day*
K. Ha.	F	25	Normal	P. Do.	M	22	Nephrotic syndrome			
B. Al.	F	76	Osteoporosis				Minimal change biopsy	148	4.4	150
N. St.	F	30	Pyelonephritis							
G. Ab.	M	34	Normal	L. Li.	F	23	Nephrotic syndrome			
P. Ra.	M	52	Chemical				Diffuse membranous			
			Diabetes mellitus				Glomerulonephritis	70	5.5	125
S. Pa.	F	51	Normal	J. Ga.	F	21	Chronic pyelonephritis	6	2.3	
D. Wi.	F	54	Osteoporosis	P. Da.	M	27	Diffuse membranous			
C. Hu.	F	31	Obesity				Glomerulonephritis	7	1.6	75
B. Si.	F	38	Obesity	M. Ra.	M	17	Chronic glomerulonephritis	8	1.7	

a single sharp elution peak. Homogeneity of the injected substance is indicated by these findings. Denaturation of the proteins by these procedures was highly unlikely, since identical labeling procedures did not abolish the crystallizable properties of an abnormal γ-globulin. Each lot of labeled proteins was given to three or four individuals and all preparations were given to patients within 48 h after labeling.

Quantitation of serum IgG and L-chain levels. Serum IgG levels were determined by radial immunodiffusion utilizing commercially available plates specific for IgG-Fc fragment (Meloy Laboratories, Springfield, Va.). All sera were assayed in triplicate at two dilutions of patient sera; levels were determined by comparison to the precipitin ring diameters obtained for various standards containing known concentrations of purified human IgG.

Serum L-chain levels were determined by complement fixation utilizing two rabbit antisera, each specific for either κ or λ light-chain determinants. In order to obtain serum fractions free of contamination by intact immunoglobulins, 1 cm³ of the patient's serum was fractionated on a Sephadex G-100 column equilibrated with pH 7.4 phosphate-buffered saline (Fig. 1). The column utilized had been previously calibrated with radiolabeled L-chain and IgG markers. The regions from the serum fractionation which corresponded in elution position to the L-chain markers were pooled and checked for lack of contamination by other immunoglobulins by immunodiffusion or complement fixation. These fractions were then tested via C′ fixation for total quantity of L-chains present and the κ-λ ratios. All L-chain quantitations were performed at least twice. Further, in order to obtain precise quantitative data, determinations were performed within 1 wk after obtaining the L-chain serum pools.

Identification of the labeled substances in plasma and urine. In order to determine the catabolic products of the injected material, various serum and urine samples from each patient were placed on Sephadex G-100 columns. Further, precipitation of the protein-bound radiolabel in the urine and serum in 5% trichloroacetic acid was performed on all samples. Samples of urine were placed on columns of ion exchange resins to determine if the recovered urine radioactivity was due to iodine bound to amino acids or to free iodine. In this manner it was determined that the intravascular radiolabeled protein remained intact during the period of the study and that the radiolabel in the urine existed as free iodine in normal individuals. These two parameters have not been previously checked.

Method of calculation. While all radioactivity given with labeled L-chain was recovered as inorganic urinary iodine, it became apparent early in the study that excreted inorganic iodide would not account for 100% of the injected dose of radioactivity given with γG globulin in the time interval of the study. It was also noted that the rate of urinary excretion of inorganic iodine over long time did not parallel the plasma radioactivity decay curve (Fig. 2). Such observations led us to examine methods of calculation which would lead to valid interpretation. Exhaustive study of the interpretation of tracer protein curves has been done by Anderson (6), Nosslin (7), McFarlane and Jeejeebhoy (8), Vitek (9), and Mathews (10), which has been applied most extensively to iodinated albumin. In general, four analytical methods have been used with tracer data by various investigators to determine the amount of protein and its rate of turnover, i.e., (*a*) biological half-life as determined from the final decay rate, (*b*) metabolic clearance or fractional catabolic rate which estimates the fraction of the plasma pool metabolized per day via the urinary excretion of inorganic iodine, (*c*) compartmental analysis of the plasma disappearance curve, and (*d*) integration of the decay curves to determine synthesis and pool size. An excellent review and critique of these methods has recently been published by Waldmann and Strober (11). While it has been clearly recognized that a basic assumption of the urinary clearance method is intravascular catabolism, most data in the literature have been derived from this method since collection of specimens over only a few days' time have been felt to be sufficient. The integrative approach, however, requires no assumption as to model or site of catabolism, but does necessitate full expression of the plasma radioactivity decay curve which may require 30–40 days. Thus, we expressly obtained sufficient early samples as well as long time data to precisely define the total curve. The 2, 3, 5, and 10 min samples allowed extrapolation back to zero time for the amount of radioactivity in 1 cm³ of plasma at time 0. Then the injected dose divided by the radioactivity per milliliter at time 0 gave an initial volume of distribution. Although the initial disappearance of L-chain was far more rapid (about 30% in 10 min) than γ-globulin (about 6% in 10 min), there was very close agreement for the initial volume of distribution of the two substances (see Tables II and III) and this volume was felt to be close to plasma volume. Then the plasma concentration times initial volume of distribution gave the size of the intravascular pool in milligrams. The plasma radioactivity disappearance curve was resolved into three exponentials by the technique of curve stripping and could be represented by $A_1e^{-\lambda_1 t} + B_1e^{-\lambda_2 t} + C_1e^{-\lambda_3 t}$. The production rate or irreversible disposal rate was determined as: intravascular pool

