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# METABOLISM OF RADIO-IODINATED IgG IN PATIENTS WITH ABNORMAL SERUM IgG LEVELS

## I. HYPERGAMMA-GLOBULINAEMIA

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SUMMARY

Metabolic turnover studies were performed with radio-iodinated IgG in twelve patients with a serum IgG level greater than 1600 mg/100 ml (six with monoclonal gammopathy and six with a polyclonal increase in IgG associated with liver disease). The six patients with an IgG monoclonal protein comprised four multiple myeloma, one benign monoclonal gammopathy and one biclonal gammopathy presenting as Waldenström's macroglobulinaemia. The six patients with liver disease comprised two patients with cirrhosis, two with infective hepatitis and two with chronic active hepatitis. The injected IgG was either autologous normal IgG (five cases), autologous monoclonal IgG (five cases), homologous normal IgG (one case) or therapeutic intravenous HGG (two cases).

The plasma volume was increased in six patients; the plasma IgG pool in nine; and the total body IgG pool in seven. The plasma $T\frac{1}{2}$ was normal in one patient with monoclonal and one patient with polyclonal gammopathy but shortened in the other ten studies with mean values of 11·3 and 11·0 days in monoclonal and polyclonal gammopathy respectively. The fractional turnover rate was normal in two studies in polyclonal gammopathy and increased in the other ten with mean values of 13·6% per day in both groups of patients. The IgG synthesis rate was significantly increased in all studies except for a reduced synthesis of normal IgG in one patient with multiple myeloma. The mean synthesis rates in monoclonal and polyclonal gammopathy were respectively 6·7 and 4·1 times the mean synthesis rate in normal controls.

The pattern of increased synthesis and increased catabolism in such patients confirms published reports in some diseases and demonstrates a similar pattern in chronic active hepatitis. The findings are consistent with the 'concentration-catabolism' effect.

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## INTRODUCTION

An increase in the serum level of IgG is seen in many disorders including auto-immune diseases, infections and γG monoclonal gammopathy (e.g. Schultze & Heremans, 1966). The serum IgG level alone, however, does not convey sufficient information on the processes involved in its regulation. Measurements of the IgG synthesis rate, fractional catabolic rate, rate of loss from abnormal channels, and distribution between plasma and extra-vascular pools are necessary in the individual patient to indicate the relative contribution of these various factors to the serum IgG level. An increased serum IgG level may result from increased synthesis, decreased catabolism with prolonged plasma survival, increased plasma localization, or combinations of these factors. Moreover, there is a significant and direct correlation between the serum IgG level and the fractional catabolic rate (Waldmann & Strober, 1969).

The metabolism of IgG in various diseases was recently reviewed by Waldmann & Strober (1969). Many earlier studies, however, employed homologous normal IgG rather than autologous IgG (Andersen, 1964) and may not detect individual abnormalities in metabolism. The present study was undertaken to examine the patterns of IgG metabolism in patients with a monoclonal or polyclonal increase in serum IgG (>1600 mg/100 ml). Turnover studies were performed with radio-iodinated IgG in six patients with a monoclonal IgG protein (four patients with multiple myeloma and one each with benign gammopathy and biclonal gammopathy presenting as Waldenström's macroglobulinaemia) and in six patients with polyclonal gammopathy associated with liver disease (two patients each with cirrhosis, acute infective hepatitis and chronic active hepatitis).

## MATERIALS AND METHODS

### *Subjects*

The clinical and laboratory data for the twelve patients are summarized in Table 1. Patients 1. AJ, 2. RS, 3. JH and 4. JH all had proven multiple myeloma with anaemia, osteolytic lesions and bone marrow plasmacytosis associated with an IgG monoclonal protein. Patient 5. WM had polycythemia vera but no indication of multiple myeloma with monoclonal protein, i.e. benign monoclonal gammopathy. Although patient 6. SF had biclonal gammopathy with IgG and IgM monoclonal proteins, he presented with clinical features of Waldenström's macroglobulinaemia (WM) viz., hepatomegaly, lymphadenopathy, anaemia, hypervolaemia and bone marrow lymphocytosis. This clinical presentation has been noted in other patients with both IgG and IgM monoclonal proteins (Wang *et al.*, 1969).

Patients 7. AR and 8. LN were studied simultaneously 7 days after admission to the hospital with acute infective hepatitis. They were icteric for approximately the first 10 days of their turnover studies and were on a low fat diet with restricted activity. They had significant falls in serum levels of bilirubin and SGOT but the serum IgG levels did not alter significantly during the three weeks of their studies.

