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J Clin Invest. 1966;45(9):1467-1478. https://doi.org/10.1172/JCI105454.

Research Article

Journal of Clinical Investigation
Vol. 45, No. 9, 1966

Metabolism of Human Immunoglobulin D (IgD) *

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In addition to the three commonly recognized classes of human immunoglobulins (IgG, IgA, and IgM), a fourth immunoglobulin class was recently identified and designated IgD (γD) (1, 2). Serum values in normal subjects ranged from less than 3 μg to greater than 100 μg IgD per ml of serum (2). The median serum IgD concentration was 30 μg per ml, about 1/400 of the serum IgG concentration. IgD is the least plentiful member of the immunoglobulin family.

The immunoglobulin system is interesting from a metabolic standpoint. The biological half-life of normal IgG is about 23 days and its synthetic rate 40 mg per kg per day (3, 4). In contrast, the half-lives of IgA and IgM are about the same (4 to 5 days), but the synthetic rate of 20 mg per kg per day for IgA compared to 4 mg per kg per day for IgM explains in part the higher serum level of IgA (5–7).

Nothing was known, however, about the metabolic behavior of immunoglobulin D. We therefore undertook this study using radioactive iodine-labeled IgD hoping to elucidate reasons for the relatively low serum concentration and to determine whether the metabolism of IgD is related to that of the other immunoglobulin classes.

The metabolism of labeled IgD was investigated in 28 patients selected so as to cover a wide range of immunoglobulin disorders. These included patients with *a*) increased synthesis of specific immunoglobulins, *b*) deficient immunoglobulin synthesis, *c*) increased catabolism of certain immunoglobulins, and *d*) no discernible abnormalities of the immunoglobulin system. The results of these studies indicate that the synthetic rate is the major factor controlling the serum IgD concentration and that IgD is metabolized independently of the other immunoglobulins.

Methods

Preparation of labeled IgD. IgD was isolated from whole human serum or plasma by DEAE cellulose chromatography followed by Sephadex G-200 gel filtration according to the method of Rowe and Fahey (2).

Two separate studies were done with radioiodine-labeled IgD. Different sources of IgD were used for each study; therefore, the preparation and characterization of the labeled purified proteins will be discussed separately.

IgD preparation no. 1. This protein was prepared from sterile frozen plasma obtained 1½ years previously from a patient with an immunoglobulin deficiency syndrome (8) who had 0.6 mg per ml IgD of both Types K and L and abundant serum IgM, but only trace quantities of IgG and IgA. Thirty ml of plasma was applied to each of two 20-g DEAE cellulose columns at 24° C equilibrated with 0.01 M phosphate buffer, pH 8.0, with a gradient of phosphate buffer to 0.30 M final buffer. Effluent fractions were tested for presence of specific protein fractions by Ouchterlony analysis with specific anti-IgD, IgG, IgA, and IgM antisera prepared in rabbits (1, 9), goat antihuman transferrin,[1] and a horse antinormal human serum.

Effluent fractions containing IgD were pooled and ultrafiltered to about 2 ml, made 3% in sucrose, and applied to a Sephadex G-200 column equilibrated at 24° C with 0.2 M Tris buffer, pH 8.0, in 0.2 M NaCl. The effluent containing IgD was again identified and ultrafiltered to about 2 ml to yield IgD preparation no. 1. Total protein content of this ultrafiltrate was 3.6 mg per ml as estimated from the OD at 280 mμ, assuming an extinction coefficient of 13.5. Antibody-in-agar ring diffusion analysis (2, 10) revealed an IgD concentration of 4.1 mg per ml with a standard reference serum of IgD concentration 0.33 mg per ml.

Ouchterlony analysis of this concentrated preparation showed a heavy precipitin line with specific anti-IgD antisera. Transferrin was also detected and found to be 0.05 mg per ml with commercial antitransferrin agar plates,[1] i.e., about 1.3% of the total protein was transferrin. Antisera specific to IgA and IgG failed to react with preparation no. 1. Anti-IgM antisera did yield a

* Submitted for publication February 28, 1966; accepted June 9, 1966.

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[1] Hyland Laboratories, Los Angeles, Calif.

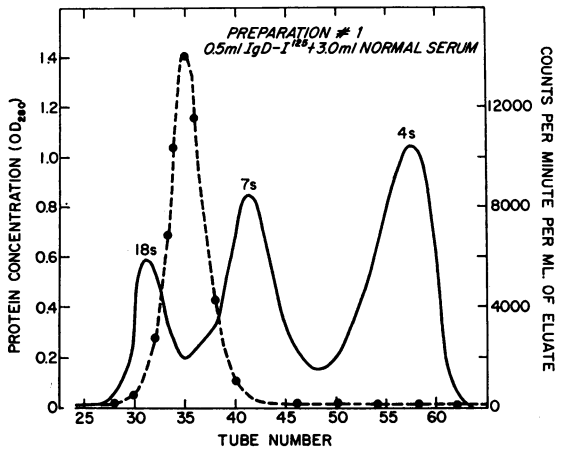

FIG. 1. SEPHADEX G-200 GEL FILTRATION CHARACTERISTICS OF IgD-^{125}I, PREPARATION NO. 1. Labeled IgD was added to normal carrier serum. The protein concentrations (OD 280) are represented by the unbroken curve, radioactivity by the broken curve.

precipitin line. This was a straight line that did not have the concavity toward the antigen well typical of IgM. This component showed a reaction of identity with normal serum IgM detected by these antisera. Absorption of these antisera by IgG and by transferrin did not alter the appearance of this line. We concluded that it represented a form of IgM of reduced molecular weight. Quantitation of this component was not possible, but the precipitin line with anti-IgM antiserum was much less pronounced than that detected by anti-IgD antiserum, and we concluded that the low molecular weight IgM probably constituted a minor part of preparation no. 1.

The IgD preparation was dialyzed against pH 8.0 borate buffer and labeled with ^{125}I by the iodine monochloride technique of McFarlane (11). After dialysis against saline to remove unbound ^{125}I, normal human serum albumin was added to prevent self-irradiation damage of the IgD. The product was calculated to have 0.7 mole iodine per mole of IgD. It was then sterilized by filtration, pyrogen tested, and stored at 4° C before use.

