OROFACIAL GRANULOMATOSIS: CLINICAL AND IMMUNOLOGICAL STUDIES

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by

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ABSTRACT

Orofacial granulomatosis (OFG) is assuming increasing importance in the practice of Oral Medicine. This is particularly true in the west of Scotland where over 400 patients with the condition have been identified over a 10 year period. This study has analysed the clinical and immunological parameters in 301 patients with orofacial granulomatosis in an attempt to understand the underlying aetiopathogenesis and to develop a protocol for the investigation and management of such patients.

During the study, 140 patients were identified with OFG, 45 patients with gastrointestinal Crohn's disease (CD) (20 having a pre-existing diagnosis; 25 having the diagnosis established during the study), ten patients with Melkersson-Rosenthal Syndrome (MRS), and six patients with sarcoidosis.

Gastrointestinal symptoms were found to be of practical value in identifying patients who may have gastrointestinal Crohn's disease. Similarly, systemic examination of patients yielded findings which aided differentiation of CD and OFG.

An immunological (allergic) basis for OFG has been postulated in this study. Despite similar compliance scores and follow-up periods between the two groups (CD and OFG), dietary and environmental exclusion advice for substances identified on positive skin testing led to statistically significant differences in changes of both final symptom and sign scores.

Further weight to the allergic basis for OFG was added with the overall levels of IgE demonstrated by RAST testing being significantly higher in the OFG group (82.4%) over the CD group (58.6%). In addition, levels of IgG directed against unidentified proteins in the sera of the OFG group, comparable to coeliac disease and significantly higher than control groups, further strengthen the likelihood of immunological reactivity in the OFG group.

The consistent HLA haplotype (A2/3 B7 DR2/3/4) and results of lymphocyte studies suggest an immunological mechanism to the clinical presentation in OFG patients.

The results of this study would suggest that the antigen may be dietary in origin. However, the negative findings in searching for *Mycobacterium paratuberculosis* require further analysis; the use of fresh tissue from orofacial biopsies instead of paraffin-processed sections would be a most appropriate next step using PCR technology.

Laboratory findings were also of practical value in differentiating the disease categories. Whereas haemoglobin concentrations and serum vitamin B12 levels were not statistically different between the groups, the mean corpuscular volume (lower in CD), ESR (higher in CD), whole blood folate, and serum ferritin (mean lower in CD) were statistically significant in highlighting differences between CD and OFG.

Biochemical parameters were largely redundant, with urea and electrolytes and calcium, albumin and phosphate levels revealing no real differences between the diagnostic groups.

Analysis of histological data revealed that patients with CD were much more likely to have lymphoedema ($p=0.004$) and/or granulomata ($p=0.012$), alone or in combination, on mucosal biopsy than patients with OFG. However, 20.0% of CD biopsies and 30.9% of OFG biopsies had no granulomas present; but only 6.7% of CD biopsies and 16.7% of OFG biopsies had no lymphoedema present. It therefore seems inappropriate to persist with the title Orofacial Granulomatosis, a histopathological term used to describe a clinical entity, and the alternative of Orofacial Lymphoedema is suggested on the basis of clinical findings alone.

The clinical findings in patients with OFG led to the helpful concept of Major and Minor diagnostic criteria for Orofacial Lymphoedema, with Major criteria being present in >30% of patients and Minor criteria being present in 7-29% of patients as follows:

Major:	Chronic lower lip swelling	52.2%
	Chronic upper lip swelling	51.2%
	Mucosal oedema and cobblestoning	48.8%
	Aphthous ulceration	39.2%
	Mucosal tags	31.2%
	Full-thickness gingivitis	30.2%

Minor:	Chronic facial swelling	27.0%
	Angular cheilitis	26.2%
	Fissured tongue	11.3%
	Non-aphthous ulceration	8.0%
	Papillary hyperplasia	7.3%

The diagnosis would be based on 2 major criteria (i.e. present in at least 60.4% of patients) or 1 major plus 3 minor criteria (i.e. present in at least 56.8% of patients).

On the basis of this study, OFG, sarcoidosis and Crohn's disease are distinct clinical entities. OFG, or more appropriately Orofacial Lymphoedema, OFL, would appear to be primarily allergic in its pathogenesis (Type IV or delayed hypersensitivity), as would Melkersson-Rosenthal Syndrome.

Overall, 45 out of 301 patients in this study population had, or developed, evidence of gastrointestinal Crohn's disease - some 15% over a 10-year period. The successful use of technetium-labelled leucocyte scanning of the gastrointestinal tract to identify gastrointestinal CD in patients with OFL in the paediatric population has been demonstrated in this study.

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"The fear of the Lord is the beginning of knowledge, but fools despise wisdom and discipline." Proverbs 1: 7

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INTRODUCTION & AIMS

Orofacial granulomatosis (OFG) is assuming increasing importance in the practice of Oral Medicine. This is particularly true in the west of Scotland where over 400 patients have been identified with the condition over a 10 year period. Such figures cannot, it would seem, be equalled anywhere else in the UK or abroad - hence the importance of this study based in Scotland.

However, in quoting such numbers of patients, one is immediately aware of the difficulties in rational diagnostic criteria since, as a chronic granulomatous disorder, some clinicians may be labelling this entity as Crohn's disease, *oral* Crohn's disease, sarcoidosis, Melkersson-Rosenthal Syndrome, cheilitis granulomatosa of Miescher or the remote possibilities of leprosy and tuberculosis. Some authors clearly use OFG as an "umbrella" term encompassing all the above granulomatous disorders and more; others use OFG, as Wiesenfeld *et al* (1985) first intended, as a term to describe a constellation of signs resembling those of Crohn's disease clinically and histologically in patients who do not appear to have abnormalities at any other site in the gastrointestinal tract. But what evidence is there that these patients do not indeed have Crohn's disease at other sites; or that these are simply local manifestations of some other granulomatous disorder such as sarcoidosis? What account is taken of the increasing evidence that OFG may be a Type IV hypersensitivity reaction quite distinct from other granulomatous disorders; or indeed that these are the orofacial manifestations of Crohn's disease, which itself may be a gastrointestinal manifestation of allergy? Add to this the recently developed science of molecular biology with its claims about *Mycobacterium paratuberculosis* as an aetiological factor in Crohn's disease and the scene is set for a life-long study into the possible aetiology of OFG.

Indeed the term OFG itself is hugely inadequate, since it uses a histopathological term to describe a clinical entity. This, then, is clearly a mine-field of mixed information, poorly applied nomenclature and inappropriately applied conclusions. How can we steer a course ahead which will take us into better charted waters?

Firstly, a number of logical assumptions *must* be made. OFG may be a "rag-bag" of assorted conditions, all simply presenting in the same or similar ways. However, its

histology is consistently that of a non-caseating granulomatous disorder and hence tuberculosis would appear not to be worthy of consideration.

Similarly, leprosy is unlikely to be a major contender since it is primarily a disease of the tropics *not* of the West of Scotland. That leaves sarcoidosis, Crohn's disease and the local entities of Melkersson-Rosenthal Syndrome and cheilitis granulomatosa of Miescher. Clearly, OFG could be a descriptive term for the orofacial manifestations of any or all of these entities; or it could be a disease state in its own right. The purpose of this thesis is to attempt to ascertain which is correct.

The null hypothesis is therefore stated **that OFG is simply a clinically descriptive term for the orofacial manifestations of the numerous non-caseating granulomatous disorders which affect man, and is NOT a disease entity in its own right.**

This thesis therefore aims to examine the clinical presentation of patients with OFG, the results of numerous investigations (both clinical and laboratory), the effects of therapeutic intervention, and outcome measurements in an attempt to disprove the above hypothesis.

AIMS

- To describe the clinical presentation of patients with OFG, detailing the prevalence of anatomical site involvement in each patient group.
- To analyse haematological, biochemical and immunological parameters in each patient group to evaluate any consistent differences.
- To determine the clinical response to the exclusion of identified dietary and environmental allergens in each patient group.
- To establish an investigative protocol for all patients presenting with OFG in an attempt to identify the correct diagnostic label and prognosis for each individual patient.

CHAPTER 1

REVIEW OF THE LITERATURE PERTAINING TO CHRONIC GRANULOMATOUS DISORDERS AND OROFACIAL GRANULOMATOSIS

1.1 Historical review

1.1.1 Chronic granulomatous disorders

Chronic granulomatous disorders have been evident in man for thousands of years. Earliest confirmed examples of caseating variants (primarily tuberculosis) come from studies in skeletal tissues from Egyptian and Peruvian mummies (Meachen, 1936). Similarly, non-caseating variants are reported in Old Testament times with references to the Hebrew word *sara'at*, translated as "leprosy", in Leviticus chapter 13 and elsewhere.

1.1.2 Leprosy

There is a description of leprosy in the Indian text *Charaka Samhita*, written between 600 and 400 BC. It is thought that the disease was brought from India to Greece and on to continental Europe by the armies of Alexander the Great in the fourth century BC. The disease was termed *elephantitis graecorum*, probably due to the ichthyosis and wood-hard oedema of the legs of some sufferers. Other Greek names for the disease were *leontiasis* and *satyriasis* due to the facial abnormalities present in some sufferers (Grange, 1988).

1.1.3 Tuberculosis

There is evidence that tuberculosis has existed from earliest times. Ancient Chinese writings from as far back as 2698 BC describe "lung cough and lung fever" which are probably tuberculosis or lao-ping. In the *Rig Veda* (2500 BC) there is a hymn on the cure of Yakshma or consumption whilst Susruta in an ancient work on Hindu medicine, the *Ayer Veda*, speaks of the difficulty of curing the disease and even blames physicians for not treating it early enough. He also advised walking, horse or carriage exercise and good feeding (Meachen, 1936).

Osseous lesions of tuberculosis have been confirmed from the vertebral columns in an Egyptian mummy of about 1000 BC (Meachen, 1936). Hippocrates (460-377 BC) coined the term "phthisis" which he applied to "a diminution or shrinking of the body, following incurable ulcers of the lungs, accompanied with a small fever". Aristotle

(384-322 BC) first suggested that consumption might be a contagious disease (Meachen, 1936).

Gaspard Laurent Bayle (1774-1816) was the first to employ the term tuberculosis. He described the relationship between pulmonary tuberculosis and tuberculosis of other organs. However, it was Rene Theodore Hyacinthe Laennec (1781-1826), the inventor of the stethoscope and himself a consumptive, who laid the foundation of our knowledge of the pathological anatomy of tuberculosis. He showed clearly the caseative nature of the lesions and recognised the latency of the disease. Rudolf Virchow (1821-1902) affirmed that the inflammation in phthisis might terminate in caseation. Klencke (1843) showed that cow's milk could transmit tuberculosis but it was Jean Antoine Villemin (1827-1892) who conducted the well-known experiment demonstrating that human pulmonary tubercle could be inoculated into rabbits, showing clearly that tuberculosis was a specific infection (Meachen, 1936).

The Ziehl-Neelsen stain to identify the causative bacillus in sputum was introduced in 1882 and, despite modification by various workers, remains a standard way of demonstrating *Mycobacterium tuberculosis* today (Meachen, 1936).

1.1.4 Sarcoidosis

Sarcoidosis has been known for over 100 years in increasingly protean manifestations. The first report of sarcoidosis is credited to Robert Willan who introduced the term "erythema nodosum" in a patient who also had oral manifestations. The description is in his classic work *On Cutaneous Disorders* published in parts between 1798 and 1808, and among the fine copperplate engravings in colour is an illustration of erythema nodosum (Sharma, 1985). His vivid description has never been bettered :

"In erythema nodosum, many of the red patches are large and rounded. The central parts of them are very gradually elevated and on the 6th or 7th day, form hard and painful protuberances. From the 7th to the 10th day, they constantly soften and subside without ulceration. On the 8th or 9th day, the red colour changes to bluish or livid.....I have only seen it in females, most of whom were servants. It is preceded by irregular shiverings, nausea, headaches and fretfulness with a quick unequal pulse and a whitish fur on the tongue." (Sharma, 1985)

In January 1869, a 58-year old coal wharf worker was seen by Jonathan Hutchinson at the Blackfriars Hospital for Diseases of the Skin complaining of purple skin plaques on his legs and hands. Hutchinson's account of this patient appeared under the title "Case of Livid Papillary Psoriasis" in his *Illustrations of Clinical Surgery* (1877). The patient died in 1875 from kidney disease and Hutchinson linked the skin and kidney diseases after treating the patient at King's College Hospital, London (James, 1984). It is now recognised that patients with sarcoidosis, particularly those with chronic skin lesions, have disordered calcium metabolism leading to renal calculi and renal failure.

In 1889, Ernest Besnier coined the term "lupus pernio" to describe the cutaneous manifestation of sarcoidosis at the Saint Louis Hospital, Paris when he presented a paper entitled "Lupus Pernio de la Face - Synovites Fongueuses Symmetriques des Extremities Superieures" at a weekly hospital conference (Besnier, 1889). In 1892, Tenneson described the granulomatous histology of lupus pernio (Tenneson, 1892). Then, in 1899, Caesar Boeck as Professor of Dermatology in Oslo described "the sarcoid nodule" and used the term sarcoidosis for the first time (Boeck, 1899). The term sarcoid derives from two Greek words "sark" and "aid" which translate as "flesh-like". Heerfordt, a Danish ophthalmologist, in 1909 drew attention to a condition characterised by uveitis and enlargement of the parotid glands, frequently complicated by seventh cranial nerve palsies.

The Kveim test was developed by Ansgar Kveim in Oslo. He made the observation that sarcoid lymph node tissue inoculated intradermally gave rise to sarcoid papules in 12 of his 13 patients with sarcoidosis. Simultaneous control injections of Frei antigen and tuberculin did not produce this response. Since this reaction did not occur in normal subjects, or in patients with lupus vulgaris, he concluded that the papules were specific lesions due to an unknown agent and that the test differentiated sarcoidosis from tuberculosis (Kveim, 1941). The Kveim test is occasionally referred to in the literature as the Kveim-Siltzbach test because of a chronological association between the work of the two authors and a refinement of Kveim's methodology by Siltzbach (1974). For simplicity, the test will be referred to as the Kveim test.

1.1.5 Crohn's Disease

In 1761 a description appeared by Morgagni of ulceration and erosion of the terminal ileum and adjacent colon in his *De Sedibus et Causis Morburum* (Morgagni, 1769). The following is an abstract from Benjamin Alexander's translation of this work dated 1769:

"In that part (the extremity of the ileum and the nearest part of the colon besides) the intestines were eroded and ulcerated on their internal surfaces ever affected with a gangrene so that you see they might be easily perforated. Near to this tract some of the mesentery had grown into a tumour, wherein was ichor, not unlike that which had burst forth into the cavity of the abdomen; but the very substance of this tumour was soft and flaccid and seemed to incline to corruption."

In 1806 a case presented to the Royal College of Physicians of ileal stricture by Combe and Saunders and a report by Abercrombie in 1820, of a 13-year old girl with a thickened and ulcerated terminal ileum and ascending colon, may have been the first documented cases of what is now known as Crohn's disease. At the time, these cases were likely considered as being due to tuberculosis. In 1901, however, Lartigua recognised the absence of caseative necrosis and other typical changes of tuberculosis.

In 1901 Sir T. Kennedy Dalziel, a Glasgow surgeon, operated on a colleague suffering from diarrhoea and "obstruction of the bowels of a fortnight's duration". At the operation, the small and large intestines were "rigidly fixed" and "chronically inflamed". He could do nothing to help his physician colleague who died a few days later. Dalziel operated on another five similar cases over the next few years and published his findings in 1913 in a paper entitled "Chronic Interstitial Enteritis". He commented on the pathology of these cases as showing "mucosa replaced by granulomatous tissue with a notable number of eosinophils throughout, and a few giant cells present". He continued, "the cases gave the impression they were probably tuberculosis yet from the uniform character of the affection it evidently is not so. The affected bowel gives the consistence of an eel with rigor mortis and the glands, though enlarged, are evidently not caseous." In 1932, Burrill Crohn published his landmark paper describing the disease which now bears his name.

Dalziel was aware of the work of Johne and Frothingham (1895) describing Johne's disease in cattle and noted the similar naked-eye appearance and histological features of the two diseases. Dalziel's dilemma was that in the inflamed tissue of the human disease, no acid-fast bacteria could be seen. Despite this, he considered the "histological characters so similar that...the diseases may be the same." (Dalziel, 1913)

Since Crohn's disease was first reported as affecting the terminal ileum, the same disease has been reported everywhere in the gastrointestinal tract - in small bowel, colon and rectum (Lockhart-Mummery and Morson, 1960); in the stomach (Comfort *et al.,* 1950); and in the oesophagus (Gelford and Krone, 1968). The first case of Crohn's disease affecting the mouth was recorded by Dudeney and Todd in 1969, closely followed by Issa in 1971. Since then, numerous case reports and studies have demonstrated the fact that Crohn's disease may occur at any point in the gastrointestinal tract from mouth to anus. These cases, however, were reported in patients with pre-existing intestinal Crohn's disease. Subsequently, the assumption arose in the literature that such orofacial manifestations could exist without any evidence of intestinal Crohn's disease. Such assumptions preclude the possibility that this may be an entirely separate disease process and not simply "oral Crohn's disease" (Williams *et al.,* 1990). This assumption may have hindered substantially the investigation of the aetiology of granulomatous inflammation of the orofacial region.

The first link between orofacial and gastrointestinal involvement may have been made some 200 years previously by Dr. Archibald Pitcairn (1652-1713) who was an eminent member of the medical profession in Edinburgh and a founder member of the Royal College of Physicians of Edinburgh. As Pitcairn was a well known supporter of the Jacobite cause, it would seem appropriate that a physician of these sympathies would be consulted by the Maxwell Stuart family, residing at Traquair House, a notable Scottish border property. The correspondence at Traquair discusses the health (or lack of it) of Lord Traquair, his Countess and their large family. In one particular letter, dated 1703, advice on the health of their daughter, Lady Anne, the eldest of 17 children, is given. She is described as suffering from "a little of a bloody flux" and an "old swelling on her lip". For both of these symptoms, a prescription of *cavew* was offered by Pitcairn (MacFadyen and Ferguson, 1996).

Cavew, cafew or *catechu* is the resin extracted from the acacia or uncaria tree (Salmon, 1706). This material was widely used as an astringent to "stop all sorts of fluxes of the bowels, whether of blood or humours". In addition, it was used to treat oral lesions, ulcers or a particular condition of a relaxed uvula or sore throat found in delicate females. In this description of Lady Anne's illness, it is interesting to note the combination of bowel lesions and lip swelling. The relatively recent account by Dudeney and Todd (1969) may have been pre-empted by Pitcairn who, in 1703, related the bowel and lip disorders to a single disease for which one prescription sufficed. Unfortunately, there are no portraits of Lady Anne extant (Marshall, 1979). She died in 1755 and her only records are embroideries and colfichets (fine silk needle painting on a paper background) (Swain, 1984).

1.1.6 Orofacial granulomatosis (OFG)

Wiesenfeld *et al* (1985) introduced the term orofacial granulomatosis (OFG) to describe a constellation of signs resembling those of Crohn's disease clinically and histologically in patients who do not appear to have abnormalities at any other site in the gastrointestinal tract. However, Wiesenfeld's definition of OFG is wholly unsatisfactory since it fails to take into account the underlying pathophysiology of the condition and discern which granulomatous disorder is manifesting in each case. This has resulted in the term OFG being used in the literature as an "umbrella term" for all granulomatous disorders around the head and neck. This has led to confusion and little progress in developing a scientific understanding of the pathophysiology of the disease processes.

Similarly, the term OFG itself is unfortunate since it uses histopathological terms to define a clinical entity. For this reason, the term orofacial lymphoedema may be preferred and this new concept will be addressed in this thesis.

1.1.7 Melkersson-Rosenthal Syndrome (MRS)

Hubschamann (1894) and Rossolimo (1901) presented case reports of patients in whom a transient lower motor neurone facial nerve palsy and facial oedema occurred. Melkersson (1928) reported an additional case and established the link between the facial palsy and facial oedema. Rosenthal (1931) reported a series of patients who had lingua plicata (fissured tongue) in addition to facial palsy and facial oedema. The triad of

recurrent facial swelling, unilateral or bilateral facial palsy and fissured tongue comprise the Melkersson-Rosenthal syndrome (MRS). Numerous cases of MRS have been reported in the world literature (Worsaae *et al.,* 1980; Levenson *et al.,* 1984; Pisanty and Sharav, 1969; Nally, 1970; Vistnes and Kernahan, 1971; Alexander and James, 1972; Hornstein,1973; Worsaae and Pindborg, 1980; Azaz and Nitzan,1984; Worsaae *et al.,* 1982; Storrs, 1975).

1.1.8 Cheilitis granulomatosa of Miescher

Miescher (1945) independently described cheilitis granulomatosa, a chronic enlargement of the lips that is a result of granulomatous infiltration and resultant lymphatic channel obstruction. Numerous cases of cheilitis granulomatosa of Miescher without facial palsy or fissured tongue have been reported (Shaikh *et al.,* 1989; Doku *et al.,* 1965; Laymon, 1961; Rhodes, 1965; Alpert and Nelson, 1974; Krutchkoff and James, 1978a; Hernandez *et al.,* 1986).

Some investigators have suggested that cheilitis granulomatosa of Miescher is an oligosymptomatic manifestation of MRS (Eisenbud *et al.,* 1971; Bishop and Garcia, 1979). However, although macrocheilia is a component of MRS, it may also be a sign of Crohn's disease, sarcoidosis or other disseminated granulomatous disease such as tuberculosis (Wiesenfeld *et al.,* 1985). This lack of clarity in the literature has hindered the furtherance of scientific scrutiny of these granulomatous disorders.

1.1.9 GLUS syndrome (granulomatous lesions of unknown significance)

Finally, mention must be made of the GLUS syndrome (granulomatous lesions of unknown significance). Brincker in 1989 suggested the existence of a specific syndrome where certain clinical features occurred with great regularity (Brincker, 1989). He highlighted the fact that all studies of granulomatous disease include a residual group of unclassifiable cases in which the presence of the granulomata (chiefly in liver, bone marrow or lymph nodes) remains unexplained in spite of all relevant histopathological, microbiological, biochemical and serological studies. Brincker further pointed out that such cases are often characterised by prolonged fever, a benign course and a tendency to recurrence (Brincker, 1990; Brincker and Pedersen, 1989). The existence of the GLUS

syndrome has been ratified by several other authors in the 1990's (Telenti and Hermans, 1989; Friedland *et al.,* 1990).

Because of the caseating nature of the granulomata in tuberculosis, the mixed histological picture in leprosy and the fact that the infective aetiology is well established for each of these disease entities, this thesis, having placed them in the historical context of a literature review, will now dismiss them from further discussion in the presentation and aetiology of orofacial granulomatosis.

1.2 Clinical and Laboratory aspects

1.2.1 Sarcoidosis

Sarcoidosis is a relatively common multi-system disorder of unknown aetiology. It is an extraordinary disease, not only in the very variable presentation and spectrum of tissues it may affect, but also in its duration. Although transient and asymptomatic in some individuals, it may become chronic for others, affecting multiple systems, causing organ dysfunction and failure, debilitating symptoms and premature death (Scadding, 1987).

A statement prepared in 1975 by the International Committee on Sarcoidosis serves as the most appropriate definition of the disease:

"Sarcoidosis is a multisystem granulomatous disorder of unknown aetiology most commonly affecting young adults and presenting most frequently with bilateral hilar lymphadenopathy, pulmonary infiltration, and skin or eye lesions. The diagnosis is established most securely when clinical or radiographic findings are supported by histological evidence of widespread non-caseating epithelioid cell granulomata in more than one organ or a positive Kveim-Siltzbach skin test. Immunological features are depression of delayed type hypersensitivity, suggesting impaired cell-mediated immunity and raised or abnormal immunoglobulins. There may also be hypercalciuria, with or without hypercalcaemia. The course and prognosis may correlate with the mode of onset. An acute onset with erythema nodosum heralds a self-limiting course and spontaneous resolution, while an insidious onset may be followed by relentless, progressive fibrosis. Corticosteroids relieve symptoms and suppress inflammation and granuloma formation." (Mitchell, 1975)

To this may be added that the organs most frequently affected are the lymph nodes, lungs, skin, eyes, liver, spleen and salivary glands, although every organ has been reported to be involved, albeit at the level of case reports (Mayock *et al.,* 1963). There are even reports of extra-pulmonary sarcoid nodules making an appearance in skin tattoos (Collins *et al.,* 1994).

There is an extremely diverse number of clinical presentations and recent UK experience is summarised in Table 1.1. The percentage frequency of organ and tissue involvement in sarcoidosis is summarised in Table 1.2.

Table 1.1 First presenting symptoms of patients with sarcoidosis in the UK. Combined male and female data. Figures are percentages.

	Ethnic Group		
Feature	White	Black	Asian
Abnormal chest radiograph	34	7	10
Respiratory symptoms	25	57	55
Constitutional symptoms	5	57	55
Erythema nodosum	20	8.5	17
Ocular symptom	7	12	3
Superficial lymphadenopathy	3	34	17

Based on Scadding and Mitchell (275 patients, London 1946-66); BTTA (567 patients in four geographical areas of the UK, 1961-66); Edmonstone and Wilson (156 patients, South London 1969-82)

Table 1.2 Percentage frequency of some clinical manifestations of sarcoidosis from various centres.

	World Centre					
Feature	London (275)	London (537)	Paris (379)	New York (311)	Los Angeles (150)	Tokyo (282)
Hilar lymphadenopathy	98	84	92	90	93	87
Peripheral lymphadenopathy	31	29	23	37	31	23
Eyes	14	27	11	20	11	14
Skin	17	25	12	19	27	17
Spleen	11	12	6	18	15	1
Parotid	2	6	6	3	6	5
Central Nervous System	1	7	6	4	6	5
Bones	4	4	3.5	9	4	2
Erythema Nodosum	14	31	6.5	11	9	4

This data is summarised from the work of Siltzbach *et al* (1974).

(Patient numbers are given in parenthesis).

Two recognised entities are worthy of mention (James *et al.,* 1976): Lofgren's syndrome describes erythema nodosum and bilateral hilar lymphadenopathy in combination (Lofgren, 1953); Heerfordt-Waldenstrom syndrome (or uveoparotid fever) describes an uncommon condition which presents acutely and runs a chronic course with parotid gland enlargement, uveitis, fever and cranial nerve palsies (Heerfordt, 1909b; Hagerstrand and Linell, 1964). The facial nerves are particularly involved. Uveitis (redness of the eye, epiphora, cloudy vision and photophobia; circumcorneal congestion, pupillary irregularity, and keratotic precipitates in the anterior chamber) is a common manifestation of sarcoidosis, occurring in around 30% of patients, while parotid gland enlargement and nerve palsies are uncommon and occur in less than 5% of patients (James, 1994a).

Laboratory investigations are important in sarcoidosis. Full blood count may show a moderate reduction in peripheral blood lymphocyte numbers in active disease; thrombocytopoenia is also reported (James, 1994b). The erythrocyte sedimentation rate (ESR) is commonly elevated; C-reactive protein (CRP) shows no consistent pattern and is seldom elevated in sarcoidosis. Hypercalcaemia and hypercalciuria due to abnormal synthesis of vitamin D are well recognised in active sarcoidosis and occur in about 2-10% of patients (Scadding, 1987). The picture is similar to hypervitaminosis D with elevation of serum calcium, normal serum phosphate, and normal or slightly elevated alkaline phosphatase (Fuss *et al.,* 1992). Hypercalciuria is commoner than hypercalcaemia (James, 1994a).

Total serum globulin and specific immunoglobulin levels are often raised above normal. In active disease, there is polyclonal increase in immunoglobulin levels, particularly in blacks and more often in females than males. High IgM levels are associated with erythema nodosum and other immunoglobulins variously raised, with IgA and IgG more commonly raised in blacks than whites. IgD is often depressed, the opposite to the common finding in tuberculosis. Circulating immune complexes are commonly detected in the early acute stage of the disease (James, 1994a; Scadding, 1987).

With respect to biochemical tests, such as liver function tests, and urea and electrolytes, there are no special features reported although alkaline phosphatase may be elevated as a consequence of bone or hepatic involvement (James *et al.,* 1976).

Some two-thirds of patients with active sarcoidosis have raised levels of serum angiotensin-converting enzyme, with the highest levels in those with clinically florid or radiographically extensive lung disease. This enzyme is produced by epithelioid cells and its activity is therefore a biochemical marker of cellular activity in the formation of granulomata (James, 1994b).

The management of sarcoidosis is determined by disease staging but is chiefly by oral corticosteroids (Klesper *et al.*, 1994).

Orofacial lesions of sarcoidosis have assumed increasing importance in the literature. Reports of lymph node involvement around the head and neck region, as might be expected, outweigh all other reported manifestations (van Maarsseveen *et al.*, 1982). The most frequently involved (extrathoracic) lymph nodes are the posterior cervical group and these tend to be bilaterally enlarged, mobile and non-tender (Martinez and Amedee, 1993). Head and neck manifestations are present in 10-15% of patients with sarcoidosis (Martinez and Amedee, 1993). Oral mucosal involvement has been reported in the lips, tongue, buccal mucosa, gingivae, palate and floor of mouth (Sharma, 1990). Oral lesions are usually described as well-circumscribed nodules or papular eruptions, sometimes ulcerated, of a purple or brownish-red colour (Lazarus, 1982). Oropharyngeal involvement is rare with the commonest manifestation being tonsillar hypertrophy (Dash and Kimmelman, 1988). Involvement of the major salivary glands occurs in 6% of patients (Hildebrand *et al.*, 1990). The usual presentation is gland enlargement bilaterally and a dry mouth may be reported (Ellison and Canalis, 1986). Parotid gland disease is generally transient and self-limiting in 40% of cases (Hoggins and Allan, 1969).

The oral lesions may be placed in four distinct categories :

(1) *Patients with previously proven systemic sarcoidosis who also had lesions in the orofacial region:*

In some patients with a previous diagnosis of sarcoidosis, oral involvement consisted of one or more nodules of the mucosa without preference for any specific site (Covel, 1954; Hobkirk, 1969; Orlean and O'Brien, 1966; Samitz *et al.*, 1953). A few cases have been reported of involvement of the jaw bones (Betten and Koppang, 1976; Hillerup, 1976;

Poe, 1943). Involvement of salivary gland tissue is a fairly common finding (Kalman, 1954; Narang and Dixon, 1975). A decreased level of α-amylase and increased levels of albumin and lysozyme have been observed by Beeley and Chisholm (1976) in parotid saliva from a patient suffering from sarcoidosis with salivary gland involvement. Some years earlier, the value of salivary gland function tests in diagnosing sarcoidosis and in monitoring the response to possible therapy was described by Chisholm *et al* (1971).

(2) *Patients with previously proven systemic sarcoidosis in which an oral biopsy of apparently healthy tissue was taken:*

Cahn *et al* (1964) described a group of 23 patients with known sarcoidosis in which palatal biopsies of clinically normal areas were performed. They demonstrated the presence of sarcoid granulomata in 38% of the cases. Tillman (1964) described a 66-year old male with sarcoidosis in whom a biopsy was performed of clinically normal gingival tissue to exclude amyloidosis. The histological findings were, in fact, consistent with sarcoidosis. The involvement of minor salivary gland tissue with sarcoid granulomata is well recognised (Chisholm *et al.,* 1971; Tannenbaum *et al.,* 1974; Tarpley *et al.,* 1972). One study reported non-caseating granulomata in the labial gland biopsies of 58% of 75 patients with known sarcoidosis (Nessan and Jacoway, 1979). The presence of non-caseating granulomata in salivary tissue is a well recognised specific feature of sarcoidosis (Kerr, 1965).

(3) *Apparently healthy patients in whom the findings of the oral lesions of sarcoidosis led to additional examination and the diagnosis of systemic sarcoidosis:*

A number of cases have been described in which an oral lesion, diagnosed histologically as sarcoidosis, led to the detection of generalised sarcoidosis (Greet and Sanger, 1977; MacDonald *et al.,* 1969; Roche *et al.,* 1967; Schroff, 1942). Vijay *et al* (1995) reported a healthy female who presented with a ranula related to the sublingual gland. This was diagnosed histologically as a sarcoid ranula and the patient progressed to widespread sarcoidosis over a 7 month period.

(4) *Apparently healthy patients in whom the findings of "sarcoid" granulomata in the orofacial region led to additional examination but the diagnosis of sarcoidosis remained unproven :*

Schroff (1942) described a 48-year old female with a full-thickness mucosal swelling affecting the cheek which revealed "tuberculoid changes" histologically. All other tests in this patient were negative for tuberculosis but a definitive diagnosis of sarcoidosis cannot be assumed from the case report. Other reports from buccal mucosa (Hoggins and Allan, 1969), tongue (Tillman *et al.,* 1966) and gingivae (Watts, 1968) raise suspicions of sarcoidosis but no definitive diagnosis. Nitzan and Azar (1975) described a 62-year old female with an indurated submandibular mass, measuring about 3cm in diameter. Radiography revealed a sialolith and the submandibular gland was removed together with an adjacent lymph node. The lymph node showed features of sarcoidosis. No Kveim test was performed but all other tests were negative for sarcoidosis. The Mantoux test was also negative. The patient was also suffering from arthritis, xerophthalmia, dyspnoea and hypertension and so a diagnosis of systemic sarcoidosis was assumed. The authors mention that "specific treatment for sarcoidosis was of great benefit" and consider this as another supporting statement for their diagnosis. Orlian and Birnbaum (1980) described a 43-year old female with a swelling in the lower left anterior muco-buccal fold. There was no evidence of lymphadenopathy and histological assessment was consistent with the diagnosis of sarcoidosis. All additional tests were negative. It may well be that many of these latter reports of orofacial sarcoidosis would now be reclassified as orofacial granulomatosis and an alternative formal diagnosis and aetiology sought.

A search of the English literature revealed a plethora of well-documented cases of oral involvement of sarcoidosis supported by histological findings. Ten cases were in the buccal mucosa or vestibule (sulci) (Campbell, 1944; DeLuke and Sciubba, 1985; Gold and Sager, 1976; Greet and Sanger, 1977; Hobkirk, 1969; Hoggins and Allan, 1969; Kolas and Roche, 1960; Orlian and Birnbaum, 1980). Lesions at this site usually appeared as an irregular firm submucosal swelling fixed to the periosteum. In two cases, the lesions were encapsulated (Campbell, 1944). In eight, oral involvement was the first manifestation of the disease.

Six cases (Altman and Robinson, 1984; Caudill, 1988; Hayter and Robertson, 1988; Hogan, 1983; Sloan *et al.,* 1983; Watts, 1968) were in the gingival tissues and manifested as red swellings, sometimes with superficial ulceration located mainly in the anterior labial gingivae. The enlarged gingivae occasionally exhibited a nodular pattern. In all cases, the lesions were the first clinical manifestation of the disease.

Of the five cases in the lips (Bourgeois-Droin *et al.,* 1993; Calderon *et al.,* 1990; Steinberg and Mueller, 1994), two presented as a swelling and three as numerous submucosal nodules. In the floor of mouth, five cases (Narang and Dixon, 1975; Orlean and O'Brien, 1966; Roche *et al.,* 1967; Takimoto *et al.,* 1989; Vijay *et al.,* 1995) presented as ranulas of the sublingual gland. In three, it was the first manifestation of the disease (Roche *et al.,* 1967; Takimoto *et al.,* 1989; Vijay *et al.,* 1995). Monasebian *et al* (1997) described a recurrent, painful swelling in the submental region of a 32-year-old black woman. This was the first presentation of sarcoidosis in this patient who also had florid pulmonary involvement.

In the tongue, four cases (Macleod *et al.,* 1985; Mendelsohn *et al.,* 1992; Tillman *et al.,* 1966; van Maarsseveen *et al.,* 1982) clinically appeared as a submucosal induration. In all cases, the tongue lesions were the first clinical manifestation of the disease. In the hard and soft palate, three cases (Cohen *et al.,* 1981; Hildebrand *et al.,* 1990; van Maarsseveen *et al.,* 1982) presented as multiple nodules. All patients were known to have sarcoidosis. A more recent case described tongue involvement in a 56-year-old white woman with pre-existing pulmonary sarcoidosis (Soto *et al.,* 1997). This was a 2 x 3 cm erosive area on the ventral surface and was untypically asymptomatic. Biopsy confirmed the diagnosis of sarcoidosis.

With bony involvement, there were six cases in the maxilla (Aragon *et al.,* 1988; Hildebrand *et al.,* 1990; Kalman, 1954; Klesper *et al.,* 1994; Rubin *et al.,* 1991; van Maarsseveen *et al.,* 1982), four in the body of the mandible (Betten and Koppang, 1976; Cohen and Reinhardt, 1982; Hillerup, 1976; MacDonald *et al.,* 1969), one in the condyle (Thomas *et al.,* 1976) and one in both jaws (Cohen *et al.,* 1981). Radiographic examination revealed a radiolucency in the alveolar bone with ill-defined borders. The lesion eroded the cortical bone and never expanded the cortex. Teeth in the area of the lesion were usually vital and root resorption was not observed. In nine cases of the twelve recorded, the lesions in the jaws appeared in patients with known sarcoidosis.

The literature supports a true association between sarcoidosis and Sjogren's syndrome (James and Sharma, 1985). Indeed, various autoimmune disorders have been linked with Sjogren's syndrome and sarcoidosis such that the acronym TASS syndrome (thyroiditis, Addison's disease, Sjogren's syndrome, sarcoidosis) has been proposed (Seinfeld and Sharma, 1983). However, Cox and McCrea (1996) have postulated that the autoimmune

link with sarcoidosis extends even further and may be even more memorable, coining the term TOASSUC syndrome (thyroiditis, other autoimmunity, Sjogren's syndrome, sarcoidosis, ulcerative colitis).

Cranial neuropathies may be seen in sarcoidosis when the leptomeninges are involved in the granulomatous inflammatory process - the so-called neurosarcoidosis or cerebrospinal sarcoidosis (James, 1996). Although neurological manifestations of sarcoidosis occur in only 5% of cases, any part of the nervous system may be involved (Delaney, 1977). Peripheral neuropathies may be sensory, motor or mixed lesions. Colover (1948) reported on 115 cases of neurosarcoidosis and found that 58 patients had evidence of lower motor neurone palsies affecting the facial (seventh) cranial nerve. This was bilateral in 22 cases. The optic (second) nerve was the next most frequently involved cranial nerve. Other reviews have confirmed Colover's findings (Delaney, 1977; Wiederholt and Siekert, 1965; Silverstein *et al.,* 1965). When present, cranial neuropathies tend to be an early feature of sarcoidosis and the prognosis is generally good; facial nerve palsies in particular often remit quickly and spontaneously (Boucher *et al.,* 1994). In addition to CNS sarcoidosis, the possibility that a facial palsy is the result of parotid gland sarcoidosis should also be considered (Colover, 1948; Wiederholt and Siekert, 1965; Silverstein *et al.,* 1965). Sarcoidosis involves the major salivary glands, primarily the parotid, in 6% of cases (Siltzbach *et al.,* 1974).

Ng *et al* (1997) recently described 2 cases of patients who developed sarcoid-like foreign body reactions at the sites of body piercing performed for religious purposes. The granulomatous reaction involved the buccal mucosae in both cases and neither had any evidence of systemic granulomatous disorders.

1.2.2 Crohn's Disease

The manifestations of Crohn's disease are protean and are determined largely by the anatomical location of the disease. The majority of patients complain of diarrhoea (70-90%), abdominal pain (45-66%) and weight loss (65-75%) (Bozdech and Farmer, 1990). Fever is also common (30-49%) (Farmer *et al.*, 1975). Obstructive symptoms (colic and vomiting) are much more commonly associated with ileal disease than colonic disease (Nugent and Roy, 1989). Colonic disease causes rectal bleeding more commonly than ileal disease but even so, it is present in only about 50% of patients with Crohn's colitis (Farmer *et al.*, 1985). Colonic disease is also associated with perianal disease in about one-third of patients and with extra-intestinal manifestations which are uncommonly seen when the disease is confined to the ileum (Fielding *et al.*, 1970). Symptoms of anaemia are common and usually occur as a result of iron deficiency from intestinal blood loss or, less frequently, from vitamin B_{12} or folate malabsorption (Mekjian *et al.*, 1979). Other nutritional deficiencies may also be present, for example magnesium, zinc, ascorbic acid and the B vitamins, but these are uncommon and usually due to inadequate intake rather than malabsorption (Lind *et al.*, 1985a).

A few patients present with the clinical features of acute appendicitis, but at operation are found to have acute terminal ileitis (Farmer *et al.*, 1972). A minority of these patients prove to be due to Crohn's disease. Diagnostic difficulties may also occur when the disease presents without gastrointestinal symptoms. These include patients presenting with fever, weight loss and anaemia without diarrhoea or abdominal pain, and those with ileo-caecal disease presenting with urinary frequency and dysuria due to ureteric involvement (Bozdech and Farmer, 1990).

Physical examination may be normal but many patients will show signs of anaemia, for example glossitis, aphthous ulcers in the mouth or pharynx, beaking or frank clubbing of the nails, evidence of weight loss, and a tachycardia are common features (Farmer *et al.*, 1975). Abdominal examination usually reveals tenderness over the affected bowel, which may feel thickened. A mass is frequently palpable when small intestinal disease is present. Anal examination often shows the presence of fleshy skin tags, which have a characteristic violaceous hue. Anal fissures, perianal fistulae and abscesses are particularly associated with colonic disease (Farmer *et al.*, 1985). The extra-intestinal manifestations of Crohn's disease are shown in Table 1.3 (Hastings and Weber, 1993).

Table 1.3 Extra-intestinal manifestations of Crohn's disease

Manifestation	Frequency (%)	Comment
Related to disease activity		
Aphthous ulceration	20	
Erythema nodosum	5-10	
Pyoderma gangrenosum	0.5	
Acute arthropathy	6-12	Large joints affected; transient, non-destructive
Eye complications (conjunctivitis, episcleritis, uveitis)	3-10	
Unrelated to disease activity		
Sacroileitis	15-18	Usually asymptomatic; may be present in up to 50% using isotope scanning; unrelated to HLA-B27
Ankylosing spondylitis	2-6	75% have the HLA-B27 phenotype
Primary sclerosing cholangitis	5-6	
Gallstones	very common	Due to malabsorption of bile salts from ileum
Chronic active hepatitis	2-3	
Cirrhosis	2-3	
Fatty change of liver	6	Very common in ill patients requiring surgery
Amyloid	rare	

Carcinoma of the colon may complicate Crohn's colitis. The incidence is about 3-5%, a frequency similar to that associated with ulcerative colitis. The risk factors are not yet established, however, although histological dysplasia has been reported in some cases of Crohn's disease (Munkholm *et al.,* 1993). Small bowel carcinomas have been reported in association with ileal Crohn's disease (Munkholm *et al.,* 1993).

Examination of the oesophagus, stomach and duodenum is best done endoscopically because the radiological appearances are often non-specific and biopsies are required for histological confirmation (Tanaka and Riddell, 1990). The small intestine may be examined with a standard barium meal and follow-through, but more information is obtained with the barium infusion technique (small bowel enema). The classical features of Crohn's disease of the small intestine (early) are thickening of the valvulae coniventes and small, discrete aphthoid ulcers. In more severe cases, cobblestoning, fissure ulcers and thickening of the wall occur. Longitudinal ulcers, areas of stenosis, dilatation, sinus tracts and fistulae may also be evident (Morson, 1990).

Radiological examination of the colon is made with double-contrast barium enema after a thorough but gentle preparation. Characteristically, there is rectal sparing but otherwise the radiological appearance is similar to that of the small intestine (Bozdech and Farmer, 1990).

Sigmoidoscopy and rectal biopsy tend to be performed routinely in all patients (Tytgat and Lygidakis, 1990). Although the rectal mucosa is frequently normal or shows evidence of a granular proctitis, histological examination often shows an inflammatory infiltrate which is focal and may contain granulomata (Morson, 1990). Endoscopically, the earliest signs of Crohn's disease are small aphthoid ulcers surrounded by normal mucosa. In more severe disease, the mucosa is erythematous and penetrated by fissuring ulcers to give a cobblestone appearance. The ulcers are often linear and eventually become confluent (Tytgat and Lygidakis, 1990). Upper gastrointestinal endoscopy is not routinely required and pertinent only if the symptoms dictate or abnormalities are detected on barium meal. Although Crohn's disease of the stomach and duodenum may occur as an isolated phenomenon, most cases are associated with disease elsewhere in the gastrointestinal tract. Deep, longitudinal ulcers may occur in the stomach together with rugal hypertrophy and a cobblestone appearance. Multiple biopsies are usually helpful although granulomata are found infrequently (Fielding *et al.,* 1970).

From a laboratory viewpoint, anaemia is common and often due to mixed deficiencies. Iron deficiency from gastrointestinal blood loss is the most common but serum folate and vitamin B_{12} may also be low. A neutrophil leucocytosis is usually associated with active disease and there may also be a thrombocytosis. The total lymphocyte count and absolute numbers of circulating T-lymphocytes may be reduced (Bartholomeusz and Shearman, 1989).

Hypokalaemia is associated with severe diarrhoea. Serum albumin is reduced in active disease, largely due to down-regulation of albumin synthesis by cytokines such as interleukin IL-1 and IL-6, and tumour necrosis factor (TNF) but studies with ^{51}Cr-labelled albumin often demonstrate a protein-losing enteropathy (Farmer *et al.,* 1985). Serum immunoglobulins are normal or mildly elevated but there may be a rise in the α_2-globulins. A low serum calcium, when corrected for albumin, is unusual unless there is extensive small bowel disease. Liver function tests are frequently abnormal, consisting of mild elevations of aspartate transaminase and alkaline phosphatase. Persistent abnormality of the liver function tests suggest associated liver disease and should be investigated by liver biopsy and radiographic visualisation of the biliary tree (Mekjian *et al.,* 1979).

There is no satisfactory method of assessing disease activity (Lind *et al.,* 1985a). Symptoms such as fever or weight loss are obvious indicators but severe disease can be present in the absence of any major symptoms. Laboratory evidence of activity includes a reduced serum albumin, a rise in the acute-phase reactants (such as C-reactive protein) and in the ESR. A number of disease activity indices (e.g. American Crohn's disease activity index and the Dutch activity index) have been developed to standardise assessment but they are too complex for routine use and tend to measure different aspects of disease activity (Issenman *et al.,* 1993).

Much interest recently has centred around the use of indium-labelled neutrophil scanning to assess disease activity (Giaffer, 1996). The labelled cells preferentially migrate to inflamed mucosa and the increased uptake of the isotope can be detected using a gamma-camera. Labelling leucocytes with technetium-99 using hexamethylpropylemeamine oxime (HMPAO) as a chelator is gradually replacing indium since it is easier, cheaper and quicker. It appears to provide similar sensitivity and specificity (Li *et al.,* 1994) and is now endorsed for use in the paediatric patient population (Charron, 1997).

The treatment of Crohn's disease is complex and requires attention to patient education, psychosocial support, diet and nutrition, cancer surveillance and drug therapy (Shanahan and Targan, 1992). The commonly used drugs are (Selby, 1993):

salicylates (sulphasalazine, mesalazine (5-ASA), olsalazine, and coated preparations of mesalazine), corticosteroids (topical, oral or parenteral - hydrocortisone, prednisolone, prednisone, and budesonide), immunosuppressive agents (azathioprine, 6-mercaptopurine, cyclosporin, and methotrexate), antibiotics (metronidazole, ciprofloxacin, co-trimoxazole, vancomycin, and gentamycin) and anti-tuberculous agents. Recently, the use of thalidomide has been investigated with success (Wettstein and Meagher, 1997).

Other therapies currently undergoing evaluation are oral fish oil preparations (Belluzzi *et al.,* 1994), lymphocyte apheresis (Lerebours *et al.,* 1994), and hyperbaric oxygen (Brady, 1993). A major area of international interest is nutritional therapy and diet modification in the treatment of Crohn's disease (Russell, 1991). Nutritional therapy can be considered from two viewpoints: (1) adjunctive, aimed at maintaining adequate nutrition and replacing losses; and (2) primary, having a direct modifying effect on the disease itself (Selby, 1993).

