IgM turnover in Crohn's disease¹

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SUMMARY Simultaneous turnover studies with radioiodine-labelled IgM and IgG were made in 12 patients with Crohn's disease. Intestinal protein loss was estimated by means of ⁵⁹Fe-labelled iron dextran.

The serum levels of IgM, IgG, and IgA were normal in most cases. The catabolic rate of IgM was increased in all but one case. A positive correlation was present between the catabolic rate and serum concentration of IgM, an observation which, so far, has been made only in Crohn's disease.

The synthetic rate of IgM was raised or high normal in four cases with an intraabdominal abscess. It was normal in the remaining cases. A strong positive correlation was found between the synthetic rates of IgM and IgG.

The size of the protein loss was unrelated to the raised catabolic rates of IgM and IgG.

Faecal radioiodine excretion from labelled IgM and IgG bore no relation to faecal ⁵⁹Fe excretion, nor did it indicate the site of the intestinal lesion. However, a close correlation was observed between faecal excretion of the labels from IgM and IgG.

Immunological mechanisms may be involved as a primary cause of Crohn's disease, the aetiology of which is still unknown. Once established, the disease is commonly associated with abnormal immunological phenomena.

Granuloma formation is frequently encountered in the intestinal lesions. It is generally held as a token of a state of cellular hypersensitivity. However, attempts to demonstrate directly in white blood cells a tissue-specific cellular hypersensitivity to colon or small intestine antigen have failed (Bendixen, 1967). Furthermore, patients with Crohn's disease have a depressed delayed hypersensitivity reaction to dinitrochlorobenzene (Jones, Housley, Ashurst, and Hawkins, 1969).

IgG metabolism is often profoundly altered. Whereas the serum concentration is normal in the majority of cases, the synthetic and catabolic

rate is markedly increased (Bendixen, Jarnum, Søltoft, Westergaard, Weeke, and Yssing, 1968; Weeke and Bendixen, 1969).

For theoretical reasons the turnover of IgM in Crohn's disease is of considerable interest. If it is altered similarly to that of IgG it would imply that both IgG- and IgM-producing plasma cells are stimulated in this condition.

In the present study of 12 patients with Crohn's disease simultaneous studies were made of the size of abnormal protein loss by means of ⁵⁹Fe-labelled iron dextran and IgG and IgM degradation. The results indicate that IgM metabolism is markedly altered and that intestinal protein loss *per se* is of minor importance for the alteration.

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Patient No.	Age (yr) and Sex	Height (cm) Weight (kg)	Site of Lesion	Duration of Disease (yr)	Hb (g/100 ml)	ESR (mm/hr)	Blood in Stools (Benzidine)
1	26 M	167 54	Terminal ileum	10	13.2	23	0
2	24 M	168 58	—	5	13.8	6	0
3	20 M	185 49	—	2	11.9	45	+++
4	19 F	170 41	—	3	11.1	40	+0
5	37 F	159 42	—	9	10.2	20	+++
6	49 M	170 58	Ileum + colon	6	12.8	7	00+
7	26 F	171 45	—	5	14.3	30	0
8	20 M	181 46	—	1	12.9	37	0
9	27 F	162 41	—	2	9.5	75	0+
10	38 F	163 42	—	11	11.0	33	0
11	40 F	158 55	Colon	6	10.2	10	+0
12	16 F	156 37	—	2	8.5	13	++

Table I Localization of the lesion and some laboratory data in 12 cases of Crohn's disease

Case Material

Twelve patients (seven females and five males), aged from 16 to 49 years were studied (Table I). Intestinal resection had been performed in six cases (nos. 4, 6, 8, 10, 11, 12) and the diagnosis confirmed by the pathoanatomical findings. In one of these (no. 10) a sigmoidostomy had been established.

In the remaining six cases the diagnosis was based on sigmoidoscopy, radiography, and clinical symptoms. In one case (no. 1) an exploratory laparotomy had been performed.

Five patients suffered from ileocolitis, five from terminal ileitis, and two from regional colitis.

In four patients (nos. 4, 9, 10, 12) the disease was highly active (profuse diarrhoea, necessitating partial intravenous feeding). In the remaining eight cases the disease was moderately active.

Four patients (nos. 3, 4, 6, 9) had developed an abscess in the right iliac fossa, and in one of these patients (no. 9) the abscess was associated with a cutaneous fistula.

Three patients (nos. 7, 9, 10) had elevated alkaline phosphatase levels. In the remaining nine cases the hepatic function appeared normal according to the findings for alkaline phosphatases, serum bilirubin, and glutaminicpyruvic transaminase.