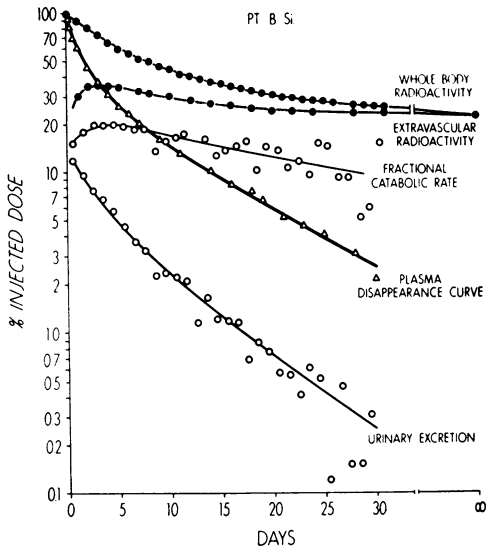

FIGURE 2 Curves of plasma radioactivity disappearance, rate of urinary excretion of inorganic iodide, whole body retention, and extravascular radioactivity in patient B. Si. It can be seen that during the 40 day interval, none of the final slopes are equal. The point depicted at ∞ is the injected dose minus the total urinary excretion (as determined from the integral of the excretion curve). The "fractional catabolic rate" as shown is the rate of urinary excretion of iodide per plasma radioactivity.

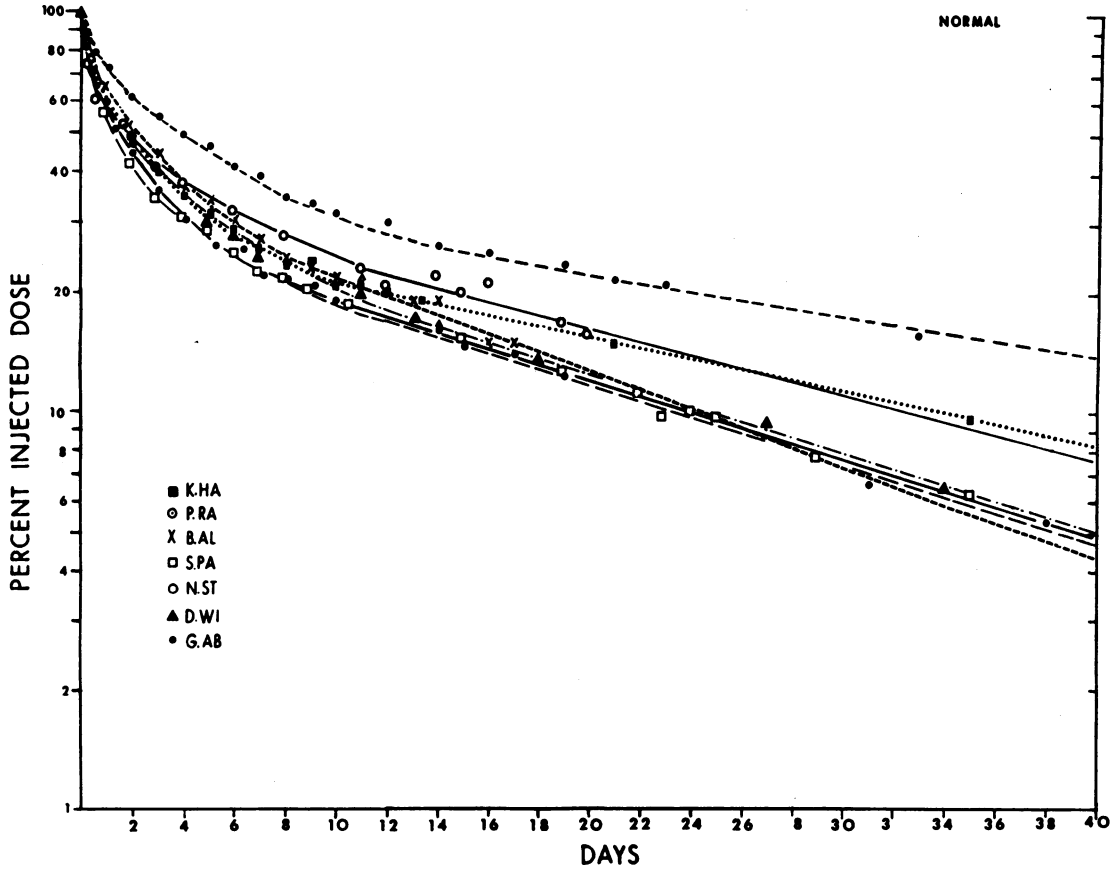

FIGURE 3 Plasma radioactivity disappearance curves of ^{131}I-labeled γ-globulin in seven control subjects. With the exception of P. Ra., there is very little spread of the data and 4–8% of the injected radioactivity remains in the plasma at 40 days.

(milligrams)/$(A_1/\lambda_1 + B_1/\lambda_2 + C_1/\lambda_3)$.[1] Concurrent data on the rate of urinary excretion of inorganic iodide allowed graphic representation of the total radioactivity remaining in the body at any time t. From our data this curve could be represented as $A_2e^{-\alpha_1 t} + B_2e^{-\alpha_2 t} + C_2e^{-\alpha_3 t} + K$, the constant K being determined from the integral of the rate of urinary excretion.

RESULTS

γ-Globulin metabolism. The disappearance curves of the ^{131}I-labeled γG globulin from the plasma are shown in Figs. 3 and 4. As noted by others, the final disappearance rate is not manifest until 7 days at the earliest and 21 days at the latest with a mean of 11.7 days ± 0.83 SEM (6, 14–17). The final decay rate is also quite variable with the $t_{\frac{1}{2}}$ varying from 9 to 29 days with a mean of 17.8±1.78 SEM. Other than the fact that the final disappearance is more rapid in the obese subjects there appears to be little that is unique for any group of subjects. The irreversible disposal rate (IDR) (Injected dose/$\int_0^\infty$ radioactivity per mass) which may be equated with production rate in these experiments is shown in Table II. In general, this value was positively correlated with the amount of intravascular globulin and our normal subjects (including obese) synthesized 35–62 mg/kg per day, with a mean of 47 mg/kg per day, a figure somewhat higher than usually quoted in the literature (11).