The hepatic cirrhosis in patients 9. JL and 10. RM was thought to be alcoholic in aetiology. An episode of decompensation secondary to a drinking bout had led to the admission of 9. JL with oedema and ascites 4 weeks before her study. Both had cleared before her study commenced. Patient 10. RM had been admitted with pneumonia, which cleared before his study commenced.

TABLE 1. Clinical and laboratory data in twelve patients with serum IgG > 1600 mg/100 ml in whom IgG turnover studies were performed. Subjects 1–6 had monoclonal gammopathy and subjects 7–12 had polyclonal hypergamma-globulinaemia

| Patient | Sex | Age (yrs) | Weight (kg) | Diagnosis | Serum proteins (g/100 ml) | | | Serum electrophoresis and immunoelectrophoresis | Serum immunoglobulins (mg/100 ml) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Total | Albumin | Globulin | | IgG | IgA | IgM |
| 1. AJ | F | 63 | 49 | Multiple myeloma | 8·6 | 4·0 | 4·6 | γ1 M-band (γG-L) | 2700 | 233 | 155 |
| 2. RS | M | 55 | 70 | Multiple myeloma | 12·0 | 3·0 | 9·0 | γ2 M-band (γG-K) | 5750 | 200 | 20 |
| 3. JH | M | 55 | 75 | Multiple myeloma | 9·8 | 3·8 | 6·0 | γ1 M-band (γG-L) | 3720 | 155 | 46 |
| 4. JH | M | 70 | 61 | Multiple myeloma | 11·0 | 3·3 | 7·7 | γ1 M-band (γG-K) | 4350 | 10 | 8 |
| 5. WM | M | 72 | 52 | Benign monoclonal gammopathy | 7·1 | 3·5 | 3·6 | β2 M-band (γG-K) | 1640 | 130 | 90 |
| 6. SF | M | 72 | 60 | Biclonal gammopathy | 10·4 | 2·6 | 7·8 | γ1; γ2 M-bands (γM-K; γG-K) | 3300 | 170 | 2600 |
| 7. AR | M | 30 | 71 | Acute infective hepatitis | 6·6 | 3·2 | 3·4 | Polyclonal increase | 1950 | 270 | 600 |
| 8. LN | M | 19 | 66 | Acute infective hepatitis | 8·7 | 3·7 | 5·0 | Polyclonal increase | 2200 | 300 | 530 |
| 9. JL | F | 54 | 45 | Hepatic cirrhosis (alcoholic) | 5·3 | 1·5 | 3·8 | Polyclonal increase | 1680 | 645 | 570 |
| 10. RM | M | 60 | 63 | Hepatic cirrhosis (alcoholic) | 8·4 | 3·1 | 5·3 | Polyclonal increase | 2380 | 600 | 164 |
| 11. AB | M | 42 | 66 | Chronic active hepatitis | 7·5 | 3·7 | 3·8 | Polyclonal increase | 1700 | 325 | 90 |
| 12. GT | M | 24 | 70 | Chronic active hepatitis | 10·3 | 3·1 | 7·2 | Polyclonal increase | 4350 | 316 | 69 |
| Normal Controls | | | | | 6·0–8·0 | 3·8–5·5 | 2·0–3·5 | | 600–1600 | 60–250 | 60–200 |

The diagnosis of chronic active hepatitis was confirmed in patients 11. AB and 12. GT with microscopical examination of specimens obtained by aspiration liver biopsy demonstrating liver cell necrosis, regeneration, fibrosis, and infiltration with mononuclear cells (Mistilis & Blackburn, 1970). Each had been treated with Prednisone for 3 weeks before the studies and was receiving 20 mg/day. There were no significant alterations in their disease parameters during their studies, including SGOT and serum protein levels.

*Serum proteins*

The serum levels of total protein, albumin and total globulin were measured in an AutoAnalyzer (Technicon) with the Biuret and bromcresol methods (Bartholomew & Delaney, 1966). Serum levels of IgG, IgA and IgM were measured by radial immune-precipitation in antibody-agar plates by the method previously described (Stiehm, Vaerman & Fudenberg, 1966). The values in normal controls are listed in Table 1.

