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THE METABOLISM OF AUTOLOGOUS IgM AND 19S RHEUMATOID FACTOR IN RHEUMATOID ARTHRITIS

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SUMMARY

The turnover of autologous preparations of radio-iodine labelled IgM and 19S rheumatoid factor was studied and compared in patients with severe rheumatoid disease. The IgM was isolated by block electrophoresis and column chromatography. 19S rheumatoid factor was isolated by a combination of euglobulin precipitation, column chromatography, and absorption onto and acid-elution from solid aggregated IgG.

Ten studies were made in seven patients, five with IgM, and five with 19S rheumatoid factor. In two patients the turnovers of 19S rheumatoid factor and IgM were studied simultaneously.

The turnover of IgM was similar to that reported for normal subjects and patients with other diseases: fractional catabolic rate 0·14–0·18, plasma and whole body $T_{\frac{1}{2}}$ 3·7–6·5 days, with 65–77% intravascular localization. The absolute catabolic rate for IgM was elevated (8–60 mg/kg/day).

The turnover of 19S rheumatoid factor isolated from serum was comparable in fractional catabolic rate (0·15–0·19) plasma and whole body $T_{\frac{1}{2}}$ (3·9–6·0 days) and intravascular localization (62–88%). No evidence of rapid catabolism of the 'immune' elimination type was obtained.

INTRODUCTION

The group of serum proteins collectively known as rheumatoid factors were first described by Waaler (1940) following the observation that sera of some patients with rheumatoid disease inhibited the haemolysis of sheep red cells sensitized with rabbit γ-globulin in complement fixation tests. Subsequent work (Rose *et al.*, 1948; Grubb, 1956; Waller & Vaughan, 1956; Vaughan, 1956) established that rheumatoid factor (RF) had activity directed against rabbit and human γ-globulins, and that activity might be species specific.

Most rheumatoid factors are 19S proteins of the IgM class of immunoglobulins (Franklin *et al.*, 1957) but examples are now known to exist of low molecular weight (7S) rheumatoid

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factors of the IgA (Heimer & Levin, 1964; Allen, 1966) and IgG (Schrohenloher, 1966) classes of immunoglobulins.

The majority of patients with rheumatoid arthritis have demonstrable rheumatoid factors in their serum and in the ultracentrifuge an extra component, a 22S complex, may be visible. This consists of 19S rheumatoid factor combined with five or six molecules of 7S IgG (Franklin *et al.*, 1957; Normansell & Stanworth, 1966). A high titre of rheumatoid factor has frequently been associated with a poor prognosis (Rose *et al.*, 1948; Pike *et al.*, 1953; Shard *et al.*, 1964) and with manifestations of vasculitis, (Sokoloff & Bunim, 1957; Bywaters & Scott, 1963; Dixon, 1963) but the role of rheumatoid factors is far from clear. The association with vasculitis may be related to the known effect of soluble antigen–antibody complexes on vessel walls (Edelman, Kunkel & Franklin, 1958; McCluskey *et al.*, 1960), the latter resulting from recombination of rheumatoid factor with denatured IgG, or with IgG complexed to antigen (Edelman *et al.*, 1958). On the other hand, rheumatoid factors may represent an expression of increased sensitivity to altered γ-globulin in rheumatoid arthritis and frequently in other diseases (Kunkel, Simon & Fudenberg, 1958; Williams & Kunkel, 1962) but may not have a pathogenic role in producing vasculitis.

The antibody present in soluble antigen–antibody complexes formed *in vivo* in rabbits (Benacerraf, Sebestyen & Cooper, 1959) is more rapidly catabolized than the uncomplexed antibody. The purpose of these studies was to see whether or not autologous 19S RF was catabolized at a different rate from IgM in patients with severe rheumatoid arthritis. The results of these studies suggest that 19S RF has the same fractional catabolic rate as IgM.

MATERIALS AND METHODS

For each study, 19S rheumatoid factor or IgM was prepared from serum freshly obtained from the subject. All the reagents used were sterile and had been prepared with pyrogen-free distilled water. All the apparatus was sterilized by autoclaving, dry heat, ethylene oxide or gamma-ray irradiation.

Preparation of 19S rheumatoid factor

Stage 1. *Euglobulin precipitation.* Blood was allowed to clot at room temperature, then kept at 4°C overnight. The serum obtained by centrifugation was diluted with 14 volumes of distilled water at 4°C. After standing for 2 hr, the precipitate was collected by centrifugation in sterile polyallomer cups at 15,000 ***g*** and 4°C. The precipitate was redissolved in a small volume of 0·2 M-NaCl, pH 7·2, so that the final volume of euglobulin was approximately one-tenth the amount of serum.

Stage 2. *Sephadex G-200 gel filtration.* The euglobulin solution was applied to a column 100 × 5 cm. Phosphate–citrate buffer, 0·05 M, containing 0·15 M-NaCl, pH 4·5, was used as eluant. Those fractions of the first optical density peak were pooled which contained IgM alone, as detected by Ouchterlony analysis techniques. The pH was adjusted to 7·2 by the addition of concentrated buffer.

Stage 3. *γ-Globulin column.* The first two preparations were passed through a γ-globulin column before being labelled with radio-iodine. In the remaining three preparations, the 19S euglobulins were labelled prior to passage through the column. The latter method avoided the use of another stage to remove the protein bound iodine from the non-protein bound iodine.