Urinary excretion of inorganic iodine was obtained daily in all patients for at least 13 days and in three subjects for 30 days. All had periodic 24-h urine values determined after daily collections were discontinued so that we could extrapolate for total urinary excretion. In the control subjects where daily collections were made for 30 days, we had a total of 78, 75, and 54% of the injected dose excreted and final 24-h urines contained < 0.5% of the injected dose. Most subjects at this time interval had < 5% of the injected dose in the plasma, thus suggesting the possibility of sequestration of some

[1] In several recent papers (12, 13), $1/(A_1/\lambda_1 + B_1/\lambda_2 + C_1/\lambda_3)$ has been called "fractional catabolic rate" or "fractional turnover rate." By definition this term is the mean time a radioactive particle spends in the plasma compartment. It can be equated with the fraction of the plasma pool turning over per unit time, but carried no connotation concerning the site of catabolism.

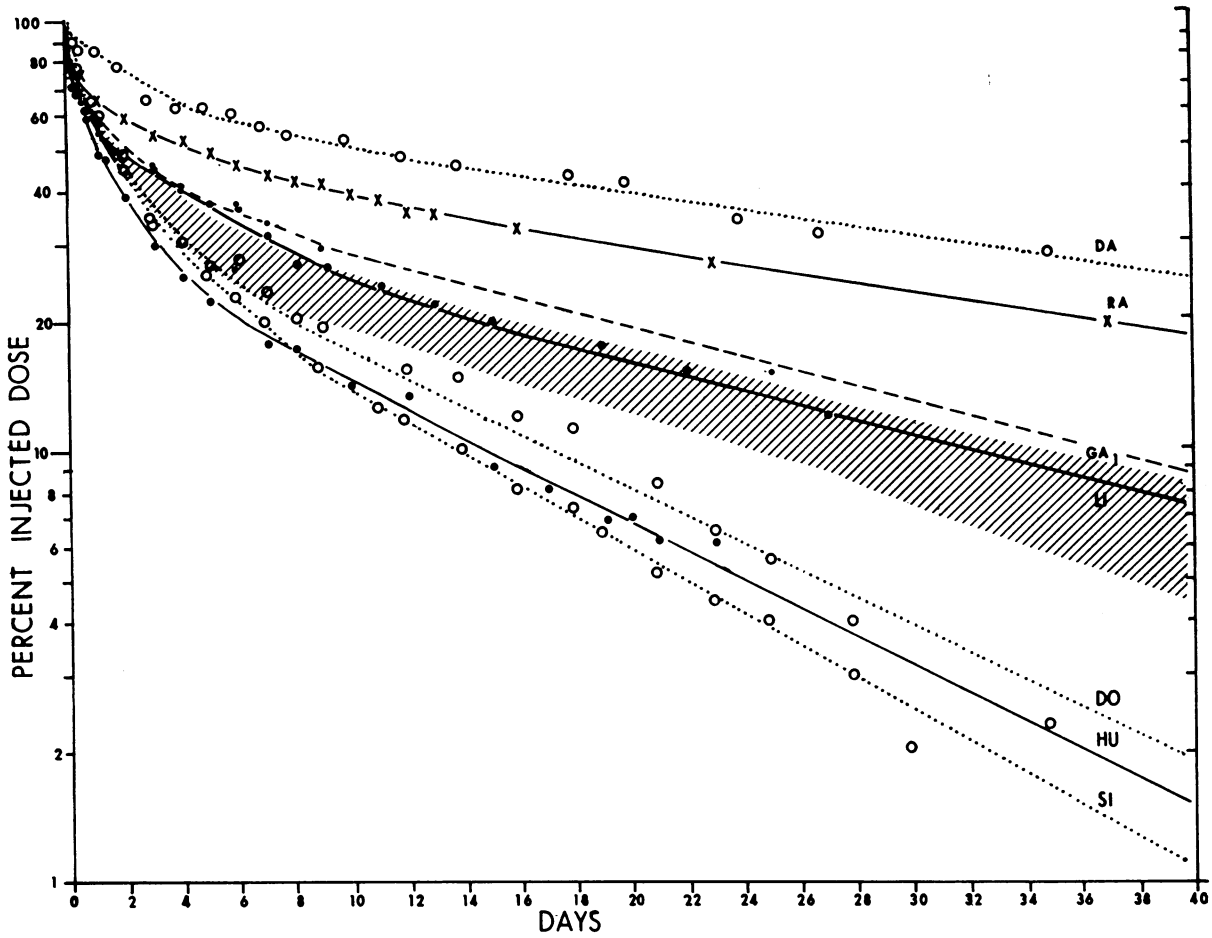

FIGURE 4 Plasma radioactivity disappearance curves of ^{131}I-labeled γ-globulin of two very obese subjects, the two nephrotic subjects on Imuran, and the uremic patients. Shaded area represents data from six of the seven control subjects (P. Ra. excluded). The rapidity of the final disappearance rate in the obese patients is obvious. In general, the disappearance is slower than normal in the uremic subjects.

of the labeled material outside the vascular compartment. Fig. 2 shows graphic representation of the data in subject B. Si. The amount of radioactivity remaining in the body, and the amount of radioactivity outside the vascular compartment showed a very slow decay, a consistent characteristic in nearly all subjects. Also the urinary radioactivity decreased faster than vascular, extravascular, or total body activity. These findings suggest at least two extravascular γ-globulin pools, one of which exchanges very slowly with the vascular compartment. The changing metabolic clearance when referred to the plasma pool probably indicates more than one catabolic site for the injected protein.