This radioiodinated protein was characterized by Sephadex gel filtration, radio-Ouchterlony analysis, and radioimmunoelectrophoresis. One-half ml (2.6 μc) IgD-^{125}I was added to 3.0 ml of normal carrier serum and put on a Sephadex G-200 column (Figure 1). The radioactivity appeared as a single symmetrical peak midway between the first and second optical density peaks, the typical elution position for IgD (2). There was no significant labeling of the transferrin contaminant which, if labeled, would have appeared in the third optical density peak.

Radioimmunoelectrophoresis of IgD-^{125}I plus carrier serum showed the IgD precipitin line to be heavily labeled. Labeling of the transferrin contaminant was not observed. Radio-Ouchterlony analysis demonstrated a heavily labeled IgD precipitin line and also indicated labeling of IgM (Figure 2). The bulk of the radioactivity, however, appeared to be associated with IgD. Attempts to quantitate the labeled IgM by precipitation of radioactivity with specific antisera were unsuccessful.

IgD preparation no. 2. This protein was obtained from subject B.M., who had clinical and laboratory evidence of multiple myeloma. This patient had 8.6 mg per ml of serum IgD myeloma protein, Type L. Eighteen ml of serum, freshly drawn, was fractionated on a DEAE cellulose column under the same conditions as described for preparation no. 1. The IgD-containing effluent fractions were pooled, ultrafiltered, and applied to a Sephadex G-200 column equilibrated with 0.2 M Tris buffer, pH 8.0, in 0.2 M NaCl. The two fractions (about 5.5 ml) containing most of the IgD were pooled. The total protein concentration of this pool was 3.5 mg per ml by optical density estimation at 280 mμ and 4.1 mg per ml by antibody-in-agar quantitation. The pool contained no IgG, IgM, or IgA by Ouchterlony analysis, but did give a very faint precipitin line in antibody excess with antiwhole human serum. After dialysis against pH 8.0

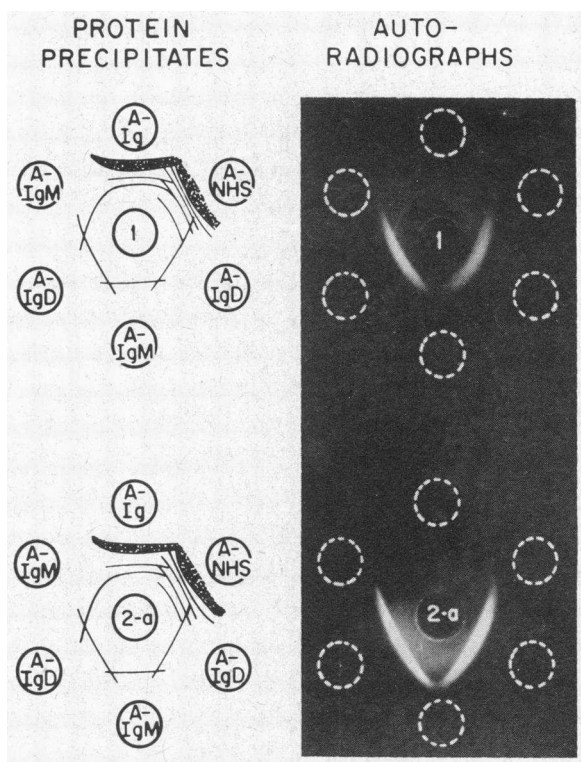

FIG. 2. RADIO-OUCHTERLONY ANALYSES OF IgD-^{125}I, PREPARATIONS 1 AND 2-A. The center well was charged with IgD-^{125}I plus normal human serum, which added abundant unlabeled IgD (to aid in precipitation of labeled IgD). Antisera were placed in the outer wells (A-Ig = polyvalent antihuman immunoglobulins; A-NHS = antiwhole normal human serum).

TABLE I

Clinical data on the 28 patients studied with radioiodinated IgD

Patient	Sex	Age	Total serum protein	Albumin	γ-Globulin	Clinical status and therapy
		years	*g/100 ml*	*g/100 ml*	*g/100 ml*	
G myeloma						
C.H.	M	57	6.3	2.9	1.7	G myeloma, Type K. 320 mg chlorethyl nitrosourea 3 weeks before first study.
A.J.	F	55	9.2	3.3	4.2	G myeloma, Type L.
W.H.	M	64	6.2	2.9	2.3	G myeloma, Type K. Prednisone 15 mg per day. See text.
M.M.	F	42	9.3	3.5	4.1	G myeloma, Type K. Radiation to spine and melphalan 5 mg per day before study, melphalan 2 mg per day during study.
A myeloma						
G.C.	M	72	9.4	3.0	4.9	A myeloma, Type L. Testosterone enanthate 500 mg im per week, melphalan 1 mg per day. Renal damage. See text.
A.Ho.	M	67	6.5	3.5	1.5	A myeloma, Type K. Tryptophan mustard 5 mg every third day for preparation no. 1, no drugs for preparation no. 2-a.
D myeloma						
B.M.	M	45	6.5	4.2	0.8	D myeloma, Type L. Radiation to cervical spine, melphalan. Proteinuria. See text.
Myeloma with Bence Jones proteinemia and proteinuria						
F.M.	M	37	6.7	3.4	0.3	Type K Bence Jones protein. Prednisone 35 mg per day for preparation no. 1, melphalan 3 mg per day for preparation no. 2-a. Renal damage. See text.
M-macroglobulinemia						
K.I.	M	58	8.5	2.8	4.4	Type K macroglobulin. Whole body radiation 5 rads per day.
A.C.	M	63	7.0	2.5	3.3	Type L macroglobulin. Cytoxan 200 mg per day for last 3 days of study. Renal damage. See text.
J.B.	M	61	8.4	3.9	3.1	Type K macroglobulin. Plasmapheresis; see text.
H.Hi.	M	74	6.9	3.5	1.9	Type L macroglobulin. Chlorambucil 2 mg every other day.
Agammaglobulinemia						
P.B.	M	20	5.6	3.8	0.1	Congenital hypogammaglobulinemia. Chronic bronchitis and bronchiectasis, mild. See text.
Ataxia telangiectasia						
G.L.	F	19	7.5	4.1	1.4	Decreased synthesis and accelerated catabolism of IgA. See text.
Myotonic dystrophy						
W.J.	M	48	5.9	3.4	0.6	Accelerated IgG catabolism. See text.
J.C.	M	39	6.5	3.5	0.6	Accelerated IgG catabolism. See text.
Others						
P.J.	M	23	5.4	3.3	0.6	Paroxysmal nocturnal hemoglobinuria. Prednisone 5 mg per day.
O.K.	F	44	5.9	3.9	0.5	Mycosis fungoides.
A.W.	F	70	6.3	3.4	1.4	Mycosis fungoides; see text.
E.S.	F	31	7.2	2.0	3.1	Biliary cirrhosis; see text.
D.S.	M	67	6.9	4.3	0.8	Reticulum cell sarcoma, stage III-A. Methylhydrazine 167 mg per day, prednisone 60 mg per day, vincristine 2.1 mg per week, nitrogen mustard 9 mg per week.
H.A.	F	48	6.2	3.7	1.0	Lymphoblastic lymphoma.
A.L.	F	60	6.8	2.8	1.2	Hodgkin's disease, stage III-B; on same therapy as D.S. for 2 weeks before start of study but not during study.
C.R.	M	61	6.6	3.4	1.2	Hodgkin's disease, stage III-A; same therapy as D.S.
S.T.	M	54	6.2	3.9	0.7	Primary amyloidosis. 6-Mercaptopurine 185 mg per day.
H.Ha.	M	50	5.7	2.8		Darier's disease. Methotrexate 67 mg per week.
A.Ha.	F	59	6.1	3.2	1.1	Rheumatoid arthritis and quiescent pulmonary tuberculosis. Prednisone 45 mg per day. Renal damage. See text.
N.S.	M	51	6.2	3.6	1.0	Bronchial carcinoma. Radiation to lung.