The column used to separate 19S rheumatoid factor from other 19S euglobulins contained a finely ground preparation of solid human γ-globulin dispersed in Sephadex G-25. The γ-globulin had been prepared at the Lister Institute, London by ether fractionation, and for column use it was denatured by heating at 75°C for 10 min. Phosphate–citrate buffer (0·05 M) containing NaCl, pH 7·2, was used as eluant. The effluent was monitored by an ultraviolet absorptiometer and recorder (LKB Model 4701A+6520A). When no more protein, and in the last three preparations no more radioactivity, was detected in the eluate, the pH of the eluant (0·05 M-phosphate–citrate, containing 0·15 NaCl) was changed to 3·6, and RF, demonstrable by sensitized sheep cell agglutination (SSCA) tests, was eluted from the column usually in the pH range of 5·0–4·0. Fig. 1 shows an example of this.

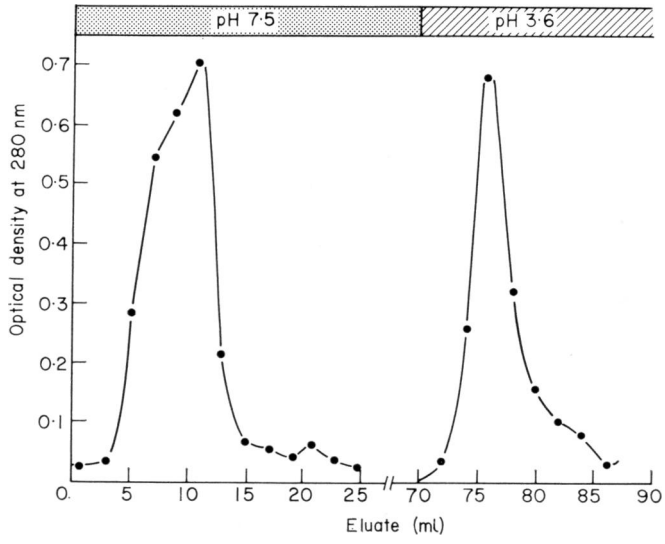

FIG. 1. Optical density of fractions eluted from a Sephadex G-25–human γ-globulin column. The 19S euglobulin fraction of serum from a patient with rheumatoid arthritis was eluted with phosphate buffer of pH7·2 –7·5 and 3·6. SSCA titre of serum was 1024, of first peak (7·2) was < 16, of second peak (3·6) was 1024.

Preparation of IgM

The method of isolation used was similar to that of Barth *et al.* (1964). This consisted of zone electrophoresis of serum on a polyvinyl chloride–polyvinyl acetate block using 0·06 M-barbitone buffer, pH 8·6, at 4°C. Strips of the block were eluted, and those containing immunoglobulin only, as detected by Ouchterlony gel diffusion tests, were pooled. This pool was subjected to Sephadex G-200 gel filtration to obtain fractions which contain IgM alone.

Iodination

All the preparations were labelled with carrier free ^{125}I (IMS.3) or ^{131}I (IBS.3) obtained from the Radio-chemical Centre, Amersham. The iodine monochloride method of McFarlane (1958) was used. Assuming a molecular weight of 1×10^6 for IgM and 19S rheumatoid factor, the calculated ratio of iodine atoms per molecule of protein was always less than 1:1, and was frequently between 0·6 and 0·8:1. The non-protein bound radio-iodine was

removed by Sephadex G-25 gel filtration, or occasionally in the case of the IgM preparation only by Amberlite IRA 400 exchange resin. Apart from the first two preparations (F.A., M.H.) of 19S rheumatoid factor, more than 98% of the radioactivity in the injected preparation was precipitable with 10% trichloracetic acid.

Throughout the isolation of 19S rheumatoid factor and IgM the protein solutions were kept at 4°C, and not frozen.

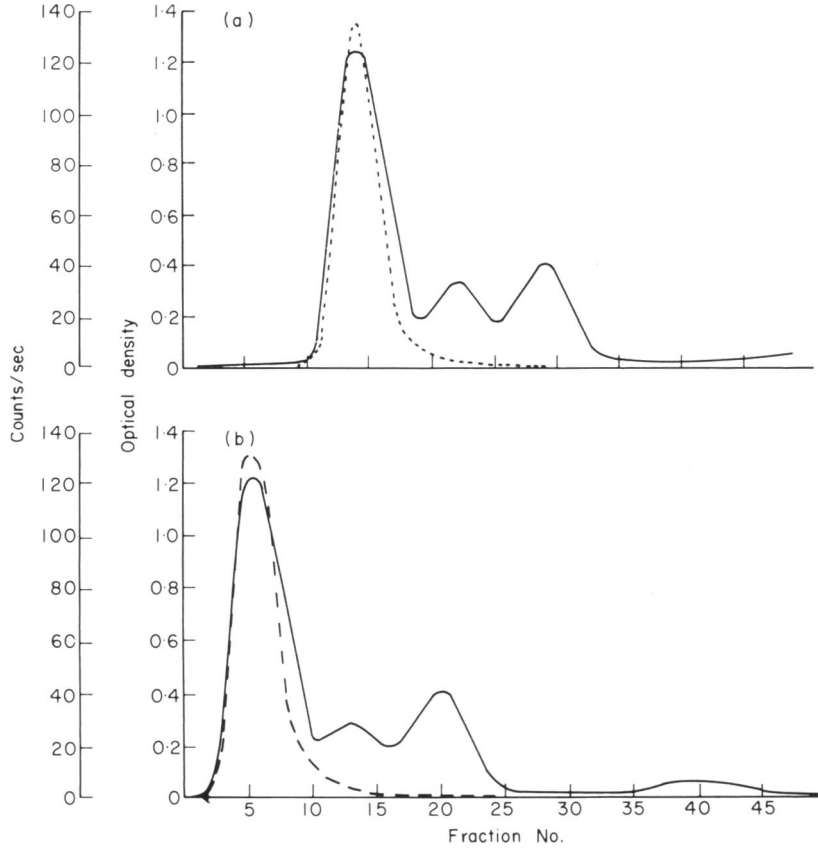

FIG. 2. Radioactivity present in fractions produced by Sephadex G-200 filtration of subjects, serum 10 min (a) and 23 hr (b) after injection of ^{131}I labelled protein. ———, Optical density; – – – –, counts/sec.