*Preparation of radio-iodinated IgG*

Autologous IgG was prepared from serum and homologous pooled normal serum by ion-exchange chromatography on DEAE-cellulose with phosphate buffer, pH 6·3. 0·0175 M (Levy & Sober, 1960). HGG (CSL) is a preparation of human gamma-globulin developed for therapeutic intravenous administration by the Commonwealth Serum Labs., Melbourne and turnover studies with this preparation have been reported previously (Wells & Penny, 1969). Each protein was labelled with carrier-free $^{125}I$ or $^{131}I$ (The Radiochemical Centre,

TABLE 2. Details of the thirteen radio-iodinated preparations of IgG injected into the respective patients for the turnover studies. In patients 1. AJ, 2. RS[1], 4, JH, 5. WM and 6. SF the protein was the autologous monoclonal IgG

| Patient | Preparation | Labelling efficiency (%) | Non-protein bound activity (%) | Gamma-globulin activity (%) | Injected dose ($\mu$Ci) | $Q_{U,\,1+2}$ (%) |
|---|---|---|---|---|---|---|
| 1. AJ | $^{125}I$-IgG (autologous) | 54·6 | 0·6 | 95·0 | 8 | 5·6 |
| 2. RS* | $^{125}I$-IgG (homologous) | 67·9 | 1·0 | 97·8 | 5 | 10·4 |
| 2. RS[1] | $^{131}I$-IgG (autologous) | 63·4 | 1·0 | 96·2 | 20 | 13·4 |
| 3. JH | $^{131}I$-HGG (C.S.L.)† | 75·0 | 1·6 | 99·1 | 20 | 18·7 |
| 4. JH | $^{131}I$-IgG (autologous) | 45·1 | 0·8 | 96·4 | 16 | 4·8 |
| 5. WM | $^{131}I$-IgG (autologous) | 74·8 | 1·1 | 95·0 | 15 | 10·4 |
| 6. SF | $^{131}I$-IgG (autologous) | 63·3 | 0·7 | 98·0 | 18 | 6·3 |
| 7. AR | $^{125}I$-IgG (autologous) | 71·2 | 0·4 | 99·0 | 6 | 16·3 |
| 8. LN | $^{125}I$-IgG (autologous) | 39·8 | 0·6 | 98·8 | 7 | 10·5 |
| 9. JL | $^{131}I$-HGG (CSL)+ | 69·0 | 1·2 | 98·0 | 16 | 23·7 |
| 10. RM | $^{131}I$-IgG (autologous) | 50·1 | 1·0 | 98·5 | 18 | 5·3 |
| 11. AB | $^{125}I$-IgG (autologous) | 51·7 | 1·1 | 98·9 | 6 | 19·0 |
| 12. GT | $^{131}I$-IgG (autologous) | 66·1 | 0·5 | 97·9 | 20 | 14·9 |
| Mean | | 60·9 | 0·9 | 96·0 | | |
| Controls‡ | | 30–90 | <2·0 | >95·0 | | <12·0 |

* Two studies performed simultaneously in 2. RS.

† HGG (CSL) is an intravenous preparation of human gamma-globulin.

‡ Labelling data from Wells (1969a).

Amersham) by the iodine monochloride of McFarlane (1958). Free iodine was removed by passage through Deacidite resin columns; the preparations were dialysed against 0·9% saline at 4°C overnight to remove non-protein-bound isotope; human serum albumin was added to prevent autoirradiation and denaturation; and the preparations passed through millipore filters and sealed in ampoules for subsequent injection. An aliquot of each preparation was tested by electrophoresis on cellulose acetate to measure the percentage of activity located in the electrophoretic gamma-zone; by immunoelectrophoresis to confirm the immunological purity of the IgG; and by precipitation with 10% trichloracetic acid (TCA) to measure the non-protein-bound activity (Wells, 1969a). The results of these tests on the thirteen labelled preparations injected into the twelve patients are summarized in Table 2. Patient 2. RS was injected simultaneously with two preparations: $^{131}$I-labelled autologous IgG monoclonal protein and $^{125}$I-labelled homologous IgG from pooled normal serum.

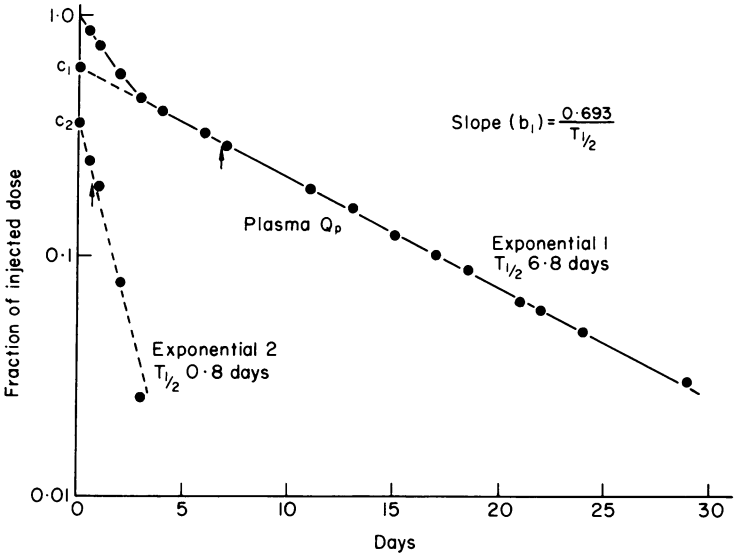


FIG. 1. Graph of the survival of autologous $^{131}$I-IgG in patient 12. GT (chronic active hepatitis).

