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The Immunoglobulins in Hodgkin's Disease

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Summary. Immunoglobulins have been measured in fifty patients with Hodgkin's disease. Levels of γG were slightly raised as compared with a control population, but γA tended to be low and γM was generally at or below our lower limit of normal. These findings are discussed in relation to the duration of the disease, its clinical staging, and the possible effects of radiotherapy and chemotherapy.

It is suggested that the reduced γM levels are due to decreased synthesis in the reticulo-endothelial system rather than to increased catabolism. Reference is made to the depression in delayed-type hypersensitivity found early in Hodgkin's disease and this and reduction in γM levels may both reflect the same derangement in lymphoid cells.

The detection of α_3-globulin in the sera of 62 per cent of the patients is referred to and its association with other neoplastic reticuloses is emphasized.

INTRODUCTION

The immunological status of patients with Hodgkin's disease is an intriguing subject that has attracted much research work in recent years. It has been well reviewed by Aisenberg (1964a, 1966). There is general agreement that delayed-type hypersensitivity is impaired early in Hodgkin's disease and the degree of impairment is said to correlate to a certain extent with the clinical course of the disease. Chase (1966) has made a detailed comparison of the delayed-type hypersensitivity reaction in Hodgkin's disease and sarcoidosis and concludes that the anergy seen in these two conditions is basically similar and to be clearly distinguished from the 'cachectic anergy' seen in the terminal stages of many fatal diseases.

Homograft reactions have been studied in Hodgkin's disease. Out of a total of thirty-three patients with Hodgkin's disease grafted by various workers (Green, Inkelas and Allen, 1960; Kelly, Lamb, Varco and Good, 1960; Miller, Lizardo and Sanderman, 1961) delayed homograft rejection has been observed in twenty. Beilby, Cade, Jelliffe, Parkin and Stewart (1960) have reported prolonged survival of a bone marrow graft in a patient with Hodgkin's disease.

In contrast to the early loss of delayed-type hypersensitivity, it appears generally agreed that the capacity for antibody formation is relatively well preserved. Normal amounts of antibody are produced in response to mumps and tularaemia, and to injected blood group substances. In addition, good antibody response has been shown (Aisenberg and Leskowitz, 1963) to pneumococcal polysaccharide types I and II in thirteen out of nineteen Hodgkin's patients whose depression of delayed type hypersensitivity had previously been established by skin testing with dinitrochlorobenzene.

With the qualifications that he sets out, Aisenberg (1964b) concludes that antibody formation to most antigens is normal in Hodgkin's disease. As a further guide to the antibody forming status, we have estimated serum immunoglobulin levels in fifty patients with proven Hodgkin's disease. We have at the same time examined the sera for excess α_3-globulin as described by Sunderman (1964).

METHODS

Patients

Most of the fifty patients investigated were seen in routine follow-up in one out-patient clinic specializing in the treatment of malignant lymphomas. At the time the blood sample was taken, approximate clinical staging was attempted according to the categories laid down by Peters (1950) and modified by Kaplan (Karnofsky, 1966).

Stage 0: No detectable disease following excisional lymph node biopsy.

Stage I: Disease localized to a single node and continuous structures.

Stage II: Disease limited by the diaphragm to the upper or lower half of the body, but at more than a single site.

Stage III: Disease above and below the diaphragm.

Stage IV: Disease demonstrated in any one of the following tissues: bone marrow, lung, skin, gastro-intestinal tract or bone.

Stages II and III are subdivided into A and B (with no symptoms and with symptoms of generalized disease, respectively).

To have made use of the assessment of clinical stage made at the time of diagnosis would have been more elegant, for accurate clinical staging was not always possible in patients attending the out-patient department. Involvement of abdominal lymph nodes, for example, in an otherwise Stage II patient could not be confirmed without recourse to lymphangiography. Nevertheless, an attempt to assess clinical stage at the time of the immunoglobulin estimation seemed more appropriate.

Diagnosis was confirmed by lymph node biopsy in every case; the majority of patients fitted the histological diagnosis of Hodgkin's granuloma; two had been classified by the examining pathologist as Hodgkin's paragranuloma (Jackson and Parker, 1947) and one was termed 'benign Hodgkin's'.