Characterization

The purity of the labelled preparations was checked by; (a) Ouchterlony and radio-Ouchterlony gel diffusion; (b) radio-immunoelectrophoresis; (c) Sephadex G-200 gel filtration of the labelled preparation in non-radioactive serum (see Fig. 2); and (d) Zonal centrifugation (Stanworth, 1964). In addition, the labelled 19S rheumatoid factor was incubated with 5 mg solid heat aggregated human γ-globulin for 1 hr at 37°C, then kept at 4°C for several hours. This removed an amount of radioactivity equivalent to the amount that was precipitable by trichloracetic acid.

Study protocol and calculation of data

Each subject received 200–300 mg potassium iodine orally each day in divided doses for at least 3 days before, and during the entire period of the study. Between 12 and 50 μC of labelled protein was injected intravenously, and plasma samples were obtained 10 min after the injection, several times during the first 24 hr, then daily thereafter. Urine was collected in 24-hr lots. In some studies 24-hr collections of stools were made for the first 5 days of the study.

The serum, urine and stool specimen were counted in a twin channel, automatic, gamma ray, well type scintillation counter (Nuclear Enterprises Gammamatic) which utilized a 2-in. thallium activated sodium iodide crystal. The total body radioactivity was determined by a whole body monitor in the following studies:

(i) ^{131}I labelled 19S RF in patients M.H. (experiments 1 and 2), F.B. and C.H.
(ii) ^{131}I labelled IgM in patients M.A., C.C. and F.A.

Details of the whole body monitor are given elsewhere (Directory of Whole Body Radiation Monitors, 1964).

Plots were constructed on semi-logarithmic paper of plasma radioactivity, trichloracetic acid precipitable radioactivity in the plasma, and of total body radioactivity. The latter was obtained directly from the whole body monitor and/or indirectly from the cumulative subtraction of urinary and stool radioactivity from the total injected.

Two methods were employed to analyse the data from the 19S RF and IgM studies. The first, Matthews' method (1967), uses the plasma radioactivity measurements only. A model system with one extravascular pool was used. In this system the following relationships exist:

(i) Fraction of intravascular pool catabolized per day:

$$K_{12} = \frac{1}{\frac{C_1}{b_1} + \frac{C_2}{b_2}}.$$

(ii) Fraction of intravascular pool transferred to extravascular pool per day:

$$K_{13} = \frac{C_1 C_2 (b_2 - b_1)^2}{C_1 b_2 + C_2 b_1}.$$

(iii) Fraction of extravascular pool transferred to intravascular pool per day:

$$K_{31} = C_1 b_2 + C_2 b_1.$$

The values of C_1, C_2, b_1, b_2 were obtained by computer programmed analysis of the plasma curve into exponentials, the functions used being those that gave the best fit to the experimental data by the least squares criterion. The proportion of labelled protein remaining intravascularly after equilibration was calculated from:

$$\frac{K_{31}}{K_{31} + K_{13}}.$$

The second method of analysis was that of Berson *et al.* (1953) and fractional catabolic rate (FCR):

$$\frac{U}{P} = \frac{\text{Total urinary radioactivity in collection period}}{\text{Radioactivity remaining in Plasma}} =$$

$$\frac{\text{Percentage of injected dose excreted in urine in collection period}}{\text{Mean plasma activity as \% of that 10 min after injection of labelled protein}}.$$

The proportion of protein that was intravascular was also determined by the equilibrium time method of Campbell *et al.* (1956).

The plasma volume was calculated on the initial distribution of administered radioactivity expressed as:

$$\frac{\text{Total counts/min injected}}{\text{Counts/min/ml plasma 10 min after injection}}.$$

The total intravascular protein = serum concentration (mg/ml) × plasma volume. The total body protein was calculated from the total intravascular protein and the fraction of protein that was intravascular.

The total quantity of IgM catabolized per day was calculated from:

(i) The total intravascular IgM × FCR. This assumes that all catabolism occurs in the intravascular compartment.

(ii) The total body IgM $\times \frac{0{\cdot}693}{\text{Half life of total body IgM in days}}$.

The absolute catabolic rate (mg/kg/day),

$$= \frac{\text{Total body IgM catabolized per day}}{\text{Body weight in kilograms}}.$$

Because it was not possible to measure the amount of 19S RF in the serum accurately, values for FCR, $T_{\frac{1}{2}}$ and distribution between intra- and extra-vascular compartments only were obtained from the 19S studies, and total intravenous 19S rheumatoid factor, total body 19S RF, and absolute catabolic rate could not be calculated.