borate buffer, one half was frozen and sent by air express from Birmingham, England, to Bethesda, Md., where it was thawed and labeled with ^{125}I as described above, yielding a ratio of 2 moles iodine per mole protein (preparation no. 2-a). The other aliquot was labeled in Birmingham with ^{131}I by McFarlane's iodine monochloride technique yielding 0.8 mole ^{131}I per mole of protein (preparation no. 2-b). Both labeled products were then handled in the same manner as described for preparation no. 1. Radioimmunoelectrophoresis of preparation no. 2-a added to normal carrier serum revealed only a single heavily labeled IgD precipitin line. Radio-Ouchterlony analysis also revealed only a heavily labeled IgD precipitin line (Figure 2). Radioimmunoelectrophoresis of preparation no. 2-b demonstrated a strongly labeled IgD precipitin line plus two very faintly radioactive α_2-globulin lines. Both these preparations were subjected to Sephadex G-200 gel filtration as described for labeled preparation no. 1 and behaved identically, yielding a sharp symmetrical radioactivity peak in the IgD elution region.

Patients. A total of 28 patients received labeled IgD;

16 received preparation no. 1, 14 received preparation no. 2-a, and 4 preparation no. 2-b (6 patients received both preparations 1 and 2-a). The pertinent clinical data are presented in Table I.

Eight patients had multiple myeloma. Of this group only one (A.J.) had never received either cytotoxic drugs or steroids. Six of the eight were receiving drugs during the study. Renal disease was evident in two patients, G.C. [proteinuria, 0.5 g per day; blood urea nitrogen (BUN), 30; creatinine clearance, 79 L per 24 hours] and F.M. (Bence Jones proteinuria, BUN of 25, hypercalcemia). Although patient B.M. had Bence Jones proteinuria (3.7 g per day), he had no other laboratory evidence of renal disease. Patient W.H. had a drop in hemoglobin of 2 g per 100 ml on the third day of the study due in part to gastrointestinal blood loss and also to a Coombs' positive hemolytic anemia. Oral potassium iodide was stopped because of possible gastric irritation, and 1,250 ml of packed red blood cells was transfused over the fourth and fifth days of the study. Patient F.M. received 500 ml of packed red blood cells on the second day of one turnover study (preparation no. 2-a).

Four patients had macroglobulinemia as confirmed by serum immunoelectrophoresis and ultracentrifugation. One, A.C., had, in addition, amyloidosis involving the peripheral nerves and renal disease of uncertain etiology with a creatinine clearance of 74 L per 24 hours. All patients had received some form of chemotherapy in the past, but only two (K.I., H.Hi.) were on therapy during the major part of the study. Patient J.B. was treated with plasmapheresis before each study but not for 2 days before or during the study.

Four other patients with known abnormalities in immunoglobulin metabolism were included: P.B., congenital agammaglobulinemia, who was suffering from mild chronic bronchitis and bronchiectasis, but received no γ-globulin during the study or during the 2 weeks before study; G.L., ataxia telangiectasia, with known decreased synthesis and increased catabolism of IgA (6); and W.J. and J.C., both with myotonic dystrophy and accelerated IgG catabolism (12). J.C. had evidence of mild hepatic disease [mildly elevated serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactic dehydrogenase (LDH), and alkaline phosphatase].

Twelve patients with a variety of other diseases were studied. One (A.Ha.) had renal disease (BUN, 23; creatinine clearance, 94 L per 24 hours). Seven had cancer; six were receiving chemotherapy. One, A.W., developed parotitis on the fourth day of the study necessitating stopping potassium iodide. Four had liver disease: A.L. [sulfobromophthalein (BSP) retention of 20% at 45 minutes], H.Ha (BSP 16%, mildly elevated SGOT and SGPT), E.S. (biliary cirrhosis with the typical laboratory findings), and P.J. (moderately elevated SGOT, SGPT, and LDH).

All patients were considered suitable for study. None was considered critically ill; only one (N.S.) had a fever of over 99.5° F during the study.

The transfusions (subjects W.H. and F.M.), gastrointestinal bleeding (subject W.H.), and the cessation of iodide administration (subjects W.H. and A.W.) did not change the respective plasma radioactivity curves (see below).

Study protocol and calculation of data. Each patient received 0.5 ml of Lugol's solution or a saturated solution of potassium iodide three times a day before and during the study. In two patients iodides were stopped during the study (see above). Approximately 15 to 35 μc (0.1 to 1.2 mg) of labeled IgD was injected intravenously from calibrated syringes. Plasma samples were obtained at 10 minutes after injection, several times during the first 24 hours, twice and then only once daily. Urine was collected in 24-hour pools throughout the study. In some patients, 24-hour lots of stool (days 1 through 4 of the study) were examined for radioactivity. Serum and urine samples were counted in an automatic gamma-ray well-type scintillation counter employing a thallium-activated sodium iodide crystal. For three patients (M.M., N.S., and B.M.) total body radioactivity was measured daily by whole body counters. Exhaustive dialysis or trichloroacetic acid precipitation of the urine samples revealed 4% or less protein-bound radioactivity in all patients tested, even in those (B.M., F.M., G.C.) with proteinuria. Stools were collected in paint cans and homogenized. The sample cans brought to the same weight with water and appropriate standards were counted in a Tobor [2] large sample gamma counter. In each patient tested less than 0.25% of the injected radioactivity appeared in any 24-hour stool sample.