The duration of illness varied widely: four patients had been diagnosed ten or more years previously and one of these had had symptoms for more than 18 years. We decided to calculate the duration of illness from the date of first symptoms considered to be definitely attributable to Hodgkin's disease irrespective of the actual date of subsequent diagnostic biopsy. Twelve patients had a duration of illness of 1 year or less. Only seven of the patients had had no radiotherapy and two had been irradiated on as many as five occasions. Nineteen of the patients had had chemotherapy, mostly after courses of radiotherapy, and the usual drug was nitrogen mustard. Cyclophosphamide, chlorambucil and vinblastine were also used. Four patients had been treated with corticosteroids, but none had had splenectomy.

Serum studies

Blood samples were usually drawn early in the day and serum was separated at 37° within 1 or 2 hours in order that no cold agglutinin or cryoprotein should be lost. Serum

total protein was measured by a biuret method using bovine albumin (Armour) as a reference standard. Scanning the dye uptake after cellulose acetate electrophoresis (Hobbs, 1965) enabled estimation of the fraction cathodal to the β_2-position to an accuracy of $\pm 0{\cdot}2$ g/100 ml. This has been designated the strip γ-globulin. It serves as a check on the total of the immunological measurements of immunoglobulins, for less than 0·2 g/100 ml of these is likely to have a mobility faster than β_2.

α_3-Globulin was sought in the interval between α_2 and the crystal clear β_1 band on cellulose acetate. This interval normally contains little protein and for this reason the application is routinely made five-eighths along the strip towards the anode. The forward movement to the anode is countered by the endosmotic flow to the cathode so that albumin, α_1- and α_2-globulins make a net movement towards the anode and β_1 and γ make a net movement towards the cathode. Thus any scratches or denatured protein remain at the application site now in the α_3-position. Furthermore, any haemoglobin freed by *in vitro* haemolysis of the sample is initially bound to haptoglobins and changes their mobility so as to end up close to α_3. In fact, α_3-globulin, on cellulose acetate is most often an artefact. By using fresh non-haemolysed sera, however, and by checking that no scratch artefacts have occurred, it becomes possible to identify any genuine excess of protein in the α_3 position (Fig. 1); α_3-globulin has thus been recorded in Table 1 as follows:

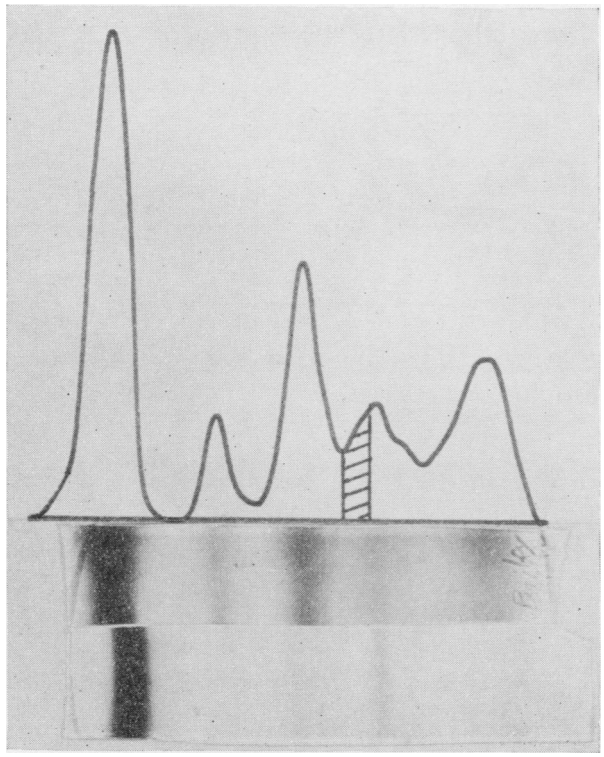

FIG. 1. Fresh serum from a patient with Hodgkin's disease after electrophoresis on cellulose acetate compared with a normal serum underneath. The raised α_1, α_2 and slightly raised γ-globulins are well recognized. The shaded portion of the scan, just in front of β_1, shows α_3-globulin which is not normally seen.