Serum proteins were determined by the biuret method. Serum immunoglobulin levels were determined by the antibody in agar, radial diffusion method (Fahey & McKelvey, 1965). Serum IgM was estimated by the same method using rabbit anti-human IgM and compared to a standard human serum. The IgM content of the latter was calibrated against two macroglobulinaemic sera. Frequent determinations of the IgM content and sensitized sheep cell agglutination (SSCA) titre of the subjects plasma were made during the course of these studies. There was no significant alteration in either during the studies in any patient.

RESULTS

Characterization of 19S *RF preparation*

Sephadex G-200 gel filtration of the subjects' serum demonstrated that virtually all the SSCA reactivity was confined to the first optical density peak (see Fig. 3). After isolation, the reactivity of 19S RF was confirmed by several tests:

(1) *SSCA reactivity*: The preparations tested had titres of 1:128 to 1:4096. It was not possible to relate the titre of this preparation to the titre of the subjects' serum because of inability to quantitate accurately the recovery of 19S RF during isolation procedures.

(2) Adsorption of radioactivity by γ-globulin solidified by heating to 75°C for 10 min.

(3) Radio-immunoelectrophoresis of the preparation of labelled RF in non-radioactive serum revealed radioactivity in the β_2 to γ_2 regions unless an anti-IgM component was present in the antiserum when the IgM precipitin line only was radioactive (Fig. 4). In those

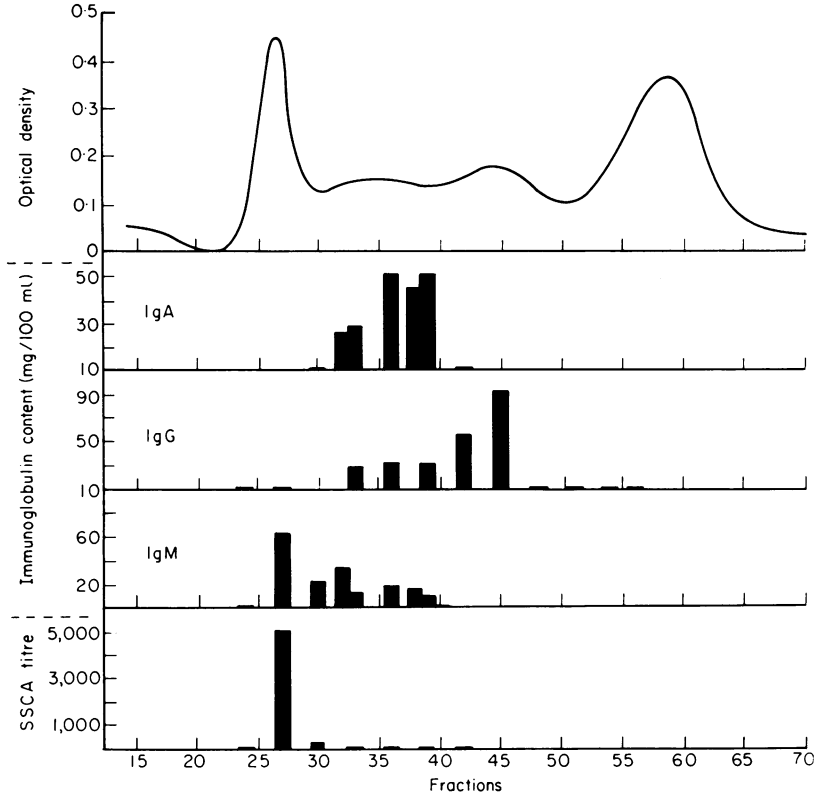

FIG. 3. Sephadex G-200 filtration of serum from subject with classic rheumatoid arthritis showing distribution of immunoglobulins with respect to rheumatoid factor activity.

cases where no anti-IgM was present the attachment of radioactive, unprecipitated RF to antigen–antibody complexes in the electrophoretic region of IgM can explain this feature. Zone ultracentrifugation confirmed that all radioactivity was confined to the 19S components, and none was present in the 7S region.

(4) Sephadex G-200 gel filtration of the preparation diluted with non-radioactive serum demonstrated radioactivity only in the first optically dense peak.

Patient-studies

These were made with ten autologous preparations injected into seven subjects: five IgM preparations injected into five subjects, and five 19S RF given to four subjects. Table 1 gives details of the subjects.

IgM studies

In one patient with primary macroglobulinaemia (M.A.) the FCR of IgM was 0·17, and the plasma half-life was 6·1 days. These values were similar to those already reported for such subjects (Barth *et al.*, 1964) (Fig. 5).

Three subjects with rheumatoid arthritis (F.A., M.H. and W.G.) had SSCA titres of 1024 or greater and IgM serum levels of 95–630 mg/100 ml. One subject (C.C.) was SSCA negative