*Metabolic turnover study*

The theory and use of isotope tracers in metabolic protein studies have been reviewed by Robertson (1957), Matthews (1957), and Andersen (1964). The present methods have been reported in detail (Wells, 1969a, b; Wells & Penny, 1969; Wells & Jeremy, 1971). To summarize, a known amount of the preparation was injected intravenously and specimens of plasma and urine collected for three weeks. The $^{125}$I and $^{131}$I gamma-ray activities in 5 ml aliquots of these specimens were measured in an automatic scintillation counter (Packard Inst. Inc., Illinois) with corrections for background, decay during counting, and channel cross-over. Zero time plasma activity ($Q_{P_0}$) was estimated graphically from values for plasma activity in 6-min and 12-min samples. The graph for plasma activity ($Q_P$) is reproduced on 2-cycle semi-logarithmic paper in Fig. 1 for the study in patient 12. GT. After resolution of the plasma curve into its component exponentials (Matthews, 1957), the

turnover parameters were calculated from a series of equations for the mamillary model (Table 3).

In patients in whom the urine collections appeared complete for the 21-day duration of the study (1. AJ, 6. SF, 7. AR, 8. LN, 11. AB and 12. GT), the FTR and IgG synthesis rate were also calculated by the urinary clearance method (Campbell *et al.*, 1956). In this method, the FTR is calculated for each day from day 3 to day 21 as the ratio of activity excreted in the urine to the average plasma activity ($Q_P$) for the corresponding 24 hr. The mean of these individual FTRs is taken as the FTR for the whole study.

All patients understood the nature of the studies and gave their free and informed consent. Lugol's iodine (0·5 ml three times daily) was given to each patient throughout the study to block thyroid uptake of radio-iodine after its release from catabolized protein.

## RESULTS

### *Assessment of denaturation*

An important prerequisite to the interpretation of metabolic studies with labelled proteins is the assessment of protein denaturation by laboratory and biological studies. For all preparations used in the present studies, more than 95·0% of protein-bound activity was located in the electrophoretic gamma-zone and more than 98·0% of total activity was bound to protein (Table 2). An average of 1·1 atoms of iodine were attached per molecule of IgG

TABLE 3. List of definitions for calculating parameters of IgG metabolism (Matthews, 1957)

| Term | Unit | Definition |
|---|---|---|
| 1. Labelling efficiency | % | Percentage of isotope attached to protein during the labelling procedure |
| 2. Gamma-globulin activity | % | Percentage of total protein-bound activity located in the electrophoretic gamma-zone |
| 3. $Q_{U,\ 1+2}$ | % | Percentage of total injected activity excreted in urine in free form in the initial 48 hr of the study |
| 4. Plasma $T\frac{1}{2}$ | days | Graphical half-life of final slope of curve $Q_P$. |
| 5. Plasma volume (PV) | ml/kg | $\frac{\text{Activity of standard} \times \text{volume injected} \times 100}{Q_{P_0}}$ |
| 6. Plasma IgG pool | g/kg | $\frac{\text{PV} \times \text{serum concentration (mg/100ml)}}{\text{body weight (kg)}}$ |
| 7. Distribution ratio (DR) | | Fraction of total body IgG located in plasma $DR = \frac{\text{plasma IgG pool}}{\text{total body IgG pool}} = \frac{b_1}{K_{12}}$ |
| 8. Total body IgG pool | g/kg | $\frac{\text{Plasma IgG pool}}{DR}$ |
| 9. Fractional turnover rate (FTR) | %/day | Percentage of plasma pool catabolized and cleared into urine per day. $FTR = K_{12} \times 100$ |
| 10. Absolute IgG catabolic rate | g/day<br>mg/kg/day | Mass of protein catabolized per day = FTR × plasma pool |
| 11. IgG synthesis rate | g/day<br>mg/kg/day | IgG synthesis rate = IgG catabolic rate, in conditions of equilibrium with steady serum protein levels and body weight |