Fractional catabolic rates as determined by urinary clearance from days 5–8 are listed in Table II. With a few exceptions, the values of absolute catabolism derived from this method were not far different from those derived by integration (IDR). However, if days 13–17 are used, considerably smaller rates are seen. Thus, we feel the use of urinary excretion as related to plasma radioactivity for calculation of turnover may lead to inconsistent values.

Some mention should be made of the studies in P. Ra. who had a high concentration of γG (and also a low concentration of γA), but no specific disease process such as liver disease, rheumatoid arthritis, etc. to which this could be related. In contrast to other studies (12, 18) the survival half-time was long. We have no explanation for this finding though the suggestion of an increased number of protection sites is attractive (19).

L-chain metabolism. Labeled L-chains disappeared rapidly from the plasma in all subjects except those with uremia (Figs. 5 and 6). Only 1–2% of the total radioactivity was present in the intravascular L-chain population at the end of 24 h and all injected radioactivity was recovered in the urine in 3–4 days. This rapid ca-

TABLE II

γG Globulin Metabolism in All Subjects

Patient	Weight	γ-Globulin	IVD*	$t_{\frac{1}{2}}$	IDR‡	Fractional catabolic rate§	(Days 5–8)
	kg	*mg/100 ml*	*liters*	*days*	*mg/kg per day*	*%/day*	*mg/kg per day*
K. Ha.	47	990	2.07	21	42	8.3	36
B. Al.	52	700	2.37	14	36	10.5	33
N. St.	57	1210	2.29	18	48	9.0	43
G. Ab.	80	1065	3.09	15	55	8.3	34
P. Ra.	65	2200	3.00	28	55	5.3	54
S. Pa.	88	885	2.61	15	35	10.5	24
D. Wi.	72	740	2.80	15	35	7.8	16
C. Hu.	124	960	3.87	9	53	12.2	36
B. Si.	128	1100	3.50	8	62	18.8	60
P. Do.	80	315	3.50	10	24	11.4	12
L. Li.	63	450	2.63	18	19	12.0	22
J. Ga. no. 1	46	550	2.68	16	30	4.5	14
J. Ga. no. 2	46	725	2.53	23	50		
P. Da.	73	395	5.30	29	8	3.1	9
M. Ra.	65	1020	3.24	29	26	6.0	31

* IVD, Initial volume of distribution.

‡ IDR, Irreversible disposal rate as determined from the plasma pool/$\int_0^\infty$ plasma radioactivity curve.

§ Fractional catabolic rate as determined from rate of urinary excretion per plasma radioactivity.

tabolism invariably resulted in temporary retention of inorganic iodine in the blood.

As previously described (4, 20, 21), the uremic subjects had a slower decay curve for L-chains and significant radioactivity remained in the plasma L-chains for a week or more (see Fig. 6).

L-chain concentrations are shown in Table III and are quite variable even in the control population. Highest

TABLE III

L-Chain Concentrations and Metabolism in All Subjects

Patient	IVD*	L-Chain	Vascular pool	IDR‡		Fractional metabolic rate§	Fractional proteinuric rate§
	cm³	*γ N/ml*	*mg/kg*	*mg/day*	*mg/kg per day*	*%/h*	*%/h*
K. Ha.	1900	6.11	1.5	715	15.9	41.0	
B. Al.	2670	4.98	1.6	514	9.9	26.0	
N. St.	2610	4.32	1.23	606	10.6	36.0	
G. Ab.	2900	8.30	1.87	1140	14.2	31.5	
P. Ra.	3170	6.16	1.87	509	7.8	17.4	
S. Pa.	2690	7.9	1.51	1328	15.1	42.0	
D. Wi.	2880	7.5	1.88	1350	18.7	42.0	
C. Hu.	3910	8.71	1.72	2128	17.2	42.0	
B. Si.	3660	5.76	1.03	1050	8.1	33.0	
P. Do.	3150	4.04	0.99	625	7.8	33.0	
L. Li.	2610	2.17	0.56	221	3.5	26.0	
J. Ga. no. 1	2600	17.22	0.61	445	9.6	6.6	0.85
J. Ga. no. 2	2600	11.01	3.9	460	10.0	10.6	
P. Da.	5290	5.02	2.3	484	6.7	12.1	0.14
M. Ra.	3200	11.23	3.46	449	6.9	8.3	0.59

* IVD, Initial volume of distribution.

‡ IDR, Irreversible disposal rate as determined from plasma pool/$\int_0^\infty$ plasma radioactivity curve.

§ Fractional metabolic and proteinuric rates are defined as follows: IDR/pool and urinary excretion of protein/pool.