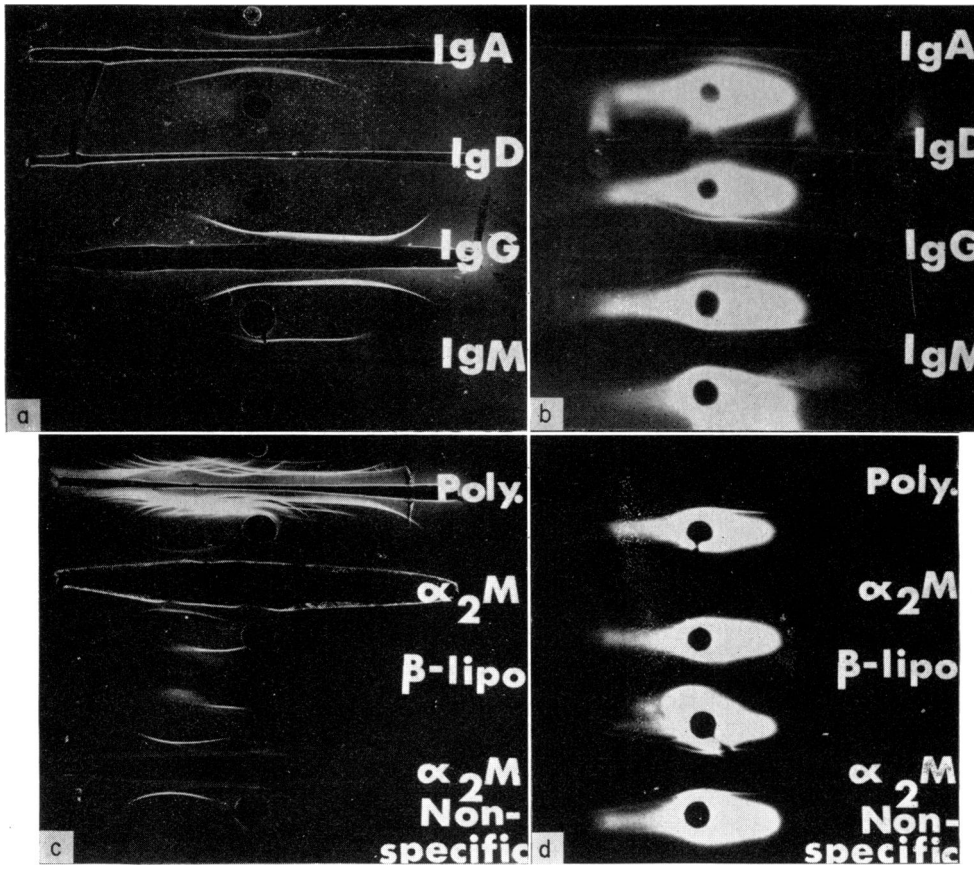

FIG. 4. Radioimmunoelectrophoresis of ^{131}I RF from subject F.B. in presence of non-radioactive normal serum. (a) and (c) Immunoelectrophoresis; (b) and (d) Radioautograph of plates shown in (a) and (c).*

and her IgM concentration was 250 mg/100 ml. The mean FCR of these patients was 0·17 (0·13–0·21) and mean plasma $T_{\frac{1}{2}}$ was 4·7 days (3·7–6·5). These values are similar to those reported for normal subjects and for patients with other diseases (Barth *et al.*, 1964). The absolute catabolic rate (ACR) in these four subjects, calculated from the product of FCR

* When an anti-IgM was present, as in poly antiserum, the only radioactive line is that of IgM. However, when no anti-IgM was present the IgA and IgG precipitin lines are radioactive and this is assumed to be due to the attachment of 'free' RF to antigen–rabbit antibody complexes.

(U/P) and total intravascular IgM, ranged between 8 and 48 mg/kg/day. When an attempt was made to calculate the ACR from the formula:

$$ACR = \frac{\text{Total i.v. IgM} \times 100}{\% \text{ of IgM i.v.}} \times \frac{0{\cdot}693}{\text{Whole body } T_{\frac{1}{2}} \text{ IgM in days}}.$$

TABLE 1. Patients investigated

	Rheumatoid arthritis						Macro-globulinaemia
Patient	F.A.	F.B.	C.H.	W.G.	M.H.	C.C.	M.A.
Age	54	60	56	56	62	42	58
Sex	M	F	F	M	F	F	F
Weight (kg)	58	58	87	58	50	40·5	45
Height (in.)	70	61	65	68	62	65·5	63
Venous haematocrit (%)	47·2	39·0	39·7	38·2	35·0	36·0	32·9
Plasma volume (litres)	3·15	2·45	2·46	2·93	2·61	1·67	2·70
Plasma volume (ml/kg)	54·5	42·3	28·3	51·3	52·3	41·3	60·5
Serum albumin (g/100 ml)	3·0	2·1	3·3	3·2	2·7	2·4	2·2
Serum IgA (mg/100 ml)	555	222	308	283	210	300	186
Serum IgD (mg/100 ml)	ND	3·3	3·3	ND	3·3	3·6	ND
Serum IgG (mg/100 ml)	1240	800	1660	1040	1600	1510	890
Serum IgM (mg/100 ml)	630	360	220	95	340	250	2100+
RA Latex test	+	+	+	+	+	+	0
SSCA titre	8102	1024	2048	1024	1024	<16	0
LE cells	+	0	0	0	0	0	0
Serum urea (mg/100 ml)	32	34	30	36	42	23	50
Creatinine clearance	90	65	74	ND	40	90	45
Serum complement normal: 35±4·7 units/ml	30	45	26	23	22	41	ND
Westergren sedimentation rate (1 hr)	110	124	50	65	90	52	120
Disease duration (years)	12	26	6	6	15	8	10
Rheumatoid nodules	++	++	+	++	++	++	0
Peripheral neuropathy	0	0	0	0	+	0	0
Episcleritis	0	0	0	0	+	0	0
Proteinuria (g/day)	0	1·5	0	0	0	0	0
Corticosteroid therapy (years)	0	13	0	5	0	1	0

ND, Not determined.

the results were similar to those obtained from plasma values except in the case of M.H. (Table 2) where the most likely cause of difference was incomplete urinary collections. The percentage of IgM in the intravascular compartment was between 57 and 77%.