Elemental diets have been used for the treatment of Crohn's disease since their efficacy was first noticed in patients being prepared for surgery. Their benefit has subsequently been confirmed in a number of controlled trials comparing them with corticosteroids - in adults (Gorard *et al.,* 1993; Riordan *et al.,* 1993) and children (Ruuska *et al.,* 1994). Elemental diets consist of nutrients in their simplest forms - amino acids, glucose and fatty acids. The initial trial comparing these elemental diets with polymeric diets, which contain intact proteins, was not able to demonstrate a consistent benefit (Giaffer *et al.,* 1990). The overall comment from these studies is that an hypothesis emerges - Crohn's disease may be caused by allergens in food or drink (Koretz, 1994). Similarly, resting the gut may reduce the antigenic load (Woolner *et al.,* 1998).

Despite these medical therapies, 80% of patients with Crohn's disease will require surgery at some stage in the disease process (Selby, 1993).

The extra-intestinal manifestations of Crohn's disease are myriad and multi-system (Hyams, 1994). With respect to lesions affecting the head and neck, the pharyngolaryngeal localisations and temporomandibular joint arthropathies are well recognised (Wilder *et al.,* 1980) but nasal manifestations are extremely rare (Pochon *et al.,* 1995). Other specific entities worthy of note are reports linking Crohn's disease with Sjogren's syndrome (Gainey *et al.,* 1985), oral T-cell lymphocytic lymphoma (fatal) (Scully *et al.,* 1993), and vulvitis granulomatosa, with labial swelling at two anatomical sites (Guerrieri *et al.,* 1995). The aggressive nature of Crohn's disease in many patients is demonstrated by the report of the disease affecting a hitherto "normal" myocutaneous (rectus abdominis) flap after surgical closure of an abdominoperineal defect (Reed *et al.,* 1993).

Orofacial lesions are common in patients with proven Crohn's disease of the intestine and case reports in the international literature now run to several hundred in number (Plauth *et al.,* 1991). The lesions include recurrent aphthous stomatitis (Croft and Wilkinson, 1972; Basu, 1976; Emery Jnr *et al.,* 1979; Simpson *et al.,* 1974; Estrin and Hughes Jnr, 1985; Tydesley, 1983; Bernstein and McDonald, 1978; Tyldesley, 1979; Weiss *et al.,* 1991; Fedotin *et al.,* 1974; Stankler *et al.,* 1972), diffuse swellings of the cheek and/or lips (Basu, 1976; Varley, 1972; Snyder and Cawson, 1976; Kano *et al.,* 1990; Talbot *et al.,* 1984; Estrin and Hughes Jnr, 1985; Tydesley, 1983; Tyldesley, 1979; Kolansky *et al.,* 1993; Clayton, 1975), cobblestoning of the oral mucosa (Issa, 1971; Eisenbud *et al.,* 1972; Tydesley, 1983; Weiss *et al.,* 1991; Clayton, 1975), mucosal tags (Basu, 1976; Eisenbud *et al.,* 1972; Irvine and Fisher, 1982; Simpson *et al.,* 1976; Ghandour and Issa, 1991; Bishop *et al.,* 1972; Tydesley, 1983), vertical fissures of the lips (Schiller *et al.,* 1971; Tydesley, 1983), full-thickness (extending to the muco-gingival margin or beyond) gingivitis (Frankel *et al.,* 1985; Eisenbud *et al.,* 1972; Frost *et al.,* 1981; Ghandour and Issa, 1991; Bottomley *et al.,* 1972; Tydesley, 1983; Tyldesley, 1979) and gingival swelling (Misra and Ament, 1996; Holmes and Smith, 1985; Giller *et al.,* 1997), angular cheilitis (Field and Tyldesley, 1989; Estrin and Hughes Jnr, 1985; Tydesley, 1983), perioral erythema and scaling of the skin (Field and Tyldesley, 1989), and persistent cervico-facial lymphadenopathy (Field and Tyldesley, 1989). Erythema migrans has also been described in association with Crohn's disease (Basu *et al.,* 1975; Issa, 1971; Simpson *et al.,* 1976) as has altered taste sensation (Frankel *et al.,* 1985) and minor salivary gland enlargement (Schnitt *et al.,* 1987). The prevalence of oral

manifestations of inflammatory bowel diseases is higher in the paediatric than adult population (Barnard and Walker-Smith, 1994).

Four types of stomatitis have also been described in Crohn's disease :

1. Pyostomatitis vegetans was first described by McCarthy in 1949. It affects all age groups with a male to female ratio of 3:1 (Hansen *et al.,* 1983). Affected patients characteristically have multiple miliary pustules overlying erythematous and oedematous oral mucosa (McCarthy, 1949). The labial gingivae and buccal and labial mucosae are most frequently involved. The floor of mouth and tongue are rarely affected (Calobrisi *et al.,* 1995). These pustules often rupture leading to erosions and ulceration, with fissuring of a pattern described as snail track ulceration (Forman, 1965). The histopathological features are hyperkeratosis, acanthosis, dense cellular infiltrates (neutrophils and eosinophils) in the lamina propria and the epithelium which aggregate to form small abscesses with necrosis and ulceration (Forman, 1965). Immunofluorescence studies are negative which distinguishes the disease from oral forms of pemphigus (Chan *et al.,* 1991). Pyostomatitis vegetans is a highly specific marker for inflammatory bowel disease (van Hale *et al.,* 1985) and all of the 33 case reports to date have occurred in patients with Crohn's disease or ulcerative colitis (Calobrisi *et al.,* 1995; Ficarra *et al.,* 1993).

2. Pseudo-pyostomatitis vegetans was described by Lewis and Beutner in 1995, making the first recorded association between Crohn's disease and mucous membrane pemphigoid. They described a 45 year-old white female with biopsy-proven Crohn's disease of the colon who developed "classical" pyostomatitis vegetans with snail track gingivitis affecting the anterior maxillary and mandibular gingivae. However, direct immunofluorescence of lesional tissue revealed deposits of IgG in the basement membrane zone in a linear pattern. Indirect immunofluorescence performed on the patient's serum also revealed IgG reactive to the basement membrane zone at a titre of 1:1280. It is clear therefore that pyostomatitis vegetans cannot simply be diagnosed clinically but immunofluorescence studies are essential. The authors state that misdiagnoses between these two conditions may have occurred previously (Lewis and Beutner, 1995).

3. Stomatitis gangrenosum was first described by Margoles and Wenger in 1961 who noted atypical oral (stomal) ulceration in two patients with ulcerative colitis and pyoderma gangrenosum. Fourteen years elapsed before the next description (Basu *et al.,* 1975) of irregular, deep ulcers of varying sizes. They are deep with rolled margins and a greyish fibrinous base. They are often foul-smelling. Histopathological findings are ulceration with a fibrino-purulent membrane. The lamina propria is heavily infiltrated by chronic inflammatory cells with histiocytes and giant cells (Basu and Asquith, 1980).

4. Chronic stomatitis was introduced by Dunlap *et al* in 1997 to describe the finding in an 11 year-old boy of generalised erythema of the oral mucosa, with chronic ulceration (of three months' duration) affecting the hard palate. Microbiological and viral cultures from the lesions were consistently negative but a biopsy of the palatal mucosa revealed non-caseating granulomata. This patient was subsequently shown to have asymptomatic Crohn's disease of the caecum.

Any of these lesions may occur individually or together and may antedate bowel symptoms by several years (Scully *et al.,* 1982). Indeed, according to some reports, the mouth and peri-oral tissues may be the only identifiable site of the disease process - the so-called "orofacial Crohn's disease" (Williams *et al.,* 1990). However, it is unclear on the evidence presented whether these patients genuinely have Crohn's disease or some other granulomatous disorder. Several case reports make the observation that the mouth and anus are often involved in the Crohn's disease process (not infrequently to a severe degree) in isolation with no apparent involvement of the gastrointestinal tract in between (Ward *et al.,* 1985).

The orofacial lesions of Crohn's disease may be classified (Talbot *et al.,* 1984) as :

1. Specific lesions of Crohn's disease (e.g. mucosal cobblestoning; stomatitis gangrenosum)
2. Non-specific lesions of Crohn's disease (e.g. lip swelling)
3. Lesions related to nutritional deficiencies (e.g. aphthous stomatitis; erythema migrans)

Several studies have investigated the dental health status of patients with Crohn's disease of the gastrointestinal tract. One study (Meurman *et al.,* 1994) found higher scores of

gingivitis in patients with active disease over those with inactive disease. Lactobacillus counts and numbers of decayed tooth surfaces are consistently higher in patients with Crohn's disease (Sundh *et al.,* 1993). Similarly, periodontal disease (Lamster *et al.,* 1978; Halme *et al.,* 1993) and periapical periodontitis (Halme *et al.,* 1993) are commoner in patients with Crohn's disease than control populations - as is the consumption of refined carbohydrate in the diet (Halme *et al.,* 1993).

Some patients with only oral lesions - between 10% and 48% in various studies - are reported to have symptomless intestinal Crohn's disease (Wiesenfeld *et al.,* 1985). However, the investigations used in some such studies to arrive at this understanding are not without criticism. It has been suggested that 55% of patients with oral Crohn's disease have involvement at four or more oral sites and that such multifocal oral involvement is more commonly associated with systemic disease (gastrointestinal Crohn's disease) (Barnard and Challacombe, 1995).

1.2.3 Orofacial Granulomatosis

OFG can present as recurrent facial swelling which may affect the lips, cheeks, eyelids and forehead (Henry, 1994). Facial swelling - affecting the upper half of the face - has been described as extensive but this appears to be rare with only a few cases being reported (Patton *et al.,* 1985; Hornstein, 1973). However, prominent enlargement of the lips is the most common sign with little difference in prevalence between the upper and lower lips (Wiesenfeld *et al.,* 1985). Lip swelling may initially occur unilaterally. Because the lip swelling is soft, non-tender, non-pitting and initially of sudden onset, it may in the acute phase resemble angioedema (Nally, 1970; Vistnes and Kernahan, 1971; Hornstein, 1973). However, with repeated episodes, progressive chronic and permanent enlargement occurs, secondary to fibrosis (Alexander and James, 1972).

The prevalence of facial palsy associated with OFG has ranged from 13% to 50%, depending on the series reported (Wiesenfeld *et al.,* 1985; Vistnes and Kernahan, 1971; Worsaae and Pindborg, 1980). The facial paralysis is probably a result of direct granulomatous infiltration of the facial nerve or sheath, or it may be secondary to compression of the nerve by oedema within the bony canal of the temporal bone or at the stylomastoid foramen (Wiesenfeld *et al.,* 1985; Nally, 1970). Some authors suggest that the palsy is due to sarcoidosis of the pia-arachnoid (Cohen *et al.,* 1981; van Maarsseveen *et al.,* 1982). Facial nerve palsy may occur alone when it may precede or follow orofacial swelling by years (Worsaae and Pindborg, 1980; Worsaae *et al.,* 1982; Hornstein, 1973).

The incidence of lingua plicata has varied between 40% and 77% according to the series reported (Worsaae *et al.,* 1980; Worsaae *et al.,* 1982; Alexander and James, 1972; Hornstein, 1973).

The study conducted by Wiesenfeld *et al* (1985), which coined the term orofacial granulomatosis, examined 60 West of Scotland patients with the condition. The clinical features at presentation are listed in Table 1.4.

Table 1.4 Clinical features in 60 patients presenting with orofacial granulomatosis (Wiesenfeld *et al*, 1985)

CLINICAL FEATURES	No. OF PATIENTS	% OF PATIENTS
Facial swelling	28	47
Total lip swelling	41	68
Upper lip swelling	27	45
Lower lip swelling	30	50
Mucosal oedema	14	23
Mucosal tags	12	20
Gingival lesions	13	22
Angular cheilitis	11	18
Oral ulceration	19	32
Geographic tongue	3	5
Fissured tongue	1	2
Facial nerve palsy (unilat)	8	13

The extent and duration of facial swelling was variable; in some cases there was diffuse enlargement of the entire lower half of the face, whereas in others swelling was restricted to just one lip or to a localised patch on the cheek. The lower lip was more commonly involved than the upper lip. Many patients with swollen lips developed painful vertical fissures from which a range of organisms were isolated. Of the 11 patients with angular cheilitis, 10 could not be attributed to nutritional deficiency or inadequate vertical occlusal dimension.

The nature of the mucosal abnormality ranged from widespread thickening to small, firm tags. Buccal mucosa was most frequently affected with the tissue developing into broad folds. Gingival involvement had an appearance quite distinct from non-specific inflammatory gingivitis. The gingivae were erythematous and enlarged, often with a patchy distribution, with the anterior region most commonly affected. The gingival changes extended from the free gingivae to the non-keratinised mucosa of the sulci - a "full thickness" pattern.

One patient had oral ulceration typical of aphthae but the other patients with ulceration had more irregular lesions which were predominantly superficial, although some became deep and persistent (Wiesenfeld *et al.*, 1985).

Sainsbury *et al* (1987) reported the findings in 8 children (all under 15 years of age at presentation) with OFG. Their findings are summarised in Table 1.5.

Table 1.5 Clinical features of eight children with orofacial granulomatosis. (Sainsbury *et al*, 1987)

CLINICAL FEATURES	No. OF PATIENTS	% OF PATIENTS
Swollen lips	7	88
Swollen cheeks	6	75
Angular cheilitis	3	38
Oral ulceration	6	75
Swollen gingivae	6	75
Lymphadenopathy	4	50
Facial palsy (unilateral)	0	0
Anal fissure/Skin tag	3	38
Intestinal involvement	1	13

Sainsbury *et al* highlighted the fact that half of the patients observed in South Wales with OFG have been children. This study also demonstrated, for the first time, the involvement of dental pulp with granulomatous inflammation (Sainsbury *et al.*, 1987).

One study (James and Ferguson, 1986) reported a 19-year-old Indian male (resident in UK since one year of age) who presented with the classical lip swelling and histological features of OFG. However, 3 years previously he had presented with enlarged lymph nodes in the posterior triangles of the neck bilaterally, and the left submandibular region. Histopathology of the lymph nodes showed discrete granulomata with multinucleate giant cells, histiocytes and lymphocytes. Minimal central necrosis was present: no acid/alcohol-fast bacilli were demonstrated in tissue sections, and inoculation of homogenate into a guinea pig failed to grow any mycobacteria. A chest radiograph was also normal. Despite this, the patient was treated with anti-tuberculous chemotherapy for 11 months and no further adenitis was noted.

Enlarged cervical lymph nodes have been reported in patients with OFG (Tyldesley, 1979; Hornstein, 1973). In 9 cases described by Tyldesley (1979), 5 were found to have enlarged and indurated submandibular lymph nodes. Lewis and Morley (1969) documented a case with intermittent enlargement of the cervical nodes over a period of 18 years.

The presentation of cervical lymphadenitis raises the possibility of atypical mycobacterial infection (Saitz, 1981). Atypical mycobacteria (AMB) infection is an important cause of lymph node enlargement in children. Akhtar *et al* (1997) recently

reviewed the casenotes of 17 children seen in Glasgow with lymphadenitis due to AMB. The mean age at presentation was 5.37 years (range 1.5-10.6 years). The patients had a short history (1-11 weeks) of unilateral single focus, usually cervicofacial (16/17) disease. The tuberculin skin test was negative in 14/15 patients. Primary excision was curative (11/11). Incision and drainage of an abscess or drainage with partial excision led to chronically discharging sinuses in all cases (6/6). AMB were seen on staining of drained or excised material in 11/17 cases and cultures were positive in 9 cases. The AMB cultured were *avium intracellulare* or *AMB avium complex*. The diagnosis was made in the remaining cases on the basis of clinical features and particular histopathological pattern (granulomatous inflammation with or without caseous necrosis).

Haase *et al* (1994) have described facial lymphadenitis in a 15-month-old boy which had histological features compatible with the diagnosis of AMB lymphadenitis. The AMB was subsequently identified, by DNA probing, as the recently described *M. celatum.*

James (1991a) made a comparison of the clinical findings in sarcoidosis and OFG (see Table 1.6) and drew the conclusion that these were two distinct disease entities.

Table 1.6 A comparison of Sarcoidosis and OFG (James, 1991)

FEATURES	**SARCOIDOSIS**	**OFG**
Gender	Equal	Equal
Age at presentation	20-45	20 (3-60)
Swelling of lips	No	Characteristic
Buccal mucosal oedema	No	Frequent
Oral ulceration	No	Yes
Gingival hyperplasia	No	Yes
Fissured tongue	No	Occasional
Facial palsy	4% (may be bilateral)	13% (unilateral)
Oral biopsy helpful	$\pm$	+
Associated Crohn's	Very rare	Frequent
Ocular lesions	Yes	No
Skin lesions	Yes	No
Chest radiograph	Abnormal	Normal
Kveim test	Positive	Negative
Serum ACE	Raised	Normal

Worsaae *et al* (1982) studied 16 patients with established OFG and found none with gastrointestinal Crohn's disease. Scully *et al* (1982) reviewed 19 patients with OFG and found 7 (37%) to have gastrointestinal Crohn's disease. These authors suggested that an elevated ESR, in conjunction with haematological evidence of malabsorption (iron studies, whole blood folate, serum albumin and calcium), were suggestive of gastrointestinal Crohn's disease (Scully *et al.,* 1982). Taylor and Smith (1975), having reviewed the world literature and presented 1 case report, make the bold statement on the inevitability of patients with OFG subsequently developing gut Crohn's disease. This view is clearly not endorsed by other authors.

Wiesenfeld *et al* (1985) found 6 patients (10%) had definite gastrointestinal Crohn's disease. A further 3 patients with possible Crohn's disease were followed-up for 5 years and remained well. Importantly, all 9 patients with positive gastrointestinal investigations had symptoms referable to the gastrointestinal tract at the time of presentation. A further 13 patients with gastrointestinal symptoms had no evidence of Crohn's disease on investigation. Furthermore, in 22 control patients with no gastrointestinal symptoms, no evidence of gastrointestinal disease was found.

In the study by Wiesenfeld *et al* (1985), no patients had a history suggestive of systemic sarcoidosis. Two patients had cutaneous lesions suggestive of sarcoidosis and a Kveim test in both supported this diagnosis. A total of 8 patients had Kveim tests performed and 34 had serum ACE levels estimated - all were within normal limits. However, the 2 patients who were diagnosed with sarcoidosis did not have serum ACE levels measured. Indeed, it is not clear how the diagnosis was arrived at.

A recent case report has linked OFG and syringomyelia (Sabroe and Kennedy, 1996). A 66-year-old male patient presented 15 years after a diagnosis of syringomyelia with marked right-sided facial swelling, particularly around the orbit. Histology showed granulomatous inflammation and all investigations for Crohn's disease and sarcoidosis were negative. The authors propose that syringomyelia may be a further neurological abnormality associated with OFG, to join those previously reported - facial palsy, headache, migraine, other cranial nerve palsies, Horner's syndrome, salivary gland dysfunction, psychoses, epilepsy, and abnormalities in the electroencephalogram (EEG) (Hornstein, 1973; Greene and Rogers, 1989; Zimmer *et al.,* 1992; Worsaae *et al.,* 1982).

In the localised form of OFG, it is unusual to encounter any other systemic manifestations. A few studies have commented on a general malaise or joint pains in patients in association with their facial swelling, which suggests the presence of circulating immune complexes (Ferguson and MacFadyen, 1986a). Six patients with joint pain and seropositive or seronegative arthritis have been described with OFG (Sabroe and Kennedy, 1996; Greene and Rogers, 1989; Zimmer *et al.,* 1992; Worsaae *et al.,* 1982; Eggelmeijer *et al.,* 1989).

Management of patients with OFG should include biopsy of the lesion and exclusion of intestinal Crohn's disease by haematological and biochemical investigations; if there are abdominal symptoms, radiographic bowel imaging with possible subsequent endoscopy and biopsy are indicated (Anonymous, 1991b). Sarcoidosis should also be excluded by chest radiography, serum ACE measurements and possibly a Kveim test (Anonymous, 1991b).

Treatment of symptoms in patients with OFG is often unsatisfactory (Miele,1994). In dealing with children, Sainsbury *et al* (1987) considered the natural history of OFG to be often one of gradual improvement. For ulceration they recommended chlorhexidine mouthwash (0.2%); 1% hydrocortisone ointment for angular cheilitis and lip fissuring. Intralesional corticosteroids were not well tolerated by children and a short course of systemic prednisolone could only be justified in children with severe disease (Sainsbury *et al.,* 1987).

Patients with minor symptoms may not require active treatment, apart from encouragement and clinician support (Armstrong and Burrows, 1995). Intralesional and systemic corticosteroids generally have a beneficial effect although relapse is common on cessation of therapy (Zimmer *et al.,* 1992; Allen *et al.,* 1990). In one study (Sakuntabhai *et al.,* 1993), five patients with biopsy-proven OFG (aged 10-24 years) were injected with high-volume intra-lesional triamcinolone (3-10ml of 10mg/ml; mean 60mg) after first numbing the lips with infra-orbital and mental local anaesthesia nerve blocks. Lip swelling immediately after injection was dramatic but started to subside after 5-6 days. After 6 weeks, the lip size returned to normal in 4 patients and was reduced in the fifth. One patient was injected on 4 occasions over a 2-year period; in the 4 other patients treated once, lip size remained reduced for over 10 months (Sakuntabhai *et al.,* 1993; Sakuntabhai *et al.,* 1992).

In adult patients, the use of low dose prednisolone (5mg daily) may reduce symptoms to cosmetically acceptable levels (Ingram, 1993). Other agents for which some success has been claimed include clofazimine, hydroxychloroquine and danazol (Zimmer *et al.*, 1992; Allen *et al.*, 1990; Podmore and Burrows, 1986). Treatment with sulphasalazine, metronidazole, azathioprine and cyclosporin has been disappointing (Armstrong and Burrows, 1995).

Ferguson and MacFadyen (1986b) suggested, on reviewing their cases of OFG over a 10 year period, that the mainstay of management should be identifying potential allergens rather than loading patients up with anti-inflammatory drugs, simply to control symptoms.

Several authors have reported good responses to the introduction of very low-allergen diets or elimination diets that identify and exclude putative provoking dietary factors (McKenna *et al.*, 1994; Haworth *et al.*, 1986). Armstrong and Burrows (1995) report that improvement in symptoms with dietary manipulation seems to involve only a sub-group of patients and they contend that this may reflect the heterogeneity in the aetiology of the condition.

Sanderson *et al* (1996) have reported the ileo-colonoscopic findings in ten patients with OFG and no gut symptoms. Abnormalities were detected in the ileum or colon (by direct vision and/or histology) in 8/10 cases. Macroscopic abnormality was present in only two cases (ileal aphthae and ileal erythema). Microscopic inflammation was detected in a further six cases and seven cases had identifiable granulomata. They concluded that ileocolonoscopy detected previously undiagnosed gut inflammation in the majority of patients with OFG, especially those presenting at a younger age.

The vast differences between studies in reporting gut inflammation in OFG is a concerning feature of the literature and requires clarification and standardisation of assessment.

1.2.4 Melkersson-Rosenthal Syndrome

The Melkersson-Rosenthal Syndrome (MRS) is a triad of lower motor neurone (facial nerve) palsy, facial oedema and fissured tongue (lingua plicata). The original report linking lower motor neurone facial nerve palsies and facial oedema came from Hubschamann in 1894. This was further clarified by a similar case described by Rossolimo in 1901 who also noted that the patient suffered from migraine. However, the syndrome is credited to Melkersson (1928) and Rosenthal (1931) and numerous case reports of MRS have been reported in the world literature since (Worsaae *et al.,* 1980; Levenson *et al.,* 1984; Cohen *et al.,* 1994; Pisanty and Sharav, 1969; Nally, 1970; Vistnes and Kernahan, 1971; Alexander and James, 1972; Hornstein, 1973; Worsaae and Pindborg, 1980; Azaz and Nitzan, 1984; Worsaae *et al.,* 1982; Storrs, 1975; Dhar and Kanwar, 1995).

In keeping with many such entities described in the medical literature, the syndrome has been modified and "downgraded" by subsequent authors who failed to acknowledge the strict diagnostic criteria for the syndrome as originally described. Thus, the literature becomes confused with references to "monosymptomatic" and "oligosymptomatic" MRS (Levenson *et al.,* 1984; Jain *et al.,* 1990; Cheng *et al.,* 1993; Stosiek *et al.,* 1992; Minelli *et al.,* 1991; Mainetti *et al.,* 1994; Rubino and Ficarra, 1994; Mendez *et al.,* 1991; Archibaldo and Alfredo, 1995; Mahler and Kiesewetter, 1996; Marques *et al.,* 1994; Labarthe *et al.,* 1995; Pellegrino *et al.,* 1993; John *et al.,* 1992; Benavides, 1990; Orlando and Atkins Jnr, 1990).

Further confusion enters the discussion with case reports clearly referring to sarcoidosis with a raised angiotensin converting enzyme level (Orlando and Atkins Jnr, 1990). Indeed, in 1984 without any justification whatever, Azaz and Nitzan stated that "..not all the symptoms (of MRS) need appear (together)" - presumably in a feeble attempt to justify their case report of a man with "MRS" in whom "(the neurological examination) disclosed no facial paresis or neurologic disorders". This lack of stringency is endorsed by Winnie and DeLuke in 1992 who state that "the presence of 2 (of the original three) findings *should* suggest a clinical diagnosis of MRS, subject to histopathologic confirmation." Similar lack of stringency of definition is demonstrated by other authors (Mahler *et al.,* 1995) allowing "*trigeminal* nerve paralysis" as part of the Syndrome.

Hornstein (1973) stated that MRS "is not so much a rare disease as one seldom diagnosed". An extensive review of the literature suggests that 262 cases have been reported since the syndrome was originally described. Combining the data from these 262 cases, the orofacial manifestations are listed in Table 1.7.

Table 1.7 Orofacial manifestations during the course of MRS.

MANIFESTATION	NUMBER	PERCENTAGE
Labial swelling	216	82
Upper	157	60
Lower	108	41
Facial swelling*	68	40
Facial palsy	65	25
Migraine/headache	63	24
Gingival swelling	28	11
Gingival pain	5	2
Gingival erosions	2	<1
Buccal mucosal swelling	41	16
Buccal mucosal erosions	3	1
Palatal swelling	22	8
Lingua plicata	154	59
Lingual swelling	27	10
Lingual dysaesthesia	11	4
Alteration of taste	7	3
Hyposalivation	5	2

Note * Only 127 patients from the literature review were included because of insufficient data in the original reports.

In most cases, the facial or lip swelling occurs before the facial palsy, but in others, it may occur some time after or simultaneously with it (Bataineh *et al.,* 1995; Kettel, 1949). The disease may have an acute, a recurrent, or a chronic course (Daoud and Rogers III, 1995). On the strength of combined data from various studies, Daoud and Rogers (1995) have suggested major and minor criteria for the diagnosis of MRS as follows:

Major criteria

Recurrent or persistent orofacial swelling

Minor criteria

Relapsing facial paralysis

Fissured tongue (lingua plicata)

Histological evidence of granulomatous inflammation

However, on this basis it would be difficult or impossible to differentiate MRS from the orofacial manifestations of systemic diseases such as sarcoidosis and Crohn's disease.

Greene and Rogers (1989) commented on the laboratory findings in 36 patients with MRS. Leucocytosis or eosinophilia were not present in any case; nor were titres of anti-streptolysin O and anti-deoxyribonuclease B. No abnormalities in serum concentrations of calcium, creatinine, or phosphate were recorded. Liver function tests were normal. Three patients had increased levels of IgM (unspecified) and one patient had an elevated level of IgE at 3089 IU/ml (normal 20-367 IU/ml). This elevated IgE was unspecified in terms of target antigen but could easily indicate an allergic state. Other isolated findings (each in one patient) were: raised ESR; a positive ANA (speckled 1:80); iron deficiency anaemia, and hypokalaemia. These last two findings were not expanded upon but could easily be found in patients with advanced Crohn's disease. Elevated blood glucose levels were present in two patients with previously diagnosed diabetes mellitus, a systemic disease which could account for the neurological palsies.

MRS has been reported in association with other local conditions such as gustatory sweating, epiphora, tinnitus, and disturbed taste sensation (Bataineh *et al.,* 1995), geographic tongue (Nally, 1970), masseteric myopathy (Saito *et al.,* 1994), laryngeal swelling with obstruction and respiratory arrest (Jayamaha, 1993), and granulomatous blepharitis (Manganaro and Holmes, 1997). MRS has also been linked with systemic diseases: distant-site neoplasia (Nifosi and Scassa, 1997); facial rosacea (Bose, 1996);

malignant pharyngeal lymphoma (Kanda, 1996); vulvitis granulomatosa (Samaratunga *et al.*, 1991); perivulvitis granulomatosa (Knopf *et al.*, 1992); Horton's temporal arteritis (Stäbler *et al.*, 1990); juvenile rheumatoid arthritis (Eggelmeijer and Dijkmans, 1990); sero-negative oligoarthritis (Eggelmeijer *et al.*, 1989); Crohn's disease (Lloyd *et al.*, 1994; Worsaae *et al.*, 1980); oral Crohn's disease (Diamond *et al.*, 1990); orofacial granulomatosis (Rogers III, 1996); and sarcoidosis (Alexander and James, 1972; Altman and Robinson, 1984). Basal sarcoid arachnoiditis has been postulated as an aetiological factor in MRS and would certainly explain the facial nerve palsy (Graff-Radford, 1981).

James (1994b) made a comparison of the clinical findings in Melkersson-Rosenthal Syndrome (MRS) and sarcoidosis (Table 1.8). He made the comment that, on the basis of these clinical findings, MRS and sarcoidosis must be two separate disease entities (James,1994b).

Table 1.8 Comparison of Melkersson-Rosenthal Syndrome and Sarcoidosis

Feature	**MRS**	**Sarcoidosis**
Male:Female	equal	equal
Age of onset (years)	any	20-40
Multiple Granulomata	+	+
Facial Paralysis	+	+
Facial Oedema	+	-
Swollen Lips	+	-
Fissured Tongue	+	-
Angular Cheilitis	+	-
Granulomata of :		
Face	+	+
Oral Mucosa	+	-
Gingivae	+	+
Lips	+	+
Eyelids	+	+
Tongue	+	-
Pharynx	+	+
Vulva	+	-
Salivary Gland Dysfunction	+	+
Uveitis	-	+
Abnormal Chest X-ray	-	+
Positive Kveim Test	-	+
Angiotensin Convertase	Normal	Elevated

There is some concern expressed in the literature about long-term follow-up of patients with MRS, or indeed any chronic lymphoedematous state (Kanda, 1996). This is due to the recognised association of MRS and subsequent development of lymphoma (Breuchat *et al.,* 1985; Kanda, 1996). The literature clearly highlights established links between lymphoedema and subsequent lymphomatous change (Breuchat *et al.,* 1985; Tatnall and Mann, 1985; Waxmann *et al.,* 1984; d'Amore *et al.,* 1990; Peyron *et al.,* 1993) - the so-called Stewart-Traves syndrome (d'Amore *et al.,* 1990).

Treatment of MRS is largely symptomatic given that the aetiology of the syndrome is unknown (Tausch and Sönnichsen, 1992). In addition, the confusion in the literature over diagnostic criteria for MRS make it likely that a number of different granulomatous disorders are being classified as MRS, leading to an erroneous assessment of treatment outcome measures. Similarly, partial or complete spontaneous remission is reported in around 27% of patients (Sussman *et al.,* 1992) and this will also confuse treatment outcome scores.

The mainstay of treatment is corticosteroids - topical, oral, parenteral, or intra-lesional (Greene and Rogers, 1989), although effects are often temporary (Daoud and Rogers III, 1995). Multiple injections over weeks or months are often required (Greene and Rogers, 1989).

The elimination of odontogenic foci of infection was followed in one study by regression or disappearance of swelling in 11 of 16 patients (Worsaae *et al.,* 1982), suggesting that foci of infection may play some role in aetiopathogenesis.

Henderson and Tschen (1988) reported a patient whose MRS responded well to treatment with intra-lesional corticosteroids and a hydroxyquinolone antibiotic. Other authors have suggested the use of tetracycline (500mg daily) in combination with prednisone (10mg alternate days) in the treatment of the full triad of MRS (Fisher, 1990). The patient described continued this treatment regime for 2.5 years and the lip oedema improved by 60%; the tongue remained unaltered and the facial palsy did not recur. Tetracycline has been used successfully in the treatment of certain dermatoses that produce non-specific sarcoid-like histopathological patterns (Falk, 1985), such as perioral dermatitis, periocular dermatitis (Fisher, 1987), and sarcoid-like ochronosis

(Fisher, 1988). The combination of prednisone and tetracycline together appears to work better than either alone (Fisher, 1990).

The first report using the anti-leprosy drug clofazimine appeared by Neuhofer and Fritsch in 1984. Seven cases were treated with clofazimine in a dosage of 100mg daily for 10 days, then 200-400mg weekly. Of the seven cases, three had the complete triad and four had cheilitis only. There was an excellent initial response after two weeks in those patients in whom the swelling was still fluctuant, but in those cases with persistent swelling the response was slower, continuing for up to 3 months. Three cases relapsed on discontinuing treatment, but responded on re-introduction of the drug. The overall duration of treatment was 5-7 months. Other reports have claimed partial or complete remission with low dose clofazimine therapy with low toxicity and good side-effect profile (Tausch and Sönnichsen, 1992; Sussman *et al.,* 1992; Amézaga *et al.,* 1991). Pre- and post-treatment biopsy results in a trial of four patients with clofazimine revealed histological evidence of resolution of granulomatous inflammation but persistence of oedema (Podmore and Burrows, 1986).

Clofazimine is a phenazine iminoquinone derivative which is metabolised to a red dye-stuff with oxygen (Podmore and Burrows, 1986). It is taken up by the reticulo-endothelial system and fatty tissue. The mechanism of action is not fully known but is thought to be related to its ability to stimulate phagocytosis (Neuhofer and Fritsch, 1984). Clofazimine has proved effective in the treatment of pyoderma gangrenosum, discoid lupus erythematosus, leprosy and pustular psoriasis (Sarracent and Finlay, 1982).

Treatment of MRS with thalidomide was suggested in 1995 (Safa *et al.,* 1995). Two patients with MRS were treated with thalidomide 100mg daily for 3 and 6 months respectively. In the first case, there was suppression of episodes of facial oedema and reduced lip swelling but the treatment was stopped after 3 months since the patient could not guarantee adequate contraception. In the second case, there was complete clinical and histological resolution. Thalidomide is thought to work by suppressing the formation of tissue necrosis factor (TNF-α) (Powell, 1996).

Other drugs have been tried in the management of MRS with varying degrees of success. These include hydroxychloroquine, dapsone and colchicine (Rey *et al.,* 1996), sulfasalazine, penicillin, erythromycin, clindamycin, ranitidine, and diphenhydramine

(Zimmer *et al.*, 1992), ketotifen, and cromoglycate (Pachor *et al.*, 1989). All have been noted at the level of case reports and none have been subjected to rigorous scientific trial.

The option of surgical reduction cheiloplasty has been investigated by several authors (Ellitsgaard *et al.*, 1993; Glickman *et al.*, 1992; Rey *et al.*, 1996). This is only recommended when conservative treatments have failed and the lip swelling has become stabilised and associated with permanent aesthetic deformity (Ellitsgaard *et al.*, 1993). Intralesional corticosteroid injections after a cheiloplastic procedure have been recommended to minimise the tendency to recurrence (Krutchkoff and James, 1978b).

Decompression of the facial nerve is indicated for the treatment of long-standing facial nerve paralysis, following failure of systemic corticosteroid therapy (Daoud and Rogers III, 1995). This procedure was effective in 11 of 13 patients reported by Kettle (1959) without evidence of recurrence.

Hornstein (1997) has recently appealed for multi-disciplinary professional involvement to ascertain the most appropriate way to treat patients with MRS.

1.2.5 Cheilitis Granulomatosa of Miescher

When Miescher first reported his granulomatous cheilitis in 1945, in the proceedings of 26th Congress of the Swiss Society of Dermatology and Venereology held in Zurich in September 1944, he described six cases of macrocheilia. One or both lips were affected and, in some cases, the inner surface of the cheeks. There was no local septic focus and microscopic examination revealed peri- and paravascular aggregations of a tuberculoid character as the cause of the swelling. He stated that aetiology and pathogenesis were unknown but the possibility of a localised tuberculous lesion should be borne in mind, although he could not identify active tuberculosis (pulmonary, gastrointestinal or miliary) in any of the six patients.

Since that original description, numerous case reports have been presented world-wide of cheilitis granulomatosa of Miescher (CG) (Allen *et al.,* 1990; Shaikh *et al.,* 1989; Williams and Greenberg, 1991; Hirshberg *et al.,* 1989; Kuno *et al.,* 1992; Alpert and Nelson, 1974; Hernandez *et al.,* 1986; Tatnall and Dodd, 1987; Brook, 1984; Carr, 1974; Brook *et al.,* 1983; Kano *et al.,* 1990; Guerrieri *et al.,* 1995; Bourgeois-Droin *et al.,* 1993; Kano *et al.,* 1993; Veller Fornasa *et al.,* 1992; Eisenbud *et al.,* 1971; Miralles *et al.,* 1995; Liu, 1994; Takeshita *et al.,* 1995; Liu, 1993a; Liu, 1993b; Creus *et al.,* 1994; Shehade and Foulds, 1986). However, great confusion exists in the literature as to whether CG is a disease entity in its own right or whether it is a "monosymptomatic" variant of MRS or orofacial granulomatosis (Hornstein, 1973; Wiesenfeld *et al.,* 1985; Field and Tyldesley, 1989; Hernandez *et al.,* 1986). It is important to differentiate CG from cheilitis glandularis (enlargement of the lower lip due to hyperplasia of the mucous glands) by histological assessment, since the clinical presentation may be similar (Doku *et al.,* 1965). Similarly, the condition known as actinic cheilitis granulomatosa should be excluded (Kuno *et al.,* 1992). This is an entity similar to CG in clinical and histological appearance but caused by UVB-photosensitivity

It is now clearly accepted in the literature that many authors consider CG to be a monosymptomatic variant of MRS (Worsaae *et al.,* 1982; Allen *et al.,* 1990), although this has been a subtle erosion of the clear diagnostic criteria established by Melkersson (1928) and Rosenthal (1931). Indeed, some authors now blatantly assert that "a diagnosis of oligosymptomatic Melkersson-Rosenthal syndrome is accepted as being synonymous with CG." (Allen *et al.,* 1990).

CG is characterised by diffuse, non-tender, soft to firm swelling of one or both lips; the swelling may be unilateral or symmetrical (Allen *et al.,* 1990). Initially, the swelling may be episodic but eventually the enlargement persists (Shaikh *et al.,* 1989). Gingival involvement was noted in 21% of patients in a Danish study (Worsaae *et al.,* 1982) and in 33% of patients in a North American study (Allen *et al.,* 1990). Depending on the view of the authors, additional oral, peri-oral, or facial manifestations may be described as part of CG, MRS or orofacial granulomatosis.

Vesicular lesions have been described in association with CG and the assumption made that these were associated with Herpes simplex infection (Hornstein, 1973). However, microscopic examination of these vesicles has demonstrated that they are actually superficial dilated lymphatic vessels (Allen *et al.,* 1990).

CG has been described in association with advanced periodontitis with the periodontium and lip biopsies exhibiting non-caseating epithelioid granulomata histologically (Takeshita *et al.,* 1995). Interestingly, the lip swelling resolved following periodontal therapy and antibiotics.

Despite the obvious confusion in the literature over establishing the diagnosis of CG, clear links have been established in some cases with sarcoidosis and Crohn's disease. In a French study, two children with CG were subsequently found to have sarcoidosis (Bourgeois-Droin *et al.,* 1993). Similarly, there are a number of reports where patients with CG have a positive Kveim test (Shehade and Foulds, 1986; Nelson and Stevenson, 1988). In most cases, there is a preceding history of multiple symptoms prior to the onset of CG. Shehade and Foulds (1986) report a 43-year-old Caucasian female with a long-standing history of multi-system complaints who subsequently developed cheek and lip swelling. Her Kveim test was positive, giving a well defined dermal epithelioid granulomatous response on the right forearm. Her chest radiograph, serum angiotensin converting enzyme level, Mantoux test and gastrointestinal investigations were all within normal limits. A Kveim test is regarded as specific for sarcoidosis (Sharma, 1984); however, cross-reactivity between sarcoidosis and Crohn's disease can occur (Mitchell *et al.,* 1970), and in other conditions - notably tuberculous lymphadenitis, non-specific lymphadenitis and lymphomas (Israel and Goldstein, 1971). In view of the fact that Kveim test reactivity is low among patients in whom the manifestation of sarcoidosis is confined to a single organ (Bradstreet *et al.,* 1976), and that the reactivity decreases

sharply with the passage of time (Mikhil and Mitchell, 1970), a positive Kveim test response obtained at this early stage in the disease and a negative Mantoux test (possibly due to cutaneous anergy) strengthen the possibility that this patient had cutaneous sarcoidosis. The authors suggest that, recognising the limitations of Kveim test reactivity and taking into account the occasional spontaneous remission of sarcoidosis, more cases of CG, MRS and orofacial granulomatosis may have eluded a diagnosis of cutaneous sarcoidosis in the past (Shehade and Foulds, 1986).

If numbers of publications are indicative of strength of association, then there are even stronger links between CG and Crohn's disease (Tatnall and Dodd, 1987; Brook, 1984; Carr, 1974; Brook *et al.,* 1983; Kano *et al.,* 1990; Guerrieri *et al.,* 1995). In cases of gastrointestinal Crohn's disease, treatment of the gut condition has been reported to lead to an improvement in the lip swelling (Brook *et al.,* 1983) although this is not always so (Carr, 1974). Brook *et al* (1983) make the recommendation that patients with CG be screened at presentation and at 6-month intervals for malabsorption as a warning of development of granulomatous inflammation in the gut. Kano *et al* (1990) reported five cases of CG which subsequently developed gastrointestinal Crohn's disease. They make the recommendation that patients presenting with CG require gastrointestinal investigations and long-term follow-up.

CG would appear to be particularly prevalent in patients who have widespread or metastatic Crohn's disease - notably of perineum, vulva (Guerrieri *et al.,* 1995), and skin (Tatnall and Dodd, 1987).

A rational approach to treatment for CG is problematic because the cause is unknown and the lip swelling may be a manifestation of various disease states, for example Crohn's disease (Kano *et al.,* 1990) or sarcoidosis (Bourgeois-Droin *et al.,* 1993). Although removal of odontogenic foci of infection may elicit a good response in some patients (Worsaae *et al.,* 1982; Rintala *et al.,* 1973), most therapeutic regimes include corticosteroid therapy, either systemic or intra-lesional, as an empirical approach to the inflammatory infiltrate (Williams and Greenberg, 1991; Hernandez *et al.,* 1986; Bishop and Garcia, 1979; Eisenbud *et al.,* 1971; Krutchkoff and James, 1978a; Levenson *et al.,* 1984). Eisenbud *et al* (1971) are credited with the first recorded use of triamcinolone injections to an upper lip affected with CG. They used 20mg of triamcinolone at intervals of 2 weeks to 1 month for a total of 20 doses. Efforts were made to distribute

the solution over a broad area of the lip from a single puncture site. Total treatment time extended over 1 year. Reduction of the swelling became evident after several weeks and complete resolution was the result. No side-effects were reported, except for a small haematoma on one occasion. Follow-up continued for 9 months after cessation of treatment with no recurrence of swelling or discomfort.

The response to such treatment is generally favourable but temporary and requires multiple injections for months or even years. It is reported that the patients' acceptance of the intra-lesional therapy is increased if local anaesthetic blocks are given before the corticosteroid injections (Allen *et al.,* 1990; Eisenbud *et al.,* 1971).

Intralesional corticosteroids may cause degenerative changes in skeletal muscle (William, 1959), necrosis of granulomata, and scar tissue formation (Krutchkoff and James, 1978a). In addition, some reports have suggested that a threshold may be reached beyond which no further reduction in lip size can be achieved (Krutchkoff and James, 1978a).

Hydroxychloroquine sulphate has been used with limited effect (Allen *et al.,* 1990). One patient responded well to 200mg daily for three months, increasing to 400mg daily for an additional three months. However, retinopathy is an established side-effect of anti-malarial agents and so an ophthalmologic assessment is required at baseline and every 4-6 months during treatment (Portnoy and Callen, 1983). The rationale for using hydroxychloroquine is based on the well-documented improvement of the specific cutaneous lesions of sarcoidosis with anti-malarial therapy (Gibson and Winkleman, 1986). Some authors consider CG and sarcoidosis to be indistinguishable (Veien, 1986), although a world authority on sarcoidosis has claimed that lip enlargement is not a feature of sarcoidosis (James, 1994b).

The use of antibiotics in the treatment of CG has generated some interest in the literature (Veller Fornasa *et al.,* 1992; Kano *et al.,* 1992; Miralles *et al.,* 1995). This is on the basis that, in addition to their antibacterial properties, antibiotics may act as biological response modifiers (Anonymous, 1991a). One study (Veller Fornasa *et al.,* 1992) found minocycline at a dose of 100mg daily for 4-6 months to be ineffective in reducing lip swelling in five patients with CG; however it was effective in one patient with chronic granulomatous disease (type 1, X-linked form).

The response of CG in some patients to metronidazole (Kano *et al.,* 1992; Miralles *et al.,* 1995) has prompted some authors to draw comparisons between CG and gastrointestinal Crohn's disease - the latter often responding to metronidazole when other therapies have failed (Brandt *et al.,* 1982; Duhra and Paul, 1988). Kano *et al* (1992) reported a female patient whose CG had failed to respond to ketotifen and triamcinolone. She was found to have colonic and rectal changes diagnostic of Crohn's disease and was treated with oral metronidazole - 500mg twice daily for 3 months. Complete resolution of lip swelling and gastrointestinal ulceration was achieved with no recurrence at follow-up after 2 years.

The successful use of metronidazole in the treatment of CG in patients without gastrointestinal Crohn's disease has been emphasised in one study (Miralles *et al.,* 1995). A black female with CG (upper and lower lips) and no evidence of Crohn's disease on sigmoidoscopy, barium meal and follow-through, and rectal biopsy was treated unsuccessfully with oral doxycycline and triamcinolone injections. She was then commenced on oral metronidazole - 750mg daily for 1.5 months, increased to 1g daily for three months. This was then tapered down to complete eight months of treatment and the lip enlargement has improved progressively.

Wiesenfeld *et al* (1985) did not detect any improvement in 2 patients with CG treated with metronidazole. However, in this study neither the exact clinical circumstances nor the dose of the drug were described by the authors.

The mechanism of action of metronidazole is unknown but may be related to anti-inflammatory rather than antibiotic properties. It is known to suppress granuloma formation around parasite eggs and to inhibit cell-mediated immunity (Grove *et al.,* 1977).

In addition to dental and medical management, surgical reduction of the lips has also been advocated (Krutchkoff and James, 1978a; Shaikh *et al.,* 1989). Most authors recommend that this procedure is carried out only after failed medical management and when the lip has reached a quiescent phase (Rintala *et al.,* 1973). In addition, intra-lesional triamcinolone injections or suppressive medical treatment should continue after surgery for an indefinite period to reduce the considerable risk of recurrence (Krutchkoff and James, 1978a).

It has recently been stated, without much evidence, that CG is a pre-malignant condition with between 20% and 35% of patients eventually developing squamous cell carcinoma of the lip (Manganaro and Holmes, 1997). This would appear to be another myth borne out of much case reporting and little proper research.

1.2.6 GLUS Syndrome (Granulomatous Lesions of Unknown Significance)

Cases of hepatic granulomata, often termed "granulomatous hepatitis", collectively represent one of the largest biopsy-defined groups of granulomata of various aetiologies. Collective data from six studies, shown in Table 1.9, demonstrate that amongst such patients, 92 cases of GLUS presented with one or more of the following features : fever (43%), anorexia/weight loss (34%), abdominal pain (28%), hepatomegaly (28%), splenomegaly (28%), and lymphadenopathy (7%). There were no consistent biochemical abnormalities, but hypergammaglobulinaemia was frequent (66%). Liver function tests showed abnormalities in the following parameters: bilirubin (21%), alkaline phosphatase (51%) and aminotransferases (51%) (Harrington *et al.*, 1982).