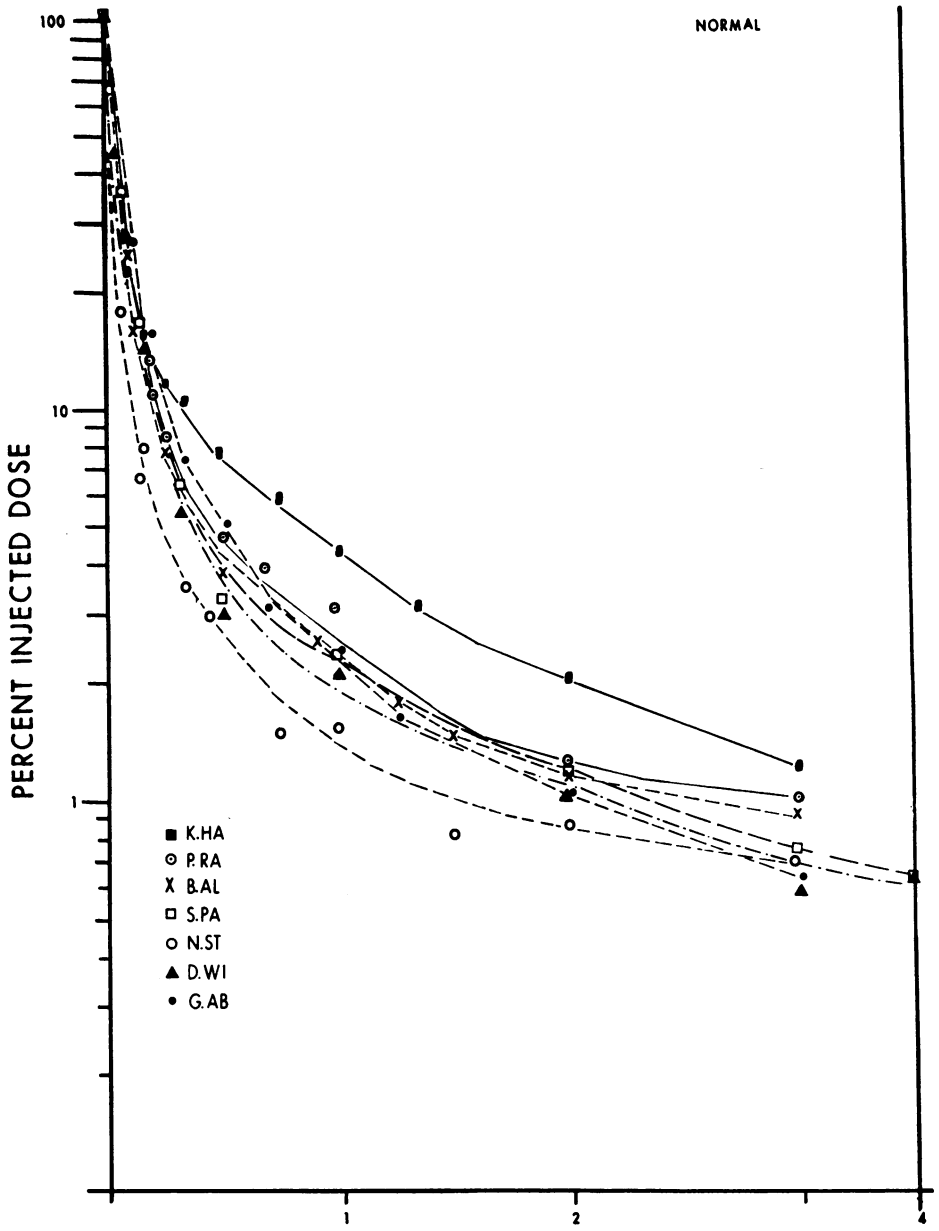

FIGURE 5 Plasma radioactivity disappearance curves of ^{125}I-labeled L-chains in the seven control subjects. In contrast to γG globulin, there is only 1–2% of the radioactivity left in the serum after 48 h. It should be noted that these curves have been corrected for the retained inorganic iodide in plasma and represent only the protein associated radioactivity.

levels were seen in the uremic subjects while the lowest were seen in the patients with the nephrotic syndrome receiving Imuran (Burroughs Wellcome Co., Research Triangle Park, N. C.).

Integration of these disappearance curves is less accurate than that for γ-globulin because of the extremely rapid disappearance, but probably is accurate to ±10%. Determination then of IDR or production rate shows values of 7.8–18.7 mg/kg per day of excess L-chains. A positive correlation with blood concentration may be seen in all but the uremic subjects. Of considerable interest are the relatively normal synthetic rates in this latter group. Thus, the prolonged survival time of these low molecular weight proteins in uremic subjects, as noted previously (4), reflects only the increased pool size and carries no connotation for single molecules.

We mention in passing that all subjects had column separation (Sephadex G-100) of the urinary proteins