TABLE 4. Results of thirteen IgG turnover studies in the twelve patients

| Patient | Serum IgG level (mg/100 ml) | Plasma volume (mg/kg) | Plasma IgG pool (g/kg) | Distribution ratio (D.R.) | Total body IgG pool (g/kg) | Plasma $T_{\frac{1}{2}}$ (days) | $Qu_{,1+2}$ (%) | Fractional turnover rate (%/day) | IgG synthesis (g/day) | IgG synthesis (mg/kg/day) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. AJ | 2700 | 38·3 | 1·03 | 0·50 | 2·07 | 8·9 | 5·6 | 15·5 | 7·85 | 160 |
| 2. RS | 250 | 53·5 | 0·13 | 0·56 | 0·24 | 8·8 | 10·4 | 14·0 | 1·31 | 19 |
| 2. RS[1] | 5500 | 53·5 | 2·94 | 0·63 | 4·67 | 9·0 | 13·4 | 12·3 | 25·31 | 362 |
| 3. JH | 3720 | 39·2 | 1·46 | 0·34 | 4·29 | 10·5 | 18·7 | 19·4 | 21·20 | 283 |
| 4. JH* | 4350 | 57·7 | 2·51 | — | — | — | 4·8 | — | — | — |
| 5. WM | 1640 | 57·2 | 0·94 | 0·36 | 2·60 | 19·0 | 10·4 | 12·1 | 5·65 | 109 |
| 6. SF | 3300 | 78·7 | 2·60 | 0·54 | 4·81 | 11·3 | 6·3 | 11·4 | 17·83 | 296 |
| 7. AR | 1950 | 33·8 | 0·65 | 0·41 | 1·59 | 9·7 | 16·3 | 17·2 | 7·98 | 112 |
| 8. LN | 2200 | 43·8 | 0·96 | 0·47 | 2·05 | 15·3 | 10·5 | 9·6 | 6·11 | 93 |
| 9. JL | 1680 | 48·2 | 0·81 | 0·49 | 1·65 | 11·2 | 23·7 | 13·5 | 4·92 | 109 |
| 10. RM | 2380 | 53·6 | 1·28 | 0·65 | 1·96 | 11·5 | 5·3 | 9·2 | 7·39 | 117 |
| 11. AB | 1700 | 38·2 | 0·65 | 0·37 | 1·75 | 11·6 | 19·0 | 16·0 | 6·86 | 104 |
| 12. GT | 4350 | 51·3 | 2·23 | 0·65 | 3·44 | 6·8 | 14·9 | 15·8 | 24·70 | 352 |
| Normal range | 600–1600 | 33–50 | 0·28–0·82 | 0·32–0·64 | 0·57–2·05 | 14–28 | < 12·0 | 4·3–9·8 | — | 20–60 |
| Mean | 989 | 41 | 0·50 | 0·50 | 1·03 | 21 | | 6·9 | | 36 |

* 4. JH died on the 7th day of his study.

(range 0·3–2·0), excluding over-iodination as a potential cause of denaturation (Johnson, Day & Pressman, 1960). The detection of minor degrees of denaturation relies on observations of their biological behaviour since denatured preparations are rapidly cleared by the reticulo-endothelial system (e.g. Benacerraf *et al.*, 1955) and there is a marked increase in urinary excretion of free isotope in the first 12–24 hr (Freeman, 1959). The interpretation of high values for $Q_{U,\ 1+2}$ (see Table 2) in patients is complicated by the fact that patients with a disease associated with rapid catabolism have a higher initial excretion of free isotope than normal subjects, in the absence of any denaturation in their labelled preparation. In the present studies, six of the thirteen preparations were associated with a value for $Q_{U,\ 1+2}$ greater than 12·0%. In patients 3. JH and 9. JL the high values were ascribed to denaturation from pepsin treatment of human gamma-globulin in the production of HGG (CSL) (Wells & Penny, 1969). The other four preparations with high values for $Q_{U,\ 1+2}$ (2. RS[1], 7. AR, 11. AB and 12. GT) were all preparations of autologous IgG and in each case the excretion of isotope was higher during the second day than during the first day of their study. It was therefore felt that this higher value for $Q_{U,\ 1+2}$ was a reflection of individual rapid catabolism rather than denaturation.

The results of thirteen turnover studies in twelve patients are summarized in Table 4. The values in normal controls are taken from seven personal and sixty-two published studies which used satisfactory and comparable methods and calculations (Cohen & Freeman, 1960; Cohen, 1963; Birke *et al.*, 1963; Solomon, Waldmann & Fahey, 1963; Andersen, 1964; Waldmann & Schwab, 1965; Ahlinder *et al.*, 1968; Wells, 1969a, b; Wells & Penny, 1969). The study in 4. JH was incomplete as the patient died on the seventh day of his study before equilibrium of the injected IgG had been attained. Full data were analysed from twelve studies in eleven patients, including two studies in patient 2. RS.