STUDY/FEATURES	**1**	**2**	**3**	**4**	**5**	**6**	**TOTAL**
No. of cases	13	10	13	18	14	24	92
Male patients	6	4	9	10	8	9	50%
Anorexia/ Weight loss	5	0	8	?	3	9	34%
Fever	5	10	13	3	6	3	43%
Abdominal pain	1	0	6	?	7	7	28%
Hepatomegaly	10	2	7	6	8	10	47%
Splenomegaly	3	1	7	4	4	7	28%
Lymphadenopathy	5	0	0	0	0	1	7%
Hypergamma-globulinaemia	7/7	6/10	5/13	10/14	10/14	?	66%
Kveim test Positive	?	?	0/13	0/5	0/?	0/8	0%

Table 1.9 The frequency of various clinical features in six studies of granulomatous lesions of unknown significance, diagnosed by liver biopsy.

Studies : 1=Guckian and Perry, 1966; 2=Terplan, 1971; 3=Simon and Wolff, 1973; 4=Mir-Madjlessi *et al*, 1973; 5=Neville *et al*, 1975; 6=Cunningham *et al*, 1982.

A minority of cases had a relatively short, benign, self-limiting, "mononucleosis-like" course with peripheral lymphocytosis (Eliakim *et al.,* 1968; Gelb *et al.,* 1970). These cases were EBV-negative but were not tested for CMV. The majority of the remaining cases did not exhibit a lymphocytosis and often showed a prolonged course sometimes of several years' duration, with exacerbations and remissions. The latter type generally responded well to treatment with systemic corticosteroids (Simon and Wolff, 1973).

In a study of granulomatous lesions in peripheral lymph nodes in 85 children, no obvious aetiology could be found in 39 cases (46%). The proportion of GLUS varied according to body site: there were 19/60 cases of GLUS in the head and neck and 20/25 cases in peripheral lymph nodes (Benjamin, 1987). The majority of cases of GLUS occurred in children over 10 years old; atypical mycobacterial infections predominated in younger children and among the head and neck cases. If cases of atypical mycobacteria are excluded, the frequency of GLUS in the head and neck rises to 76% - the same level as in peripheral lymph nodes (80%). Apparently, the cases showed no distinctive clinical features apart from lymphadenopathy, and no instance of recurrence of GLUS was recorded; however, two cases of recurrent lymphadenopathy with GLUS were observed in another study (Brincker, 1990).

In 1989, Telenti and Hermans described 20 patients with prolonged fever of unknown origin, associated with idiopathic granulomatosis of the liver, lymph nodes, spleen or bone marrow. Initially, half the patients also had anorexia, arthralgia or myalgia, and a few had unspecified skin rashes. Granulomata were found in 6/9 spleens, 14/16 livers, 8/14 lymph nodes and 11/16 bone marrows. Nevertheless, there was only minor enlargement of liver, spleen and lymph nodes. Half the patients had abnormal liver function tests and hypergammaglobulinaemia. Serological tests were negative for EBV and CMV in 8/8 and 8/10 patients so studied. Fourteen of 15 patients responded favourably to corticosteroid treatment. In about half the patients, complete resolution occurred within a few years but after 5-10 years of follow-up, six patients still required corticosteroids for control of symptoms; however, the disease was not lethal in any patient. An aetiological diagnosis was established in five patients : Crohn's disease, sarcoidosis, temporal arteritis, CMV and hypergammaglobulinaemia; in the remaining 15 patients, the lesions remained unexplained.

In 1990, Friedland *et al* described nine cases of GLUS. In addition to granulomata in liver, spleen, lymph nodes and bone marrow, they were also present in kidney and skin. A total of 5/5 patients had a negative Kveim test, and 4/4 had normal levels of serum angiotensin converting enzyme. The syndrome had a prolonged history of episodic exacerbations; in some patients it resolved spontaneously and in others it responded to immunosuppressive therapy with corticosteroids and alkylating agents. One patient died of renal failure, probably unrelated to the granulomatous disorder.

In summary, several studies support the existence of a febrile, granulomatous, multi-system disease with moderate abnormalities of liver function, hypergammaglobulinaemia, responsive to immunosuppressive therapy and splenectomy, and a favourable prognosis. Although the combination of features described conforms to a diagnosis of extra-pulmonary sarcoidosis, it is distinguished from the latter by the absence of hypercalcaemia, elevated serum ACE and negative Kveim test.

There are no specific orofacial features described in any studies related to GLUS although clearly the lymphadenopathy may affect any body site.

Generally, the prognosis appears to be good in GLUS with spontaneous remission in most cases; in others, however, intermittent signs of disease activity may be present for months or even years. In such cases, corticosteroid therapy is almost always helpful (Simon and Wolff, 1973; Telenti and Hermans, 1989; Friedland *et al.,* 1990). Responses to cytotoxic drugs (Friedland *et al.,* 1990) and splenectomy have also been reported (Kuo and Rosai, 1974).

1.3 The epidemiology of chronic granulomatous disorders

1.3.1 Sarcoidosis

There are large numbers of epidemiological studies showing the prevalence and incidence of sarcoidosis (most commonly related to the respiratory system since that is the likeliest system to be affected) among various population groups world-wide. See Table 1.10.

Table 1.10 The prevalence of pulmonary sarcoidosis (per 100 000 of the population)

Country	Prevalence
Sweden	64
Denmark	48
West Germany	43
East Germany	41
Ireland	40
USA (New York)	39
England	27
Norway	27
Holland	22
Switzerland	16
Yugoslavia	12
France	10
Italy	9
Scotland	7
Finland	5
Japan	2.5
Spain	1.2

The true prevalence of sarcoidosis in any country is uncertain since many cases are known to be asymptomatic. Necropsy studies on 6706 patients in Malmo, Sweden revealed evidence of sarcoidosis completely unrelated to the cause of death with a prevalence of 640 per 100 000, ten times the local prevalence of sarcoidosis as determined by mass miniature radiography (MMR) (Hagerstrand and Linell, 1964).

Since most cases of sarcoidosis show characteristic chest radiographic abnormalities, MMR (often taken for purposes of screening for pulmonary tuberculosis) gives some indication of its frequency within the population. Average findings for MMR in 1959 for England and Wales suggested sarcoidosis in 13.8 men and 19.8 women per 100 000 population, with the highest prevalence, irrespective of sex, in the age group 25-34 years (British Thoracic and Tuberculosis Association, 1969).

MMR screening in London in 1958 showed a high prevalence of pulmonary sarcoidosis in the immigrant populations with rates per 100 000 of 197 for West Indian men and 170 for West Indian women; and 97 for Irish men and 213 for Irish women (Edmonstone and Wilson, 1985). This compared with an overall rate of 27 per 100 000 for those born in the UK (with similar gender rates) and a rate of 39 per 100 000 for UK women over the age of 16 years (Edmonstone and Wilson, 1985).

Four areas of the UK were studied in depth during the years 1961-66 to ascertain the annual incidence of sarcoidosis. The incidence was found to increase from north to south and was highest in the age group 24-34 years. The annual incidence was 2.1-4.1 per 100 000 men and 3.5-4.5 per 100 000 women (British Thoracic and Tuberculosis Association, 1969).

Two recent studies have demonstrated the variation in incidence and course of the disease in different racial groups in the same geographical area (Scadding and Mitchell, 1985; Edmonstone and Wilson, 1985). A ten-fold increase in the incidence of sarcoidosis in West Indian and Asian immigrants living in London has been reported in comparison with the indigenous white population. The immigrant patients also had an increased incidence of extra-pulmonary disease, a greater need for corticosteroid treatment and full recovery was less likely in comparison to their white neighbours.

Sarcoidosis is common in South Africa with a prevalence of 23 per 100 000 in the black population, 11.6 per 100 000 in the mixed race population and 3.7 per 100 000 in the white population (Siltzbach *et al.,* 1974; James *et al.,* 1976).

In the USA, sarcoidosis is at least ten times commoner in the black than in the white population, regardless of birthplace or residence with prevalence rates per 100 000 variously estimated at 8.7-81.8 and 0.5-7.5 for the black and white populations respectively in military and veterans administration studies (Siltzbach *et al.,* 1974; James, 1994b). Sarcoidosis is rarely reported in Middle Eastern Arabs, Chinese, Southeast Asians, Eskimos or North American Indians (Siltzbach *et al.,* 1974).

The prevalence in age groups at the extremes of life is difficult to ascertain. It is rare in European children who tend to present with extra-pulmonary symptoms; Japanese children, however, show a moderate frequency of asymptomatic pulmonary sarcoidosis, as reported by MMR (Siltzbach *et al.,* 1974; Scadding and Mitchell, 1985). Frequency increases in later childhood and from adolescence onwards, presentation and prognosis are similar to the adult population. Interestingly, sarcoidosis is commoner in non-smokers than in smokers (Siltzbach *et al.,* 1974; James, 1994b).

With respect to orofacial manifestations of sarcoidosis, there is a slight female preponderance (ratio 1.5:1). Patients ranged from 5 to 69 years with the highest prevalence between 30 to 40 years (Blinder *et al.,* 1997).

1.3.2 Crohn's Disease

A review of the current world literature suggests that Crohn's disease is most common in North America and northern Europe, emerging in southern Europe and least common in other parts of the world. Scandinavian studies have produced the highest prevalence figures (75 and 54 per 100 000 of the population) (Kraft, 1975; Basu, 1976) while high British figures are between 26 and 56 per 100 000 (Henry, 1994; James, 1991a). These are demonstrated in Figure 1.11.

Table 1.11 Studies of the incidence (cases/10^5/year) and prevalence (cases/10^5 of the population) of Crohn's disease

Place of study	Reference	Incidence	Prevalence
UNITED KINGDOM			
Oxford	(Evans and Acheson, 1965)	0.8	9
London	(Wright, 1970)	-	13
Gloucester	(Tresadern *et al.*, 1973)	1.5	-
Nottingham	(Miller *et al.*, 1974)	3.6	26.5
North East Scotland	(Kyle,1971; Kyle and Stark, 1980)	2.1	32.5
Clydesdale	(Smith *et al.*, 1975)	1.5	-
Northern Ireland	(Humphreys and Parks, 1975)	1.3	-
1 Belfast	(Humphreys and Parks, 1975)	3.5	-
2 County Down	(Humphreys and Parks, 1975)	0.3	-
North Tees	(Devlin *et al.*, 1980)	5.3	35
Cardiff	(Mayberry *et al.*, 1979)	4.8	56
NORTH AMERICA			
Baltimore White male	(Monks *et al.*, 1967)	2.5	-
Baltimore White female	(Monks *et al.*, 1967)	1.2	-
California	(Gelpi,1978)	-	13
USA - 15 towns study	(Garland *et al.*, 1981)	2.4	-
Olmsted County, Minn	(Sedlack *et al.*, 1980)	6.6	106
Sherbrooke, Quebec	(Nootens and Devroede, 1972)	0.7	6.3
NORWAY			
Norway	(Myren *et al.*, 1971)	1.03	-
Bergen	(Skarstein *et al.*, 1982)	3.5	-
SWEDEN			
1 Uppsala and Vastmanland	(Norlen *et al.*, 1970)	3	27
2 Gothenburg	(Kewenter *et al.*, 1974)	6.3	-
3 Malmo	(Brahme *et al.*, 1975)	6	75.2
4 Stockholm	(Hellers, 1979)	5	54.2

Place of study	Reference	Incidence	Prevalence
DENMARK			
Copenhagen	(Hoj *et al.*, 1973; Binder *et al.*, 1982)	2.7	32
FINLAND			
Turku	(Havia and Thomasson, 1972)	0.27	-
SWITZERLAND			
Basle	(Fahrlander and Baerlocher, 1971)	2.6	-
ITALY			
Bologna	(Lanfranchi *et al.*, 1976)	0.8	-
SPAIN			
1 Galicia	(Ochoa, 1977)	0.14	1.22
2 Madrid	(Paredes and Garcia, 1981)	0.7	-
CZECHOSLOVAKIA			
Northern Bohemia	(Bitter and Zuvacova, 1981)	1.6-2.0	12
SOUTH AFRICA			
1 Western Cape Jewish	(Novis *et al.*, 1975; Wright *et al.*, 1981)	7.2	-
2 Western Cape White	(Novis *et al.*, 1975; Wright *et al.*, 1981)	1.2	-
3 Western Cape Black	(Novis *et al.*, 1975; Wright *et al.*, 1981)	1.3	-
4 Pretoria White	(Mieny *et al.*, 1981)	1.1	-
5 Pretoria Black	(Mieny *et al.*, 1981)	0.2	-
NEW ZEALAND			
1 Whole Country	(Couchman and Wigley, 1971)	-	49
2 Auckland Caucasians	(Tasman-Jones *et al.*, 1982)	1.8	-
3 Auckland Polynesians	(Tasman-Jones *et al.*, 1982)	0	-
ISRAEL			
Tel-Aviv	(Rozen *et al.*, 1979)	1.3	12.3
Beersheba	(Rozen *et al.*, 1979)	1.8	12.3

It is clear that the incidence of Crohn's disease is rising steeply in the Western world for reasons that remain largely unexplained. In Wales, between 1931 and 1985, the incidence has increased from 0.18 per 100,000 population per year to 8.3 per 100,000 population per year (Rhodes, 1988). This study also highlighted a biphasic age distribution with peaks in young adults and the elderly.

The type of health care available in terms of investigative and diagnostic protocols, particularly in Scandinavia and the United Kingdom, may partly account for the high figures from these geographical areas. The health care is largely free and information technology may facilitate collection of epidemiological data. However, the type of health care provision alone cannot explain the high prevalence figures since comparable countries in the southern hemisphere, such as Australia (Newcombe *et al.,* 1983), have apparently low figures although good data to substantiate this is lacking. The incidence of Crohn's disease in New Zealand (Couchman and Wigley, 1971; Tasman-Jones *et al.,* 1982) and South Africa (Novis *et al.,* 1975; Wright *et al.,* 1981; Mieny *et al.,* 1981) is lower than in Europe despite many of their citizens being of European extraction. The major difference between countries is therefore unlikely to be due to ethnic factors alone. Attempts to examine the disease in different racial groups within the same country do suggest that prevalence figures are higher in subjects of northern European origin (Novis *et al.,* 1975; Wright *et al.,* 1981; Mieny *et al.,* 1981; Couchman and Wigley, 1971; Tasman-Jones *et al.,* 1982; Rozen *et al.,* 1979). Figures of prevalence from developing countries are less reliable for various reasons. Diarrhoea of unclassified aetiology and gastrointestinal tuberculosis are common among such populations and in areas with a limited medical service, the true incidence of Crohn's disease would be masked.

Few cases have been reported from Africa (Segal *et al.,* 1981). Similarly, there is only a single series of 44 cases from India (Gupta *et al.,* 1962), and small groups of cases have been reported from Chile (Castillo, 1959; Quintana *et al.,* 1978). There appears to be relatively low incidence figures for West Indian (O'Donoghue and Clark, 1976) and Asian populations resident in the UK (Burke and Zafar, 1975).

The incidence of Crohn's disease in children in the UK is around 10 per 100 000 and increasing (Ferguson *et al.,* 1986). Interestingly, Crohn's disease is never seen in infants less than two years of age and only rarely in children less than ten. The incidence increases rapidly through childhood and adolescence so that the peak incidence of

around 20 per 100 000 occurs during late adolescence and early adulthood (Ekbom *et al.*, 1991).

Studies from different parts of the world support the view that Crohn's disease is commoner in towns than country areas as shown in Table 1.12. This has been demonstrated in Wales where prevalence was examined throughout the country, involving 1100 patients (Mayberry *et al.*, 1980). Similar findings were also reported in Ireland (Humphreys and Parks, 1975), Scotland (Kyle, 1971), New Zealand (Couchman and Wigley, 1971), the USA (Sedlack *et al.*, 1980), Spain (Paredes and Garcia, 1981) and Italy (Lanfranchi *et al.*, 1976). These differences, however, have not been observed in central Sweden where the incidence is particularly high (Norlen *et al.*, 1970; Hellers, 1979). The differences observed in Aberdeen (Kyle, 1971) and Minnesota (Sedlack *et al.*, 1980) were against a background of a marked rise in incidence during the previous decade, which was most marked in urban areas.

Table 1.12 Urban-rural distribution of Crohn's disease

Place	Measurement	Urban	Rural
Wales	Period prevalence (cases/10^5)	47.6	34
Northern Ireland	Incidence (cases/10^5/yr)	3.5	0.29
Aberdeen	Prevalence (cases/10^5)	49	29
Olmstead County, USA	Prevalence (cases/10^5)	116.7	84.2
New Zealand	Mean rates/yr	119	59
Madrid Province, Spain	% Composition	94.2	5.8
Bologna, Italy	% Composition	77.8	22.2

Therefore, most studies show that Crohn's disease is commoner in urban than rural areas and one explanation for this might be an environmental factor.

With respect to oral Crohn's disease, the largest group studied is that of Plauth *et al* (1991) with 79 patients in a West German study. Their data relied on histological confirmation from intestinal biopsies before the patients were included for analysis. They noted a male preponderance of 1.85:1 which increased to 3.0:1 in the 16-30 year-old age group. Almost two-thirds of patients had experienced oral lesions in the first three decades of life as shown in Table 1.13.

Table 1.13 Clinical data on 79 patients with oral Crohn's disease (Plauth *et al.*, 1991).

M:F	50:27 (1.85:1)
<16 years	18:12 (1.50:1)
16-30 years	15:5 (3.00:1)
>30 years	16:10 (1.60:1)
Age at presentation of oral lesions (years)	(%)
<16	30/76 (39)
16-30	20/76 (26)
>30	26/76 (34)
Median (and range)	22 (6-57)
Mean $\pm$ SD	4.4 $\pm$ 12.9

For 2/79 cases, data on sex, and, for 3/79 cases, data on age at presentation were not available

1.3.3 Orofacial Granulomatosis

OFG is assuming increasing importance in the world literature - immunological, dermatological and dental. The epidemiology is not well established with isolated case reports being the order of the day. However, a number of UK centres have published data on groups of patients (Patton *et al.,* 1985; Sweatman *et al.,* 1986; Field and Tyldesley, 1989; Sainsbury *et al.,* 1987) and there is growing evidence of increased reporting world-wide. For example, two cases in Nigeria (Odukoya, 1994), two cases in Italy (Rubino and Ficarra, 1994) and six cases in India (Dhar and Kanwar, 1995).

Sainsbury *et al* (1987) reported their series of patients in South Wales and noted that half of the sixteen cases occurred in children under the age of 15 years. Of eight patients, six were male and two were female with a mean age of 8.5 years (range 4-14 years). Four of the children came from Social Class I, the parents of three children being in the medical profession (Sainsbury *et al.,* 1987).

In the West of Scotland study by Wiesenfeld *et al* (1985) 60 patients were examined and an equal gender distribution was noted. The median age at presentation was 20 years (range 3-61 years). The mean time interval between onset of symptoms and presentation was two years (range one month to eight years). Fifty-nine patients were white Caucasians and one was of Indian origin.

Armstrong and Burrows (1995) in reviewing the literature state that the onset of OFG is highly variable with the median in most series in the second and third decades; the gender distribution was approximately equal.

1.3.4 Melkersson Rosenthal Syndrome

MRS generally appears in the second to fourth decades of life but a wide age range at onset of symptoms has been reported (2 to 81 years) (Zimmer *et al.,* 1992; Minor *et al.,* 1987; Grosshans and Pfeffer, 1991). An overall female preponderance has been reported in some studies - particularly in Spain (Amézaga *et al.,* 1991; Hernandez *et al.,* 1987; Seasone *et al.,* 1990). A most extensive recent study (Zimmer *et al.,* 1992) supported a slightly increased prevalence among females. However, MRS is commoner in females during the first, second and sixth decades of life, and it usually occurs in males in the other decades (Hornstein, 1973).

MRS would appear to be without racial preference (Worsaae *et al.,* 1982; Grosshans and Pfeffer, 1991; Levenson *et al.,* 1984; Seasone *et al.,* 1990) although most cases have been reported in northern Europe from a white Caucasian population(Minor *et al.,* 1987; Meisel-Stosiek *et al.,* 1990; Patton *et al.,* 1985). This may simply represent a reporting bias or a lack of uniform criteria for diagnosing MRS.

A report from Germany calculated the incidence of MRS to be 1:2100 cases referred to a dermatology clinic (Hornstein, 1973). Muller (1952) followed 209 cases of facial palsy for 15 years and found 29 patients with symptoms of recurrent facial palsy. Four of these 29 patients had facial oedema and one patient had lingua plicata.

In one study, MRS had a median duration of 6.5 years with a range of 6 months to 31 years (Worsaae *et al.,* 1982).

1.3.5 Cheilitis Granulomatosa of Miescher

Due to the diagnostic confusion over MRS, CG and OFG, the world literature reveals very little direct information on the epidemiology of this condition. In a North American study looking solely at CG, the median age of onset was 28.5 years, with an equal gender ratio (Allen *et al.*, 1990).

1.3.6 GLUS syndrome

The epidemiology of GLUS syndrome is a little difficult to pursue. Since routine biopsies are often obtained from liver, bone marrow, lung and lymph nodes, it is not surprising that the occurrence of granulomata of various aetiologies has been reported in these sites. Less commonly studied sites include the spleen, nasal and gastric mucosa, and connective tissue. In many cases, granulomata can be accounted for by evidence of granulomatous disease elsewhere in a patient with a known granulomatous condition. However, in almost all studies, there is a residual group of cases in which the presence of granulomata remains unexplained. It should be remembered, however, that biopsies are not generally taken from any tissue unless the patient has symptoms or signs indicating disease involvement. Thus, any figure on the incidence of granulomatous lesions in any body site will be imprecise and based on highly selected biopsy material.

With that in mind, an indication of the observed frequency of GLUS in various sites was summarised by Brincker (1994). In liver, bone marrow and lung, where more than 300 cases of granulomata have been reported at each site, the overall percentages of GLUS were 14%, 19%, and 21% respectively. Thus overall, from 15 to 20% of histologically verified granulomatous lesions may be characterised as GLUS for these three sites (Brincker, 1994). The incidence of GLUS syndrome in mucosa is unknown since so few series have been reported.

Thus, the epidemiology of GLUS syndrome in patients with granulomatous inflammation of mucosa is largely under-researched and poorly documented in the scientific literature.

1.4 The histology of chronic granulomatous disorders

1.4.1 Sarcoidosis

The characteristic feature histologically in sarcoidosis is the presence in affected tissues of non-caseating epithelioid granulomata (Hagerstrand and Linell, 1964). In the early stages, the granulomata consist of focal, close-packed collections of macrophages and epithelioid cells which often fuse to form multi-nucleate Langhans' type giant cells. A peripheral ring of lymphocytes is commonly seen around the granuloma and a few lymphocytes may be present in the central portion (Thomas and Hunninghake, 1987). Monoclonal antibody studies show that B lymphocytes are present in small numbers; CD4 helper cells predominate over CD8 suppressor cells. CD4 helper cells and activated macrophages penetrate to the centre of the granuloma where the latter coalesce into epithelioid and multinucleate giant cells. In the peripheral mantle, CD8 cells lie adjacent to numerous antigen-presenting macrophages (Hagerstrand and Linell, 1964). Central fibrinoid necrosis may occur in florid granulomatous reactions but true caseation is never seen, a finding that differentiates sarcoidosis from tuberculosis (Hagerstrand and Linell, 1964).

Cytoplasmic inclusions are not infrequently seen within the cells of the granulomata, particularly the multi-nucleated giant cells (Hagerstrand and Linell, 1964). Three types of inclusion bodies are described: crystalline, conchoidal and asteroid. Crystalline inclusions are composed of calcium carbonate and are birefringent to polarised light. Conchoidal (Schaumann's) bodies are densely basophilic, stain with haematoxylin, and are probably formed when lipoglycoproteins and amorphous calcium and iron salts become deposited around a small birefringent crystalline focus. Conchoidal and crystalline bodies are more commonly identified in the granulomata of sarcoidosis than in other granulomatous disorders, but they are not diagnostic. Star-shaped asteroid bodies are composed of lipoprotein, occur within giant cells, and are present in many granulomatous diseases (Thomas and Hunninghake, 1987).

When the disease remits, either spontaneously or with corticosteroid therapy, the granulomata disperse and the mononuclear infiltrate settles. The granulomata are capable of complete resolution but those that remain are usually slowly replaced with

featureless hyaline scar tissue. Granulomata resolve by dispersion of cells or by centripetal proliferation of fibroblasts from the periphery of the granuloma inwards to form a scar which may either disappear or result in fibrosis with permanent tissue damage (Siltzbach *et al.,* 1974).

1.4.2 Crohn's disease

Microscopic assessment of tissue from the gastrointestinal tract reveals transmural inflammation and ulceration. Non-caseating granulomata, once thought to be pathognomonic, are seen in only 10-40% of cases overall (Brinberg and Berkeley, 1989) and in two-thirds of resected specimens and a smaller proportion of mucosal biopsies (Thompson, 1990). The granulomata vary in appearance, ranging from rather loose collections of epithelioid macrophages through sarcoid-like densely cellular aggregates to larger tuberculoid granulomata with Langhans' type multinucleate giant cells. Small foci of central necrosis and occasional clusters of neutrophils or eosinophils may be present but areas of caseation are not seen (Brinberg and Berkeley, 1989).

The submucosa is usually oedematous and contains dilated lymphatics and blood vessels. Fibrosis, which may be present throughout the bowel wall, is usually maximal in the submucosa. The regional lymph nodes in Crohn's disease show granulomata in 25-50% of cases (Cook, 1972); those without granulomata show non-specific changes such as follicular hyperplasia or sinus dilatation. Nodal granulomata without intestinal granulomata are almost never seen in Crohn's disease.

There is now some evidence that Crohn's disease is a heterogeneous condition and that subdividing it on histopathological grounds may help with determining prognosis, requirements for surgery and likely response to anti-mycobacterial chemotherapy (Prantera *et al.*, 1991).

1.4.3 Orofacial granulomatosis

Wiesenfeld *et al* (1985) obtained 58 mucosal biopsies from their series of 60 patients. In only one case was no abnormality detected but this was clinician error. The remaining 57 cases showed a range of histological features. The most frequent change was oedema of the superficial corium with prominent dilated lymphatic vessels. In 47 biopsies granulomata were recorded and these varied considerably in appearance, number and location within the tissue. Most granulomata were small and ill-defined consisting of epithelioid histiocytes and lymphocytes. Multinucleate giant cells were seen in many granulomata but were not always present - this being particularly the case in the more superficial lesions in the lamina propria. In only one case was central necrosis seen in the granulomata but special stains failed to reveal any acid/alcohol fast bacilli in this or indeed in any other case in the series.

The morphology of the granulomata was variable ranging from follicular with multinucleate giant cells to more loosely formed types, comprised of epithelioid cells, macrophages and lymphocytes. Occasional birefringent intracellular foreign material was noted in some granulomata. The numbers of granulomata varied from specimen to specimen and in some cases were only seen on examining multiple sections. The location of granulomata varied from the superficial lamina propria, throughout the corium to small numbers of granulomata in the minor salivary glands and the striated muscle. These granulomata are histologically similar to those found in Crohn's disease and systemic sarcoidosis (James, 1991a).

Sainsbury *et al* (1987) reported the histological features in OFG affecting the pulp of a deciduous tooth. They make the assertion that the intensity of the lymphocytic infiltrate and foreign body giant cell reaction present in the biopsies from patients with OFG would point to a possible allergic reaction to a dietary or topical allergen (Sainsbury *et al.*, 1987).

The granulomata are identified in 46-81% of oral mucosal biopsies and vary in size, number and location; they are normally non-caseating epithelioid type, small and ill-defined, and may be sparsely distributed (Armstrong and Burrows, 1995).

1.4.4 Melkersson-Rosenthal Syndrome

It should be emphasised at the outset that although granulomatous inflammation is considered a typical histological finding in MRS, it is not required to establish a diagnosis since MRS is a clinical syndrome (Zimmer *et al.,* 1992). However, on the basis of histological findings, Bazex and Dupre (1957) subdivided MRS into two types - the sarcoid type and the lymphoedematous type. The *sarcoid* type is characterised by granulomata with chronic inflammation of varying degrees; Langhans' giant cells are found perivascularly and are surrounded by a connective tissue capsule. The *lymphoedematous* type is characterised by marked oedema and cellular infiltration. Oedematous fibrous connective tissue may replace the muscle fibres in the longer term and thus the term idiopathic fibroedema was suggested by Stevens (1954).

Similarly, the granulomata were described by Hornstein (1973) as two distinct types. First, *tuberculoid-type* granulomata appear as tiny epithelioid cell granulomata surrounded by lymphocytes and other mononuclear cells with a diffuse oedema of the interstitial connective tissue. Second, *lymphonodular-plasmocytic-type* granulomata appear as central lymphocytic nodules surrounded by plasma cells and histiocytes in an oedematous connective tissue. These two types of granulomata were found in 67% of Hornstein's biopsy specimens and non-specific inflammation in 33%.

In a Danish study of 30 patients with MRS, gingival biopsy specimens were found to have non-caseating epithelioid granulomata, dominated by lymphocytes with a varying number of plasma cells and epithelioid cells interspersed in an oedematous connective tissue with several dilated vessels (Worsaae and Pindborg, 1980). In addition, peri-vascular aggregations of lymphocytes, plasma cells and histiocytes were found - thought by the authors to represent early granuloma formation. Multinucleated giant cells of the Langhans' type were seen occasionally, but no birefringent material was observed in any of the specimens.

In a sizeable and up-to-date study of 42 patients with MRS (Zimmer *et al.,* 1992), granulomatous inflammation was noted in 46% of lip biopsy specimens; 36% showed non-specific inflammation; 11% showed incidental findings (such as solar elastosis); 7% were histologically normal. Zimmer *et al* (1992) noted that some patients had obvious

clinical MRS although biopsy specimens showed non-specific histological features, whereas other patients showed only mild symptoms although the histology revealed typical granulomatous inflammation. Zimmer and his colleagues then go on to say "it is reasonable to state that the histologic picture of typical tuberculoid granulomata confirms the clinical diagnosis even when the clinical picture is *monosymptomatic*. Negative histologic findings in the presence of typical clinical symptoms, on the other hand, do not refute the diagnosis". They base this supposition on the basis that granulomata may form and vanish within days to weeks and do not strictly coincide with the clinical course of swelling (Zimmer *et al.*, 1992; Hornstein, 1973). However, this fails to recognise that MRS is a purely *clinical* syndrome - a triad of clinical entities. This further lack of diagnostic stringency may have set back the emergence of a new clinical entity such as orofacial lymphoedema by some years.

1.4.5 Cheilitis granulomatosa of Miescher

In a North American study of six patients concerned solely with CG, all patients histologically showed non-caseating granulomatous inflammation in the submucosal connective tissue of the lip (Allen *et al.,* 1990). Special stains for mycobacterial and fungal organisms were negative in each case. In one case, the granulomata were exceedingly sparse and were identified only after examining multiple sections. In a recent study of six Greek patients with CG, all patients had lip biopsies performed (Kolokotronis *et al.,* 1997). One biopsy showed non-specific chronic inflammation with hyperplasia of the overlying squamous epithelium. In all other cases, the formation of non-caseating granulomata, consisting in some areas solely of epithelioid giant cells and in others of epithelioid cells and Langhans' giant cells, were observed.

1.4.6 GLUS syndrome

Histopathologically, granulomata in the GLUS syndrome have been found to be remarkably uniform when found in the liver (Mir-Madjlessi *et al.,* 1973). However, some series of hepatic granulomata include both caseating and non-caseating granulomata (Guckian and Perry, 1966), whilst others have excluded cases with caseating necrosis on the assumption that these were tuberculous (Mir-Madjlessi *et al.,* 1973).

Using immunohistochemical methods, it has been demonstrated that granulomatous lesions occurring in lymph nodes can be divided into two different families according to the presence or absence of B lymphocytes in the granulomata (Brincker and Pedersen, 1991). Old, chronic granulomata associated with a high rate of fibrosis and a high rate of transformation of macrophages into giant cells are B-cell negative; this family includes sarcoidosis and mycobacterial infections. The B-cell positive family of young, inflammatory granulomata include GLUS, tumour-related sarcoid lesions, toxoplasmosis, and Crohn's disease. Thus, sarcoid granulomata are rather loose and less densely cellular whereas GLUS and Crohn's granulomata are tightly packed and densely cellular.

Among the various diseases studied by Brincker and Pedersen (1991), only the granulomata of mycobacterial infections and GLUS demonstrated significant degrees of necrosis. It was also demonstrated that the granulomata of GLUS contained NK cells in addition to B lymphocytes, whereas sarcoid granulomata did not (Brincker and Pedersen, 1989).

1.5 Aetiological factors in chronic granulomatous disorders

1.5.1 Sarcoidosis

The occasional occurrence of familial sarcoidosis, predominantly in the Irish and West Indian populations, has suggested possible genetic influences. Sharma *et al* (1976) reported 16 families in whom 33 persons had sarcoidosis and demonstrated it was commoner in monozygotic than dizygotic twins. However, sarcoidosis in spouses is not unique, suggesting a common environmental basis. Sarcoid arthritis and erythema nodosum are most likely to occur in patients who are HLA-B8, A1, CW7, and DR3 positive, whereas chronic disease is commoner in HLA-B13 type.

In 1961, Mankiewicz reported that bacteriophages, lytic for mycobacteria, could be isolated with great frequency from stool and resection specimens from patients with tuberculosis and sarcoidosis, whereas patients with other diseases were seldom found to harbour mycobacteriophages. Chapman and Speight (1964) reported the high incidence of serum anti-mycobacterial antibodies in sarcoidosis patients.

More recently, the polymerase chain reaction (PCR) has been used to detect mycobacterial DNA in clinical samples from patients with sarcoidosis (Saboor *et al.,* 1992). Broncho-alveolar lavage samples, bronchial washings and tissue specimens were assayed by PCR to detect DNA from *M. tuberculosis* and other mycobacteria. *M. tuberculosis* DNA was found in half the sarcoidosis patients and non-tuberculous DNA in 70% of sarcoidosis patients. However, these results are fiercely contested by Scottish (Thakker *et al.,* 1992) and French (Bocart *et al.,* 1992) investigators. The Glasgow group failed to detect mycobacterial DNA in sarcoid lymph nodes (Thakker *et al.,* 1992). The French workers rarely found DNA from *M. tuberculosis* in sarcoid tissue (Bocart *et al.,* 1992). The Danish group also discounted the role of *M. paratuberculosis* in sarcoidosis by enzymatic gene amplification techniques (Lisby *et al.,* 1993).

Other suspected causal agents have been put forward but without convincing evidence (James, 1991b). These are shown in Table 1.14.

Table 1.14 Proposed aetiological factors in sarcoidosis (after James, 1991).

Class of Aetiological Factor	**Type of Aetiological Factor**
Bacterial	*Mycobacteria*
	Streptococcal cell wall
	Propionibacterium acnes
	Borrelia burgdorferi
	Mycoplasma
	Nocardia
Viral	EBV (including Herpes group, CMV)
	Rubella
	Measles
	Coxsackie B
	Retrovirus
Chemicals	Beryllium
	Zirconium
	Pine pollen
	Peanut dust
	Clay eating

There is considerable evidence accumulating that some antigen (or antigens), as yet unidentified, induces a cell-mediated immune response involving a macrophage-CD4 cellular axis, perpetuated by a cascade of cytokine production progressing to granuloma formation (James, 1991b). The inciting antigens, as the above list might suggest, may be diverse exogenous stimuli with differing regional localisation; and indeed an internal auto-antigen clearly cannot yet be excluded. Thus the co-factor may be *Borrelia burgdorferi* (Bing *et al.,* 1992) in China and clay in the USA (Comstock *et al.,* 1961). Regardless, the granulomata may be the result of an antigen-driven process together with an exaggerated cell-mediated immune response. Herein may lie a unifying hypothesis for the range of granulomatous disorders.

Kiely and Rees (1994) make the suggestion that Crohn's disease and sarcoidosis are part of the same disease spectrum, triggered by the same (possibly mycobacterial) pathogen in immunogenetically similar subjects, disease expression being dependent on the route of entry of the triggering pathogen. They base this supposition on a 42-year-old female Sri Lankan patient who presented initially with Crohn's disease of the small bowel and developed renal and multi-system sarcoidosis 9 years later. The absence of the usual pulmonary manifestations of sarcoidosis, and profound gut symptoms and signs, at initial presentation made Crohn's disease the most likely diagnosis; the subsequent recurrence of symptoms with additional granulomatous renal impairment and hypercalcaemia represented a shift "along the disease spectrum" towards sarcoidosis. This, they postulate, may have followed reactivation of gut infection (possibly related to the withdrawal of sulphasalazine), or alternatively reinfection via the lungs (Kiely and Rees, 1994).

Oakley *et al* (1983) described a further association between sarcoidosis and Crohn's disease. A 32-year-old Caucasian female patient presented initially with Crohn's disease of the ileum which was biopsy-proven. One year later, she developed sarcoidosis with bilateral hilar lymphadenopathy on chest radiograph and a positive Kveim test. Her sarcoidosis gradually resolved but she presented 3.5 years later with Crohn's disease affecting the mouth and oesophagus, but without active gut involvement.

1.5.2 Crohn's disease

Crohn's disease is an idiopathic inflammation of the gastrointestinal tract anywhere from mouth to anus, but predominantly ileocaecal. There are currently three main theories for the causation of Crohn's disease :

- *Mycobacterium paratuberculosis* infection
- Measles virus-induced vasculitis causing mucosal ischaemia
- Local immune-mediated response to an unidentified allergen (e.g. normal flora, food substances) - the so-called mucosal immunological dysregulation.

There are also other theories, such as food hypersensitivity or a reaction to toothpaste or cornflakes but the evidence is scant (Sullivan, 1990). Interestingly, a recent large study (Wurzelmann *et al.,* 1994) has indicated that persons with Crohn's disease in adulthood were more likely to report an increased frequency of childhood infections in general and pharyngitis specifically. The same study (Wurzelmann *et al.,* 1994) noted that urban living in childhood also increased the risk for Crohn's disease.

1.5.2.1 *Mycobacterium paratuberculosis* infection

Following his original description of the disease in 1932, in which Crohn addressed the histopathological similarities between the eponymous disease and intestinal tuberculosis (Crohn *et al.,* 1932), interest in the putative mycobacterial origin of the disease was rekindled in 1984 by the isolation of an organism by Chiodini, later identified as a slow-growing *Mycobacterium paratuberculosis*, from two North American children with Crohn's disease (Chiodini *et al.,* 1984c). Chiodini subsequently gave the bacteria to 4 infant goats by the oral route and all the goats acquired intestinal lesions resembling those of Johne's disease (Van Kruiningen *et al.,* 1985). Johne's disease is considered by some authors to be the animal equivalent of Crohn's disease and was described by Johne and Frothingham in 1895. *Mycobacterium johnei* (subsequently renamed *M. paratuberculosis*) was isolated from affected animals and was deemed the infectious cause of this condition (Morgan, 1987).

The similarities between Johne's disease and Crohn's disease have long been noted. In

1913, Sir Thomas Kennedy Dalziel, a surgeon at Glasgow's Western Infirmary, made the connection when describing the pathological features in nine patients whose clinical features would now be recognised as Crohn's disease (Dalziel, 1913). That he should draw this conclusion was doubtless due to the fact that his knowledge of cattle farming rivalled that of surgery. In 1906 he had purchased the estate of Nether Kinnedar, near Dunfermline, and bred his own herd of shorthorn cattle there (Hampson and MacFadden, 1987). Granulomatous enteritis due to AMB infection has recently been reported in a pig (Sigurdardóttir *et al.,* 1994) and *Mycobacterium paratuberculosis* infection in a colony of stumptail macaques (McClure *et al.,* 1987) - the latter extending the natural host range of *Mycobacterium paratuberculosis* to include non-human primates, adding support to current suggestions that *Mycobacterium paratuberculosis* may be pathogenic for humans.

M. paratuberculosis is part of the atypical mycobacterial (AMB) group. According to the Runyon Classification, AMB are divided into four groups (Akhtar *et al.,* 1997) as shown in Table 1.15.

Table 1.15 The Runyon Classification of atypical mycobacteria.

CLASSIFICATION OF AMB ACCORDING TO RUNYON
I Photochromogens: M. kansasii, M. marinum
(Do not produce pigment in the dark; become bright yellow on exposure to light)
II Scotochromogens: M. scrofulaceum
(Produce bright orange pigment in dark as well as light)
III Non-photochromogens: M. avium intracellulare complex
(Do not produce pigment in dark or light)
IV Rapid growers: M. chelonei, M. fortuitum
(Visible growth within several days)

In 1984, unidentified mycobacteria were isolated from 3/14 patients with CD but none of 6 patients with ulcerative colitis and other disease controls. The organism was fastidious, mycobactin dependent and required at least 18 months for growth in primary culture (Chiodini *et al.,* 1984a). The organisms were postulated to be a sub-species of the *Mycobacterium avium-intracellulare* (MAI) complex - most likely *M. paratuberculosis.* In a follow-up paper later in the same year (Chiodini *et al.,* 1984b), the organisms were identified as belonging to Runyon group III and did not conform to any already recognised mycobacterial species but were most closely related to *M. paratuberculosis.* Inoculation of the organisms revealed a pathogenicity for mice and goats but not rats, chickens or guinea pigs (Chiodini *et al.,* 1984b). Thorel (1989) stated his frustration for current bacteriological methods, believing them sub-optimal for primary isolation of putative Crohn's disease mycobacteria. However, he considered the cultural and biochemical characteristics so akin to the mycobacteria of Johne's disease that he was confident to name the causative bacterium of Crohn's disease in humans as *Mycobacterium paratuberculosis* (Thorel, 1989).

With the advent of molecular biological techniques, samples could be studied at the DNA level and the confirmation came that Chiodini's isolates were in fact *M. paratuberculosis* (Green *et al.,* 1989). The molecular biology of *Mycobacterium*

paratuberculosis continued to be investigated and breakthrough came with the discovery of the complete nucleotide sequence of a unique insertion element IS 900 (Green *et al.*, 1989). This paved the way for the application of the newly established polymerase chain reaction (PCR) for the detection of *Mycobacterium paratuberculosis* in CD tissue samples. The PCR is a way of amplifying or making multiple copies of any desired piece of nucleic acid (Tyrrell, 1997). Thus it would appear ideal for identifying *Mycobacterium paratuberculosis* which may be in tissue samples in small numbers.

The first signs of progress in the molecular biological search for *Mycobacterium paratuberculosis* came with the paper by Vary *et al* (1990). They used DNA sequences from IS900 to prepare DNA primers for detection and identification of *Mycobacterium paratuberculosis* by PCR. Highly specific direct detection of *Mycobacterium paratuberculosis* DNA in faeces from cattle with Johne's disease was obtained - taking only hours compared with 6 to 12 weeks for culture of the organism (Vary *et al.*, 1990).

Then in 1992 came a perceived breakthrough with the identification of *Mycobacterium paratuberculosis* DNA in intestinal lesional tissue from human subjects with Crohn's disease (Sanderson *et al.*, 1992). This came from the laboratory of Hermon-Taylor - a prominent name in the scientific search for mycobacteria in Crohn's disease. These workers used PCR technology based on the 5' region of IS900 and capable of the specific detection of a single *Mycobacterium paratuberculosis* genome. This was applied to DNA extracts of fresh (unfixed) full thickness samples of intestine removed at surgery from 40 patients with CD, 23 patients with UC, and 40 control patients without inflammatory bowel disease. Stringent precautions were taken to exclude contamination artefact. *Mycobacterium paratuberculosis* DNA was detected in 26/40 (65%) CD, 1/23 (4.3%) UC, and 5/40 (12.5%) control tissues. All PCR internal control reactions were negative. These findings, the authors contended with much excitement, were consistent with an aetiological role for *Mycobacterium paratuberculosis* in Crohn's disease (Sanderson *et al.*, 1992).

Close on the heels of this study came reports from another centre, using DNA probes, to identify the Wood Pigeon strain of *Mycobacterium avium* and *Mycobacterium paratuberculosis* from fresh (unfixed) human intestinal tissue (McFadden *et al.*, 1992). Although the results were from a small group of 4 patients, they added further independent support to Hermon-Taylor's work. Hermon-Taylor pleaded with the

scientific community to move on from "static bewilderment" (Sanderson and Hermon-Taylor, 1992). And move on they did.

Fidler *et al* (1994) used IS900 PCR technology in a double-blind control study on paraffin-embedded tissue. Four of 31 Crohn's disease tissues and none of the 30 control and UC derived tissues amplified *Mycobacterium paratuberculosis* DNA. Crohn's disease tissues containing granulomata were significantly more likely to amplify *Mycobacterium paratuberculosis*-specific DNA than tissues without granulomata (Fidler *et al.,* 1994).

Berche's group in Paris used PCR to detect the presence of IS900 DNA sequences specific to *Mycobacterium paratuberculosis* genomes in biopsies and surgical resections (fresh, frozen tissue) from 53 children with various gastrointestinal diseases and disorders (Dell'Isola *et al.,* 1994). IS900 sequences were found in13/18 samples from patients with Crohn's disease (72%; $p<0.01$ versus samples from patients without CD), in 1/5 with UC, in 2/6 with severe unclassified colitis, and in 7/24 with other gastrointestinal disorders. This generated further evidence to support the hypothesis that *Mycobacterium paratuberculosis* is involved in the pathogenesis of CD.

Lisby *et al* (1994) in Denmark used a more sensitive variant of PCR technology - nested primer PCR - on fresh lesional tissue and found *Mycobacterium paratuberculosis* DNA in 11/24 patients with CD, in 2/10 patients with UC, and in 3/28 patients with other colonic disorders. Treatment before surgery with prednisolone did not affect detection levels. DNA extracted from paraffin-embedded intestinal tissue was also analysed and 4/58 patients with Crohn's disease (and no control patients) produced a specific signal at the expected size. The authors conclude that the lower occurrence of *Mycobacterium paratuberculosis* DNA in paraffin-embedded intestinal resections from patients with Crohn's disease may be explained by a general loss of DNA during the extensive extraction procedure applied to paraffin-embedded tissue (Lisby *et al.,* 1994). The authors further postulate the following possibilities from their results :

1 Crohn's disease is directly caused by a toxic property of *Mycobacterium paratuberculosis* and, since direct microscopy carried out in several studies has failed to reveal mycobacteria in lesional tissue, very small numbers of bacteria are required to exert this toxic effect;

2 *Mycobacterium paratuberculosis* is only present in a sub-population of CD patients and causes an immune response in such patients. This is analogous to the pathologic findings in tuberculoid-type leprosy;

3 *Mycobacterium paratuberculosis* is present initially in all patients developing CD but may be cleared in some patients, although the immunological reaction persists and perpetuates the disease;

4 The presence of *Mycobacterium paratuberculosis* DNA in CD patients could be a mere coincidence, not reflecting any aetiological relationship to CD; to which could be added,

5 The current PCR techniques are not yet sufficiently sensitive to identify *Mycobacterium paratuberculosis* DNA in all tissue specimens, or this could be a fault of specimen handling.

The source of *Mycobacterium paratuberculosis* in the human food chain is believed by some authors to be cows' milk, and that current pasteurisation regimes allow the bacterium to pass through unaffected (Millar *et al.,* 1996). Shedding of *Mycobacterium paratuberculosis* occurs in the milk of asymptomatic infected cows but, apparently, less frequently than in symptomatic cows (Sweeney *et al.,* 1992).

Van Kruiningen *et al* (1993) described the clustering of Crohn's disease in two families in northern France. In the first family, the father, mother, and all children (3 sons and 1 daughter) developed Crohn's disease. One of the sons who developed Crohn's disease in 1974 met a girl 3 years later whom he subsequently married in 1983. She developed Crohn's disease in 1991. In the second family, neither the mother nor the father had Crohn's disease; however, four of the five sons and three of the six daughters developed Crohn's disease. These events represent the most concentrated clustering of CD ever reported and suggests a role for an infectious agent (Hermon-Taylor, 1993).