TABLE 1

Patient No.	Sex	Age	Date	Serum globulins (mg/100 ml) γG	γA	γM	γD	γ-Globulin of strip (g./100 ml)	α_3	Disease duration (years)	Radiotherapy: Treatments (No.)	Radiotherapy: Time since last course (years)	Chemotherapy: each symbol* represents one course	Stage and clinical details
1	F	31	24.6.66	1300	135	120	<1	1·5	+	18·4	1	7	–	0–I Paragranuloma
2	M	35	1.6.66	420	110	15	<1	0·5	0	14·4	2	1·8	–	0 'Benign Hodgkin's'
3	M	41	31.5.65	1200	800	200	5	2·1	+++	11·5	4	0	M, M	III A/B
			16.2.66	1300	270	50	2	1·4	+++					
			10.6.66	1600	280	40	<1	1·6	+++					
			1.7.66	1400	255	30	<1	1·5	+++					
			26.8.66	1350	240	10	<1	1·4	+++					
4	F	29	8.7.66	1450	210	55	<1	1·6	0	11·0	2	6	M, N, Ch, Cy	0–I
5	M	35	4.7.66	1700	130	40	<1	1·9	+	9·9	4	0·10	–	IIIA–IV
6	M	47	15.8.66	750	335	35	<1	1·0	+	9·3	2	1·2	M	IIIB–IV
7	F	31	8.7.66	1200	165	70	<1	1·2	+	8·9	5	2·0	M, Ch, Cy	IV
8	M	51	8.7.66	1400	55	80	<1	1·4	++	8·9	2	6·0	–	I
9	M	60	25.7.66	1000	115	80	<1	1·1	0	8·6	1	6·0	–	IIIB
10	F	26	23.6.66	800	295	75	<1	1·2	0	7·6	5	1·2	Ch	IV
11	M	35	1.7.66	750	75	15	<1	0·9	0	6·7	2	3·2	M, Cy	IIB–IIIB
12	F	30	4.7.66	800	110	30	25	0·9	0	6·2	2	3·5	M, Ch, V	IV Steroids
13	F	28	2.8.66	900	160	60	<1	1·0	0	6·2	3	0	M, Cy	II A/B
14	F	27	18.8.66	1050	110	35	<1	1·0	++	6·0	2	5	Cy, Ch, V	IV
15	F	63	27.11.64	1000	560	50	2	1·5	+++	5·7	1	4·2	M	*Died* 2 months later
			3.2.65	1200	640	10	<1	1·5	+++					
			2.3.65	900	280	10	<1	1·0	+++					
16	F	65	7.9.66	300	35	40	<1	0·4	+	5·5	1	1·2	–	II–IIIB
17	F	44	31.5.65	1300	160	20	<1	1·5	++	5·1	2	1·8	M, M, Cy	*Died* 5 months later
			8.10.65	1100	140	10	<1	1·2	++					
18	M	28	19.8.66	1200	210	115	2	1·3	0	4·11	1	4·7	–	0–I
19	M	29	12.8.66	1250	195	5	<1	1·2	+	4·6	1	1·9	Cy	IIIB
20	F	36	21.10.66	1600	370	90	<1	1·7	0 0	3·11	3	0	–	II
21	F	56	21.10.66	1000	600	70	2	1·6	+	3·9	1	3·0	–	0