The PV was normal in six patients and increased in the other six patients, the increased values occurring in four patients with monoclonal gammopathy, one with cirrhosis (10. RM, 53·6 ml/kg) and one with chronic active hepatitis (12. GT, 51·3 ml/kg). The highest value was seen in the patient with biclonal gammopathy (6. SF, 78·7 ml/kg). The mean PV in monoclonal gammopathy (54·1 ml/kg) was significantly higher than the mean PV in polyclonal gammopathy (44·8 ml/kg) and in normal controls (41·0 ml/kg).

The plasma IgG pool was increased in nine patients (six with monoclonal gammopathy and three with polyclonal gammopathy) and to our surprise, normal in three. The mean plasma IgG pool was significantly increased in both monoclonal and polyclonal gammopathy (1·91 g/kg and 1·10 g/kg respectively) compared to the normal value of 0·50 g/kg. The plasma pool of normal IgG in 2. RS was significantly decreased to 0·13 g/kg while the plasma pool for monoclonal IgG in the same patient was the highest in the present study (2·94 g/kg).

The DR varied from 0·34 to 0·65 with mean values of 0·49 and 0·51 in monoclonal and polyclonal gammopathy respectively (normal mean DR 0·50). Increased plasma localization was noted in one patient with cirrhosis (10. RM, 0·65) and in one patient with chronic active hepatitis who was receiving corticosteroid therapy (12. GT, 0·65).

Total body IgG was increased in the five patients with monoclonal gammopathy, ranging from 2·07 to 4·81 g/kg with a significantly reduced level of normal IgG in 2. RS (0·24 g/kg). The total body IgG pool was significantly increased in one patient with chronic active hepatitis (12. GT, 3.44 g/kg) and ranged from 1·59 to 3·44 g/kg in the six patients with polyclonal gammopathy. The mean value in monoclonal gammopathy (3·69 g/kg) was

significantly higher than the mean value in polyclonal gammopathy (2·07 g/kg) and both were significantly higher than the mean value in normal controls (1·03 g/kg).

The plasma $T\frac{1}{2}$ was normal in one patient with monoclonal gammopathy (5. WM, 19·0 days) and in one with polyclonal gammopathy (8. LN, 15·3 days) and shortened in all other subjects. The mean values for plasma $T\frac{1}{2}$ were 11·3 days and 11·0 days in the two groups.

A normal FTR was observed in two patients with polyclonal gammopathy (8. LN and 10. RM) and an increased FTR in all other studies. The mean FTR was 13·6%/day in both groups.

The synthesis of normal IgG in 2. RS was decreased to 19 mg/kg/day while IgG synthesis rates in all other studies were significantly increased. The mean values of 242 mg/kg/day (range 109–362 mg/kg/day) in monoclonal gammopathy and 148 mg/kg/day (range 93–352 mg/kg/day) in polyclonal gammopathy were respectively 6·7 and 4·1 times the mean IgG synthesis rate in normal controls (36 mg/kg/day).

TABLE 5. Comparison of IgG turnover data in six patients calculated by Matthews' method (1957) and by the urinary clearance method (Campbell *et al.*, 1956)

| Subject | Matthews' method | | | Urinary clearance method | | | Fractional turnover rate |
|---|---|---|---|---|---|---|---|
| | Fractional turnover rate (%/day) | IgG synthesis rate (g/day) | IgG synthesis rate (mg/kg/day) | Fractional turnover rate (%/day) | IgG synthesis rate (g/day) | IgG synthesis rate (mg/kg/day) | Ratio: Matthews / Urinary clearance |
| 1. AJ | 15·5 | 7·85 | 160 | 14·4 | 7·25 | 148 | 1·08 |
| 6. SF | 11·4 | 17·83 | 296 | 10·6 | 16·56 | 276 | 1·08 |
| 7. AR | 17·2 | 7·98 | 112 | 14·3 | 6·60 | 93 | 1·20 |
| 8. LN | 9·6 | 6·11 | 93 | 8·6 | 5·48 | 83 | 1·12 |
| 11. AB | 16·0 | 6·86 | 104 | 13·3 | 5·68 | 86 | 1·20 |
| 12. GT | 15·8 | 24·70 | 352 | 13·3 | 20·79 | 297 | 1·19 |
| Mean | 14·3 | — | 186 | 12·4 | — | 167 | 1·15 |

No significant loss of intact radio-labelled protein was found via the gastrointestinal or urinary tracts in any patient during the turnover study.