19S rheumatoid factor studies

Four patients with severe rheumatoid disease received five preparations of autologous 19S RF. All patients had classic rheumatoid disease (American Rheumatism Association Criteria, 1956). One patient had Sjögrens syndrome, peripheral neuropathy, gangrene of

the fingertips, skin ulceration and had developed aortic incompetence of the type described by Zvaifler & Weintraub (1963). To make a direct comparison of the catabolic rates of IgM and 19S RF autologous preparations of each were injected simultaneously in this patient. Patient F.A. received IgM and 19S RF consecutively. The two other patients, F.B. and C.H., received 19S RF only.

The FCR of the 19S RF varied between 0·12 and 0·18. The plasma $T_{\frac{1}{2}}$ was 3·9–6·0 days, and where the total body radioactivity was determined by whole body counting the whole body $T_{\frac{1}{2}}$ was in close agreement with the plasma value (see Fig. 6). The percentage of 19S RF in the intravascular compartment was between 57 and 82%.

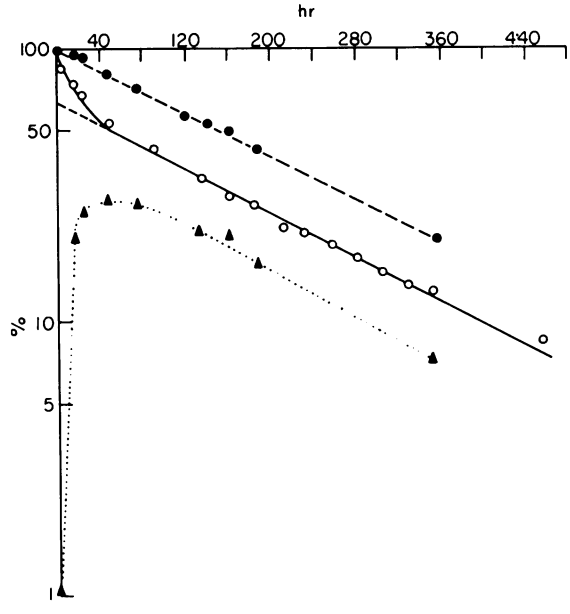

FIG. 5. Survival of ^{131}I. IgM in M.A. (primary macroglobulinaemia). ●, Total body radioactivity determined by whole body counter (WBC); ○, plasma radioactivity (P); ▲, apparent extra-vascular radioactivity derived from WBC–P. Values expressed as a percentage of value obtained 10 min after injection of labelled protein. Plasma $T_{\frac{1}{2}}$, 145 hr (6·1 days); whole body $T_{\frac{1}{2}}$, 144 hr (6·0 days).

The percentage of radioactivity that was precipitable by 10% trichloracetic acid was determined throughout both the IgM and 19S RF studies, and the plasma survival curves of TCA precipitable radioactivity accepted as that of the labelled protein. However, the presence of diffusible non-protein bound radioactivity may invalidate the whole body counts, and because the first preparation of 19S RF injected into M.H. contained non-protein bound radioactivity a second preparation which had more than 98% protein bound activity was isolated. This was because M.H. had widespread evidence of vasculitis and, therefore, was the subject most likely to demonstrate a difference between the metabolism of 19S RF and IgM.

In this second study the daily FCR (U/P) for the first 3 days were 0·34, 0·33 and 0·37, but thereafter were in the region of 0·18. The total body radioactivity contained two components for this period, and the intercept at T for the slower component was 85%. Thus there was

evidence of a higher rate of catabolism for approximately 15% of the preparation. Whether this was due to specific removal of RF or non-specific catabolism of denatured protein will be discussed later.

No evidence of more rapid catabolism initially was seen in the studies F.B. (Fig. 6) and C.H. The plasma and whole body radioactivity curves and the urinary radioactivity excretion curves were those of catabolism of a metabolically homogeneous protein.

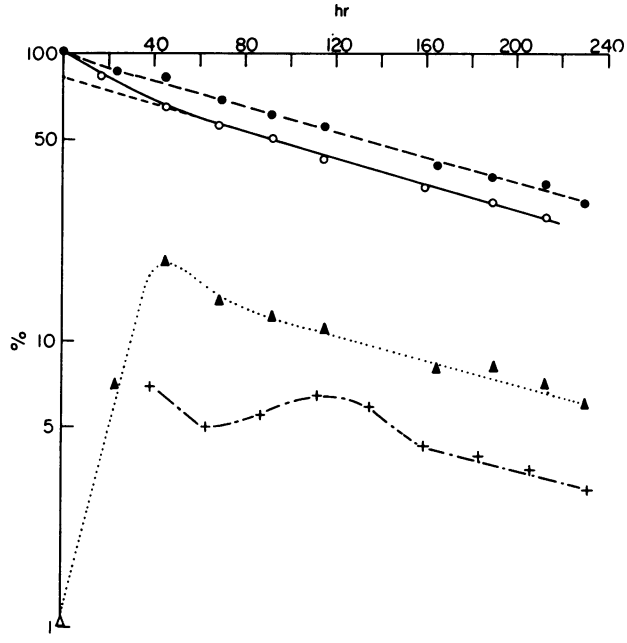

FIG. 6. Survival of ^{131}I. RF in F.B. (classical rheumatoid arthritis). ●, Total body radioactivity determined by whole body counter (WBC); ○, plasma radioactivity (P); ▲, apparent extravascular radioactivity derived from WBC–P (EV); +, percentage of injected radioactivity excreted per day in urine (U). WBC, P, and EV expressed as a percentage of value obtained 10 min after injection of labelled protein. Plasma $T_{\frac{1}{2}}$, 138 hr (5·8 days); whole body $T_{\frac{1}{2}}$, 140 hr (5·8 days).

