IMMUNOLOGICAL STUDIES IN COELIAC DISEASE

by

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S T A T E M E N T

This thesis contains no material which has been accepted for any other degree or diploma in any University and does not contain any material previously published or written by another person, except where due reference is made to such material in the text.

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C O N T E N T S

SUMMARY

CHAPTER I: THE HISTORY OF COELIAC DISEASE

Since Gee's first description of the entity which he termed the coeliac affection, a welter of terms has been used for coeliac disease: non-tropical sprue, celiac sprue, coeliac syndrome, idiopathic steatorrhoea, idiopathic sprue and gluten enteropathy.

In early times, diagnosis was difficult and mortality high. Dicke's observations implicating gluten had profound effects both on the development of new knowledge and on the mortality of coeliac disease.

CHAPTER II: THE CURRENT CLINICAL CONCEPT OF COELIAC DISEASE

The study of coeliac disease has been made difficult through the lack of unanimity regarding a definition. However, most workers now agree on certain basic aspects of the disease:

(1) A flat jejunal mucosa is a diagnostic prerequisite.

(2) Dietary restriction of gluten results in histological and clinical improvement.

The Clinical Features

Diarrhoea, is common but not invariable. Hematological disturbances are due to malabsorption of Vitamin K and folate and to iron deficiency which results mainly from excessive epithelial cell loss. A variety of skin conditions have been

described, the most important of which is Dermatitis Herpetiformis. The central and peripheral nervous system may be involved. A few reports suggest an association between coeliac disease and diffuse interstitial lung disease. Patients with long standing coeliac disease on a normal diet may develop lymphoma of the small bowel.

CHAPTER III: THE PATHOLOGY AND PATHOGENESIS OF COELIAC DISEASE

The Histological Lesion

Following Paulley's description of villous atrophy in jejunal mucosa taken at laparotomy from patients with coeliac disease, Shiner's modification of Woods biopsy capsule for small bowel biopsy provided an impetus to the study of the small intestine. In 1960 Rubin's group provided proof of the toxicity of gluten, by demonstrating that instillation of gluten into the proximal ileum resulted in mucosal damage in two patients with coeliac disease.

The Nature of the Toxic Substance

Following the early work by Dicke and his associates, considerable effort has been directed towards the isolation of the toxic substance. Recent studies suggest that the N-pyrrolidone carboxyl peptides are implicated but the exact nature of the toxic substance is still unknown.

The Enzyme Deficiency Theory

In coeliac disease the fundamental problem involves the digestion of a protein - gluten. Frazer's classic experiment demonstrating the toxicity of a peptic-tryptic digest of gluten (Gluten Fraction III) and the detoxification of this product

by hog intestinal mucosa strongly suggested the existence of an enzyme deficiency.

Numerous studies have demonstrated low peptidase activity in the damaged small bowel mucosa but there is a return to normal with histological improvement. Recent work in Australia by Townley's group demonstrated that both "treated" and untreated coeliac mucosa showed only a partial ability to digest Fraction 9 obtained from a peptic-tryptic "cotazym" digest of gliadin.

This study needs confirmation but is a promising recent development in the quest for an enzyme deficiency.

The Immunological Aspects

The documentation of gliadin shock, the efficacy of steroids, and the presence of circulating dietary antibodies were early pointers suggestive of an immunological basis for coeliac disease. Changes in serum immunoglobulins and complement, the presence of antireticulin antibodies, of circulating antibodies to wheat constituents and of circulating immune complexes suggest an enhanced humoral immune response. Alterations in the ratios of plasma cells, changes in intestinal immunoglobulin levels and precipitins to dietary antigens have been documented in the small bowel of patients with untreated coeliac disease.

Studies on lymphocytes have indicated a possible depressed response to PHA and a weak response to gluten fraction III. Recent studies document the undue frequency of HL-A8 leucocyte antigen type in coeliac disease. Though the significance of this is still not clear, it has been suggested that the HL-A8

gene may be involved in the immune response in coeliac disease.

CHAPTER IV: MATERIAL AND METHODS

The main purpose of the present study was to investigate the role of immunological mechanisms in coeliac disease.

Fifty patients with coeliac disease were studied and 23 of these were investigated before and after gluten restriction. Patients with Crohn's disease (15), Ulcerative colitis (31) and the Irritable Colon Syndrome (23) were included in the study. The following tests were applied although not to all patients:-

A. Tests on Serum
   1. serum immunoglobulin levels
   2. serum complement levels
   3. autoantibodies including antireticulin antibodies
   4. dietary antibodies
   5. antibody responses to immunisation with tetanus toxoid and S.typhi

B. Tests on Lymphocytes
   1. B cells
   2. lymphocyte transformation

C. Tests on Small Bowel
   1. jejunal mucosal plasma cells
   2. jejunal juice
      (i) immunoglobulin levels
      (ii) dietary antibodies

D. Delayed Hypersensitivity
   1. intradermal skin tests

CHAPTER V: RESULTS AND DISCUSSION

The results of the tests relevant to immunological mechanisms can be summarised as follows:-

A. TESTS ON SERUM

Serum Immunoglobulin Levels

There were no major alterations in serum IgG levels. Serum IgA levels were elevated in 24% of patients on a normal diet, while low IgM levels were found in 19% in the same group. A comparison of immunoglobulin levels in the group of 23 patients before and after a mean period of 15 months of gluten restriction, showed no significant change in IgA and IgG levels but a significant rise in IgM.

Serum Complement

C_3 and total haemolytic complement were not significantly altered in the groups studied and were unaffected by exclusion of gluten from the diet. Thus in the present study there was no evidence to suggest involvement of the complement system.

Serum Autoantibodies

The prevalence of anti nuclear factors (ANF) and auto-antibodies to smooth muscle (SMA) were higher in patients on a normal diet than in those on a gluten free diet. The prevalence of SMA fell after exclusion of gluten from the diet.

Antireticulin Antibodies

Antireticulin antibodies (ARA) were detected in 56% of untreated patients and 5% of patients on a gluten free diet. The present study thus confirms the high incidence of ARA in untreated coeliac disease.

Dietary Antibodies

Precipitins to wheat products were present in 28% of patients on a normal diet and in a much smaller proportion of patients on a gluten free diet and in subjects with other types of gastrointestinal disease. Their prevalence fell after gluten restriction.

Antibody Responses to Immunisation with Tetanus Toxoid and S.typhi

Only one of 12 patients studied failed to respond to both antigens and one other patient failed to respond to S.typhi. Although comparison with normal controls showed no significant difference, the findings suggest that the humoral immune response may be impaired in a small proportion of patients with coeliac disease.

B. TESTS ON LYMPHOCYTES

B Lymphocytes

The proportion of B cells in the peripheral blood of patients on a normal diet was significantly higher than in control subjects. This also applied to absolute numbers of B cells. Untreated patients had significantly higher numbers of lymphocytes bearing IgG receptor sites than did control subjects or patients on a gluten free diet. There was a correlation between the presence of antireticulin antibodies and increased numbers of B cells of IgG type. Peripheral blood lymphocytes bearing receptor sites to fluoresceinated gluten fraction III were not detected.

PHA Induced Lymphocyte Uptake of ^{3}HT

There was no significant difference between patients - irrespective of dietary treatment - and control subjects.

The Effect of GF III on PHA Induced Uptake of ^{3}HT

Two hundred microgrammes of GF III caused a decrease in lymphocyte uptake of ^{3}HT in patients on a normal diet, patients taking a gluten free diet and in control subjects.

C. TESTS ON SMALL BOWEL

Jejunal Plasma Cells

In the present study the numbers of immunoglobulin containing plasma cells were studied in a larger number of both patients (28) and control subjects (29) than previously reported. The number of IgM containing plasma cells was significantly increased but fell with dietary treatment. The reason for the increase in IgM containing cells remains speculative.

Intestinal Juice

(i) Immunoglobulin Levels

IgA levels were significantly lower in the 15 untreated patients studied than in control subjects. The finding of normal intestinal juice IgM levels despite an absolute increase in IgM containing plasma cells appears incongruous. It may be that active immunoglobulin synthesis does not occur in all the IgM cells.

(ii) Dietary antibodies were not detected in the intestinal juice of 15 patients.

D. DELAYED HYPERSENSITIVITY

Intradermal Skin Tests

One of 12 patients failed to react to all three antigens used, but as a group the patients did not differ significantly from age and sex matched control subjects.

Thus the studies on lymphocytes and delayed hypersensitivity in patients with coeliac disease did not provide evidence of impaired cellular immunity.

SIGNIFICANT CONTRIBUTIONS OF THIS THESIS

The mechanism by which gluten damages the small bowel in coeliac disease is not known. However, the observations of gliadin shock, the beneficial effect of steroids, the presence of circulating dietary antibodies and evidence of lymphoreticular dysfunction were early pointers to a possible immunological mechanism.

In recent times, there has been added information to suggest involvement of the immune system. Changes in serum immunoglobulin and complement, antireticulin antibodies and circulating immune complexes have been described. Studies on the jejunal mucosa and intestinal juice have demonstrated alterations in the number of plasma cells and immunoglobulin levels and the presence of dietary antibodies.

While many of these findings were confirmed in the present study, changes in serum complement were not observed. The findings pertaining to the small bowel mucosa and juice were at variance with the results of some previous workers.

In order to investigate whether the immunological changes observed were of a primary nature and thus possibly related to the pathogenesis of coeliac disease, 23 of the 50 patients studied were investigated both whilst on a normal diet and again after gluten restriction. The changes in serum immunoglobulins, the antireticulin and smooth muscle antibodies and the circulating antibodies to dietary antigens did not persist after gluten exclusion. In the small bowel there was a significant change in the plasma cell distribution.

In addition, studies of humoral immunity showed a mean increase in total 'B' cell numbers and IgG bearing receptor cells only in untreated patients. There was a positive correlation between the presence of antireticulin antibodies and increased numbers of 'B' cells of IgG type. The role of the 'T' lymphocyte which participates in cell mediated immunity was also investigated by measuring PHA induced lymphocyte uptake of ^{3}HT. This proved to be normal as were delayed hypersensitivity skin tests.

The findings in this study thus suggest that the immunological changes are not primary but rather epiphenomena of the disease process. They are therefore unlikely to be concerned in its pathogenesis, but may nevertheless be involved in the perpetuation of the disease process. Moreover, this study has exonerated immunological mechanisms as initiating events only within the limits of the tests used and more sophisticated methods may well substantiate the existence of a primary immunological mechanism at a future date.

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CHAPTER I: THE HISTORY OF COELIAC DISEASE

I.A. INTRODUCTION

The word coeliac is derived from the Greek, Coeliakus, meaning abdominal. Aretaeus, the Cappadocian, in the second century A.D. is credited with the first use of the term coeliac disease. He described a condition characterised by diarrhoea, flatulence, abdominal pain, generalised weakness and emaciation (Major, 1948).

In 1888, an English physician, Samuel Gee published the first account of coeliac disease. Gee's paper "On the Coeliac Affection" appeared in St. Bartholomew's Hospital Reports. The term coeliac disease naturally did not have the same significance as it does now and Gee was probably describing both coeliac disease as we know it today and also tropical sprue (though he does not use this term) as a single entity. He wrote: "sometimes from India Englishmen return sick with the coeliac affection: seldom is it met in adults who have never left our island". He posed this question: "Errors in diet may be a cause but what error?". With prophetic insight he concluded his article: "But if the patient can be cured at all it must be by means of diet".

I.B. TERMINOLOGY

The study of coeliac disease has been bedevilled to the present day by a multiplicity of terms. Parsons (1932) reviewing the literature on coeliac disease notes that the following synonyms were used: "acholia", "intestinal infantilism", "Schwere Verdauung-sinsuffizienz", "coeliac infantilism" and "chronic intestinal indigestion". Following Herter's (1908) monograph on infantilism from chronic intestinal infection, in America and Heubner's (1909) paper dealing with marked digestive insufficiency in infants, the

term "Heubner-Herter disease" came into use. In addition, in Europe, the term "Gee-Herter disease" was in vogue.

The word sprue, which has embraced many and varied forms of diarrhoea, is derived from the Dutch Sprouw, meaning diarrhoea. The word, no longer used in Holland, was introduced in 1669 by a Dutch physician, Vincent Ketelaer, to describe a disease in which widespread aphthous ulceration and diarrhoea occurred (Ketelaer, 1715). However, an English physician, William Hillary is credited with the first clear description of tropical sprue which he called Aphthoides Chronica. His accurate description of tropical sprue was published in 1759.

In 1880 Manson anglicized the term to sprue when he reported cases of diarrhoeal disease in China.

With time, while some of the older synonyms fell into disuse, new terminology arose. It is interesting that as late as 1957, there was no clear concept of what coeliac disease, non-tropical sprue and tropical sprue was. At a symposium on the malabsorption syndrome at the Mt. Sinai Hospital many participants felt that tropical sprue and non-tropical sprue were variants of the same disease. (Bossak, Wang and Adlersberg, 1957).

In 1950 Dicke made his classical observation relating coeliac disease to wheat and rye flour and later to gluten (Dicke, Weijers and van de Kamer, 1953). Non-tropical and tropical sprue are now recognised as separate entities. However, there is still a plethora of terms for the former: coeliac disease, coeliac sprue, coeliac syndrome, idiopathic steatorrhoea, gluten enteropathy, idiopathic sprue. On the Continent the term Herter-Heubner disease is still in use.

I.C. MORTALITY AND TREATMENT

In the late nineteenth century and early twentieth century, the noxious effect of gluten was unknown; the existence of a mucosal lesion was in doubt and there were no biopsy methods to arrive at a conclusive diagnosis. It is thus conceivable that many diseases giving rise to a malabsorption syndrome may well have been labelled as coeliac disease. However, from early reports of what was described as coeliac disease it appears that it was associated with a high mortality.

While Gee felt that "death is a common end", Parsons (1932) reviewing the literature gives a rough estimate of the mortality; "Gibbons also thought that the majority of sufferers died from the disease; Lehndroff and Mautner are of the opinion that the prognosis of the severe type of the disease is not good and Knopfelmacher spoke of a 50 per cent mortality".

From Parsons' figures, the best results were obtained by Howland who reported no mortality at all in his series. In 1921, Howland instituted a dietary regime in which fat was not excluded but complex carbohydrates were strictly withheld. In effect he had instituted a gluten free diet. Clinical improvement was also observed by Haas (1924) who advocated a banana diet and Fanconi (1928) whose patients were placed on a vegetable-fruit diet.

In England, there was an intensive preoccupation with the problem of steatorrhoea and fat restriction. The situation was well summed up by Webster who said: "...it is unwise perpetually to starve the patient of fat, treating the stools and not the child" (Miller, Webster and Perkins, 1920).

Both Still (1918) and Parsons (1932) laid considerable stress on fat restriction. Parsons (1932) claimed that a "cure can usually be promised if treatment is carried out efficiently". It is unlikely that patients cured by this diet were coeliacs. Parsons gives a mortality rate of 10.6 per cent, close to Still's (1918) figure of 14 per cent. In an excellent study on the prognosis of seventy three cases of coeliac disease admitted to the Hospital for Sick Children, London between 1923 and 1938, Hardwick (1939) gives a mortality figure of 35 per cent. Hardwick reviewed 544 cases reported since 1909; the mortality in this large series was 15 per cent.

In 1949 Sheldon noticed that starch restriction caused clinical improvement in children on a normal fat intake. Although Sheldon's interpretation on the efficacy of starch restriction later proved incorrect, his observation contributed to a major change in therapy. By withdrawing starch and therefore all flour, he too, like Howland in 1921, had introduced a gluten free diet.

The management of coeliac disease was transformed by Dicke's research in Holland on the harmful effects of certain types of cereal in patients with coeliac disease. This work formed his doctoral thesis. In 1950, Dicke's crucially important observation linking coeliac disease with wheat received international attention when he presented a paper at the Sixth International Congress of Paediatrics at Zurich.

Dicke's observations were soon confirmed in England by Frazer's group (Anderson, Frazer, French, Gerrard, Sammons and Smellie, 1952), Sheldon and Lawson (1952), in Canada

by McIver (1952) and by Ruffin and his colleagues (Ruffin, Carter, Johnston and Baylin (1954), in the U.S.A.

Although many questions relating to the toxic substance, its mechanism of action and the possible role of hereditary transmission, still remain unanswered, a milestone had been reached with the dietary exclusion of gluten. Coeliac disease is no longer associated with appreciable mortality or morbidity, provided the patient adheres to a gluten-free diet (Table I). In a long term follow up study of 57 patients at the Hospital for Sick Children at Great Ormand Street between 1951 and 1968, the mortality rate was 0.4 per cent, (Sheldon, 1969).

TABLE I MORTALITY IN COELIAC DISEASE

(Modified from Hardwick, 1939)

NAME	DATE	NUMBER OF CASES	MORTALITY PER CENT
Heubner	1909	10	10
Still	1918	41	14
Lichtenstein ..	1921	9	22
Howland	1921	30	0
Hablutzel-Weber ..	1923	26	23
Pipping	1924	6	50
Schaap	1926	114	11
Sauer	1927	25	4
Thaysen	1929b	23	22
Parsons	1932	94	10.6
Neale	1935	93	12
Hardwick	1939	73	36
Sheldon	1969	57	0.4

CHAPTER II: THE CURRENT CLINICAL CONCEPT OF COELIAC DISEASE

II.A. THE DIAGNOSTIC CRITERIA

A definition of coeliac disease acceptable to all workers in the field has yet to be formulated. At the conclusion of an International Conference on coeliac disease in London, Rubin's group suggested two essential criteria for the diagnosis (Weinstein, Shimoda, Brow and Rubin, 1970).

(i) A characteristic flat mucosa at the duodeno-jejunal junction in a patient with malabsorption.

(ii) An unequivocal clinical response to a gluten free diet.

The European Society for Paediatric Gastroenterology, at a meeting in Interlaken in 1969, discussed the diagnostic criteria in coeliac disease (Meeuwisse, 1970). The answers to a questionnaire completed by paediatric gastroenterologists were the basis for the discussion and reflect the lack of unanimity in many areas (Visakorpi, 1970). Surprisingly, five of the thirty respondents to the questionnaire felt that a small bowel biopsy was not a necessary diagnostic procedure.

There was, however, unanimity at both conferences that a flat mucosa is the first essential criterion, though not the only one for the diagnosis of coeliac disease.

A diagnosis of coeliac disease cannot be made in the absence of histological evidence of villous atrophy. A clinical diagnosis can be fraught with error in view of the fact that some patients may have no diarrhoea at all, (Hamilton, Lynch and Reilly, 1969; Mann, Brown and Kern, 1970; Young and Pringle, 1971). Egan - Mitchell and McNicholl (1972) describe 12 of 112 children with biopsy proven coeliac

disease who had constipation. Nine of the twelve first presented with fecal impaction.

The second criterion, that of an unequivocal clinical response to gluten withdrawal is necessary because a flat mucosa does not occur in coeliac disease alone. Thus, villous atrophy may be seen in Whipple's disease (Trier, Phelps, Eidelman and Rubin, 1965), Zollinger-Ellison syndrome (Shimoda, Saunders and Rubin, 1968), Eosinophilic gastro-enteritis (Leinbach and Rubin, 1970), Intestinal lymphoma (Eidelman, Parkins and Rubin, 1966) and in infants with gastroenteritis (Barnes and Townley, 1973).

These conditions are distinguishable from coeliac disease on other grounds. However, mucosal appearances in Dermatitis herpetiformis, Kwashiorkor and tropical sprue, are identical to those in coeliac disease (Rubin, Eidelman and Weinstein, 1970).

The second criterion of an unequivocal clinical response to gluten withdrawal though necessary, poses problems. Some patients with coeliac disease have neither diarrhoea nor steatorrhoea (Hamilton et al., 1969, Mann et al., 1970, Walker-Smith, 1970). This criterion cannot therefore be applied to all patients. A further complication is introduced by the fact that approximately 25% of patients show no response to gluten free diets, (Pink and Creamer, 1967). In this category of patients it may be difficult to be certain whether the diagnosis is indeed coeliac disease or whether the gluten free diet is adhered to.

The panel members at the European Paediatric Conference were of the opinion that a relapse after the reintroduction of gluten was a necessary diagnostic criterion. At the

London conference many participants felt that gluten challenge posed ethical questions and there was no unanimity regarding the necessity for gluten challenge.

At Interlaken the panel agreed on the following diagnostic criteria: "the diagnosis should be limited to patients with permanent gluten intolerance. A patient with sub-total villous atrophy who shows improvement on a gluten free diet cannot be designated as having coeliac before he has been proved to normalize entirely (or almost so) on dietary treatment, not only clinically but also histologically and subsequently to relapse after the reintroduction of gluten". It was felt that in children on a strict gluten free diet it may take one or two years for the mucosa to return to normal (Meeuwisse, 1970).

In spite of the conflicting views on coeliac disease it would appear that most workers now agree on the following:

1) A flat jejunal mucosa is a diagnostic prerequisite.
2) There should be histological and clinical improvement after gluten exclusion.
3) Gluten intolerance is permanent and therefore coeliacs should be on a life-long gluten free diet.

For the purposes of this study a diagnosis of coeliac disease was made if the following criteria were fulfilled.

1) Histological evidence of villous atrophy.
2) Clinical and/or biochemical evidence of altered small bowel function.
3) Histological and/or clinical and biochemical improvement on a gluten free diet.

II.B. THE INCIDENCE

The figures on the incidence of coeliac disease are open to question as the diagnostic criteria vary in different centres even today.

The presence of a flat jejunal biopsy in symptomless relatives of patients with coeliac disease and the fact that diarrhoea is not a complaint in many patients, would suggest a significant number of unrecognised cases in the general population.

The peroral jejunal biopsy technique though introduced by Shiner in 1956, was not readily available until a few years later. Diagnoses made earlier on purely clinical grounds may well be erroneous. Mortimer, Stewart, Norman and Booth (1968) studied ten adults in whom a clinical diagnosis had been made in childhood and found normal biopsy appearances in one patient only. However, in a similar study, reappraisal of the clinical diagnosis in 12 children revealed the characteristic histological lesion in only six (Cook, Evans, Lloyd and Stewart, 1971). Of these six children, five were asymptomatic on a normal diet. These studies indicate the difficulty of making a clinical diagnosis of coeliac disease. Thus there are likely to be many patients either asymptomatic and therefore undiagnosed or incorrectly labelled as having coeliac disease. The earlier view that a flat jejunal mucosa was diagnostic only of coeliac disease would have also contributed to many errors.

The clinical entity known as transient gluten intolerance has been well documented, (Walker-Smith, 1970). The (damaged) intestinal mucosa recovers on a gluten free diet and suffers no change on subsequent gluten challenge. It is the view

of some workers that such cases, though initially satisfying both the clinical and the histological diagnostic criteria should not be included in the category of coeliac disease. The inclusion of this group as coeliacs would make a statistical evaluation of incidence inaccurate.

These possible sources of error should be borne in mind when considering the available estimates of the frequency of coeliac disease. McCrae (1970) estimated that the mean annual incidence (1948-1962) of coeliac disease in Glasgow was 1:1850. In England, the figures are thought to be between 1:2000 to 1:1500 (Smits, 1971). Cunningham (1973) attempted to arrive at an accurate figure by obtaining the number of prescriptions for gluten free products but found this avenue to be unreliable. The Coeliac Society has 6,000 members with a monthly increase of approximately 100 - 120 new members.

In the U.S.A. Kowlessar and Phillips (1970) set the figure similar to that in England. The incidence of coeliac disease in Australia is not known. In Sydney, New South Wales, Walker-Smith (1969) estimates a prevalence of 1:5000. Attempts to estimate the prevalence in Adelaide, were abandoned due to the absence of histological evidence in many patients labelled as having coeliac disease.

II.C. THE CLINICAL FEATURES

Coeliac disease is not a common illness. It does however constitute an important and remediable cause of malabsorption. Moreover, there is increasing evidence that most systems in the body may be involved. Some of the manifestations of coeliac disease are attributable directly to malabsorption, but for others the patho-physiological

basis is still unknown.

DIARRHOEA

Diarrhoea is the commonest presenting symptom in coeliac disease, but not invariable (Hamilton et al., 1969, Mann et al., 1970).

Several mechanisms have been held responsible for the diarrhoea. Impaired carbohydrate absorption is thought to lead to inefficiency of the sodium pump and consequently to decreased water absorption. The osmotic activity of the solute and the reduced permeability of the jejunum to solute, are added mechanisms which result in the colon receiving a load of fluid which is beyond its capacity to absorb (Low-Beer and Read, 1971).

Lassitude and weakness and weight loss are common and failure to thrive is a common mode of presentation in children. "Coeliac dwarfs" have been described (Sheldon, 1969).

HAEMATOLOGICAL

Haemorrhagic manifestations, most frequently in the skin, occur as a result of decreased vitamin K absorption leading to hypoprothrombinemia in 26 to 36 per cent of patients (Menendez-Corrada, 1968).

The proximal jejunum is most severely involved in coeliac disease and since folate absorption occurs mainly at this site, serum levels of folate are frequently low. Delamore (1972) reported abnormal folate levels in 90% of patients.

Apart from causing anaemia, folic acid deficiency during pregnancy may be associated with an increased incidence of

foetal abnormalities (Hibbard and Smithells, 1965). In addition, low folate levels have been thought to be responsible for some cases of abruptio placentae (Hibbard and Hibbard, 1963) and for recurrent abortion (Martin, Harper and Kelso, 1965). In 1970, Morris, Ajdukiewicz and Read, reported three women with untreated coeliac disease in whom infertility was cured by a gluten free diet. It was thought that the infertility was related to folate deficiency.

Vitamin B_{12} deficiency occurs far less frequently, as the absorptive site of B_{12}, the distal ileum, is usually not involved. Menendez-Corrada (1968) reviewing the literature, reported abnormal vitamin B_{12} absorption in 12-43 per cent of patients with coeliac disease whilst Delamore (1972) quoted a 40 per cent incidence of low serum B_{12} levels in coeliac disease.

Low levels of serum iron in coeliac disease are probably due more to the excessive epithelial cell loss in the coeliac mucosa than due to iron malabsorption (Croft, 1970). Studies using labelled iron administered intravenously have demonstrated a continuous loss of endogenous non-haem iron in up to 3 per cent of the injected dose in coeliacs and only 0.3 per cent in control subjects (Singh, 1970).

SKIN MANIFESTATIONS

Marks and her colleagues (Marks, Shuster and Watson, 1966) documented jejunal mucosal atrophy indistinguishable from that in coeliac disease in 60 per cent of cases of dermatitis herpetiformis (D.H.). There is also an increased incidence of jejunal mucosal abnormalities in close relatives (Marks and Shuster, 1971).

In two studies of dermatitis herpetiformis Rubin's

group (Brow, Parker, Weinstein and Rubin, 1971; Weinstein, Brow, Parker and Rubin, 1971) demonstrated that 21 of 22 patients had an abnormal small intestinal mucosa. Multiple biopsies of the jejunum revealed patchiness of the lesion in six patients, thus indicating the need for more than one biopsy. Gluten withdrawal may result in a reversion of the intestinal lesion to normal (Weinstein et al., 1971).

Similar immunological abnormalities exist in both coeliac disease and dermatitis herpetiformis. Low serum IgM levels (Fry, Keir, McKinn, Cowan and Hoffbrand, 1967, Hobbs and Hepner, 1968) and antireticulin antibodies are found in both conditions (Seah, Fry, Hoffbrand and Holborow, 1971a).

Seah, Fry, Stewart, Chapman, Hoffbrand and Holborow (1972) showed immunoglobulin bound to the dermal reticulin in D.H., and suggested there is an abnormality in the reticulin of the skin and small bowel in D.H., while in coeliac disease the abnormality is confined to the small bowel.

Although evidence of overt malabsorption is absent in D.H., a gluten free diet has been recommended in view of the reversibility of the lesion and the hazard of lymphoma formation in coeliac patients on a normal diet.

Coeliac disease may be associated with pigmented psoriasiform eczema responsive to gluten restriction, icthyosis and generalised melanosis similar to Addison's Disease (Wells, 1970).

NERVOUS SYSTEM

Disorders of affect in untreated coeliac disease have been well documented in children (Still, 1918; Parsons, 1932; Daynes, 1956) and in adults (Cooke, Peeney and Hawkins, 1953; Benson, Kowlessar and Sleisenger, 1964; Morris, et al., 1970).

It is thought that the association between depressive illness and coeliac disease is not a casual one (Goldberg, 1968). Epileptiform attacks are more frequent than in the general population (Cooke and Smith, 1966 and Morris et al., 1970). About 30 per cent of patients have parasthesiae (Benson et al., 1964; Morris et al., 1970). Peripheral neuropathy has been reported by Cooke's group (Cooke and Smith, 1966; Cooke, Johnson and Woolf, 1966) but has not been confirmed in later studies (Morris et al., 1970).