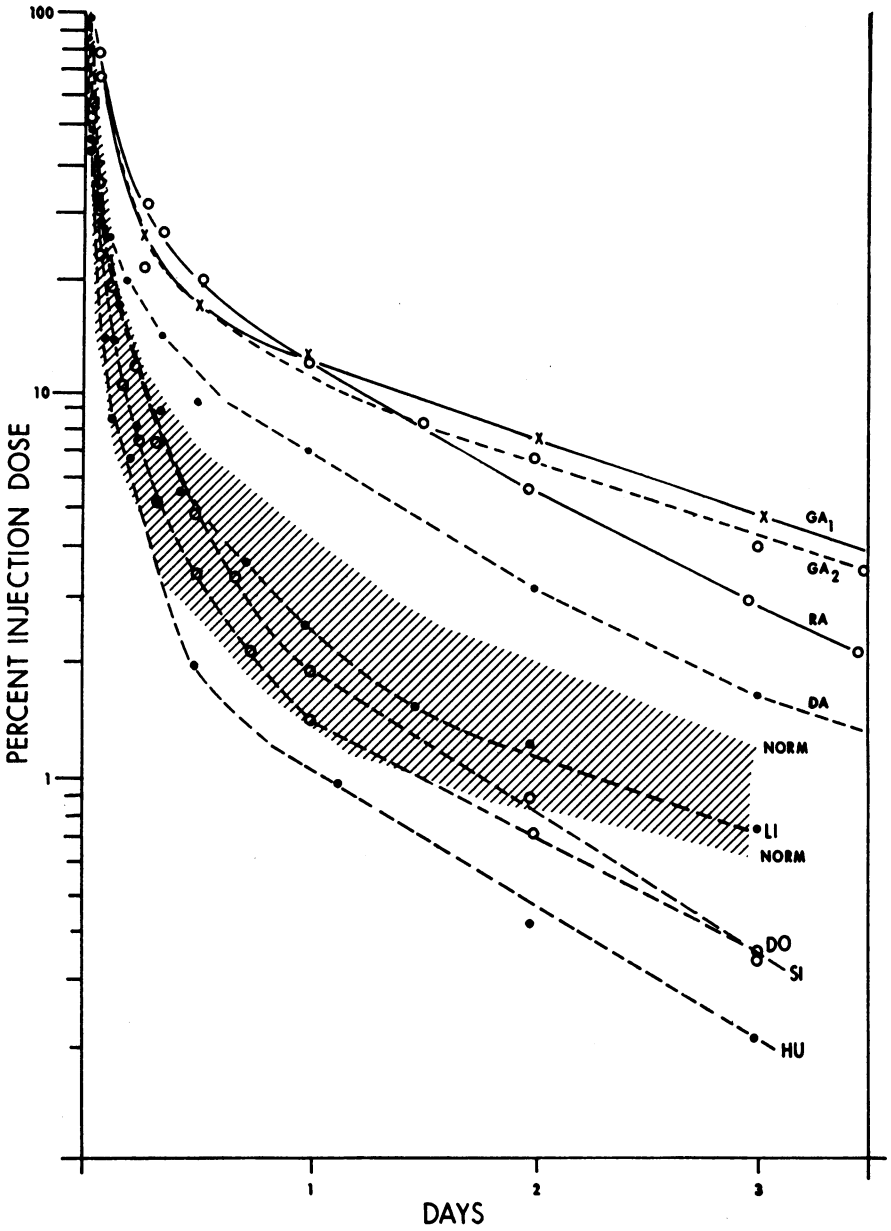

FIGURE 6 Plasma radioactivity disappearance curves of ^{125}I-labeled L-chains in the two obese subjects, the patients with nephrotic syndrome and those with uremia. The shaded area represents data from the control subjects. As with γG globulin, the terminal disappearance in the obese subjects is rapid. The slow disappearance of L-chain from the plasma in uremia is readily seen.

done. Neither individual with the nephrotic syndrome had any detectable L-chains in the urine. Those with uremia (P. Da., J. Ga., and M. Ra.) all had small amounts of L-chain in the urine (see Table I).

DISCUSSION

Our data allow conclusions to be drawn concerning the relationship of excess L-chain and γ-globulin metabolism only in normal subjects. We do not have sufficient data as to the effects of Imuran or uremia on this relationship and view our hypotheses in these areas as purely tentative until a larger series of subjects is available for study.

It has been generally accepted that degradation of plasma proteins occurs intravascularly or at a rapidly equilibrating site (11). The evidence for this is the apparent constant fractional catabolic rate as determined

TABLE IV

Radioactivity in Counts Per minute on Isolated Leukocytes and External Counts Over Equal Areas of the Precordium, Spleen, Liver, and Thigh

Blood					External counting				
Subject	Time	Plasma	RBC	WBC	Time	Precordium	Spleen	Liver	Thigh
G. Ab.	8 min	12,830	4	389	2 days	6780	13,583		1466
	5 h	10,593	64	703	3 days	5276	8804	7758	1169
	12 h	9853	10	186	4 days	3658	7109	6259	1060
	24 h	8247	4	180	7 days	2794	3773	3375	608
	48 h	5903	3	105	9 days	1752	1880	1900	595
	72 h	5270	570	18					
J. Ga. no. 2	8 min	14,942	5	77	2 h	15,023	13,383		3016
	5 h	12,572	14	352	6 h	15,975	11,600		2933
	24 h	9313	10	1083	24 h	14,431	10,121		2859
					96 h	8057	5614		1685

by urinary clearance over 3–14 days (6, 14). Other investigators have noted a slow decline in this parameter with time in patients, (22, 23) and short-term early studies in rabbits have failed to show constancy (24). Furthermore, in studies with [^{131}I] albumin where much more extensive data are available, model solutions for extravascular pool size which allow only for intravascular catabolism are not in accord with actual measurements of extravascular pool size (25). Our data have the advantage of a long time span (about 40 days) and urine collections of accuracy since patients were hospitalized on the Clinical Research Center for at least the first half of the study. In nearly all instances we found decreasing fractional catabolic rates with time, though this only became truly evident when intervals of 8 days or more of urinary excretion are compared. Further confirmation is found in the curve of the rate of urinary excretion of iodide which did not contain the same exponents as found in the plasma disappearance curve.

We have repetitively examined the question of the preparation of the administered protein and asked ourselves if our results could be the result of use of a denatured protein. Early high urinary excretion of iodide as well as rapid disappearance from the plasma are known to occur under such a circumstance (14). Conclusive evidence that no denaturation of the administered proteins has occurred during either isolation or labeling is difficult to obtain. We feel the following data make this an unlikely possibility. (*a*) Some protein preparations made by cold methanol or ethanol precipitation have been found to behave differently in vivo from proteins prepared with sodium sulfate (27, 28). In order to test the integrity of our starting protein as obtained from Cohn Fraction II, we prepared a sample of pooled fresh sera with sodium sulfate precipitation which was then treated identically with the Cohn Fraction II. Simultaneous administration of the two protein preparations to a patient resulted in similar plasma radioactivity disappearance curves and urinary excretion. (*b*) The availability and use of an unusual γG globulin which spontaneously crystallizes in solutions when the temperature is less than 30°C (26) has permitted an evaluation of our preparation techniques on the integrity of the molecules. The parameters which influence the crystallization of this protein have been previously described in detail.[2] Briefly, subtle changes in configuration of the protein not reflected by changes in such parameters as changes $S_{20}w$ or isoelectric point totally abolish the ability of the protein to crystallize. These conformational changes are caused by such procedures as brief dialysis against 1 M propionic or acetic acid, or 5 M urea. But purification of this protein by fractionation in 18% Na_2SO_4, DEAE column chromatography, iodination by the method utilized here, and preparative ultracentrifugation or passage through an ultrafilter does not alter the ability of this protein to spontaneously crystallize. (*c*) Our control subjects excreted from 7.6 to 15.1% of the injected dose on the first day. While 15% excretion during the first 24 h is somewhat higher than quoted by other investigators (14), we purposely gave each lot of labeled protein to a group of three or four patients in the hope that they might serve as internal control for one another. We have had wide spread of the percent of labeled protein excreted in the first 24 h within each of the five groups, e.g. 9–24%, 2–8%, 5–12%, 11–15%, and 14–23%. (Not all patients studied have been reported in this paper since they had disease processes not under consideration here.) Thus, we feel that some patients (particularly those with disease) will excrete a relatively large percentage of the injected dose rapidly and this need not be equated with the administration of a denatured protein.