Comes *et al* (1994) reported 10 pairs of husband-wife couples with Crohn's disease in the Nord Pas de Calais region of France and in Liege county of Belgium. In 9/10 couples, neither spouse had symptoms before marriage but Crohn's disease subsequently developed in both. In the final couple, one spouse had CD before marriage and the other

partner experienced symptoms afterwards. This clustering adds further weight to a transmissible agent in Crohn's disease.

Some studies would appear to support the role of *Mycobacterium paratuberculosis* in the aetiology of CD by the response to anti-mycobacterial chemotherapy. Prantera *et al* (1989) reported the use of dapsone at a dose of 100mg daily in 5 patients with Crohn's ileocolitis. The therapy was effective in 2/5 patients - one patient showing clinical improvement and the other showing complete healing of all cutaneous and rectal ulcers. In the two responders, antibody levels to a soluble extract of *Mycobacterium paratuberculosis* were significantly higher than in the other three patients; moreover, in the first patient there was a rise of 39% in antibody titres following treatment. Such a rise, as may occur following the death of a pathogen with release of antigen, is similar to that observed after treatment of tuberculosis.

Other studies are somewhat scathing of the role of anti-mycobacterial chemotherapy in Crohn's disease and demand further stringently controlled trials (Pallone *et al.,* 1992). One such study (Swift *et al.,* 1994) ran a double blind randomised controlled trial with rifampicin, isoniazid, and ethambutol or placebos with 126 patients over 2 years. The conclusion was that the active treatment group derived little tangible benefit from the trial treatment (Swift *et al.,* 1994). Another smaller study (Prantera *et al.,* 1994) of 40 patients randomised to treatment with rifampicin, ethambutol, clofazimine and dapsone, or placebo over 9 months, showed effective relief of symptoms and maintenance of remission in some patients. Substantial endoscopic or radiographic healing did not occur (Prantera *et al.,* 1994).

Recently, the concept of a mycobacterial susceptibility gene has gained credence (Levin *et al.,* 1995; de Groot *et al.,* 1995) and this might explain why, if an external pathogen is involved, not everyone exposed to the pathogen subsequently manifests the disease.

However, not all studies have agreed with the *Mycobacterium paratuberculosis* theory in the causation of Crohn's disease and the literature exemplifies some vociferous opponents (Wu *et al.,* 1991; Suenaga *et al.,* 1995; Morgante *et al.,* 1994). Other commentators accept the growing volume of evidence but state the need for further rigorous molecular biological and microbiological studies (Jones, 1994; Hawkey, 1994; Thompson, 1994).

1.5.2.2 Measles virus-induced vasculitis causing mucosal ischaemia

It has been recognised for many years that there are vascular changes in the mucosa and submucosa in active Crohn's disease, although it was assumed that these were secondary to the inflammation originating in the mucosa. However, extremely detailed studies by Wakefield *et al* (1989) have suggested that vascular injury and focal arteritis were early events, even occurring in the submucosa underlying apparently normal mucosa. In subsequent studies, it was shown that many of the granulomata were associated with vessels in the mucosa (Wakefield *et al.,* 1991), and that microthrombi and fibrin deposition was evident in small vessels in the lamina propria in apparently normal mucosa from patients with disease elsewhere (Sankey *et al.,* 1993).

However, blood flow is higher in the proximal bowel (Ottaway and Parrott, 1980) so why should a vasculitis affect the ileocaecum? Wakefield *et al* felt they had found the answer in 1993 when they demonstrated measles virus-like particles in a cell adhering to the vascular endothelium in Crohn's affected intestine. Hermon-Taylor *et al* (1995) contended that measles virus could not be responsible for Crohn's disease since the incidence of measles infection was continuing to fall while the incidence of Crohn's disease continued to rise throughout the UK. Thompson *et al* (1995) then suggested that it may be the measles vaccine itself which is causing the problem. They followed a cohort of 3545 people who had received live measles vaccine in 1964 and found that there was a significantly increased risk of developing Crohn's disease and ulcerative colitis, but not coeliac disease or peptic ulceration, in the vaccinated cohort compared with their unvaccinated partners.

Of further interest is the recent report from the Israel defence force soldiers (Katz *et al.,* 1996). It is reported that during an outbreak of measles in 1994, 20% of patients presented during their illness with severe oral ulceration, similar to that seen in Crohn's disease and easily distinguishable from Koplik's spots. The authors suggest this may be a further hint of an association between measles virus and Crohn's disease (Katz *et al.,* 1996). However, this attempt to diagnose a systemic disease from transient oral manifestations defies scientific scrutiny.

More recently, there have been vociferous cries in the literature that the original link between Measles virus and inflammatory bowel disease was entirely artefactual (Fisher

et al., 1997; Metcalf, 1998). Furthermore, it has been clearly stated that there is a great need for rigorous methodological reviews when causal associations are proposed (Metcalf, 1998).

1.5.2.3 Immunological hypersensitivity disease

This notion is not incompatible with the a distinct infectious aetiological agent, such as that considered above, since the local immune reaction has to be driven by antigen(s), which may be of microbiological, dietary, or endogenous origin.

The intestinal immune system is only a few micrometers away from the lumen of the gut and increased permeability, either genetic or as a result of pathogenic influences, might allow ingress of antigen(s) and initiate chronic mucosal inflammation. There is good evidence that patients with CD have increased intestinal permeability as do their relatives (Hollander *et al.,* 1986), thereby providing a mechanism whereby immunological sensitisation to lumenal antigens could occur (May *et al.,* 1993).

There is no doubt that the tissue damage and mucosal ulceration in CD are due to immunological hyperactivity and every branch of the immune system is activated (Brandtzaeg, 1991). Examination of normal areas of the intestine, distant from ulcers, in sufferers of CD shows focal accumulations of mononuclear cells (T cells and macrophages) in the lamina propria (Rickert and Carter, 1980). When these underlie epithelium there may be granuloma formation and disruption of the epithelium (Rappaport *et al.,* 1951). These focal accumulations are presumably in response to antigen in the lamina propria being processed and presented by dendritic cells to lamina propria CD4+ T-cells. These then release cytokines which upregulate endothelial adhesion molecules in the adjacent vessels (Dogan *et al.,* 1993) and monocytes and neutrophils move into the mucosa from the blood. Granuloma formation requires persistent antigen and therefore antigens must be constantly crossing the epithelium, or be persistent in the lamina propria.

Diseased mucosa contains large numbers of activated T-cells and macrophages (Schreiber *et al.,* 1992), together with large numbers of IgG plasma cells (Kett *et al.,* 1987); there is extensive local complement activation (Haltensen *et al.,* 1992) and non-

specific effector cells such as mast cells, eosinophils and neutrophils are abundant and functionally active (Oshitani *et al.,* 1993).

Pathogens such as *Yersinia enterocolitica* and *Mycobacterium paratuberculosis* in animal models clearly enter the mucosa from the lumen and cause transmural intestinal inflammation, setting up chronic infection with antigen persistence and the result of this is a Crohn's-like mucosa. Repeated feeding of enterotoxin-secreting staphylococci to dogs also produces a terminal ileitis (Prohoska, 1963). Therefore, it is clear that transmural inflammation can result from antigen persisting in the mucosa. It has been demonstrated recently that an ongoing T-cell-mediated immune response is functionally present in the mucosa of CD, but not UC, by quantitative PCR and functional lymphokine analysis (Mullin *et al.,* 1992; Breese *et al.,* 1993). This observation that activated T-cells are not seen in the mucosa in UC shows that T-cell activation in CD is not a non-specific secondary effect due to increased antigen uptake across a damaged epithelium, but a primary event. However, until the specificity of these T-cells is identified, the stimulus for the local T-cell hypersensitivity will remain unknown. That antigenic specificity is likely to come from the faecal stream. Recent work has shown that after resection of diseased bowel, there is no disease in the neoterminal ileum if the segment is bypassed. After reconstruction to the faecal stream, aphthous-like ulceration and inflammatory changes develop (Rutgeerts *et al.,* 1991).

1.5.3 Orofacial granulomatosis

The aetiology of OFG, in the absence of Crohn's disease or sarcoidosis, is largely unknown. However, in view of the obvious confusion in the literature over nomenclature and diagnosis of the various clinical entities, orofacial granulomatosis, Melkersson-Rosenthal syndrome and cheilitis granulomatosa of Miescher will be considered here together under the heading of orofacial granulomatosis.

Allergic, infectious and hereditary causes have been proposed (James *et al.,* 1986; Pachor *et al.,* 1989; Pachor *et al.,* 1989; Lygidakis *et al.,* 1979). Elimination diets to diagnose food intolerance and allergy have been used with some success, suggesting an immunological mechanism (Hernandez *et al.,* 1986; Pachor *et al.,* 1989). However, the great criticism of such studies is that they generally lack definitive control groups. Morales *et al* (1995) subjected 6 patients with MRS to extensive skin prick tests and patch tests under the Standard European Series with uniformly negative results. When asymptomatic, the patients were subjected to double-blind oral challenge, under placebo control, with various food additives (monosodium glutamate, tartrazine, sulphites, erythrosine, paraoxybenzoate, sodium benzoate, lactose, aspirin and annate) with negative results. In no case did patients relate their condition to exposure to dietary or environmental agents and the course of the disease was unaffected by exclusion diets and the elimination of environmental agents. Interestingly, three patients had circulating immune complexes identified and two had elevated C-reactive protein (CRP) levels. The immune complexes were unspecified and the degree of elevation of CRP not recorded; neither finding is specific nor indicative of any disease process or abnormality.

Pachor *et al* (1989) reported a male patient with upper lip swelling, gingival hypertrophy and fissured tongue whom they labelled as having "MRS", despite no neurological abnormality. A lip biopsy did show granulomatous inflammation. Sodium benzoate and tartrazine were identified as precipitants of the swellings by direct oral challenge. Elimination of these substances from the patient's diet brought about a progressive improvement in his symptoms until complete resolution which lasted more than a year (at the time of the report).

An association between OFG and atopy was established by James *et al* (1986) when 60% of a group of 75 patients with OFG were found to be clinically atopic (having infantile

eczema, hay fever or extrinsic asthma) compared with 15% of a control group of 200 patients drawn from the same geographical area. Patton *et al* (1985) suggested an association between OFG and allergy to foodstuffs with 14 out of 80 patients with OFG reported having "intolerance to foods or flavourings", all but one of whom clinically were atopic. In five cases the history of a specific provoking factor was so clear that patch testing "was not deemed necessary". The chief "allergen" identified on patch testing was cinnamon or its principal constituent, cinnamonaldehyde. The authors concluded that OFG may be related to some form of cell-mediated allergic response, with the allergen being derived from food, toothpaste flavourings or micro-organisms.

The role of food additives in OFG was further endorsed by Sweatman *et al* (1986) who reported a female child with OFG and clinical atopy in whom a relapse of her condition was shown to be related to exposure to the food additives carmoisine, sunset yellow and monosodium glutamate (MSG). This was shown with double-blind diet provocation tests and the use of an elemental diet. Oliver *et al* (1991) again endorsed the role of MSG in OFG with a positive scratch test result to MSG in a 15-year old female with OFG who experienced significant clinical improvement following institution of a low-allergen exclusion diet.

Sakuntabhai *et al* (1993) reported nine patients (six males and three females; aged 10-47 years) with OFG. No evidence of an allergic cause was found using patch or contact urticaria tests and yet eating chocolate produced lip swelling in one man, and his lip shrank in size after avoiding this for 12 months.

Reed *et al* (1993) reported two cases of Australian patients who responded to elimination diet. A 37-year-old female Pakistani had "plasma cell gingivitis" and used a combination spice product containing cinnamon, cloves, cardamon, cumin seeds, coriander seeds and leaves, green and red chillies, ginger, and mint leaves. Eliminating this product from her diet produced marked decrease in gingival swelling and resolution of pain. A 47-year-old white Caucasian male presented with upper lip swelling which showed non-caseating granulomatous inflammation. His diet analysis revealed significant consumption of carbonated drinks and chewing gum. When these were eliminated from the diet, the lip swelling resolved. The authors assume the provoking allergen to be aspartame (Reed *et al.,* 1993), but these foods also contain cinnamon and

benzoic acid (McKenna *et al.*, 1994; Patton *et al.*, 1985). This patient's swollen lip returned 1 year later following lapses in his dietary avoidance regime.

The antipodean patient profile with OFG continued in 1993 with reports from New Zealand (Frisken, 1993). However, no histological confirmation is reported for the patients in this series. All were female (aged 19, 20 and 70 years); the first had upper and lower lip swelling and gingivitis; the second had lip swelling and mucosal tags; the third had profound upper lip swelling. The first patient identified dairy products as a precipitant to the swelling and her symptoms resolved rapidly and completely with dietary exclusion. The second patient could identify no dietary precipitants but her signs resolved completely after cessation of ACE -inhibitor drug therapy for hypertension. The third patient's problems resolved completely when she stopped rubbing sage, rosemary and parsley from her neighbour's garden onto her labial gingivae. The neighbour was using substantial quantities of pesticides in his garden (Frisken, 1993).

Pryce and King (1990) introduced non-dietary "allergens" into the arena by reporting OFG in an 8-year old male who had delayed hypersensitivity to cobalt (1% cobalt chloride) demonstrated on patch testing. He had a habit of sucking plastic pens and crayons and cobalt is present in polyester plastics as cobalt naphthenate, an accelerator; cobalt is also present in crayons as a dye.

More recently, Armstrong *et al* (1997) investigated 48 patients with OFG and subjected them to patch-testing to the European Standard Series and an "oral battery" of test substances. Ten patients showed positive skin reactions on patch-testing and, of these, 7 showed improvement on an elimination diet. Adequate control subjects were lacking in this study of an Irish population. The major problem of absence of controls is a prominent feature throughout the literature on OFG, particularly in studies claiming to investigate hypersensitivity reactions, and too much store has been put upon the volume of unsatisfactory case reports. The desire for controlled studies in food allergy generally has been expressed recently in the medical community (Sampson, 1997).

Henry (1994) presented a patient with OFG in whom the lymphocyte CD4/CD8 ratio was decreased. He suggested that this demonstrated evidence for involvement of the cellular immune system in the disease process. However, many diseases are known to

affect lymphocyte subpopulations, including sarcoidosis and Crohn's disease (Siegel, 1984; Romer *et al.,* 1984; Carney *et al.,* 1981).

Ivanyi *et al* (1993) introduced the possibility of an infective origin for OFG by building on the work of Elsaghier *et al* (1992) who had demonstrated the presence of elevated IgG antibody levels to mycobacterial stress protein (with a molecular weight of 65kDa) in 52% of patients with Crohn's disease. Stress proteins are produced in response to many forms of cellular stress, including viral and bacterial infections, cytokines and temperature change and have been implicated in the pathogenesis of autoimmune diseases and bacterial inflammation. IgG antibody titres to the mycobacterial stress protein (mSP65) were determined by ELISA in sera from 10 patients with OFG. Seven patients had titres ranging from 180-950, whilst no serum antibody to this antigen could be detected in 3 patients (Ivanyi *et al.,* 1993). Although the aetiology of gastrointestinal Crohn's disease remains largely unknown, the involvement of mycobacteria has been suspected for several years and much scientific evidence supports this view (Morgan, 1987; Hampson *et al.,* 1988; Prantera *et al.,* 1991).

A family study on MRS (73 patients) suggested a multifactorial origin to the syndrome, based on an hereditary predisposition and a genetic basis is postulated since several cases among related individuals are recorded (Meisel-Stosiek *et al.,* 1990). Some authors describe a high, but not significant, level of HLA B16 and CW3 antigens in cases thought to have a genetic basis (Stosiek *et al.,* 1992). An autosomal dominant inheritance with variable expression has been well documented (Carr,1966; Lygidakis *et al.,* 1979). Work by Smeets *et al* (1994) further categorised MRS as an autosomal dominant disorder with variable expression and suggested that the "Melkersson-Rosenthal gene" is located at 9p11.

Hornstein (1973) characterised MRS as a polyaetiological syndrome in which an hereditary or acquired disposition to a functional disturbance of the autonomic nervous system occurs, with a granulomatous reaction in the oedematous tissue resulting from an allergic response to different non-specific circulating antigens.

Recently, Lim *et al* have examined the lymphocytes from peripheral blood and lesional tissue in a 12-year-old boy with OFG to determine the T cell receptor (TCR) Vß gene

usage of the T cell infiltrate associated with the primary lesion (swollen right buccal mucosa) (Lim *et al.,* 1997). They used a molecular method involving reverse transcriptase (RT)-polymerase chain reaction (PCR), DNA cloning, single strand conformation polymorphism (SSCP), length analysis, and nucleotide sequencing. Compared with the peripheral blood, lesional lymphocytes had notably restricted TCRVß gene usage. Only three of the 24 major TCRVß gene families were represented in the repertoire. There was preferential usage of the Vß6 gene. In addition, more than 20% of the Vß6 TCR transcripts exhibited an identical V-D-J junctional sequence, suggesting a local antigen driven Vß6 T cell clonal expansion in vivo, a phenomenon not observed in normal oral mucosa. Although these data were presented from only one patient with OFG, they provide a unifying hypothesis on the immunopathology of OFG - namely, genetics playing a role in the development of abnormal T cell clonal expansion, yet driven by a local antigen (whether microbiological, food or environmental).

Challacombe *et al* (1997) found that there were detectable disturbances in mucosal immunity in patients with OFG with significantly greater serum titres of both IgA and IgG against *Candida albicans* ($p<0.002$; $p<0.01$) and *Saccharomyces cerevisiae* (bakers' yeast) ($p<0.05$; $p<0.005$) compared with the control group.

1.5.4 Melkersson-Rosenthal syndrome

The possibility that MRS is a variant of sarcoidosis is discussed elsewhere in this literature review but Kveim tests were reported as negative in seven patients with MRS, making sarcoidosis unlikely (Lindelöf *et al.,* 1985). In the same study, levels of serum angiotensin converting enzyme and calcium were normal.

The elimination of odontogenic infection has also been reported to cause regression and disappearance of the lip swelling in some cases of MRS (Worsaae *et al.,* 1982). The presence of herpetic eruptions on the lips and oral mucosa of some patients has been used by some authors to suggest a viral aetiology (Pisanty and Sharav, 1969; Nally, 1970; Alexander and James, 1972; Worsaae *et al.,* 1982) but such findings are by no means universally reported.

1.5.5 Cheilitis granulomatosa of Miescher

The cause of CG remains unknown, except for its association with sarcoidosis (Bourgeois-Droin *et al.,* 1993) and Crohn's disease (Brook *et al.,* 1983). It has been observed in persons who had been in contact with sodium silicate, which was believed to be of aetiological significance (Forman and Shuttleworth,1956). Infectious factors have also been proposed and lesions similar to CG occurring on the vulva and penis (preceded by recurrent infection) have been described (Larsson and Westermarch, 1978; Westermark and Henriksson, 1979), suggesting a common pathogenic mechanism (Larsson and Westermarch, 1978). Westermark and Henriksson (1979) indicated that bacteria of low-grade pathogenicity could play a role in the development of CG - although if this were the case then immunosuppressive states (e.g. HIV) might be expected to bring an increased prevalence of CG; this has not been reported.

Other infections of the face, such as chronic parotitis (Bishop and Garcia, 1979), Herpes simplex (Poex *et al.,* 1974), and paranasal infections with anaerobic bacteria (Frederick and Burde, 1974) have been observed before or after the development of CG.

An association with CG and food allergy was demonstrated in a patient allergic to almonds and hazelnuts (Hernandez *et al.,* 1986). Oral rechallenge with the identified food substances resulted in a recurrence of lip swelling; histological confirmation of granulomatous inflammation was recorded. The authors suggested a Type I or Type IV hypersensitivity reaction - the latter would explain the presence of granulomata and giant cells; the former would explain the degree of oedema evident on biopsy. An immunological basis would be further substantiated with GC followed by Crohn's disease (Carr, 1974), Hodgkin's disease (Mulvehill *et al.,* 1973) and Anderson-Fabry disease (Young *et al.,* 1978) - all of which have had an immunological basis established. Other authors suggest that the obstruction of lymphatic vessels by the granulomata may be a factor in the pathogenesis and a constitutional tendency to form epithelioid cell granulomata in chronic infections has also been suggested (Westermark and Henriksson, 1979).

Hornstein (1973) suggested that the granulomatous reaction was an allergic reaction to circulating antigens (non-specific); and the oedema a result of disturbance in the

autonomic nervous system, with an increase in vascular permeability. The non-specific allergy theory in CG was taken up by Liu and colleagues (1994;1993a) but then focused more specifically on the spirochaetal cause of Lyme's disease, *Borrelia burgdorferi* (BB) (Liu, 1993b). They reported the serum anti-BB antibody titres in 18 patients with CG and 5 patients with MRS at 77.8% and 80.0% respectively. They conclude that these results, coupled with histological features suggestive of spirochaetal involvement, suggest that CG and MRS are caused by BB.

Creus and colleagues (1994) reported a case of ulcerous CG in a 43-year-old immunocompetent male patient. The saprophytic fungal species Scopulariopsis brevicaulis was cultured from the labial swelling and submaxillary lymph nodes. The patient experienced an excellent response to oral itraconazole.

A report from Japan (Kano *et al.,* 1993) has made the suggestion that all patients with CG are genetically predisposed to Crohn's disease - an idea extrapolated from HLA antigen status in three patients! These three patients with CG and no evidence of gastrointestinal disease had the following HLA antigens in common : HLA-DR4, DRw53, and DQw3. In Japanese patients with Crohn's disease, 94% express HLA-DR4 (or DRw9), DRw53, and DQw3 (Kano *et al.,* 1990).

1.5.6 GLUS syndrome

If a specific GLUS syndrome is to be differentiated from sarcoidosis, then the features of GLUS - the occurrence of fever and constitutional symptoms, multisystem involvement, and evidence of immunological upset leading to granuloma formation - suggest an infectious aetiology (Benjamin, 1987). The GLUS syndrome demonstrates many features of infection caused by DNA viruses such as EBV and CMV, and one study suggests that cases of GLUS may be particularly frequent at a young age, coincident with a viral aetiology (Kuo and Rosai, 1974). This is shown in Table 1.16.

Table 1.16 Comparison of features of EBV and CMV infection with GLUS.
EBV = Epstein-Barr virus; CMV = Cytomegalovirus

FEATURE	EBV/CMV INFECTION	GLUS SYNDROME
Occurrence	Children/young adults	Children
Subclinical course	May occur	May occur
Latency/reactivation	Characteristic	Prolonged course
Granuloma formation	May occur	Defining feature
Constitutional symptoms (e.g. fever)	Yes	Yes
Multisystem disease	Yes	Yes
Skin rash	Yes	Occasional
Abnormal liver function tests	Yes	Yes
Hypergammaglobulinaemia	Yes	Yes
Peripheral lymphocytosis	Characteristic	Occasional
Prognosis	Good	Good

There is good evidence to suggest that infections with EBV and CMV are associated with granuloma formation, but apparently in such a way that the granulomata in EBV tend to be localised to bone marrow, whereas those of CMV preferentially affect the liver (Cohen and Corey, 1985; Fiala *et al.*, 1987). Furthermore, mononucleosis-like conditions without serological evidence of EBV or CMV infection may also be associated with granuloma formation (Telenti and Hermans, 1989; Krause and Kaplan, 1982). Both EBV and CMV are associated with a decrease in the normal ratio of CD4:CD8 T lymphocytes in the peripheral blood, as in sarcoidosis (Carney *et al.*, 1981). It could be speculated that the occurrence of natural killer cells within the granulomata of GLUS (Brincker and Pedersen, 1989) might indicate an immunological response directed against cells carrying the aetiological agent - and, on the strength of current literature,

that agent may well be a DNA virus or other infectious agent - such as toxoplasmosis, catch-scratch disease and *Yersinia* (Weitberg *et al.,* 1979; Lenoir *et al.,* 1988).

For the sake of completeness, the other granulomatous disorders which may present around the orofacial region should be mentioned - Wegener's granulomatosis, foreign body reactions, toothpaste reactions, myiasis, pulse (vegetable) granuloma, deep mycoses and syphilis - and discounted due to aetiological factors having been established previously (Eveson, 1996).

CHAPTER 2

PATIENT AND CONTROL GROUPS

2.1 Patients

A total of 443 patients with a diagnosis of orofacial granulomatosis attended the Department of Oral Medicine at Glasgow Dental Hospital & School during the twenty year period 1978-1998. These patients were referred from general dental and medical practitioners, consultant physicians (including dermatology, gastroenterology and general medicine), hospital dental practitioners (including the institution's Accident and Emergency Department, previously called the Receiving Clinic). Initially, data from historical case notes were assessed retrospectively and were deemed to be incomplete in 142 patients; these data were discarded. Useful data were available on 301 patients (consecutive referrals) – 50 of whom were initially retrospective assessments (from the eight year period 1978-1986) and 251 of whom were prospective assessments (from the twelve year period 1986-1998). Thus, the patients were selected historically (50 patients) only on the basis of completeness of data; all patients analysed prospectively were entirely unselected.

All the patients enrolled in the clinical aspects of this study were attending the Department of Oral Medicine at Glasgow Dental Hospital & School, later called Glasgow Dental Hospital & School NHS Trust, and/or the Contact Dermatitis Investigation Unit, Belvidere Hospital, later housed at Glasgow Royal Infirmary University NHS Trust. Of the 301 patients enrolled, 50 had historical data collated from casenotes (with 36 subsequently being contacted personally by telephone or letter to clarify some aspects of their history and presentation; 28 patients' general medical practitioners and/or hospital specialists were contacted personally by telephone or letter to clarify some aspects of their history, investigations or laboratory results). The remaining 251 patients were interviewed and examined personally by the investigator.

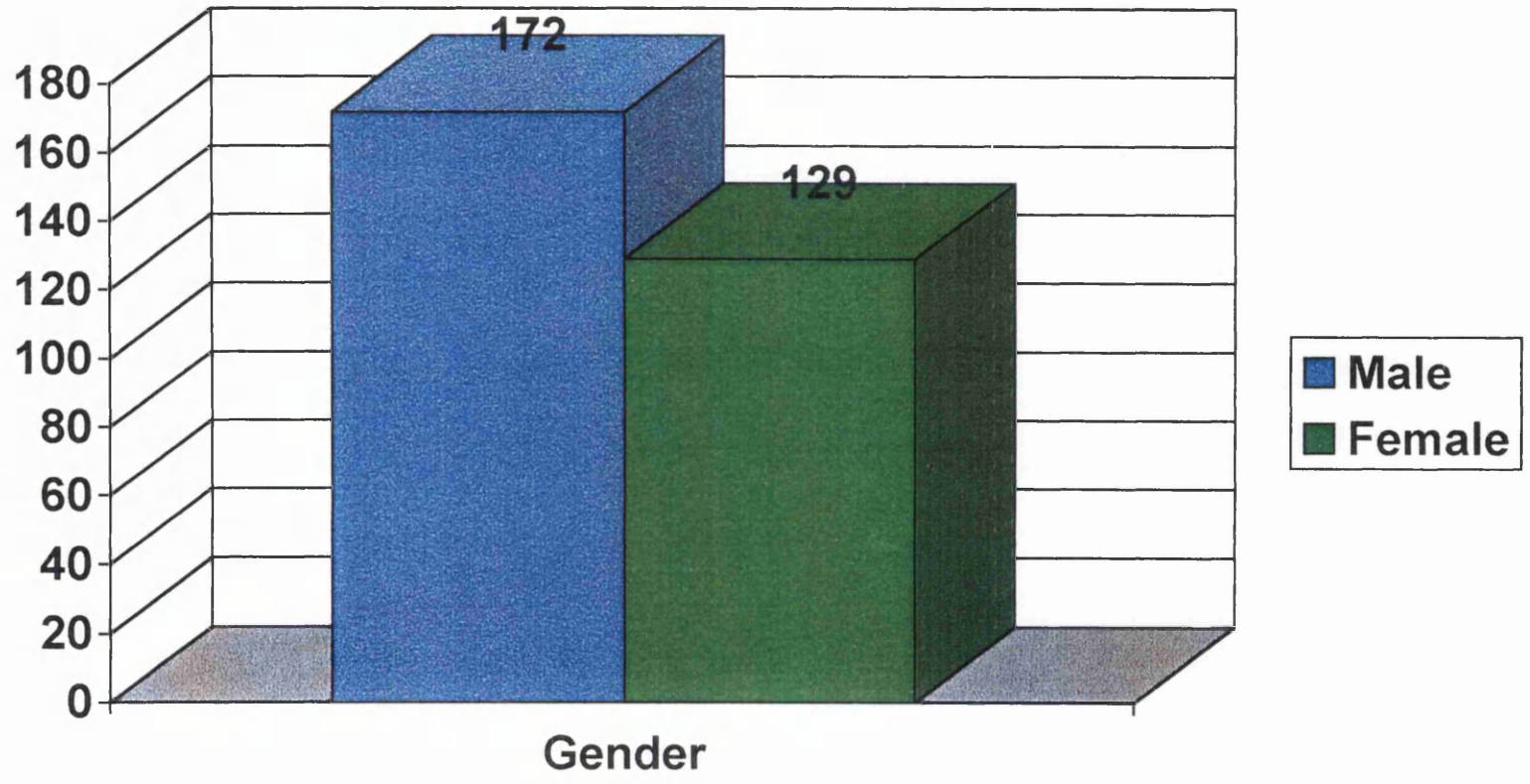
The total of 301 patients included in the clinical aspects of the study all had symptoms and signs constituting a diagnosis of orofacial granulomatosis (Anonymous, 1991b; Wiesenfeld *et al.,* 1985). The patients were primarily from the west of Scotland but included a wide geographical referral area from the islands of Barra and Lewis in the west, the towns of Fort William in the north west, Inverness in the north, Edinburgh and the Borders in the east, Newcastle in the south and Castle Douglas in the south west.

The demographic details are shown in Table 2.1 and Figure 2.1.

Table 2.1 Demographic details of patients with OFG

(total no/age in years)	**Male**	**Female**
Total (%)	172 (57.1)	129 (42.9)
Mean age at presentation	24	
Age range at presentation	3-66	

Figure 2.1 Gender profile of patients with OFG (absolute numbers)



Where patients underwent invasive procedures (including venepuncture) beyond those deemed necessary for normal patient management, informed, written consent was obtained and the necessary documentation signed by patient, parent or guardian as appropriate. Application was made to, and granted by, the Ethics Committees of each of the participating hospitals, viz. Glasgow Dental Hospital & School NHS Trust, Glasgow Royal Infirmary NHS Trust, and Yorkhill Hospitals NHS Trust.

2.2 Controls

Where appropriate, informed written consent was obtained and the necessary documentation signed by the control subject, parent or guardian. Application was made to, and granted by, the Ethics Committees of each of the participating hospitals, viz. Glasgow Dental Hospital & School NHS Trust, Glasgow Royal Infirmary NHS Trust, and Yorkhill Hospitals NHS Trust.

Control subjects were allocated to various groups :

2.2.1 Haematological, biochemical and immunological studies - whole blood, plasma and serum analysis

The reference ranges for these parameters were obtained from the diagnostic laboratories used in West Glasgow Hospitals NHS Trust and Glasgow Royal Infirmary NHS Trust. Reference was also made to the results of the Scottish Health Survey 1995.

2.2.2 Technetium-99m-HMPAO leucocyte labelling

Fifteen patients (8 male, 7 female; mean age 153 months, range 93-202 months) with objective evidence of inflammatory bowel disease (radiological +/- histological) were used as controls for this study. These were patients attending the clinic of Dr. John Evans, Consultant Paediatric Gastroenterologist, at the Royal Hospital for Sick Children, Glasgow.

2.2.3 Patch-Testing and Contact Urticaria Testing

To standardise methodology, all patch-testing and contact urticaria testing for the control population was carried out by the same clinician throughout, Dr. Shiona Rees, who had been trained and calibrated by Dr. Angela Forsyth, Consultant Dermatologist. Dr. Forsyth patch-tested the entire patient population.

One hundred and fourteen volunteers made up of staff and students at Glasgow Dental Hospital & School NHS Trust, and their friends and family members, applied to be enrolled in the study. Volunteers were provided with an Information Sheet and signed a

Consent Form. A short questionnaire was completed by each volunteer to determine age, gender, area of residency, occupation and past medical history.

Control subjects were excluded for the following reasons:

- Previous exposure to patch-testing or currently awaiting patch-testing.
- History of, or clinical evidence of, mucosal or cutaneous lichen planus or lichenoid reaction.
- History of recurrent aphthous stomatitis (defined as an episode of oral ulceration affecting the non-keratinised mucosa, occurring more than twice per year).
- History of orofacial granulomatosis or inflammatory bowel disease.
- History of erythema multiforme.
- History of angioedema.

As a result, 14 volunteers were excluded and 100 were formally enrolled as control subjects (29 male, 71 female; mean age 33.3 years, range 19-60 years). All were patch-tested in the Department of Oral Medicine, Glasgow Dental Hospital & School NHS Trust between July and December 1996.

2.2.4 Detection of anti-gliadin antibodies

Seven patients (4 female, 3 male; mean age 28 years, range 14-43 years) with coeliac disease (proven by jejunal biopsy), and 11 patients (8 female, 3 male; mean age 25 years, range 22-42 years) with no gastrointestinal or oral mucosal disorders acted as controls for this study. The coeliac disease group was attending the gastroenterology clinic of Dr. Robin I. Russell, Department of Medicine, Glasgow Royal Infirmary; the disease-free group was made up of staff of the Department of Oral Medicine, Glasgow Dental Hospital & School.

2.2.5 Molecular Biological Studies

Twelve patients (6 male, 6 female; mean age 36 years, range 22-63 years) with a history of recurrent aphthous stomatitis (minor variant) attending the Department of Oral Medicine, Glasgow Dental Hospital & School NHS Trust, for investigation and

management of their oral disease acted as controls for this study. An aphthous ulcer (less than 24 hours old) was excised under local anaesthesia from each patient, following the signing of informed consent documentation.

CHAPTER 3

CLINICAL METHODS I :
INVESTIGATION OF PATIENTS & CONTROLS

3.1 Introduction

This thesis focuses on the clinical aspects of orofacial granulomatosis and, as such, the author's personal involvement was chiefly in the clinical aspects of the patients' care, namely haematological, biochemical, immunological, microbiological and histopathological sampling, and patch-testing and contact urticaria testing. As a result, a number of diagnostic NHS laboratories were involved in processing the specimens, relying on the expertise of many support and technical staff. Due recognition is made of this throughout the text. Some aspects of the investigative protocol were part of a service commitment; others were entirely research orientated. Again, due recognition is made of this throughout the text.

For the 301 patients included in the study, history and examination were recorded for each on a proforma which was updated at each clinic attendance, and subsequently analysed. A diagnostic label was given to each patient on the basis of clinical, histological, haematological, biochemical, immunological, and radiographic findings as follows:

1 orofacial granulomatosis (with no evidence of systemic granulomatous disease).
2 gastrointestinal Crohn's disease (pre-existing and subsequently diagnosed).
3 sarcoidosis.
4 Melkersson-Rosenthal syndrome.

The diagnosis for each group followed recognised guidelines laid down in the current medical scientific literature as follows:

1 orofacial granulomatosis

Clinical appearance of chronic soft tissue swelling of the orofacial region at one or more sites with, ideally, histological confirmation of non-caseating granulomatous inflammation and/or lymphoedema. No clinical, haematological, biochemical, immunological and/or radiographic evidence of other systemic granulomatous disorders (Wiesenfeld *et al.*, 1985).

However, many case reports in the literature to date rely on clinical appearance only, with no histological findings reported. Therefore, it would be appropriate to use the clinical term Orofacial Lymphoedema (OFL) as presumptive OFG; i.e. "histological" OFG is a subset of "clinical" OFL. This convention will be developed throughout this study.

2 gastrointestinal Crohn's disease

Assessment by a consultant physician in gastroenterology to demonstrate clinical features associated with the disease and, directly, transmural chronic granulomatous inflammation of the gut or, indirectly, the results of this process (deep ulceration, fistulae and strictures of the gut; haematological and/or biochemical abnormalities associated). Whilst accepting that a histological assessment is the gold-standard for diagnosis, radiographic imaging of the gut may supply supportive information, particularly in children where direct visualisation of the gut may be a hazardous process (Bozdech and Farmer, 1990).

3 sarcoidosis

Assessment by a consultant physician, including (a) full history and detailed clinical examination, including ophthalmoscopy and slit-light examination of the eyes; (b) chest radiography and/or CT or MRI imaging of the thorax and cranium; (c) haematological (including ESR) and biochemical (including serum calcium and 24 hour urine collection; serum angiotensin converting enzyme assay) tests; (d) tuberculin skin reactivity test; (e) sputum sampling and/or bronchoalveolar lavage; (f) histological confirmation by specific organ biopsy, bronchial or transbronchial lung biopsy, or a Kveim skin test; (g) respiratory function tests (James *et al.,* 1976).

4 Melkersson-Rosenthal syndrome

This is a clinical diagnosis of a triad of signs, namely chronic swelling of the soft tissues of the face, fissured tongue, and unilateral or bilateral lower motor neurone facial palsy (Rosenthal, 1931).

Such a diagnosis was therefore subject to review for each patient as they progressed through investigations and follow-up. However, for the purposes of final analysis, these four patient groups were employed.

A standard haematological, biochemical and immunological profile was performed on all patients, except the first 50 for whom incomplete test data was obtained. Similarly, incomplete information on patch-testing was available on some of the earlier patients.

Patients with evolving evidence of systemic disorders, such as sarcoidosis and gastrointestinal Crohn's disease on the basis of clinical findings and/or the results of investigations, were further investigated to establish a correct diagnosis. Such medical evaluations were performed by the Department of Medicine at Glasgow Royal Infirmary NHS Trust or, in the case of paediatric patients, at The Royal Hospital for Sick Children, Glasgow.

3.2 Clinical History

A clinical history was obtained from each patient (or parent) with regard to age of onset and duration of the condition, medical history (with specific questioning on atopy), dental history, drug history (including disease modifying drugs such as immunosuppressants), social history (including family history, smoking and alcohol consumption, and dietary fads), and systemic enquiry (with specific questioning on facial palsy, weight loss, altered bowel habit, number of stools passed daily, rectal bleeding, and abdominal pain).

Patients were asked to grade their orofacial symptoms at presentation and throughout the study using a visual linear analogue scale. Patients were asked to imagine a line 10cm long with graded marks at centimetre intervals, giving a possible score of 0-10. Patients were advised that 0 meant no symptoms at all, and 10 meant their symptoms could not be worse.

3.3 Physical Examination

Patients were assessed at initial consultation for evidence of local and systemic disease. General examination was carried out selectively at initial consultation where indicated on the basis of symptoms; thereafter, all patients attending the joint dental-dermatology clinic after patch-testing at Glasgow Royal Infirmary had a full medical examination performed. This included the presence or absence of abdominal pathology, perianal and general skin changes, lymphadenopathy, and finger clubbing. A more detailed examination was performed where indicated by symptoms, signs or the results of haematological or other special investigations, correlated with the findings of a consultant physician when referral was instituted.

Orofacial examination included the presence or absence of lymphadenopathy, facial and perioral skin changes, angular cheilitis, facial swelling, lip swelling, oral ulceration, full-thickness gingivitis, mucosal tags, mucosal oedema (cobblestoning), fissured tongue, and papillary hyperplasia of the palate (Figures 3.1 – 3.12).

Patients were assessed at initial consultation and throughout the study using a scale graded from 0 (minimum) to 16 (maximum). This scale simply demonstrated the presence (1 mark) or absence (0 mark) of disease at various sites as shown in Table 3.1. This was deemed to be the most objective method of assessing disease activity, rather than attempting to quantify a graded record of changes in each individual clinical sign.

Table 3.1 Sign score used in assessing patients with OFG.

SITE/CONDITION	GRADE
ANGULAR CHEILITIS - LEFT	0 or 1
ANGULAR CHEILITIS - RIGHT	0 or 1
LIP SWELLING - UPPER	0 or 1
LIP SWELLING - LOWER	0 or 1
FACIAL SWELLING	0 or 1
ULCERATION – APHTHOID	0 or 1
ULCERATION – OTHER	0 or 1
FULL THICKNESS GINGIVITIS (UPPER ARCH)	0 or 1
FULL THICKNESS GINGIVITIS (LOWER ARCH)	0 or 1
MUCOSAL TAGS (RIGHT SIDE OF MOUTH)	0 or 1
MUCOSAL TAGS (LEFT SIDE OF MOUTH)	0 or 1
MUCOSAL OEDEMA (RIGHT SIDE OF MOUTH)	0 or 1
MUCOSAL OEDEMA (LEFT SIDE OF MOUTH)	0 or 1
FISSURED TONGUE	0 or 1
PAPILLARY HYPERPLASIA	0 or 1
FACIAL PALSY	0 or 1