RESPIRATORY SYSTEM

Simultaneous occurrence of coeliac disease and diffuse intestinal lung disease has been reported. (Hood and Mason, 1970; Scadding, 1970; Smith, Benson and Strickland, 1971). Abnormalities in serum immunoglobulin levels in some of these patients and the presence of serum autoantibodies have suggested the possibility of a common immunological basis.

COELIAC DISEASE AND MALIGNANCY

There is now ample evidence that long standing coeliac disease may be complicated by malignancy. In 1962, Gough, Read and Naish, described the occurrence of a lymphoma of the small bowel in four coeliac patients. They also suggested that of the 29 cases of lymphoma of the small intestine reported in the literature, 16 may have had coeliac disease. In the large group of 202 patients with coeliac disease studied by Harris, Cooke, Thompson and Waterhouse (1967) there was a significantly higher incidence of malignant lymphoma (6.9 per cent) compared with that of the general population. Harris et al., (1967) and Austad, Cornes, Gough, McCarthy and Read (1967) have confirmed the higher incidence of reticulin cell sarcoma and Hodgkin's disease involving

the jejunum in the coeliac group, while in the general population, lymphoma occur more commonly in the ileum.

Carcinoma may also be more frequent in coeliacs. Read's group (Ajdukiewicz, McCarthy, Austad, Cornes, Harrison and Read, 1966) reported twenty six cases of carcinoma complicating coeliac disease, the commonest sites involved were the oesophagus in nine cases and the small bowel in six cases, although in the general population the colon and the stomach are the commonest sites for cancer of the G.I. tract. Harris et al., (1967) found 13 carcinomas in patients with coeliac disease, six in the oesophagus and three in the stomach, whilst the tongue, colon, rectum and anus were involved in one patient each. According to Harris et al., malignant disease was less frequent in patients on a gluten free diet.

Although in the association between malabsorption and malignancy there is still controversy as to which is the primary event the data at hand would suggest that long standing coeliac disease predisposes to malignancy.

CHAPTER III: THE PATHOLOGY AND PATHOGENESIS OF COELIAC DISEASE

III.A. THE HISTOLOGICAL LESION

Gee (1888) in his article "On The Coeliac Affection", stated that naked eye examination of the dead bodies threw no light upon the coeliac affection and that "nothing unusual can be seen in the stomach, intestines or other digestive organs".

In 1890, Thin described the mucosa of the small intestine in sprue as being entirely destroyed. Beneke (1910) and Justi (1913) described the same specimen of small intestine in a case of sprue. Beneke noted a loss of intestinal villi while Justi described the villi as being broad, short and swollen.

In 1915, Bahr published the post-mortem appearances in seven cases of sprue, in Ceylon. Five of the cases clearly demonstrated abnormal villous architecture.

In 1929, Mackie and Fairley in a careful post-mortem study, reported "withering of villi" in patients with sprue in Bombay. They said: "we incline to the view that sprue is essentially a disease of the intestinal tract and that the atrophy of the epithelium is at least a contributory factor in the evolution of the disease".

In 1932, Thayssen published his monograph on "Non-tropical Sprue". Of the 34 published cases of non-tropical sprue which he reviewed, histological examination of the small bowel had been performed in five. On the basis of the histological findings in these five cases, only one of which case was his own, Thayssen dismissed the findings of earlier workers. With a dogmatism disproportionate to the meagre evidence, he declared: "I wish to point out emphatically that in well preserved material (formaldehyde

injection) the intestinal epithelium of the villi as well as that of the Lieberkuhn crypts shows nowhere, outside the actual area of ulceration, any sign whatever of desquamation, degeneration or atrophy; in such specimens the only changes in the mucous membrane are those found in the connective tissue".

In 1936, Fairley repudiated his previous findings in sprue in Bombay. He felt that the changes which Mackie and he had reported were due to autolysis. Following the assertions of Thayssen and Fairley, the question of histological abnormalities in sprue was not re-opened for the next eleven years.

In 1947, Suarez, Spies and Suarez Jr., reported Koppisch's post-mortem findings in 16 cases of sprue in Puerto Rico. In half of the cases there was evidence of shortening and bluntening of the villi and plasma cell infiltration. In the same year, Adlersberg and Schein reported abnormal villous histology and increased cellular infiltration of the lamina propria in a study of six autopsied cases of "secondary sprue".

The abnormal histological findings described in patients with sprue thus far were made post-mortem. Due to the possibility of autolysis contributing to or even being solely responsible, they were regarded with some doubt.

In the wake of Dicke's (1950) observations associating wheat and rye and later gluten with coeliac disease, there was a renewed interest in a possible mucosal lesion.

Almost a quarter of a century after Thayssen's rejection of a characteristic lesion in non-tropical sprue,

Paulley's studies on jejunal mucosa obtained at laparotomy from patients with non-tropical sprue clearly established that the villous changes were due primarily to the disease process. Paulley's observations were made initially in 1949, (Cullinan, Gill and Paulley, 1949). Similar findings were documented by (Milanes, Leon and Causa, 1951) in tropical sprue. It was only after further documentation by Paulley in 1954 that there was general acceptance of this view. Following Dicke's discovery, Paulley's findings may well be regarded as the second milestone in the understanding of coeliac disease.

Margot Shiner's (1956) modification of Wood's gastric biopsy capsule for obtaining duodenal and subsequently, jejunal mucosa gave impetus to the study of the small intestine. It was now possible to adequately document the histological appearances of normal intestinal mucosa (Doniach and Shiner, 1957) and to provide material to establish conclusively the abnormalities in coeliac disease and other disorders of the small intestine. Numerous studies confirmed Paulley's observations (Shiner, 1957, Himes and Adlersberg, 1957).

In 1958, Butterworth and Perez-Santiago described findings from Puerto Rico similar to those of Paulley (1954) in six cases of sprue. Thus in both tropical and non-tropical sprue, the intestinal lesion appeared to be similar. Tropical sprue is endemic in tropical and sub-tropical climates and epidemics have been reported. Gluten has no harmful effect. Folic acid and antibiotic therapy results in rapid relief of symptoms, in a proportion of patients (Baker and Mathan, 1970).

Shiner and Doniach (1960) suggested that the mucosal abnormalities in coeliac disease were primary and irreversible and that the deleterious effects of gluten were superimposed. Rubin and his colleagues (Rubin, Brandborg, Phelps, Taylor, Murray, Stemler, Howrie and Volwiler, (1960) agreed with this concept, though with reservations as there was some histological improvement in two patients. They also suggested that the lesion may persist throughout life.

However, numerous workers reported contrary findings. Anderson (1960) showed that the mucosal lesion improved with gluten withdrawal and this was confirmed by Cameron, Astley, Hallowel, Rawson, Miller, French and Hubble, (1962). Electron-microscopic studies (Ashworth and Chears, 1962) showed histological improvement of the microvilli and diminished cellular infiltration of lamina propria. Rubin and co-workers (MacDonald, Brandborg, Flick, Trier and Rubin, 1964) agreed that the mucosal lesion was reversible but made the observation that in a few cases the small bowel damage may be irreparable in the face of clinical improvement.

The final step of actually demonstrating the toxicity of gluten was carried out by Rubin's group (Rubin, Brandborg, Flick, Parmentier, Phelps and van Niel, 1960). In two patients with histological remission gluten was instilled into the proximal ileum three times a day for nine days. Mucosal damage resulted and was most marked near the site of instillation whilst the damage was less pronounced more distally. In 1962 Bayless, Yardley, Norton and Hendrix established that epithelial degeneration could occur as quickly as 24 hours after the administration of gluten.

These studies established the toxicity of gluten and the reversibility of symptoms, biochemical and mucosal abnormalities on gluten withdrawal.

The characteristic small bowel lesion of villous atrophy in coeliac disease is now beyond doubt. There is increased depth of the intervillous crypts and an inflammatory response in the lamina propria.

Increased mitotic activity has been noted in the intestinal crypts (Padykula, Strauss, Ladman and Gardner, 1961; Yardley et al., 1962). There has however been uncertainity as to whether it reflects a maturation arrest (Creamer, 1962) or whether there is increased cell turnover (Croft, Loehry and Creamer, 1968). Clark and Senior (1969) measured the levels of pyrimidine precursor enzymes, which reflect epithelial cell turnover and concluded that the increased mitotic activity in coeliac disease is due not to maturation arrest but due to increased cell renewal.

Electron-microscopic studies have unfortunately not been of great benefit in coeliac disease although the scanning electron-microscope has added a new dimension to the assessment of villous architecture.

The numerous studies carried out have served to confirm the findings of light microscopic studies, though in greater detail (Trier and Rubin, 1965).

There is a consistent derangement of the brush border. The microvilli are fewer in number and are wider and shorter (Ashworth and Chears, 1962; Shiner and Birbeck, 1961). At times total absence of the brush border has been reported (Zetterqvist and Hendrix, 1960). Disappearance of the

terminal web has been reported (Ashworth and Chears, 1962; Nunez-Montiel, Bazua, Brunser and Sepulveda, 1963). It is thought that significant alterations do not occur in the surface coat though changes in the microvilli are present (Shiner, 1967).

III.B. THE NATURE OF THE TOXIC SUBSTANCE

Further work on the toxicity of wheat by Dicke, Weijers and van de Kamer (1953) established that wheat starch was innocuous. The toxicity lay in a substance which they termed "wheat factor". In the same year the "wheat factor" was identified as gluten, a mixture of proteins obtained by removal of starch from wheat flour by washing with water. Gluten was further separated into an alcohol soluble fraction gliadin and an alcohol insoluble fraction glutenin. Clinical experiments indicated that gliadin was more toxic than glutenin (van de Kamer, Weijers and Dicke, 1953).

Amino acid analysis of gliadin revealed a high glutamine content of 43% (van de Kamer and Weijers, 1955). Further interest was therefore centred on the possible toxic role of glutamine. Following gliadin ingestion, Weijers and van de Kamer (1955) were able to demonstrate a 90% rise in blood glutamine levels in patients with coeliac disease, while in control subjects there was a rise of only 15% and similar findings were reported by others (Alvey, Anderson and Freeman, 1957; Payne and Jenkinson, 1958). Acid hydrolysis was found to render gliadin harmless (van de Kamer and Weijers, 1955). This important fact further implicated glutamine as a toxic substance. The clinically non-toxic end product of acid hydrolysis was a protein with the same amino acid composition as gliadin except that it contained 43% of glutamic acid

instead of 43% glutamine. Alvey et al. (1957) have also shown that complete acid hydrolysis detoxifies gluten. Although from these experiments glutamine appeared to be toxic, oral administration of glutamine caused no ill effects. Because of this fact and because of the wide distribution of glutamine in foods which are well tolerated by patients with coeliac disease the interest in the toxicity of glutamine decreased.

However, there is an association between milk sensitivity and coeliac disease (Lietze, 1969) and antibodies to both milk and wheat proteins occur in coeliac disease, (Heiner, Lahey, Wilson, Gerrard, Shwachman and Khaw (1962), Alarcon-Segovia, Herskovic, Wakim, Green and Scudamore (1964). The high content of glutamine in both milk and gluten may therefore not be fortuitous.

In 1957, Alvey et al. demonstrated the deleterious effects of a pancreatic digest of gliadin. Two years later Frazer's group (Frazer, Fletcher, Ross, Shaw, Sammons and Schneider, 1959) showed that gluten fraction III - a peptic-tryptic digest of gluten, retained the toxicity. Krainick, Mohn and Fischer, (1959) also showed that peptic-tryptic digestion did not render gliadin non-toxic.

Woychik, Boundy and Dimler (1961), have demonstrated that gliadin contains several electrophoretically distinct components. More recently, studies by Kasarda, Nimmo and Kohler (1971) have distinguished possibly more than 30 components in whole gliadin. Cornell and Townley (1973 a) using a peptic, tryptic, pancreatin digest of gliadin, were able to obtain 13 fractions by ion-exchange chromatography. Fraction 9 was digested to a lesser extent by

duodenal mucosa of coeliac children in remission than by normal mucosa. The residue which remained after digestion of fraction 9 by coeliac mucosa, had a high content of glutamine/glutamic acid, proline and serine. On re-chromatography, fraction 9 could be further fractionated into two major components, each composed of peptides containing leucine and glutamine as the major N-terminal amino acids.

Kowlessar's group has made valuable and continuous contributions in clarifying the nature of the toxic substance. The studies on the ultrafiltrate of a peptic-tryptic digest of gliadin indicated that the low molecular weight acidic peptides which were eluted with water, were N-pyrrolidone carboxyl peptides. Further fractionation yielded glycopeptides and a group of peptides with a high glutamine content which were ninhydrin negative but became ninhydrin positive on mild alkaline hydrolysis, indicating a cyclization of the N-terminal glutaminyl residues (Bronstein, Haeffner and Kowlessar, 1966).

Although there is as yet insufficient evidence to designate firmly the N-pyrrolidone carboxyl peptides as the toxic factor, it would appear that they are implicated in the toxicity of gluten.

III.C. THE ENZYME DEFICIENCY THEORY

In coeliac disease, the fundamental problem resides in the digestion of a protein - gluten. The possibility that coeliac disease is due to a primary enzyme deficiency was suggested by the observation that although a peptic-tryptic digest of gluten is still toxic, digestion of this product with Hog intestinal mucosa, renders it non-toxic (Frazer, 1956). Since then, many workers have confirmed that pre-

digestion of gluten using pepsin, trypsin or pancreatin, does not remove the toxicity of gluten. (Alvey et al., 1957; Frazer et al., 1959).

From this observation it would appear that pepsin and trypsin have no role in the detoxifying process of gluten and that the abnormality in the digestion of the peptides produced resides in the peptidase enzymes.

In most patients with coeliac disease peptic and tryptic activity is normal. In 1957, Dreilling demonstrated that the secretin test, performed on 36 patients, was abnormal in only three. Normal carboxypeptidase A (which acts on the peptide bonds phenylalanine, tyrosine or tryptophan as the C-terminal amino acids) activity and normal chymotrypsin activity have been demonstrated in coeliac patients (Shwachman, Leubner and Catzel, 1955). Messer and Anderson (1961) investigated both carboxypeptidase A and carboxy-peptidase B which split bonds adjacent to lysine, arginine or ornithine in the C-terminal position in eight patients with coeliac disease and found no abnormality.

In a group of 49 patients with coeliac disease, Cooke, Fone, Cox, Meynell and Gaddie (1963) found free gastric acid in 49 patients and in five, no free gastric acid following maximum stimulation. In four of these patients marked gastric atrophy was present with atrophic gastritis in the other.

Confirmation of normal peptic and tryptic activity in patients with coeliac disease and Frazer's observation that an "enzyme is deficient or inadequate in the mucous membrane of a patient with gluten induced enteropathy" (Frazer, 1956) generated a considerable degree of research for an abnormality

in peptide digestion at the mucosal level.

The observation that digestion of gliadin with papain renders it almost non-toxic (Krainick et al., 1959) is extremely suggestive of a peptidase deficiency.

In 1963, Messer writing to 'Nature', made a series of important observations in relation to the deamidation of L-glutamine with papain. He demonstrated that crude papain would convert glutamine to a compound not stainable with ninhydrin. Ammonia was liberated in the process. A similar change could not be effected using pure papain, crude papain pre-heated to 90^{o}C or chymopapain, an enzyme in crude papain.

A year later it was demonstrated clinically that a crude papain digest of gluten was non-toxic as opposed to a pure papain digest (Messer, Anderson and Hubbard, 1964). These workers suggested that crude papain, in addition to the two known proteases (papain and chymopapain) may also contain an additional enzyme having a detoxifying action of gluten. It was demonstrated that crude papain acted on two glutamine-containing peptides (L-glutaminyl-L-asparagine and L-glutaminyl-L-leucine) converting them to the corresponding pyrrolidone carboxyl peptides with the liberation of ammonia. These products were unreactive to ninhydrin. Although similar N-glutaminyl peptides were not sought in the crude papain digest of gluten, the liberation of ammonia and the presence of pyrrolidone carboxyl peptides as end products justified the extrapolation that crude papain detoxified gluten by converting N-glutaminyl peptides to pyrrolidone carboxyl peptides. The converting enzyme was named glutamine cyclotranferase.

It is now known that protein digestion is a function

of the enterocyte thus confirming the view of Florey, Wright and Jennings (1941) that the enzymes of the succus entericus were derived from cast off epithelial cells and not from the mucosa by secretion. Following the demonstration of peptidase enzymes in the cytoplasm of amoebae (Holter, 1954), Newey and Smyth (1959, 1960) demonstrated the intracellular transport of dipeptides and also showed that most dipeptidase was intracellular, thus indicating that protein digestion occurred in the cell.

Recently Peters (1970) has demonstrated peptidase activity in both the brush border and the cytoplasm of the enterocyte. It has been proposed that the peptides produced by peptic and tryptic digestion are hydrolysed by amino-peptidases of the brush border and that dipeptides are transported and hydrolysed by the intracellular dipeptidases.

There is ample evidence that peptidase levels are below normal in untreated coeliac disease. However, normal digestion of peptide substrates by the jejunal mucosa of coeliac patients has been demonstrated following treatment with a gluten free diet, suggesting that enzyme deficiency is not primary, (Berg, Dahlqvist, Lindberg and Norden, 1970; Douglas and Peters, 1970; Douglas and Booth, 1970).

Although these studies seem to weigh against the hypothesis that coeliac disease is due to a primary enzyme defect, it may be premature to dismiss this hypothesis completely. Cornell and Townley (1973 a) have recently investigated enzyme activity in coeliac disease using a peptic-tryptic "cotazym" digest of gliadin. Homogenates of duodenal mucosa from six patients with treated coeliac disease, three patients on a normal diet and 14 normal control

subjects were incubated with fractions of the gliadin digest. Fraction 9 was only partially digested by the mucosa, in both groups of coeliacs suggesting that a primary enzyme abnormality was present. The two major subfractions of fraction 9 were also not digested to the same extent by coeliac mucosa as by "normal" mucosa. Moreover, even damaged coeliac mucosa was able to digest all other fractions except fraction 9.

Townley's group further demonstrated the toxicity of fraction 9 by electronmicroscopic studies. Lysosomal damage followed incubation of coeliac small bowel mucosa with fraction 9 but could be prevented by pre-digestion of fraction 9 with normal mucosa (Cornell and Townley, 1973 b).

Cohen, McNamara, Blumenfeld and Arias (1970) have demonstrated the presence of gamma-carboxyl-amide linkages in gliadin and its fractions. These linkages are broken down by the enzyme gamma-glutamyl-transpeptidase and the levels of this enzyme are low in treated and untreated patients with coeliac disease.

It would thus appear that the question of a primary enzyme defect in coeliac disease is still unresolved. However, progress can be expected in this field as the refined methods of digestion and separation which are now available are producing peptides of small molecular weight and making possible more accurate evaluation of the digestion potential of the mucosa.

III.D. THE IMMUNOLOGICAL ASPECTS

There is accumulating evidence that coeliac disease may have an immunological basis, although the proof is by no means conclusive. It is still uncertain whether patients

with coeliac disease react abnormally to a normally digested product of gluten, or whether an abnormal reaction is a secondary phenomenon which is elicited by an undigested or partially digested peptide.

Nevertheless there are good criteria that the immune system is involved and local hypersensitivity has been suggested as a possible mechanism. Two early observations bear on this - "gliadin shock" and the effect of steroids on the disease. In 1958 "Gliadin Shock" was described by Krainick, Debatin, Gautier, Tobler, Velasco and Schwenk, who reported the occasional occurrence of an acute reaction when very small amounts of gliadin were ingested by children with coeliac disease on a gluten free diet. Administration of steroids prior to the ingestion of gliadin, in some cases, prevented the vomiting, colic, diarrhoea and circulatory collapse, which were the hallmarks of "gliadin shock".

The beneficial effects of Prednisolone on the clinical status of patients with sprue were described by Adlersberg, Colcher and Drachman (1951) and also by Lepore (1958). Significant improvement in histology, enzyme levels and intestinal absorption occurs in patients on a normal diet taking 40 mg of Prednisolone a day. Cessation of steroids produced a histological relapse. The mechanism by which steroids affect the coeliac mucosa is not clear, (Wall, Douglas, Booth and Pearse, 1970).

LYMPHORETICULAR DYSFUNCTION

As early as 1923, Blumgart reported splenic atrophy in idiopathic steatorrhoea. McCarthy, Fraser, Evans and Read (1966) showed that many patients with adult coeliac disease have a small spleen. It was also demonstrated that the spleen

in patients with coeliac disease, even in the absence of splenic atrophy is less efficient in removing Rhesus injured ^{51}Cr - labelled red blood cells from the circulation. The fact that red cell removal time in patients with coeliac disease is even longer than that seen in patients after surgical splenectomy suggests that factors other than loss of splenic function are contributary. Comparison of lymph node biopsy material from coeliac disease patients and control subjects indicated the presence of lymphoid atrophy.

SERUM IMMUNOGLOBULIN LEVELS

Because of the possibility that immunological mechanisms may be concerned in the pathogenesis of coeliac disease, both humoral and cellular immune functions have received attention in recent years. One finding, now confirmed by many workers, is that of abnormal serum immunoglobulin levels.

Hobbs and Hepner (1968) in a study of 44 untreated adults and five children with coeliac disease showed that IgG and IgA levels were relatively normal. However, over half of the adults and all children were deficient in IgM. The low IgM rose significantly in most patients after institution of a gluten free diet. The initially high IgA levels found in six adults also returned to normal after treatment.

Cooke and his co-workers (Asquith, Thompson and Cooke, 1969) made a large survey of 110 patients. They found that IgG levels were significantly reduced both before and after treatment. In contrast, IgA levels were significantly raised in patients taking a normal diet than in the control group and did not change significantly after gluten restriction. IgM was significantly reduced and remained unaltered by a

gluten free diet. A sequential study of ten patients at monthly intervals over thirteen months following dietary treatment showed a significant rise in IgG, no significant change in IgA and a fall in IgM in a proportion of patients.

Kenrick and Walker-Smith (1970) found abnormalities in immunoglobulin levels in 15 of 24 children with untreated coeliac disease. IgG was low in two patients and raised in one. Eight had high IgA and eight low IgM but there was no correlation between the patients with low IgM and high IgA. Eight of the patients were investigated while on a gluten free diet for a mean period of seven months. The immunoglobulin levels returned to normal in all but one case.

Thus wide variations in immunoglobulin levels have been reported in coeliac disease. IgG levels may be normal (Hobbs and Hepner, 1968) elevated or decreased (Blecher, Ajdukiewicz, McCarthy and Read, 1969, Kenrick and Walker-Smith, 1970, Asquith et al., 1969). IgA levels (except in cases of IgA deficiency) are frequently elevated though only in a small proportion of cases (Hobbs and Hepner, 1968, Blecher et al., 1968, Asquith et al., 1969, Kenrick and Walker-Smith, 1970).

Most patients in Hobbs and Hepner's (1968) series and about a third of the patients studied by Asquith et al. (1969) and Kenrick and Walker-Smith (1970) had significantly reduced levels of IgM.

On a gluten free diet (mean four and seven months respectively) normal levels were attained by most of the patients studied by Hobbs and Hepner (1968), Kenrick and Walker-Smith (1970) but Cooke's group (Asquith et al., 1969) which studied their patients for a longer period found that the IgM levels rose and subsequently showed a fall in 30 per

cent of patients, still significantly lower than the control group. With regard to the more recently discovered immunoglobulin classes, Mietens, Johansson and Bennich (1971) reported that most of the patients had IgE levels which were below normal or within the normal range. Twenty per cent had raised IgE levels. IgD levels were normal in most patients and were not altered by dietary treatment.

DIETARY ANTIBODIES

The presence of circulating antibodies to gluten or its derivatives suggests that an immunological mechanism may be operative in coeliac disease. However, their significance is still unclear and antibodies to other dietary products may also be present.

Taylor, Truelove, Thomson and Wright (1961) found that coeliac sera contained antibodies not only to GF III but also to purified cow's milk, casein, L-lactalbumin and B-lactoglobulin. The prevalence and titre of antibodies was significantly higher in coeliac disease than in the control group. In 1964, in a similar study, (Kivel, Kearns and Liebowitz) though using a less sensitive double diffusion in gel method confirmed these findings. Using the tanned red cell technique Alarcon-Segovia et al. (1964) found antibodies to GF III and casein in approximately half of the patients and antibodies to B-lactoglobulin in three quarters. By contrast none of the 90 control subjects and antibodies to GF III and antibodies to the other two dietary proteins were found only infrequently.

The presence of antibodies to gluten and other dietary products is not confined to coeliac disease. Although they are significantly more common in coeliac disease, antibodies

to GF III are also present in patients with aphthous ulceration, pernicious anaemia, duodenal ulcer and ulcerative colitis (Taylor, Truelove and Wright, 1964).

More recently, Ferguson and Carswell (1972) studied a group of 71 children including 33 patients with coeliac disease. A micro-gel-diffusion technique was used to detect the presence of antibodies to cereal (wheat flour, gluten, oatmeal, rice flour and corn flour) and animal protein (cow's milk, bovine calf serum, sheep serum, egg white and egg yolk) antigens. In the coeliac sera, precipitins were detected to all the antigens except corn flour. There was a significantly higher incidence of antibodies to wheat flour, gluten, oatmeal, cow's milk, bovine calf serum, sheep serum, egg white and egg yolk in the patients with coeliac disease than in the control group.

The findings of Taylor et al. (1964), Kivel et al. (1964), Alarcon-Segovia et al. (1964) and Ferguson and Carswell (1972) differ from those of Rossipal (1970) who detected precipitins to wheat and barley in all 15 coeliac sera studied. Precipitins were not detected in the sera of 160 control subjects which included 40 children with gastroenterological disease.

Although different techniques and different antigens make valid comparisons between the various studies difficult, it is evident that patients with coeliac disease have a greater prevalence of antibodies to wheat products, though antibodies to many other dietary antigens are also present. Moreover antibodies to gluten and other dietary antigens are present not only in coeliac disease, but also in other gastrointestinal disorders (Taylor et al., 1964).

The evidence that circulating antibodies to gluten or

its digestion products reflects an immunological mechanism uniquely concerned in the pathogenesis of coeliac disease is thus not convincing.

SERUM AUTOANTIBODIES

The presence of serum autoantibodies in coeliac disease associated with other illnesses has been doucmented (Goudie, Boyle, Stuart-Smith and Ferguson, 1969). Smith and Strickland (1971) found autoantibodies in seven of 23 patients with coeliac disease. Rheumatoid factor was present in three patients and the thyroglobulin tanned-red-cell haemagglutination test was positive in three others. Two patients had thyroid microsomal antibodies and one, antibodies to gastric parietal cells. Two of the seven patients had associated lung disease and another two had features suggestive of Sjogrens syndrome. Brown, Ferguson, Carswell, Horne and Macsween (1973), detected anti-nuclear factor (ANF) in one of 48 children with coeliac disease and smooth muscle antibody (SMA) in another.

ANTIRETICULIN ANTIBODIES

Antireticulin antibodies (ARA) were described in coeliac disease by Seah et al. (1971 a) and Alp and Wright (1971). The former group found ARA in one third of the patients with adult coeliac disease and in the majority of patients with childhood coeliac disease. Further studies indicated that ARA were more common in children on a normal diet (Seah, Fry, Rossiter, Hoffbrand and Holborow, 1971 b), similar findings were reported by Alp and Wright (1971). The latter workers also reported that a quarter of the 59 patients with Crohn's disease had ARA whilst the occurrence of the antibodies in the control group was negligible. Von Essen,

Savilahti and Pelkonen (1972) detected ARA in the sera of 68% of patients. Similar figures were reported by Brown et al. (1973).

DELAYED HYPERSENSITIVITY

Host defence can be effected either by the humoral defence system or by cell mediated mechanisms. Although the former has been studied in some detail in coeliac disease the latter has received less attention.

LYMPHOCYTE STUDIES

Winter, McCarthy, Read and Yoffey, 1967 studied phytohaemagglutinin (PHA) - induced lymphocyte transformation in 13 patients with coeliac disease and found it to be normal in only three. The fact that three of the patients with reduced transformation were on a gluten free diet and clinically well was thought to indicate an intrinsic abnormality of the lymphocyte. Two patients on repeated testing showed no transformation at 72 hours. Cross over studies suggested that an inhibitory serum factor might also be present. Blecher et al. (1969) reported impaired PHA - induced lymphocyte transformation in about half of the ten patients studied. Cooke's group (Asquith, Housley and Cooke, 1970 a) studied the lymphocyte response to GF III, autoclaved and filtered GF III (AF), egg albumin and casein in 15 patients, 11 of whom were on a gluten free diet. Peripheral blood lymphocytes from patients on a gluten free diet showed a significantly greater stimulation by GF III than those from control subjects and those from patients on a normal diet. Neither GF III (AF) nor egg albumin elicited this response.