Plots were constructed on semilogarithmic paper of the plasma radioactivity and of the remaining total body activity as plotted against time. Total body activity was calculated by cumulative subtraction of radioactivity excreted in urine and stool from that originally administered or by the whole body counting technique. After initial equilibration the plasma curves for both preparations 1 and 2 declined as a single exponential function, each having a slope comparable to the total body curves (Figure 3). The biological half-life ($t_{\frac{1}{2}}$) was calculated from the plasma curve. Since some of the urine collections were incomplete, adequate total body curves could not be obtained for many subjects. Therefore Matthews' method, which uses only plasma radioactivity measurements, was employed to further analyze the data (13). This method assumes that *a*) all catabolism occurs in the intravascular pool,[3] *b*) any new protein synthesized is added only to the intravascular compartment, and *c*) rates of breakdown and rates of transfer of pro-

[2] Nuclear-Chicago Corp., Des Plaines, Ill.

[3] The assumption was valid for this study. Complete urine collections were available for some subjects. The daily urinary radioactivity for these subjects was expressed as a per cent of the mean intravascular, extravascular, or total body activity for that day. A constant figure for fractional catabolic rate was obtained only when the urinary activity was expressed as a fraction of intravascular radioactivity, indicating that catabolism of IgD was in the intravascular pool or in a compartment closely associated with it (14).

tein between intravascular and extravascular compartments are constant.

We were able to analyze our data using a model system with one extravascular pool. Matthews' equations describing this system are as follows: K_{12} (fractional catabolic rate or fraction of total intravascular IgD catabolized per day) $= 1/(c_1/b_1 + c_2/b_2)$, K_{13} (fraction of intravascular pool transferred to extravascular pool per day) $= [c_1\ c_2\ (b_2 - b_1)^2]/(c_1\ b_2 + c_2\ b_1)$, and K_{31} (fraction of extravascular pool transferred to intravascular pool per day) $= c_1\ b_2 + c_2\ b_1$. The values for c_1, c_2, b_1, and b_2 can be obtained by analysis of the plasma curve. The values for b_1 and b_2 are the decay constants of the two single exponential functions obtained from the plasma radioactivity curve, and c_1 and c_2 are the respective Y axis intercepts of these functions.

Plasma volume was calculated on the basis of initial distribution of administered radioactivity, with the specific activity of the 10-minute plasma sample. Plasma volume (milliliters) = counts per minute administered/10-minute plasma activity (counts per minute per milliliter). The proportion of administered radioiodinated IgD remaining intravascular after equilibration with the extravascular space could be calculated from K_{13} and K_{31} values: fraction intravascular IgD $= K_{31}/(K_{31} + K_{13})$. Total intravascular content of IgD was calculated from the serum concentration and plasma volume. Total intravascular IgD = serum concentration (milligrams per milliliter) × plasma volume. Dividing the total intravascular IgD by the fraction intravascular yielded total body IgD.

The total quantity of IgD catabolized per day was calculated from the total intravascular IgD and the fractional catabolic rate constant, K_{12}, assuming that all catabolism occurs in the intravascular compartment. With the further assumption of a steady state, this is equivalent to the synthetic rate. Turnover rate (milligrams per kilogram per day) = total intravascular IgD (milligrams) × K_{12}/body weight (kilograms).

Total serum proteins were determined by the biuret method. Serum paper electrophoresis was also performed. Serum immunoglobulin levels were determined by the antibody-in-agar method (2, 10). Serum IgD was estimated by the same method employing rabbit anti-IgD myeloma protein antisera from three different pools. The antisera used for analysis of subjects receiving preparations 1 and 2-b could detect IgD concentrations of greater than 0.003 and 0.008 mg per ml, respectively, whereas the antiserum used for preparation no. 2-a could detect IgD concentrations of greater than 0.017 mg per ml. Sera were analyzed for IgD content at various times in a single study and found to vary less than 10% from the mean for that subject. Thus, the assumption of the steady state seemed realistic. The data were analyzed for significant correlations using Spearman's rank-order correlation coefficient method corrected for ties (15).

Results

A total of 34 studies in 28 patients was accomplished with two preparations of radioiodine-labeled IgD (Table II). Six patients (C.H., G.C., A.Ho., F.M., J.B., and O.K.) received two preparations (no. 1 and 2-a). In these patients the mean biological half-life was 82 hours for preparation 1 and 55 hours for preparation 2-a. The mean fractional catabolic rates were 0.33 and 0.48, respectively. The reasons for this discrepancy are unclear but may reflect a difference in metabolism between a myeloma IgD protein (preparation 2-a) and a nonmyeloma IgD protein (preparation 1). These results could also be explained by the minor contamination of preparation no. 1 with radioiodine-labeled low molecular weight IgM, which may have a longer half-life if it is metabolized like 18 S IgM (7). Analysis of the urine or whole body radioactivity in all patients given both preparations revealed no evidence of a significant amount of early degradable, i.e., denatured, labeled IgD. Despite these differences in behavior in six patients, the full range of values obtained with both preparations overlapped extensively for each parameter of metabolism studied (Table III). The results for each preparation will be discussed separately.

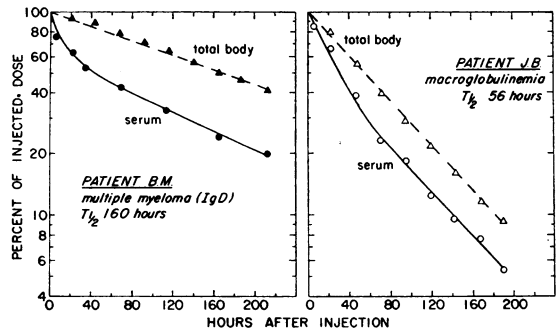

FIG. 3. SURVIVAL OF RADIOIODINATED IgD MYELOMA PROTEIN (PREPARATION NO. 2) IN TWO PATIENTS. The unbroken curve represents the serum (or plasma) radioactivity and the broken curve the total body radioactivity. Patient B.M. was the source of the labeled IgD. The plots of the survival of preparation no. 1 are similar and demonstrate single exponential decay.

IgD preparation no. 1. This ^{125}I-labeled protein was given to 16 subjects. The intravascular distribution of the labeled protein varied from 63 to 86% of the total body pool with a median of 73%.