Figure 3.1 Patient with OFG and angular cheilitis

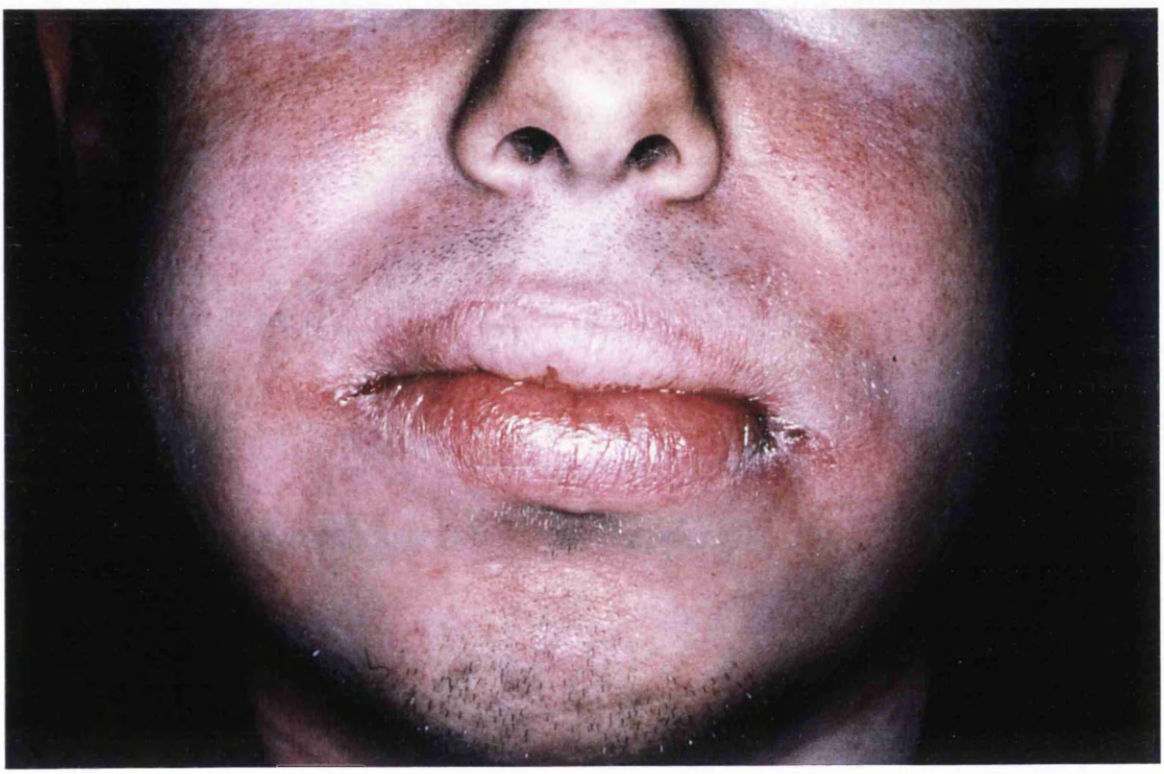


Figure 3.2 Patient with OFG and upper lip swelling

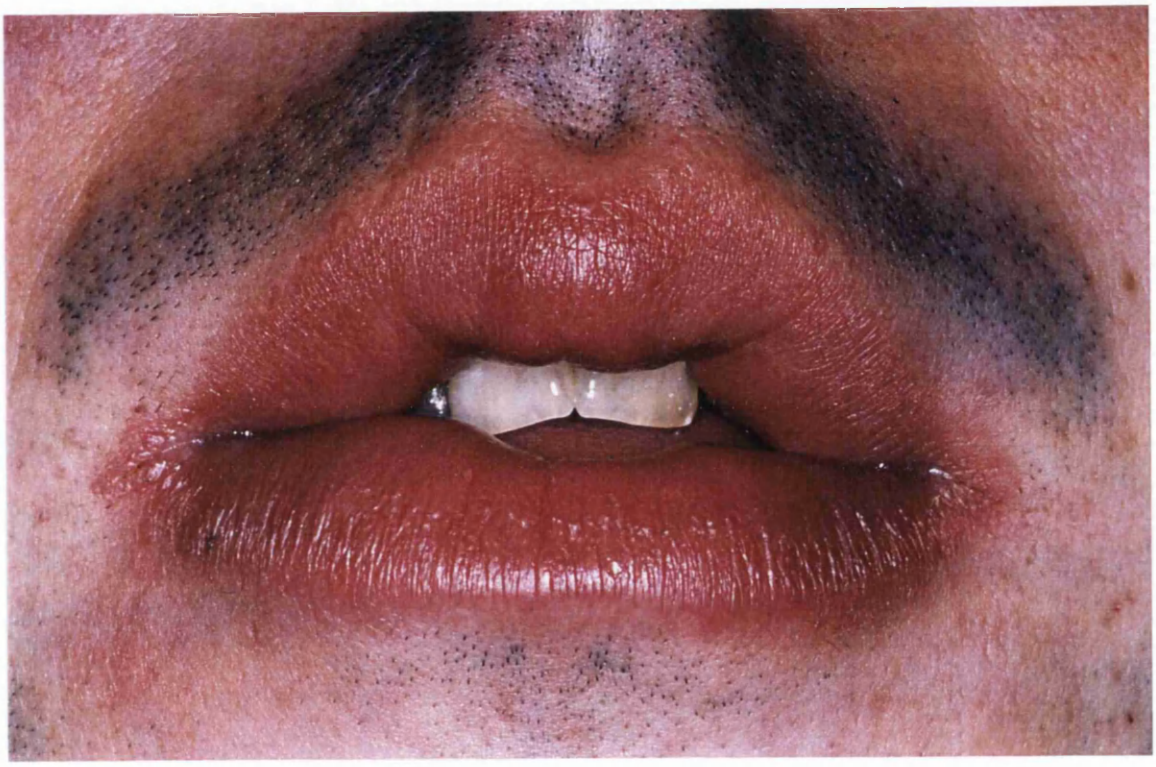


Figure 3.3 Patient with OFG and lower lip swelling

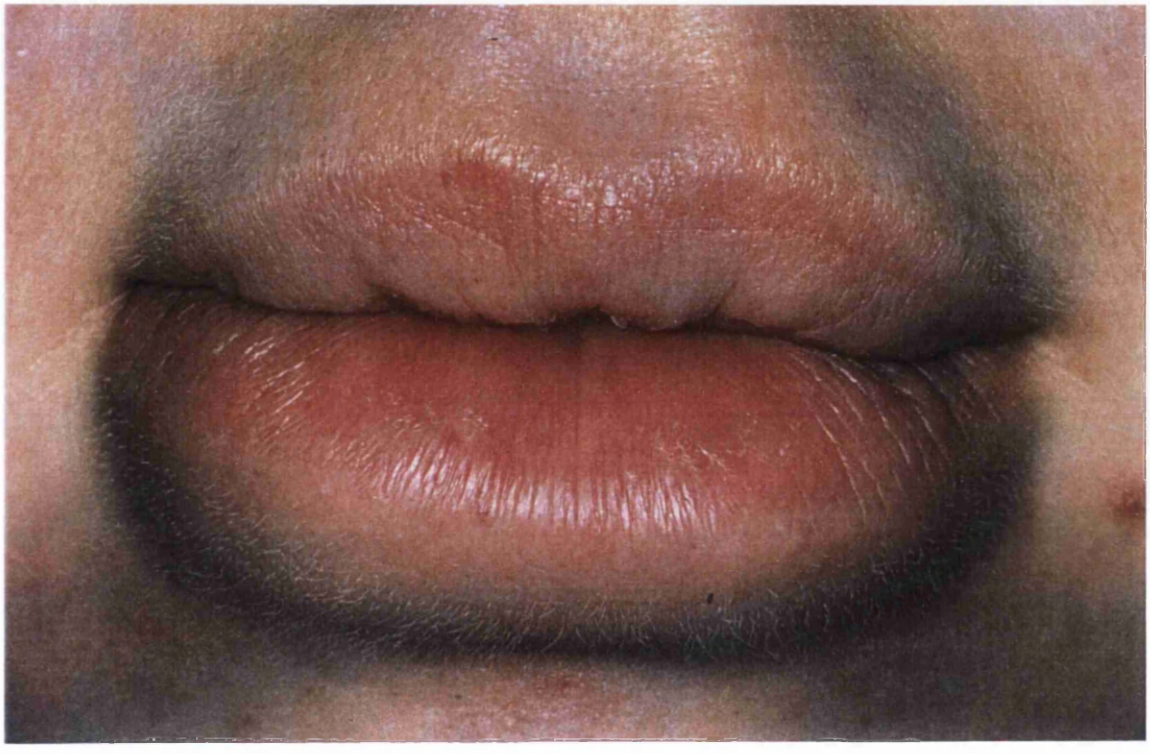


Figure 3.4 Patient with OFG and facial swelling

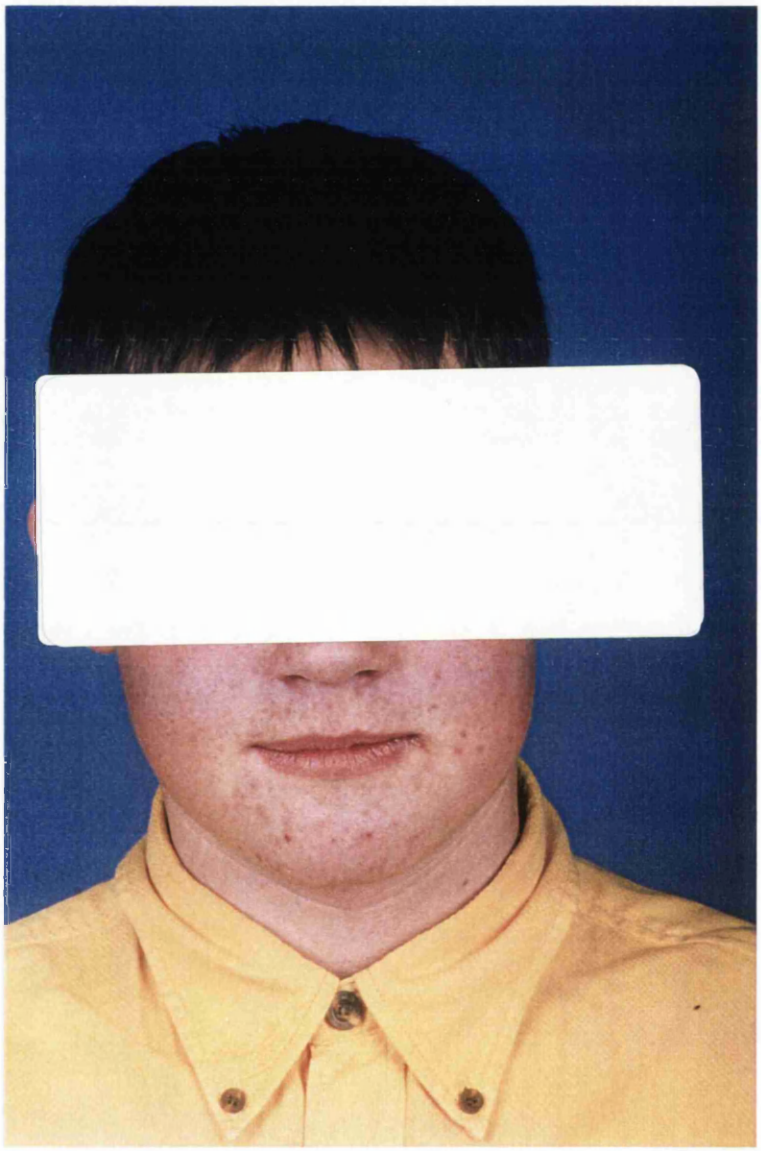


Figure 3.5 Patient with OFG and recurrent aphthous ulceration

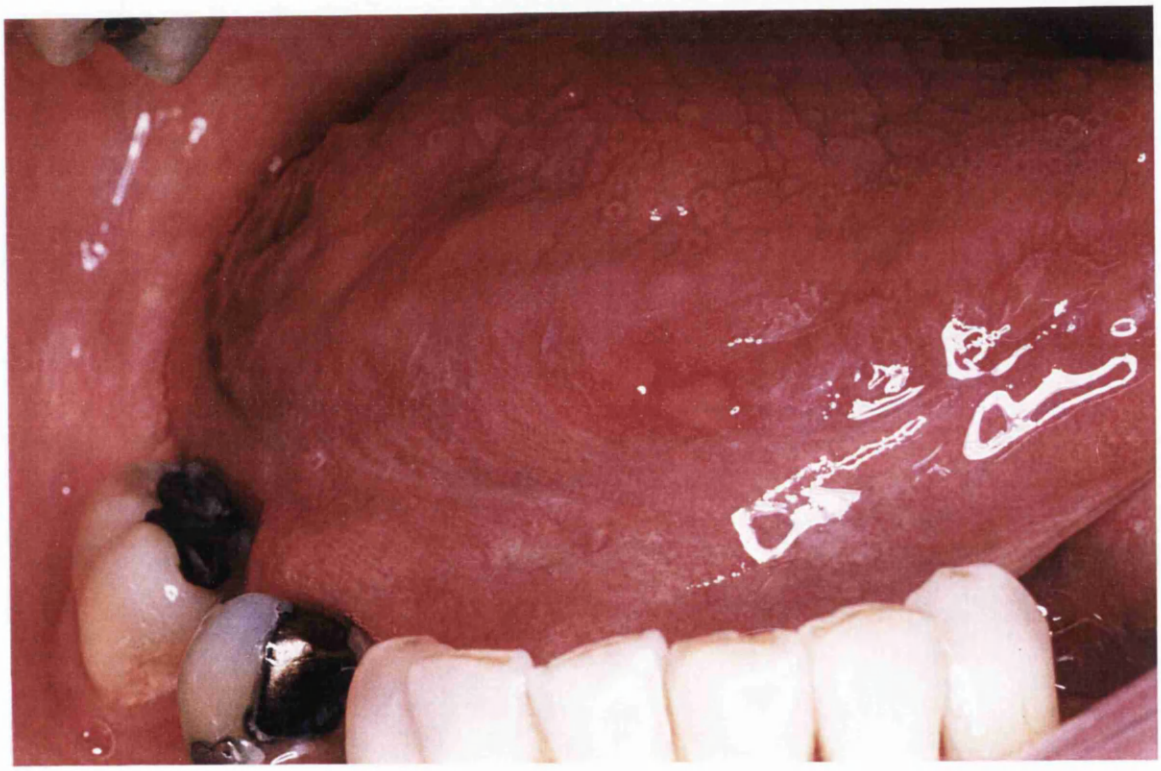


Figure 3.6 Patient with OFG and non-aphthoid oral ulceration

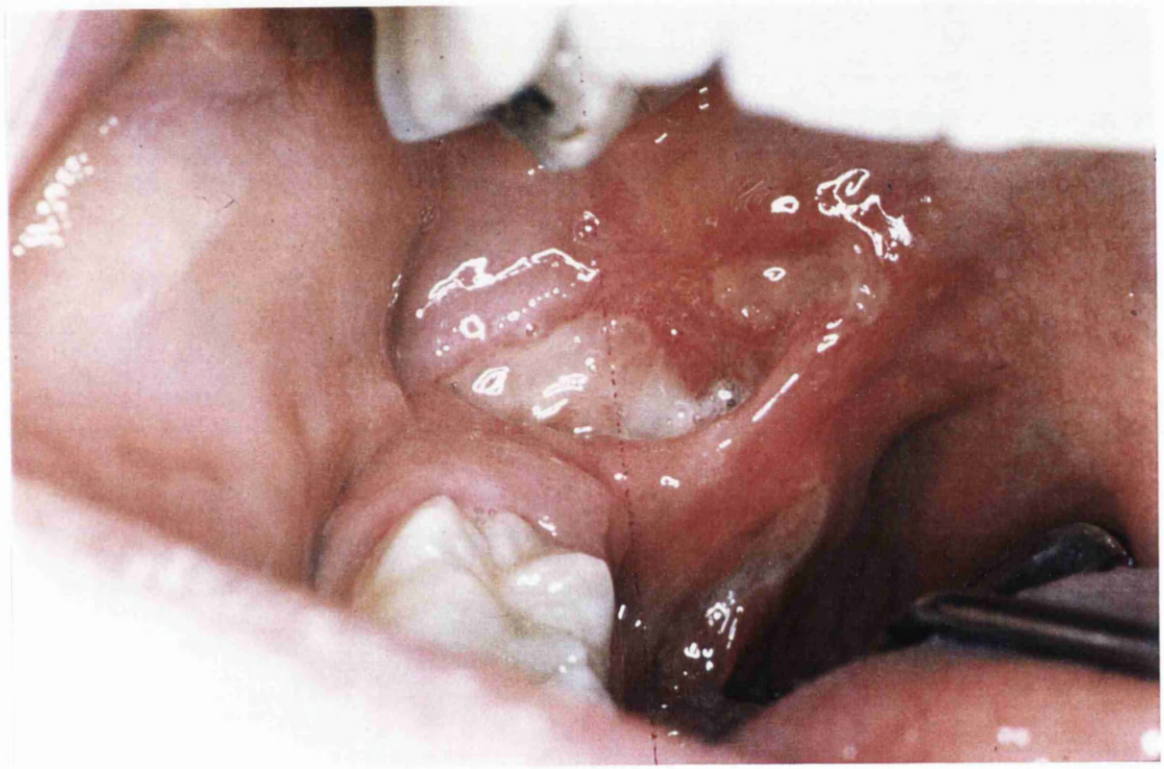


Figure 3.7 Patient with full thickness gingivitis

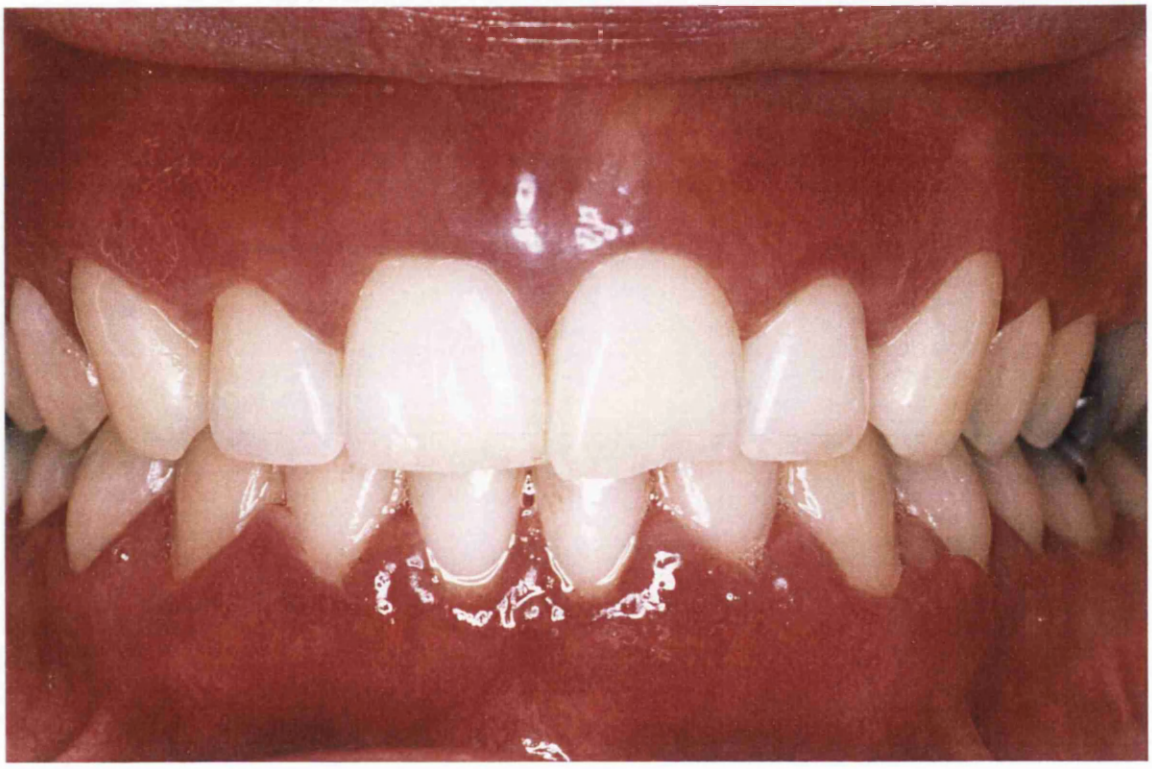


Figure 3.8 Patient with buccal mucosal tags

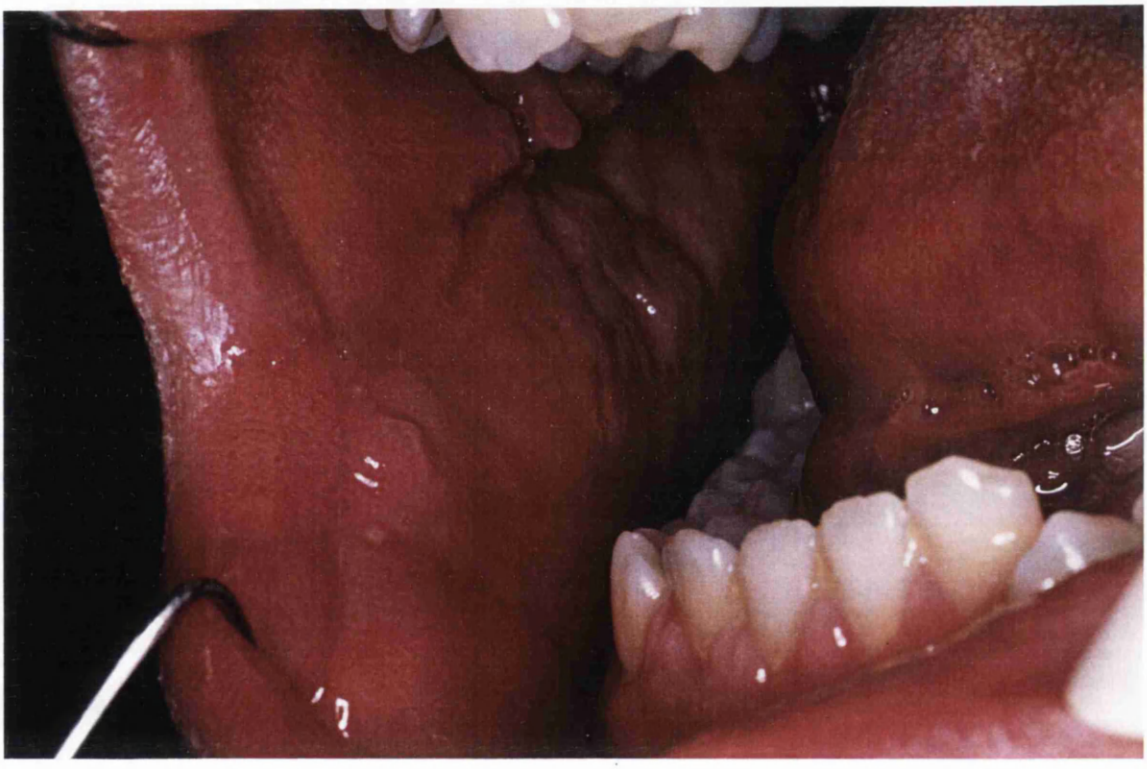


Figure 3.9 Patient with OFG and buccal mucosal oedema

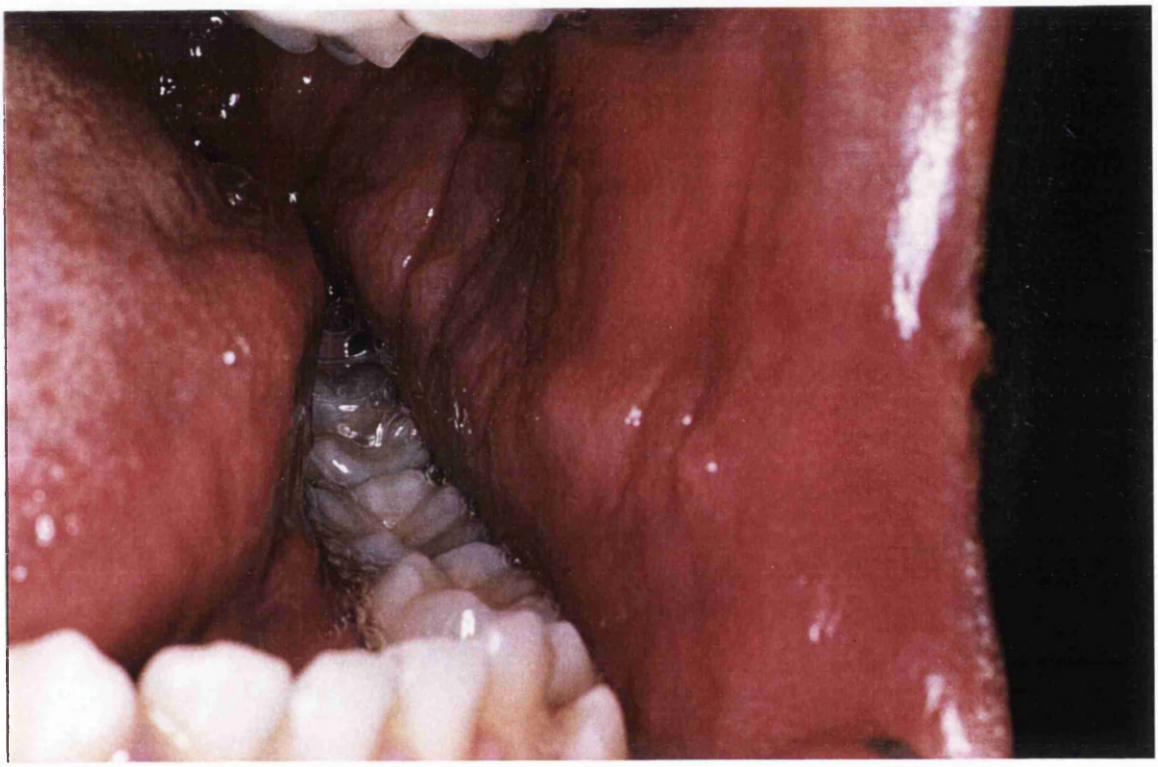


Figure 3.10 Patient with OFG and fissuring of the tongue

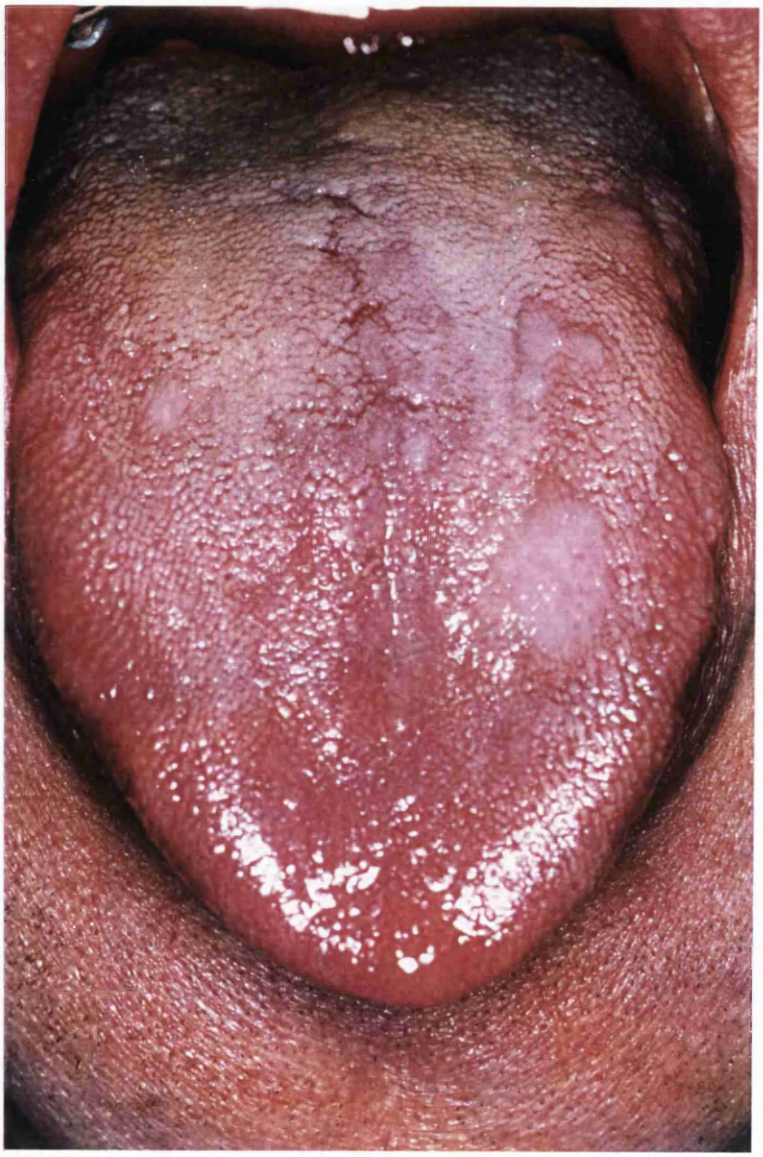


Figure 3.11 Patient with OFG and papillary hyperplasia

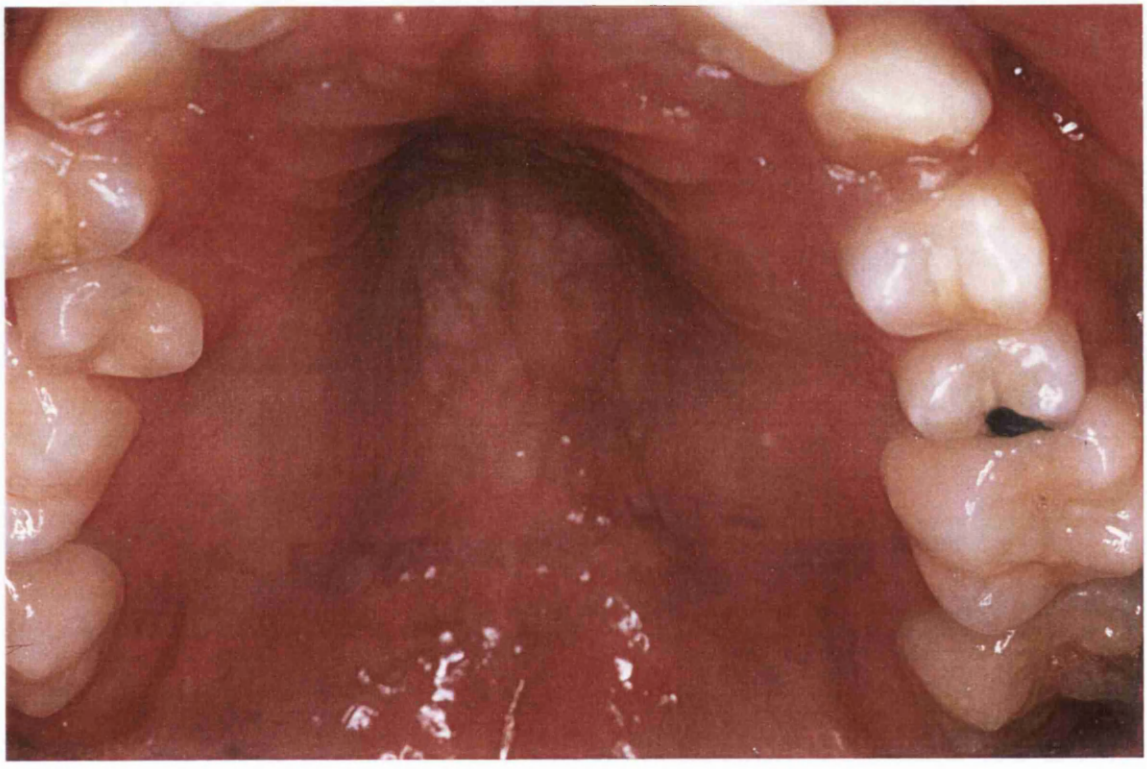
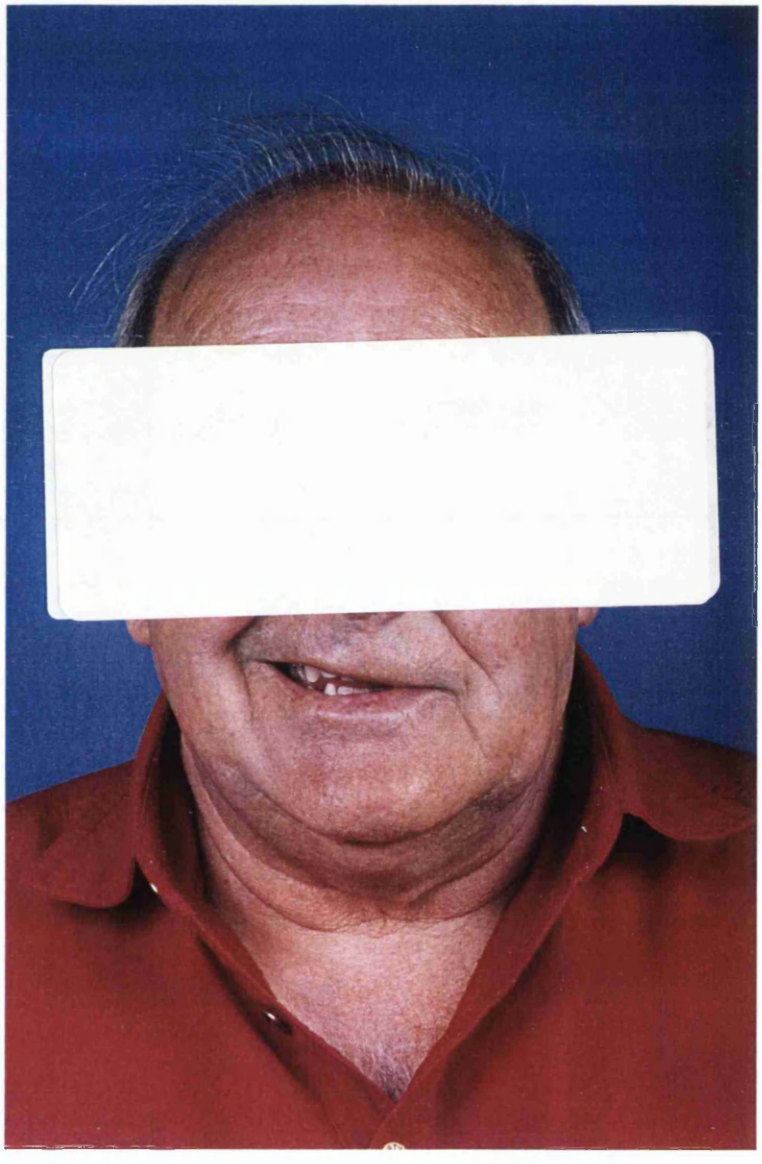


Figure 3.12 Patient with OFG and lower motor neurone facial (VII) palsy



3.4 Haematological Investigations

Two hundred and twenty-seven patients had venous blood removed at the first or second clinic visit and sent to the Department of Haematology at the Western Infirmary, Glasgow, for measurement of haematological parameters. This was repeated, if required, to verify results at subsequent visits. The haemoglobin concentration (Hb) and Mean Cell Volume (MCV) were determined by automated techniques and stained blood films were examined according to standard methods. Total white and red blood cell counts, and differential white cell counts and platelets numbers were obtained by automated cell counting techniques.

Serum ferritin, whole blood folate, and serum vitamin B_{12} were measured by radioimmunoassay. Erythrocyte sedimentation rates were measured by the Westergren method.

The normal ranges of these haematological parameters are shown in Table 3.2. Subsequent statistical analysis took in to account the age- and gender-matched differences in haematological indices between adult and paediatric populations.

Patients were regarded as being deficient in iron, folate or vitamin B_{12} only if they showed two consecutive results below the normal range.

Table 3.2 Normal ranges of haematological parameters measured.

Laboratory Test	Normal Range
Haemoglobin (Hb)	Male 13-18 g/dl
	Female 12-16 g/dl
Mean Corpuscular Volume (MCV)	80-100 fl
Red Cell Count	4.6-6.2 x 10^{12}/l
White Cell Count	4.0-11.0 x 10^{9}/l
Neutrophils	2.5-7.5 x 10^{9}/l
Lymphocytes	1.5-3.5 x 10^{9}/l
Monocytes	0.2-0.8 x 10^{9}/l
Eosinophils	0.04-0.44 x 10^{9}/l
Basophils	0.015-0.1 x 10^{9}/l
Platelets	150-400 x 10^{9}/l
Serum Ferritin	25-300 ng/ml
Serum Vitamin B_{12}	150-900 pg/ml
Whole Blood Folate	160-640 ng/ml
Erythrocyte Sedimentation Rate (ESR)	Age and Sex dependant

3.5 Biochemical Investigations

One hundred and forty-six patients had venous blood removed at the first or second clinic visit and sent to the Department of Biochemistry at the Western Infirmary, Glasgow, for measurement of biochemical parameters. This was repeated, if required, to verify results at subsequent visits. Estimation of urea and electrolytes, calcium, albumin and phosphate, liver function tests, and serum angiotensin converting enzyme was carried out by standard laboratory methods.

The normal ranges of these biochemical parameters are shown in Table 3.3.

Patients were regarded as being abnormal in any or all of these parameters only if they showed two consecutive results outwith the normal range.

Table 3.3 Normal ranges of biochemical parameters measured.

Laboratory Test	Normal Range
Calcium	2.2-2.65 mmol/l (corrected)
Albumin	36-50 g/l
Phosphate	0.80-1.45 mmol/l
Alkaline Phosphatase	70-260 IU/l
Alanine-amino Transferase (ALT)	10-50 U/L
Aspartate-amino Transferase (AST)	10-35 U/l
Bilirubin	3-18 μmol/l
Gamma-glutamyl Transferase (γGT)	5-50 IU/l
Protein (total)	60-77 g/l
Serum Angiotensin Converting Enzyme (SACE)	<80 IU/l

3.6 Immunological Investigations

One hundred and forty-six patients had venous blood removed at the first or second clinic visit and sent to the Department of Immunopathology at the Western Infirmary, Glasgow, for measurement of immunological parameters. This was repeated, if required, to verify results at subsequent visits. Quantitative estimation of immunoglobulins (IgA, IgG and IgM) was carried out by standard laboratory methods. Qualitative estimation of IgE was carried out by Radio-allergo-sorbent Testing (RAST). Quantitative assessment of Complement C_3 and C_4 and C_1 esterase inhibitor was carried out by routine immunological assay. Where abnormal results were found in any or all of these estimates of complement cascade activity, a functional (qualitative) assessment of C_1 esterase inhibitor was carried out to exclude hereditary angioedema as a cause of orofacial swelling.

The normal ranges of these immunological parameters are shown in Table 3.4.

Patients were regarded as being abnormal in any or all of these parameters only if they showed two consecutive results outwith the normal range.

Table 3.4 Normal ranges of immunological parameters measured.

Laboratory Test	Normal Range
Immunoglobulin A (IgA)	0.8-5.0 g/l
Immunoglobulin G (IgG)	7.2-19 g/l
Immunoglobulin M (IgM)	0.5-2.0 g/l
Immunoglobulin E (RASTs)	Grades 1-4
Complement C_3	0.739-1.69 g/l
Complement C_4	0.218-0.588 g/l
C_1-esterase Inhibitor Level	0.187-0.392 g/l
C_1-esterase Inhibitor Function	80-110%

3.7 Oral mucosal biopsy

Oral mucosal biopsies were carried out by the staff of the Department of Oral Medicine, Glasgow Dental Hospital and School on 165 patients. Biopsies were carried out under local anaesthesia with the exception of three, which were carried out under general anaesthesia. Areas of obvious mucosal involvement were selected as appropriate biopsy sites – chiefly lip and buccal mucosae. Biopsy material was placed in formalised saline and paraffin processed prior to staining with Haematoxylin and Eosin. For the purposes of histological assessment, biopsies were considered adequate if a full-thickness sample (down to, and including, muscle) was evident microscopically.

Interpretation of histological material was carried out by Professor Gordon MacDonald and Dr. James Rennie, Consultant Oral Pathologists, Glasgow Dental Hospital & School.

Biopsy reports were assessed for:

1 presence or absence of granulomata
2 presence or absence of caseation in the granulomatous inflammatory infiltrate
3 presence or absence of lymphoedema

These features were graded on a score of 1 (present) or 0 (absent).

3.8 Patch-testing and contact urticaria testing

3.8.1 Introduction

Patch-testing is a recognised and accepted way of identifying allergens responsible for Type I and Type IV allergic reactions of the skin and aerodigestive tracts (Cronin, 1980; Malling, 1993).

Two hundred and seventy-one patients were subjected to patch-testing for delayed hypersensitivity reactions (Type IV) and contact urticaria reactions (Type I). All were tested with the modified European Standard Series plus food additives, perfumes and flavourings, and chocolate. Most patients were also tested with other allergens e.g. dental materials, medicaments or other substances identified in the initial consultation as potentially playing a role in the patient's disease process.

All allergens were mixed with petrolatum as the carrying vehicle, except for formaldehyde and one of the chocolate essence preparations which were carried in aqueous solution. Both the carrying vehicles have low antigenic, irritant and sensitisation potential and a petrolatum test site was included on all subjects as a control.

The Standard European Series was used in all subjects for both the delayed hypersensitivity and contact urticaria tests. This consists of 23 test allergens and a control (petrolatum, PMF). See Table 3.5 for the concentrations, carrying vehicle and manufacturers of the various allergens.

The food additives used in both the delayed hypersensitivity and contact urticaria tests were: Benzoic acid, salicylic acid, tartrazine, glutamic acid, butylated hydroxytoluene, propylene glycol, sorbic acid and sodium metabisulphite. See Table 3.6 for the concentrations, carrying vehicle and manufacturers of the various allergens.

The perfumes and flavourings used in both the delayed hypersensitivity and contact urticaria tests were: Cinnamyl alcohol, cinnamaldehyde, eugenol, amyl cinnamaldehyde, hydroxycitraonella, geraniol, isoeugenol, oak moss absolute, benzyl alcohol and musk ambrette. Sorbitan sesquiolate was included as an independent test substance as it was

added as an emulsifier to the fragrance mix in the European Standard Series. See Table 3.7 for the concentrations, carrying vehicle and manufacturers of the various allergens.

Essence of chocolate was tested with 2 different carrying media in both the delayed hypersensitivity and contact urticaria tests. See Table 3.5 for the concentrations, carrying vehicle and manufacturers of the chocolate test substances

All patients attending for patch-testing at the Contact Dermatitis Investigation Unit were interviewed and examined by a consultant dermatologist with many years experience in the field of contact dermatitis. They were asked detailed questions about symptoms and exposure to dietary and environmental allergens. In particular, dietary fads and occupational exposure were explored in depth. Patients were questioned about their general medical, allergy and dermatological history. Informed consent was obtained from each patient prior to testing.

3.8.2 Delayed hypersensitivity testing

Petrolatum (Paraffin Molle Flavum or PMF) was used as the carrying vehicle of choice where possible since this facilitates handling and avoids the "edge effect" which can complicate the reading of patch-tests carried out with solutions (Fyad *et al.*, 1987). The test substances suspended in petrolatum were dispensed from plastic disposable syringes and placed in Finn chambers™ (Epitest, Hyrylä, Finland) – 8mm diameter aluminium wells – and arranged on Scanpor™ surgical tape (Scanpor, Norgesplaster, Oslo, Norway). Figure 3.13 demonstrates the testing system prior to application to a patient.

A 4mm length of the test substance was expressed into the aluminium well. Those carried in aqueous solution were dispensed from plastic dropper bottles and placed on a 5mm disc of filter paper (Bio Diagnostics Ltd, London, UK) in the bottom of the well. One drop was used to wet the filter paper immediately prior to the placement of the tests. The tapes were applied to the upper back, avoiding the vertebral column, and a hypoallergenic skin marker was used to mark the skin immediately adjacent to each test site. Figure 3.14 demonstrates the testing system in place. The tapes remained in place for 48 hours and patients were instructed not to wash or otherwise disturb the test sites.

Patients were instructed to remove the tapes and attached chambers one hour prior to their clinic attendance at 48 hours. Figure 3.15 shows a positive skin reaction at 48 hours. The test sites were examined and the Swedish Multicenter Study System (SMCS) grading system applied (Bruze *et al.*, 1995). This system uses scores as shown in Table 3.8.

Table 3.8 The scoring system for patch-test results.

Score	Appearance
0	Negative
1 (allergic)	Erythema (with inflammatory infiltration around the test site)
2 (allergic)	Erythema plus papules
3 (allergic)	Erythema plus papules plus vesicles
4	Irritant
E	Equivocal

Figure 3.13 Patch-testing system prior to skin application.

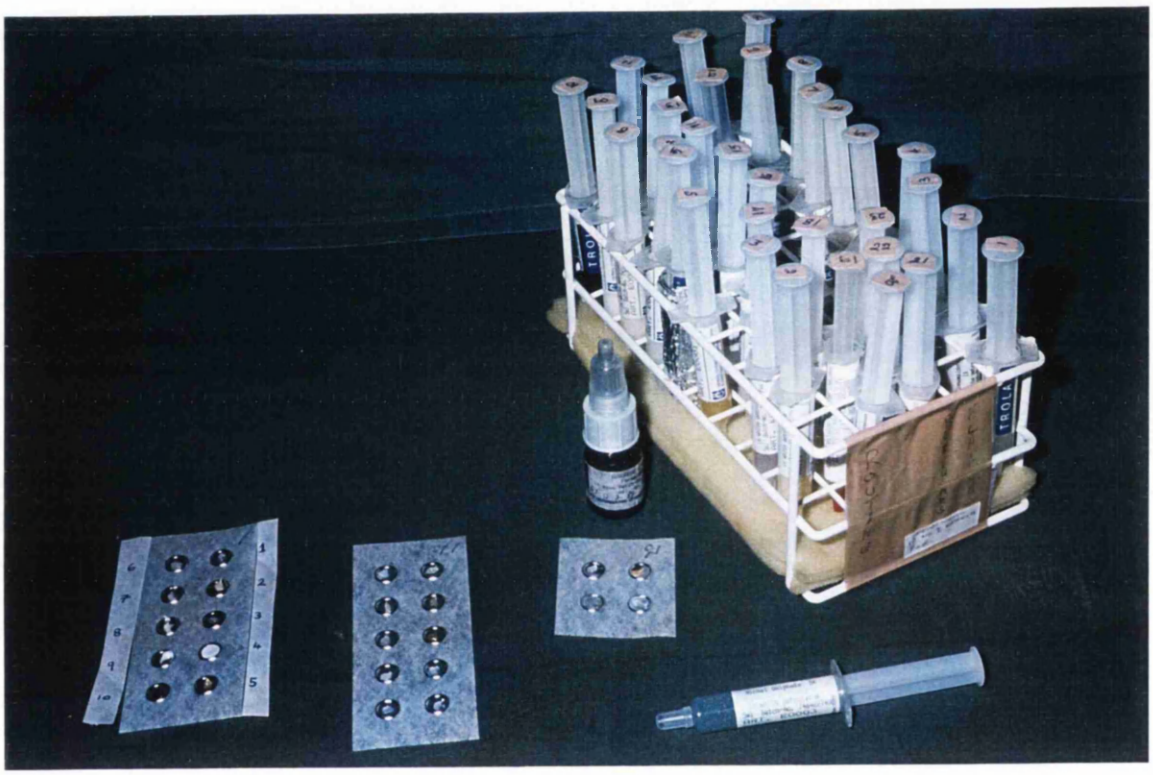


Figure 3.14 The patch-testing system in place.

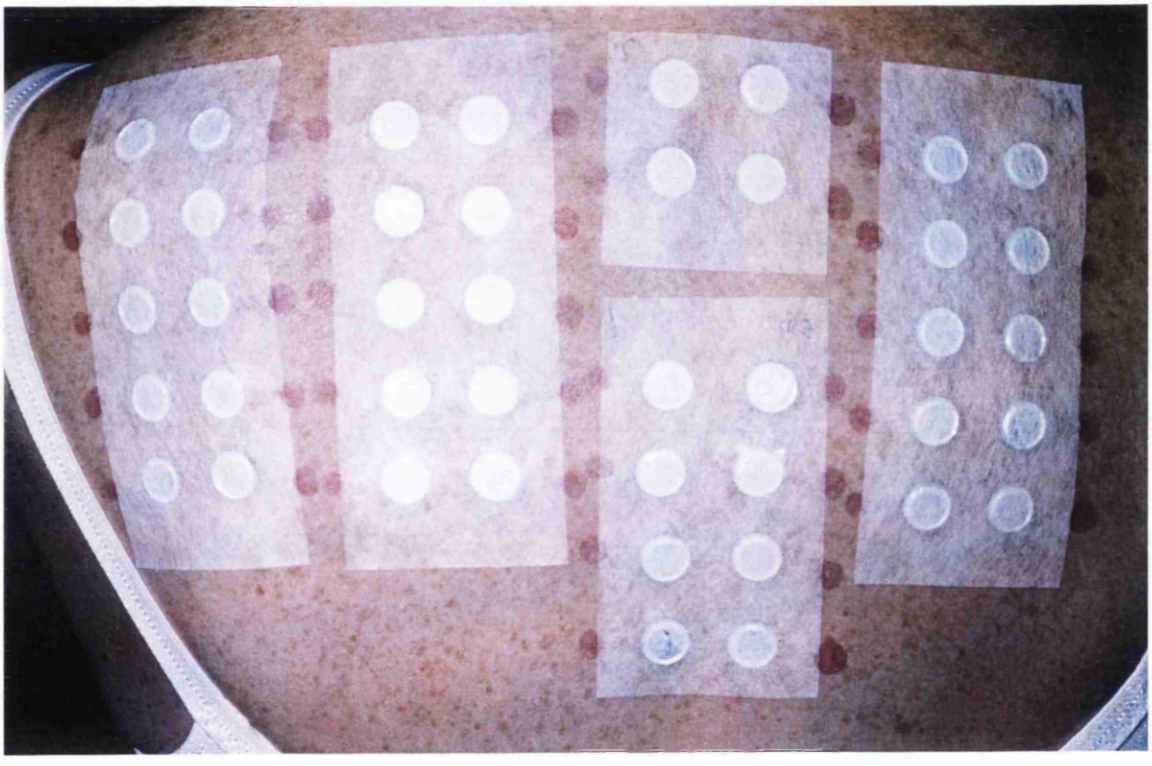
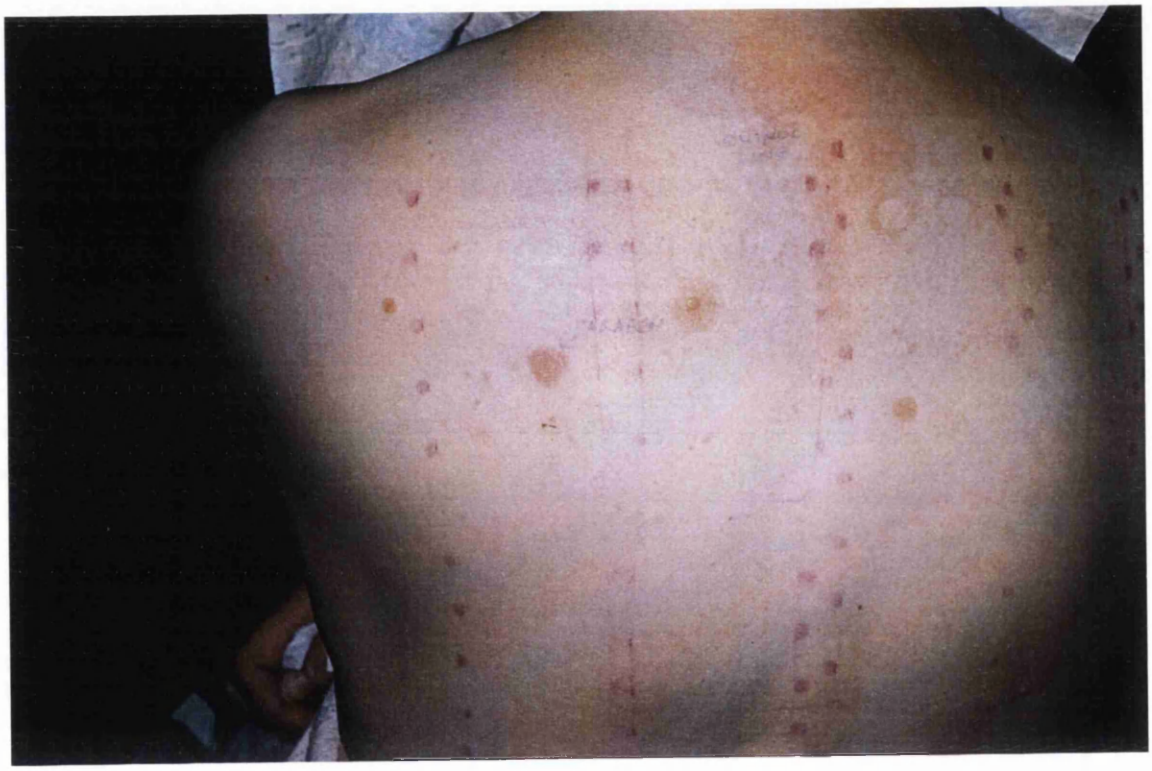


Figure 3.15 Patient showing a positive skin reaction at 48 hours.



Irritant responses to cutaneous patch tests can be misleading and cause the examiner to treat these reactions as allergic in nature (Nater and Hoedemaker, 1976). Irritant reactions were identified if the appearance was as follows (Klas *et al.*, 1996):

- a smooth glazed appearance or slight erythema; or
- sharp borders confined to the patch-test site; or
- cracked superficial surface; or
- minimal induration or no induration.

The test sites were re-examined and scored as above on Day 5, a total of 96 hours after initial exposure to the allergens.

3.8.3 Contact urticaria testing

Patch-testing was also used to elicit immediate (Type I) hypersensitivity reactions in patients. The same technique as above was used with a smaller number of test substances (food additives, perfumes and flavourings, and chocolate). On Day 1, the test substances were placed on the anterior surface of the patient's forearms. On the skin next to each test site, a number was clearly marked with a skin marking pen to identify each test substance clearly. After 20 minutes, the Scanpor™ surgical tape and test chambers were removed and the test sites examined. A score of 0 (no reaction) or 1 (reaction clearly visible or palpable) was given for each test substance. The patient was instructed to observe the test sites at hourly intervals over the subsequent 6 hours and record any redness or itchiness associated with any of the sites. At the 48 hour clinic visit, the patient's written record of the test sites was examined and a final score allocated as above i.e. 0 (no reaction) or 1 (reaction clearly visible or palpable).

3.8.4 Control Group

Patch-testing and contact urticaria testing of the control group was carried out by Dr. Shiona Rees. Her contribution to this part of the study is gratefully acknowledged. Thus to standardise methodology, all patch-testing and contact urticaria testing for the control population was carried out by the same clinician throughout.

The demographic details and selection criteria were described in Chapter 2.

A total of 48 different allergens (46 suspended in yellow soft paraffin; 2 suspended in an aqueous solution) were placed on the backs of volunteers on Day 1 of the study. The patches were reviewed at 48 and 72 hours.