Lower values for FTR and IgG synthesis rate were obtained with calculations by the urinary clearance method in six patients (Table 5) with a mean value of 1·15 for the ratio of $K_{12}$ to FTR (urinary clearance). This pattern resembles that reported from other turnover studies, with mean ratios of 1·10 in albumin studies in patients with cirrhosis (Dykes, 1968) and 1·13 and 1·27 in albumin and IgG studies respectively in patients with renal homografts (Wells, 1969b; Wells & Jeremy, 1971). The differences in values for turnover parameters calculated by various methods were examined by Ahlinder *et al.* (1968). They stated that with complete urine collections, these differences indicate a loss of up to 17% of eliminated free isotope by routes other than urine, e.g. saliva and sweat. Subsequent discussion of the present data will therefore refer to values obtained by analysis of the plasma curve (Matthews, 1957) which eliminates this possible error.

## DISCUSSION

The finding of an expanded PV in four of the present six patients with monoclonal IgG

proteins confirms previous reports of hypervolaemia in such patients, both in the presence (Smith, Kochwa & Wasserman, 1965) and absence (Kopp, MacKinny & Wassen, 1969) of hyperviscosity (MacKenzie, Fudenberg & O'Reilly, 1970). The increased PV, together with the high serum level of monoclonal protein produces a markedly increased value for the plasma IgG pool and this subsequently affects metabolism of the protein.

The metabolism of IgG in patients with monoclonal gammopathy has been reviewed by Waldmann & Strober (1969) but detailed comparisons of reported studies are difficult since (a) early studies were performed with denatured preparations, (b) the selection of patients is unlikely to yield closely similar groups for investigation, (c) various proteins have been labelled and injected, and (d) different methods have been employed to analyse the data. Reported studies have been performed with normal IgG from the patient or from pooled normal serum (Lippincott *et al.*, 1960; Solomon *et al.*, 1963; Andersen, 1964), autologous monoclonal IgG (Berson & Yalow, 1957; Gabuzda, 1962; Spiegelberg, Fishkin & Grey, 1968; Morell, Terry & Waldmann, 1970) or with both (Cohen, 1963; Alper, Freeman & Waldenström, 1963; Birke *et al.*, 1963).

The present data clearly support the view that the high serum levels of monoclonal IgG in such patients are due primarily to an increased synthesis rate and not to prolonged survival of the IgG with reduced catabolism (Waldmann & Strober, 1969). Most of the studies cited above describe a shortened plasma $T\frac{1}{2}$ and an increased FTR in patients with monoclonal gammopathy. Drivsholm (1961) observed in his study with *in vivo* $^{14}C$-labelling that the half-life of monoclonal protein decreased with the duration of the disease and Andersen (1964) suggested an increased FTR indicated a poor prognosis. Although the longest plasma $T\frac{1}{2}$ of a monoclonal protein in the present study occurred in the patient with benign gammopathy (5. WM, 19·0 days), his FTR was significantly increased at 12·1%/day; further studies in such patients are obviously required to clarify this point.

Two recent reports described the results of turnover studies with $\gamma$G monoclonal proteins of known subclass and revealed that $\gamma G_3$ subclass proteins were catabolized at a significantly faster rate than proteins of subclasses $\gamma G_1$, $\gamma G_2$, and $\gamma G_4$ (Spiegelberg *et al.*, 1968; Morell *et al.*, 1970). The fact that rapid catabolism was an inherent feature of the protein and not merely an expression of denaturation during isolation and labelling was verified by demonstrating similar values for plasma $T\frac{1}{2}$ with the protein labelled with $^{131}I$ and biosynthetically labelled *in vivo* with $^{14}C$ (Morell *et al.*, 1970).

A decreased serum level of normal IgG is a frequent finding in monoclonal gammopathy and is a combination (as in patient 2. RS) of an increased PV, decreased synthesis and increased FTR (Andersen, 1964; Waldmann & Strober, 1969). Solomon *et al.* (1963) suggested that these factors differ in their effects with different monoclonal proteins; decreased synthesis of normal IgG being the main factor with $\gamma$A monoclonal proteins and B-J light chain disease, and increased catabolism being the main factor with $\gamma$G monoclonal proteins. The mechanisms of these changes are unknown.

Serum immunoglobulin changes in liver disease include increases in IgG, IgA and especially IgM in infective hepatitis (Wollheim, 1968), and increases in one or more immunoglobulin in different combinations in cirrhosis (Feizi, 1968). Patients 7–12 in the present study give similar findings. However no consistent pattern diagnostic for a particular disease has been confirmed and metabolic studies are required to analyse these abnormalities. IgG turnover studies have been performed in many patients with polyclonal gammopathy including fifty-five in auto-immune or connective tissue disorders (Cohen, 1963; Andersen,

1964; Wochner, 1970) and over ninety with liver cirrhosis (Eisenmenger & Slater, 1953; Havens *et al.*, 1954; Cohen, 1963; Birke *et al.*, 1963; Andersen, 1964).