Four patients (nos. 3, 7, 11, 12) were treated with salicylazo-sulphapyridine. No blood was given and no patients received steroids during the investigation.

Methods

IgM from normal serum (used in case nos. 9 and

11) or from serum containing an IgM M component was isolated by way of Sepharose 4B gel filtration (Jensen, 1970) and IgG by means of DEAE cellulose chromatography (Peterson and Sober, 1960). The radioiodine labelling of the immunoglobulins was performed according to McFarlane's ICI method (1958). On average the preparations contained one iodine atom per protein molecule, and more than 99.5% of the radioactivity was precipitable by trichloroacetic acid. Purity of the labelled preparations was checked by means of paper electrophoresis and immunoelectrophoresis (Jensen, 1970).

⁵⁹Fe-labelled iron dextran was used for the quantitative estimation of gastrointestinal protein loss (Jarnum, Westergaard, Yssing, and Jensen, 1968).

Serum albumin and immunoglobulin concentrations were determined by Laurell electrophoresis (Weeke, 1968).

EXPERIMENTAL PROCEDURE

Throughout the study potassium iodide, 50 mg daily, was given orally to prevent thyroid uptake of radioactive iodide.

A weighed amount of the labelled IgM and IgG (about 0.15 μ Ci of ¹²⁵I and 0.40 μ Ci of ¹³¹I per kg body weight) and ⁵⁹Fe-labelled iron dextran (2-3 μ Ci) was given intravenously. Blood was withdrawn into heparinized tubes 10 minutes after the injection and at daily intervals for a period of two to three weeks. In addition total body activity was recorded by means of whole body counting (Jarnum *et al*, 1968).

Stools were collected until they became red after the oral administration of 1 g of carmine 96 hours after the injection. Urine was collected in 24-hour specimens for eight to 10 days.

Fig. 1 Summary of clinical findings in 12 cases of Crohn's disease.

Fig. 2 Fractional catabolic rate of IgG and IgM.

Fig. 4 Rates of synthesis of IgM and IgG in Crohn's disease.

Fig. 3 Fractional catabolic rate of IgG and IgM concentration in Crohn's disease.

The amount of radioactivity in 3 ml samples of plasma and urine was measured in a three-channel gamma spectrometer (Autogammaspectrometer, Packard) (Jarnum *et al*, 1968). Weighed homogenized 24-hour stool samples

were counted in an Armac 2-channel gamma spectrometer (Packard). Serum protein was determined in all plasma samples (biuret method) and the values were used for estimation of the specific activity (plasma

Patient No.	Serum Concentration (g per l)				Type of IgM Used in Study M (monoclonal) or P (polyclonal)	Fractional Catabolic Rate (% per day)	
	Albumin ^a (37.7-55.5)	IgA (0.74-3.06)	IgM (0.23-1.33)	IgG (7.2-15.1)		IgM (8-15)	IgG (4.0-9.6)
1	38.4	0.02	0.40	15.5	M	19.9	10.5
2	40.4	0.67	0.14	5.7	M	15.7	7.3
3	26.4	3.55	0.86	12.2	M	28.9	13.1
4	23.0	1.20	0.51	8.7	M	25.6	11.8
5	22.5	1.07	0.29	13.5	M	19.5	14.3
6	39.0	0.52	0.74	7.6	M	18.5	12.1
7	41.4	3.41	0.61	16.1	M	19.1	15.0
8	34.6	2.16	0.67	15.5	M	17.7	17.1
9	22.3	0.69	0.91	13.3	P	36.3	20.4
10	35.3	2.40	0.38	11.1	M	22.8	14.4
11	39.7	0.96	0.63	12.8	P	14.6	16.1
12	26.5	0.67	0.29	8.3	M	21.8	18.5

Table II Summary of results

^aNormal range

radioactivity in counts per minute per unit of serum protein) which was used in calculations.

Steady state condition was checked by daily determination of serum protein and body weight and biweekly haemoglobin estimation.

CALCULATIONS

The fractional catabolic rate of IgG was calculated by mathematical analysis of the plasma radioactivity recording after Nosslin's method (1966) and that of IgM by a method according to which the fractional catabolic rate (percentage of intravascular mass per day) is equal to the slope constant of the 'whole body' and the plasma curves multiplied by the ratio between their intercepts (Berson, Yalow, Schreiber, and Post, 1953). The faecal excretion of ⁵⁹Fe was calculated as described by Jarnum *et al* (1968).

Results

The results are summarized in Table II.