Lymphocytes from the axillary, inguinal and mesenteric nodes of patients with coeliac disease were also stimulated

by GF III. However the results of similar studies in patients who did not have coeliac disease were not uniform. There was stimulation in lymphocytes from the axillary gland of one patient and the mesenteric glandsof two patients.

Morganroth, Watson and French (1972) studied cell mediated immune responses to gluten fraction III and gliadin by means of lymphocyte transformation of peripheral blood lymphocytes. Three groups of 12 subjects each were studied. Group I comprised patients with coeliac disease on dietary treatment. Patients with miscellaneous gastro-intestinal disorders constituted group II while group III contained healthy controls.

In each subject, the transformation response to gluten fraction III, gliadin and PHA was studied.

In none of the three groups was there a significant difference in response to either of the wheat products, or PHA.

THE GASTROINTESTINAL TRACT AND IMMUNE RESPONSES

The human gastrointestinal tract being well endowed with plasma cells is capable of both effecting and experiencing an immune response. It is therefore of importance in the function of the immunological system as a whole. (Taylor, 1965; Watson, 1969; Shearman, Parkin and McClelland, 1972; Doe, 1972 and Jones, 1972).

The local humoral immune response in the gut is mediated through the production of coproantibodies, a concept suggested as early as 1919 by Besredka. In 1922, Davies demonstrated the presence of antibodies in stools of patients with bacillary dysentery before the occurrence of serum antibodies. Studies on germ-free mice using orally

given ferritin (Crabbe, Nash, Bazin, Eyssen and Heremans, 1969) showed that the plasma cells containing specific antibody in the gut was of the IgA class. Parenterally given ferritin elicited an IgM response in the lymph nodes and spleen and an IgA response in the small bowel.

Numerous studies indicated that the secretory antibody against viruses is of IgA class. Secretory IgA antibody is produced to orally given live attentuated polio virus, but not in response to parenteral polio virus. Both modes of administration lead to formation of serum antibody (Ogra, Karzon, Righthand and MacGillivray, 1968).

Further work by Ogra and Karzon (1969) has supported the concept that the major local immune response of the small bowel is mediated by the production of antibody of the IgA class.

Thus initial defence of the small bowel mucosa is provided by the production of the IgA antibodies which unlike IgM antibodies do not bind complement and mediate an Arthus-type reaction with subsequent local tissue damage (Beale, Douglas, Parish and Hobbs, 1971).

In 1964, Gowans and Knight studying the recirculation of lymphocytes in the rat found that large lymphocytes from thoracic duct lymph localise mainly in the lymphoid tissue of the intestinal mucosa. They suggested that this may be because large lymphocytes originate from gut lymphoid tissue and undergo sensitisation to gut antigens. Griscelli, Vassalli and McCluskey (1969) showed that labelled cells from rat lymph nodes injected into syngenic recipients behaved according to their site of origin. Cells obtained form peripheral lymph nodes localised preferentially in

peripheral nodes and cells obtained from the thoracic duct and mesenteric nodes localised mainly in the intestinal mucosa. These workers suggested that cells which had been sensitised to gut antigens were trapped in the intestinal mucosa and lymphoid tissue where they ultimately developed into plasma cells.

In the neonate, the intestinal mucosa is almost devoid of plasma cells (Bridges, Condie, Zak and Good, 1959). The numbers progressively increase with age. This increase reflects local antigenic stimulus since both germ-free (Abrams, Bauer and Sprinz, 1963) and antibiotic treated (Sprinz, 1962) animals have fewer plasma cells in the intestinal mucosa than control animals.

Crabbe, Carbonara and Heremans (1965) studied the distribution and numbers of IgA, IgM and IgG plasma cells in histologically normal duodenal and jejunal mucosa. Using fluorescein conjugated monospecific antisera they showed that IgA cells outnumbered IgG and IgM cells by a ratio of 10:1.6:1. Subsequently IgD cells (Rowe, Crabbe and Turner, 1968) and IgE cells (Tada and Ishizaka, 1970) have also been identified.

In coeliac disease Soltoft and Weeke (1969) found the total number of plasma cells in the small bowel increased. The predominant cell type was IgA. There was no significant difference between the number of IgA cells either before or after dietary treatment when compared to the controls. However, the IgG and IgM containing cells were significantly increased and unaffected by dietary treatment. This predominance of IgA plasma cells in the coeliac mucosa is in agreement with the findings of Rubin, Fauci, Sleisenger and

Jeffries (1965).

In 19 children with coeliac disease, Savilahti (1972) demonstrated that IgA was the predominant cell type and was significantly increased compared to control values. The numbers of IgM and IgG cells were also significantly increased in coeliac disease. Gluten exclusion resulted in a fall of numbers of all three types of cells to within normal.

However, Douglas, Crabbe and Hobbs (1970) found the IgM cells to be increased markedly in both treated and untreated coeliac disease. The number of IgA cells was not increased and often less than in the control group. Prolonged gluten restriction was accompanied by a decrease of IgM plasma cells toward normal.

Pettingale (1971) investigated the distribution of immunoglobulin containing cells in six control subjects, six untreated patients with coeliac disease and one patient on a gluten free diet. The mean density of the IgA containing cells was significantly reduced in patients with coeliac disease. The predominant cell type was IgM.

There is an association between IgA deficiency and coeliac disease (Asquith et al., 1969), but this only occurs in approximately one in 50 patients, (Beale et al., 1971). Most studies show no quantitative deficiency of IgA immunocytes but rather the reverse. There is however a suggestion of a quantitive defect of the IgA response to antigenic stimulation. Beale et al. (1971) gave oral polio vaccine to five patients with coeliac disease on a gluten free diet and to five patients on a normal diet. The antibody titre was measured four weeks later. Seven of the ten patients had a subnormal response to at least one of the three types of oral polio

vaccine. However the antibody titre was not measured within the different immunoglobulin subclasses so that this study provides suggestive evidence but not conclusive proof of an impaired IgA response.

In coeliac disease Loeb, Strober, Falchuk and Laster (1971) showed increased incorporation by intestinal plasma cells, of L - leucine - ^{14}C into IgA and IgM after gluten challenge. They suggest that a primary immune response may occur.

Beale et al. (1971) suggest that an Arthus-type reaction may occur in coeliac disease. Evidence of immune complex deposition would substantiate this suggestion. Shiner and Ballard (1972) demonstrated deposition of IgA and IgM in the jejunal mucosa in ten of 14 patients with coeliac disease. In three patients there was evidence of concomitant fixation of complement and the findings were interpreted as evidence of an Arthus-reaction.

Relevant to these findings was the study of Agnello, Winchester and Kunkel (1970) who described the C1q precipitation test for the detection of circulating antigen/antibody complexes. Of 91 patients with coeliac disease 30% had a positive test. However the test is also frequently positive in other gastrointestinal disorders such as Crohn's disease and ulcerative colitis (Doe, Booth and Brown, 1973).

In normal subjects the intestinal fluid contains IgA, IgM and IgG in concentrations proportional to the distribution of the IgA, IgM and IgG plasma cells in the mucosa (Tomasi, 1970). IgE and IgD immunoglobulins have not yet been detected in the intestinal juice (Doe, 1972). In patients with coeliac disease there is conflicting evidence concerning

the amounts of immunoglobulin in the intestinal juice.

Savilahti (1972) found no abnormalities in the immunoglobulin content in patients with coeliac disease while Douglas et al. (1970) reported raised IgM levels. Asquith et al. (1970 b) found elevated IgA and IgG levels.

Intestinal antibodies to wheat antigens have been reported by Katz, Kantor and Herskovic (1968) and this finding was confirmed by Ferguson and Carswell (1972). The immunoglobulin class of the antibodies was not demonstrated in either study.

Thus, although, Rubin et al. (1965) were unable to demonstrate the presence of antibodies to gluten in the jejunal mucosa in coeliac disease, the presence of antibodies to gluten in the intestinal juice (Katz et al., 1968; Ferguson and Carswell, 1972) and the demonstration of immune complexes in the small bowel mucosa (Shiner and Ballard, 1972) suggest strongly that immune mechanisms play an important role in the pathogenesis of coeliac disease.

HL - ANTIGEN TYPE IN COELIAC DISEASE

In 1972, Falchuk, Rogentine and Strober, reported the association of HL - A8 leucocyte antigen type and coeliac disease. Evans (1973) made a statistical comparison of the association and was able to conclude that it was highly significant and that the presence of HL - A8 antigen increased the risk of having coeliac disease by a factor of ten.

Gebhard, Katz, Marks, Shuster, Trapani, Rogentine and Strober (1973) suggested that the HL -A8 gene may be linked to another gene such as an "immune response gene" capable of producing anti-gluten antibodies with consequent tissue damage. A second postulate was that the HL - A8 antigen may be the

determinant of a binding site in the gut mucosa, which may be necessary for the production of toxic antibodies.

CHAPTER IV: MATERIAL AND METHODS

IV. A. PURPOSE AND PLAN OF INVESTIGATION

The mechanism by which gluten damages the small bowel is not known. While it is well recognized that immunological abnormalities occur in coeliac disease, there is no unanimity regarding their frequency. There is little evidence on whether these changes are of a primary nature or are secondary.

It was therefore decided to study a group of patients with coeliac disease to ascertain what immunological differences, if any, existed between untreated patients and patients taking a gluten free diet. The patients were compared with normal control subjects and patients with other gastrointestinal disorders. The changes in serum immunoglobulin levels, the frequency of autoantibodies, including antireticulin antibodies and dietary antibodies to wheat proteins were studied. Since there has been only one brief communication regarding the complement system in coeliac disease, C_3 and total haemolytic complement levels were measured.

There have been conflicting reports concerning the predominant immunoglobulin containing cell in the mucosa of the small bowel and the immunoglobulin levels in the jejunal juice. In the present study the distribution of plasma cells in the small bowel mucosa was studied in a larger number of patients and control subjects than previously reported.

Evidence of functional impairment of the humoral and cellular immune systems in patients with coeliac disease was sought by measuring the numbers of circulating lymphocytes bearing immunoglobulin receptors , the antibody responses to S.typhi and Tetanus toxoid, phytohaemagglutinin induced

lymphocyte transformation and the reaction to intradermal skin tests.

The antigenicity of gluten was assessed by tests for dietary antibodies in peripheral blood and jejunal juice and the effect of gluten fraction III on lymphocyte transformation.

IV.B. MATERIAL

One hundred and nineteen patients with gastrointestinal disorders were studied. They comprised the following categories:

1. Coeliac Disease (C.D.) (50)
2. Crohn's Disease (Cr.D.) (15)
3. Ulcerative Colitis (U.C.) (31)
4. Irritable Colon Syndrome (I.C.S.) (23)

1. COELIAC DISEASE

The case notes were examined of all patients in whom a diagnosis of coeliac disease had been made, from 1965 onwards, at The Queen Elizabeth Hospital and at the Adelaide Children's Hospital. The patients (or their parents) were contacted either by the consultant physician in whose care they were or by letter (Appendix A). Home visits were made in a majority of cases included in this study. The nature of coeliac disease, the purpose of the study, and the importance of a gluten free diet were discussed with the families. In some patients the diagnosis of coeliac disease had been made only on clinical evidence. The importance of making a histological diagnosis was explained.

Some patients were referred by general practitioners, physicians, or by other patients already taking part in the study.

Patients on a gluten free diet, in whom a histological diagnosis had already been made were informed that the studies were for purposes of research.

In this study the patients with coeliac disease were divided into 3 groups.

GROUP I:

Ten patients were investigated while on a normal diet only. There were three males and seven females; eight were children (Fig. 1).

GROUP II:

Ten males and thirteen females, with a mean age of 32 years were studied before and after the institution of a gluten free diet (Fig. 2). The mean duration of treatment before the study was repeated was 15 months.

GROUP III:

This group consisted of 17 patients studied while on a gluten free diet only, all of whom had been on this diet for more than two years. The mean age of the eight male and nine female subjects was 24 years (Fig. 3).

CROHN'S DISEASE (Cr.D.)

There were fifteen patients (males 5 and females 10) with Crohn's Disease (Fig. 4). The diagnosis had been made on radiological and/or histological grounds. Seven of the 15 patients had involvement of the small bowel.

ULCERATIVE COLITIS (U.C.)

The thirtyone patients in this group consisted of 13 males and 18 females (Fig. 5). The diagnosis had been made on the basis of sigmoidoscopy, radiology and histology. The mean age of the patients in this group was 44 years.

IRRITABLE COLON SYNDROME (I.C.S.)

Twentythree patients (9 males and 14 females) had the

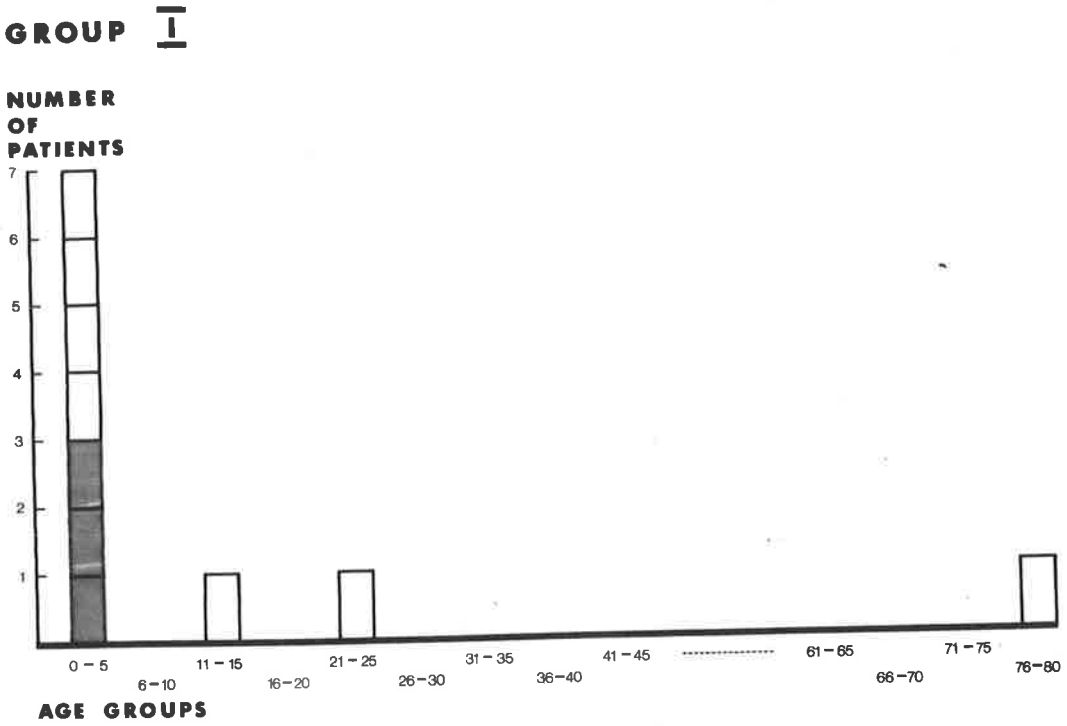

Figure 1. The age and sex distribution of ten patients with coeliac disease (Group I). Male patients are represented by hatched columns.

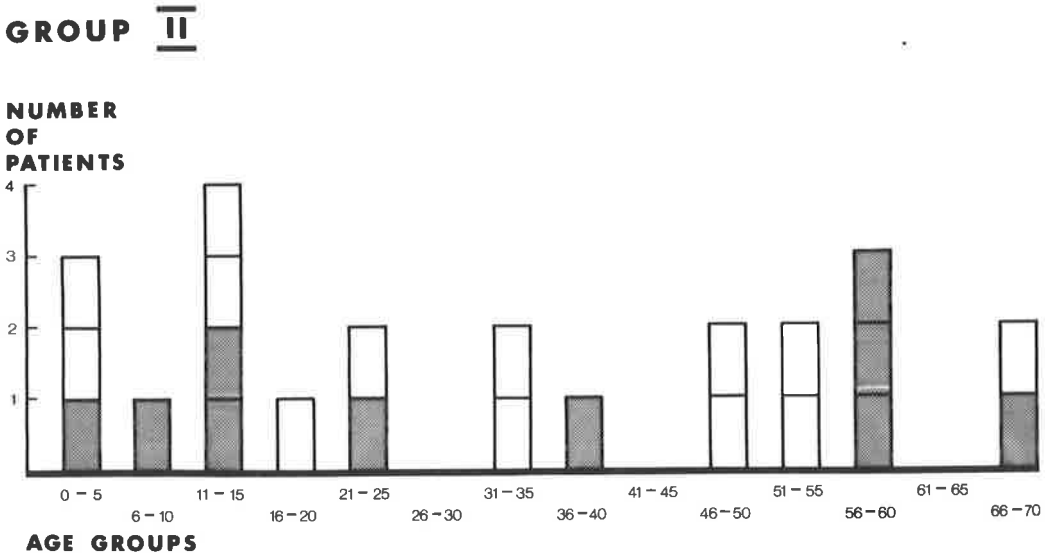

Figure 2. The age and sex distribution of 23 patients with coeliac disease (Group II). Male patients are represented by hatched columns.

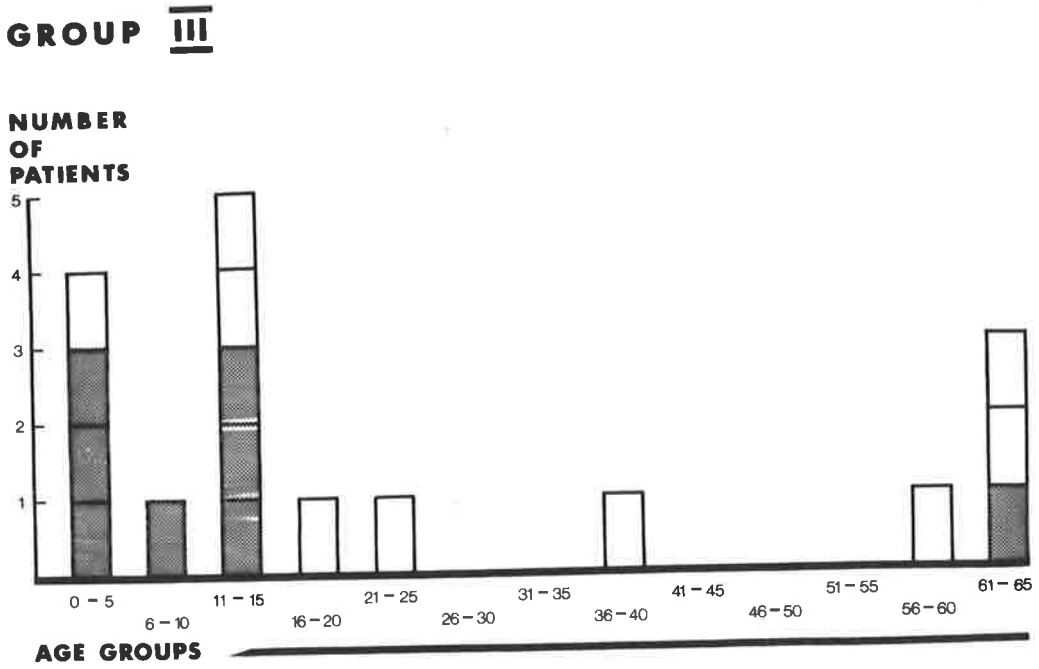

Figure 3. The age and sex distribution of 17 patients with coeliac disease (Group III). Male patients are represented by hatched columns.

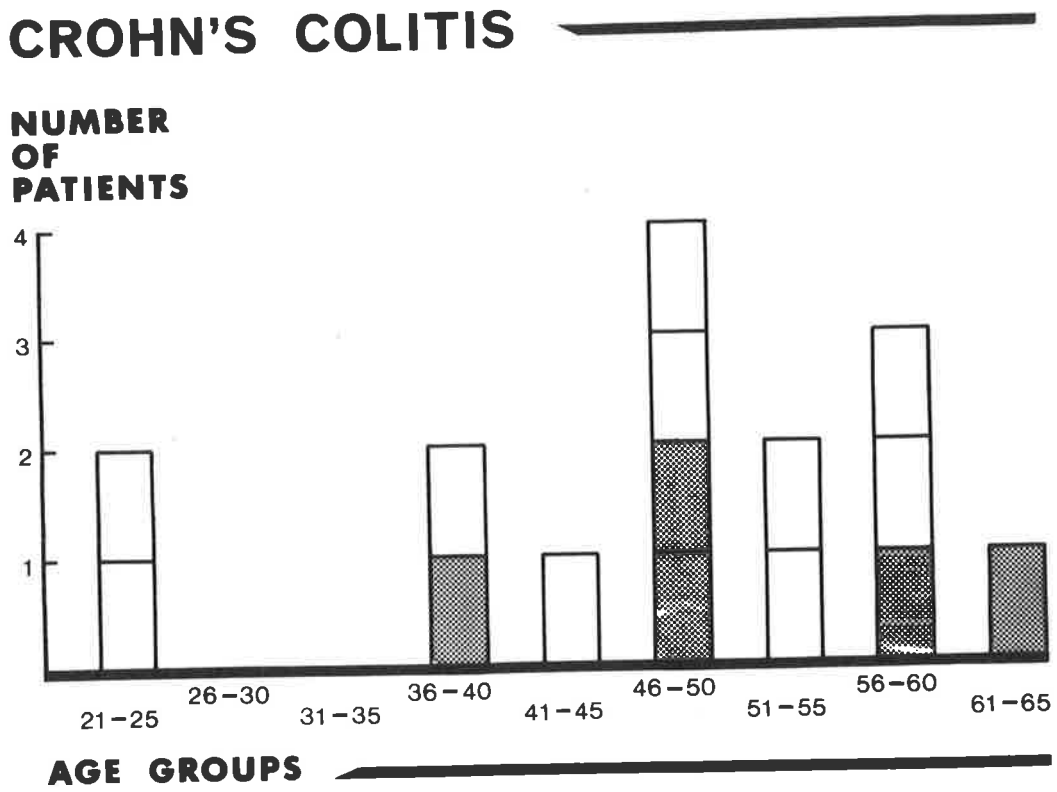

Figure 4. The age and sex distribution of 15 patients with Crohn's Disease. Male patients are represented by hatched columns.

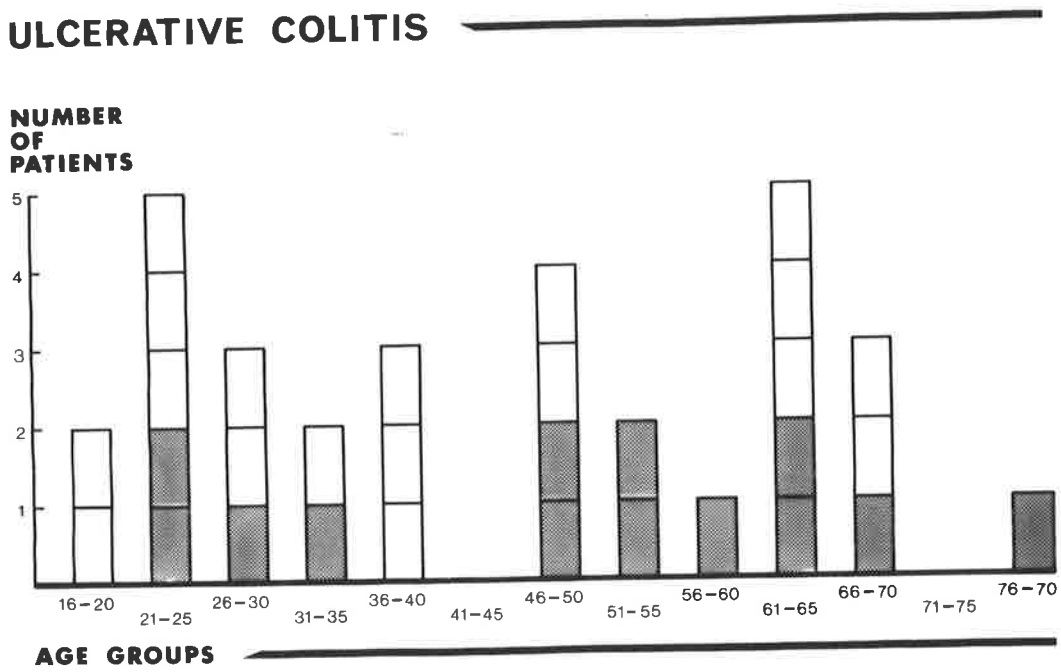

Figure 5. The age and sex distribution of 31 patients with ulcerative colitis. Male patients are represented by hatched columns.

irritable colon syndrome (Fig. 6). The mean age of the group was 45 years. These patients had abdominal pain and either diarrhoea or constipation. The presence of organic disease had been excluded by extensive investigation.

For practical reasons it was not possible to carry out all the immunological tests on every patient. Listed below are the numbers of patients in whom each test was performed.

A. TESTS ON SERUM

1. Serum immunoglobulin levels

(a)	Coeliac Disease	- 50
(b)	Crohn's Disease	- 15
(c)	Ulcerative Colitis	- 31
(d)	Irritable Colon Syndrome	- 23
(e)	Control subjects	- 246

2. Serum Complement

(i) Total haemolytic complement

(a)	Coeliac Disease	- 46
(b)	Crohn's Disease	- 11
(c)	Ulcerative Colitis	- 28
(d)	Irritable Colon Syndrome	- 20

(ii) Serum Complement (C_3)

(a)	Coeliac Disease	- 47
(b)	Crohn's Disease	- 15
(c)	Ulcerative Colitis	- 31
(d)	Irritable Colon Syndrome	- 23
(e)	Control subjects	- 246

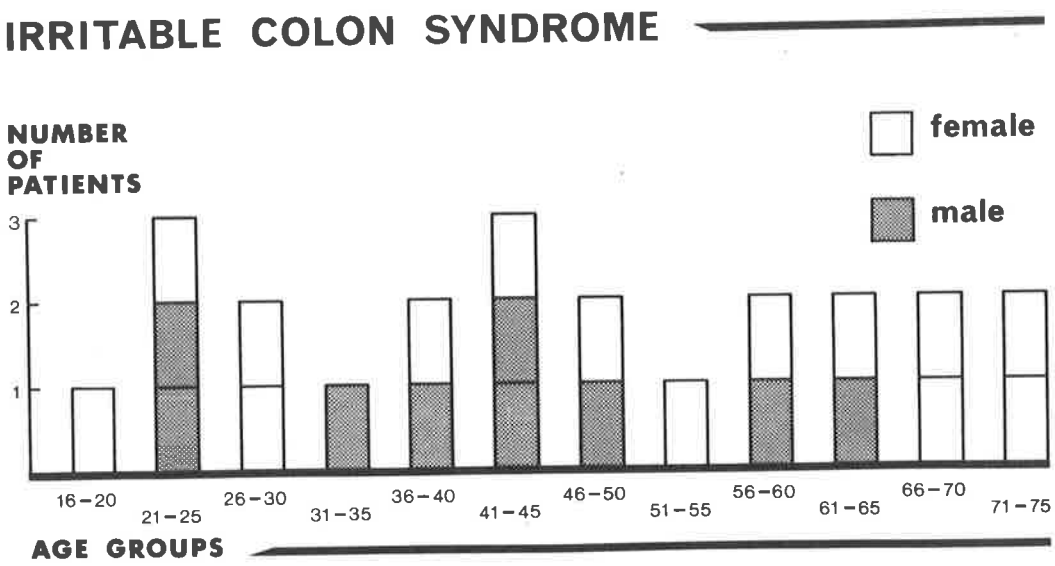

Figure 6. The age and sex distribution of 23 patients with the irritable colon syndrome.

3. Auto antibodies including Antireticulin antibodies.

(a) Coeliac Disease - 49

(b) Crohn's Disease - 15

(c) Ulcerative Colitis - 31

(d) Irritable Colon Syndrome - 23

4. Dietary Antibodies

(a) Coeliac Disease - 49

(b) Crohn's Disease - 15

(c) Ulcerative Colitis - 31

(d) Irritable Colon Syndrome - 23

5. Antibody responses to immunisation with Tetanus toxoid and S.typhi.

(a) Coeliac Disease - 12

(b) Control Subjects - 12

B. TESTS ON LYMPHOCYTES

1. B cells

(a) Coeliac Disease - 15

(b) Control Subjects - 29

2. Lymphocyte transformation

(a) Coeliac Disease - 18

(b) Control Subjects - 84

C. TESTS ON SMALL BOWEL

1. Jejunal Mucosal Plasma Cells

(a) Coeliac Disease - 29

(b) Control Subjects - 28

2. Jejunal Juice

(1) Dietary antibodies

(a) Coeliac Disease - 15

(b) Control Subjects - 29

(2) Immunoglobulin levels

(a) Coeliac Disease - 15

(b) Control Subjects - 29

D. DELAYED HYPERSENSITIVITY

1. Intradermal skin tests

(a) Coeliac Disease - 12

(b) Control Subjects - 11

IV.C. METHODS

1. HAEMATOLOGICAL EXAMINATION

1.1 Haemoglobin and packed cell volume were measured in a Model S Coulter Counter.

1.2 Peripheral blood films were stained with May - Grünwald - Giemsa stain.

2. ASSESSMENT OF NUTRITIONAL STATUS

2.1 Serum vitamin B_{12} was estimated by the micro-biological assay method of Hutner, Bach and Ross (1956) using Euglena gracilis 'Z' strain.