In order to identify Type I hypersensitivity reactions, test substances (food additives, chocolate, perfumes and flavourings) were placed on the volunteers' forearms on Day 1 of the study. On the skin adjacent to each forearm site, a number was clearly marked with a skin marker pen to identify each individual test. The test substances and carriers were removed after 20 minutes and the test sites examined. The volunteers were instructed to observe the test sites at hourly intervals over the following 6 hours and to record any changes in sensation (e.g. pruritus), colour or texture associated with any of the sites. At the 48-hour visit, the volunteers' records of the test sites were examined and a further score allocated.

Table 3.5 Concentrations and carrying vehicles of the dietary allergens used in patch-testing

ALLERGEN	CONCENTRATION (%)	VEHICLE	MANUFACTURER
Food Additives			
Benzoic Acid	5	PMF	Crawford
Butylated Hydroxytoluene	2	PMF	Trolab
Butylated Hydroxyanisole	2	PMF	Trolab
Glutamic Acid	2	PMF	GRI
Propylene Glycol	5	PMF	Crawford
Salicylic Acid	1	PMF	GRI
Sodium Metabisulphite	1	PMF	Trolab
Sorbic Acid	2	PMF	Trolab
Tartrazine	0.1	PMF	GRI
Perfumes and Flavourings			
Amyl Cinnamaldehyde	1	PMF	Trolab
Benzyl Alcohol	1	PMF	Crawford
Cinnamonaldehyde	1	PMF	Trolab
Cinnamyl Alcohol	1	PMF	Trolab
Eugenol	1	PMF	Trolab
Geraniol	1	PMF	Trolab
Hydroxy Citraonella	1	PMF	Trolab
Isoeugenol	1	PMF	Trolab
Musk Ambrette	5	PMF	Trolab
Oak Moss Absolute	1	PMF	Trolab
Sorbital Sesquiolate	20	PMF	Trolab
Chocolate			
Essence of Chocolate	5	Aqueous	GRI
Essence of Chocolate	5	PMF	GRI

PMF = Paraffin Molle Flavum (Yellow Soft Paraffin)

Crawford Pharmaceuticals
71a High Street
Stony Stratford
Milton Keynes
MK11 1BA

GRI Glasgow Royal Infirmary
Department of Pharmacy
84 Castle Street
Glasgow
G4 0SF

Trolab Patch Test Allergens
Hermal Diagnostics Department
D-21462 Reinbek
Germany

Table 3.6 Concentrations and carrying vehicles of the environmental allergens used

ALLERGEN	CONCENTRATION (%)	VEHICLE	MANUFACTURER
Balsam of Peru	25	PMF	Trolab
Caine Mix	12	PMF	Trolab
Carba Mix	3	PMF	Trolab
Cobalt Chloride	1	PMF	Trolab
Colophony	20	PMF	Trolab
Epoxy Resin	1	PMF	Trolab
Ethylene Diamine	1	PMF	Trolab
Formaldehyde	1	Aqueous	Trolab
Fragrance Mix	8	PMF	Trolab
Mercaptomix	2	PMF	Trolab
Neomycin	20	PMF	Trolab
Nickel Sulphate	5	PMF	Trolab
Parabens Mix	16	PMF	Trolab
Paraphenylene Diamine (PPD)	0.5	PMF	Trolab
Paratertiarybutyl Phenol (PTBP) Formaldehyde Resin	1	PMF	Trolab
Potassium Dichromate	0.5	PMF	Trolab
PPD Mix	0.6	PMF	Trolab
Primin	0.01	PMF	Trolab
Quaternium 15	1	PMF	Trolab
Quinoline	6	PMF	Trolab
Thiomersal	0.1	PMF	Trolab
Thiuram Mix	1	PMF	Trolab
Wool Alcohols	30	PMF	Trolab
Control PMF	100	-	Trolab

Table 3.7 Concentrations and carrying vehicles of the mixed preparations used

(a) Mercapto-mix supplied by Trolab ready mixed.

Allergen	Concentration (%)	Vehicle	Manufacturer
Dibenzothiazyl Disulphide	1	PMF	Trolab
Morpholinyl-mercaptobenzo-thiazole	0.5	PMF	Trolab
N-Cyclohexyl-benzothiazyl Sulphenamide	1	PMF	Trolab

(b) Paraben-mix supplied by Trolab ready mixed.

Allergen	Concentration (%)	Vehicle	Manufacturer
Butyl Parahydroxy-benzoate	3	PMF	Trolab
Ethyl Parahydroxy-benzoate	3	PMF	Trolab
Methyl Parahydroxy-benzoate	3	PMF	Trolab
Propyl Parahydroxy-benzoate	3	PMF	Trolab

(c) Thiuram-mix supplied by Trolab ready mixed.

Allergen	Concentration (%)	Vehicle	Manufacturer
Dipentamethylene-thiuram Disulphide	0.25	PMF	Trolab
Tetraethylthiuram Disulphide	0.25	PMF	Trolab
Tetramethylthiuram Disulphide	0.25	PMF	Trolab
Tetramethylthiuram Monosulphide	0.25	PMF	Trolab

(d) PPD-mix prepared by GRI pharmacy.

Allergen	Concentration (%)	Vehicle	Manufacturer
Cyclohexylphenyl PPD	1	PMF	Crawford
Diphenyl PPD	0.25	PMF	Trolab
Isopropylphenyl PPD	1	PMF	Trolab

(e) Fragrance-mix supplied by Trolab ready mixed.

Allergen	Concentration (%)	Vehicle	Manufacturer
Amylcinnamaldehyde	1	PMF	Trolab
Cinnamonaldehyde	1	PMF	Trolab
Cinnamyl Alcohol	1	PMF	Trolab
Eugenol	1	PMF	Trolab
Geraniol	1	PMF	Trolab
Hydroxycitraonella	1	PMF	Trolab
Isoeugenol	1	PMF	Trolab
Oak Moss Absolute	1	PMF	Trolab

(f) Carba-mix prepared by GRI pharmacy.

Allergen	Concentration (%)	Vehicle	Manufacturer
Dibutyldithiocarbamol	1	PMF	Trolab
Diethyldithiocarbamol	1	PMF	Trolab
Diphenylguanidine	1	PMF	Trolab

(g) Caine-mix prepared by GRI pharmacy.

Allergen	Concentration (%)	Vehicle	Manufacturer
Benzocaine	5	PMF	Trolab
Cinchocaine	5	PMF	Trolab
Lignocaine	15	PMF	Trolab
Tetracaine	1	PMF	Trolab

3.9 HLA typing

The discovery of associations between certain diseases and the major histocompatibility complex (MHC) represents one of the most important recent advances in medicine (Lund and Festenstein, 1991). The human MHC is known as the HLA (human leucocyte antigen) locus and is located on chromosome 6, and spans 3000 kbp. There are at least four blocks of genes within the complex:

- MHC class I genes, which are expressed on all nucleated cells;
- MHC class II genes, expressed on cells which may present antigens to $CD4^+$ T-lymphocytes;
- MHC class III genes, which include the complement components of C4, C2 and Factor B, and the isoenzyme of 21-hydroxylase;
- MHC class IV genes, which encode molecules with a similar structure to class I, but with restricted distribution. They are thought to act as differentiation antigens during embryogenesis.

There are three loci, or genes, encoding class I molecules in the HLA region, termed HLA-A, HLA-B and HLA-C. Class I antigens are all defined by serological reactions and typing is performed using standard serological (antigen-antibody) techniques. The antibodies may bind uniquely to a particular molecule from a single locus, or they may bind to a group of molecules which share some common structures. Many specificities have been found to consist of different subtypes - the original haplotype then said to be split. Subtypes are identified by isoelectric focusing. International guidelines have been laid down for the nomenclature of genes and alleles of class I and II based on nucleotide or amino acid sequencing (Bodmer *et al.*, 1989).

There are at least three class II regions: HLA-DP, HLA-DQ and HLA-DR. The term DR was originally used to describe the HLA tissue types detected by antibody testing which approximate to those lymphocyte activating determinants (LADs) assigned by their ability to stimulate T-cell proliferation in mixed lymphocyte reactions (MLR). The LADs were originally termed alleles of the HLA-D locus, and the HLA-DR specificities were related to the particular HLA-D allele. Thus antibody which identified HLA-DR4

recognised the HLA-D4 allele identified in MLR. The LADs of the HLA-DR subregion belong to an independently segregated series known as the HLA-D types. Since the D determinants activate a large subpopulation of helper T-cells, they are critical in controlling the basis of immunological disease.

The MHC region on chromosome 6 is referred to as the haplotype and is inherited in a Mendelian manner. The likelihood of a recombination event occurring between the parental chromosomes within the MHC region is dependent on the size of the MHC region. Thus with the standard 3000 kbp size of the MHC region, the likelihood is estimated at 1%.

There is a substantial difference in the frequency of the main alleles at each of the HLA loci, and in some cases considerable variation between different populations. HLA-A2 is present at relatively high frequency (27%) in all populations, however HLA-A1 and HLA-A3 are present in most ethnic groups but absent in the Japanese. In African blacks, A11 is absent, whereas HLA-Aw43 and -Aw42 are present and seem to be specific for that one ethnic population (Bodmer *et al.,* 1989).

On the basis of the importance of HLA type in immunologically-mediated disease, 16 caucasian patients with OFG (and no evidence of other systemic granulomatous diseases) underwent HLA typing. There were 8 male patients (mean age 30 years; age range 9-55 years) and 8 female patients (mean age 32 years; age range 17-58 years).

Twenty millilitres of venous blood were withdrawn into potassium EDTA vials and transported to the Tissue Typing (Clinical Immunology) Laboratory at Glasgow Royal Infirmary. HLA phenotypes were identified on the basis of antigen-antibody reactions and, where appropriate, by isoelectric focusing.

3.10 Lymphocyte subpopulation studies

The expert technical assistance of Mr. Matthew Small, Chief Technician, Department of Oral Medicine, Glasgow Dental Hospital & School, in this part of the study is gratefully acknowledged

Eighteen patients (9 male, 9 female; mean age 22 years, range 12-30 years) were selected for this part of the study. All had biopsy-proven OFG of at least 1 year's duration with no evidence of other systemic granulomatous disorders. None were taking topical or systemic corticosteroid preparations, immunosuppressant drugs or anti-histamines.

Thirty-four age control subjects (20 male, 14 female; mean age 22 years, range 16-37 years) were selected from staff in the Department of Oral Medicine and Pathology, Glasgow Dental Hospital & School. All haematological and biochemical indices were normal.

Ten millilitres of venous blood were obtained in a lithium-heparin blood vial. No attempt was made to standardise the time of day of venepuncture. Personal or parental consent was obtained in each case. The 10ml blood sample was added to a universal container with 15ml of tissue culture medium. The cells were separated by standard density gradient techniques (Boyum, 1968). A 1:20 dilution of cells in phosphate-buffered saline (PBS) was added to a haemocytometer slide and dilution adjustments made to give a count of 5×10^6 cells/ml.

Lucham tubes (Neil *et al.,* 1994) were then prepared in duplicate with monoclonal antibody preparations as follows:

OKT_3	(Ortho-Mune)	5µl
OKT_4	(Oxoia)	5µl
OKT_8	(SAPU)	20µl
HLA-DR	(SAPU)	10µl
PBS control		10µl

To each of these, 200µl of the cell suspension were added. The contents of the tubes were mixed gently and incubated on ice for 60 minutes. The cells were then washed twice with PBS, centrifuging at 1,400 rpm for 10 minutes. The cells were further incubated on ice for 60 minutes with 100µl (1:10 dilutes) of FITC-conjugated rabbit anti-mouse immunoglobulin.

Further washing occurred twice with PBS, as above, centrifuging again at 1,400 rpm for 10 minutes. Finally, the washed cells were stored in 150µl 1% formalin at 4°C and quantified in the fluorescent activated cell sorter (Neil *et al.,* 1994).

3.11 Technetium-99m-HMPAO leucocyte labelling

Leucocyte labelling with ^{99}Tc-HMPAO (hexamethylpropylemeamine oxime) is an established way of investigating patients who may have inflammatory bowel disease (Charron, 1997).

Ten consecutive paediatric patients (9 male, 1 female; mean age 166 months, range 109-204 months) with OFG underwent ^{99}Tc scanning. Fifteen paediatric patients (8 male, 7 female; mean age 153 months, range 93-202 months) with objective evidence of inflammatory bowel disease (radiological +/- histological) were used as positive controls.

The assistance in this part of the study of the consultant and technical staff of the Department of Nuclear Medicine, Glasgow Royal Infirmary University NHS Trust, is gratefully acknowledged. The sequential steps in this part of the study are shown in Figures 3.16 – 3.19.

To a tissue culture flask were added 10ml of Plasmasteril or Hespan starch solution and 2ml of heparin (5,000 units per 5ml). The solutions were mixed gently and used to coat the internal surface of the flask.

A 19G butterfly needle was inserted to the patient's hand or arm vein and, with the open end of the butterfly tube in the flask, blood was dripped into the mixture until the 50ml mark was reached. The blood and anti-coagulant/starch solution were gently mixed throughout the procedure.

The flask was placed in the laminar flow cabinet and the cells allowed to settle for 30 minutes at room temperature. The resultant leucocyte-rich plasma (LRP) was removed with a sterile pipette and placed in a sterile container, marked with the patient's name and study number, for centrifuging. As much as possible of the LRP was removed without disturbing the red cell layer, and this was centrifuged for 10 minutes at 1000rpm.

800 MBq of Technetium pertechnetate (1ml of 99mTc) were added to a commercially available vial of hexamethylpropylemeamine oxime (HMPAO) and the resultant mixture allowed to stand for a maximum period of 30 minutes while the LRP was centrifuging.

The supernatant platelet-rich plasma (PRP) was decanted into a sterile universal container and kept for preparation of cell-free plasma (CFP). The resultant white cell pellet was resuspended in 0.5ml of sterile normal saline.

The 99mTc-HMPAO mixture was added to the white cell suspension, mixed gently and incubated at room temperature for 10 minutes. The flask was placed within a lead shield in the laminar flow cabinet.

During this 10 minute period, the PRP was centrifuged for 5 minutes at 3,000rpm. The resultant supernatant was CFP. After 10 minutes incubation, 3ml of CFP was added to the labelled white cell suspension, mixed gently and centrifuged at room temperature for 10 minutes at 700rpm. The resultant supernatant was decanted and kept to assess activity.

The white cell pellet was further resuspended in 3ml of CFP as before. The activity of the 99mTc was measured in the supernatant and WBC suspension by Geiger counter and the percentage labelling efficiency calculated as follows:

$$\frac{\text{WBC activity X 100}}{\text{WBC activity + Supernatant activity}}$$

Labelling efficiency <40% was deemed to be unacceptable and the procedure repeated on a subsequent occasion.

The labelled WBC suspension was then drawn into a 5ml syringe and injected intravenously.

Scanning of the abdomen took place at 1 hour, 2 hours and 4 hours post-injection using a gamma camera. Interpretation of the scans was carried out blindly (without reference to the patient's clinical details) by Dr. Brian Neilly, Consultant Physician, Department of Nuclear Medicine, Glasgow Royal Infirmary NHS Trust.

The resultant images were graded on a scale 0 – 4 (Li *et al.*, 1992) as follows:

Grade 0	No uptake
Grade 1	Uptake less than bone marrow
Grade 2	Uptake equal to bone marrow
Grade 3	Uptake greater than bone marrow
Grade 4	Uptake equal to, or greater than, spleen

Figure 3.16 Patient having blood sample removed for leucocyte labelling.

Figure 3.17 Patient having labelled leucocytes reintroduced.

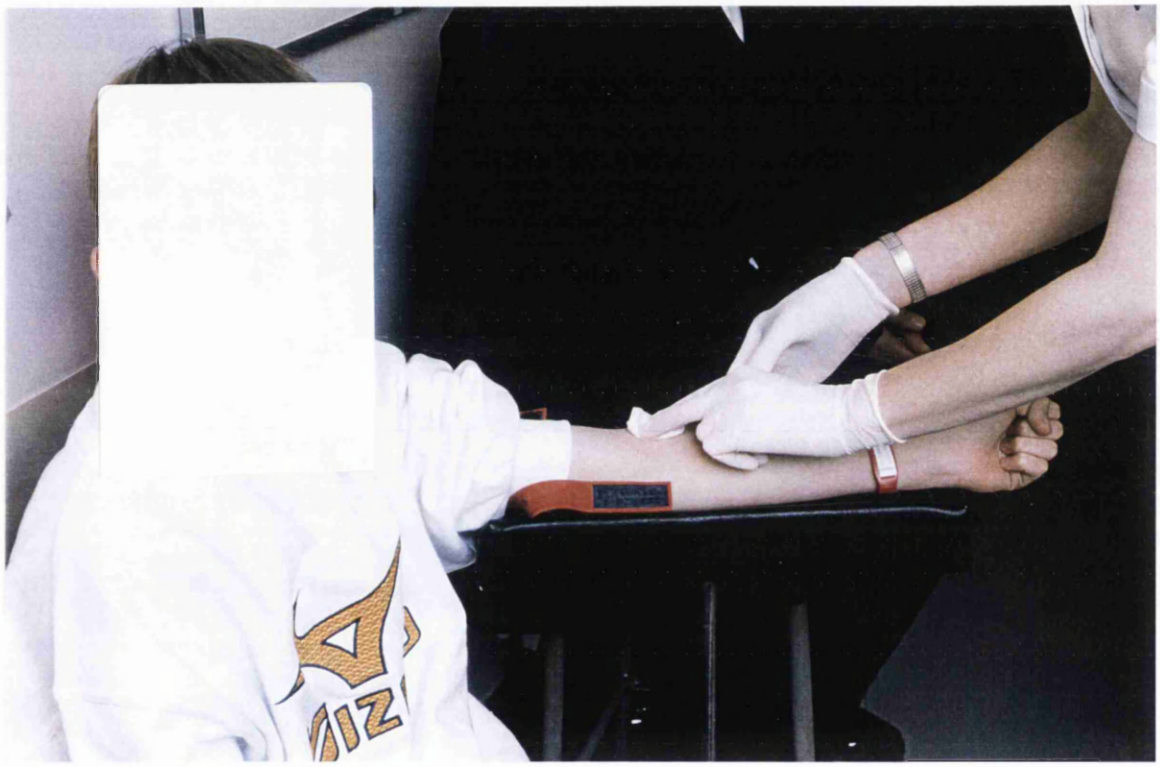


Figure 3.18 Gamma camera in place.

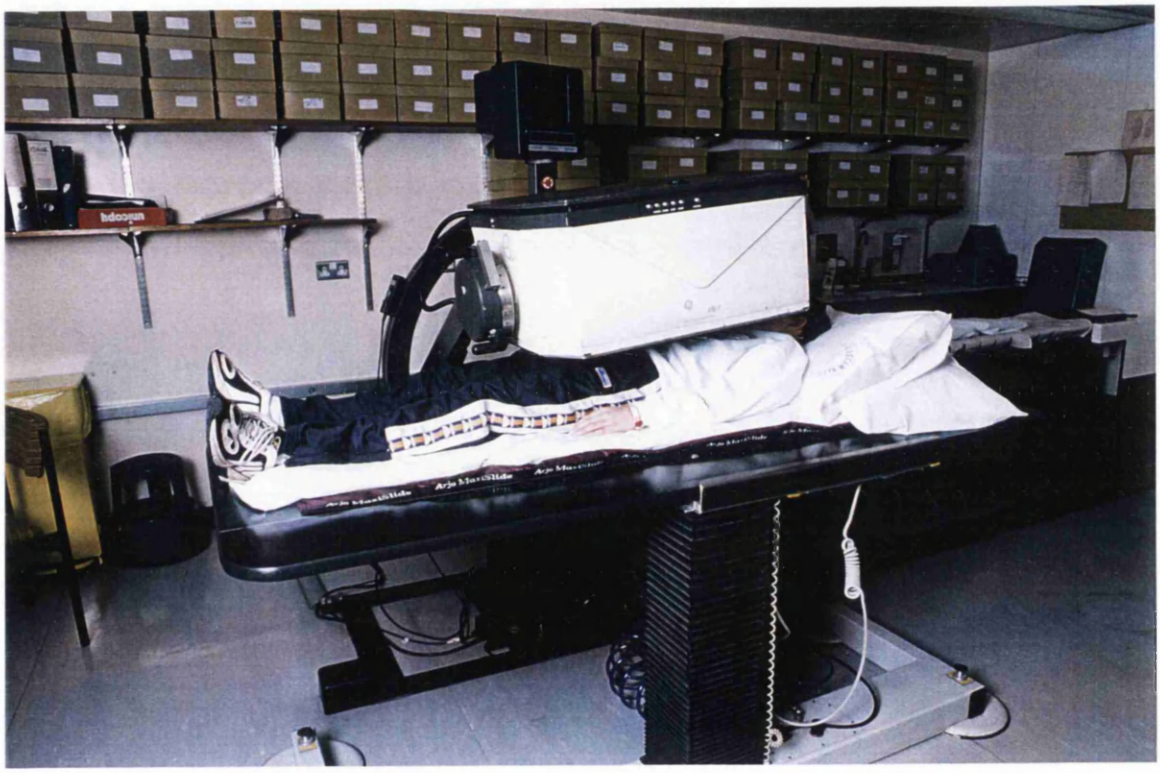


Figure 3.19 Patient relaxing after imaging at four hours.

3.12 Endoscopy and barium studies

Endoscopy and barium studies were considered, at the planning stage, an essential and integral part of the study in order to identify which patients had gastrointestinal Crohn's disease and which did not.

However, it became apparent early on that the Ethical Committees of participating hospitals were not prepared to allow patients (whether paediatric or adult) to be subjected to endoscopy or barium studies as part of a routine investigative protocol. This view was endorsed by the participating consultant physicians in paediatric and adult gastroenterology.

This part of the study is therefore flawed by the fact that only those patients considered by consultant gastroenterologists to have clear indications for barium studies and/or endoscopy were subjected to these tests. The "clear indications" were symptoms and signs of gastrointestinal disorders and/or abnormalities in haematological and biochemical parameters.

It is accepted that this is unsatisfactory in terms of seeking objective scientific evidence but, on this occasion, compromise was unavoidable and governed by the decision of the relevant Ethical Committees.

Where clinical and/or laboratory parameters indicated the requirement for barium studies of the upper or lower gastrointestinal tract, these were carried out as "barium meal and follow-through" and "barium enema" studies respectively. These were conducted according to accepted national protocols (Bozdech and Farmer, 1990; Winship *et al.*, 1979) and the radiographic films were reported by consultant radiologists.

Where clinical and/or laboratory parameters indicated the requirement for endoscopy of the upper or lower gastrointestinal tract, this was carried out as simple endoscopy and colonoscopy/sigmoidoscopy respectively. Such procedures were normally performed subsequent to barium studies when an abnormality had been evident on radiographic films.

These procedures were conducted according to accepted national protocols (Bozdech and Farmer, 1990; Winship *et al.,* 1979), under general anaesthesia in the paediatric population and under sedation and/or topical anaesthesia in the adult population.

On the basis of clinical findings, 57 patients (excluding the 20 patients who had entered the study with a pre-existing diagnosis of gastrointestinal Crohn's disease) underwent imaging of the gastrointestinal tract.

Of these 57 patients, 37 underwent barium studies ("meal and follow-through" and enema) alone; 18 patients underwent barium studies and endoscopic procedures; and two patients underwent endoscopic procedures alone.

CHAPTER 4

CLINICAL METHODS II :
TREATMENT AND MANAGEMENT

4.1 Exclusion of allergens

4.1.1 Background

Preventive therapy in the management of the patient group with OFG centred around the identification of dietary and environmental allergens by skin reactivity testing (patch-testing and contact urticaria testing).

Two hundred and seventy-one patients underwent patch-testing and contact urticaria testing to identify putative dietary and environmental allergens according to the methods described previously.

When dietary and/or environmental allergens were identified in this group, exclusion advice was offered to the patient (and accompanying adult, in the case of children). Such dietary and environmental exclusion advice was issued verbally and reinforced with written advice sheets. Dietary exclusion advice was issued by the Senior Dietician in the Contact Dermatitis Investigation Unit, Glasgow Royal Infirmary, Mrs. Karen Milligan. The dietary exclusion advice sheets are published in Appendix II, courtesy of Mrs. Karen Milligan and with her permission.

Environmental exclusion advice was issued by the nursing staff in the Contact Dermatitis Investigation Unit, Glasgow Royal Infirmary, Sister Jean Reid and her team of three staff nurses. Dietary and environmental exclusion advice was reinforced and expanded upon, where required, at each subsequent review appointment by the medical, nursing and dietetics staff.

4.1.2 Compliance scores

Compliance with dietary and environmental exclusion advice was determined at the end point of individual patient follow-up using the subjective self-rating score shown in Table 4.1.

Table 4.1 Score of patient compliance with dietary and/or environmental exclusion advice.

COMPLIANCE SCORE	DESCRIPTION
0	Patch-test not done or negative
1	Poor compliance
2	Compliance (incomplete)
3	Good compliance

4.2 Follow-up time

All patients were excluded from using symptomatic therapies (such as topical and systemic corticosteroid preparations) until patch-testing had been performed and, where appropriate, until the effect of allergen exclusion had been determined. This period varied considerably from patient to patient.

Patients were followed-up over varying periods of time (mean 3.8 years; minimum 0.5 years, maximum 23 years) with periodic recall assessment visits to the joint Dental-Dermatology Clinic at Glasgow Royal Infirmary and/or the Department of Oral Medicine, Glasgow Dental Hospital & School NHS Trust.

4.3 Assessment of response

4.3.1 Exclusion of allergens

The effect of dietary and/or environmental exclusion advice was determined by direct comparison, on enrolment into the study and at the end of the follow-up period, of:

- The patient's subjective symptom score
- The investigator's objective sign score.

These scoring systems are described fully in Chapter 3.

CHAPTER 5

LABORATORY METHODS

5.1 Detection of anti-gliadin antibodies

5.1.1 Background

The validity of using raised serum IgG to alpha-gliadin as a serological screening test for Coeliac disease is well documented (O'Farrelly *et al.,* 1983; Watson *et al.,* 1986; Kelly *et al.,* 1987). Similarly well documented is the lack of IgG response to alpha-gliadin in patients with inflammatory bowel disease (Paganelli *et al.,* 1985).

There is some evidence to suggest that alpha-gliadin antibody levels may be elevated in patients with chronic lip enlargement in an Irish population (12 out of 20 cases; McCartan, BE, Personal Communication, 1995). The diagnosis underlying the lip enlargement in this population was unspecified.

5.1.2 Methodology

Pooled sera from 4 patients (2 female, 2 male; mean age 24.5 years, range 14-35 years) with OFG (and no evidence of other systemic granulomatous or gastrointestinal disorders) were used for this part of the study. Pooled sera from 7 patients (4 female, 3 male; mean age 28 years, range 14-43 years) with coeliac disease (proven by jejunal biopsy), and 11 subjects (8 female, 3 male; mean age 25 years, range 22-42 years) with no gastrointestinal or oral mucosal disorders acted as controls for this study. The coeliac disease group was attending the gastroenterology clinic of Dr. Robin I. Russell, Department of Medicine, Glasgow Royal Infirmary; the disease-free group was made up of staff from the Department of Oral Medicine, Glasgow Dental Hospital & School.

The laboratory work was carried out in the Oral Biochemistry Laboratory, Glasgow Dental Hospital & School. I am indebted to the laboratory training given by Mr. Daniel Sweeney, Senior Technician.

Immunoblot techniques were employed to identify antibodies to crude gliadin components in each of the pooled sera according to the following technique. Crude gliadin (from wheat gluten), and antiserum to gliadin conjugated to peroxidase were

obtained from Sigma (Fancy Road, Poole, Dorset, UK). Sheep anti-human IgG conjugated to horseradish peroxidase, sheep anti-human IgG, donkey anti-sheep/goat IgG, and sheep peroxidase anti-peroxidase complex (PAP) were obtained from The Scottish Antibody Production Unit (Law Hospital, Carluke, UK).

SDS (sodium dodecyl sulphate) polyacrylamide gel electrophoresis was carried out in 12.5% gels according to the method of Laemmli (Laemmli, 1970). Crude gliadin was dissolved in sample buffer (2% w/v) SDS, 25ml of dithiothreitol (DTT) and boiled for 2 minutes prior to loading onto the gels. Molecular weight markers (14,400-94,000) were used and electrophoresis carried out at 60v for 18 hours at 10°C. Tracks from the gel were stained with 0.1% Coomassie Blue R250 (Sigma, Fancy Road, Poole, Dorset) in 50% (v/v) ethanol and 10% (v/v) acetic acid for at least 3 hours and destained in 10% acetic acid.

The SDS gels were blotted onto nitrocellulose (NC) membranes (Hybond-C, Amersham International, Aylesbury, Bucks, UK) on an LKB Multiphor II Nova Blot according to the manufacturer's instructions. After blotting, the NC membranes were blocked for 1 hour at room temperature in 5% skimmed milk powder (Marvel, Premier Brands UK Ltd., Birmingham, UK) in phosphate-buffered saline-Tween (PBST; NaCl 8.0g/l, KCl 0.2g/l, Na_2HPO_4 0.2g/l, Tween 20 5g/l, pH 7.4), cut into triplicate tracks which were placed in test sera diluted 1:5 with the same solution. After standing overnight at 4°C, the membranes were washed in three changes of PBST and placed in sheep anti-human IgG diluted 1:200 with PBST. The use of anti-human IgG peroxidase conjugate followed by addition of substrate produced only faint immunostaining and so further amplification was carried out to increase the sensitivity. After standing for 2 hours at room temperature, the membranes were washed as before and placed in a solution of 1:200 donkey anti-sheep/goat IgG for 2 hours. After washing, the membranes were transferred to 1:200 solution of sheep PAP for 2 hours and further washed.

Sequential addition of donkey anti-sheep/goat and sheep PAP was repeated twice more. Finally, the washed membranes were placed in peroxidase substrate solution of hydrogen peroxide (0.3% w/v) and 4-chloro-1-naphthol (0.015% w/v) in phosphate/citrate buffer (pH 6.0, prepared using 0.2M Na_2HPO_4 and 0.1M citric acid) until bands developed. The reaction was stopped by washing the membrane in purified water.

5.2 Molecular biological studies – the polymerase chain reaction

5.2.1 Background

The polymerase chain reaction (PCR) is an *in vitro* technique which allows the amplification of a specific deoxyribonucleic acid (DNA) region that lies between two regions of known DNA sequence ("primers"). This allows for sensitive and specific detection of particular genetic sequences against a large background of irrelevant DNA (Newton and Graham, 1994). Nested PCR primers are ones that are internal to the first primer pair. The larger fragment produced by the first round of PCR is used as a template for the second round of PCR. The sensitivity and specificity of both DNA and RNA amplification can be dramatically increased by using the nested method, since it almost always eliminates any spurious non-specific amplification products (Newton and Graham, 1994).

"Hot start" is a modification of PCR whereby there is an initial separation of one or more important components of the reaction by a wax barrier. Thus, when the wax melts and all the components mix together, the template is already denatured and the reaction well underway. This causes a more efficient PCR reaction (Newton and Graham, 1994).

Polymerase chain reaction (PCR) is a highly sensitive and specific technique which has been successfully used to detect *M. paratuberculosis* DNA in Crohn's disease tissue (Sanderson *et al.,* 1992; Dell'Isola *et al.,* 1994; Lisby *et al.,* 1994; Fidler *et al.,* 1994). In this part of the study, PCR using primers directed against the multicopy IS900 DNA insertion element of the *M. paratuberculosis* genome (Green *et al.,* 1989) was carried out on DNA extracted from paraffin-embedded tissue sections of oral mucosal biopsies from 30 patients (24 male, 6 female; mean age 30.8 years, range 9-66 years) with OFG (and no evidence of other granulomatous or gastrointestinal disorders) and oral mucosal biopsies from 7 patients (6 male, 1 female; mean age 21.2 years, range 10-48 years) with biopsy-proven gastrointestinal Crohn's disease.

Twelve patients (6 male, 6 female; mean age 36 years, range 22-63 years) with a history of recurrent aphthous stomatitis (minor variant) attending the Department of Oral Medicine, Glasgow Dental Hospital & School NHS Trust, for investigation and

management of their oral disease acted as controls for this study. An aphthous ulcer was excised under local anaesthesia from each patient, following the signing of informed consent documentation. These tissue specimens were paraffin processed to ensure uniformity of tissue handling.

The expertise of Dr. Marcello Riggio, Lecturer in Molecular Biology, Glasgow Dental Hospital & School, is gratefully acknowledged. This work was carried out in his laboratory.

5.2.2 Tissue processing and DNA extraction

Samples were paraffin-embedded sections of oral tissue, which had been examined histopathologically and non-caseating granulomata identified. In each case the paraffin block showing the best demonstration of granulomata was selected for study. In all cases duplicate samples were obtained from the paraffin blocks, cut on two separate occasions.

For each sample to be analysed by PCR, five 10 μm sections were cut. The microtome knife blade was thoroughly cleaned between cutting of each different sample with xylene to prevent sample-to-sample contamination. The paraffin-embedded sections were placed in 1.5 ml centrifuge tubes and DNA was extracted using a method developed specifically for obtaining mycobacterial DNA from paraffin-embedded tissue sections as previously described (Cook *et al.,* 1994). Tissue sections were deparaffinised in xylene, resuspended in 200 μl proteinase K (200 μg/ml)/50mM Tris-HCl pH 8.3 and incubated overnight at 37°C. Samples were frozen in dry ice for 1 minute, boiled for 8 minutes, placed on ice for 5 minutes and centrifuged at 700rpm for 2 minutes to remove insoluble debris. 40 μl of the supernatant was used for each PCR reaction.

5.2.3 PCR Primers

The primers used for PCR (P90+ and P91+) targeted the IS900 DNA insertion element of *M. paratuberculosis* as previously described (Millar *et al.,* 1995), and were similar to primers P90 and P91 used in another study (Sanderson *et al.,* 1992) except that each primer contained an additional 6 or 7 bases at its 5' end. The primer sequences were 5'-GAAGGGTGTTCGGGGCCGTCGCTTAGG-3' (P90+; IS900 nucleotides 15-41) and 5'-GGCGTTGAGGTCGATCGCCCACGTGAC-3' (P91+; IS900 nucleotides 427-401).

The expected size of the amplification product using primer pair P90+/P91+ is 413 base pairs (bp). PCR was also used to generate an internal IS900 probe for use in subsequent Southern blot hybridisation. The sequences of the primers used for probe generation were 5'-CCAGGGACGTCGGGTATGGC-3' (P25; IS900 nucleotides 53-72) and 5'-GGTCGGCCTTACCGGCGTCC-3' (P26; IS900 nucleotides 281-262), which give an expected amplification product of 229 bp.

5.2.4 Nested PCR

PCR was carried out in a total reaction volume of 100 µl, with conditions essentially as previously described (Sanderson *et al.*, 1992; Millar *et al.*, 1995). Each PCR consisted of 10 µl of extracted DNA and 90 µl of PCR reaction mixture comprising 1 x PCR buffer (10 mM Tris-HCl, pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 1% Triton X-100), 2.0 U Dynazyme I DNA polymerase (Flowgen Instruments Ltd., Lichfield, UK), 0.2 mM of each of the four deoxynucleotide triphosphates and primers P90+ and P91+ each at 6 ng/µl. The primers were separated from the other components of the reaction mixture by a layer of wax (DynaWax; Flowgen Instruments Ltd.). This 'hot start' PCR method improves the specificity and yield of reaction products by preventing the reaction from starting until the wax has melted following the commencement of thermal cycling. PCR was carried out in an OmniGene thermal cycler (Hybaid Ltd., Teddington, UK). The cycling conditions comprised an initial denaturation step at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 5 minutes, primer annealing at 58°C for 2 minutes and extension at 72°C for 3 minutes, and a final extension step at 72°C for 10 minutes. A second round of PCR was then carried out using identical conditions, except that 5 µl of the first round product were used as a template (nested PCR).

For generation of the internal 229 bp probe, PCR was set up as described above except that a $MgCl_2$ concentration of 1.0 mM and the primer pair P25/P26 were used in a single round of PCR. Target DNA was 10 ng of plasmid pPN14 which contains the cloned *M paratuberculosis* IS900 DNA insertion element. After an initial denaturation step at 94°C for 5 minutes, 30 cycles of denaturation at 94°C for 1 minute, annealing of primers at 50°C for 1 minute and extension at 72°C for 2 minutes was carried out, followed by a final extension step at 72°C for 10 minutes. The 229 bp PCR product was purified using the Wizard PCR Preps Purification System (Promega Corporation, Southampton, UK).

5.2.5 Sensitivity of PCR assay

The sensitivity of the PCR assay was determined by spiking of DNA extracted from paraffin-embedded tissue sections of OFG, which were PCR-negative for *M. paratuberculosis* DNA, with serial ten-fold dilutions of *M. paratuberculosis* DNA in the range 100 pg to 1 fg. PCR was carried out as described above.

5.2.6 PCR quality control

Several anti-contamination procedures were employed when carrying out PCR. Setting up of PCR reactions, thermal cycling and post-PCR analysis of reaction products was carried out in separate rooms. Pipette filter tips were used at all stages, except when adding template DNA in which case positive displacement tips were used. Positive and negative PCR controls were included with each batch of samples being analysed; the positive control used was 1 pg of *M. paratuberculosis* DNA instead of sample, whereas the negative control contained sterile molecular biology grade water instead of sample.

In order to serve as an internal control for the successful isolation of PCR-amplifiable DNA from tissue sections, amplification of the ß-haemoglobin gene was carried out for each sample analysed using nested primer PCR as previously described (Frank *et al.*, 1992).

5.2.7 Agarose Gel Electrophoresis

PCR reaction products were fractionated by electrophoresis of 20 µl aliquots on 2% agarose gels containing ethidium bromide (0.5 µg/ml) and visualised under ultraviolet (UV) illumination. A 100 bp DNA ladder (Pharmacia Biotech, Milton Keynes, UK) was used as a size marker.

5.2.8 Southern Blot Hybridisation

Amplified products were electrophoresed on 2% agarose gels as described above and transferred to positively charged nylon membranes (Boehringer Mannheim, Lewes, UK)

by Southern blotting. Briefly, gels were prepared for blotting by soaking in denaturation solution (0.5 M NaOH/1.5 M NaCl) for 2 x 20 minutes followed by soaking in neutralisation solution (0.5 M Tris-HCl, pH 7.4/3.0 M NaCl) for 2 x 20 minutes. DNA was transferred to membranes using a capillary transfer blotting unit (Anachem Ltd., Luton, UK) with 20 x SSC (3.0 M NaCl, 0.3 M sodium citrate, pH 7.0) as transfer buffer. Following transfer, membranes were rinsed in 2 x SSC and DNA immobilised by exposure to an optimal dose of UV energy in a crosslinker (UVC-508, Anachem Ltd.).

Membranes were hybridised overnight at 68°C with the 229 bp internal IS900 PCR product labelled with digoxigenin (DNA Labelling and Detection Kit; Boehringer Mannheim) at 25 ng/ml in standard hybridisation buffer (5 x SSC, 1% blocking reagent, 0.1% N-laurylsarcosine, 0.02% sodium dodecyl sulphate [SDS]). Membranes were washed at room temperature in 2 x SSC/0.1% SDS for 2 x 5 minutes, and at 68°C in 0.1 x SSC/0.1 % SDS for 2 x 20 minutes. Immunological detection was carried out according to the manufacturer's instructions using an anti-digoxigenin antibody conjugated to alkaline phosphatase and colorimetric detection with 4-nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate as a colour substrate.

CHAPTER 6

STATISTICAL ANALYSIS

6.1 Statistical analysis

6.1.1 Software data handling

Statistical analysis was carried out using the Minitab® for Windows® PC package for data handling and statistical analysis.

6.1.2 Data analysis

Categorical data were analysed using the Chi-square test. Continuous variables showing normal distribution were analysed using the Analysis of Variance (ANOVA) test. Continuous variables not showing normal distribution were analysed using the Kruskal-Wallis test. One set of results, the lymphocyte sub-population study, was analysed using the student t-test.

6.1.3 Independent statistical analysis

Due to the large quantity of data collected, independent data analysis was deemed to be appropriate. This was carried out by staff at the Robertson Centre for Biostatistics, University of Glasgow. A logistic regression analysis was used to model the data as the outcome variable was essentially binary (namely, the patient had gastrointestinal Crohn's disease or not). The prime objective of the analysis was to obtain the best fitting and most parsimonious, yet biologically reasonable model to describe the relationship between outcome and the set of independent variables. The presence of gastrointestinal Crohn's disease versus not having Crohn's disease was used as the outcome measure. The patients with sarcoidosis (n=6) and Melkersson-Rosenthal syndrome (n=10) were excluded from the regression analysis due to the small numbers. Therefore, the patients studied either had Crohn's disease or allergic orofacial granulomatosis. Hence, if a patient failed to have the outcome event (Crohn's disease), by default the patient had orofacial granulomatosis.

CHAPTER 7

CLINICAL RESULTS I :
CLINICAL OBSERVATIONS

7.1 Introduction

Of the 301 patients, at the time of presentation, 20 had a pre-existing diagnosis of gastrointestinal Crohn's disease, and 10 had clinical features sufficient to make the diagnosis of complete Melkersson-Rosenthal syndrome. It was deemed inappropriate, for analytical purposes, to make the diagnosis of incomplete Melkersson-Rosenthal syndrome or cheilitis granulomatosa of Miescher.

Thus, for the purposes of analysis, four diagnostic groups emerged: orofacial granulomatosis, Crohn's disease, Melkersson-Rosenthal syndrome, and sarcoidosis. Patients were allocated to these diagnostic categories according to the clinical and laboratory-based criteria given in Chapter 3.

The following key will apply throughout the chapters containing results:

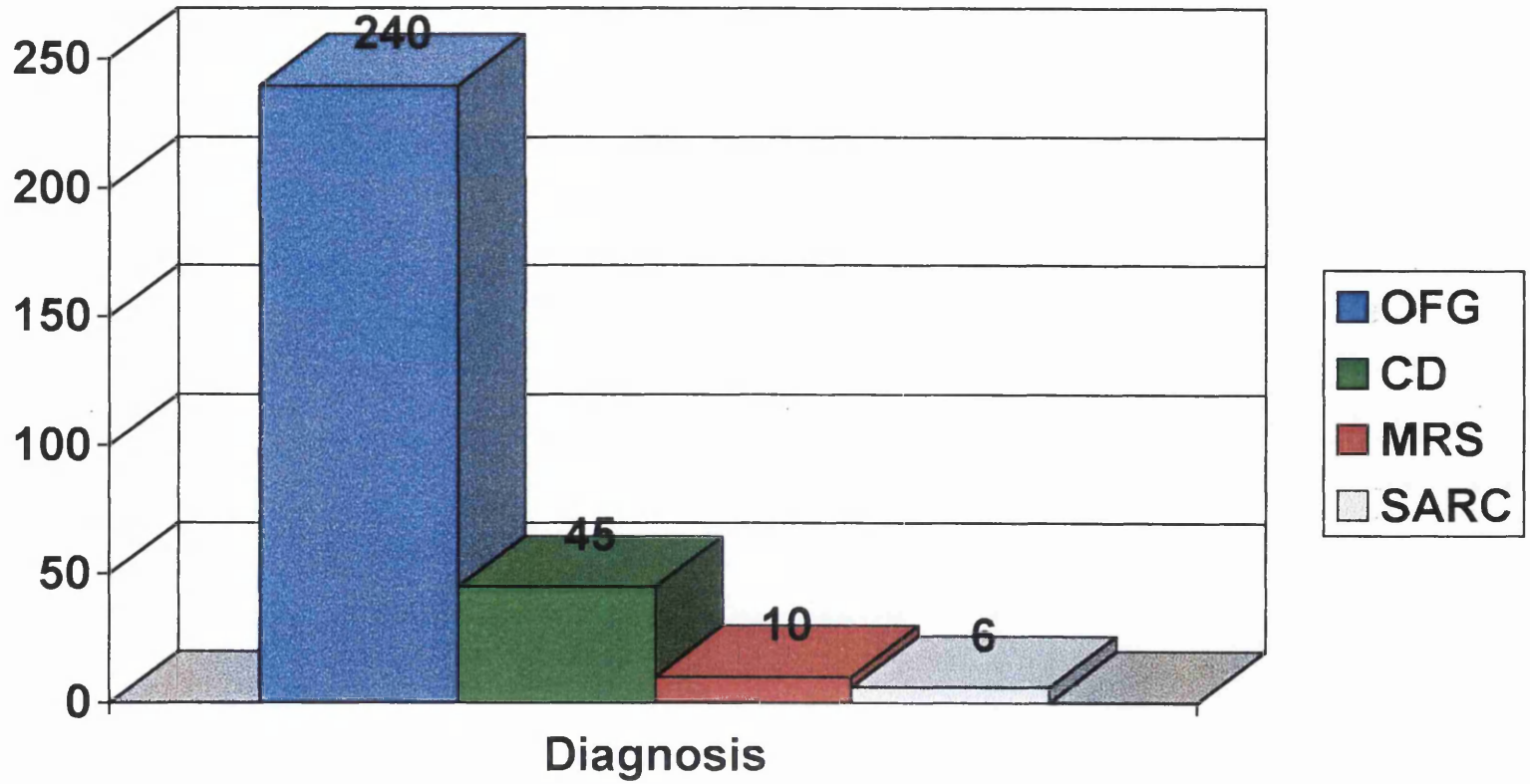
OFG	Orofacial Granulomatosis
CD	Gastrointestinal Crohn's disease
MRS	Melkersson-Rosenthal syndrome
SARC	Sarcoidosis
ALL	All patient groups together

Patient numbers were as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
No. of patients (%)	240 (79.7)	45 (15.0)	10 (3.3)	6 (2.0)	301 (100.0)

These data are presented graphically in Figure 7.1.

Figure 7.1 Patient numbers in each diagnostic category (absolute numbers)



7.2 Clinical history

7.2.1 Age

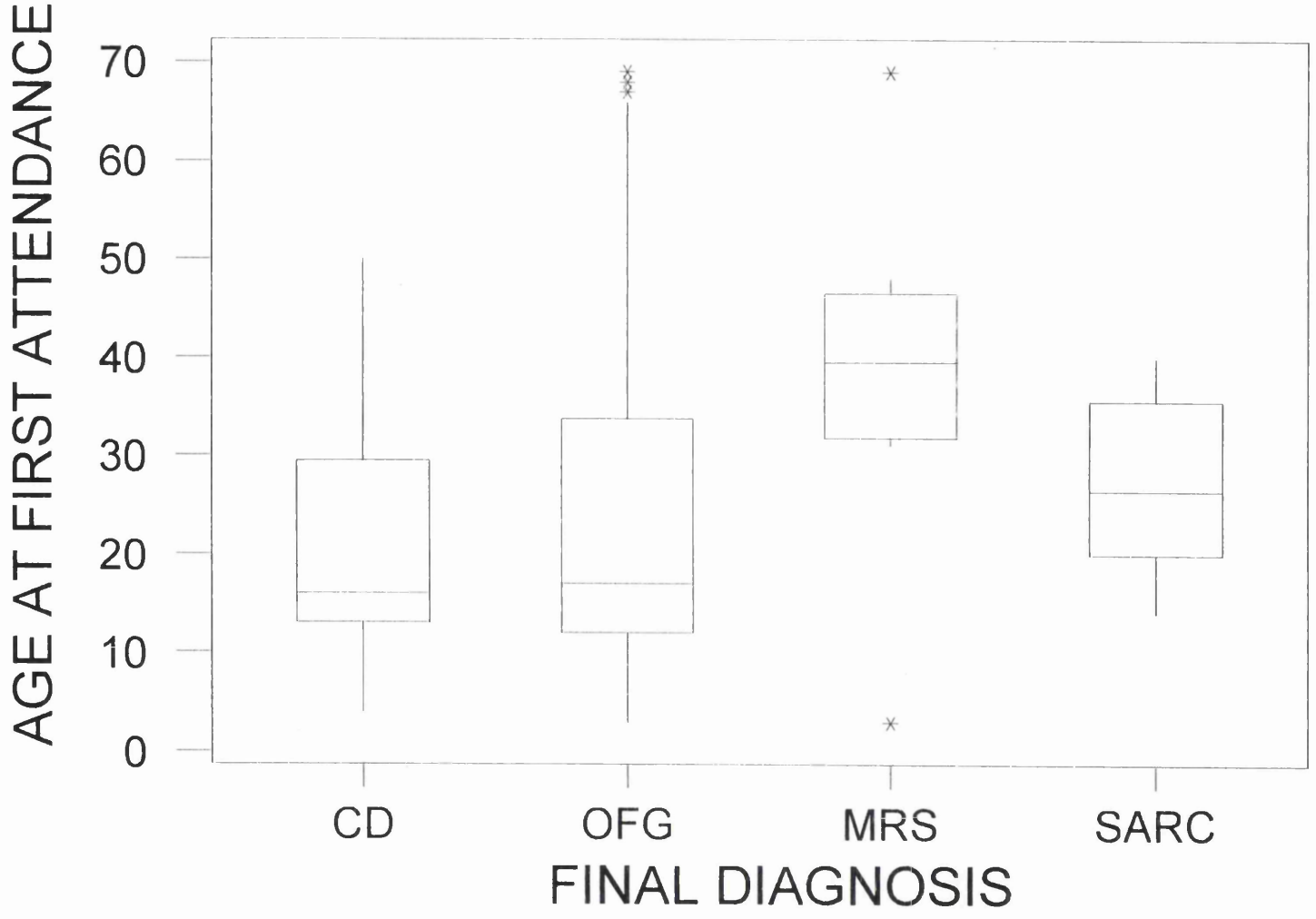
The ages of patients at presentation were as follows:

Diagnosis / **Age in years**	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Mean	24.0	20.8	38.5	27.2	24.0
Minimum	3.0	4.0	3.0	14.0	3.0
Maximum	69.0	50.0	69.0	40.0	69.0

This is represented graphically in Figure 7.2.