Serum IgD concentrations in these patients varied from ⩽ 0.003 mg per ml to 0.141 mg per ml, a 47-fold difference. In general the lower levels

TABLE II

^{125}I- and ^{131}I-labeled IgD turnover in 28 patients

Patient	Serum IgD	Plasma volume	% intravascular IgD	Total intravascular IgD	Total body IgD	Plasma $t_{\frac{1}{2}}$	Fractional catabolic rate (K_{12})	IgD turnover	Other serum immunoglobulins		
									IgG	IgA	IgM
	mg/ml	*ml/kg*	%	*mg/kg*	*mg/kg*	*hours*	*fraction of total intravascular IgD/day*	*mg/kg/day*	*mg/ml*	*mg/ml*	*mg/ml*
Preparation no. 1 (IgD-^{125}I)											
G myeloma											
C.H.*	0.048	51.0	73	2.45	3.35	94	0.26	0.637	17.0	2.70	0.37
A.J.	⩽0.003	40.0	63	⩽0.132	⩽0.210	80	0.37	⩽0.050	35.1	0.90	0.24
A myeloma											
G.C.*	⩽0.003	48.0	72	⩽0.158	⩽0.219	70	0.36	⩽0.057	1.69	113.0	0.08
A.Ho.*	⩽0.003	34.1	71	⩽0.113	⩽0.158	71	0.35	⩽0.040	2.60	8.10	0.21
Myeloma with Bence Jones proteinemia and proteinuria											
F.M.*	⩽0.003	41.3	69	⩽0.136	⩽0.197	58	0.46	⩽0.063	4.48	0.17	0.12
M-macroglobulinemia											
K.I.	⩽0.003	57.8	75	⩽0.191	⩽0.255	82	0.28	⩽0.053	6.05	0.56	19.0
A.C.	⩽0.003	52.0	75	⩽0.172	⩽0.229	66	0.43	⩽0.074	4.48	0.14	32.0
J.B.*	0.024	63.5	75	1.52	2.03	106	0.25	0.380	6.53	0.61	38.5
Agammaglobulinemia											
P.B.	⩽0.003	36.8	76	⩽0.121	⩽0.160	72	0.32	⩽0.039	1.51	⩽0.01	⩽0.05
Ataxia telangiectasia											
G.L.	0.033	24.6	75	0.812	1.08	75	0.31	0.252	9.3	0	1.96
Myotonic dystrophy											
W.J.	0.051	38.5	77	1.96	2.55	86	0.28	0.549	4.48	3.78	0.68
J.C.	⩽0.003	23.5	64	⩽0.078	⩽0.121	74	0.36	⩽0.028	8.95	1.62	0.64
Others											
P.J.	⩽0.003	51.2	86	⩽0.169	⩽0.196	68	0.29	⩽0.049	5.32	0.72	0.53
O.K.*	0.040	32.0	66	1.28	1.94	92	0.31	0.396	6.05	2.25	0.82
A.W.	0.022	29.6	63	0.651	1.03	90	0.31	0.201	10.9	2.07	1.68
E.S.	0.141	58.7	73	8.28	11.34	138	0.18	1.49	24.2	6.21	2.20
Preparation no. 2-a (IgD-^{125}I)											
G myeloma											
C.H.*	0.057	41.4	69	2.36	3.42	63	0.43	1.01	17.0	2.70	0.37
W.H.	0.023	52.3	81	1.20	1.48	46	0.49	0.588	20.6	1.12	1.10
A myeloma											
G.C.*	⩽0.017	63.2	74	⩽1.07	⩽1.45	56	0.45	⩽0.481	1.69	113.0	0.08
A.Ho.*	⩽0.017	41.9	81	⩽0.712	⩽0.879	50	0.46	⩽0.327	2.60	8.10	0.21
Myeloma with Bence Jones proteinemia and proteinuria											
F.M.*	⩽0.017	47.8	68	⩽0.812	⩽1.19	48	0.60	⩽0.487	4.48	0.17	0.12
M-macroglobulinemia											
J.B.*	0.080	34.5	80	2.76	3.45	56	0.41	1.13	6.53	0.61	38.5
H.Hi.	0.019	42.0	79	0.798	1.01	50	0.45	0.359	4.48	0.68	34.0
Others											
O.K.*	0.052	33.0	72	1.72	2.39	56	0.50	0.860	6.05	2.25	0.82
D.S.	0.030	42.1	81	1.26	1.56	50	0.50	0.630	6.05	2.25	0.92
H.A.	0.048	45.4	73	2.18	2.99	55	0.45	0.981	5.80	3.15	0.82
A.L.	⩽0.017	38.8	72	⩽0.659	⩽0.915	47	0.54	⩽0.355	5.80	1.12	1.0
C.R.	0.027	43.7	72	1.18	1.64	50	0.49	0.578	12.7	4.50	0.48
S.T.	0.045	43.0	77	1.94	2.52	66	0.35	0.679	7.74	1.40	0.39
H.Ha.	0.027	42.1	78	1.77	2.27	66	0.36	0.637	38.7	5.28	0.88
Preparation no. 2-b (IgD-^{131}I)											
G myeloma											
M.M.	⩽0.008	54.0	70	⩽0.432	⩽0.618	78	0.34	⩽0.147	50.0	0.90	0.22
D myeloma											
B.M.	8.60	46.6	65	401	617	160	0.16	64.0	3.3	0.15	<0.04
Others											
A.Ha.	0.026	40.8	73	1.06	1.45	62	0.42	0.445	6.5	5.10	1.31
N.S.	0.030	47.4	70	1.42	2.03	62	0.43	0.611	5.3	5.80	0.62

* Patients who received both preparations 1 and 2-a.