22	M	60	20.6.66	1100	450	30	<1	1·5	+	3·8	1	3·1	–	I–II
23	F	37	12.8.66	1250	400	95	4	1·6	++	3·8	1	3·1	–	0–I Paragranuloma
24	M	54	24.6.66	465	180	10	<1	0·6	+	3·6	2	1·5	–	IIIB–IV
			29.7.66	450	160	10	<1	0·5	+					
25	F	18	10.6.66	1150	170	90	<1	1·2	++	3·0	1	2·5	–	II–IIIB
26	F	39	10.6.66	1150	235	135	2	1·3	0	3·0	3	0·2	–	III–IV
27	M	18	24.6.66	800	180	25	25	1·1	+	2·7	2	0·5	–	I
			26.8.66	800	170	20	25	0·9	+					
28	M	51	19.8.66	900	105	200	<1	1·0	++	2·6	2	0·4	–	III–IV Steroids
29	F	45	22.7.66	850	145	80	<1	1·0	++	2·5	2	1·3	–	I Steroids
30	M	22	10.6.66	1300	170	155	15	1·4	0	2·5	1	2·2	–	0–I
31	M	67	26.8.66	1050	190	160	<1	1·3	0	2·4	1	2·0	–	0–I
32	M	47	1.6.67	500	80	20	<1	0·6	0	2·1	0	–	–	IIA
			24.7.66	450	80	35	<1	0·5	0					
33	M	19	21.10.66	1350	230	40	<1	1·5	+++	1·1	1	1·3	–	0
34	M	35	1.7.66	80	110	40	<1	1·1	++	1·9	1	0·4	Ch, Cy	IIB–III 'Uncommon form of Hodgkin's'
35	M	32	8.7.66	1500	410	50	3	2·0	+	1·5	2	0·1	–	I
36	F	42	29.7.66	1500	270	85	<1	1·5	+	1·4	0		M	IIIB
37	M	61	26.7.66	850	195	35	<1	0·9	0	1·1	2	0	–	I
38	M	69	11.2.66	800	160	45	<1	0·9	+	1·1	2	M	M, Cy	IIB *Died* 5 days later
39	F	79	20.6.66	850	190	55	<1	1·0	++	1·0	0		–	IIIA
40	M	37	7.3.66	350	50	10	5	0·3	+	1·0	1	0·1	Cy, M	Crohn's disease, colectomy, hepatitis, steroids. *Died*
41	F	60	31.3.64	1200	190	35	<1	1·1	+++	0·1	1	0·6	–	IV *Died* 3 days later
42	M	54	8.7.66	1600	165	45	<1	1·6	+	0·1	2	0·1	M	IIIA
43	M	28	31.8.66	900	65	65	<1	1·0	+	0·9	1	0	–	IIA
44	M	25	8.7.66	1800	220	75	<1	2·0	0	0·9	1	0·3	–	IV
45	M	59	30.8.66	950	210	90	<1	0·9	0	0·8	0	0	–	I–II
46	M	27	6.6.66	1100	385	70	6	1·3	+	0·7	0	0	–	IIIB
47	F	83	23.8.66	1400	210	70	<1	1·4	+	0·6	0	0	–	IIIA
			9.9.66	1450	180	60	<1	1·4	+					
48	M	12	26.7.66	1200	65	40	8	1·4	0	0·5	1	0	Cy	IIA
49	M	64	4.7.66	800	255	30	<1	1·0	0	0·3	0	0	Ch, V	IIIB
50	M	18	24.6.66	500	60	80	<1	0·6	0	0·1	1	0·1	–	IIA

* M = Mustine hydrochloride; Ch = chlorambucil; Cy = cyclophosphamide; V = vinblastine.

0, no visible difference from normal; +, present but less than half the normal β_1; ++, between + and +++; +++, equal or greater in amount than the normal β_1.

Immunoglobulins were measured by a modified Mancini method (Hobbs and Maatela, 1967) using radial immunodiffusion in agar incorporating our own mono-specific antisera to γG, γA and γM. Anti-γD serum was kindly supplied by Dr D. Rowe of Birmingham. Perfectly flat plates with a single well-cutter used throughout, ensured a reproducibility of ±10 per cent. Absolute standards were freshly purified solutions of γG, γA, γM and γD calibrated by their absorption at 280 mμ and used within 4 hours to check a working reference standard normal serum. Later their purity was proved by immunoelectrophoresis and gel diffusion to be at least 98 per cent.

Immunoglobulins measured in fifty healthy adults and in fifty-seven hospital patients without disease known to affect immunoglobulin levels were found to be almost identical. The data were therefore pooled to give a normal range for 107 adult patients. The normal distributions were all skewed, so that they are best described as γG, 600–1600 mg/100 ml; γA, 125–425 mg/100 ml; γM, 50–175 mg/100 ml; γD, 0–15 mg/100 ml. The first three normal ranges are illustrated in Fig. 2.

RESULTS

SERA

Serum γ-globulin and immunoglobulins were estimated in fifty patients with Hodgkin's disease. Twenty-four patients showed γM levels at some time at or below our lower limit of normal of 50 mg/100 ml. The γA levels also tended to be low but the γG values were, if anything, raised. Increased γG values, however, were not sufficient to correlate with the incidence of hypergammaglobulinaemia commented on by Arends, Conrad and Rundle (1954) and Miller (1962). The results are illustrated in Fig. 2. None of these consecutive patients showed monoclonal immunoglobulins, although we have observed these in three other patients with Hodgkin's disease.

DURATION OF ILLNESS

The patients in Table 1 are arranged in order of the duration of their illness. We considered the possibility that there might be a correlation between immunoglobulin level and duration of illness, but this is not apparent.