No relation existed between the concentrations of the various immunoglobulins (Figure 1). Nor were they related to serum albumin concentration or the size of the intestinal protein loss estimated by means of the ⁵⁹Fe-iron dextran clearance (Figure 1). One patient (case 1) had agamma-A-globulinaemia and one (case 2) had diffuse hypogammaglobulinaemia. The remaining patients had normal serum IgM concentration. Serum IgG was slightly raised in three (cases 1, 7, 8), low in one (case 2), and normal in the remaining patients.

The fractional catabolic rate of IgM was increased in all but one (case 11), and all but one (case 2) had an accelerated IgG catabolism (Figure 2).

No correlation existed between the catabolic rate of the two proteins ($r = 0.42$, $0.10 > P > 0.05$, Figure 2). However, when only the four patients with abscess (Fig. 1) were considered,

a positive correlation was present ($r = 0.85$, $0.05 > P > 0.02$).

As far as IgM is concerned a slight positive correlation was noticed between serum concentration and catabolic rate ($r = 0.53$, $0.05 > P > 0.02$, Figure 3). The same correlation, although less significant, held true for IgG.

The synthetic rate of IgM (Fig. 4) was markedly raised in two patients both with an abscess (case 3 and 9). In another three patients (cases 8, 6, 4), two of whom had an abscess (nos. 6 and 4), it was high normal. In the hypogammaglobulinaemic subject (case 2) it was low normal, and in the remaining patients it was normal.

The synthetic rate of IgG was elevated in everyone except the patient with hypogammaglobulinaemia.

The correlation between the synthetic rate of the two immunoglobulins in the case material as a whole was slightly positive ($r = 0.55$, $0.05 > P > 0.02$). However, a strong positive correlation existed in the four patients with an abscess ($r = 0.99$, $P < 0.001$, Fig. 4), the equation of regression being

$$y = 0.12x + 0.97 \quad (1)$$

where y = the synthetic rate of IgM in mg per kg per day and x = the synthetic rate of IgG in mg per kg per day.

The remaining eight patients without an abscess also showed a highly significant positive correlation ($r = 0.86$, $P < 0.01$), equation of regression

$$y = 0.04x + 0.36 \quad (2)$$

The presence of abnormal intestinal protein loss was demonstrated in everyone by means of ⁵⁹Fe-iron dextran. However, the intestinal protein loss was moderate (Figure 1). The faecal ⁵⁹Fe content ranged from 1.8 to 6.4% of the injected dose and the faecal ⁵⁹Fe clearance from 2.1 to 8.3% of the intravascular ⁵⁹Fe-iron dextran pool per day.

The stool excretion of radioiodine label from IgG was increased in most patients, from 0.2 to 7.9% of the injected dose over a period of four to five days after the injection. It was not deter-

Rate of Synthesis (mg per kg per day)		Intravascular Fraction (%)		Q_F^{-1}		Faecal ^{59}Fe Imferon	
IgM (0.8-8)	IgG (10-49)	IgM (51.0-96.5)	IgG (44.2-71.9)	IgM	IgG	^{59}Fe Imferon	Clearance ² (<0.8)
4.0	86	60	55	1.2	0.8	2.3	2.6
1.0	19	82	48	0.6	0.2	1.8	2.1
15.1	110	69	59	4.0	3.7	2.9	2.9
6.9	54	78	72	2.5	5.3	3.9	7.1
3.4	116	79	71	2.1	—	5.6	7.8
6.8	49	70	59	6.5	6.0	6.4	5.2
5.2	111	72	64	8.2	7.2	3.6	4.6
7.8	175	72	57	0.4	0.4	2.8	3.5
19.3	159	68	76	1.2	2.2	2.7	2.5
3.7	69	62	50	14.4	9.7	2.8	4.9
4.6	102	79	72	3.2	4.5	3.5	3.4
3.7	100	71	73	—	—	6.0	8.3

Table II Summary of results continued

¹Faecal excretion, percentage of injected dose in five days

²Daily faecal isotope excretion as percentage of the intravascular mass

mined by the size of the intestinal protein loss since it was insignificantly correlated with the ^{59}Fe -excretion ($r = 0.48$, $p > 0.05$, Fig. 5), nor was it dependent on the site of the lesion (colon or ileum, Tables I and II).

Faecal isotope excretion from labelled IgM was also compared with the excretion of ^{59}Fe . The correlation was insignificant. However, the correlation between faecal isotope excretion from labelled IgM and IgG was highly significant ($r = 0.92$, $p < 0.001$, Fig. 6), the equation of regression being

$$y = 1.29x - 0.9 \quad (3)$$

where y = faecal radioiodine from IgM and x = faecal radioiodine from IgG.