DISCUSSION

The metabolism of IgM has been studied before in normal subjects and in patients with various diseases (Cohen & Freeman, 1960; Drivsholm, 1961; Gabuzda, 1962; Costea, Constantoulakis & Schwartz, 1962; Olesen, 1963; Barth *et al.*, 1964) but not in rheumatoid subjects. The results reported here show that autologous IgM has the same FCR, $T_{\frac{1}{2}}$ and distribution between the intravascular and extravascular compartments in rheumatoid arthritis as in other subjects (see Tables 2 and 3). Two of the patients (C.C. and W.G.) were receiving corticosteroid therapy during the period of the study, but the FCR, $T_{\frac{1}{2}}$ and distribution were in the range reported for normal subjects.

The synthetic rate, assumed in a steady state to be equivalent to the absolute catabolic rate (ACR), varied greatly with a range of 8–50 mg/kg/day in these subjects. As reported previously (Barth *et al.*, 1964), the FCR is independent of serum IgM concentration, so that the latter is a good guide to the magnitude of IgM synthesis.

TABLE 2. Radio-iodine labelled IgM and 19S rheumatoid factor in seven patients

Patient	Diagnosis	Study	Serum IgM (mg/100 ml)	Plasma volume (ml/kg)	Calculations from plasma and urine radioactivity								Calculations from whole body counter		
					% intravascular IgM		Total intravascular (mg/kg)	Total body IgM (mg/kg)	Plasma $T\frac{1}{2}$ (days)	Fractional catabolic rate		Absolute catabolic rate for IgM (mg/kg/day)	Total body ($T\frac{1}{2}$)	$0{\cdot}693/T\frac{1}{2}$	Absolute catabolic rate† (mg/kg/day)
					$\frac{K_{31}}{K_{31}+K_{13}}$	TE				K_{12}	U/P				
M.A.	Mac	IgM	2000	60·5	73·6	67·5	1210	1640	6·1	0·17	0·14	204·0	5·98	0·119	195·7
C.C.	RA	IgM	235	41·3	70.0	77 0	97·0	138·6	4·8	0·21	0·18	20·4	5 45	0·127	17·6
W.G.	RA	IgM	86	51·3	ND	65 0	44·1	67·9	3·7	0·19	0·18	8·0	ND	ND	ND
F.A.	RA	IgM	600	54·5	56·6	56·7	327·0	580	6·6	ND	0·15	48·5*	6·74	0·103	59·8
	RA	RF	630	54·5	ND	59·0	327·0	580	6·0	ND	0·13	ND	ND	ND	ND
M.H.	RA	IgM	320	52·3	ND	ND	167·4	ND	3·9	ND	0·13	ND	ND	ND	ND
	RA	RF_1	320	52·3	ND	ND	167·4	ND	3·9	ND	0·16	ND	4·66	0·149	ND
	RA	RF_2	320	52·0	57·0	62·5	166·5	ND	4·0	0·18	0·18	ND	3·93	0·176	ND
F.B.	RA	RF	360	42·3	80·0	77·0	166·5	ND	5·77	0·14	0·12	ND	5·85	0·12	ND
C.H.	RA	RF	216	28·3	82·4	80·0	61·1	ND	4·51	0·18	—	ND	4·87	0·14	ND
Normal (Barth, *et al.* 1964)		Range	57–198		ND	65–100	15·4–68·2	22·3–105	3·8–6·5	ND	14·1–25·1	3·16–16·9	ND	ND	ND
		Mean	93			76·4			5·1			16·9			

ND, Not determined.

* Urine collections known to be incomplete.

† Calculated from total body IgM value obtained from plasma curve analysis.

TABLE 3. Summary of previous data on metabolism of IgM

	Method of IGM preparation	Ratio iodine atoms to IgM	$T_{\frac{1}{2}}$ (days)	Fractional catabolic rate		Intravascular IgM (%)	Absolute catabolic rate (mg/kg/day)
				U/P	K_{12}		
Cohen & Freeman (1960)	Ultracentrifugation and zone electrophoresis		4·0	(0·35 initially) 0·15–0·18		Nearly all	
Costea *et al.* (1962)	Adsorption of IgM cold agglutinins	No details	2·6			66	
Gabuzda (1963)	Euglobulin precipitation and DEAE chromatogaphy	2·5:1	6·7		0·15	75	320·0 (in primary macroglobulinaemia)
Olesen (1963)	Euglobulin precipitation and adsorption of cold agglutinins	2·6 to 14:1	4·9–5·2 (99% pure) 9–11 (90–95% IgM)	0·18–0·20 0·09–0·14		63–81	
Barth *et al.* (1964)	Zone electrophoresis and Sephadex G-200 filtration	1:1	3·8–6·5	0·14–0·25		65–100	3·16–16·9 (normal subjects)
This study							
IgM	Zone electrophoresis and Sephadex G-200 filtration	1:1	3·7–6·5	0·17–0·21	0·14–0·18	65–77	204 (macroglobulinaemia) 8–60 (rheumatoid arthritis)
RF	Euglobulin precipitate G-200 filtration adsorption on aggregated γ	1:1	3·9–6·0	0·15–0·19	0·15–0·19	62–82	

The subjects with rheumatoid arthritis studied were not a representative group, but were chosen because their disease was more active and severe than in most patients. They were, therefore, more likely to show a difference between IgM and rheumatoid factor metabolism, if one existed. Previously, the metabolism of rheumatoid factor in normal subjects had been studied (Harris & Vaughan, 1961) by transferring plasma from rheumatoid patients with high SSCA titre into normal subjects. The SSCA titre of the normal recipients' plasma declined with a $T_{\frac{1}{2}}$ of 4–7 days.