TABLE III

Summary of data on the metabolism of IgD

	Serum IgD concentration	% intravascular IgD	$t_{\frac{1}{2}}$	Fractional catabolic rate	Turnover rate (synthetic rate)
	mg/ml	*%*	*hours*	*fraction of intravascular IgD/day*	*mg/kg/day*
Preparation no. 1					
Range	⩽ 0.003–0.141	63–86	58–138	0.18–0.46	⩽ 0.028–1.49
Mean	0.024	72	83	0.32	0.272
Median	0.003	73		0.31	0.074
Preparation no. 2*					
Range	⩽ 0.008–0.080	68–81	46– 78	0.34–0.60	⩽ 0.147–1.13
Mean	0.032	75	56	0.45	0.606
Median	0.027	73	56	0.45	0.588
Preparations no. 1 and 2*					
Range	⩽ 0.003–0.141	63–86	46–138	0.18–0.60	⩽ 0.028–1.49
Mean	0.028	74	69	0.39	0.444
Median	0.023	73	66	0.37	0.396

* Excluding patient B.M.

were found in patients with myeloma, macroglobulinemia, and agammaglobulinemia and the higher levels in those patients without such disorders of immunoglobulin production. The highest level was found in patient E.S., who had biliary cirrhosis and generalized "secondary" hypergammaglobulinemia. This wide range of serum concentrations parallels that found for 100 normal adults as reported by Rowe and Fahey (2). The range of serum concentration is reflected in total body IgD, which varied greatly from ⩽ 0.121 to 11.34 mg per kg.

IgD turnover rates (synthetic rates in the steady state) ranged from ⩽ 0.028 to 1.49 mg per kg per day. Serum IgD concentrations are plotted as a function of synthetic rates in Figure 4. A strong positive correlation is readily evident, indicating that serum IgD levels are determined largely by the IgD synthetic rate.

The biological half-life as measured in the plasma varied from 58 to 138 hours. The fractional catabolic rates varied from 0.18 to 0.46 (fraction of the intravascular pool catabolized per day) with a median value of 0.31. The highest fractional catabolic rate and shortest half-life were found in a patient with multiple myeloma (F.M.) who had a serum IgD concentration of ⩽ 0.003 mg per ml. Despite the fact that this patient had proteinuria (Bence Jones), more than 99% of his urinary radioactivity was dialyzable and therefore presumably not protein bound. The lowest fractional catabolic rate and longest half-life were found in patient E.S., who had the highest serum IgD level.

When serum IgD levels were plotted as a function of the fractional catabolic rates (Figure 5), a relationship was suggested. High serum levels were generally associated with low fractional catabolic rates (and long half-lives) and low serum levels with high fractional catabolic rates (and shorter half-lives). The correlation coefficient for this relationship is − 0.72, which is significant at a level of $p < 0.005$. The relatively narrow range of

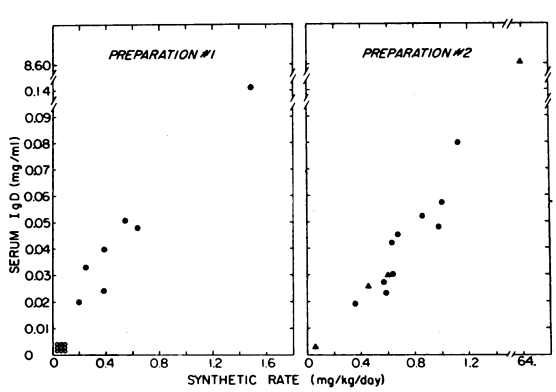

FIG. 4. RELATIONSHIP OF IgD SYNTHETIC RATE AND SERUM CONCENTRATION. For preparation no. 1 those serum values of ⩽ 0.003 mg per ml are represented by open circles. For preparation no. 2 the solid circles represent IgD-^{125}I (preparation 2-a) and the triangles IgD-^{131}I (preparation 2-b).

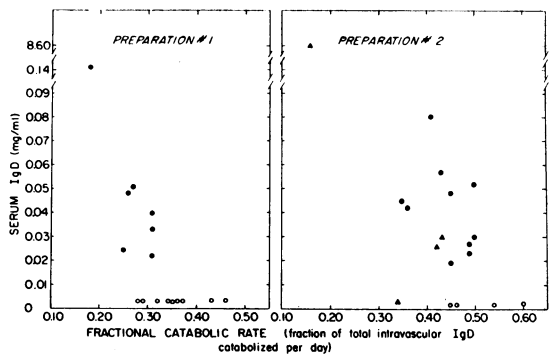

FIG. 5. RELATIONSHIP OF IgD FRACTIONAL CATABOLIC RATE AND SERUM CONCENTRATION. For preparation no. 1 serum values of $\leqslant 0.003$ mg per ml are represented by open circles. For preparation no. 2 the solid circles represent preparation 2-a, the triangles preparation 2-b. Serum values of $\leqslant 0.017$ mg per ml for preparation no. 2-a are represented by open circles.

fractional catabolic rates (about 3-fold) compared to the wide range of synthetic rates (about 50-fold), however, indicates that the latter plays the major role in determining IgD serum concentration.

Two patients with myotonic dystrophy (W.J., J.C.) were studied previously (12) and found to have accelerated catabolism of IgG. However, their IgD fractional catabolic rates were 0.28 and 0.36, respectively, quite near the median value of 0.31 found in the 16 subjects receiving this preparation. This indicated separate catabolic pathways for these two immunoglobulin classes in these patients.

No other factors including disease category, renal damage, therapy, or levels of other immunoglobulins correlated well with the fractional catabolic rate, biological half-life, or synthetic rate.

IgD preparation no. 2. This preparation was iodinated in two lots, one with ^{125}I (preparation 2-a), the other with ^{131}I (preparation 2-b). Since these two lots behaved similarly, they shall be discussed as one with the exception that patient B.M. (D myeloma, the source of preparation 2) will be considered separately.

Excluding patient B.M., 17 patients received preparation no. 2, six of whom had previously received preparation 1. Between 68 and 81% of the injected radioiodinated IgD remained intravascular, with a median of 73%.

Serum IgD levels varied within the wide range of normals previously reported. The range was from $\leqslant 0.008$ mg per ml to 0.080 mg per ml. Total body IgD varied from $\leqslant 0.618$ to 3.45 mg per kg.

IgD turnover rates ranged from $\leqslant 0.147$ to 1.13 mg per kg per day. Figure 4 demonstrates the strong positive correlation between serum IgD concentration and synthetic (turnover) rate for this preparation.

The biological half-life ranged from 46 hours to 78 hours. The fractional catabolic rate varied from 0.34 to 0.60 with a median value of 0.45. Serum concentration and fractional catabolic rate did not correlate significantly (correlation coefficient of -0.24, $p > 0.10$) (Figure 5). If subject B.M. is included, a possibly significant correlation is found ($r = -0.36$, $0.10 > p > 0.05$), but, like preparation no. 1, serum concentration (10-fold range) depended mainly on synthetic rate (8-fold range). No other parameters correlated well with biological half-life, fractional catabolic rate, or synthetic rate.