[2] Abraham, G. N., and M. Chopek. Properties of a crystalline IgG_2 globulin. II. Mechanisms of crystallization and self-association. In preparation.

Similar findings have been noted by others (12). (*d*) The decrease in fractional catabolic rate determined by urinary clearance which was seen in this study is as evident late in the study as it is during the initial days. It seems unlikely that denatured protein would still remain available for catabolism 2 wk after injection.

We did not attain 100% excretion of the injected dose of labeled γ-globulin, in the time interval studied in any of our subjects. This finding is not unique for γ-globulin, but also has been reported for albumin, though to a lesser extent (29). We made some attempt to determine the sites of the extravascular γ-globulin and also to determine the mechanism of interchange between blood and the extravascular pool. It has been suggested that circulating γ-globulin may become attached to specific leukocytes and thus leave the circulation intact (30). A specific binding site of the Fc fragment may compete for attachment. Thus, the circulating white blood cells were isolated with dextran and counted. Also counts were made over the spleen and liver at various time intervals. Representative results of these limited studies are presented in Table IV. In general, significant counts were found on washed white cells in the four cases where these studies were done. At some time during the first 24 h after the tracer was given, radioactivity associated with the white cells was 5–10% of that found in 1 cm^3 of plasma. Although the cells were isolated from 12–20 cm^3 of plasma, no attempt at quantitation of leukocyte activity can be made since the completeness of the cell harvest is questionable. In no instance was radioactivity found on the white cells for more than 3 days. Also, in four cases, external counting over the precordium, spleen, liver, and thigh was done. In J. Ga (see Table IV), splenectomy had been performed and the data on this patient served as a very rough control. At all time intervals studied in this patient, activity over the spleen area was less than that over the precordium. In all other subjects (e.g., G. Ab. in Table IV), splenic activity exceeded precordial activity. We interpret these data to mean that early in the time-course of the experiments some radioactivity can be found on circulating leukocytes and that splenic sequestration, at least for short time intervals, is present. We do not feel that we have identified the site of prolonged extravascular storage which is seen in these subjects.

The possibility that the unexcreted radioactivity did not represent whole γG globulin molecules, but sequestered inorganic iodine or partial degradation products of γG globulin was considered. Since none of the persons studied had any difficulty completely excreting inorganic iodide during the L-chain studies, only the possibility of partial degradation of γG needs consideration. This question is important in considering the possible biologic implications of the large retained extravascular pool, but the nature of our studies does not allow this distinction.

In our control subjects, an average of roughly 45% of the L-chains synthesized were not incorporated into γ-globulin, but were discharged into the circulation and rapidly destroyed. In another recent study, λ L-chain production has been estimated by administration of a labeled dimer (31). Although plasma concentration of L-chain was not measured, indirect calculation was made from the fractional proteinuric rate and measured L-chain excretion. Their mean figure for circulating λ L-chains was 0.93 mg/kg, while our mean for both λ and κ L-chains was 1.54 mg/kg. The comparable synthetic rates were 0.21 mg/kg per h vs. 0.54 mg/kg per h. In view of the fact that our studies used monemeric κ and λ L-chains and measured directly serum L-chain concentration (where the ratio of κ- and λ-chains may vary considerably), it seems to us that the resultant figures from the two studies are remarkably close. Both studies would seem to indicate the relatively inefficient use of L-chains in γ-globulin synthesis.

In all patients with renal insufficiency and/or Imuran administration, low normal or diminished γ-globulin synthesis was found. There was, in general, a parallel reduction in excess light-chain synthesis. Thus, the possibility exists that heavy-chain synthesis in some way regulates the synthesis of L-chains. Alternatively uremia may suppress independently and equally heavy and L-chain synthesis.

A final comment concerns the catabolism of L-chains. It is quite well established that destruction of L-chains normally occurs predominantly in the renal tissue after filtration and reabsorption. Our studies indicate that with renal disease, nearly normal production and catabolism can still occur but usually under the condition of a high concentration of L-chains (patients J. Ga. and M. Ra.). Since one of our patients, J. Ga., was studied before and after bilateral nephrectomy and showed almost identical degradation rates under the two circumstances, we confirm the finding that catabolism may occur at sites other than the kidney when renal disease is advanced (32).