CLINICAL STAGE

Comparison of γM levels and clinical stage appears in Fig. 3. We estimated immunoglobulins serially in a number of the patients: in one patient (No. 3) 9 years after diagnosis γA was 800 mg/100 ml and γM 200 mg/100 ml (31st May 1965). A month later he was noted to have mediastinal lymphadenopathy and was treated by radiotherapy. In March 1966 γA had fallen to 270 mg/100 ml and γM to 50 mg/100 ml; he had systemic symptoms which were treated with a course of nitrogen mustard (total dose 25 mg) and on 10th June 1966, γA was found to be 280 mg/100 ml and γM 40 mg/100 ml. He then started a further course of mediastinal radiotherapy and γA fell slightly and γM considerably over the next 10 weeks. At the time of writing he is comparatively free of symptoms. In two other patients (Nos. 15 and 17) our first γM values were low but very much lower values

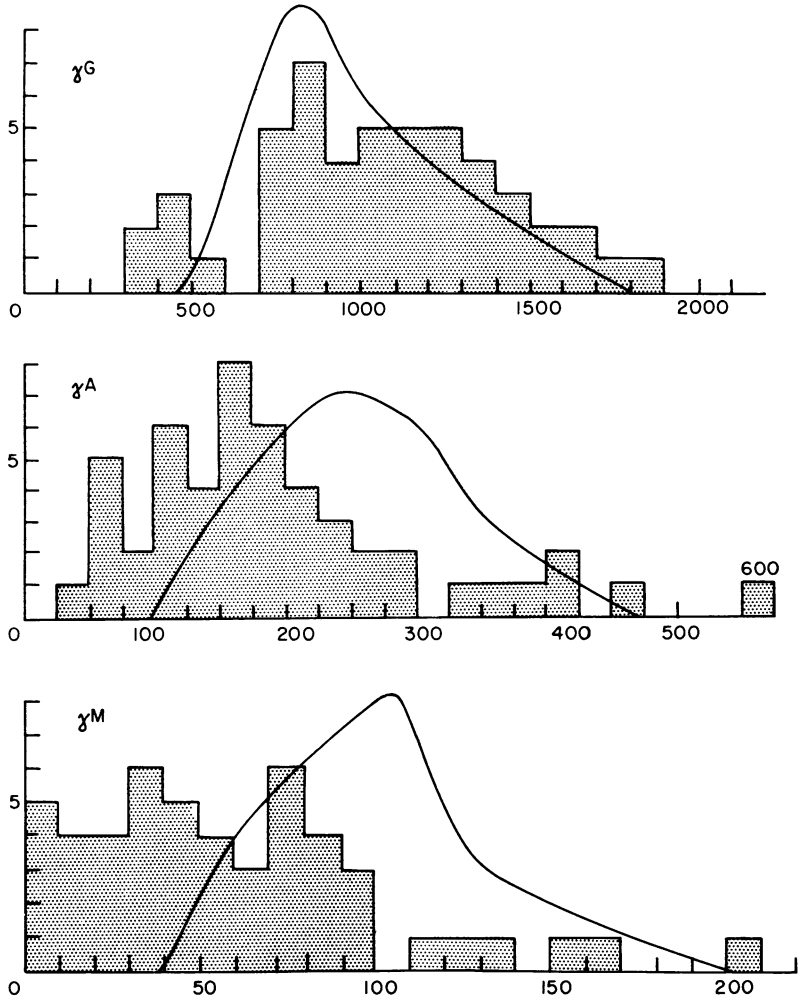

FIG. 2. Frequency distributions of serum immunoglobulin levels (mg/100 ml) in fifty patients with Hodgkin's disease. Compared with the continuous lines (distributions found in 107 normal subjects, drawn to half on the ordinates) γG in general shows slight increase, γA mild decrease and γM often marked reduction.

occurred a few months later; both these patients died shortly after the final immunoglobulin estimation. Thus the slow acquisition of a very low γM level has actually been observed in three patients.

In four other patients (Nos. 24, 27, 32 and 47) closely spaced immunoglobulin estimations were not significantly different.

EFFECTS OF RADIOTHERAPY

Very few patients will be found who have had Hodgkin's disease for any appreciable period without having at some time undergone radiotherapy. We, therefore, divided all our patients into three arbitrary groups:

Group 1: Patients who had never had radiotherapy.