Patient B.M. had clinical and laboratory evidence of multiple myeloma and was producing an IgD myeloma protein, Type L. This protein was isolated and labeled with ^{131}I and given back to the patient (preparation 2-b). Since this was the only study of the behavior of an autologous protein, it deserves special consideration. The serum IgD level (8.6 mg per ml) was the highest of any of the 28 subjects in this study. The biological half-life was 160 hours, the longest of any patient studied. The fractional catabolic rate (0.16) was the smallest of the entire series. As expected, the turnover rate was very high, 64 mg per kg per day, which in this 73-kg man amounted to 4.7 g per day. The median turnover rate for all other subjects was 0.396 mg per kg per day or about 0.028 g per day for a 70-kg man. Sixty-five per cent of the labeled protein remained intravascular, which agrees with data from the other 27 subjects of this study. Thus this subject's high serum level was the result of two factors: *a*) a greatly increased synthetic rate, and *b*) a lower fractional catabolic rate.

Discussion

The present studies show important differences between the metabolism of IgD and the other

TABLE IV

*Metabolic features of the immunoglobulins**

	IgG (3)†	IgA (6)†	IgM (7)†	IgD‡
Serum level, *mg/ml*	10.7 (6.0–15.6)	2.5 (0.19–4.95)	0.77 (0.57–1.98)	0.023 (0.003–0.141)
Synthetic rate, *mg/kg/day*	42 (16–51)	21.1 (2.67–55.0)	4.13 (3.16–16.9)	0.396 (0.028–1.49)
Fractional catabolic rate, *fraction of intravascular pool catabolized per day*	0.068 (0.058–0.078)	0.237, 0.37§ (0.144–0.338)	0.179 (0.141–0.251)	0.37 (0.18–0.60)
Intravascular distribution, *% total body pool intravascular*	44 (32–53)	40§	70 (65–100)	73 (63–86)

* Median value is presented with the range of values in parentheses below.
† Values obtained are those for control subjects only.
‡ Values presented are derived from both preparations (no. 1 and no. 2) used in this study.
§ Values obtained by Solomon and Tomasi (5).

classes of immunoglobulins, i.e., IgG, IgA, and IgM. IgD is notable in rate of synthesis, rate of catabolism, control of catabolism, and intravascular-extravascular distribution. Comparison of the metabolic features of all four immunoglobulin classes can be made with the data assembled in Table IV.

IgD has the lowest serum concentration of any of the immunoglobulins. The median value of all patients investigated in this present study was 0.023 mg per ml. This value is lower than the median value of 0.030 mg per ml for normal adults (2), due to inclusion in the present study of a large number of patients with multiple myeloma or macroglobulinemia who had low IgD levels. The serum level of IgD directly reflected the rate of IgD synthesis (Figure 4). The synthetic rate for IgD varied from $\leqslant 0.028$ to 1.49 mg per kg per day for 27 subjects without D myeloma. The median value was 0.396 mg per kg per day. In a 70-kg subject this would represent 28 mg per day. As can be seen from Table IV, these values are much below those of any other class of immunoglobulin.

The low serum concentration of IgD as compared to the other immunoglobulins cannot be explained solely by its lower synthetic rate. The median serum level of IgD in this study was only $1/465$ of the median IgG level of normal subjects reported by Solomon, Waldmann, and Fahey (3), but the quantity synthesized per day was $1/100$ of the median amount of IgG synthesized per day. Differences in catabolism of the two immunoglobulin classes are the primary cause of this discrepancy.

The immunoglobulins are catabolized in a compartment in rapid equilibrium with the intravascular pool (16). Thus, rates of catabolism can be meaningfully compared when expressed as the fraction of the intravascular pool catabolized each day. The median fractional catabolic rate for IgD was found to be 0.37. The fractional catabolic rates of IgG (median 0.07) and IgM (median 0.18) are notably lower (3, 7). Certainly the catabolism of IgG (and IgM) is different from IgD.

The fractional catabolic rate of IgD (median 0.37) is closer to the median IgA fractional catabolic rates of 0.24 noted by Strober and his colleagues (6) and 0.37 reported by Solomon and Tomasi (5). The ranges of observed fractional catabolic rates were 0.18 to 0.60 for IgD and 0.14 to 0.34 for IgA (6). These overlapping values indicate that IgD and IgA are catabolized from the intravascular compartment at similar rates. The possibility that these two proteins, IgD and IgA, might have similar catabolic mechanisms was further investigated in subject G.L. This patient had ataxia telangiectasia with a demonstrated decreased synthesis and increased catabolism of IgA (fractional catabolic rate of 5.0) (6). If IgA and IgD were catabolized by a similar mechanism, one would expect to see a rapid disappearance of labeled IgD in this subject. The IgD fractional catabolic rate, however, was 0.31. This value is identical to the median value in 16 patients who

received the same IgD preparation, demonstrating that IgD catabolism was not accelerated. These observations indicate that, at least in this situation, the catabolic pathways for IgA and IgD are different.

The ranges of fractional catabolic rates observed with the two preparations of IgD were similar. This range was checked against such factors as renal damage, disease diagnosis, specific immunoglobulin disorder, and therapy, but no correlation could be established which would indicate that these factors played a role in determining IgD fractional catabolic rate in these patients. The present data, however, do not indicate whether the observed differences reflect genetic factors or previous environmental experience.

Each of the normal immunoglobulins has a counterpart associated with plasmacytic or lymphocytic neoplasms, i.e., G (IgG) myeloma proteins, A (IgA) myeloma proteins, D (IgD) myeloma proteins, and M (IgM) macroglobulins. Of this group D myeloma is the least frequently observed clinically. This may be due in part to difficulty in detecting this myeloma protein by commonly employed clinical laboratory methods, especially serum paper electrophoresis. A case in point is patient B.M. of this study, whose total immunoglobulin concentration was only about 1.2 g per 100 ml, well within the normal limits, despite the fact that nearly 75% of this was D myeloma protein. In spite of B.M.'s high IgD synthetic rate (64 mg per kg per day), the serum IgD concentration (0.86 g per 100 ml) was not so high as commonly seen for other classes of myeloma proteins. It is interesting to calculate the IgD synthetic rates required to produce a serum concentration that would give a level of D myeloma protein comparable to G myeloma protein. A patient synthesizing a G myeloma protein at a rate similar to that of patient B.M. was reported by Cohen (17). His patient (no. 22) had a synthetic rate of 61 mg per kg per day. From the data for synthetic and catabolic rates and assuming a 70-kg man with a plasma volume of 3.5 L, the serum G myeloma protein concentration is calculated to have been 2.4 g per 100 ml for their subject. To achieve a serum concentration of 2.4 g per 100 ml D myeloma protein, patient B.M. would need a synthetic rate of 191 mg per kg per day, three times his own experimentally demonstrated rate, and also three times the IgG synthetic rate of Cohen's patient. Thus, equivalent synthetic rates of these two immunoglobulins (IgG and IgD) will not result in equivalent serum concentrations due to marked differences in their fractional catabolic rates.