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REFERENCES

1. Cohen, S., and C. Milstein. 1967. Structure and biological properties of immunoglobulins. *Adv. Immunol.* 7: 1.

2. Franklin, E. C., and B. Frangione. 1969. Immunoglobulins. *Annu. Rev. Med.* **20**: 155.
3. Waldmann, T. A., R. M. Blaese, and W. Strober. 1970. Physiological factors controlling immunoglobulin metabolism. *In* Plasma Protein Metabolism. M. A. Rothschild and T. Waldmann, editors. Academic Press, Inc., New York. 269.
4. Solomon, A., T. A. Waldmann, J. L. Fahey, and A. S. McFarlane. 1964. Metabolism of Bence Jones protein. *J. Clin. Invest.* **43**: 103.
5. Bale, W. F., R. W. Helmkamp, T. P. Davis, M. J. Izzo, R. L. Goodland, M. A. Contreras, and I. L. Spar. 1966. High specific activity labeling of protein with I^{131} by the iodine monochloride method. *Proc. Soc. Exp. Biol. Med.* **122**: 407.
6. Anderson, S. B. 1964. Metabolism of human gamma globulin. F. A. Davis Company, Philadelphia, Pa.
7. Nosslin, B. 1964. *In* Metabolism of Human Gamma Globulins. S. B. Anderson, editor. F. A. Davis Co., Philadelphia, Pa. 103.
8. McFarlane, A. S., and K. N. Jeejeebhoy. 1965. Radioisotope techniques in the study of protein metabolism. *Int. At. Energy Agency Tech. Rep. Ser.* **45**: 9.
9. Vitek, F. 1966. Labelled proteins in tracer studies. *In* Proceedings of the Conference on Problems Connected with Preparation and Use of Labelled Proteins and Tracer Studies. L. Donato, G. Milhaud, and J. Sirchis, editors. European Atomic Energy Community, Brussels. 398.
10. Mathews, C. M. E. 1966. Round table on application of tracer theory to protein turnover studies. *J. Nucl. Biol. Med.* **10**: 382.
11. Waldmann, T. A., and W. Strober. 1969. Metabolism of immunoglobulins. *Prog. Allergy.* **13**: 1.
12. Wells, J. V., and H. H. Fudenberg. 1971. Metabolism of radio-iodinated IgG in patients with abnormal serum IgG levels *Clin. Exp. Immunol.* **9**: 761.
13. Levy, A. L., and T. A. Waldmann. 1970. The effect of hydrocortisone on immunoglobulin metabolism. *J. Clin. Invest.* **49**: 1679.
14. Cohen, S., and T. Freeman. 1960. Metabolic heterogeneity of human gamma globulin. *Biochem. J.* **76**: 475.
15. Wochner, R. D., G. Drews, W. Strober, and T. A. Waldmann. 1966. Accelerated breakdown of immunoglobulin G (IgG) in myotonic dystrophy: a hereditary error of immunoglobulin catabolism. *J. Clin. Invest.* **45**: 321.
16. Solomon, A., T. A. Waldmann, and J. Fahey. 1963. Metabolism of normal 6.6S gamma globulin in normal subjects and in patients with macroglobulinemia and multiple myeloma. *J. Lab. Clin. Med.* **62**: 1.
17. Birke, G., S. O. Liljedahl, B. Olhagen, L. O. Plantin, and S. Ahlinder. 1963. Catabolism and distribution of gammaglobulin. A preliminary study with ^{131}I-labelled gammaglobulin. *Acta Med. Scand.* **173**: 589.
18. Morell, A., W. D. Terry, and T. A. Waldmann. 1970. Metabolic properties of IgG subclasses in man. *J. Clin. Invest.* **49**: 673.
19. Brambell, F. W. R. 1966. The transmission of immunity from mother to young and the catabolism of immunoglobulins. *Lancet.* **2**: 1087.
20. Wochner, R. D., W. Strober, and T. A. Waldmann. 1967. The role of the kidney in the catabolism of Bence Jones proteins and immunoglobulin fragments. *J. Exp. Med.* **126**: 207.
21. Mogielnicki, R. P., T. A. Waldmann, and W. Strober. 1971. Renal handling of low molecular weight proteins. *J. Clin. Invest.* **50**: 901.
22. Platin, L. O. 1966. Discussion of basic aspects of immunoglobulin metabolism. *In* Labelled Proteins in Tracer Studies. L. Donato, G. Milhaud, and J. Sirchis, editors. European Atomic Energy Community, Brussels. 80.
23. Mills, J. A., E. Calkins, and A. S. Cohen. 1961. The plasma disappearance time and catabolic half-life of I^{131}-labeled normal human gamma globulin in amyloidosis and in rheumatoid arthritis. *J. Clin. Invest.* **40**: 1926.
24. McFarlane, A. S., and A. Koj. 1970. Short-term measurement of catabolic rates using iodine-labeled plasma proteins. *J. Clin. Invest.* **49**: 1903.
25. Katz, J., A. L. Sellers, G. Bonorris, and S. Golden. 1970. Studies on the extravascular albumin of rats. *In* Plasma Protein Metabolism. M. A. Rothschild and T. Waldmann, editors. Academic Press, Inc., New York. 129.
26. Abraham, G. N., J. P. Leddy, J. Grossman, and J. J. Condemi. 1972. Properties of a crystalline IgG_3 globulin. *Biochem. Biophys Res. Commun.* **46**: 162.
27. McFarlane, A. S. 1956. Labelling of plasma proteins with radioactive iodine. *Biochem. J.* **62**: 135.
28. Dixon, F. J., and W. O. Weigle. 1957. The relationship of the rates of serum protein metabolism, heterologous serum protein catabolism and the time and magnitude of the antibody response. *Ann. N. Y. Acad. Sci.* **70**: 69.
29. Lewallen, C. G., M. Berman, and J. E. Rall. 1959. Studies of iodoalbumin metabolism. I. A mathematical approach to the kinetics. *J. Clin. Invest.* **38**: 66.
30. Warner, N. L., P. Byrt, and G. L. Ada. 1970. Blocking of the lymphocyte antigen receptor site with anti-immunoglobulin sera in vitro. *Nature (Lond.).* **226**: 942.
31. Epstein, W. V., P. F. Gulassy, M. Tann, and A. I. Rae. 1968. Effect of renal homotransplantation on the metabolism of the light chains of immunoglobulins. *Ann. Intern. Med.* **68**: 48.
32. Waldmann, T. A., W. Strober, and R. P. Mogielnicki. 1972. The renal handling of low molecular weight proteins. *J. Clin. Invest.* **51**: 2162.