Because the intravascular deposition of rheumatoid factor has been suggested (Franklin, Kunkel & Ward, 1958; Epstein & Engleman, 1959) as the cause of the vasculitis seen in severe rheumatoid disease differences between the metabolism of 19S rheumatoid factor and IgM in such patients were looked for. In the patient M.H. the first 19S rheumatoid factor injected contained more non-protein bound radioactivity than desirable for metabolic studies so a second preparation was isolated and injected. The FCR and $T_{\frac{1}{2}}$ of the second preparation were the same as those for the first 19S RF and for IgM, but the FCR (U/P) for the first few days was higher than subsequent values revealing the presence of a small, rapidly catabolized fraction. It is possible that this was the only part of the preparation which retained its rheumatoid factor reactivity. That this was probably not the explanation was shown by measurement of SSCA activity, by adsorption of radioactivity on heat solidified γ-globulin and by radioimmunoelectrophoresis. All of these tests indicated that at least the major part of the preparation was still reactive *in vitro*. A known hazard of isolation of IgM is the ease with which some of the protein is denatured (James, Felix-Davies & Stanworth, 1961), and this is the probable explanation for the initially high U/P observed after a second injection of 19S RF.

Injection of 19S RF into subjects F.B. and C.H. demonstrated no evidence of even a small fraction that was more rapidly catabolized than the remainder. In subject F.B. only 3% of injected radioactivity was excreted in the urine over the first 15 hr whereas 7% was excreted in the following 24 hr (see Fig. 7). This not only confirms that there was no phase of rapid 19S RF catabolism initially, but is good evidence of the lack of denaturation of the labelled protein. It was this preparation which produced radio-immunoelectrophoretic findings with the clearest evidence of radioactivity present in the precipitin lines in the β_2 to γ_2 region. This is assumed to be due to adsorption of labelled 19S RF onto antigen–antibody complexes. When the 19S RF, demonstrated to be 19S by zone-centrifugation, was precipitated by anti-IgM component in an antiserum only the IgM line was radioactive.

The metabolic studies, therefore, show that the radioactive autologous 19S RF injected into these subjects had a FCR, half-life and distribution between intra- and extra-vascular compartments similar to that of similarly labelled autologous IgM. How far can these findings be interpreted as reflecting the behaviour of 19S RF? The first qualification is that columns containing aggregated human γ-globulin will not adsorb any RF reacting specifically with rabbit IgG (Williams & Kunkel, 1963). However, solid human IgG may adsorb even this fraction. Though RF is identified with euglobulin (Ziff *et al.*, 1956) some pseudoglobulin 19S RF has been identified (Heimer & Nosenzo, 1965) but would have been excluded by the isolation process used. However, both of these factors are only a minor fraction of the total 19S RF present.

Another source of error might result from a selective release of part of RF from the aggregated γ-globulin column. The pH used for elution might release only the less avid portion and leave the more avid portion still attached to the γ-globulin. When this was

investigated the protein recovered in these studies was more than 90% of active 19S RF added to the column as judged by ultracentrifugal analysis.

By our tests the 19S RF injected was reactive with denatured IgG and representative of circulating 19S RF. Yet no evidence of rapid elimination of RF was seen in these patients three-quarters of whom had circulating 22S complexes. With careful techniques of isolation and iodination proteins labelled with radioactive iodine have been shown to behave metabolically in similar manner to biosynthetically labelled proteins (Cohen *et al.*, 1956; McFarlane, 1963). Further support for this has been provided by Gordon, Eisen & Vaughan (1966) who found that ^{14}C RF disappeared from the serum of patients with rheumatoid arthritis at approximately the same rate as IgM. It seems probable that the catabolism of labelled RF does represent that of circulating RF.

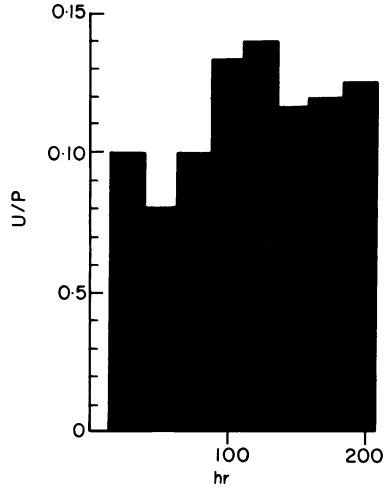

FIG. 7. Radioactivity excreted in urine of subject F.B. after injection of ^{131}I labelled RF expressed as a fraction of the mean plasma radioactivity for the same period of 24 hr. The first collection period was 15 hr and had a U/P of 0·36.

These results must be qualified. 'Active' RF may be removed from the circulation very rapidly, or may not even reach it from its site of synthesis so that any preparation of circulating 19S RF would contain little or none of this 'active' factor. Also it is possible that the antigen, altered IgG, may be sequestered in the synovial space and may not be accessible to the predominantly intravascular 19S RF. Other reports have suggested that the extravascular space may be the main site of RF action (Vaughan, 1956; Gough & Davis, 1966).

With these qualifications in mind, these studies support the concept that the great majority of circulating 19S RF has no pathogenic role since no acceptable evidence has been found of a catabolic rate greater than that of IgM group as a whole.

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