2.2 Serum folic acid was also measured by a micro-biological method using Lactobacillus casei. (Baker, Herbert, Frank, Pasher, Hutner, Wasserman and Sobotka, 1959).

2.3 Serum iron was measured using a Technicon Auto Analyser series I as was total iron binding capacity. The method described by Giovanniello, Di Benedetto, Palmer and Peters (1967) was followed.

2.4 Serum total protein and albumin were measured with a Technicon SMA 12/60 using the Biuret reaction for total protein measurement and the Bromo - cresol - green (BCG) binding method for albumin.

3. TESTS OF SMALL BOWEL FUNCTION

3.1 D-xylose absorption.

The patient fasted overnight and next morning took five grams of xylose in 300 mls of water. Urine was collected for the next five hours in plastic containers containing 5 gms of benzoic acid. Urinary xylose was estimated by the method

described by Roe and Rice (1948).

3.2 Three day faecal fat.

The patient took a diet containing 70 g of fat per day, on the day before the test was begun and for the next three days. The seventy-two hour faecal fat excretion was estimated using dried faeces (Varley, 1967).

3.3 One stage prothrombin time was measured by the method of Quick (1935).

4. SMALL BOWEL HISTOLOGY

Jejunal biopsy was performed with the Quinton, multi-purpose suction biopsy tube and capsule (Model C - 093 - 4 Quinton Instrument Company, Seattle, Washington, U.S.A.).

The specimens were taken 10 - 20 cm distal to the ligament of Treitz. The location of the capsule was verified with the aid of an image intensifier (Siemens, Type RBV7). With the Quinton capsule it was possible to obtain at least two specimens. Both were placed on filter paper and the orientation was checked with a dissecting microscope. The specimen which was to be used for immunofluorescent studies was snap frozen on dry ice, embedded in Tissue-Tek compound (Ames Company, Division Miles Laboratories Inc., Indiana, U.S.A.) and stored in airtight plastic containers at $-70^{o}C$. The specimen which was to be used for routine histology was fixed in formalin and sections 5 μ thick were stained with haematoxylin and eosin. The appearance of the small bowel mucosa was classified as follows:-

1. Within normal limits
2. Partial villous atrophy
3. Sub total villous atrophy
4. Total villous atrophy

5. IMMUNOLOGICAL METHODS

5.A TESTS ON SERUM

5.A.1 SERUM IMMUNOGLOBULIN LEVELS

Immunoglobulins, were quantitated by the single radial immunodiffusion method of Mancini, Carbonara and Heremans (1965) using Partigen Plates (Behringwerke AG, Marburg, West Germany).

5.A.2 SERUM COMPLEMENT

(i) Total Haemolytic Complement

This was measured by a modification of the method described by Kwapinski (1964), using a 2% suspension of sheep red cells sensitized with 5 MHD (Minimum Haemolytic Dose) of rabbit anti-sheep haemolysin. Patients sera were dispensed into eight tubes ranging in volume from 0.1 ml to 0.4 ml. The volume of each tube was then made up to 0.5 ml with buffered diluent. 0.5 ml of the sensitized red cells were added to each tube and to a control tube, containing 0.5 ml of buffered diluent. The tubes were mixed by hand and incubated at $37^{\circ}C$ for thirty minutes, with remixing at fifteen minutes.

Reading:

The end point tube is that which containedthe least amount of serum showing complete haemolysis.

Calculation:

The result is expressed as a percentage and calculated as follows:-

$\frac{\chi}{0.5} \times 100$ where χ is the volume of diluent used in the end point tube. 0.5 represents the volume of diluent in the control tube. The normal range is 90% - 96% of haemolysis.

(ii) C_3 Levels

The C_3 component of complement was measured by radial immunodiffusion using commercial plates (Behringwerke AG, M-Partigen - C_3 (B_1A - globulin). The serum to be tested was kept at room temperature for at least two days prior to testing so that all B_1C in the serum would be converted to B_1A.

5.A.3 AUTOANTIBODIES INCLUDING ANTIRETICULIN ANTIBODIES

An indirect immunofluorescent technique similar to that described by Taylor, Roitt, Doniach, Couchman and Shapland (1962) and Beck (1961) was used to detect antibodies to gastric parietal cells, to smooth muscle and to cell nuclei.

Method:

Six μ cryostat sections of liver tissue and gastric mucosa were placed on glass slides, pre-cleaned with alcohol. The liver sections for detection of ANF were done in duplicate and one of the sections was fixed in absolute alcohol for three minutes at room temperature.

The sections were covered with a 1:4 dilution of the serum to be tested and incubated in a moist chamber at 37°C for 30 minutes. The sections were washed three times with Coon's buffer pH 7.1, air dried and covered with fluorescein conjugated antihuman globulin (Progressive Laboratories Inc., Baltimore, U.S.A.), which had been absorbed with rat liver homogenate before use. Incubation was again carried out at 37°C for 30 minutes in a moist chamber and the slides washed thrice with Coon's buffer and air dried. The sections were mounted in a 1:1 mixture of Coon's buffer and glycerol and covered with cover slips.

They were examined under incident ultraviolet light with a Zeiss fluorescence microscope using a mercury vapour lamp, a BG12 excitation filter and a 500 mμ barrier filter.

ANTIRETICULIN ANTIBODIES (ARA)

ARA were detected by the method described by Seah, Fry, Hoffbrand and Holborow (1971, a).

Fresh, unfixed, sections of rat kidney, 2 μ thick were used as antigen. The sera were tested at a dilution of 1:10 in saline by the indirect immunofluorescence technique described above.

The fluorescein conjugated antihuman globulin (Progressive Laboratories Inc., Baltimore, U.S.A.) was absorbed with rat liver homogenate and used in a dilution of 1:5, in order to abolish non-specific fluorescence.

5.A.4 DIETARY ANTIBODIES

A modification of the method of agar gel electrophoresis and double diffusion as described by Rossipal (1971) was used.

Glass slides, 9 cm by 7 cm were washed in 5% Decon 90 concentrate solution (Decon Laboratories Ltd., Brighton 1, U.K.) for 48 hours. They were then rinsed six times in tap water, thrice in distilled water and once in deionised water. A 2% agar solution was prepared using 2 gm of agarose (Sigma Chemical Co., St. Louis, Mo. U.S.A.) in 100 ml of sodium veronal buffer, pH 8.2. The ionic strength of the solution was 0.05. The mixture was boiled at 100^{o}C in a steam bath for half an hour until all the agar had dissolved. Ten ml of the agar solution was pipetted onto each glass slide to form a thin layer. After the agar had set, 30 wells, 4 mm in diameter and 8 mm apart were punched out.

First electrophoresis was carried out for 30 minutes at 40 volts in a perspex electrophoresis tank and Heathkit variable voltage regulated power supply (model IP-17, Heath Company, Benton Harbor, Michigan). The agar plates were supported above the level of the buffer (sodium veronal pH 8.2 and ionic strength 0.05). One end of a microporous wick (Gelman Instrument Company, Ann Arbor, Michigan, U.S.A.) was placed over the edge of the slide

while the other end was immersed in the buffer. On completion of electrophoresis, the reservoirs adjacent to those which contained the serum were filled with the antigens.

The following antigens were used:-

(a) Gluten Fraction III (GF III)

(b) Gliadin

(c) Cow's milk

Gluten Fraction III, a peptic-tryptic digest of gluten (Ajax Chemicals Ltd.) was prepared as described by Frazer et al. (1959). It was used at a concentration of 5% in phosphate-saline buffer (pH 6.4). A 10% solution of gliadin (Ajax Chemicals Ltd.) was made up in phosphate-saline buffer (pH 6.4) as described by Kivel et al. (1964). Fresh cow's milk was used as spray-dried preparations have been found to be less antigenic (Freier, Kletter, Gery, Lebenthal and Geifman, 1969).

After the antigens were added the agar plates were kept in a humid atmosphere for 24 hours. They were then washed in physiological saline twice over a period of 24 hours and placed in distilled water for the next 48 hours. After air-drying, they were stained with 0.5% amido-black. Excess stain was removed by washing in Methanol/acetic acid in the ratio 9:1. Each plate was examined for precipitin lines under a bench illuminated magnifier. A positive control serum was used for each plate.

5.A.5 ANTIBODY RESPONSES TO IMMUNISATION WITH TETANUS TOXOID AND S.TYPHI

Immunisation was done with monovalent Salmonella typhi vaccine 0.1 ml. Commonwealth Serum Laboratories (C.S.L.) given subcutaneously and with 0.5 ml of soluble Tetanus Toxoid (C.S.L.) 8 Lf in 1.0 ml given intradermally. Blood was taken before and two weeks after immunisation.

Tetanus antibodies were measured by haem-agglutination, as described by Gold and Fudenburg (1967) using Group 'O' Rh negative human red blood cells, on to which Tetanus Toxoid had been attached with chromic trichloride.

Antibody to S.typhi 'H' was measured by the agglutination of bacteria using S.typhi suspensions from C.S.L.

5.B. TESTS ON LYMPHOCYTES

5.B.1 MEASUREMENT OF B CELLS IN PERIPHERAL BLOOD LYMPHOCYTES

Purified lymphocyte suspensions were prepared using a modification of the method described by Froland and Natvig (1970).

Nine per cent Ficoll (Pharmacia) and 33.9% Hypaque (Winthrop) were mixed in a ratio of 24 parts of Ficoll to ten parts of Hypaque. Blood was diluted in twice its own volume of Dulbecco phosphate buffer (CSL) and 27 ml of this suspension was layered onto 11 ml of the Ficoll/

Hypaque mixture in 50 ml glass centrifuge tubes. The tubes were centrifuged at 400 g for 40 minutes and the white cell layer was removed and washed three times in 1 ml of Dulbecco phosphate buffer. The white cell suspension contained approximately 98% lymphocytes and over 98% of these were viable as assessed by Trypan blue exclusion. A yield of approximately 5×10^6 lymphocytes per millilitre was obtained. The suspension was diluted with foetal calf serum (FCS) so that approximately 1×10^6 lymphocytes were added to each culture.

5.B.2 STUDIES OF PERIPHERAL LYMPHOCYTES USING FLUORESCEIN ISOTHIOCYANATE CONJUGATED GF III (FITC-GF III)

Conjugation of GF III

GF III was conjugated by the method described by Fothergill (1969). One hundred mls of GF III solution (200 mg/ml) were added to 200 mls of Carbonate-Bicarbonate buffer (pH 9.0) and stirred in the cold for half an hour using a magnetic stirrer. When the solutions were thoroughly mixed 100 mls of Fluorescein isothiocyanate (FITC) solution (10 mg/ml in carbonate-bicarbonate buffer pH 9.0) were added dropwise from a dropping funnel over a 16 hour period while the mixture was carefully stirred in the Cold Room.

When conjugation was complete, the solution was dialysed in the cold for three days in

phosphate buffered saline 7.4;then small aliquots were passed through G-25 Sephadex columns and the first coloured eluant was collected and kept in each case. Unconjugated FITC was retarded in the G-25 Sephadex due to its small molecular size. The volume of the conjugate collected was measured in each case. The concentration of the FITC - GF III eluant was 20 mg/ml (1:10 solution).

Immunofluorescent Technique:

Fluorescein conjugated antihuman globulin (0.3 ml) and FCS (0.1 ml) were added to 0.2 ml of the washed suspension. The antisera used were, firstly, Horse Antihuman Globulin (Progressive Laboratories Inc., Baltimore, U.S.A.) and secondly Goat Antihuman IgG, IgM and IgA (Hyland Div., Travenol Labs. Inc., California). The mono-specificity of the conjugates was verified by immuno-electrophoresis. After incubation with the fluorescein conjugated antiserum at $4^{\circ}C$ for one hour, the lymphocyte suspension was washed three times in the cold with phosphate buffer (pH 7,4, 0.018M), centrifuging each time for three minutes at 250 g. The suspension was then allowed to stand for ten minutes at $37^{\circ}C$. Cell counts were made with a Zeiss Universal Microscope, equipped for incident ultraviolet light and transmitted white light for phase contrast. The number of fluorescent cells in a high power field were counted and the total number of

lymphocytes in the same field was counted by phase microscopy.

5.B.3 LYMPHOCYTE TRANSFORMATION

(i) Phytohaemagglutinin Induced Lymphocyte Transformation

A modification of the whole blood micro-technique described by Junge, Hoekstra, Wolfe and Dienhardt (1970) was used. Phytohaemagglutinin (PHA, Wellcome reagent grade) was reconstituted with sterile saline. An aliquot was diluted with medium 199 (C.S.L.) so that 3 ml of the medium contained 0.02 ml of PHA. A whole blood suspension was prepared containing 20% blood, 40% serum (foetal calf serum (FCS); C.S.L.) and 40% medium 199. Cultures were set up in triplicate in sterile plastic culture tubes. Each culture contained 1.0 ml of the whole blood suspension described above and 3.0 mls of the PHA preparation. The cultures were incubated at 37°C for 92 hours at which time 2.5 µci tritiated thymidine (^{3}HT, Amersham, specific activity 500 mCi/m Mol) was added in a volume of 0.1 ml. The incubation was continued for a further four hours at which time DNA synthesis was terminated by holding the cultures at 4°C for 30 minutes.

(ii) Effect of GF III on PHA Induced Lymphocyte Transformation

The cultures were set up as described above. Varying amounts of GF III (10 µg to

1000 µg) were added to sets of triplicate cultures and the response to PHA was determined.

Preparation for counting:

The cultures were transferred to 10 ml plastic centrifuge tubes with 0.9% saline (2 ml) and centrifuged at 250 g for ten minutes at 4°C. The supernatant was removed and the cell button resuspended with 0.9% saline (4 ml) and re-centrifuged. After removal of the supernatant the erythrocytes were lysed by adding 4 ml of 3% acetic acid and the cultures centrifuged again. Following removal of the supernatant and a further saline wash, the tritium labelled DNA protein of the lymphocytes was precipitated by adding 4 ml of 10% trichloro-acetic acid (TCA). The cultures were allowed to stand overnight at 4°C and then centrifuged at 850 g for 20 minutes. The supernatant was removed and the precipitate washed once with 5% TCA (4 ml) and twice with absolute methanol (4 ml). The supernatant was removed and the dried residue was dissolved in 0.5 ml Soluene (Packard) and transferred to glass scintillation vials with 10.0 ml of scintillation fluid. The vials were left in the dark at 4°C overnight and counted in a Packard Tricarb liquid scintillation spectrometer (Packard Model 331-, Packard Instrument Co., Inc., U.S.A.). The counts obtained were corrected for quenching and recorded as disintegrations per minute (dpm).

5.C TESTS ON SMALL BOWEL

5.C.1 JEJUNAL MUCOSAL PLASMA CELLS

Although most of the biopsies were done with the Quinton capsule, some biopsies were performed at the Adelaide Children's Hospital using a Crosby capsule.

Fluorescein conjugated monospecific goat antisera to human IgA, IgM and IgG were obtained commercially (Hyland). The conjugates were centrifuged further and then purified by passage through a Sephadex G-25 column before use. Their monospecificity was verified by immunoelectrophoresis.

Immunocytochemical studies were carried out by the method described by Odgers and Wangel (1968). Six to twelve serial frozen sections, 6 μ thick were cut in a Harris cryostat (International Equipment, Massachusetts, U.S.A.) at right angles to the mucosal surface. The sections were air-dried. Unfixed sections and sections fixed with acetone for five minutes at room temperature gave equally satisfactory results in preliminary studies and therefore no fixation was done in the main study.

The mucosal cells were stained by the direct immunofluorescent technique (Nairn, 1969). The first three serial sections were stained with the three monospecific antisera. Incubation was done at 37^{o}C for 30 minutes. The excess conjugate was removed by three washes with

Coon's buffer (pH 7.1). The sections were air-dried, mounted with glycerol - Coon's buffer in the ratio 1:1 and covered with a cover slip. The fourth serial section was stained with haematoxylin and eosin.

Microscopy and Measurements:

A Leitz - Wetzlar ultraviolet microscope with an 'HBO 200' lamp as the light source, a 'UGI' exciting filter and a 'K 430' absorption filter, was used for counting the cells. The total number of fluorescent cells in each section was counted under high power. Initial studies showed little variation between the counts obtained by two separate observers and later counts were therefore done by a single observer.

The section which had been stained with haematoxylin and eosin was projected onto millimetre squared graph paper using a projecting microscope. The entire section was drawn by hand on the graph paper and cut out with iris scissors. The area of the paper cut out was weighed and a known area of the graph paper was weighed at the same time. The area of the image of the section was calculated and the actual area obtained by dividing this value by the magnification. The cell counts were expressed per square millimetre of mucosal surface.

5.C.2 JEJUNAL JUICE

(i) Immunoglobulin Levels

The intestinal juice was aspirated and stored as described by Plaut and Keonil (1969). The fasting subjects swallowed a polythene tube which was attached to a perforated, metal collecting tip. The tip was placed a few centimetres beyond the ligament of Treitz under fluoroscopic control. The juice was collected in a sterile glass container placed in ice. Samples which contained traces of blood as shown by testing with an impregnated paper strip (Labstix) were not used.

The concentration of IgG, IgA and IgM was measured using commercially obtained immunoplates for low level IgG, IgG, IgA and IgM assays (Hyland Division, Travenol Laboratories Costa Mesa, California, U.S.A.). The serum IgA standard used was not specific for secretory IgA.

(ii) Dietary Antibodies

The method employed to detect dietary antibodies was similar to that described in pp.72.

5.D DELAYED HYPERSENSITIVITY

5.D.1 INTRADERMAL SKIN TESTS

Skin tests were performed by injecting 0.1 ml of each of the following antigens intradermally in the forearm:-

(i) Candida albicans 0.5% (Bencoard)

(ii) Streptokinase - Streptodornase ('Varidase', Lederle) which contains Streptokinase 10 units and Streptodornase 2.5 units.

(iii) Mumps skin test antigen (Eli Lilly, Indianapolis).

Results:

The results of the skin test were read at 48 hours by measuring the diameter of both the induration and erythema in two diameters.

With the mumps antigen induration of at least 6 mm was regarded as positive. With the other two antigens both induration and erythema of at least 6 mm had to be present for the test to be read as positive.

THE RESULTS OF THE HAEMATOLOGICAL EXAMINATION, ASSESSMENT OF NUTRITIONAL STATUS, TESTS OF SMALL BOWEL FUNCTION AND SMALL BOWEL HISTOLOGY ARE GIVEN IN APPENDIX B.

6. STATISTICAL METHODS

1. The significance of differences in the means of two populations of small size (less than 200 samples) was calculated using Student's "t" test (Paradine and Rivett, 1960).

2. The paired Student's "t" test was performed as described by Bailey (1973a).

3. Chi-squared was calculated from two row contingency tables using Brandt and Snedecor's formula (Bailey, 1973b).

4. The Mann-Whitney U test was performed as described by Siegel (1956).

5. Two by two table comparisons were made using Fisher's Exact test (Bailey, 1973c).

6. Correlation co-efficients were calculated as described by Bailey (1973d).

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4. Siegel, S. (1956), in Nonparametric Statistics for the behavioural sciences. McGraw-Hill Book Co. Inc., International Student Edition, pp.116.

5. Bailey, N.T.J. (1973c), in Statistical Methods in Biology. English Universities Press Ltd., London, pp.61.

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CHAPTER V: RESULTS AND DISCUSSION

V.A. Tests on Serum

V. A. TESTS ON SERUM

A.1 SERUM IMMUNOGLOBULIN LEVELS

CONTROL SUBJECTS:

RESULTS:

The immunoglobulin levels of each patient with gastrointestinal disease were compared with 10 age and sex matched healthy control subjects, who were hospital employees or visitors. The control subjects of each sex were grouped in intervals of five years spanning 11 - 70 years. The frequency distribution of the immunoglobulin levels displayed visual asymmetry (Fig. A.1 - A.3) and were transformed logarithmically. The geometric mean (± 2 S.D.) of ranges of values at five year age intervals were obtained (Table A.I).

PATIENTS WITH GASTROINTESTINAL DISEASE

The individual values are shown in Appendix C. The immunoglobulin levels were converted to logarithms and compared with the logarithmic mean range (mean ± 2 S.D.) in ten age and sex matched control subjects. No comparisons were made of values in patients over 70 years as suitable control subjects could not be obtained and for the same reason male patients between the age of 6 - 10 years were compared with only six age matched control subjects. It was therefore possible to compare only 21 of 33 patients on a normal diet and 31 of 40 patients on a gluten free diet.

SERUM IgG (TABLE A.II)

Of the 21 patients on a normal diet two patients had

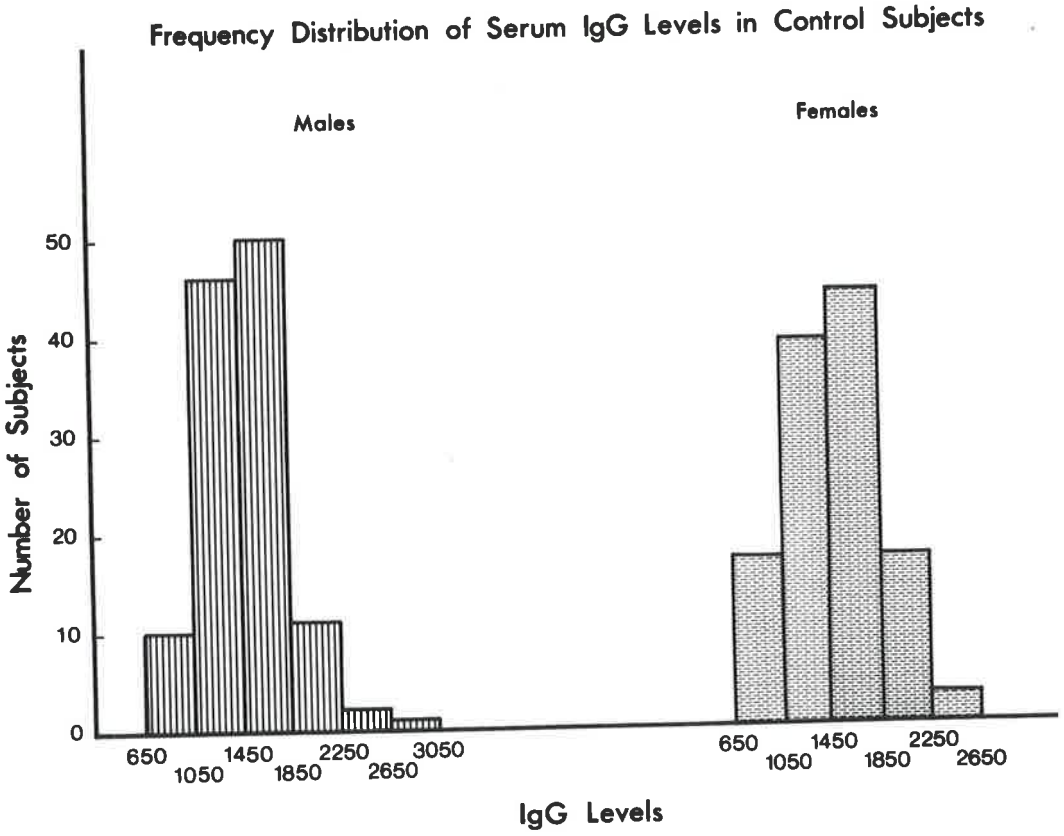

Figure A.1. Histogram of serum IgG levels in male and female control subjects.

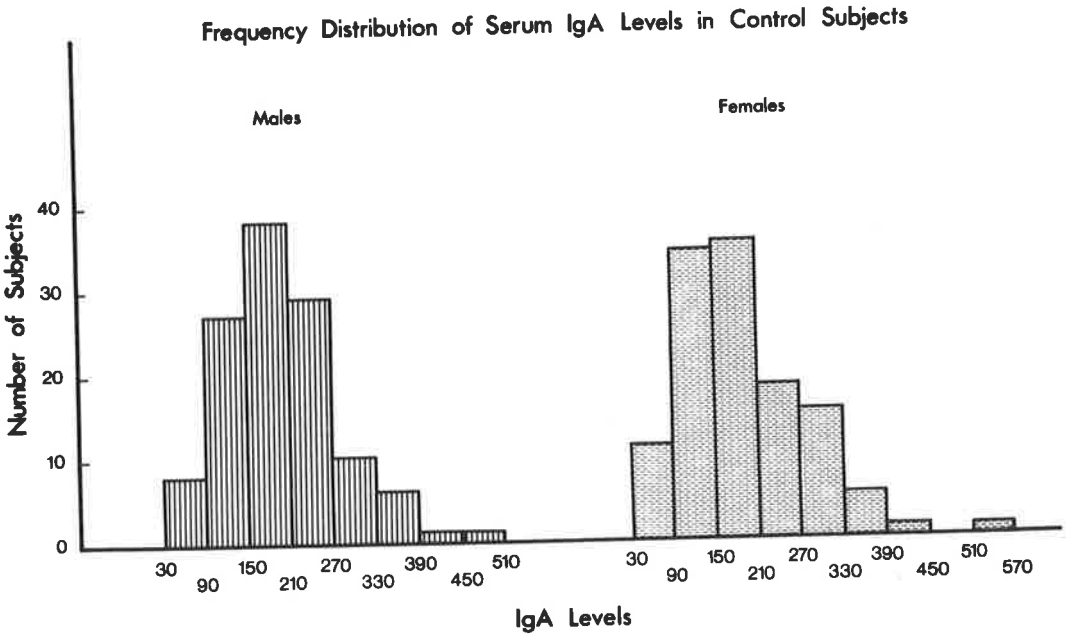

Figure A.2. Histogram of serum IgA levels in male and female control subjects.

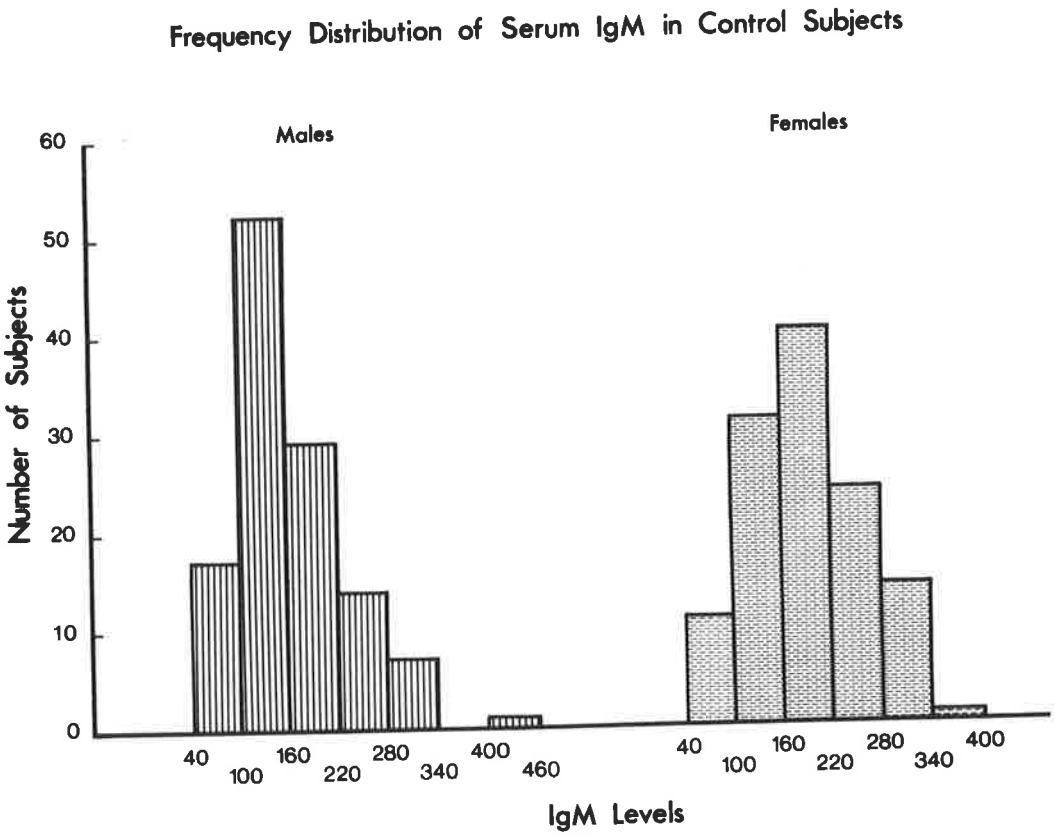

Figure A.3. Histogram of serum IgM levels in male and female control subjects.