Group 2: Patients with a single course only that did not irradiate the mediastinum.

Group 3: Patients with two or more courses of radiotherapy or with a single course including the mediastinum.

Results are illustrated in Fig. 3. In Groups 2 and 3 we calculated the period of time elapsed since completion of the most recent course of radiotherapy and compared this with immunoglobulin levels. We found no apparent correlation.

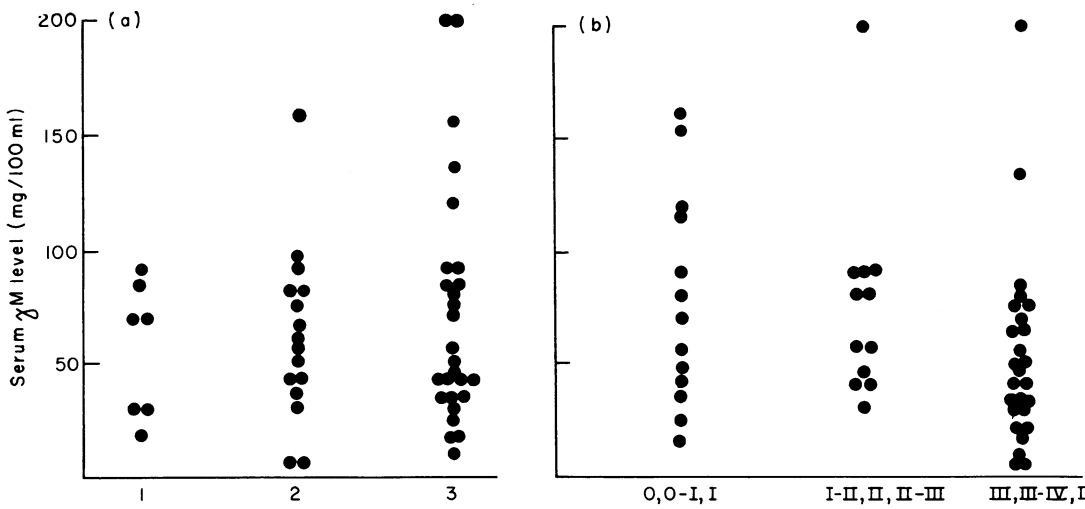

FIG. 3. The effect of deep X-ray therapy (a) and clinical stage (b) on the serum γM levels in fifty patients with Hodgkin's disease. Reduction of γM in advanced clinical stage is obvious but seems to be independent of the number of courses of radiotherapy (see text for deep X-ray therapy groups).

EFFECTS OF CHEMOTHERAPY

We divided the patients into two groups according to whether or not they had ever had cytotoxic chemotherapy. The γM levels of the two groups showed no significant difference.

INCIDENCE OF INFECTION

Infection is a frequent complication of leukaemias and lymphomas. Herpes zoster, tuberculosis and fungal disease, especially crytococcosis, have been reported to be associated commonly with Hodgkin's disease (Aisenberg, 1962). Apart from herpes zoster we could find little evidence of these diseases occurring in any of our patients after the diagnosis of Hodgkin's disease had been made. Our information about certain minor infections may have been incomplete and only five of our patients have so far died. The cause of death was marrow depression (perhaps due in part to chemotherapy) in association with widely disseminated disease in every case.

α_3-GLOBULIN

This was detected in the sera of thirty-one patients (62 per cent).

DISCUSSION

Heremans (1960) found low serum levels of γM-globulin in two out of six patients with Hodgkin's disease. McKelvey and Fahey (1965) studied the immunoglobulins in

eleven patients and noted a 20 per cent increase in γG while γA was decreased. There was no apparent change in γM. Our results from a larger series of patients show slight elevation of γG above the normal range. γA levels however were on the low side and γM levels in most cases were appreciably reduced.

Burtin, Guilbert and Buffe (1966) have suggested that γD levels can be excessive in Hodgkin's disease. In our experience, γD was lacking in most patients and the highest values at 25 mg/100 ml were encountered only twice (Nos. 12 and 27).

In three patients (Nos. 3, 15 and 21) γA was clearly raised and in three others (Nos. 6, 22 and 46) it was raised relative to the other immunoglobulins. In patients 3 and 15, the level was seen to fall as the disease progressed. We have observed a similar fall in two other patients, both with reticulo-sarcoma, who started with high initial levels.