In their studies in cirrhosis, Eisenmenger & Slater (1953) and Havens *et al.*, (1954) found markedly shortened values for plasma $T\frac{1}{2}$ in 70% of patients and Cohen (1963) and Birke *et al.* (1963) found significantly increased values for FTR in 71% of their patients. Andersen (1964) felt that denaturation of the preparation had contributed to the rapid catabolism described by Havens *et al.* (1954) and presented his own data from twenty patients with cirrhosis. He found an increased FTR in less than half his patients but an increased IgG synthesis rate in all but two. The presence or absence of oedema or ascites did not significantly alter the results. Both patients with cirrhosis in the present study (9. JL and 10. RM) had a shortened plasma $T\frac{1}{2}$ and an increased IgG synthesis rate but one had a normal and the other an increased FTR.

One previously reported IgG turnover study in acute infective hepatitis (Andersen, 1964) described an increased PV and plasma IgG pool, normal DR and FTR, and an increased IgG synthesis rate. A similar pattern of increased synthesis and turnover of an increased plasma IgG pool at a normal rate was observed in 8. LN in the present study. A different pattern of increased synthesis and rapid turnover of a normal IgG pool with short plasma $T\frac{1}{2}$ was seen in 7. AR (and also in a study of a patient BI who had infective hepatitis and a serum IgG level of 1100 mg/100 ml). These patients with infective hepatitis had similar clinical features and there are no obvious reasons for differences in their metabolic studies.

There are no published studies of IgG metabolism in chronic active hepatitis although Feizi (1968) refers to a preliminary study in an addendum. Both patients in the present study had an increased IgG synthesis rate and rapid catabolism with an increased FTR and shortened plasma $T\frac{1}{2}$. Treatment with corticosteroids would contribute to the increased FTR and also the high DR in 12. GT but the reason for the lower DR in subject 11. AB is unknown. The aetiology of chronic active hepatitis is unproven but auto-immune mechanisms have been consistently incriminated in view of the association of this disease with other auto-immune diseases; the consistent polyclonal hypergamma-globulinaemia; the frequent findings of serum auto-antibodies; the histological evidence in the liver of infiltration with lymphocytes and plasma cells; and the apparent clinical response to immuno-suppressive therapy (Mistilis & Blackburn, 1970). Cohen (1963) found increased IgG synthesis and catabolism in turnover studies in two patients with SLE while Andersen (1964) found increased IgG synthesis in eight of eleven patients and an increased FTR in seven of eleven patients with auto-immune diseases (including seven with RA). Wochner (1970) described detailed studies in forty-two patients with auto-immune diseases (including sixteen with SLE and five with RA). He stressed the presence of hypercatabolism of normal IgG in all patients and stated it was a host defect and could not be ascribed to the protein. There is no obvious explanation for this hypercatabolism in auto-immune disease.

The similar patterns of increased IgG synthesis and increased catabolism in monoclonal gammopathy and in liver disease are interesting in view of the suggestion that a polyclonal increase in IgG in liver disease can lead to the emergence of an IgG monoclonal protein (Osserman, 1968). However, other groups have not found an increased incidence of monoclonal proteins among patients with chronic liver disease (Englisova *et al.*, 1968; Ellman *et al.*, 1969).

The present data provide further confirmation of the 'concentration-catabolism' relationship which states that the serum IgG level is an important factor in the control of the rates of

catabolism of IgG (Waldmann & Strober, 1969). The relationship indicates that the survival of IgG is generally shortened with higher serum levels of IgG and other immunoglobulins or proteins do not have any effect. The pattern is seen with both normal and monoclonal IgG (as in the present study) and applies to all subclasses of IgG (Morell *et al.*, 1970). Studies of the IgG subclasses in mice have demonstrated similar results (Fahey & Sell, 1965). Further, the plasma $T\frac{1}{2}$ of IgG is lengthened in hypogamma-globulinaemia (Waldmann & Schwab, 1965; Stiehm *et al.*, 1966).

Despite this extensive knowledge of IgG metabolism the actual mechanisms and anatomical sites for metabolic degradation of IgG molecules remain unknown. A system of receptors for IgG with a protective function was postulated by Brambell, Hemmings & Morris (1964), unbound IgG molecules being available for degradation by proteolytic enzymes within some form of vacuole, perhaps in the gastro-intestinal tract. Although experiments in dogs (Andersen, Glenert & Wallevik, 1963) and mice (Bazin & Malet, 1969) have demonstrated the importance of the gastro-intestinal tract in immunoglobulin metabolism the postulated receptors have not been identified, and further studies are clearly warranted.

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