IgG levels greater than 2 S.D. above and two patients levels less than 2 S.D. below the control mean. On a gluten free diet, five of 31 patients had levels above the normal range and 10% below the normal range.

SERUM IgA (TABLE A.III)

Five of the 21 patients on a normal diet and two of 31 patients on a gluten free diet had IgA levels above normal. In the latter group, four patients had IgA values below normal and three of these, C.D.4, C.D.5 and C.D.36 suffered from IgA deficiency.

SERUM IgM (TABLE A.IV)

Of the patients on a normal diet 19% had low IgM levels compared to 23% of patients on a gluten free diet. IgM levels above the normal range were not detected in either group.

EFFECT OF GLUTEN FREE DIET ON IMMUNOGLOBULIN LEVELS

The serum immunoglobulin levels were estimated at approximately four monthly intervals in 23 patients with coeliac disease after instituting a gluten free diet. The patients were studied for a mean of 15 months. A regression analysis was done to examine the effect of time on immunoglobulin levels. Although there was a fluctuation in the immunoglobulin levels, there was no significant difference, in IgG, IgA or IgM levels ($t = 2.093$, N.S.). However comparing the IgM values in each patient before and after treatment there was a significant rise ($t = 3.5796$, $P<0.005$, paired Student's t test). There was no significant change in IgG ($t = 1.0518$, N.S.) or IgA ($t = 1.0722$, N.S.).

TABLE A.I THE GEOMETRIC MEAN (± 2 S.D.) OF SERUM IMMUNOGLOBULIN LEVELS IN CONTROL SUBJECTS (MG/100 ml.)

AGE GROUP	NO. OF SUBJECTS	MALES			FEMALES		
		IgG	IgA	IgM	IgG	IgA	IgM
6-10	6	915 (592-1411)	93 (49-175)	115 (50-263)	-	-	-
11-15	10	1309 (836-2051)	135 (63-289)	121 (87-170)	1596 (1380-1846)	131 (71-208)	180 (89-364)
16-20	10	1508 (984-2310)	153 (73-320	169 (117-244)	1329 (967-1830)	138 (51-376)	204 (120-346)
21-25	10	1375 (839-2256)	210 (109-407)	189 (91-395)	1570 (857-2876)	171 (84-346)	189 (115-311)
26-30	10	1615 (942-2768)	183 (81-415)	167 (76-365)	1381 (816-2341)	178 (80-399)	131 (39-443)
31-35	10	1526 (878-2652)	194 (108-347)	164 (94-286)	191 (553-662)	197 (94-411)	109 (74-162)
36-40	10	1373 (893-2110)	209 (96-453)	96 (74-124)	1603 (910-2822)	188 (85-415)	176 (66-472)

.../cont.

TABLE A.I THE GEOMETRIC MEAN (± 2 S.D.) OF SERUM IMMUNOGLOBULIN LEVELS IN CONTROL SUBJECTS (MG/100 ml.) (cont.)

AGE GROUP	NO. OF SUBJECTS	MALES			FEMALES		
		IgG	IgA	IgM	IgG	IgA	IgM
41-45	10	1486 (930-2398)	169 (50-572)	121 (65-226)	1406 (1028-1921)	203 (116-356)	179 (86-371)
46-50	10	1533 (932-2524)	208 (87-496)	158 (73-342)	1316 (808-2142)	152 (74-311)	179 (85-379)
51-55	10	1292 (942-1772)	194 (90-418)	115 (36-369)	1451 (619-3298)	209 (121-360)	155 (73-327)
56-60	10	1465 (900-2384)	159 (74-341)	178 (77-322)	1450 (863-2430)	203 (112-366)	177 (76-408)
61-65	10	1515 (998-2299)	191 (10-391)	188 (104-343)	1535 (910-2589)	193 (91-643)	120 (29-643)
66-70	10	1601 (1034-2478)	203 (84-494)	90 (9-929)	1529 (93-2551)	237 (78-725)	160 (106-240)

TABLE A.II SERUM IgG LEVELS IN PATIENTS WITH GASTROINTESTINAL DISEASE, COMPARED WITH TEN AGE AND SEX MATCHED SUBJECTS.

GROUP	NUMBER OF PATIENTS	IN NORMAL RANGE	ABOVE 2 S.D. LIMIT	BELOW 2 S.D. LIMIT
COELIAC DISEASE (NORMAL DIET)	21	17 (81%)	2 (10%)	2 (10%)
COELIAC DISEASE (GLUTEN FREE DIET)	31	23 (74%)	5 (16%)	3 (10%)
CROHN'S DISEASE	15	13 (87%)	1 (7%)	1 (7%)
ULCERATIVE COLITIS	30	26 (87%)	3 (10%)	1 (3%)
IRRITABLE COLON SYNDROME	21	20 (95%)	Nil	1 (5%)

TABLE A.III SERUM IgA LEVELS IN PATIENTS WITH GASTROINTESTINAL DISEASE, COMPARED WITH TEN AGE AND SEX MATCHED SUBJECTS.

GROUP	NUMBER OF PATIENTS	IN NORMAL RANGE	ABOVE 2 S.D. LIMIT	BELOW 2 S.D. LIMIT
COELIAC DISEASE (NORMAL DIET)	21	16 (76%)	5 (24%)	Nil
COELIAC DISEASE (GLUTEN FREE DIET)	31	25 (81%)	2 (6%)	4 (13%)
CROHN'S DISEASE	15	10 (67%)	3 (20%)	2 (13%)
ULCERATIVE COLITIS	30	26 (87%)	1 (3%)	3 (10%)
IRRITABLE COLON SYNDROME	21	18 (86%)	2 (10%)	1 (5%)

TABLE A.IV SERUM IgM LEVELS IN PATIENTS WITH GASTROINTESTINAL DISEASE, COMPARED WITH TEN AGE AND SEX MATCHED SUBJECTS.

GROUP	NUMBER OF PATIENTS	IN NORMAL RANGE	ABOVE 2 S.D. LIMIT	BELOW 2 S.D. LIMIT
COELIAC DISEASE (NORMAL DIET)	21	17 (81%)	Nil	4 (19%)
COELIAC DISEASE (GLUTEN FREE DIET)	31	22 (71%)	2 (6%)	7 (23%)
CROHN'S DISEASE	15	13 (87%)	1 (7%)	1 (7%)
ULCERATIVE COLITIS	30	27 (90%)	Nil	3 (10%)
IRRITABLE COLON SYNDROME	21	19 (90%)	Nil	2 (10%)

DISCUSSION

Abnormal levels of IgG have been reported in a minority of patients with coeliac disease, (Hobbs and Hepner, 1968, Blecher et al. 1969, Kenrick and Walker-Smith, 1970). The present study confirms these findings. Twentyfive per cent of patients with coeliac disease had abnormal IgG levels. A comparison of mean IgG levels in 23 patients before and after gluten restriction showed no significant change, supporting the findings of Asquith et al. (1969).

Serum IgA levels were significantly elevated in 24% of patients on a normal diet in the present study. Elevated levels of IgA were reported in 26% of untreated patients by Blecher et al (1969), while Hobbs and Hepner (1968) found 16% of patients with IgA levels significantly above normal. Kenrick and Walker-Smith (1970) and Asquith et al. (1969) reported similar findings. In the group of 23 patients studied before and after treatment there was no significant fall in the IgA levels, again supporting the findings of Asquith et al (1969).

Asquith et al (1969) drew attention to the fact that rising IgA levels in a patient on a gluten and milk restricted diet may suggest the development of a lymphoma. One patient in this study, C.D. 27, a 57 year old male with a 25 year history of diarrhoea had significantly elevated IgA levels. Although gluten and milk restriction caused a fall in IgA levels, they continued to be significantly elevated, two and four months later. The patient was shown to have a malignant lymphoma of the small bowel and died shortly after.

The association between coeliac disease and selective

IgA deficiency has been well documented (Mawhinney and Tomkin, 1971, Gelzayd, McCleery, Melnyk and Kraft, 1971). In the present study an eleven year old girl, C.D. 36, had isolated IgA deficiency, and recurrent respiratory infections. Two other subjects C.D.4 a three and a half year old boy and a nine month old infant C.D.5 had isolated IgA deficiency. It was not possible to study the immunoglobulin levels in the family members of C.D.5. Selective familial IgA deficiency, has been reported by Walker-Smith (1971) and Tomkin, Mawhinney and Nevin (1971) but the mother and two siblings of this patient (C.D. 36) had normal IgA levels. Selective IgA deficiency was detected in the mother of C.D.4.

In the present study immunoglobulin levels were measured in 50 parents and siblings of patients with coeliac disease. The findings are similar to those reported by Little (1972) who found only one case of IgA deficiency in 48 members of families which had more than one member with coeliac disease. Thus, though selective IgA deficiency occurs more frequently in patients with coeliac disease, the familial occurrence of selective IgA deficiency is not common.

Low serum IgM have been reported in one third to two thirds of patients with coeliac disease (Asquith et al 1969, Blecher et al 1969, Kenrick and Walker-Smith 1970). The prevalence in the present study was 19%.

Hobbs and Hepner (1968) and Kenrick and Walker-Smith (1970) reported a rise in IgM to normal levels following gluten withdrawal while Asquith et al (1969) who studied their patients over a longer period found that the IgM levels rose and subsequently fell in 30 per cent of the patients so that 26% of

patients on a gluten free diet still had low IgM. In the present study 23 per cent of patients on treatment had low IgM. However, on comparing the change in IgM levels in 23 patients before and after treatment (mean duration 15 months) there was a significant rise in IgM.

Brown, Cooper and Hepner (1969) demonstrated reduced IgM synthesis in the majority of untreated patients. The rise in IgM levels on gluten withdrawal may indicate an inhibitory effect of gluten at IgM producing sites. Villa, Mocarelli, Natale and Clerici (1968) demonstrated an inhibition of the IgM response in mice on injecting repeated doses of antigen above a critical concentration. A similar mechanism may occur in regard to gluten ingestion in coeliac disease.

Asquith et al (1969) suggest that the low serum IgM levels may be due to increased gastrointestinal loss and that the increased IgM in jejunal juice is indicative of this. However, serum IgM is usually normal in patients with exudative enteropathy, (Fudenberg, Good, Goodman, Hitzig, Kunkel, Roitt, Rosen, Rowe, Seligmann and Soothill, 1971).

From the present evidence it would seem that decreased IgM synthesis is mainly responsible for the low serum IgM in coeliac disease. However the suggestion by Hobbs, Hepner, Douglas, Crabbe and Johansson (1969) that the decreased synthesis is "part of the depression of lymphoreticular function" is as yet unproven.

V.A. TESTS ON SERUM

A.2 SERUM COMPLEMENT

A.2 (i) TOTAL HAEMOLYTIC COMPLEMENT

RESULTS:

Control Subjects - The range for total complement in normal subjects estimated in the serology laboratory at The Queen Elizabeth Hospital, Woodville, S.A., is 90 - 96% of haemolysis.

Patients - Appendix C showsthe total complement levels of the individual patients studied. Table A.V shows the number of subjects within the normal range. Of the 28 patients with coeliac disease on a normal diet all but one had normal total complement levels. Total complement was normal in 90% of patients on a gluten free diet, and in approximately two thirds of patients with Crohn's disease and ulcerative colitis. None of the 20 patients with the irritable colon syndrome had subnormal values.

Total complement in 21 patients with coeliac disease were compared before and after a mean duration of 13 months of gluten restriction. There was no significant difference ($t = 0.399$, N.S., Student's paired t test).

TABLE A.V TOTAL COMPLEMENT LEVELS IN PATIENTS WITH GASTROINTESTINAL DISEASE (NORMAL RANGE = 90%-96% HAEMOLYSIS)

DISEASE	COELIAC DISEASE (NORMAL DIET)	COELIAC DISEASE (GLUTEN FREE DIET)	CROHNS DISEASE	ULCERATIVE COLITIS	IRRITABLE COLON SYNDROME
NUMBER OF PATIENTS	28	39	11	28	20
NUMBER WITHIN NORMAL RANGE	27 (96%)	35 (90%)	8 (73%)	20 (71%)	20 (100%)

A.2 SERUM COMPLEMENT

A.2 (ii) C_3 (B_1A) LEVELS

RESULTS:

Control Subjects - As there is no available data on the possible relation of age and sex on C_3 levels in normal subjects, this was studied. Ten normal subjects of each sex were grouped into categories at five year intervals. There were insufficient control subjects in the age groups below 11 years and over 70 years.

Fig. A.4 shows the frequency distribution of C_3 values in male and female control subjects. Since there was a visual asymmetry in the histogram, the data were transformed logarithmically to obtain ranges of values at specified age intervals. The geometric mean (± 2 S.D.) is shown in Table A.VI.

Effects of sex on C_3 levels

The mean values for males and females within the same age group were compared using the Student's t test. Female control subjects had significantly higher values than male subjects in the 36 - 40 age group ($t = 3.2628$, $P<0.005$) and 56 - 60 age group ($t = 3.9720$, $P<0.001$). However, males had higher levels of C_3 in the age groups 26 - 30 ($t = 2.6342$, $P<0.02$) and 46 - 50 ($t = 3.8292$, $P<0.005$).

Effect of age on C_3 levels

There were fluctuations in C_3 levels with age in both males and females but no tendency to either a sustained rise

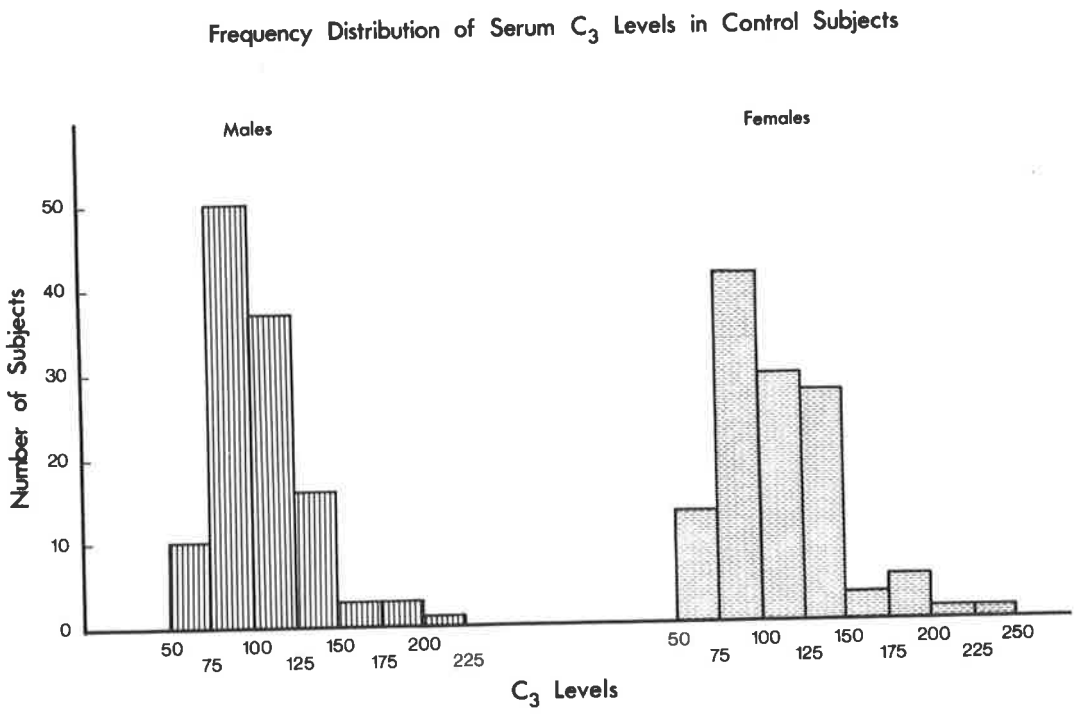

Figure A.4. Histogram of serum C_3 levels in male and female control subjects.

or fall with age was observed in either sex.

Patients - The results of the individual subjects with gastrointestinal disease are shown in Appendix C. The C_3 levels were converted to logarithms and compared with the logarithmic mean range (mean ± 2 S.D.) of ten age and sex matched control subjects. No comparisons were made in patients over 70 years as control subjects in this age range were not available. The C_3 levels in male patients between the ages of 6 - 10 years were compared with that of six age matched control subjects.

Table A.VII shows the number of patients in each group studied, above and below the logarithmic range (mean ± 2 S.D.) of the age and sex matched control subjects. In coeliac disease, irrespective of treatment approximately 75% of the patients had normal C_3 values.

C_3 values were compared in 23 patients before and after a gluten free diet of a mean duration of 15 months. There was no significant difference in the mean C_3 value on a normal diet (112 ± 54) and on a gluten free diet (103 ± 33) ($t = 0.6670$, N.S. Student's t test). The Student's paired t test also failed to reveal a significant difference before and after treatment ($t = 0.1072$, N.S.).

TABLE A.VI THE GEOMETRIC MEAN (± 2 S.D.) OF C_3 LEVELS IN CONTROL SUBJECTS (mg./100 ml.)

AGE GROUP	NO. OF SUBJECTS	MALES	FEMALES
6-10	6	122 (47 - 320)	-
11-15	10	113 (76 - 168)	134 (72 - 251)
16-20	10	106 (54 - 207)	113 (59 - 215)
21-25	10	88 (59 - 132)	100 (53 - 191)
26-30	10	104 (62 - 174)	78 (53 - 116)
31-35	10	91 (39 - 212)	110 (74 - 162)
36-40	10	96 (74 - 206)	131 (78 - 155)
41-45	10	106 (55 - 206)	111 (80 - 155)
46-50	10	120 (95 - 154)	94 (68 - 129)
51-55	10	99 (73 - 134)	106 (71 - 159)
56-60	10	106 (77 - 145)	131 (79 - 216)
61-65	10	107 (82 - 141)	120 (86 - 166)
66-70	10	105 (73 - 154)	116 (84 - 159)

TABLE A.VII C_3 LEVELS IN PATIENTS WITH GASTROINTESTINAL DISEASE COMPARED WITH TEN AGE AND SEX MATCHED CONTROL SUBJECTS

SUBJECTS	NUMBER OF PATIENTS	IN NORMAL RANGE	ABOVE 2 SD LIMIT	BELOW 2 SD LIMIT
COELIAC DISEASE (NORMAL DIET)	22	16 (73%)	2	4
COELIAC DISEASE (GLUTEN FREE DIET)	33	24 (73%)	1	8
CROHN'S DISEASE	15	12 (80%)	1	2
ULCERATIVE COLITIS	30	25 (83%)	1	4
IRRITABLE COLON SYNDROME	21	14 (67%)	4	3

DISCUSSION

The human complement system comprises a variety of proteins concerned in antigen-antibody reactions.

In coeliac disease the presence of circulating antibodies (Taylor et al 1964; Kivel et al 1964; Ferguson and Carswell 1972) immune complexes (Doe et al 1973) and the demonstration of complement-fixing complexes in the jejunal mucosa of a few (3 of 7) patients with coeliac disease (Shiner and Ballard 1972) suggest involvement of the complement system.

Carswell and Logan (1972) reported significantly lower C_3 levels in 55 patients with coeliac disease compared to 33 children who did not have coeliac disease. In the present study more rigid methods of comparison were applied. Each patient's C_3 level was compared with ten aged and sex matched control subjects. No significant differences in C_3 levels were detected either in relation to control subjects or treatment status.

Although the serum concentration of C_3 is reported to closely reflect total complement activity (Klemperer, Gotoff, Alper, Levin and Rosen, 1965) in the present study total complement levels were also estimated.

The findings showed no significant abnormalities in complement in patients with coeliac disease, either in relation to control subjects or gluten withdrawal.

V. A. TESTS ON SERUM

A.3 AUTOANTIBODIES INCLUDING ANTIRETICULIN ANTIBODIES

RESULTS:

The prevalence of antinuclear factor (ANF) and autoantibodies to smooth muscle (SMA) and gastric parietal cells (GPC) was slightly higher in the 26 patients on a normal diet than in the 40 patients on a gluten free diet (Table A.VIII). Of the six patients with SMA, anti-reticulin antibodies were present in one patient (C.D.25); precipitins to dietary proteins were not detected in these patients.

In the group of 23 patients who were studied before and after gluten restriction (mean duration 15 months) SMA was present more frequently in patients on a normal diet (4/23) than in 69 age and sex matched control subjects ($P<0.003$, Fisher's exact test). The antibody disappeared in all of the patients after a mean duration of 15 months of gluten restriction (Table A.IX).

Antibodies to GPC were found in three of the 23 patients investigated before and after treatment and persisted after gluten restriction. In two of these patients (C.D.11, and C.D.28) ARA was present. In the third patient (C.D.29) ARA and precipitins to GF III and milk were detected. The incidence of GPC was not significantly higher than in the 69 age and sex matched control subjects.

In summary, the presence of SMA was significantly greater in 23 patients studied on a normal diet than in the same patients on a gluten free diet. However, when a larger number (26) of patients on a normal diet and 40 patients on a

TABLE A.VIII PREVALENCE OF ANTI NUCLEAR FACTOR AND SERUM AUTO ANTIBODIES IN PATIENTS WITH GASTROINTESTINAL DISEASE

GROUP	TOTAL	A.N.F.	SMA	GPC
COELIAC DISEASE (NORMAL DIET)	26	1 (4%)	4 (15%)	3 (12%)
COELIAC DISEASE (GLUTEN FREE DIET)	40	Nil	2 (5%)	3 (8%)
ULCERATIVE COLITIS	31	1 (3%)	Nil	2 (6%)
CROHN'S DISEASE	15	2 (13%)	1 (7%)	1 (7%)
IRRITABLE COLON SYNDROME	23	1 (4%)	2 (9%)	1 (4%)

TABLE A. IX PREVALENCE OF ANF, SERUM AUTOANTIBODIES IN PATIENTS WITH COELIAC DISEASE BEFORE AND AFTER A GLUTEN FREE DIET.

GROUP	TOTAL	A.N.F.	S.M.A.	G.P.C.
Coeliac Disease (normal diet)	23	Nil	4	3
Coeliac Disease (gluten free diet)	23	Nil	Nil	3
Control Subjects	69	Nil	Nil	3

gluten free diet were compared, there was no significant difference in the occurrence of SMA. (P = 0.0123, N.S. Fisher's exact test)

DISCUSSION

The co-existence of coeliac disease and disease of possible autoimmune nature such as Addison's Disease (Goudie et al 1969), Sjogren's Syndrome (Pittman and Holub, 1965; Smith and Strickland, 1971) and vasculitis and cryoglobulinaemia (Doe, Evans, Hobbs and Booth 1972) may well be fortuitous.

ANF was not detected in 23 patients in the series of Smith and Strickland (1971) while Seah et al (1971) and Brown et al (1973) found positive ANF in 6% and 2% of patients respectively. These figures are of the same order as those in the present study.

Considering the total number of patients in whom tests for autoantibodies were performed the incidence of SMA was 14%, a figure higher than that reported in previous studies. The prevalence of GPC (7%) was of the same order of magnitude as that found by Seah et al (1971a) (13%) and Brown et al (1973), (2%). However, in the present study a finding previously not reported was the disappearance of antibodies to SMA following dietary treatment.

V.A TESTS ON SERUM

A.3 ANTIRETICULIN ANTIBODIES

RESULTS

Table A.X shows the prevalence of antireticulin antibodies (ARA) in the groups of patients studied. Eighteen of 32 patients (56%) with untreated coeliac disease had ARA, compared to 2 of 40 patients (5%) on a gluten free diet. In a group of 23 patients the prevalence of ARA was compared both before and after treatment and with the prevalence of ARA in 23 age and sex matched control subjects (Table A.XI). The incidence of ARA was significantly higher in the untreated patients compared to patients on a gluten free diet and normal subjects.

Antibodies to wheat antigens were detected in six of 18 patients (36%) on a normal diet with ARA and in three of 12 (24%) patients without ARA. Antireticulin antibodies were present in only two patients on a gluten free diet. Precipitins to wheat antigens were present in both patients. There was no significant relationship between the presence of ARA and precipitins to wheat antigens ($P = 0.2854$, Fisher's exact test).

DISCUSSION

The presence of an IgG class antibody in adult coeliac disease, reacting with reticulin was first reported by Seah et al (1971a), who described ARA in 36% of adult patients and 74% children (Seah et al, 1971 b) with coeliac disease. Alp and Wright (1971) reported an incidence of ARA in 34% of adult patients and 54% in childhood coeliac disease. Von Essen et al (1972) found ARA in 68% of patients and Brown et al (1973) also reported similar figures.

TABLE A.X PREVALENCE OF ANTIRETICULIN ANTIBODIES IN PATIENTS WITH GASTROINTESTINAL DISEASE

GROUP	TOTAL	POSITIVE	NEGATIVE	% POSITIVE
COELIAC DISEASE (NORMAL DIET)	32	18	14	56%
COELIAC DISEASE (GLUTEN FREE DIET)	40	2	38	5%
ULCERATIVE COLITIS	31	6	25	19%
CROHN'S DISEASE	15	1	14	7%
IRRITABLE COLON SYNDROME	23	3	20	13%

TABLE A.XI PREVALENCE OF ANTIRETICULIN ANTIBODIES IN 23 PATIENTS WITH COELIAC DISEASE BEFORE AND AFTER A GLUTEN FREE DIET

(Fisher's Exact Test*)

GROUP	TOTAL	ARA POSITIVE	ARA NEGATIVE	% POSITIVE	SIGNIFICANCE*
CONTROL SUBJECTS	23	1	22	4%	p<0.001
COELIAC DISEASE	23	14	9	61%	
COELIAC DISEASE (GLUTEN FREE DIET)	23	1	22	4%	p<0.001

In the present study ARA was detected in the serum of 56% of untreated patients and 5% of patients on a gluten free diet. The incidence of ARA (53%) in the group of children on a normal diet is less than that reported by Seah et al (1971b) who detected ARA in 9 of 10 (90%) children on an unrestricted diet. In the adult patients on a normal diet ARA was present in 63% a figure slightly higher than reported by Alp and Wright (1971) and Seah et al (1971a).

On a gluten free diet the incidence of ARA was only 5% (2 of 40 patients). Although Seah et al (1971a) reported an incidence of 55% (5 of 9 patients) the findings in the present study confirm the observations by other workers of a lower incidence of ARA in patients on dietary treatment (Alp and Wright 1971, Von Essen et al 1972). No positive correlation was found between the presence of ARA and precipitins to wheat antigens, an observation also reported by Von Essen et al (1972) and Brown et al (1973).

In the other groups of patients with gastrointestinal disease studied, 13% of patients with the irritable colon syndrome had positive ARA. Alp and Wright (1971) detected ARA in 8% of patients with ulcerative colitis, and in 25% of patients with Crohn's Disease. In the present study patients with ulcerative colitis and Crohn's disease had an incidence of ARA in 19% and 7% respectively.

The significance of ARA is not yet understood. Seah et al (1971b) suggested that the presence of ARA is specific for coeliac disease and that ARA cross reacting with gluten may be involved in the pathogenesis in dermatitis herpetiformis and coeliac disease.

V.A TESTS ON SERUM

A.4 DIETARY ANTIBODIES

RESULTS:

Table A.XIIshows the prevalence of antibodies to GF III, gliadin and cow's milk in patients with gastrointestinal disease. Precipitins to constituents of wheat were present in the sera of 9 of the 32 patients with coeliac disease on a normal diet. Antibodies to GF III, and gliadin were present in a much smaller proportion of patients with coeliac disease on a gluten free diet and in patients with other forms of gastrointestinal disease.

There was no significant difference in the number of patients with precipitins to the three antigens in any of the groups studied (chi-square = 9.58, n = 8, $p<0.30$). However the group of 23 patients who were studied before and after gluten restriction (Table A.XIII)had a significantly higher prevalence of dietary antibodies to GF III whilst on a normal diet than did 23 age and sex matched control subjects ($p<0.05$, Fisher's exact test). Dietary antibodies were also present more frequently in patients on a normal diet than after treatment ($p<0.05$, Fisher's exact test).

DISCUSSION

The presence of circulating antibodies to wheat and milk antigens has been well documented.

In the present study precipitins to GF III were detected in 28% of patients on a normal diet, a figure very similar to that obtained by Kivel et al (1964) who studied a similar group of patients using the technique of gel diffusion.

TABLE A.XII INCIDENCE OF PRECIPITINS TO THREE DIETARY PROTEINS IN PATIENTS WITH GASTROINTESTINAL DISEASE.