γG has a half-life of 25–35 days and γA 6–7 days. According to Barth, Wochner and Waldmann (1964) γM has a half-life of about 5 days. These authors reported also that serum γM levels showed a gross correlation with the rates of synthesis. The serum level was highest in the subject who had the highest rate of γM synthesis whereas degradation took place by a first-order process which seemed to be independent of serum concentrations. They suggested that low γM, was, therefore, more likely to be due to decreased synthesis than to increased rate of catabolism.

A significant reduction in γM synthesis could be due to a number of causes. Even localized radiotherapy might act generally to depress those cells of the reticulo-endothelial system responsible for the production of γM, though such depression might be expected to recover with time. We found, however, low γM levels in two of our patients (Nos. 32 and 49) who had not yet received irradiation and there seemed to be no appreciable difference in γM levels in patients irradiated once or not at all (Groups 1 and 2, Fig. 3) and in patients irradiated on two to five occasions (Groups 3). There did not seem to be any relationship between γM level and time since completion of the latest course of radiotherapy, so that if radiotherapy were an important influence in reducing γM there seemed to be little or no recovery with time. It was our impression, however, that advanced clinical stage definitely favoured the finding of a low γM. In five patients it was low before the disease was widely disseminated, but most reliably so in Stage IV patients.

In parallel studies of over 400 other patients with neoplasia of cells within the reticulo-endothelial system, (myelomatosis, lymphosarcoma, chronic lymphatic leukaemia, etc.) the experience of one of us (J.R.H.) shows that γM deficiency is not only common but appears to precede deficiency of γA and γG which can follow in that order. In this study fourteen of the fifty patients with Hodgkin's disease show a γA level below 125 mg/100 ml, and six a γG level below 600 mg/100 ml. We are, however, in agreement with Hoffbrand (1964) that γG levels below 200 mg/100 ml, a defined limit for hypogammaglobulinaemia, are rare in this disease.

Ngu, McFarlane, Osunkoya and Udeozo (1966) have estimated γM levels in sixteen patients with Burkitt's lymphoma and conclude that they are generally reduced, though not to the extent seen in patients in this series with Hodgkin's disease. They give no details of treatment of patients in their series but Burkitt (1966) refers to eighty-eight patients with jaw tumours who were treated with cyclophosphamide, methotrexate or vincristine and the treatment was usually completed within 3 weeks. Certainly there was no question of radiotherapy contributing to low γM levels in these patients.

Burkitt's lymphoma may be caused by a virus and Ngu *et al.* (1966) speculate that the entry of the virus particles into the lymphoid cells of the body may derange intracellular

functions concerned in the production of γM antibody. Clearly the same line of reasoning could be applied to Hodgkin's disease, though the pathogenic role of a virus here is much more hypothetical. It is pertinent to recall that depression in delayed-type hypersensitivity response is seen comparatively early in Hodgkin's disease and one wonders whether a similar mechanism might not be deranging both the lymphoid cells which produce γM (humoral response) and those cells normally capable of producing a fixed antibody (cellular response). Indeed the same cells may be involved in both processes. Pentycross, Lawlor and Reeves (1967) will be reporting on the chromosomes and lymphocyte transformation in many of our patients.

In 1964, Sunderman described the finding of an excess of α_3-globulin after paper electrophoresis and thought this might occur in reticuloses. On cellulose acetate a visible excess of protein in the α_3-globulin position, (with the reservations stated above and as shown in Fig. 1) was found in thirty-one of our fifty patients. When present in a given patient it was a fairly consistent finding, though crude variations in amount could not be correlated with the activity of the disease. It has been seen in twenty-seven of sixty-eight other patients with neoplastic reticuloses, and thus appears to be increased in about half of all such patients. α_3-Globulin has also been found however in twenty-six patients with sarcoidosis and other benign conditions involving the R.E.S., in seventeen patients with no evidence at all of disease affecting the R.E.S., in six patients with cancer and in five with obstructive jaundice; together these fifty-four patients account for half of all those in whom α_3-globulin was seen. It has been uncommon among the 12,000 new patients whose serum electrophoretic patterns have been scrutinized over the past 2 years (J.R.H.) and its presence has thus been a useful, if not wholly reliable, pointer to a reticulosis. We have not yet isolated this α_3-globulin or identified it by immunoelectrophoresis.

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