Discussion

A low total serum protein concentration is a regular finding in Crohn's disease. It is due to a decreased albumin concentration caused mainly by the associated abnormal intestinal plasma protein loss (Steinfeld, Davidson, and Gordon, 1957; Bendixen *et al.*, 1968). In contrast, serum immunoglobulins are rarely depressed. Serum IgG may be elevated or, probably in most cases, normal (Bendixen *et al.*, 1968), a finding which also held true as regards IgA and IgM in the present case material (Figure 1). However, the presence of a protein in normal concentration in serum does not preclude that profound alterations of its metabolism have occurred. Only dynamic studies with the labelled protein can reveal it.

In the present investigation IgM and IgG labelled *in vitro* with radioactive iodine were used for turnover studies. A prerequisite to this method is that the separation and labelling procedures do not influence the metabolic behaviour of the labelled product. Ample evidence has been furnished that labelled IgG with pooled, normal serum as starting material yields true turnover

Fig. 5 Rates of synthesis of IgM.

Fig. 6 Faecal excretion of radioiodine from labelled IgG and IgM.

data on this class of immunoglobulins (Andersen, 1964).

As far as IgM is concerned, difficulties in separation procedures are considerable. They are partly overcome when serum with a high content of a monoclonal IgM is used as starting material. We used labelled polyclonal IgM from normal serum in only two patients (cases 9 and 11, Table II) and monoclonal IgM for the remaining 10 patients. Theoretically, the degradation of monoclonal IgM may differ from that of the physiological polyclonal protein. However, the difference seems to be slight and, maybe, recognizable only in a brief period of a few days after the injection (Birke, Norberg, Olhagen, and Plantin, 1967).

Turnover studies in 28 control subjects with the IgM preparations used in the present report have been published elsewhere (Jensen, 1969). They showed an identical degradation rate of monoclonal and polyclonal IgM studied simultaneously in five subjects. Therefore we considered it justified to apply monoclonal IgM in the present series. On the other hand, the control studies almost invariably showed an initial degradation rate (estimated from urinary radioiodine excretion) which was slightly higher than in the succeeding period (from day 3 or 4 after the injection) from which time it remained constant from day to day. For this reason we used the modified formula (see Methods) which corrects for an 'abnormal' high initial degradation.

Several reports indicate that the catabolism of IgM is independent of its serum concentration (Barth, Wochner, Waldmann, and Fahey, 1964; Birke *et al*, 1967; Jensen, 1970). Over a wide range of different serum levels the fractional catabolic rate seems to be fairly constant; in the more recent reports about 10% of the intravascular mass per day (Birke *et al*, 1967; Jensen, 1969).

The results obtained in the present study indicate that the metabolism of IgM in Crohn's disease deviates from this pattern. Just as it was the case with IgG in a previous report (Bendixen *et al*, 1968), a positive correlation was found between serum concentration and catabolic rate, although significant only to the 5% level. A trend towards a covariation between the catabolic rates of IgM and IgG was also noticed. It was not statistically significant, but it suggested that Crohn's disease affects the degradation of these two immunoglobulins in a similar way.

The most conspicuous finding concerned the rate of synthesis. As visualized in Fig. 5 a marked increase of IgM synthesis was observed in two patients with an abscess and a high normal IgM synthesis in two other patients with abscess. In the remaining patients without abscess IgM synthesis was normal. In contrast, the synthesis rate of IgG was raised in all but one and the increase was unrelated to the presence or absence

of an abscess. Thus it appears as if the presence of an abscess has a pronounced stimulatory effect on IgM synthesis.

Abnormal intestinal protein loss was detected by means of ^{59}Fe -labelled iron dextran in every one of the present cases (Fig. 1), but it was moderate and it bore no relation to the catabolic rates of the two immunoglobulins studied. A positive correlation has been observed only in the case of albumin (Bendixen *et al*, 1968). The excretion of radioiodine in stools from labelled IgG and IgM was elevated in most cases. However, the amount of faecal radioiodine was not dependent on the size of abnormal protein loss, nor did it indicate the site of the intestinal lesion.

Evidence has already been presented (Søltøft and Jarnum, 1969) that IgG has a higher resistance to proteolytic enzymes in the intestinal lumen in Crohn's disease than has albumin. Since a significant positive correlation was noticed between faecal radioiodine from IgG and IgM (Fig. 7), we conclude that IgM, like IgG, in conditions *in vivo* possesses a high resistance to intestinal proteinases.

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