A Kruskal-Wallis statistical test was performed on these data with the following results: $H = 0.31$; $DF = 1$; $p = 0.579$. There is therefore no statistically significant difference between the disease groups (CD and OFG) in terms of age at presentation.

Figure 7.2 Age (in years) at first presentation, according to final diagnostic category



7.2.2 Gender

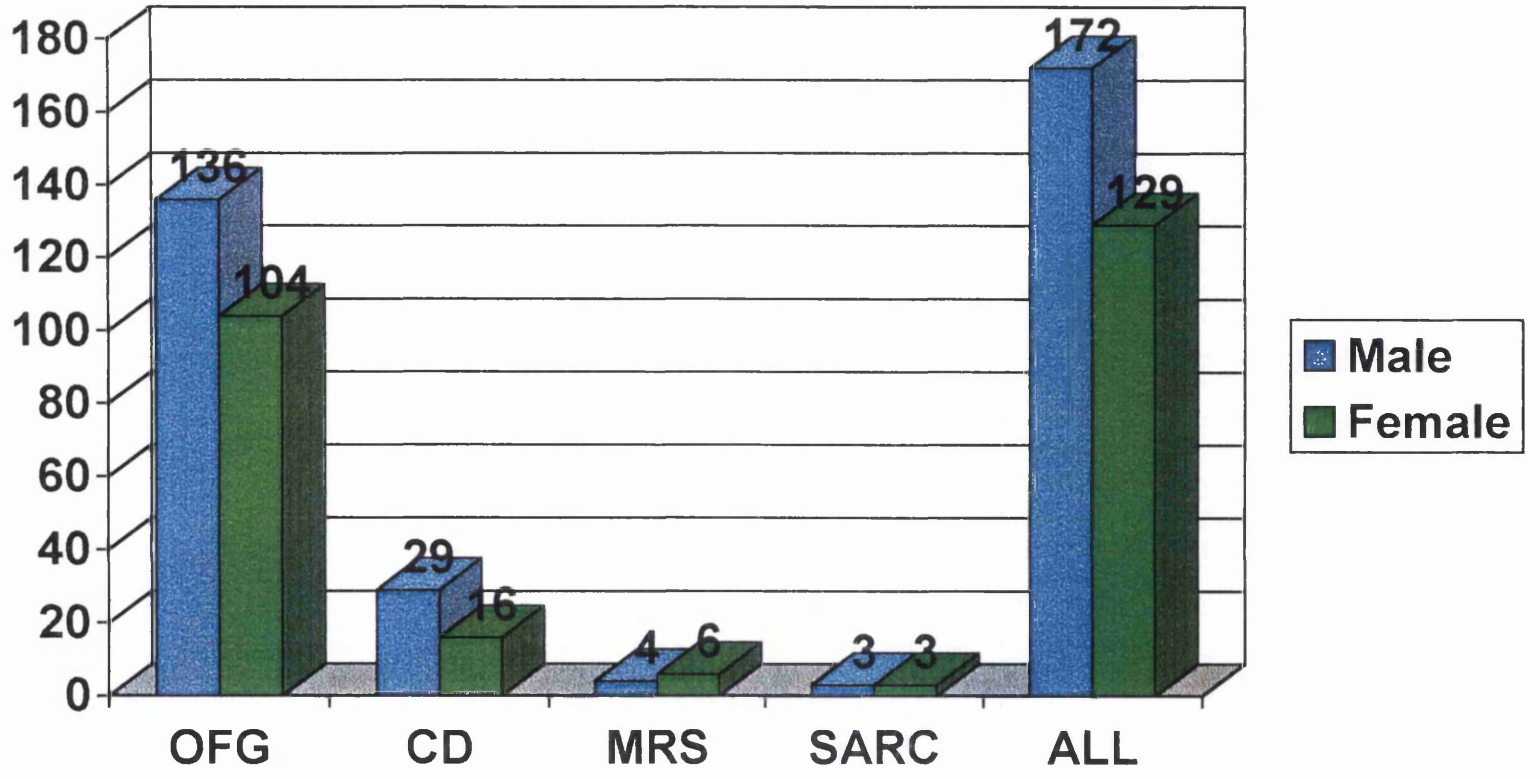
The gender of patients was as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Gender	**Patient Numbers (%)**				
Male	136 (56.7)	29 (64.4)	4 (40.0)	3 (50.0)	172 (57.1)
Female	104 (43.3)	16 (35.6)	6 (60.0)	3 (50.0)	129 (42.9)

This is represented graphically in Figure 7.3.

Chi-square analysis of these data gave a value of 0.940; DF = 1; p = 0.332. This indicated a normal distribution of gender within each disease category with no significant gender differences evident.

Figure 7.3 Patient gender (absolute patient numbers in each group)



7.2.3 Ethnic origin

The ethnic origin of patients was as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Ethnic Origin	**Patient Numbers (%)**				
White Caucasian	232 (96.7)	41 (91.1)	10 (100.0)	6 (100.0)	289 (96.0)
Asian	8 (3.3)	4 (8.9)	0 (0.0)	0 (0.0)	12 (4.0)

The data on ethnic origin were not included in overall statistical analysis due to the small numbers of ethnic groups other than Caucasians represented. The data were deemed to be simply representative of ethnic groupings within the study population (west of Scotland).

7.2.4 Geographical area

The geographical area of residence of each patient was as follows:

Diagnosis	OFG	CD	MRS	SARC	ALL
Health Board Area	**Patient Numbers (%)**				
England	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Grampian	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Fife	2 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)
Dumfries & Galloway	3 (1.3)	2 (4.4)	0 (0.0)	0 (0.0)	5 (1.7)
Lothian & Borders	5 (2.1)	1 (2.2)	0 (0.0)	0 (0.0)	6 (2.0)
Highlands & Islands	4 (1.7)	3 (6.7)	0 (0.0)	0 (0.0)	7 (2.3)
Forth Valley	26 (10.8)	6 (13.3)	1 (10.0)	0 (0.0)	33 (11.0)
Ayrshire & Arran	25 (10.4)	8 (17.8)	1 (10.0)	0 (0.0)	34 (11.3)
Lanarkshire	34 (14.2)	3 (6.7)	2 (20.0)	2 (33.3)	41 (13.6)
Argyll & Clyde	39 (16.3)	6 (13.3)	2 (20.0)	2 (33.3)	49 (16.3)
Greater Glasgow	100 (41.7)	16 (35.6)	4 (40.0)	2 (33.3)	122 (40.5)
TOTAL	240 (100.1)	45 (100.0)	10 (100.0)	6 (99.9)	301 (100.0)

The data on geographical location of patients were not included in the overall statistical analysis since referral patterns will influence the outcome. It is not known how many other Oral Medicine/Oral Surgery departments deal with patients with OFG in Scotland.

Similarly, any analysis of the data for possible geographical clustering would be biased since precise post-code data was not available on all patients. This recognises the potential variation in addresses within each Health Board area (e.g. Argyll and Clyde region stretches from Greenock in the south to Oban in the north, a distance of over 100 miles).

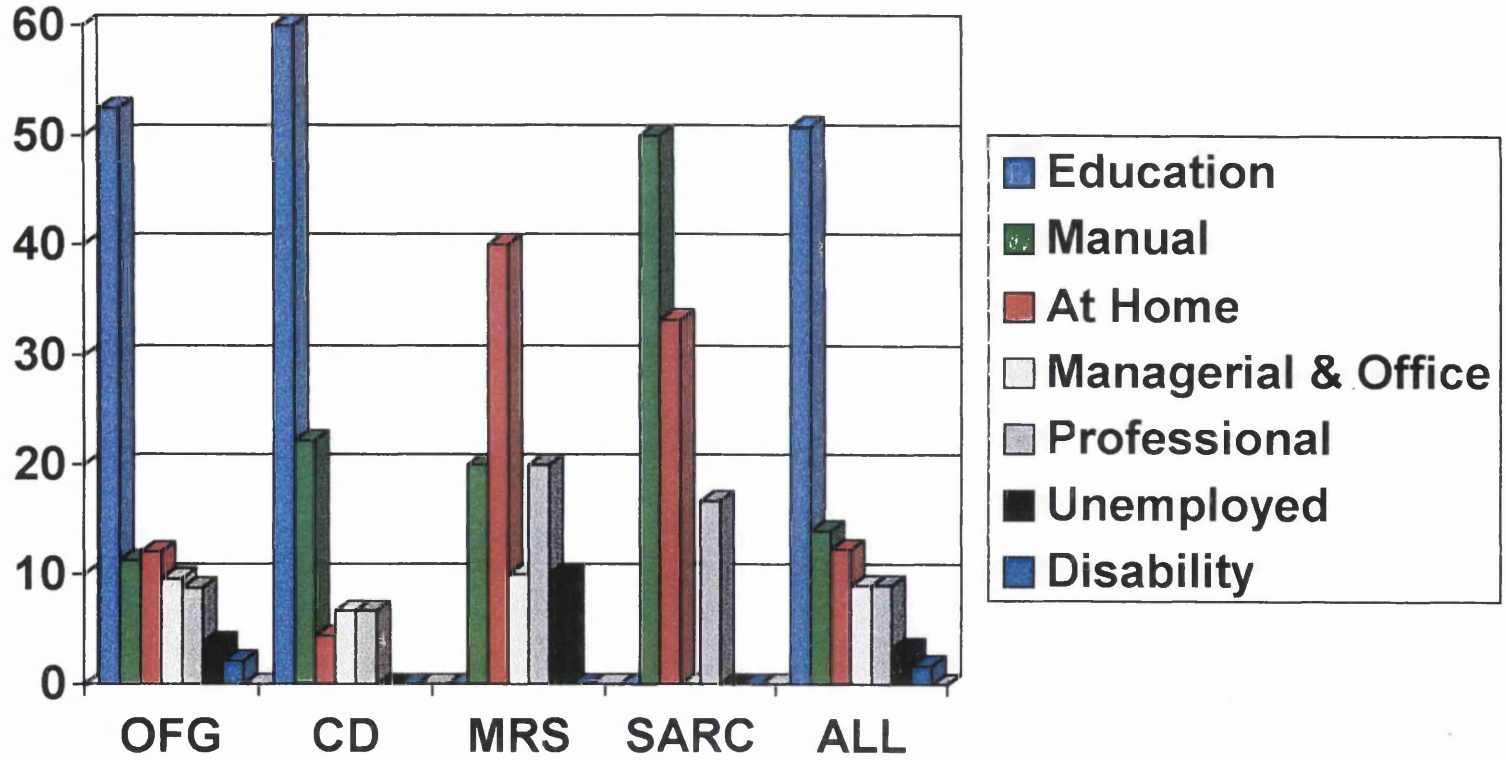
7.2.5 Occupation

The occupations of patients were as follows:

Diagnosis	OFG	CD	MRS	SARC	ALL
Occupational Category	**Patient Numbers (%)**				
Full-time education	126 (52.5)	27 (60.0)	0 (0.0)	0 (0.0)	153 (50.8)
Manual	27 (11.3)	10 (22.2)	2 (20.0)	3 (50.0)	42 (14.0)
At home (incl. Pre-school and retired)	29 (12.1)	2 (4.4)	4 (40.0)	2 (33.3)	37 (12.3)
Managerial & office	23 (9.6)	3 (6.7)	1 (10.0)	0 (0.0)	27 (9.0)
Professional	21 (8.8)	3 (6.7)	2 (20.0)	1 (16.7)	27 (9.0)
Unemployed	9 (3.8)	0 (0.0)	1 (10.0)	0 (0.0)	10 (3.3)
Disability	5 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)	5 (1.7)
TOTAL	240 (100.2)	45 (100.0)	10 (100.0)	6 (100.0)	301 (100.1)

These data are presented graphically in Figure 7.4. These data were not included in any statistical analysis since substantial numbers of patients (> 50%) were in full-time education and this fact is already represented in the age profile of patients (Section 7.2.1).

Figure 7.4 Patient occupation (% of group totals)



7.2.6 Past medical history

The past medical histories for each patient revealed that 20 had a pre-existing diagnosis of gastrointestinal Crohn's disease; all other diagnoses (OFG, CD, MRS and SARC) were made prospectively, following investigation and during follow-up.

7.2.7 Atopy

The term "atopy" was introduced by Coca and Cooke in 1923 from the Greek word meaning "out of place" to describe the difference between the anaphylactic animal and the allergic human (Brostoff and Scadding, 1991). Atopy is now used to describe the tendency of 10-15% of the UK population to suffer from allergic diseases such as asthma, eczema, hay fever, urticaria and demonstrable food allergy i.e. diseases associated with the production of specific IgE following exposure to low concentrations of allergen (Brostoff and Scadding, 1991).

For the purposes of this study, atopy was defined by a history of, or current evidence of, asthma, eczema, hay fever or urticaria.

The atopic status of patients was as follows:

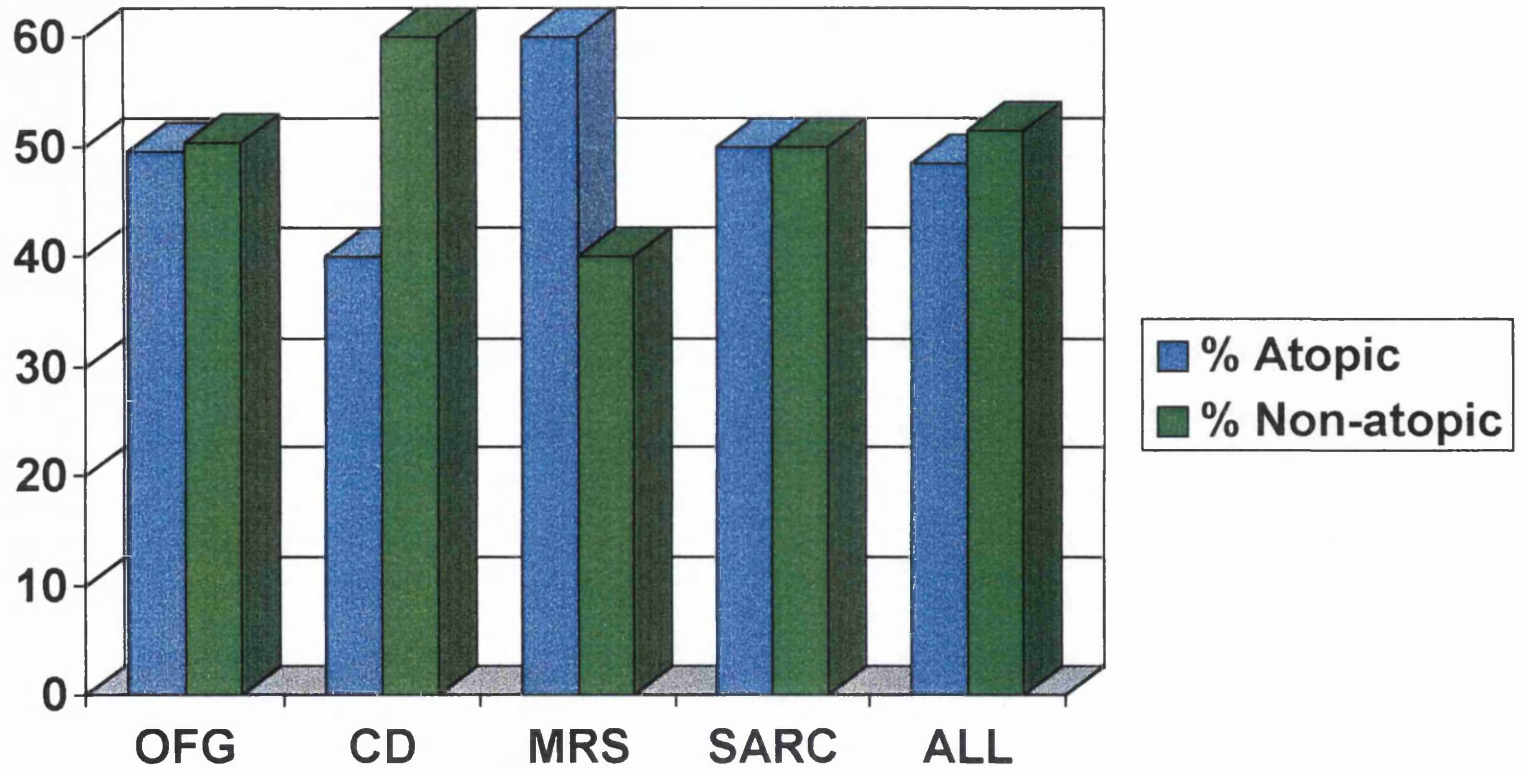
Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Atopic (%)	119 (49.6)	18 (40.0)	6 (60.0)	3 (50.0)	146 (48.5)
Non-atopic (%)	121 (50.4)	27 (60.0)	4 (40.0)	3 (50.0)	155 (51.5)

Chi-square analysis of the data gave a value of 1.394; DF = 1; p = 0.238. This indicated no differences in the atopic status of each patient group.

These data are presented graphically in Figure 7.5.

These data are at variance with that of James *et al* (1986) who found 60% of a group of 75 patients with OFG to be clinically atopic compared with 15% of a control group of 200 from the same geographical area (west of Scotland). Similarly, the figure of 40% of the CD group being atopic is substantially higher than the 15% area average and higher than the figure from other CD studies.

Figure 7.5 Atopic status of patients in each disease group (% of group totals)



7.2.8 Associated gastrointestinal symptoms

The associated symptoms revealed by systematic enquiry were as follows:

(I) Weight loss

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Weight loss (%)	9 (3.8)	11 (24.4)	0 (0.0)	0 (0.0)	20 (6.6)
No weight loss (%)	231 (96.3)	34 (75.6)	10 (100.0)	6 (0.0)	281 (93.4)

Chi-square = 24.871; DF = 1; p = 0.000

These data are presented graphically in Figure 7.6.

(II) Altered bowel habit

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Altered bowel habit (%)	16 (6.7)	25 (55.6)	4 (40.0)	0 (0.0)	45 (15.0)
No altered bowel habit (%)	224 (93.3)	20 (44.4)	6 (60.0)	6 (100.0)	256 (85.0)

Chi-square = 73.539; DF = 1; p = 0.000

These data are presented graphically in Figure 7.7.

Figure 7.6 Weight loss recorded by patients (% of patients in each group)

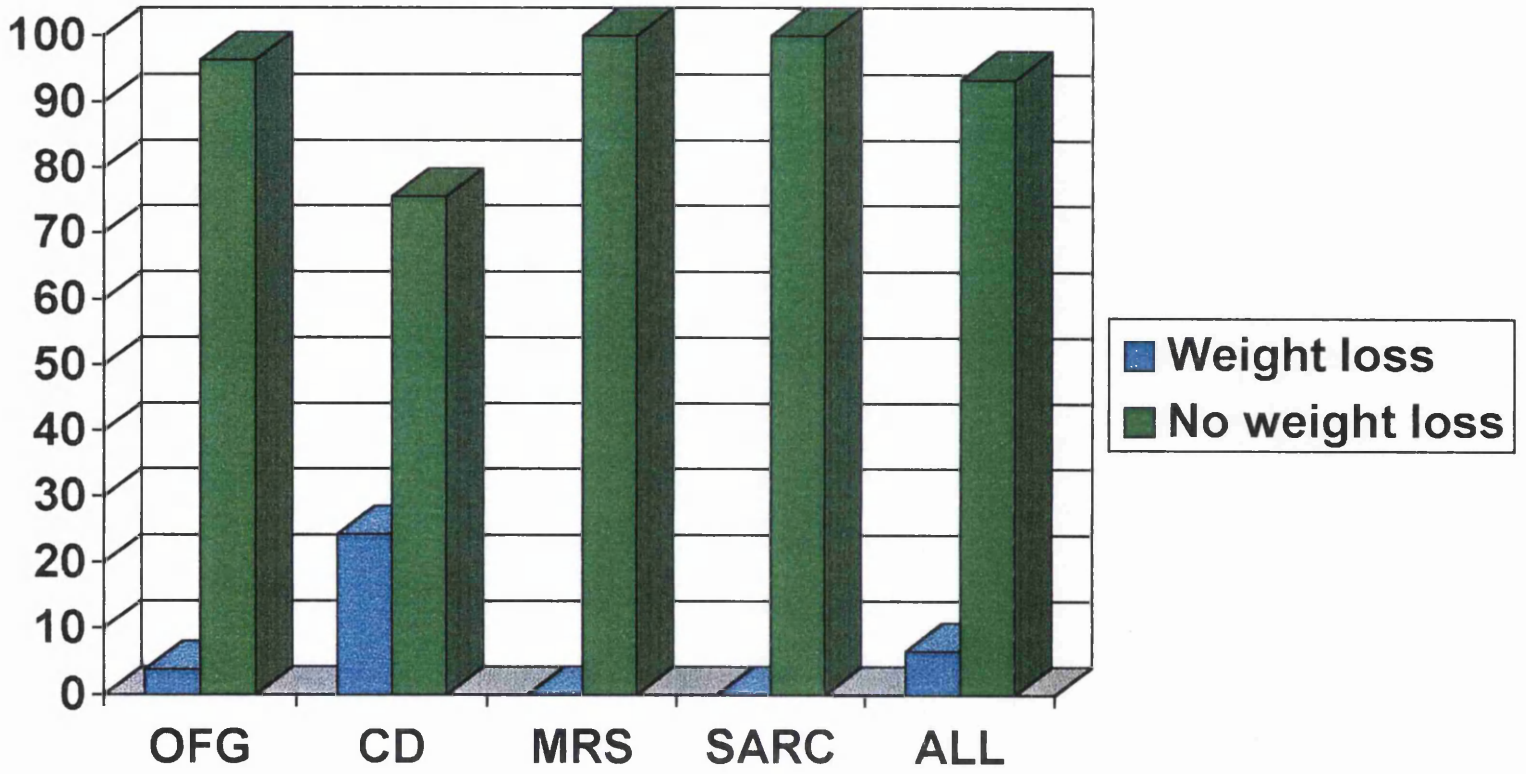
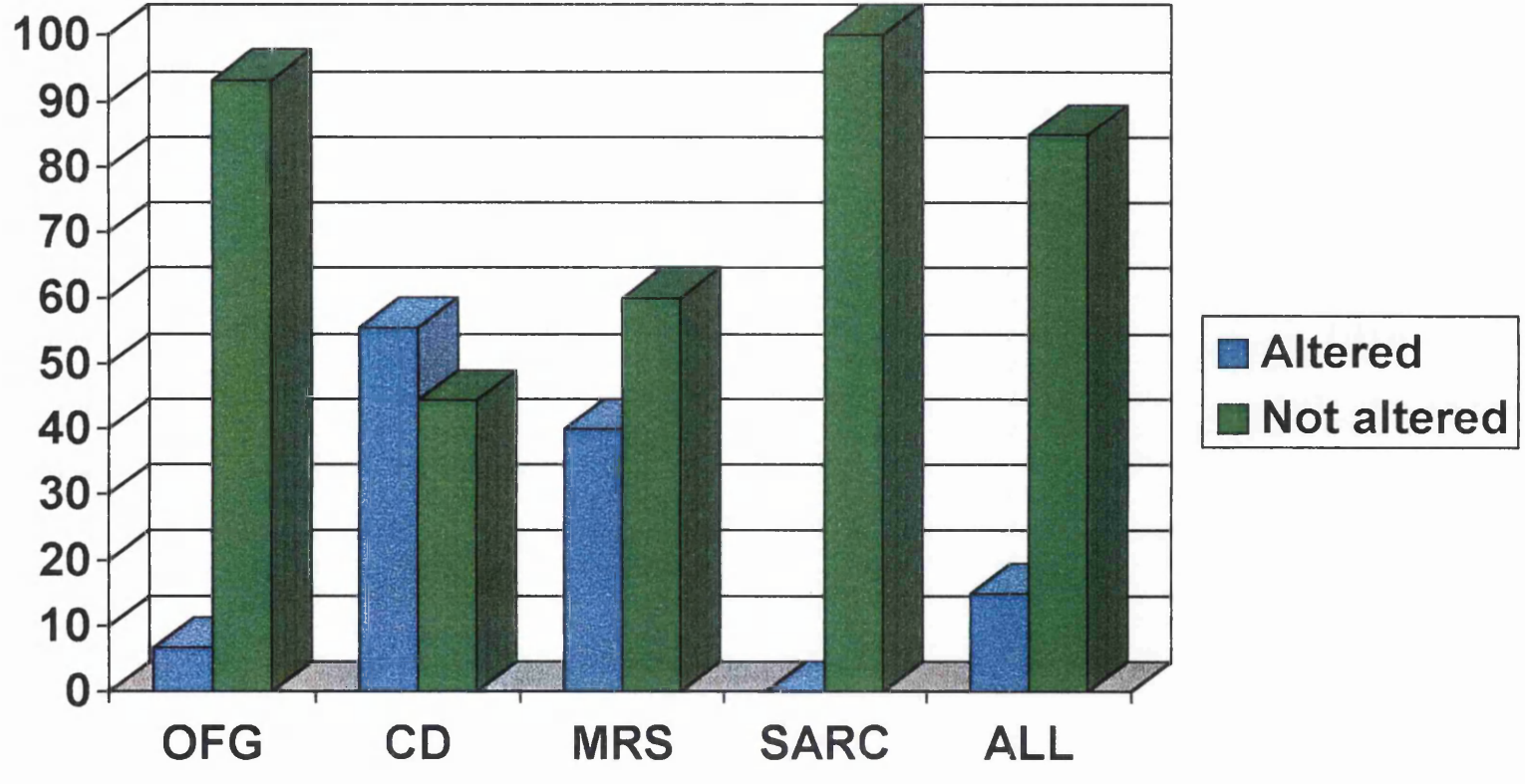


Figure 7.7 Altered bowel habits recorded by patients (% of patients in each group)



(III) Number of stools passed daily:

Diagnosis	OFG	CD	MRS	SARC	ALL
No. of stools per day	Patient Numbers (%)				
0	0 (0.0)	2 (4.4)	0 (0.0)	0 (0.0)	2 (0.7)
1	227 (94.6)	18 (40.0)	6 (60.0)	6 (100.0)	257 (85.4)
2	8 (3.3)	5 (11.1)	0 (0.0)	0 (0.0)	13 (4.3)
3	4 (1.7)	13 (28.9)	2 (20.0)	0 (0.0)	19 (6.3)
4	1 (0.4)	1 (2.2)	1 (10.0)	0 (0.0)	3 (1.0)
5	0 (0.0)	3 (6.7)	1 (10.0)	0 (0.0)	4 (1.3)
6	0 (0.0)	3 (6.7)	0 (0.0)	0 (0.0)	3 (1.0)

Kruskal-Wallis analysis gave the following values:

$H = 69.79$; $DF = 1$; $p = 0.000$

(IV) Bleeding *per rectum* (fresh blood reported):

Diagnosis	OFG	CD	MRS	SARC	ALL
Bleeding PR (%)	6 (2.5)	10 (22.2)	0 (0.0)	0 (0.0)	16 (5.3)
No bleeding PR (%)	234 (97.5)	35 (77.8)	10 (100.0)	6 (100.0)	285 (94.7)

Chi-square = 27.817; DF = 1; p = 0.000

These data are presented graphically in Figure 7.8.

(V) Abdominal pain:

Diagnosis	OFG	CD	MRS	SARC	ALL
Abdominal pain (%)	5 (2.1)	19 (42.2)	2 (20.0)	0 (0.0)	26 (8.6)
No abdominal pain (%)	235 (97.9)	26 (57.8)	8 (80.0)	6 (100.0)	275 (91.4)

Chi-square = 79.168; DF = 1; p = 0.000

These data are presented graphically in Figure 7.9.

Figure 7.8 Bleeding *per rectum* recorded by patients (% of patients in each group)

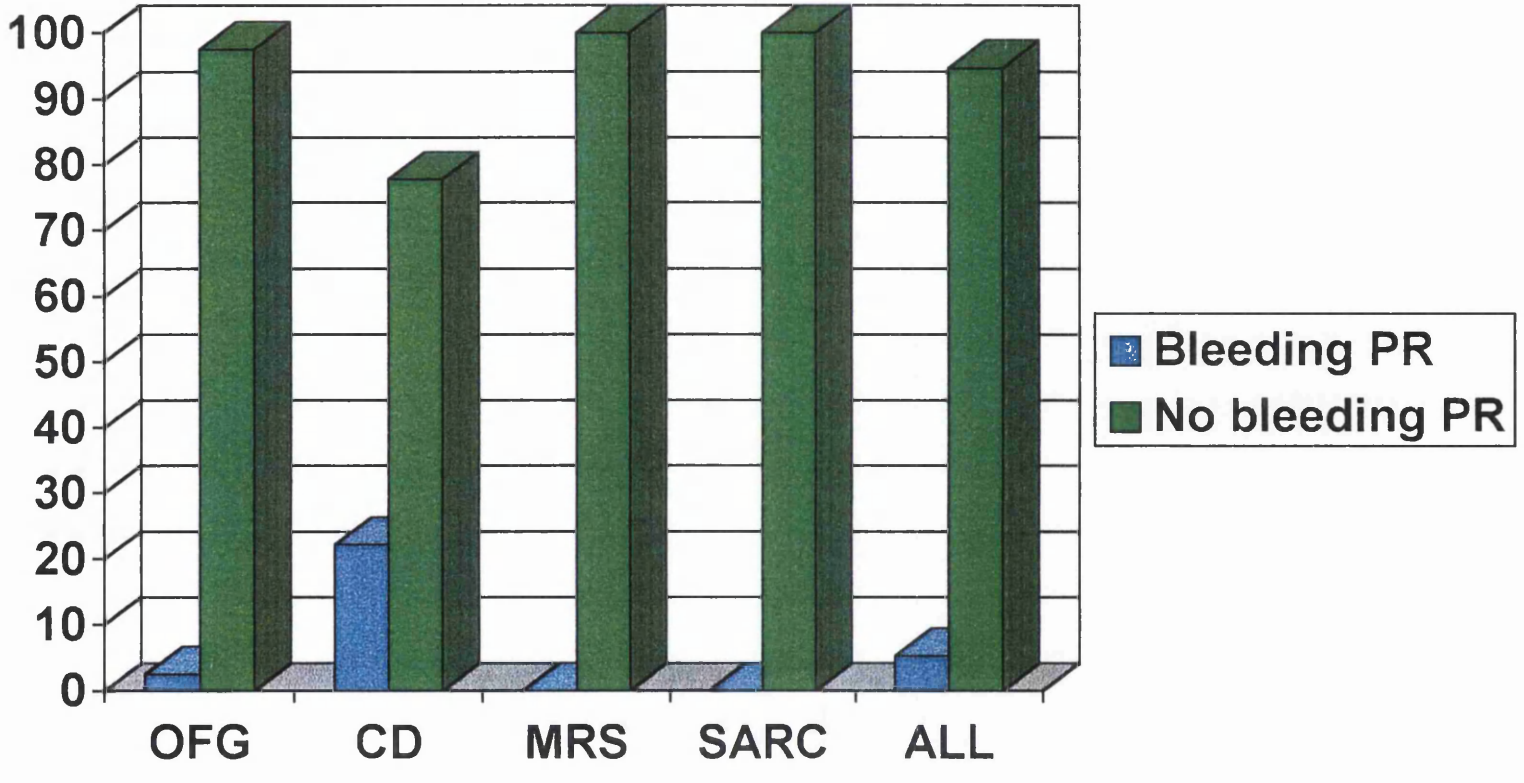
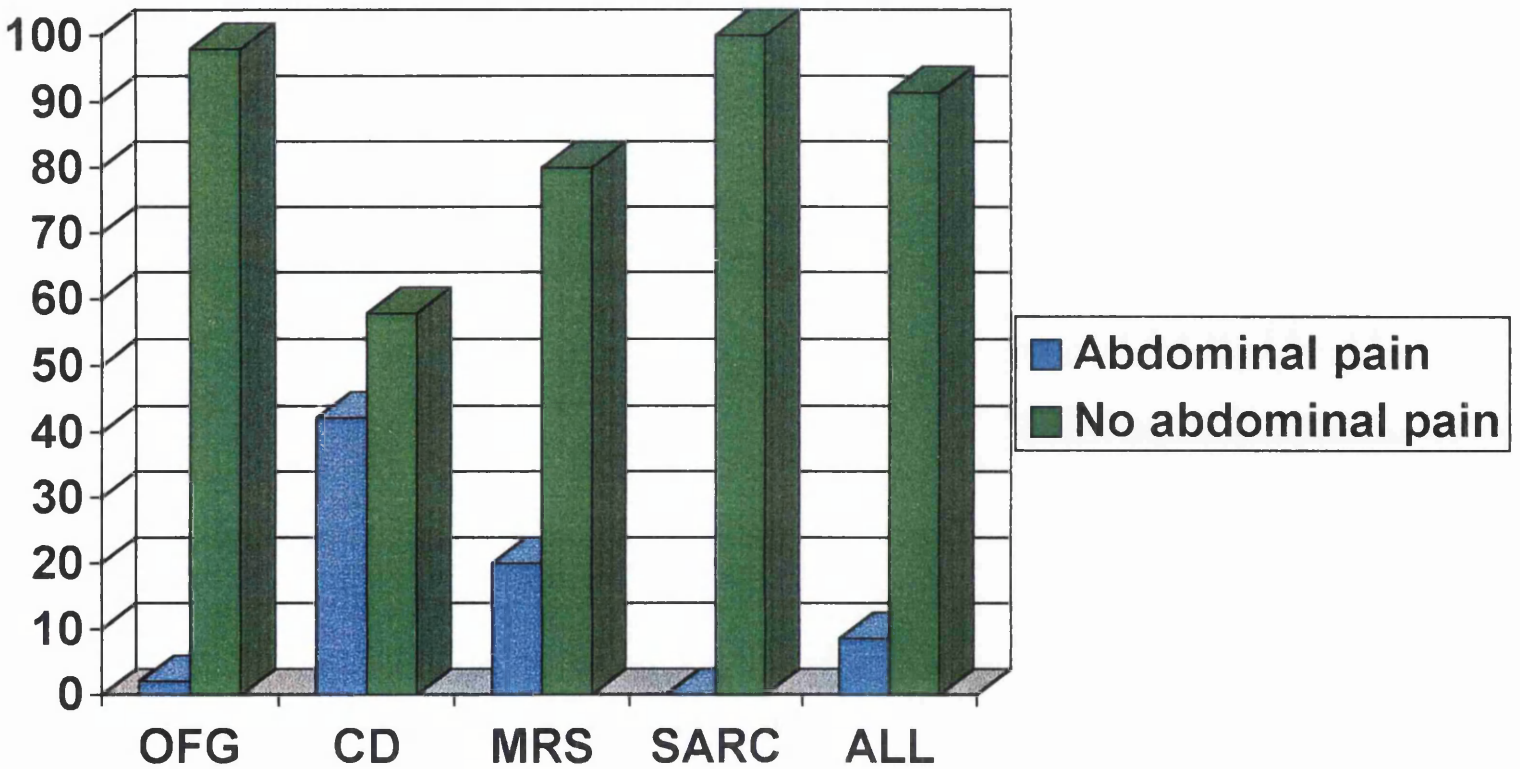


Figure 7.9 Abdominal pain recorded by patients (% of patients in each group)



7.2.9 Social habits

(I) Smoking:

The smoking habits of each patient group was recorded as follows:

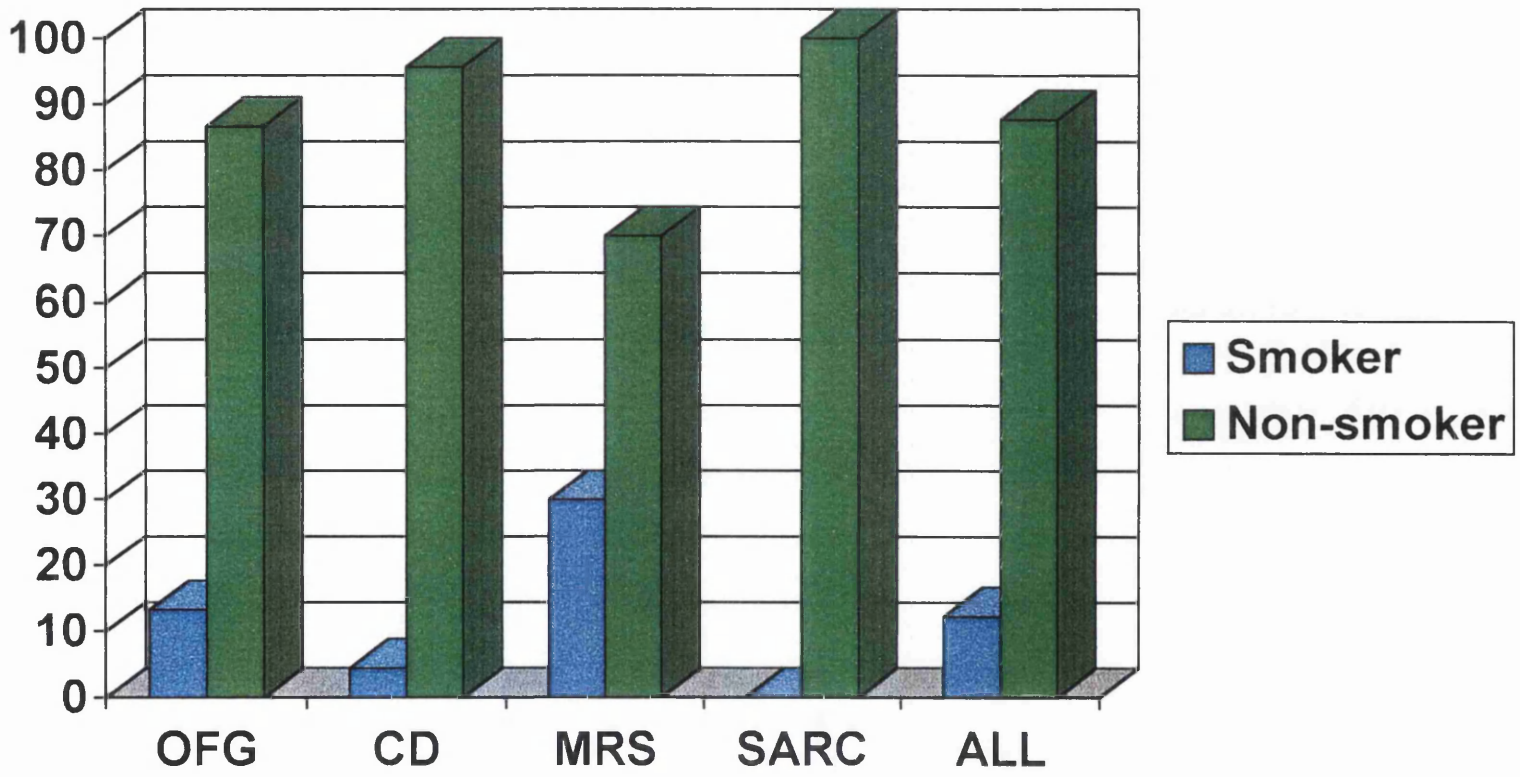
Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Smoker (%)	32 (13.3)	2 (4.4)	3 (30.0)	0 (0.0)	37 (12.3)
Non-smoker (inc. ex-smoker) (%)	208 (86.7)	43 (95.6)	7 (70.0)	6 (100.0)	264 (87.7)

Chi-square = 2.850; DF = 1; p = 0.91

These results are presented graphically in Figure 7.10.

Given the age profile of the patients in the study, perhaps these results are not surprising. Since the vast majority in each patient group was non-smoking, it is proposed that smoking plays little role in the individual disease processes, except for the patients in whom cigarette smoke was identified as an allergen (see Section 7.8).

Figure 7.10 Smoking habits of patients (% of patients in each group)



(II) Alcohol:

The alcohol consumption of each patient group was recorded as follows:

Diagnosis	OFG	CD	MRS	SARC	ALL
No. of units of alcohol per week	Patient Numbers (%)				
No record	2 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)
0	187 (78.0)	37 (82.2)	5 (50.0)	4 (66.7)	233 (77.4)
2	1 (0.4)	0 (0.0)	1 (10.0)	0 (0.0)	2 (0.7)
3	0 (0.0)	1 (2.2)	0 (0.0)	0 (0.0)	1 (0.3)
4	2 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)
5	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
10	3 (1.2)	1 (2.2)	1 (10.0)	0 (0.0)	5 (1.7)
12	4 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	4 (1.3)
14	15 (6.2)	3 (6.7)	2 (20.0)	2 (33.3)	22 (7.3)
15	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
21	24 (10.0)	1 (2.2)	0 (0.0)	0 (0.0)	25 (8.3)
25	0 (0.0)	1 (2.2)	1 (10.0)	0 (0.0)	2 (0.7)
60	0 (0.0)	1 (2.2)	0 (0.0)	0 (0.0)	1 (0.3)

7.2.10 Clinical complaint

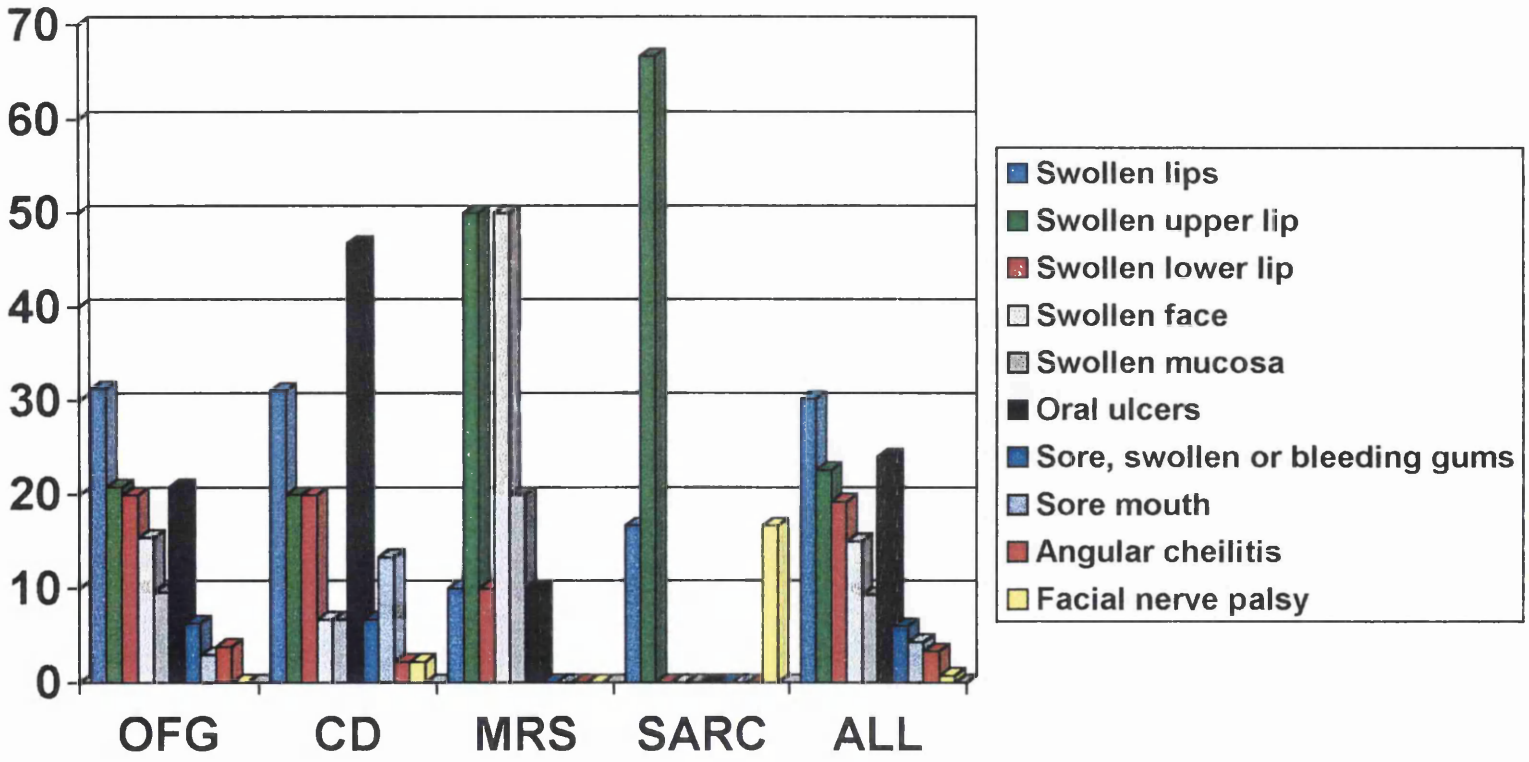
The patients' original clinical complaints (i.e. at first presentation) were recorded as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Clinical complaint	**Patient Numbers (%)**				
Swollen lips (upper and lower)	75 (31.3)	14 (31.1)	1 (10.0)	1 (16.7)	91 (30.2)
Swollen upper lip	50 (20.8)	9 (20.0)	5 (50.0)	4 (66.7)	68 (22.6)
Swollen lower lip	48 (20.0)	9 (20.0)	1 (10.0)	0 (0.0)	58 (19.3)
Swollen face	37 (15.4)	3 (6.7)	5 (50.0)	0 (0.0)	45 (15.0)
Swollen mucosa	23 (9.6)	3 (6.7)	2 (20.0)	0 (0.0)	28 (9.3)
Oral ulcers	50 (20.8)	21 (46.7)	1 (10.0)	0 (0.0)	72 (24.0)
Sore, swollen or bleeding gums	15 (6.3)	3 (6.7)	0 (0.0)	0 (0.0)	18 (6.0)
Sore mouth	7 (2.9)	6 (13.3)	0 (0.0)	0 (0.0)	13 (4.3)
Angular cheilitis	9 (3.8)	1 (2.2)	0 (0.0)	0 (0.0)	10 (3.3)
Facial nerve palsy	0 (0.0)	1 (2.2)	0 (0.0)	1 (16.7)	2 (0.7)
TOTAL	314	70	15	6	405

These data are presented graphically in Figure 7.11 and analysed statistically in Section 7.2.11.

It is evident from the Table that none of the ten patients labelled as MRS recorded a facial palsy as the main problem at presentation. However, their histories, verified from other documented sources, confirmed that facial palsies had been part of their disease experience, either as single or repeated events.