The quantity of tumor may also be a factor in the clinical detection of D myeloma. Evidence that both IgG and IgD are produced in plasma cells has been obtained by immunofluorescent studies (18). If we assume that an individual malignant IgD plasma cell produces as much immunoglobulin on a weight basis as does an individual malignant IgG plasma cell, patient B.M. would need roughly three times as many plasma cells to achieve an IgD serum level equal to that of IgG for Cohen's patient. If more tumor, i.e., more plasma cells, is necessary to produce an easily detectable serum globulin abnormality, D myeloma proteins would be recognized readily only late in the course of the disease, and might even be missed entirely.

Of all factors controlling the rate of catabolism of the various immunoglobulins, serum concentration is one of the most interesting. It is well known that the fractional catabolic rate of IgG is directly related to its serum concentration in man (3) and in the mouse (19). Subjects with high serum IgG concentrations due to myeloma, chronic infection, or hyperimmunization have increased rates of IgG catabolism (3, 19–23), and those with hypogammaglobulinemia have decreased rates of catabolism (3, 24). These catabolic adjustments to serum concentration constitute an IgG homeostatic regulatory mechanism. On the other hand, the serum levels of IgA and IgM do not influence their respective rates of catabolism. In contrast to the observations with these other classes of immunoglobulins, a significant negative correlation was observed between IgD fractional catabolic rate and serum concentration for preparation no. 1 and possibly for preparation no. 2 (Figure 5), that is, those patients with the higher serum levels had the lower fractional catabolic rates. A similar negative correlation between these two parameters has been demonstrated for transferrin (25) and haptoglobin (26) in man. The correlation of serum albumin level and fractional catabolic rate is unclear, a negative correlative having been found in the rabbit (27), a positive correla-

tion in man (28, 29). It must be emphasized, however, that we have been unable to study variations of fractional catabolic rate with changing IgD serum levels in the individual subject and that further study is necessary to confirm the fractional catabolic rate-serum concentration relationship. It is possible that serum IgD concentration and fractional catabolic rates are not causally related and that this apparent relationship may reflect only their mutual relationship to one or more still unknown factors.

IgD was distributed between intravascular and extravascular compartments differently from other immunoglobulin classes with similar ultracentrifugal sedimentation coefficients. About 75% of the total IgD (7.0 S) was found in the intravascular space after equilibration. In contrast, only about 40 to 50% of IgG (6.6 S) and IgA (7-13 S) are intravascular (3–6). Smaller molecules such as albumin (4 S) and transferrin (5 S) behave like IgG and IgA (25, 30). On the other hand, large molecules like IgM (18 S) and fibrinogen are similar to IgD in being about 70 to 80% intravascular (7, 20). The size and shape of radioiodinated IgD are the same as native IgD as shown by Sephadex gel filtration so that the purified, labeled protein used in these experiments is presumed to reflect the behavior of native IgD. Presumably the molecular weight of IgD is similar to that of IgG or 7 S IgA, but it may have a different configuration or other physicochemical features that prevent its diffusion across vascular walls. Alternatively it may be selectively retained in the intravascular space or rapidly secreted back into this space from the extravascular pool by some active process not understood at present. In any event, in this as in other features, IgD metabolism differs markedly from that of other immunoglobulins.

Summary

The turnover of radioiodine-labeled IgD was studied in 28 subjects with a variety of diseases. IgD was isolated from two sources, a patient with abundant IgD and a selective deficiency of other immunoglobulins and a patient with D myeloma. These preparations were labeled with ^{125}I or ^{131}I and used in 34 studies. Six patients received both preparations. The serum IgD level was found to be directly related to the rate of synthesis. Individuals differ markedly in the rate of IgD synthesis. Differences as great as 50-fold were found in a group of 27 subjects without neoplasms of IgD-synthesizing cells. A patient with D myeloma synthesized the protein at a rate 160 times greater than the median rate for 27 other subjects.

The median rate of IgD synthesis was 0.4 mg per kg per day. This is about 1 to 2% of the synthetic rates for IgG and IgA (and about 10% of the IgM synthetic rate).

The median serum level of IgD (0.023 mg per ml in 27 subjects studied) was only about 0.2% of the median IgG level. The difference between relative serum levels (1:465) and synthetic rates (1:100) for IgD:IgG reflects the more rapid rate of IgD catabolism.

The biologic half-life of IgD is shorter than that of other immunoglobulins. The median half-life for IgD was 2.8 days. This contrasts with the $t_{\frac{1}{2}}$ of IgM (5 days), IgA (6 days), and IgG (23 days). The fractional rates of catabolism of intravascular IgD (0.37) and IgA, however, are similar. The other immunoglobulins are catabolized at slower rates.

Studies in three patients with disordered metabolism of IgA or IgG revealed normal IgD metabolism, indicating that IgD is metabolized independently from IgA or IgG.

The fractional catabolic rate of IgD appears to be altered by the serum IgD concentration. Those subjects with high serum IgD levels tend to have relatively low fractional catabolic rates. This concentration-catabolism relationship for IgD is the opposite of that noted for IgG, where high serum levels are associated with relatively rapid IgG catabolism.

IgD is largely (73%) in the intravascular compartment. This contrasts with other 7 S immunoglobulins, for only 40 to 50% of the total body IgG and IgA is in the intravascular compartment.

Acknowledgments

The authors wish to thank Mr. William Briner of the Radiopharmaceutical Service, National Institutes of Health, Dr. John Gart of the Biometry Branch, National Cancer Institute, Drs. d'A Kok and Peter Clein of the Department of Medicine, Cambridge University, and Dr. John Davies of the Medical Research Council's Industrial Injuries and Burns Research Unit, Birmingham, for their help in these studies. We are indebted to physicians of the National Institutes of Health, to Dr.

F. G. Hayhoe, and to physicians of the United Birmingham Hospitals for permission to study patients under their care. Miss Sylvia Bryan, Miss Marjorie Emery, Miss Barbara Plocinik, and Mr. John Eustace provided expert technical assistance.

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