GROUP	TOTAL	ANTIGEN		
		GF III	GLIADIN	COWS MILK
COELIAC DISEASE NORMAL DIET	32	9 (28%)	3 (9%)	3 (9%)
COELIAC DISEASE GLUTEN FREE DIET	40	4 (10%)	1 (3%)	2 (5%)
ULCERATIVE COLITIS	31	2 (6%)	Nil	6 (19%)
CROHN'S DISEASE	15	1 (7%)	Nil	Nil
IRRITABLE COLON SYNDROME	23	1 (4%)	Nil	2 (9%)

TABLE A.XIII INCIDENCE OF PRECIPITINS TO THREE DIETARY PROTEINS IN PATIENTS WITH COELIAC DISEASE BEFORE AND AFTER A GLUTEN FREE DIET.

GROUP	TOTAL	ANTIGEN		
		GF III	GLIADIN	COWS MILK
CONTROL SUBJECTS	23	1 (4%)	Nil	1 (4%)
COELIAC DISEASE NORMAL DIET	23	7 (30%)	3 (13%)	3 (13%)
COELIAC DISEASE GLUTEN FREE DIET	23	1 (4%)	1 (4%)	2 (9%)

Precipitins to gliadin and cow's milk were detected in a larger number of patients in Kivel's (1964) study. Ferguson and Carswell (1972) who used a micro gel diffusion technique reported precipitins to gluten in 48%, and to milk in 45% of untreated patients. Taylor et al (1964) using the more sensitive tanned red cell technique detected antibodies to GF III in 65% of patients (adults and children) with coeliac disease.

Rossipal (1970) reported the findings of precipitins to wheat products in all of his 15 patients with coeliac disease. Although the technique used by Rossipal (1971) was closely followed in the present study, precipitins to wheat antigens were not detected in all patients with coeliac disease.

The present study confirms the higher incidence of antibodies to GF III in patients with untreated coeliac disease and demonstrates the lower incidence in treated patients. Confirming the findings of Taylor et al (1964) antibodies to dietary proteins were also detected in a smaller number of patients with Crohn's disease, ulcerative colitis and the irritable colon syndrome.

The present study thus confirms the findings of an increased incidence of antibodies to wheat antigens in coeliac disease. However, their presence in patients with Crohn's disease, ulcerative colitis and the irritable colon syndrome appears to indicate an epiphenomenon of disease, reflecting the passage of an undigested or partially digested product of gluten, rather than an immunological mechanism unique to coeliac disease.

V.A TESTS ON SERUM

A.5 ANTIBODY RESPONSES TO IMMUNISATION WITH TETANUS TOXOID AND S. TYPHI.

RESULTS:

Five patients on a gluten free diet and seven patients on a normal diet were immunised with Tetanus toxoid and S.typhi. Table A.XIV shows the pre and post immunisation titres for each antigen. The antibody responses of the patients were compared with those of twelve age and sex matched control subjects (Table A.XV). All control subjects responded to both antigens. Two of the patients C.D.5 and C.D.11 did not respond to Tetanus toxoid and one of these C.D.5 also failed to respond to S.typhi.

This patient C.D.5 was an eight month old male who was investigated for failure to thrive, recurrent respiratory infections and persistent diarrhoea. A small bowel biopsy revealed subtotal villous atrophy. Serum IgM was significantly elevated at 1958 mg%. The patient also failed to express delayed hypersensitivity. Exclusion of gluten was followed by weight gain and the diarrhoea ceased. However neither further immunological studies nor a repeat biopsy on a gluten free diet were performed as the family moved interstate. The second patient C.D.11 who failed to respond to Tetanus toxoid did respond to S.typhi and had normal levels of serum immunoglobulins. The responses of patients with coeliac disease were not significantly different to those of the control subjects (Table A.XVI) in regard to Tetanus toxoid ($P = 0.48$, Fisher's exact test) or S.typhi ($P = 0.5$, Fisher's exact test).

TABLE A.XIV TITRES OF ANTIBODY TO TETANUS TOXOID AND S.TYPHI IN TWELVE PATIENTS WITH COELIAC DISEASE

SUBJECT	TETANUS TOXOID		S.TYPHI	
	PRE-IMMUNISATION	POST-IMMUNISATION	PRE-IMMUNISATION	POST-IMMUNISATION
NORMAL DIET:				
CD 4	Neg.	2^5	Neg.	1/640
CD 5	Neg.	Neg.	Neg.	Neg.
CD 11	Neg.	Neg.	Neg.	1/20
CD 13	2^7	2^9	Neg.	1/80
CD 14	2^5	2^{10}	Neg.	1/160
CD 26	2^{10}	2^{10}	Neg.	1/640
GLUTEN FREE DIET:				
CD 12	Neg.	2^6	1/20	1/640
CD 16	Neg.	2^6	Neg.	1/160
CD 22	2^2	2^7	Neg.	1/320
CD 29	2^6	2^8	Neg.	1/640
CD 35	Neg.	2^5	Neg.	1/640
CD 37	2^6	2^7	Neg.	1/80

TABLE A.XV TITRES OF ANTIBODY TO TETANUS TOXOID AND S.TYPHI IN TWELVE CONTROL SUBJECTS

SUBJECT	TETANUS TOXOID		S.TYPHI	
	PRE-IMMUNISATION	POST-IMMUNISATION	PRE-IMMUNISATION	POST-IMMUNISATION
N1	Neg.	2^8	1/40	1/80
N2	Neg.	2^{10}	1/20	1/40
N3	2^4	2^{10}	Neg.	1/80
N4	2^5	2^7	1/40	1/320
N5	Neg.	2^4	Neg.	1/320
N6	2^3	2^{10}	Neg.	1/640
N7	2^5	2^{10}	1/160	1/320
N8	2^2	2^4	1/20	1/80
N9	2^5	2^7	Neg.	1/640
N10	2^5	2^9	Neg.	1/640
N11	Neg.	2^4	Neg.	1/40
N12	2^1	2^5	Neg.	1/640

TABLE A.XVI RESPONSE TO TETANUS TOXOID AND S.TYPHI H. IN COELIAC DISEASE

SUBJECTS	TOTAL	RESPONDERS	
		TETANUS TOXOID	S.TYPHI H
CONTROLS	12	12	12
PATIENTS	12	10	10

DISCUSSION:

Pettingale (1970) reported a diminished response to Tetanus toxoid in fourteen patients with coeliac disease, suggestive of an impaired humoral response. However the antibody titres were not reported and their significance was not stated. In contrast Beale et al (1971) found no abnormality of antibody response to Tetanus toxoid in ten patients, five of whom were on dietary treatment.

In the present study two patients with coeliac disease showed an impaired response to Tetanus toxoid. Only one of these patients failed to respond to S.typhi. These findings suggest that the humoral immune response may be impaired in coeliac disease but a large group of patients would need to be studied in order to establish whether such impairment is significantly more common than in normal persons.

V.B Tests on Lymphocytes PAGE

V. B. TESTS ON LYMPHOCYTES

B.1 B LYMPHOCYTES

RESULTS:

B.1 (i) Using monovalent antisera - Table B.I shows the results of the B cell counts, using monovalent antisera to IgG, IgA and IgM, in patients with coeliac disease on a normal diet (7), patients on a gluten free diet (8), and in normal subjects (7).

A comparison of the proportion of cells bearing the individual immunoglobulin receptors in the three groups (Table B.II) showed that the IgG proportion of immunoglobulin receptor bearing cells was significantly raised in patients on a normal diet compared to normal subjects ($t = 3.6134$, $P<0.005$ Student's t test) and patients on a gluten free diet ($t = 3.4575$ $P<0.005$). There were no significant differences between the groups in regard to cells bearing IgA and IgM receptors.

Table B.II also shows the mean total percentage of lymphocytes with surface bound immunoglobulin in the three groups of subjects. The mean value in the seven untreated patients was significantly higher than the value of the control group ($t = 3.1116$, $P<0.01$ Student's t test) and of the patients on a gluten free diet ($t = 2.3729$, $P<0.05$). The difference between the control group and patients on a gluten free diet was not significant ($t = 0.1789$, N.S.).

The correlation coefficient was determined between the serum IgG levels and the number of cells bearing IgG receptor sites in patients on a normal diet. There was no significant correlation

(r = 0.452).

B.1 (ii) Using Polyvalent antiserum

Immunofluorescent staining of peripheral blood lymphocytes with polyvalent antiserum is shown in Fig. B.1. The proportion of B cells in peripheral blood lymphocytes was estimated in 29 normal subjects and 15 patients with coeliac disease. Eight of the patients were on a gluten free diet. Table B.III shows the mean values and Appendix D the individual results. Untreated patients had a significantly higher proportion than did the control group (23% vs. 15%; $t = 4.0809$, $P<0.001$ Student's t test). There was no significant difference between the values of untreated patients and patients on a gluten free diet (23% vs. 16%, $t = 2.1498$, N.S.) nor between the latter and normal subjects ($t = 0.6403$, N.S.).

B.1 (iii) Absolute Numbers of B cells

Table B.IV shows the mean values of the absolute numbers of B cells in patients and in 27 control subjects. The individual values are given in Appendix D. The absolute B cell counts were significantly higher in untreated patients compared with counts in normal subjects ($t = 2.9134$, $P<0.01$ Student's t test). There was no significant difference in mean B cell values between patients before and after treatment ($t = 1.0990$ N.S.) nor between control subjects and treated patients ($t = 1.6385$, N.S.).

B.1 (iv) Using fluorescein iso-thiocyanate conjugated gluten fraction III (FITC - GF III)

The peripheral blood lymphocytes in both patients and control subjects showed no evidence of receptor sites which bound FITC - GF III.

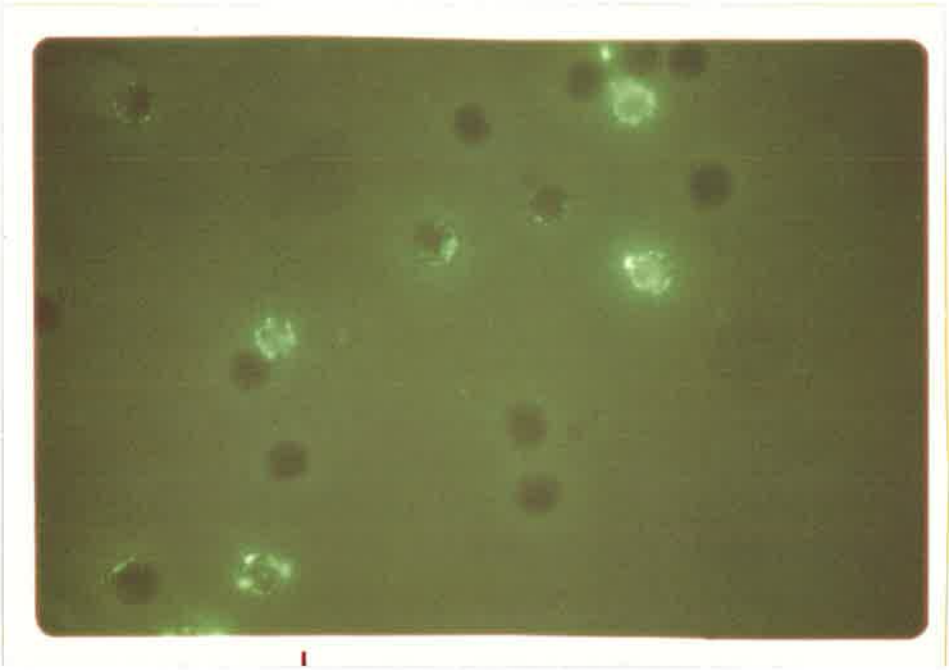

Figure B.1. Immunofluorescent staining of peripheral blood lymphocytes with polyvalent antiserum.

B.1 (v) Proportion of B cells in patients with and without Antireticulin antibodies (ARA)

Of the 15 patients, the six patients (C.D. 11, C.D. 12; C.D. 26, C.D. 30, C.D. 31 and C.D. 36) with ARA had a significantly higher proportion of IgG receptor bearing cells than the patients without ARA (18% vs. 9.9%), ($t = 4.2243$, $P<0.001$, Student's t test).

There was no significant difference in mean absolute B cell counts between ARA positive and ARA negative patients.

B.1 (vi) Proportion of B cells in patients with and without precipitins to wheat antigens

Three of the fifteen patients (C.D. 12, C.D. 31, and C.D. 36) had precipitins to gluten fraction III. They were no different in respect to IgG bearing receptor cells and mean absolute B counts, from patients who did not have precipitins.

TABLE B.I PERCENTAGE OF LYMPHOCYTES WITH SURFACE BOUND IMMUNOGLOBULIN IN PATIENTS WITH COELIAC DISEASE AND NORMAL SUBJECTS USING MONOVALENT ANTISERA

GROUP		IgG	IgA	IgM	Total %
COELIAC DISEASE (NORMAL DIET)	C.D. 11	17.2	6.0	5.5	28.7
	C.D. 12	22.2	4.1	7.0	33.3
	C.D. 18	13.9	5.3	17.6	36.8
	C.D. 21	12.3	2.7	17.2	32.2
	C.D. 26	18.4	7.6	1.0	27.0
	C.D. 30	26.8	5.2	8.5	40.5
	C.D. 31	12.6	8.5	11.4	32.5
COELIAC DISEASE (GLUTEN FREE DIET)					
	C.D. 29	15.0	11.0	7.0	33.0
	C.D. 36	10.5	6.0	11.0	27.5
	C.D. 37	11.6	9.5	13.4	34.5
	C.D. 38	10.7	8.7	8.8	28.2
	C.D. 41	6.1	2.0	3.3	11.4
	C.D. 45	4.7	4.1	5.6	14.4
	C.D. 48	8.6	1.3	9.5	19.4
	C.D. 49	10.9	6.0	10.4	27.3
NORMAL SUBJECTS	N. 2	10.0	6.0	7.0	23.0
	N. 3	9.0	7.5	11.0	27.5
	N. 4	13.0	5.4	14.0	32.4
	N. 5	9.0	6.5	9.0	24.5
	N. 6	8.0	1.3	6.0	15.3
	N. 29	12.0	7.7	8.5	28.2
	N. 30	6.0	2.0	7.5	15.5

TABLE B.II MEAN TOTAL PERCENTAGE OF LYMPHOCYTES WITH SURFACE BOUND IMMUNOGLOBULIN IN PATIENTS WITH COELIAC DISEASE AND NORMAL SUBJECTS USING MONOVALENT ANTISERA

GROUP	TOTAL	IgG	IgA	IgM	TOTAL B CELLS
COELIAC DISEASE (NORMAL DIET)	7	17.6 ±5.0	5.6 ±1.9	9.7 ±5.7	33.0 ±4.2
COELIAC DISEASE (GLUTEN FREE DIET)	8	9.8 ±3.0	6.1 ±3.3	8.6 ±3.0	24.5 ±7.9
NORMAL SUBJECTS	7	9.6 ±2.1	5.2 ±2.4	9.0 ±2.5	23.8 ±5.9

TABLE B.III MEAN TOTAL PERCENTAGE OF LYMPOCYTES WITH SURFACE BOUND IMMUNOGLOBULIN IN PATIENTS WITH COELIAC DISEASE AND NORMAL SUBJECTS USING POLYVALENT ANTISERUM

GROUP	TOTAL	MEAN ± 1 S.D.
COELIAC DISEASE (NORMAL DIET)	7	23.0 ±4.9
COELIAC DISEASE (GLUTEN FREE DIET)	8	16.2 ±6.3
NORMAL SUBJECTS	29	14.9 ±4.5

TABLE B.IV MEAN ABSOLUTE NUMBER OF LYMPHOCYTES (PER MICROLITRE) WITH SURFACE BOUND IMMUNOGLOBULIN IN PATIENTS WITH COELIAC DISEASE AND NORMAL SUBJECTS USING MONOVALENT ANTISERA

GROUP	TOTAL	MEAN ± 1.S.D.
COELIAC DISEASE (NORMAL DIET)	7	497.0 ± 160.9
COELIAC DISEASE (GLUTEN FREE DIET)	8	404.0 ± 144.2
NORMAL SUBJECTS	27	303.0 ± 150.0

DISCUSSION

Recent studies have indicated the presence of two distinct types of lymphocyte - the T lymphocyte which participates in cell-mediated immunity and the B lymphocyte which is concerned in humoral immune reactions (Roitt, Greaves, Torrigiani, Brostoff and Playfair, 1969; Raff and Wortis, 1970). B lymphocytes may be distinguished from T lymphocytes by the presence of surface bound immunoglobulin, and 10 - 34% of lymphocytes from normal persons demonstrate this phenomenon (Froland, Natvig and Berdal 1971; Papamichail, Brown and Holborow, 1971; Wilson and Nossal, 1971; Papamichail, Holborow, Keith and Currey, 1972). In this study the mean total percentage corresponded to the reported figures.

Rabellino, Colon, Grey and Unanue (1971) found that the sum of the number of cells reacting with each monospecific antiserum (48%) correlated well with the figure obtained when polyvalent antiserum was used. However in the present study the total percentage of B cells, identified with fluoresceinated monovalent antisera was higher than when polyvalent antiserum was used. The monospecificity of the antisera was verified by immunoelectrophoresis. Cross reactivity between immunoglobulin subclasses due to lack of specificity is therefore unlikely to be the cause. Absorption of serum immunoglobulin onto the lymphocyt surface is unlikely to be responsible as this should cause erroneously high counts no matter whether monovalent or polyvalent conjugates are used. On occasions, a lymphocyte may bear more than one immunoglobulin determinant, as for instance in patients with chronic lymphocytic leukemia (Papamichail, et al. 1971).

However, this is not likely to be a common phenomenon and it seems more probable that the higher counts obtained with monovalent conjugates were due to a higher avidity of their antibody.

The mean 'B' cell population was significantly higher in untreated coeliac disease than in normal subjects regardless of which type of conjugate was used but there was no significant difference between normal subjects and treated patients. As well as having significantly higher total B cell counts, untreated patients with coeliac disease also had a significantly increased proportion of IgG cells than did patients on a gluten free diet and normal subjects. There was no correlation between the increased IgG cell count and the serum IgG levels. Lymphocytes with surface bound immunoglobulins do not necessarily secrete immunoglobulins (Papamichail et al. 1971).

The high B cell population in the untreated patients with coeliac disease may be due to an increased antigenic stimulus. Rossipal (1970) reported that in coeliac disease the precipitating antibodies to aqueous extracts of flour were of the IgG class. This fact may explain the significantly higher number of IgG containing lymphocytes in the untreated patients.

Brown et al. (1973) and Gebhard et al. (1973) demonstrated that the majority of anti-reticulin antibodies belonged to the IgG class. In the present study a positive correlation was found between the incidence of ARA and IgG receptor containing cells.

Recent studies (Falchuk et al. 1972, Evans 1973) have documented the significantly high frequency of HL-A8 leucocyte antigens type in patients with coeliac disease and their role in the production of anti-gluten antibodies has been suggested.

In the present study, no lymphocytes were found which bound FITC - GF III. However, this cannot be taken as proof of the absence of such cells which might better be sought by autoradiography using I^{125} labelled GF III. The present study provided no evidence of humoral immuno-deficiency in coeliac disease. The high total B cell population in untreated patients with coeliac disease seems likely to be yet another sign of a heightened humoral immune response due to increased antigenic stimulus.

V.B. TESTS ON LYMPHOCYTES

B.2 LYMPHOCYTE TRANSFORMATION

RESULTS:

B.2 (i) Phytohaemagglutinin (PHA) induced lymphocyte uptake of ^{3}HT.

PHA induced lymphocyte uptake of ^{3}HT was measured in 18 patients with coeliac disease. Eight patients were on a gluten free diet. Eightyfour normal subjects comprised a control group. The studies on the control group were performed by Mrs. K. Holmes in association with Dr. D.I. Grove and Dr. T. Sorrell.

Table B.V shows the mean ^{3}HT uptake in the three groups of subjects investigated and Appendix D the results in individual patients with coeliac disease. There was no significant difference between normal subjects and patients on a normal diet ($t = 0.2557$, N.S. Student's t test) or patients on a gluten free diet ($t = 0.6144$, N.S.). There was no significant difference between the two groups of patients with coeliac disease ($t = 0.1782$, N.S.)

B.2 (ii) The effect of GF III on PHA induced lymphocyte uptake of ^{3}HT.

Different concentrations of GF III (10 µg, 100 µg, 200 µg and 1,000 µg) were added to lymphocyte cultures from one normal subject and one patient with untreated coeliac disease. PHA induced lymphocyte uptake of ^{3}HT was reduced when doses of 100 µg, 200 µg and 1,000 µg of GF III were added to the cultures. (Fig. B.2) Further studies were made

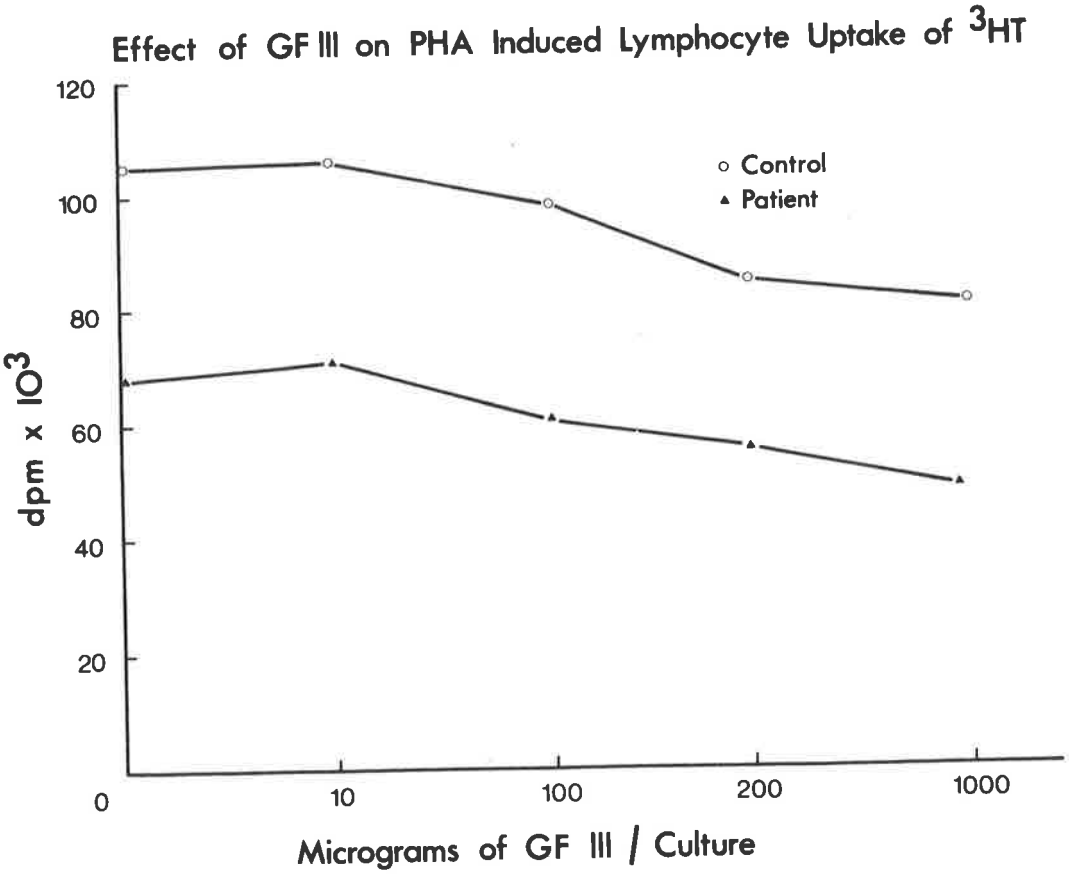

Figure B.2. The effect of varying concentrations of gluten fraction III on PHA induced lymphocyte uptake of ^{3}HT.

with 200 µg of GF III in 12 normal subjects and 13 patients with coeliac disease. Eight patients were on a gluten free diet.

The PHA induced lymphocyte uptake of ^{3}HT was measured with and without the addition of GF III. The addition of GF III caused a depression of PHA induced uptake of ^{3}HT in both normal subjects and patients with coeliac disease (Table B.VI, Fig. B.3). In the 12 normal subjects there was a decrease in mean ^{3}HT uptake (mean ± 1 S.D.) from 71167 ± 49095 to 62583 ± 43079 ($t = 2.7448$, $P<0.02$, Student's paired t test). The mean ^{3}HT uptake decreased from 73846 ± 52041 to 66077 ± 49526 ($t = 1.6114$, $P<0.20$, Student's paired t test) in the 13 patients with coeliac disease.

Table B.VII shows the PHA induced lymphocyte uptake of ^{3}HT following the addition of GF III, expressed as a percentage of ^{3}HT uptake in cultures with PHA alone. The mean percentage change (Table B.VIII) in the control subjects was not significantly different to that in the group of patients on a normal diet ($t = 0.4234$, N.S., Student's t test) or to that of patients on a gluten free diet ($t = 0.3405$, N.S.). Comparison of the mean percentage change in the two groups of patients also revealed no significant difference ($t = 0.6987$, N.S.).

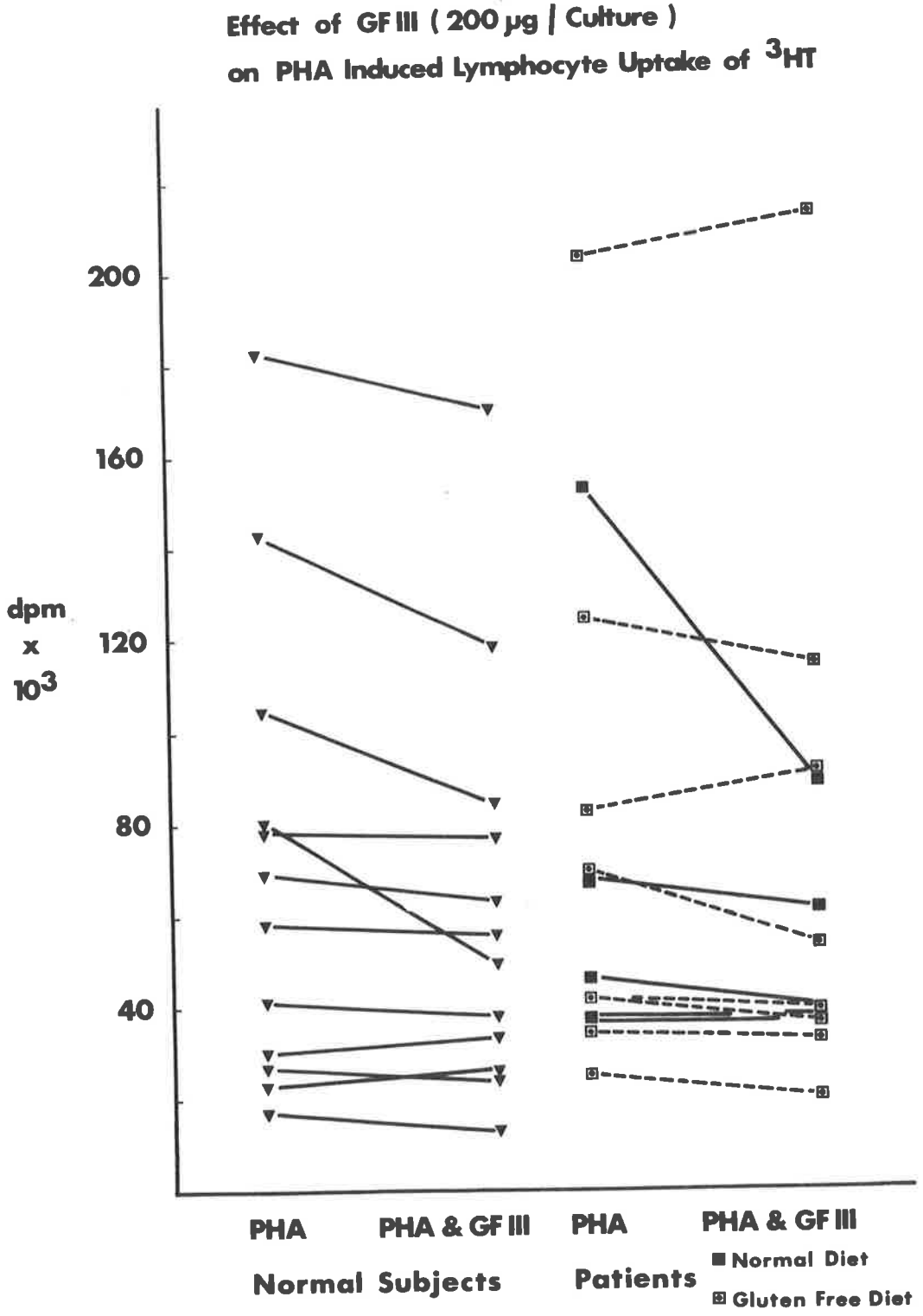

Figure B.3. PHA induced lymphocyte uptake of ^{3}HT before and after the addition of gluten fraction III (200 µg/culture) in normal subjects and patients with coeliac disease.

TABLE B.V. PHA INDUCED LYMPHOCYTE UPTAKE OF ^{3}HT IN PATIENTS WITH COELIAC DISEASE AND NORMAL SUBJECTS

GROUP	TOTAL	MEAN ± 1 S.D.
COELIAC DISEASE (NORMAL DIET)	8	72,000 ± 35,000
COELIAC DISEASE (GLUTEN FREE DIET)	10	76,000 ± 51,000
NORMAL SUBJECTS	84	69,000 ± 31,000

TABLE B.VI. EFFECT OF GF III (200 µg) ON PHA INDUCED LYMPHOCYTE UPTAKE OF ^{3}HT (d.p.m. x 10^{3})

NORMAL SUBJECTS			COELIAC DISEASE		
No.	PHA only	GF III followed by PHA	No.	PHA only	GF III followed by PHA
			NORMAL DIET		
N 1	105,000	84,000	C.D.17	46,000	40,000
N 2	17,000	13,000	C.D.23	153,000	89,000
N 3	27,000	24,000	C.D.26	68,000	61,000
N 4	23,000	26,000	C.D.28	37,000	37,000
N 5	78,000	77,000	C.D.32	37,000	37,000
N 6	58,000	56,000	GLUTEN FREE DIET		
N 7	183,000	170,000			
N 8	30,000	33,000			
N 9	80,000	49,000	C.D.16	25,000	20,000
N10	143,000	118,000	C.D.21	34,000	32,000
N11	41,000	38,000	C.D.29	82,000	90,000
N12	69,000	63,000	C.D.35	124,000	114,000
			C.D.36	41,000	36,000
			C.D.37	203,000	211,000
			C.D.45	69,000	53,000
			C.D.48	41,000	39,000

TABLE B.VII PHA INDUCED LYMPHOCYTE UPTAKE OF ^{3}HT FOLLOWING THE ADDITION OF GF III (200 µg) TO CULTURES EXPRESSED AS PERCENTAGE OF ^{3}HT UPTAKE IN CULTURES NOT CONTAINING GF III

NORMAL SUBJECTS		COELIAC DISEASE	
No.	Uptake after GF III	No.	Uptake after GF III
N 1	80.0%	NORMAL	
N 2	76.5%	DIET	
N 3	88.9%	C.D.17	87.0%
N 4	113.0%	C.D.23	58.2%
N 5	98.7%	C.D.26	89.7%
N 6	96.6%	C.D.28	100.0%
N 7	92.9%	C.D.32	100.0%
N 8	110.9%	GLUTEN FREE	
N 9	61.3%	DIET	
N 10	82.5%	C.D.16	80.0%
N 11	92.7%	C.D.21	94.0%
N 12	91.3%	C.D.29	109.8%
		C.D.35	91.9%
		C.D.36	87.8%
		C.D.37	103.9%
		C.D.45	76.8%
		C.D.48	95.0%

TABLE B.VIII MEAN PHA INDUCED LYMPHOCYTE UPTAKE OF ^{3}HT FOLLOWING THE ADDITION OF GF III (200 µg) TO CULTURES EXPRESSED AS PERCENT OF ^{3}HT UPTAKE IN CULTURES WITH PHA ONLY

GROUP	TOTAL	% CHANGE (± 1 S.D.)
NORMAL SUBJECTS	12	90.37 ± 13.6
COELIAC DISEASE (IRRESPECTIVE OF DIETARY STATUS)	13	90.3 ± 12.8
COELIAC DISEASE (NORMAL DIET)	5	86.98 ± 15.33
COELIAC DISEASE (GLUTEN FREE DIET)	8	92.4 10.32

DISCUSSION

DNA synthesis was found to be impaired in four of ten patients with coeliac disease on a normal diet in the study of Blecher et al (1969). However, Morganroth et al (1972) found no significant difference between control subjects/ patients with coeliac disease. The addition of a peptic-tryptic digest of gliadin or gliadin alone to the lymphocyte cultures did not significantly alter transformation in normal persons or patients. Although the technique employed to assess lymphocyte transformation and the antigens used were different in the present study the results confirm the normal lymphocyte response to PHA in coeliac disease.

Asquith et al (1970a) reported a significantly greater degree of stimulation of lymphocyte transformation by GF III in 11 patients on a gluten free diet, but not in four untreated patients, compared to the control group.

In contrast to the findings of Asquith et al (1970a) GF III was not found to specifically alter the DNA synthesis in patients of the present study but rather to cause an inhibition in control subjects and patients. This may suggest that GF III is toxic in the concentration used. However, even at lower concentrations, GF III did not cause increased ^{3}HT uptake by the lymphocytes from one patient with coeliac disease.

The normal lymphocyte response to PHA in patients irrespective of dietary treatment does not suggest impaired T cell function.

V.C. Tests on Small Bowel

V.C. TESTS ON SMALL BOWEL

C.1 JEJUNAL MUCOSAL PLASMA CELLS

RESULTS:

The mean number of immunoglobulin containing jejunal plasma cells in 28 control subjects and 29 patients with untreated coeliac disease are shown in Table C.I and Fig. C.1. Appendix E lists the individual cell counts per sq.mm. of jejunal mucosa. A section of jejunal mucosa after fluorescent labelling with anti-IgM antiserumis shown in Fig. C.2.

In the control subjects IgA containing cells were the most numerous and IgA counts were significantly higher than IgG counts ($t = 4.3373$, $P<0.001$, Student's t test) and IgM counts ($t = 3.6789$, $P<0.005$). The difference between the number of cells containing IgM and IgG was not significant ($t = 0.7993$, N.S.).

By contrast IgM containing cells were predominant in the 29 untreated patients with coeliac disease. They were significantly more numerous than IgG cells ($t = 4.2350$, $P<0.001$). There was no significant difference between IgM and IgA plasma cells ($t = 1.4379$, N.S.).

A comparison of patients with coeliac disease and control subjects showed there were significantly increased IgM cell counts in the former ($t = 4.9757$, $P<0.001$). There was no significant difference between mean IgA cell counts ($t = 0.2352$, N.S.) and IgG cell counts ($t = 1.9504$, N.S.).

Table C.II and figure C.3 show the mean cell counts in seven patients studied whilst on an unrestricted diet and again

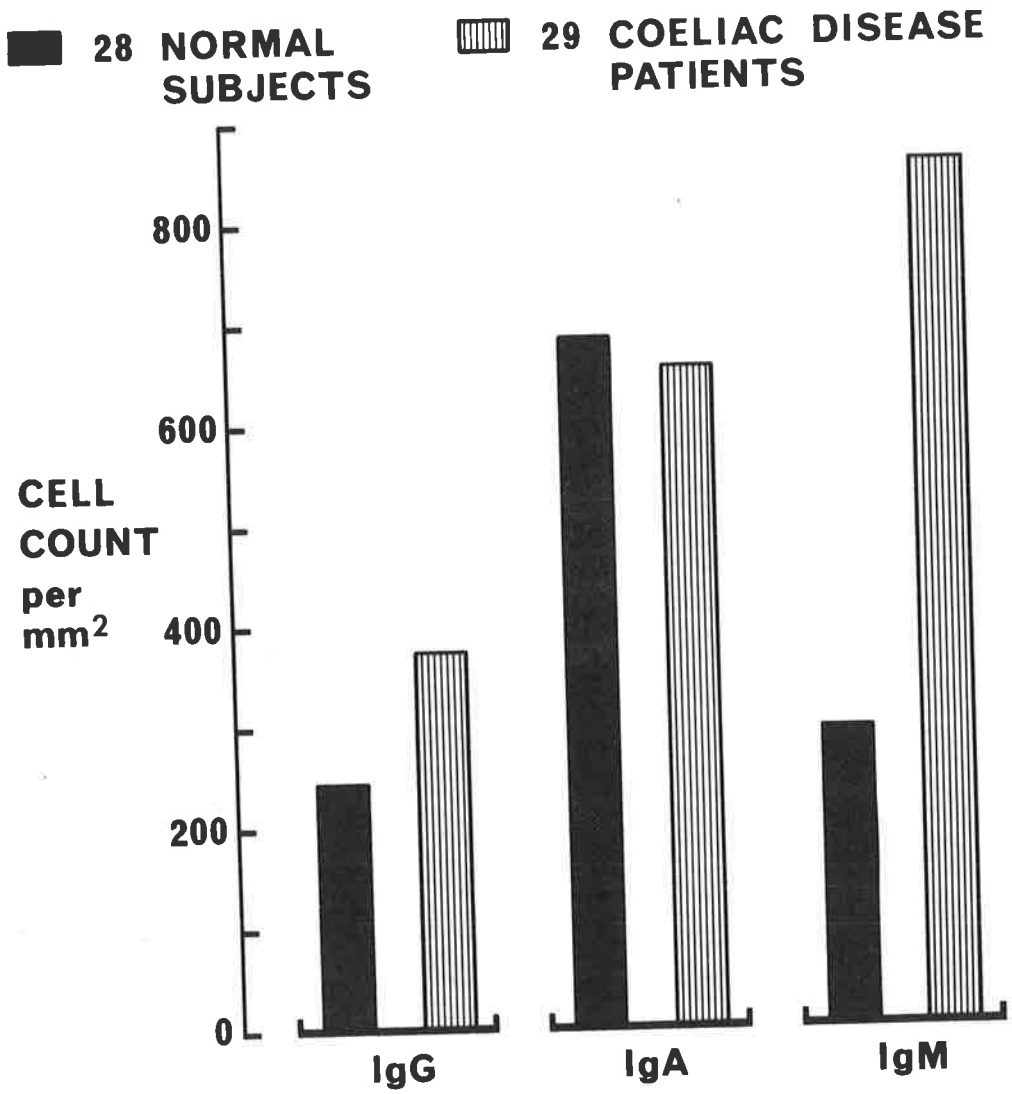

Figure C.1. Mean plasma cell counts per square mm. of jejunal mucosa in subjects with histologically normal mucosa and patients with untreated coeliac disease.

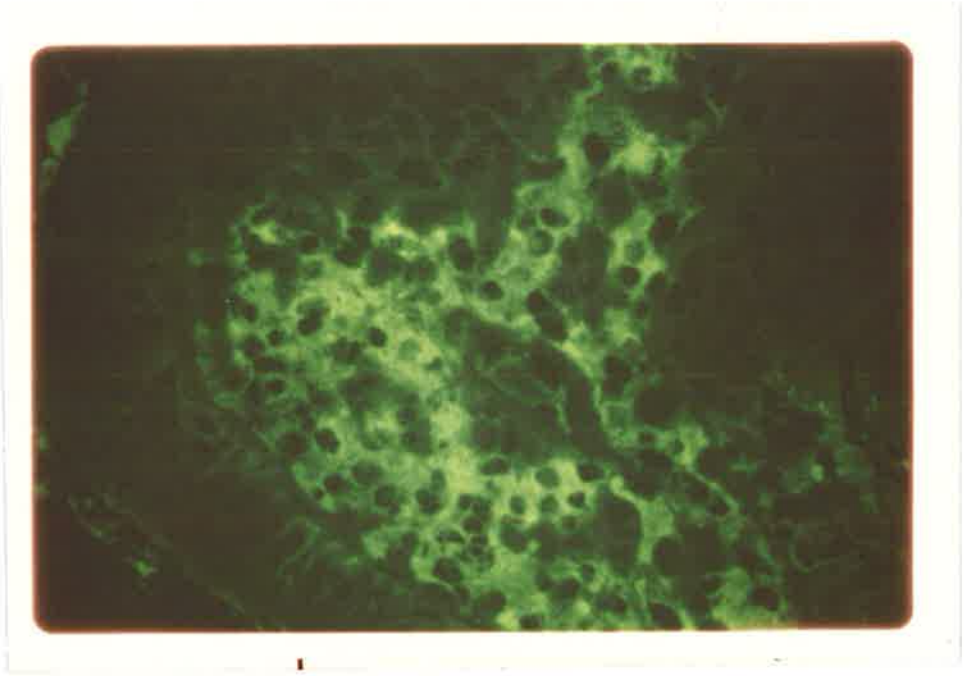

Figure C.2. Section of jejunal mucosa from a patient with coeliac disease after fluorescent labeling with anti-IgM antisera.

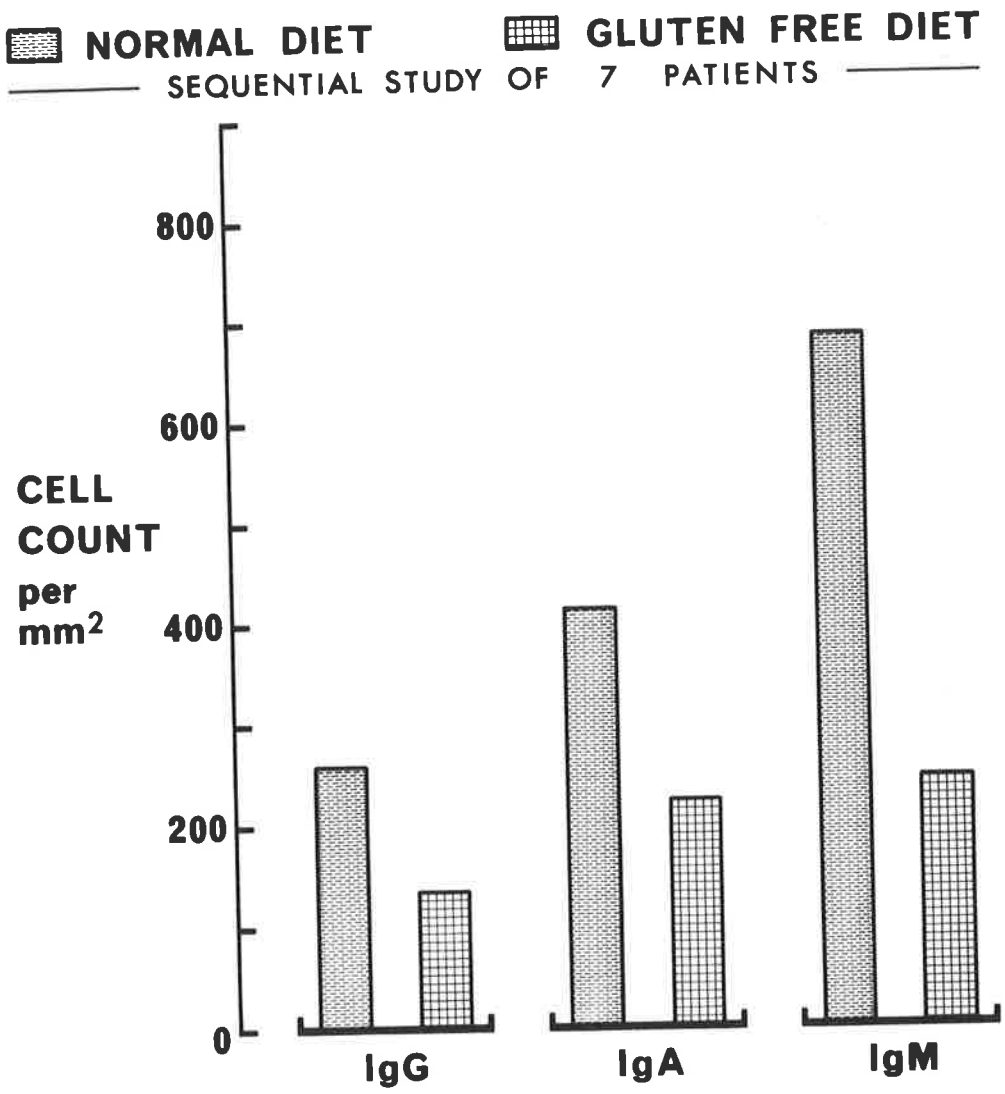

Figure C.3. Mean plasma cell counts per square mm. of jejunal mucosa in seven patients with coeliac disease before and after a gluten free diet.

TABLE C.I - JEJUNAL PLASMA CELLS (MEAN ± 1 S.D.)

IN PATIENTS WITH UNTREATED COELIAC DISEASE AND

CONTROL SUBJECTS

(Cells per/mm^2 of mucosa)

GROUP	IgG	IgA	IgM
COELIAC DISEASE (29)	374 ± 286	655 ± 516	856 ± 530
CONTROL SUBJECTS (28)	244 ± 199	687 ± 492	294 ± 257
SIGNIFICANCE (Student's t test)	N.S.	N.S.	P < 0.001

TABLE C.II - JEJUNAL PLASMA CELLS (MEAN ± 1 S.D.)

A SEQUENTIAL STUDY OF 7 PATIENTS WITH COELIAC DISEASE

(Cells per/mm^2 of mucosa)

DIETARY STATUS	IgG	IgA	IgM
NORMAL DIET	260 ± 172	414 ± 303	686 ± 404
GLUTEN FREE DIET	137 ± 85	223 ± 91	243 ± 102
SIGNIFICANCE (Student's t test)	N.S.	N.S.	P < 0.025

after a gluten free diet of 15 months mean duration. The individual cell counts are listed in Appendix E. The mean cell counts of the seven patients on a normal diet were lower than those of the group of 29 patients on a normal diet. However there was no statistically significant difference in regard to IgG (0.9829, N.S. Student's t test), IgA (1.1538, N.S.) or IgM (0.7723, N.S.) cell counts between the two groups. Although there was a reduction in the numbers of all immunoglobulin containing cells following gluten restriction this was significant only in the case of IgM ($t = 2.6042$, $P<0.025$).

On comparing the cell densities in treated patients and the control group there was a significant reduction only in the number of IgA containing cells ($t = 2.4125$, $P<0.025$).

DISCUSSION

Normal persons had significantly higher numbers of IgA cells compared to both IgG and IgM cells in the small bowel mucosa. There was no significant difference between the IgG and IgM cell counts. Similar findings were reported by Crabbe et al (1965) using a different technique. Pettingale (1971) who studied six control subjects reported similar findings in regard to IgA cells, but found the number of IgM cells to be significantly greater than the numbers of IgG cells.

In the present study the ratio of IgA : IgM : IgG was 2.8 : 1.2 : 1.0. Pettingale (1971) compared the ratios in his study (3 : 2 : 1) to the ratios which he calculated for the study of Crabbe et al (1965) of 10 : 6 : 1. However the cell ratios calculated from the figures in Crabbe's study are 10 : 1.6 : 1 and not as given by Pettingale. It is not possible

to verify the cell ratios in Pettingale's study as no mean values are stated. However, as reported by Pettingale and also in the present study, the IgA cell counts are not as markedly dominant as found by Crabbe et al (1965).

Thus the present study confirms the findings of a predominantly IgA cell population in the normal gastrointestinal tract as reported by other workers, Crabbe et al (1965), Odgers and Wangel (1968), Søltoft and Weeke (1969), Pettingale (1971).

With regard to coeliac disease, Søltoft and Weeke (1969) and Rubin et al (1965) reported that IgA containing cells were predominant. Søltoft and Weeke (1969) also reported patients with coeliac disease having significantly higher IgG and IgM cell counts than did normal subjects and that this applied regardless of dietary restrictions. Salvilahti (1972) reported significant increases in all three cell types - IgA, IgM and IgG - in the coeliac mucosa. IgA was the predominant cell type in both patients and normal subjects. Douglas et al (1970) reported findings similar to the present study in which IgM containing cells were the most frequent. Pettingale's (1971) findings of IgM cell dominance in coeliac disease are also in agreement with those of the present study.

Søltoft and Weeke (1969) compared the cell densities before and after treatment in four patients and found that they were slightly but not significantly lower after a gluten free diet. Savilahti (1972) in a sequential study of seven patients reported a significant fall in IgG, IgA and IgM cells following dietary therapy of 1 - 4 months.

In the present study seven patients were investigated

before and after dietary therapy (mean 15 months), and there was a significant fall in IgM counts compared to pre-treatment values. IgA counts also decreased, to reach a level significantly lower than that in normal subjects.

Although some comparison is possible between the present study and those of other workers, the methods used to quantitate the plasma cells differ. Søltoft and Weeke (1969), Douglas et al (1970) and Pettingale (1971) employed planimetric methods which probably underestimate the total number of cells due to the smaller size of the plasma cell in coeliac disease, which both Pettingale (1971) and Savilahti (1972) observed. Comparison of planimetric methods and the direct counting techniques showed this discrepancy which led Savilahti (1972) to conclude that the direct counting technique was superior. The direct counting technique has been criticized on the grounds that individual cells cannot be discerned clearly. This was indeed observed during the initial stage of this study. However, on using fluoresceinated antisera which was passed through a sephadex column (G-25) and centrifuged before use this problem was overcome. In the present study the total number of cells in each section were counted in duplicate and the mean value obtained.

Variation in technique, the duration of the initial disease process, dietary treatment, and the degree of histological damage may affect the plasma cell populations. Although Savilahti (1972) states that avoidance of gluten was complete in his patients, a similar claim cannot be made in regard to the seven patients studied on a gluten free diet in the present study. Patient C.D. (22) a 48-year old female admitted to gluten ingestion on social occasions. A list of all

food and drink taken over a three day period compiled by patients on a gluten free diet invariably revealed an item which the patient was unaware contained gluten. Thus, due to the ubiquitous nature of gluten a strict gluten free diet may well be impossible to achieve. However, this is likely to be a world wide problem and should not invalidate comparisons between different studies.

Gluten withdrawal had a variable effect in the seven patients studied sequentially. In one patient (C.D.12) it was unfortunately not possible to make a histological assessment due both to the small size of the biopsy specimen and cross sectioning. In four patients sub total villous atrophy was present before and after dietary treatment. The other two patients showed histological improvement (partial villous strophy and mild partial villous atrophy). Thus none of the seven patients had a histologically normal small bowel mucosa.

To summarize, the present study which comprised a larger number of normal control subjects (28) and patients (29) than previously reported, showed an absolute increase in IgM containing cells, and a significant reduction after dietary therapy. The cause for a predominance of the IgM plasma cell in coeliac disease is not known. Douglas et al (1970) speculate that the IgM containing cells are increased to compensate for an impaired response to antigenic stimulation of IgA containing cells. The in-vitro studies of Loeb et al (1971) have demonstrated significantly increased synthesis of IgA and IgM in jejunal mucosa of patients with coeliac disease. However more IgA was made than IgM although it does not necessarily follow that IgA cells outnumber IgM cells, as the rate of synthesis of IgA and IgM may

well differ.

Since the increase in IgM containing cells does not persist after gluten exclusion, it is likely to be part of an inflammatory response or due to continued antigenic stimulation rather than a primary fault.

V.C. TESTS ON SMALL BOWEL

C.2. JEJUNAL JUICE

C.2. (i) INTESTINAL IMMUNOGLOBULIN LEVELS

RESULTS:

Tables C.III and C.IV show the intestinal immunoglobulin levels in 29 normal subjects and 15 patients with coeliac disease before treatment. The 29 normal subjects consisted of members of the hospital staff who had no history of gastrointestinal disease. Values below 5.0 mg% for IgG and below 4.0 mg% for IgA and IgM were recorded as <5.0 mg or <4.0 mg as the low level Immunoplates (Hyland, Travenol Laboratories, Inc. Costa Mesa, Calif. U.S.A.) are relatively insensitive to immunoglobulin levels below these ranges. For this reason it was not possible to calculate mean values and therefore a frequency distribution of the values was obtained comparing normal subjects and patients (Tables C.V, C.VI, C.VII).

By applying the Mann-Whitney U test, it was found that there was no significant difference between controls and patients in regard to IgG and IgM levels. However, IgA levels were significantly lower in patients with coeliac disease ($z = 1.82$, $P < 0.03$).

DISCUSSION

Other authors have shown that intestinal juice IgM is raised in patients with untreated coeliac disease, but not in treated patients (Douglas et al 1970). However, neither Asquith et al (1970b) nor Savilahti (1072) found any significant difference in the intestinal juice IgM immunoglobulin levels

TABLE C.III INTESTINAL IMMUNOGLOBULIN LEVELS (mg/100 ml) IN NORMAL SUBJECTS

SUBJECT	IgG	IgA	IgM
N1	<5.0	5.2	<4.0
N2	6.2	4.5	0
N3	<5.0	5.8	<4.0
N4	0	5.2	<4.0
N5	6.2	4.5	11.0
N6	0	<4.0	0
N7	<5.0	5.9	11.0
N8	0	4.5	0
N9	6.2	5.9	4.0
N10	12.0	4.5	<4.0
N11	0	4.0	0
N12	18.0	7.9	<4.0
N13	<5.0	<4.0	5.4
N14	6.2	<4.0	11.0
N15	<5.0	<4.0	<4.0
N16	<5.0	<4.0	0
N17	<5.0	<4.0	9.0
N18	8.6	6.4	4.0
N19	<5.0	0	<4.0
N20	<5.0	0	12.0
N21	<5.0	16.0	9.0
N22	<5.0	7.0	12.0
N23	<5.0	0	0
N24	0	4.0	<4.0
N25	<5.0	0	<4.0
N26	8.6	<4.0	9.0
N27	11.5	<4.0	<4.0
N28	0	4.0	5.0
N29	<5.0	<4.0	<4.0

TABLE C.IV INTESTINAL IMMUNOGLOBULIN LEVELS (mg/100 ml.) IN PATIENTS WITH UNTREATED COELIAC DISEASE

PATIENT	IgG	IgA	IgM
C.D. 2	<5.0	4.1	<4.0
C.D. 10	<5.0	5.0	<4.0
C.D. 11	0	0	0
C.D. 12	0	5.9	<4.0
C.D. 13	<5.0	<4.0	9.0
C.D. 14	<5.0	<4.0	4.0
C.D. 15	0	0	0
C.D. 16	0	0	0
C.D. 18	18.0	<4.0	9.0
C.D. 19	0	0	9.0
C.D. 21	<5.0	<4.0	0
C.D. 23	14.0	4.0	5.0
C.D. 25	<5.0	<4.0	<4.0
C.D. 28	>120.0	0	16.0
C.D. 29	<5.0	0	0

TABLE C.V THE DISTRIBUTION OF INTESTINAL JUICE IgG LEVELS IN PATIENTS WITH UNTREATED COELIAC DISEASE AND NORMAL SUBJECTS

GROUP	TOTAL	IMMUNOGLOBULIN LEVELS mg%				
		0 - 5	>5 - 10	>10-15	>15-20	>20
COELIAC DISEASE	15	12	0	1	1	1
NORMAL SUBJECTS	29	20	6	2	1	0

TABLE C.VI THE DISTRIBUTION OF INTESTINAL JUICE IgA LEVELS IN PATIENTS WITH UNTREATED COELIAC DISEASE AND NORMAL SUBJECTS

GROUP	TOTAL	IMMUNOGLOBULIN LEVELS mg%			
		0 - 4	>4 - 6	>6 - 8	>8
COELIAC DISEASE	15	12	3	0	0
NORMAL SUBJECTS	29	16	9	3	1

TABLE C.VII THE DISTRIBUTION OF INTESTINAL JUICE IgM LEVELS IN PATIENTS WITH UNTREATED COELIAC DISEASE AND NORMAL SUBJECTS

GROUP	TOTAL	IMMUNOGLOBULIN LEVELS mg%					
		0 - 4	>4 - 6	>6 - 8	>8-10	>10-12	>12
COELIAC DISEASE	15	10	1	0	3	0	1
NORMAL SUBJECTS	29	19	2	0	3	5	0

between patients and control subjects.

The intestinal juice in this study was collected by the method described by Plant and Keonil (1969), but it is possible that some values may have been erroneously low as antitrypsin inhibitor was not added. However this should not invalidate a comparison with the studies of Savilahti and Asquith et al (1970b) since both studies do not mention addition of such an inhibitor. In this study values below the manufacturers stated lower limit of accurate measurement were not extrapolated from the graph although Savilahti (1972) reports doing this. In the present study there were many instances in both patients and control subjects of such low levels and since numerical mean values could not be calculated, it was not possible to make an accurate comparison with the results of other workers.

Although in the present study in patients with coeliac disease the predominant immunocyte was the IgM containing cell yet the IgM levels were not significantly higher than in the control group. This may suggest that all of the IgM containing cells in the jejunal mucosa are not actively synthesising IgM.

In the present investigation, untreated patients with coeliac disease had significantly lower levels of IgA compared to the control subjects. This finding is in contrast to the normal levels of IgA reported by Douglas et al (1970) and Savilahti (1972) and elevated levels detected in the study by Cooke's group (Asquith et al 1970).

It has been postulated that IgA, once synthesised in the plasma cell complexes with the secretory piece, a glyco-

protein which joins two molecules of IgA into a dimer, secretory IgA. The secretory piece which is synthesised in the epithelial cell, is thought to facilitate IgA transport across the intestinal epithelial cell and render it more resistant to both intra and extra cellular digestion (Tomasi, 1973). It may be possible that the extensive epithelial cell damage in the small bowel mucosa in untreated coeliac disease adversely affects the production or coupling of the secretary piece, thus rendering IgA more susceptible to proteolysis. This may account for the significantly decreased levels of IgA observed in untreated patients in the present study.

V.C. TESTS ON SMALL BOWEL

C.2. JEJUNAL JUICE

C.2. (ii) DIETARY ANTIBODIES

RESULTS:

Dietary antibodies were not detected in the intestinal juice of 15 patients with coeliac disease on a normal diet and 29 control subjects.

DISCUSSION

Although dietary antibodies could not be demonstrated in this study, Katz et al (1968) and Ferguson and Carswell (1972) have documented their occurrence. Both groups of workers used micro-modifications of agar gel diffusion which is less sensitive than immunoelectro diffusion which was used in this study. The collection and storage of the intestinal fluid were similar.

While Katz et al (1968) found the antibodies in a high percentage of untreated patients with coeliac disease but not in control subjects, Ferguson and Carswell (1972) found antibodies in patients with coeliac disease as well as patients with other gastrointestinal diseases. Both groups of patients also had antibodies to animal antigens. The incidence of antibodies both to cereal and animal antigens was higher in coeliac disease than in the control group suggesting that patients with coeliac disease had a heightened humoral immune response.

Loeb et al (1971) demonstrated increased synthesis of immunoglobulins in jejunal mucosa from patients with coeliac disease on stimulation with gluten but did not show that the

immunoglobulin was capable of reacting with gluten. There is thus yet insufficient evidence that a specific antibody response to gluten occurs in jejunal mucosa from patients with coeliac disease.

V.D. Delayed Hypersensitivity PAGE

V. D. DELAYED HYPERSENSITIVITY

D.1 INTRADERMAL SKIN TESTS

RESULTS:

Intradermal skin tests were performed on 12 patients with coeliac disease, five of whom were on a gluten free diet. Appendix F shows the reaction of each patient and control subject to the three antigens. One patient (C.D. 5) failed to react to all three antigens. Only eleven age and sex matched control subjects were available for comparison, none of whom failed to react (Table D.I). There was no significant difference in the reaction between the two groups (Fisher's exact test).

DISCUSSION

Although the exact mechanism whereby delayed hypersensitivity is mediated is not yet fully understood, delayed hypersensitivity reactions have proved to be an effective method of assessing cellular immunity. In this study only three antigens were used, although a larger number has been advocated by some workers (Fudenberg, Good, Goodman, Hitzig, Kunkel, Roitt, Rossen, Rowe, Seligmann and Soothill, 1971). However, Forbes (1971) detected a positive antigenic response to at least one of the antigens (Candida, Streptococcus and Mumps) in 41 of 42 random control subjects in South Australia. The possibility that a negative response might reflect absence of previous exposure to the antigens used was obviated by re-sensitization two weeks later.

Of the twelve patients with coeliac disease studied, the single non-reactor also failed to produce antibodies to both

TABLE D.I SKIN TEST REACTIVITY IN PATIENTS WITH COELIAC DISEASE AND CONTROL SUBJECTS

SUBJECTS	TOTAL	REACTORS
PATIENTS	12	11
CONTROLS	11	11

S.typhi and Tetanus toxoid, indicating a failure of both cellular and humoral responses. This is of course grossly abnormal. However, in the small group studied there was no statistically significant difference between patients with coeliac disease and normal subjects in respect of delayed hypersensitivity.

APPENDICES

APPENDIX A

APPENDIX A (1)

The Department of Medicine,
The University of Adelaide,
The Queen Elizabeth Hospital,
WOODVILLE. 5011

Dear

As you know your has been treated at the Adelaide Children's Hospital for Coeliac Disease.

We are at present studying this disease in the hope of developing better methods for its detection. Would you be prepared to help in this by asking your whether would mind a simple blood test?

In cases where the diagnosis of the disease is in doubt, we would like to help by establishing a firm diagnosis. This is important as the current view is that patients with proven coeliac disease should adhere to a strict gluten free diet. We would be happy to supply you with up-to-date diet sheets listing gluten free foods, if you should require them.

One of us would be happy to call at your home either on or after 6.00 p.m. Please indicate on the reply slip a convenient time to call and which day would be suitable and return the slip in the envelope provided.

If these dates are not convenient or if you would prefer to call in at the Hospital please contact us at 45.0222, Extension 340. You may like to show this letter to your own Doctor.

Yours sincerely,
R. N. RATNAIKE, (Lecturer).
A. G. WANGEL, (Professor).

It would be convenient for you to call on:

at.....p.m.

.....

(Signature)

APPENDIX B

APPENDIX B (1)

NORMAL RANGE

1. Haemoglobin	- Adults	: Males	14.0 - 18.0 g/100 ml.
		: Females	12.0 - 16.0 g/100 ml.
	- Children	:	11.5 - 13.0 g/100 ml.
2. Serum B_{12}	- Adults & Children		200 - 1000 pg/ml.
3. Serum Folate	- Adults & Children		3.0 - 22 ng/ml.
4. Serum Iron	- Adults	: Males	50 - 160 µg/100 ml.
		: Females	45 - 160 µg/100 ml.
	Children	:	45 - 160 µg/100 ml.
5. Serum Albumin	- Adults	:	3.5 - 5.0 g/100 ml.
	- Children	: <1 year	3.1 - 4.3 g/100 ml.
		1-4 years	3.3 - 4.5 g/100 ml.
		4-12years	3.0 - 5.0 g/100 ml.

6. The modified D-xylose (5g) absorption test as described by Kendall (1970)* was performed on patients admitted under the care of Prof. A.G. Wangel at the Queen Elizabeth Hospital. However, some patients received 25g of D-xylose. Only blood xylose levels were estimated in patients investigated at the Adelaide Children's Hospital. The results of the D-xylose absorption test are therefore expressed as normal (N) or below normal (↓N).

7. Three day faecal fat estimation

Adults	< 24 g/3 days
Children	< 15 g/3 days

8. One stage prothrombin time (OSPT)

- Adults	60 - 100%	(Queen Elizabeth Hospital)
- Children	67 - 100%	(Adelaide Children's Hospital)

* Kendall, M.J. (1970), The influence of age on the xylase absorption test. Gut, 11: 498.

APPENDIX B (2)

HAEMATOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL INVESTIGATIONS (GROUP I)

(Ten patients on whom immunological studies were performed while on a normal diet only)

No.	Age	Sex	Hb.	Serum B_{12}	Serum Folic Acid	Serum Iron	Serum Albumin	D-Xylose Absorption	3 D.F.F.	OSPT	Biopsy
C.D. 1	1	F	11.3	-	-	-	-	↓N	-	100%	PVA
C.D. 2	$12^{6/12}$	F	13.0	1400	16.8	110	4.7	N	51.0	54%	PVA
C.D. 3	$4^{6/12}$	F	11.0	-	-	50	3.9	↓N	21.7	67%	SVA
C.D. 4	$3^{6/12}$	M	11.8	-	-	-	-	N	-	65%	SVA
C.D. 5	$0^{9/12}$	M	14.0	-	-	90	3.4	↓N	14.9	56%	SVA
C.D. 6	76	F	15.1	570	1.8	100	3.5	↓N	19.3	60%	PVA
C.D. 7	24	F	13.1	460	5.0	-	4.2	N	18.0	62%	SVA
C.D. 8	2	F	11.3	-	-	-	-	↓N	10.1	77%	PVA
C.D. 9	$3^{6/12}$	M	13.6	1140	0.9	-	-	↓N	34.2	67%	SVA
C.D.10	$2^{3/12}$	F	7.4	-	-	-	3.5	↓N	-	75%	PVA

APPENDIX B (3)

HAEMATOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL INVESTIGATIONS (GROUP II)

(23 Patients on whom immunological studies were performed before and after a gluten free diet)

No.	Age	Sex	Hb.	Serum B_{12}	Serum Folic Acid	Serum Iron	Serum Albumin	D-xylose Absorption	3 D.F.F.	OSPT	Biopsy
C.D.11	70	M	11.3	400	0.4	65	2.9	↓N	32	60%	PVA
C.D.12	57	M	13.0	450	1.6	58	3.0	↓N	37.5	41%	TVA
C.D.13	8	M	12.2	850	5.1	45	3.7	↓N	18.7	41%	SVA
C.D.14	22	F	12.0	470	2.0	-	3.5	↓N	37.5	70%	SVA
C.D.15	40	M	11.8	400	2.2	-	3.0	N	22.0	60%	SVA
C.D.16	47	F	12.1	180	0.3	15	3.4	N	107.3	38%	SVA
C.D.17	12	F	13.0	800	2.6	-	2.8	N	28.0	77%	SVA
C.D.18	60	M	13.0	660	1.7	80	2.0	N	46.9	58%	PVA
C.D.19	51	F	12.0	330	-	-	2.7	↓N	83.0	25%	SVA
C.D.20	32	F	13.2	400	1.2	190	4.8	↓N	104.0	65%	SVA
C.D.21	$2^{1/12}$	M	12.6	-	-	-	2.6	↓N	37.5	25%	SVA
C.D.22	48	F	13.2	2600	7.2	70	3.4	↓N	6.0	65%	SVA
C.D.23	21	M	8.7	260	1.2	39	3.8	N	75.9	45%	SVA

APPENDIX B (4)

HAEMATOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL INVESTIGATIONS (GROUP II)

(23 Patients on whom immunological studies were performed before and after a gluten free diet)

No.	Age	Sex	Hb.	Serum B_{12}	Serum Folic Acid	Serum Iron	Serum Albumin	D-xylose Absorption	3 D.F.F.		Biopsy
C.D.24	$1^{6/12}$	F	11.3	-	6.0	-	2.9	↓N	-	90%	PVA
C.D.25	14	F	14.9	840	1.7	-	4.5	↓N	126.6	50%	SVA
C.D.26	17	F	13.2	600	4.7	-	4.4	N	14.8	57%	SVA
C.D.27	57	M	12.9	1400	1.2	34	2.5	↓N	21.0	56%	TVA
C.D.28	34	F	9.9	440	1.1	30	2.2	↓N	113.3	14%	SVA
C.D.29	11	M	13.9	1150	5.1	136	4.0	↓N	14.5	60%	SVA
C.D.30	12	M	10.2	820	8.3	130	4.4	N	39.0	70%	PVA
C.D.31	70	F	12.1	-	0.3	158	1.1	↓N	107.3	38%	SVA
C.D.32	52	F	11.9	550	3.9	106	2.5	N	42.0	65%	PVA
C.D.33	$0^{6/12}$	F	11.8	-	-	-	-	↓N	-	80%	PVA

APPENDIX B (5)

HAEMATOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL INVESTIGATIONS (GROUP III)

(17 Patients on whom immunological studies were performed while on a gluten free diet only)

No.	Age	Sex	Hb.	Serum B_{12}	Serum Folic Acid	Serum Iron	Serum Albumin	D-xylose Absorption	3 D.F.F.	OSPT	Biopsy
C.D.34	57	F	12.5	720	7.2	-	-	↓N	85.4	-	SVA
C.D.35	24	F	12.8	320	3.1	-	3.7	↓N	51.0	-	SVA
C.D.36	11	F	11.6	-	-	-	-	↓N	-	-	PVA
C.D.37	19	F	13.6	340	1.7	-	-	↓N	46.0	-	TVA
C.D.38	3	M	10.5	-	-	-	-	↓N	15.3	100%	SVA
C.D.39	8	M	11.3	1175	7.5	153	-	-	12.8	-	SVA
C.D.40	4	M	10.6	-	-	-	-	↓N	-	-	SVA
C.D.41	37	F	12.7	330	2.9	-	3.8	↓N	14.0	-	PVA
C.D.42	11	M	10.1	1300	5.8	102	2.6	↓N	26.0	-	SVA
C.D.43	65	F	13.6	1000	5.7	115	4.4	↓N	27.0	60%	PVA
C.D.44	61	M	13.0	150	0.9	48	1.7	↓N	188.0	50%	SVA
C.D.45	11	M	7.9	300	1.9	-	-	↓N	-	-	SVA
C.D.46	65	F	13.6	950	-	-	-	↓N	38.0	-	SVA
C.D.47	3	M	11.7	-	-	32	-	↓N	-	-	SVA
C.D.48	13	F	13.0	-	-	-	-	-	34.5	80%	PVA
C.D.49	5	F	11.9	-	-	70	-	↓N	28.0	63%	PVA
C.D.50	11	M	13.1	400	1.2	-	-	↓N	24.0	-	PVA

APPENDIX C

APPENDIX C (1)

SERUM IMMUNOGLOBULIN AND COMPLEMENT LEVELS IN COELIAC DISEASE.

TEN PATIENTS ON A NORMAL DIET ONLY.

NO.	IgG	IgA	IgM	C_3	C_{50}
C.D. 1	780	69	100	-	-
C.D. 2	1150	85	115	95	96
C.D. 3	645	80	105	72	96
C.D. 4	1530	7	55	100	96
C.D. 5	250	14	1958	-	-
C.D. 6	1440	380	240	-	-
C.D. 7	1240	105	100	90	94
C.D. 8	1480	140	85	195	96
C.D. 9	1280	125	85	115	96
C.D.10	950	120	65	320	94

APPENDIX C (2)

SERUM IMMUNOGLOBULIN AND COMPLEMENT LEVELS IN COELIAC DISEASE. TWENTY-THREE PATIENTS BEFORE AND AFTER GLUTEN RESTRICTION.

NO.	DURATION OF TREATMENT	NORMAL DIET			GLUTEN FREE DIET			C 3		TOTAL COMPLEMENT	
		IgG	IgA	IgM	IgG	IgA	IgM	NORMAL DIET	GLUTEN FREE DIET	NORMAL DIET	GLUTEN FREE DIET
C.D.11	6	1830	405	90	2490	455	105	240	145	94	96
C.D.12	12	1410	495	55	1700	570	75	60	75	96	96
C.D.13	24	1150	345	135	1900	195	165	130	90	96	80
C.D.14	9	1205	152	96	1240	125	160	95	90	96	96
C.D.15	12	1780	480	100	2050	295	115	75	72	96	90
C.D.16	18	1410	310	115	1530	315	185	110	185	96	96
C.D.17	1	1020	75	120	880	65	150	155	108	94	80
C.D.18	24	1410	120	45	1400	125	215	135	105	94	90
C.D.19	12	1890	290	165	1580	175	160	100	94	94	94
C.D.20	24	1410	150	45	1660	150	65	130	140	96	96
C.D.21	12	635	30	170	1240	10	125	30	82	94	96
C.D.22	24	2290	425	245	1290	285	275	100	101	-	96
C.D.23	24	1440	200	190	2020	235	185	53	60	94	90
C.D.24	20	1830	405	90	740	45	70	240	75	94	90
C.D.25	6	910	150	100	1255	150	125	72	165	96	90
C.D.26	8	1800	265	125	2150	240	140	80	90	94	90
C.D.27	5	2080	2100	110	1370	720	80	96	60	96	96
C.D.28	6	1150	130	105	1890	120	190	50	120	96	96
C.D.29	24	980	223	100	1300	245	295	120	106	96	96
C.D.30	1	1350	190	260	2600	205	290	85	134	40	60
C.D.31	6	720	275	76	1150	125	95	110	62	-	96
C.D.32	12	1300	330	80	1830	270	255	200	130	96	96
C.D.33	21	605	80	100	740	80	195	110	85	90	80

APPENDIX C (3)

SERUM IMMUNOGLOBULIN AND COMPLEMENT LEVELS IN COELIAC DISEASE

SEVENTEEN PATIENTS ON A GLUTEN FREE DIET

NO.	IgG	IgA	IgM	C_3	TOTAL COMPLEMENT
C.D. 34	1740	315	150	75	80
C.D. 35	1260	280	95	95	96
C.D. 36	1790	5	65	75	96
C.D. 37	950	150	165	115	80
C.D. 38	850	75	65	115	96
C.D. 39	865	85	100	100	94
C.D. 40	530	45	55	79	94
C.D. 41	1600	155	95	69	-
C.D. 42	1570	27	60	145	96
C.D. 43	1950	215	310	140	96
C.D. 44	1590	375	85	85	90
C.D. 45	2660	265	85	139	96
C.D. 46	1840	235	90	105	94
C.D. 47	815	60	110	55	96
C.D. 48	2140	215	175	69	80
C.D. 49	1460	145	185	88	80
C.D. 50	1230	100	70	72	96

APPENDIX C (4)

SERUM IMMUNOGLOBULIN AND COMPLEMENT LEVELS IN ULCERATIVE COLITIS.

NO.	AGE	SEX	IgG	IgA	IgM	C_3	TOTAL COMPLEMENT
UC 1	26	F	890	45	100	130	-
UC 2	67	F	1540	255	185	105	80
UC 3	30	M	1330	180	175	85	90
UC 4	63	M	1280	255	105	80	80
UC 5	22	F	1840	200	285	59	94
UC 6	24	F	1480	105	150	106	60
UC 7	47	F	1710	140	205	85	80
UC 8	57	M	2100	0	295	83	94
UC 9	67	M	1290	215	75	114	90
UC 10	24	M	1110	160	130	110	-
UC 11	36	F	2000	320	305	96	96
UC 12	27	F	1666	175	380	95	96
UC 13	76	M	3950	525	350	69	96
UC 14	16	F	1120	75	275	120	94
UC 15	38	F	1715	240	180	180	90
UC 16	64	M	1770	325	120	110	94
UC 17	63	F	3840	300	200	132	60
UC 18	55	M	2190	870	285	75	94
UC 19	24	F	1310	205	225	75	94
UC 20	70	F	1050	250	105	110	96
UC 21	65	F	1620	195	120	90	96
UC 22	19	F	2130	285	190	80	80
UC 23	33	M	1650	405	195	105	80
UC 24	62	F	2180	390	75	64	90
UC 25	24	M	1130	270	75	105	-
UC 26	46	M	515	245	85	45	90
UC 27	53	M	1180	190	70	75	90
UC 28	39	F	1400	275	85	140	90
UC 29	48	F	950	295	105	95	96
UC 30	50	M	1950	185	160	33	80
UC 31	32	F	2310	245	100	95	90

APPENDIX C (5)

SERUM IMMUNOGLOBULIN AND COMPLEMENT LEVELS IN CROHN'S DISEASE

NO.	AGE	SEX	IgG	IgA	IgM	C_3	TOTAL COMPLEMENT
Cr.D. 1	52	F	1330	300	195	125	90
Cr.D. 2	46	M	1180	155	110	144	-
Cr.D. 3	50	M	1230	230	105	68	94
Cr.D. 4	39	M	1870	290	285	110	80
Cr.D. 5	50	F	970	140	165	90	96
Cr.D. 6	55	F	2220	325	210	180	94
Cr.D. 7	60	M	1520	540	90	140	90
Cr.D. 8	39	F	1845	135	200	74	-
Cr.D. 9	47	F	1560	225	125	85	96
Cr.D. 10	23	F	1510	150	225	95	80
Cr.D. 11	21	F	1715	215	270	145	80
Cr.D. 12	59	F	620	50	50	150	-
Cr.D. 13	57	F	2015	555	305	120	94
Cr.D. 14	62	M	2430	690	100	96	-
Cr.D. 15	45	F	1800	495	125	90	96

APPENDIX C (6)

SERUM IMMUNOGLOBULIN AND COMPLEMENT LEVELS IN THE IRRITABLE COLON SYNDROME.

NO.	AGE	SEX	IgG	IgA	IgM	C_3	TOTAL COMPLEMENT
ICS 1	67	F	1200	225	110	132	90
ICS 2	27	F	1410	165	105	50	-
ICS 3	40	M	965	655	89	64	-
ICS 4	41	M	1410	140	95	96	96
ICS 5	43	M	1320	120	160	110	96
ICS 6	71	F	2150	310	240	130	96
ICS 7	57	F	1600	265	75	100	96
ICS 8	61	M	1480	185	145	124	90
ICS 9	51	F	2030	540	160	130	-
ICS 10	63	F	1360	175	95	175	96
ICS 11	36	F	1110	280	230	160	94
ICS 12	42	F	1310	155	110	115	96
ICS 13	68	F	1010	125	35	150	94
ICS 14	18	F	1200	200	170	125	96
ICS 15	21	M	1740	180	205	160	96
ICS 16	48	F	510	105	195	135	98
ICS 17	32	M	1440	170	150	85	96
ICS 18	71	M	1410	255	105	130	96
ICS 19	23	M	1900	225	170	230	96
ICS 20	50	M	2050	215	145	115	96
ICS 21	29	F	1120	3	130	200	96
ICS 22	56	M	1400	325	270	64	90
ICS 23	23	F	2490	235	215	106	90

APPENDIX D

APPENDIX D (1)

PERCENTAGE OF LYMPHOCYTES WITH SURFACE BOUND IMMUNOGLOBULIN IN PATIENTS WITH COELIAC DISEASE, USING POLYVALENT ANTISERUM

SUBJECT		% B CELLS
NORMAL DIET	C.D. 11	25.4
	C.D. 12	28.8
	C.D. 18	26.8
	C.D. 21	14.0
	C.D. 26	19.4
	C.D. 30	26.6
	C.D. 31	20.1
GLUTEN FREE DIET	C.D. 29	16.7
	C.D. 36	20.0
	C.D. 37	30.0
	C.D. 38	13.7
	C.D. 41	10.0
	C.D. 45	8.3
	C.D. 48	13.7
	C.D. 49	16.9

APPENDIX D (2)

PERCENTAGE OF LYMPHOCYTES WITH SURFACE BOUND IMMUNOGLOBULIN IN NORMAL SUBJECTS USING POLYVALENT ANTISERUM

SUBJECT	% B CELLS
N 1	10.0
N 2	13.0
N 3	20.0
N 4	20.0
N 5	12.0
N 6	15.5
N 7	22.3
N 8	10.9
N 9	11.8
N 10	12.6
N 11	12.6
N 12	11.5
N 13	15.6
N 14	18.0
N 15	18.5
N 16	5.7
N 17	17.4
N 18	10.4
N 19	22.0
N 20	11.4
N 21	21.0
N 22	12.9
N 23	14.8
N 24	23.0
N 25	18.7
N 26	16.4
N 27	15.3
N 28	6.7
N 29	13.0

APPENDIX D (3)

ABSOLUTE NUMBER OF LYMPHOCYTES (PER MICROLITRE) WITH SURFACE BOUND IMMUNOGLOBULIN IN PATIENTS WITH COELIAC DISEASE

SUBJECT	NO. OF LYMPHOCYTES	NO. OF B CELLS
NORMAL DIET:		
C.D. 11	2262	574.0
C.D. 12	2883	830.0
C.D. 18	1872	500.9
C.D. 21	2880	403.0
C.D. 26	1624	315.0
C.D. 30	1920	510.0
C.D. 31	1725	346.7
GLUTEN FREE DIET:		
C.D. 29	2808	468.9
C.D. 36	1679	335.8
C.D. 37	2000	600.0
C.D. 38	3906	535.0
C.D. 41	1500	150.0
C.D. 45	4028	334.0
C.D. 48	2016	276.0
C.D. 49	3150	532.0

APPENDIX D (4)

ABSOLUTE NUMBER OF LYMPHOCYTES (PER MICROLITRE) WITH SURFACE BOUND IMMUNOGLOBULIN IN NORMAL SUBJECTS

SUBJECT	NO. OF LYMPHOCYTES	NO. OF B CELLS
N 2	1377	179
N 3	2560	512
N 4	2142	428
N 5	1464	176
N 6	1890	293
N 7	3300	736
N 8	855	93
N 9	1708	202
N 10	2030	256
N 11	2400	302
N 12	1012	116
N 13	1457	227
N 14	2196	395
N 15	2639	488
N 16	3296	187
N 17	1560	271
N 18	1173	122
N 19	1449	319
N 20	1887	215
N 21	1820	382
N 22	1848	238
N 23	2268	335
N 24	836	192
N 25	2880	538
N 26	2542	417
N 27	2730	417
N 28	2013	135

APPENDIX D (5)

PHA INDUCED LYMPHOCYTE UPTAKE OF ^{3}HT IN PATIENTS WITH COELIAC DISEASE

SUBJECT	d.p.m. x 10^3
NORMAL DIET	
C.D. 4	72,000
C.D. 11	89,000
C.D. 17	46,000
C.D. 23	153,000
C.D. 26	68,000
C.D. 28	37,000
C.D. 30	71,000
C.D. 32	37,000
GLUTEN FREE DIET	
C.D. 16	25,000
C.D. 21	34,000
C.D. 22	51,000
C.D. 29	82,000
C.D. 35	124,000
C.D. 36	41,000
C.D. 37	203,000
C.D. 41	86,000
C.D. 45	69,000
C.D. 48	41,000

APPENDIX E

APPENDIX E (1)

PLASMA CELLS IN HISTOLOGICALLY NORMAL JEJUNAL MUCOSA

(Cells per mm.^{2} of mucosa)

NO.	IgG	IgA	IgM
N 1	362	511	159
N 2	227	1889	227
N 3	117	1537	184
N 4	258	1408	267
N 5	713	580	487
N 6	235	260	260
N 7	70	146	97
N 8	161	912	298
N 9	158	450	225
N 10	343	436	31
N 11	36	66	42
N 12	24	57	9
N 13	10	46	10
N 14	472	1290	559
N 15	110	587	176
N 16	320	840	508
N 17	111	235	181
N 18	297	859	534
N 19	774	1297	997
N 20	331	618	505
N 21	641	1108	890
N 22	110	465	81
N 23	137	346	227
N 24	166	478	307
N 25	331	1246	693
N 26	79	235	170
N 27	28	286	26
N 28	198	1061	74

APPENDIX E (2)

PLASMA CELLS IN JEJUNAL MUCOSA OF TWENTY-NINE PATIENTS WITH COELIAC DISEASE

(Cells per mm.^{2} of mucosa)

NO.	IgG	IgA	IgM
C.D. 1	366	744	981
C.D. 2	886	1341	1640
C.D. 3	439	1062	1859
C.D. 4	372	980	1228
C.D. 6	314	959	617
C.D. 7	276	316	747
C.D. 8	594	292	856
C.D. 9	182	53	734
C.D. 10	301	1144	1295
C.D. 11	758	919	1310
C.D. 12	625	983	1339
C.D. 13	41	106	153
C.D. 14	33	96	400
C.D. 15	176	550	458
C.D. 16	264	353	420
C.D. 18	121	161	232
C.D. 19	165	114	182
C.D. 20	50	112	132
C.D. 21	69	140	214
C.D. 22	318	716	832
C.D. 23	733	1845	1771
C.D. 24	305	436	507
C.D. 25	1203	1985	1774
C.D. 26	306	569	467
C.D. 28	162	405	458
C.D. 29	252	228	1172
C.D. 30	115	313	477
C.D. 32	797	1328	1035
C.D. 33	631	758	1527

APPENDIX E (3)

PLASMA CELLS IN THE JEJUNAL MUCOSA OF PATIENTS WITH COELIAC DISEASE ON A GLUTEN FREE DIET

(Cells per mm^2 of mucosa.)

SUBJECT	DURATION IN MONTHS	IgG	IgA	IgM
C.D. 12	12	291	299	317
C.D. 14	9	194	328	380
C.D. 16	18	93	236	197
C.D. 19	12	171	235	284
C.D. 22	24	33	74	124
C.D. 28	6	140	289	316
C.D. 29	24	36	102	83

APPENDIX F

APPENDIX F (1)

SKIN TEST REACTIVITY IN TWELVE PATIENTS WITH COELIAC DISEASE

SUBJECTS	CANDIDA	VARIDASE	MUMPS
NORMAL DIET			
C.D. 4	-	-	+
C.D. 5	-	-	-
C.D.11	-	+	-
C.D.13	+	-	+
C.D.14	-	+	+
C.D.26	-	+	+
GLUTEN FREE DIET			
C.D.12	+	+	-
C.D.16	+	+	+
C.D.22	+	+	+
C.D.29	+	+	+
C.D.35	-	+	-
C.D.37	-	+	-

APPENDIX F (2)

SKIN TEST REACTIVITY IN ELEVEN CONTROL SUBJECTS

SUBJECTS	CANDIDA	VARIDASE	MUMPS
N 1	+	+	+
N 2	-	-	+
N 3	-	+	-
N 4	-	+	-
N 5	-	+	+
N 6	-	+	-
N 7	+	+	+
N 8	+	+	+
N 9	-	+	+
N 10	-	+	+
N 11	+	-	-

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