Figure 7.11 Clinical complaint (% of patients in each group)



7.2.11 Symptom scores

The patients' initial symptom scores (i.e. at first presentation), using a possible score from 0 to 10 on a Visual Linear Analogue Scale, were recorded as follows:

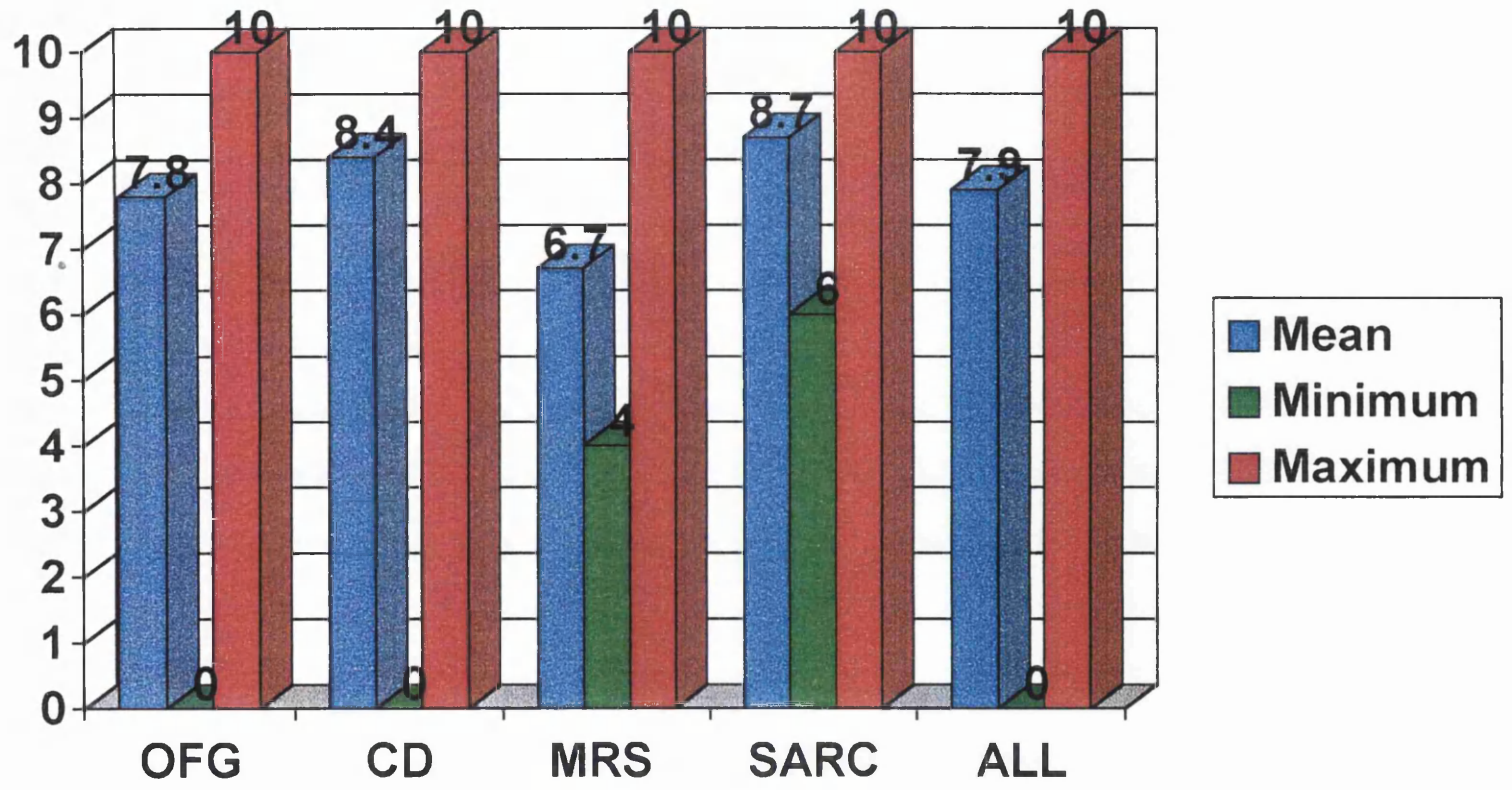
Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Original symptom score (0-10)					
Mean	7.8	8.4	6.7	8.7	7.9
Minimum	0	0	4	6	0
Maximum	10	10	10	10	10

Kruskal-Wallis analysis gave the following results:

$H = 2.98$; $DF = 1$; $p = 0.069$

These data are presented graphically in Figure 7.12.

Figure 7.12 Initial symptom scores (absolute values for each patient group)



7.3 Physical examination

7.3.1 Findings

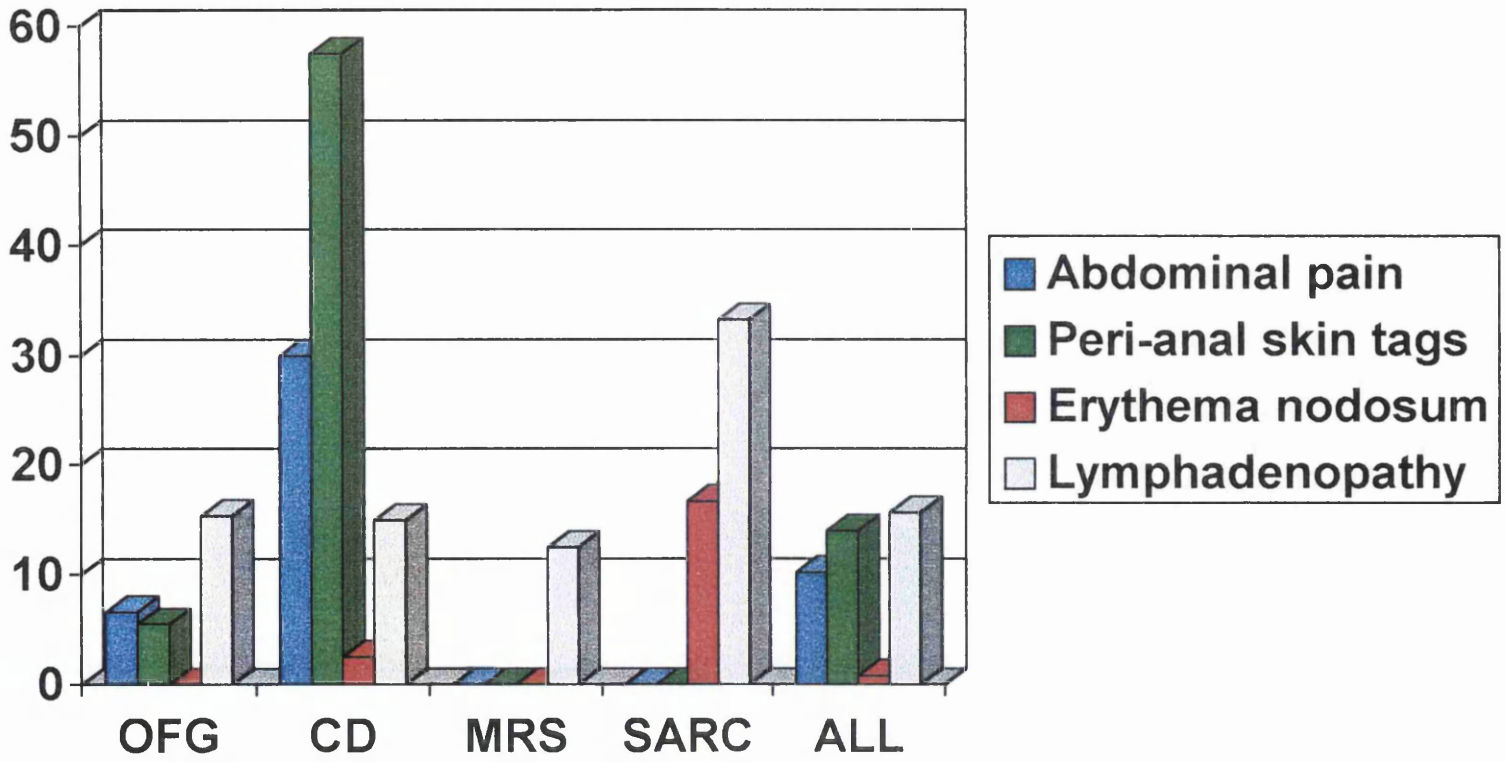
7.3.1.1 Systematic examination

Systematic examination of patients was largely unrewarding in terms of clinical signs elicited. Summary data is as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
No. of patients (%)	182 (75.8)	40 (88.9)	8 (80.0)	6 (100.0)	236 (78.4)
Clinical sign	**Patient Numbers (%)**				
Abdominal pain	12 (6.6)	12 (30.0)	0 (0.0)	0 (0.0)	24 (10.2)
Peri-anal skin tags	10 (5.5)	23 (57.5)	0 (0.0)	0 (0.0)	33 (14.0)
Erythema nodosum	0 (0.0)	1 (2.5)	0 (0.0)	1 (16.7)	2 (0.8)
Lymphaden-opathy	28 (15.4)	6 (15.0)	1 (12.5)	2 (33.3)	37 (15.7)

These data are presented graphically in Figure 7.13.

Figure 7.13 Findings on clinical examination (% of patients in each group)



7.3.1.2 Staphylococcal mucositis

During the course of the study, four patients (2 OFG; 2 CD), three male and one female, presented with severe non-ulcer oral mucosal discomfort. Examination findings were as follows:

Patient 1 - Crohn's disease
Panoral mucosal erythema and bilateral angular cheilitis; on Prednisolone 10mg/day and ferrous sulphate.

Patient 2 - OFG
Panoral mucosal erythema and bilateral angular cheilitis; no current medication.

Patient 3 - OFG
Panoral mucosal erythema; no angular cheilitis; no current medication.

Patient 4 - Crohn's disease
Panoral mucosal erythema and unilateral angular cheilitis; taking Prednisolone 10mg/day.

The appearance was highly suggestive of a radiation-induced mucositis and so microbiological investigations were instituted. Assessment of the oral flora was carried out using the oral rinse technique, whereby the patient was supplied with 10ml of sterile phosphate buffered saline (PBS: 0.1M, pH 7.2) in a universal container and requested to rinse the mouth vigorously for 60 seconds (Samaranayake *et al.*, 1986). The patient then returned the mouth rinse to the universal container which was sent the Oral Microbiology Laboratory, Glasgow Dental Hospital & School for microbiological analysis.

The assistance in this part of the study of the consultant and technical staff of the Oral Microbiology Laboratory is gratefully acknowledged.

The oral rinse was concentrated by centrifugation for 10 minutes at 1,700G. The deposit was resuspended in 1ml of sterile PBS and the resultant solution mechanically dispensed

onto appropriate media by a spiral plater (Spiral Systems Marketing Ltd, Maryland, USA) in an Archimedian spiral. The spiral plater delivered 25µl of each rinse sample onto the following media: Sabouraud's dextrose agar (candida count), mannitol salt agar (*S. aureus* count) and MacConkey's agar (coliform count).

The Sabouraud's plates were incubated aerobically for 48 hours while the other plates were incubated aerobically for 24 hours at 37°C. The number of colony forming units (cfu) of yeasts or bacteria in each plate was enumerated using a Gallenkamp Colony Counter (Gallenkamp, Leicestershire, England) and assessed by a Consultant Microbiologist. The counts were reported as mild, moderate or heavy growths of the relevant organism. Coliforms and *Candida* species were identified by standard sugar assimilation and fermentation techniques respectively while Staphylococci were identified using the coagulase test.

Results and outcome were as follows:

Patient 1 - Oral rinse: heavy growth of *Staphylococcus aureus*.
Treated with Flucloxacillin (250mg four times daily) orally with prompt resolution of oral symptoms and signs.

Patient 2 - Oral rinse: heavy growth of *Staphylococcus aureus* and a scanty growth of a Group B beta-haemolytic streptococcus.
Treated with Erythromycin (250mg four times daily) orally with prompt resolution of symptoms and signs.

Patient 3 - Oral rinse: heavy growth of *Staphylococcus aureus*.
Treated with Flucloxacillin (250mg four times daily) orally with prompt resolution of oral symptoms and signs.

This patients symptoms and signs returned four weeks later and the patient's mother was unhappy for a further course of antibiotics to be prescribed. The patient was then left untreated for two weeks until his symptoms prompted further assessment. The symptoms and signs resolved promptly with a further course of Flucloxacillin (250mg four times daily) orally.

Patient 4 - heavy growth of *Staphylococcus aureus* and *Hafnia alvei.*
Treated with Flucloxacillin (250mg four times daily) orally with prompt resolution of oral symptoms and signs.

These four cases would appear to be the first documented events in the world literature of staphylococcal mucositis in patients with OFG and Crohn's disease. The CD cases were both taking immunosuppressive drugs in the form of oral prednisolone - which may predispose to the development of bacterial infections. However, the two cases of OFG were taking no form of medication prior to presentation. None of the four cases had any demonstrable defects in their full blood counts.

7.3.2 Sign scores

The patients' initial sign scores in each group were recorded as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Clinical sign	**Patient Numbers (%)**				
Angular cheilitis (L)	65 (27.1)	12 (26.7)	0 (0.0)	0 (0.0)	77 (25.6)
Angular cheilitis (R)	67 (28.0)	13 (28.9)	0 (0.0)	0 (0.0)	80 (26.6)
Angular cheilitis (bilateral)	64 (26.7)	12 (26.7)	0 (0.0)	3 (50.0)	79 (26.2)
Upper lip swelling	119 (49.6)	23 (51.1)	6 (60.0)	6 (100.0)	154 (51.2)
Lower lip swelling	128 (53.3)	25 (55.6)	2 (20.0)	2 (33.3)	157 (52.2)
Facial swelling	66 (27.5)	7 (15.6)	5 (50.0)	3 (50.0)	81 (27.0)
Aphthous ulceration	85 (35.4)	31 (68.9)	2 (20.0)	0 (0.0)	118 (39.2)
Non-aphthous ulceration	6 (2.5)	18 (40.0)	0 (0.0)	0 (0.0)	24 (8.0)
Full-thickness gingivitis	71 (29.6)	19 (42.2)	0 (0.0)	1 (16.7)	91 (30.2)
Mucosal tags	73 (30.4)	19 (42.2)	2 (20.0)	0 (0.0)	94 (31.2)

Diagnosis	OFG	CD	MRS	SARC	ALL
Clinical sign	**Patient Numbers (%)**				
Mucosal oedema	107 (44.6)	33 (73.3)	5 (50.0)	2 (33.3)	147 (48.8)
Fissured tongue	21 (8.8)	3 (6.7)	10 (100.0)	0 (0.0)	34 (11.3)
Papillary hyperplasia	12 (5.0)	8 (17.8)	1 (10.0)	1 (16.7)	22 (7.3)
Facial palsy (lower motor)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (0.3)
TOTAL	884	223	33	19	1159

In addition to the lower motor neurone facial palsy recorded in the table in one patient with sarcoidosis as a genuine sign evident on examination, the histories of all ten patients with MRS, two patients with CD, and two patients with OFG were sufficiently convincing to include them as part of the scoring system since they were documented as observed events in their case notes.

Chi-square statistical analysis revealed the following results for each of the above parameters, comparing OFG and CD:

Clinical Finding	Chi-square	DF	p-value
Angular cheilitis	0.000	1	1.000
Upper lip swelling	0.035	1	0.851
Lower lip swelling	0.075	1	0.784
Facial swelling	2.838	1	0.092
Ulceration - aphthoid	17.591	1	0.000*
Ulceration - non-aphthoid	69.100	1	0.000*
Full-thickness gingivitis	2.802	1	0.094
Mucosal tags	2.416	1	0.120
Mucosal oedema	12.533	1	0.000*
Fissured tongue	0.213	1	0.644
Papillary hyperplasia	9.482	1	0.002*

* statistically significant at the 5% level.

The patients' initial sign scores at first presentation (mean, minimum and maximum), using a possible score from 0 to 16, were recorded as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Original sign score (0-16)					
Mean	3.7	5.0	3.8	3.8	3.9
Minimum	0	1	2	1	0
Maximum	11	13	6	6	13

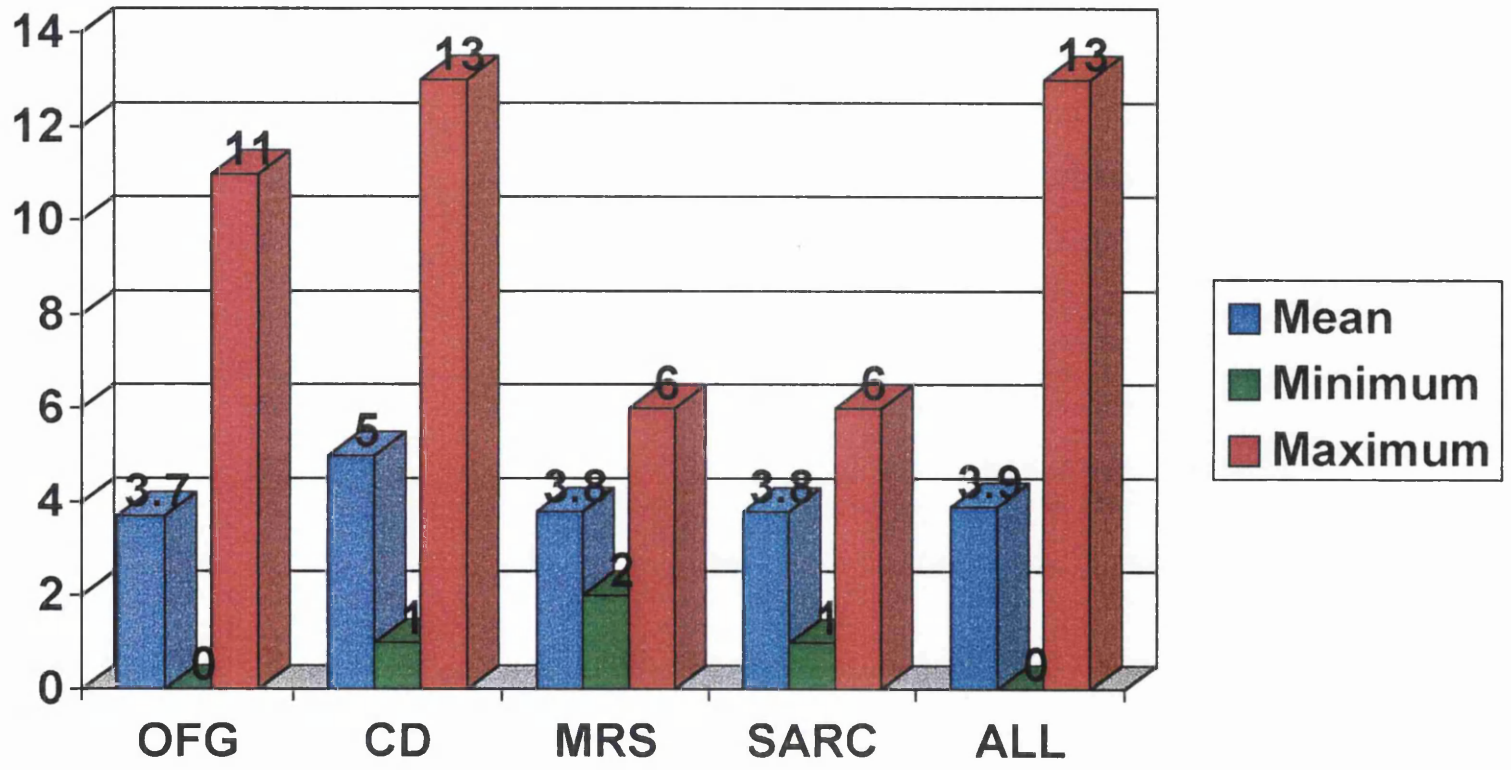
Kruskal-Wallis analysis revealed the following results:

$H = 13.25$; $DF = 1$; $p = 0.000$.

Thus, there was a statistically significant difference between OFG and CD in terms of the mean original sign scores, prior to intervention.

These data are presented graphically in Figure 7.14.

Figure 7.14 Initial sign scores (absolute values for each patient group)



7.4 Haematological investigations

7.4.1 Full blood count

The full blood counts yielded results for haemoglobin concentration, mean corpuscular volume, total and differential white cell count, red cell count, and platelet count as follows:

(A) Haemoglobin concentration

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	180 (75.0)	36 (80.0)	10 (100.0)	1 (16.7)	227 (75.4)
Haemoglobin Concentration (g/dl)					
Mean	13.7	13.2	15.1	15.0	13.7
Minimum	11.0	9.3	13.6	15.0	9.3
Maximum	18.2	17.0	18.3	15.0	18.3

Analysis using the Kruskal-Wallis test revealed the following results:

$H = 2.63$; $DF = 1$; $p = 0.105$

These data are presented graphically in Figures 7.15 and 7.16.

Figure 7.15 Haemoglobin concentration (g/dl) (absolute values for each patient group)

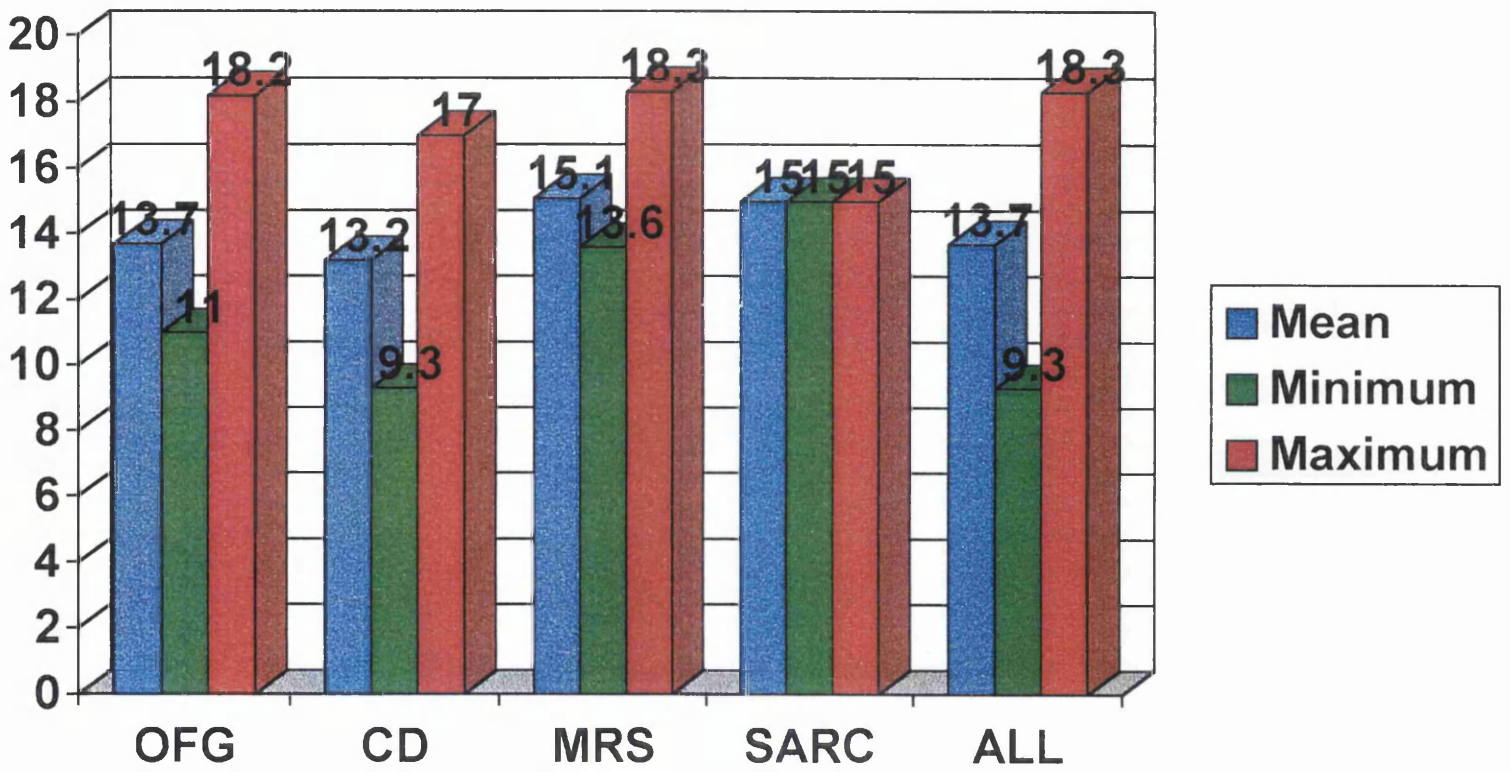
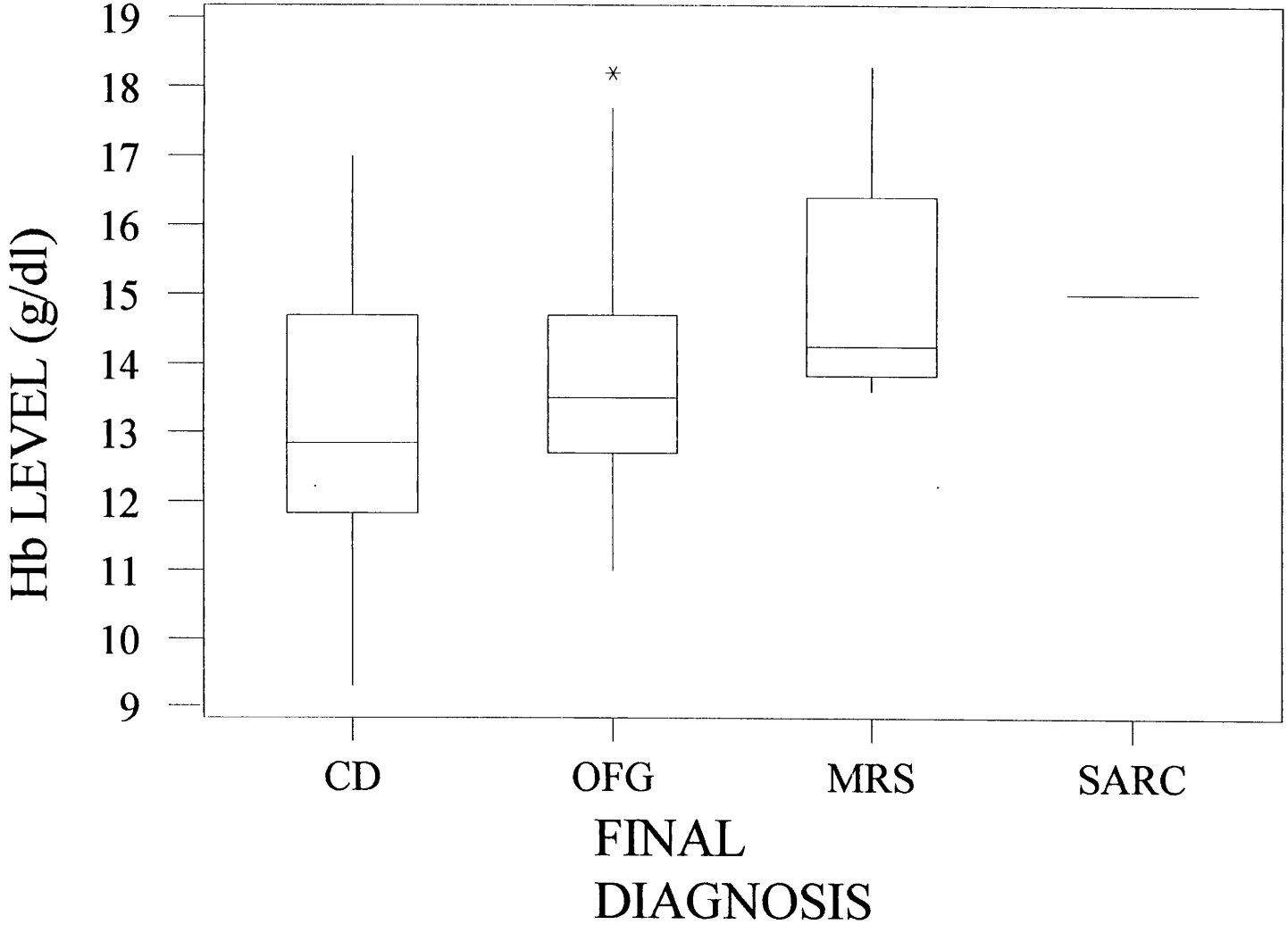


Figure 7.16 Haemoglobin concentration (g/dl), according to final diagnostic category



(B) Mean corpuscular volume (MCV)

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	178 (74.2)	36 (80.0)	10 (100.0)	1 (16.7)	225 (74.8)
Mean Corpuscular Volume (fl)					
Mean	86.0	82.8	90.1	96.0	85.7
Minimum	70.4	63.0	82.4	96.0	63.0
Maximum	100.0	103.7	98.0	96.0	103.7

Analysis using the Kruskal-Wallis test revealed the following results:

$H = 6.45$; $DF = 1$; $p = 0.011$

Thus, there was a statistically significant difference between patients with OFG and CD in terms of their initial mean corpuscular volume (MCV).

These data are presented graphically in Figures 7.17 and 7.18.

Figure 7.17 Mean corpuscular volume (fL) (absolute values for each patient group)

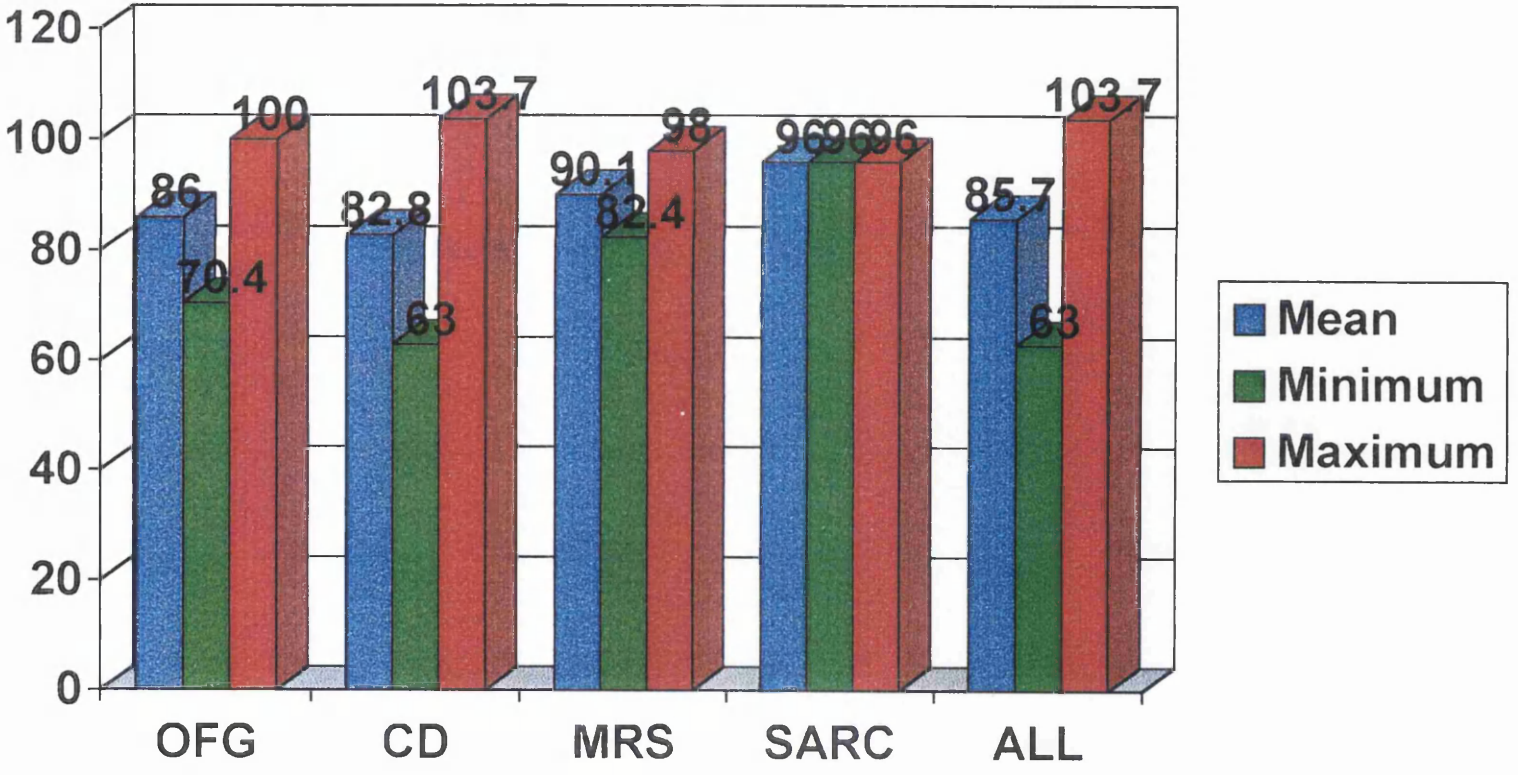
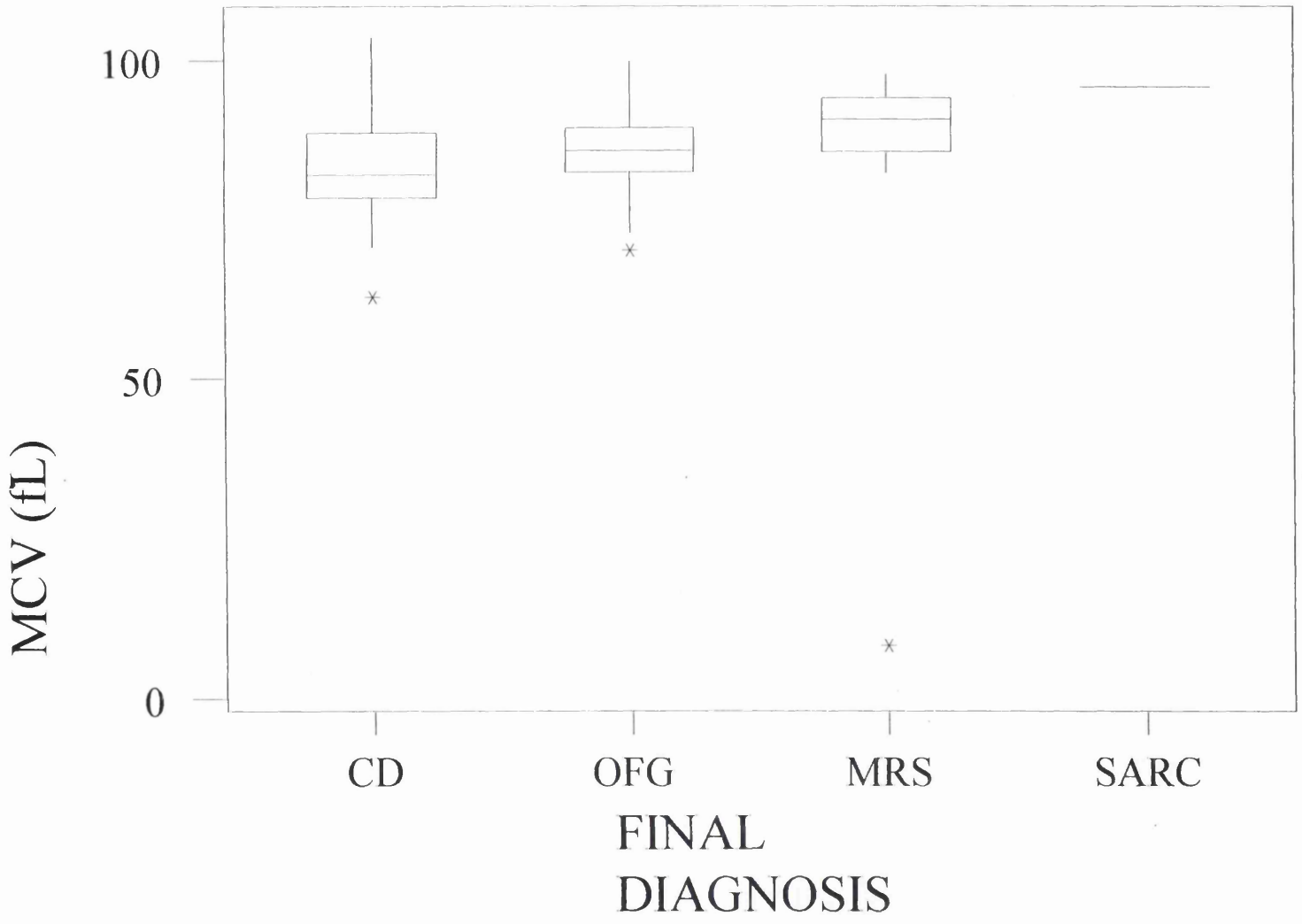


Figure 7.18 Mean corpuscular volume (fL), according to final diagnostic category



(C) Differential White Cell Count

The differential white cell counts are demonstrated graphically in Figures 7.19, 7.20, 7.21, 7.22, 7.23 and 7.24.

(D) Red Cell Count

The red cell counts are demonstrated graphically in Figure 7.25.

(E) Platelet Count

The platelet counts are demonstrated graphically in Figure 7.26.

Figure 7.19 White cell count, according to final diagnostic category

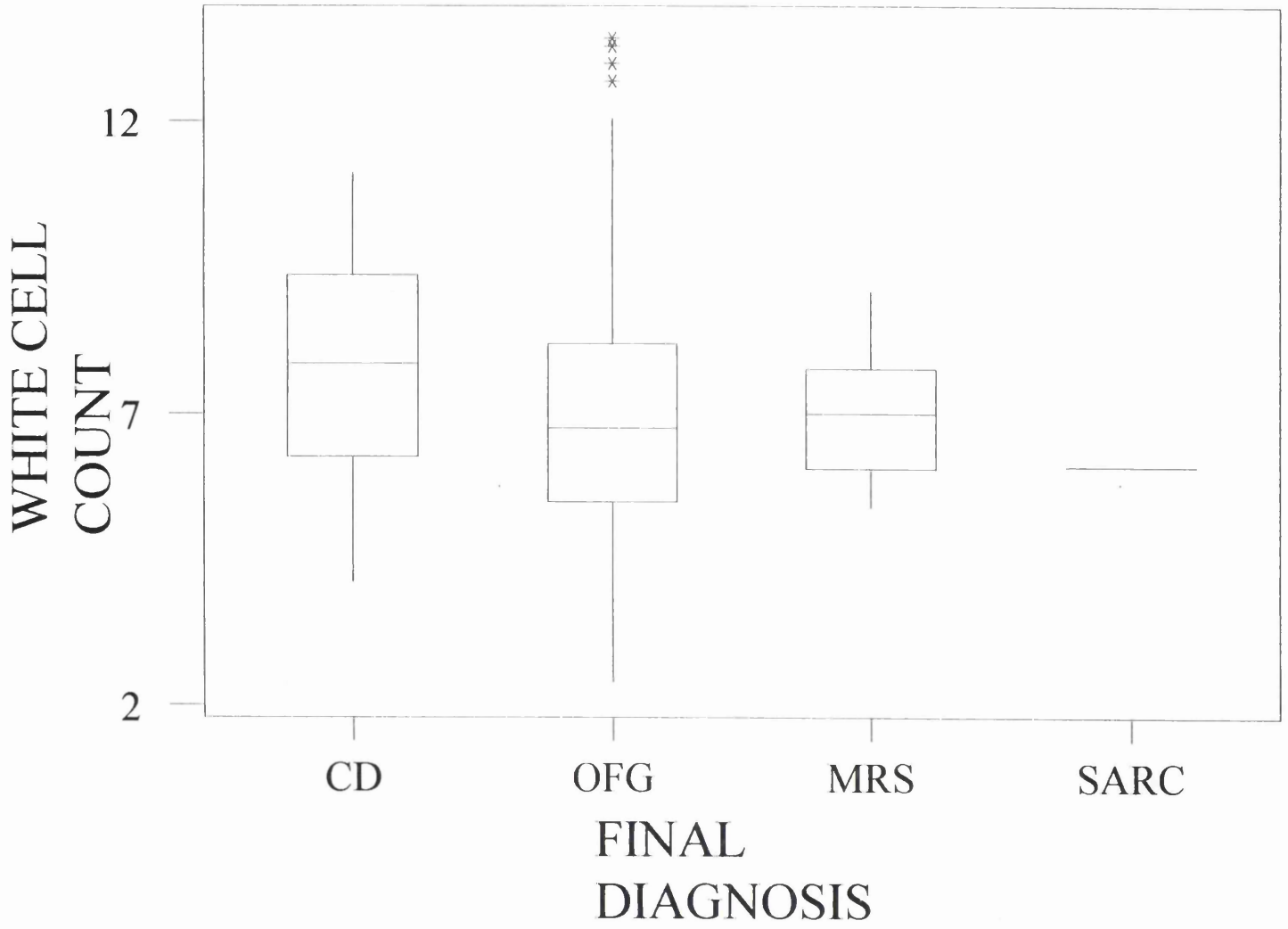


Figure 7.20 Neutrophil count, according to final diagnostic category

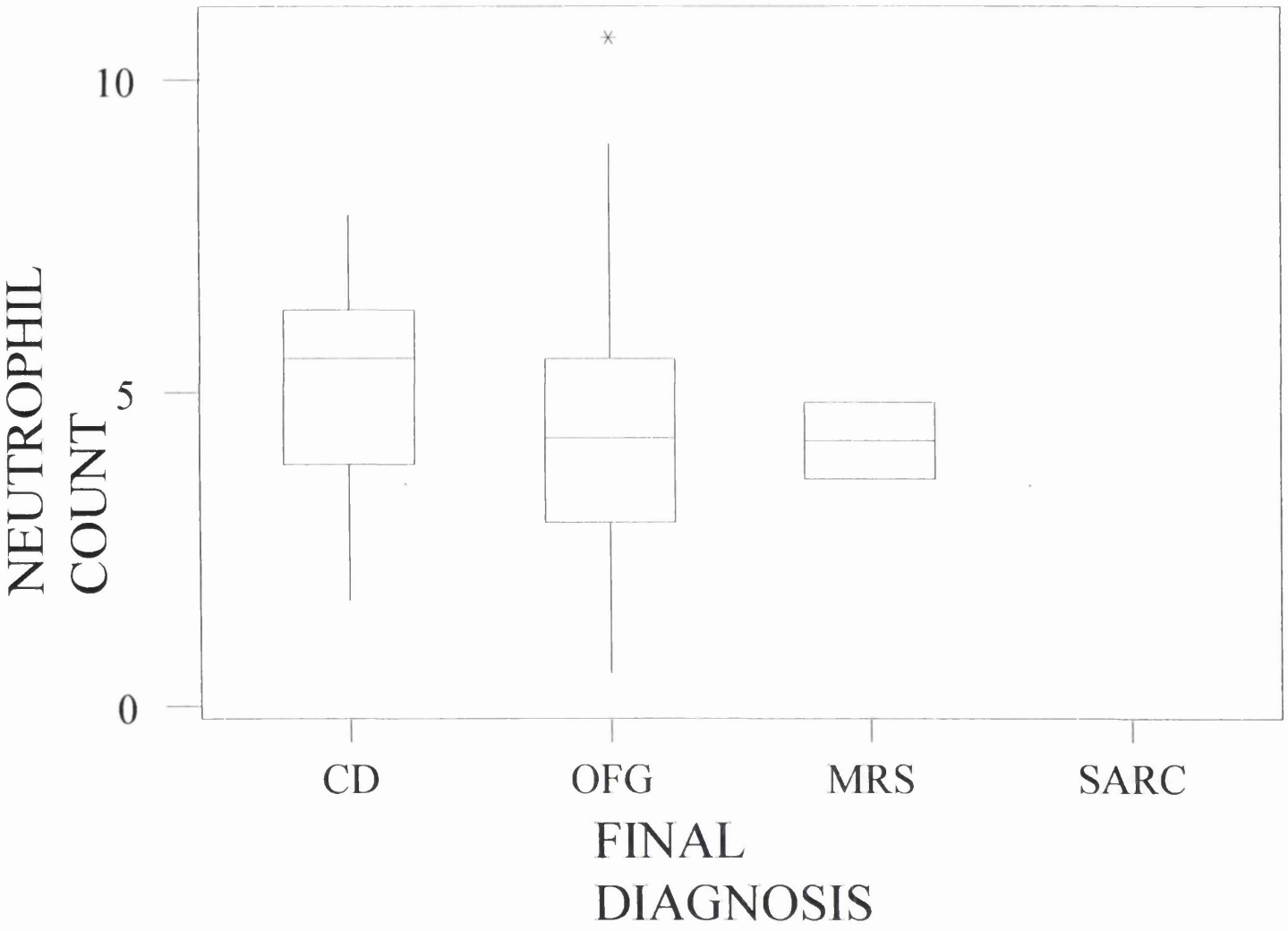


Figure 7.21 Lymphocyte count, according to final diagnostic category

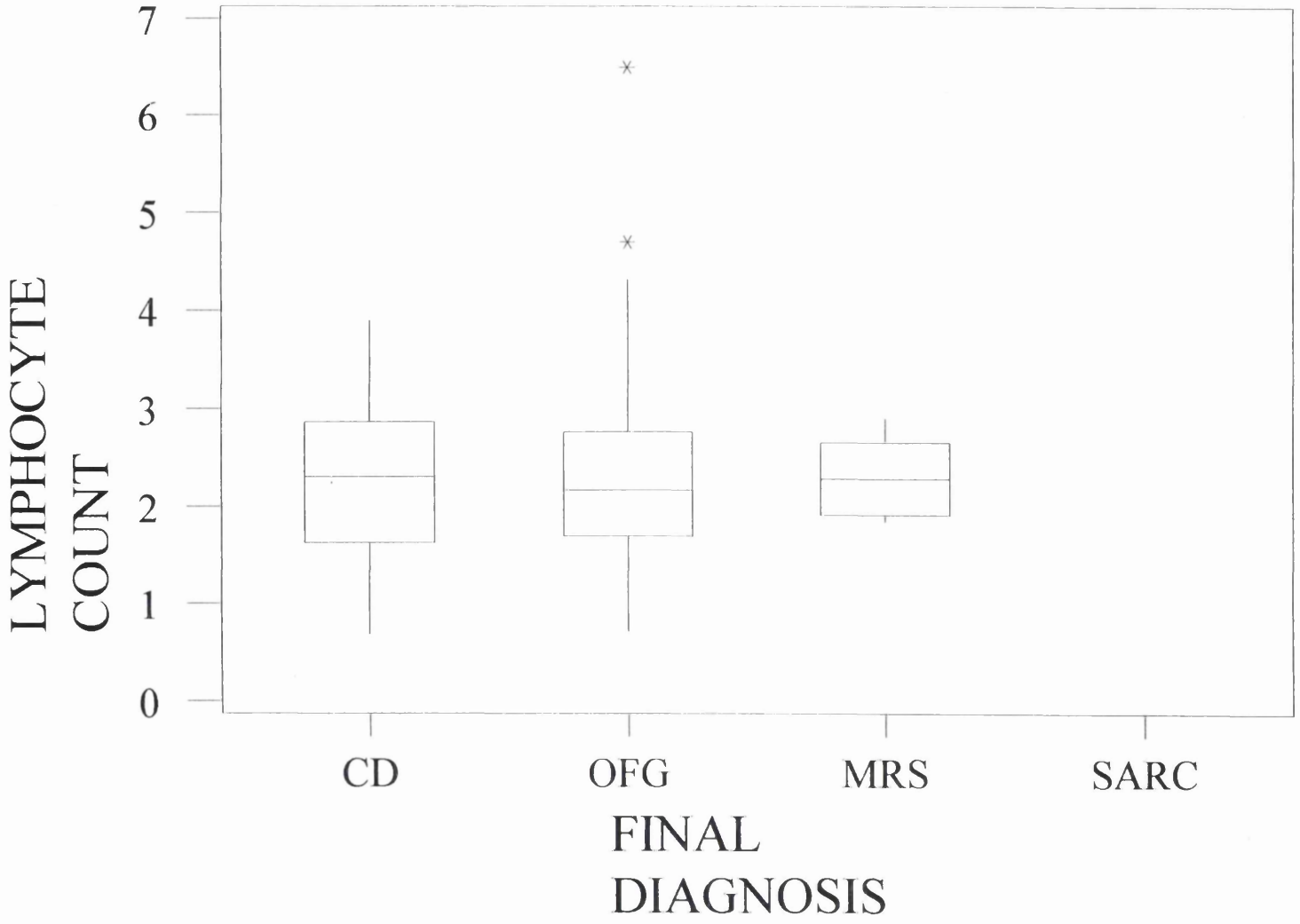


Figure 7.22 Monocyte count, according to final diagnostic category

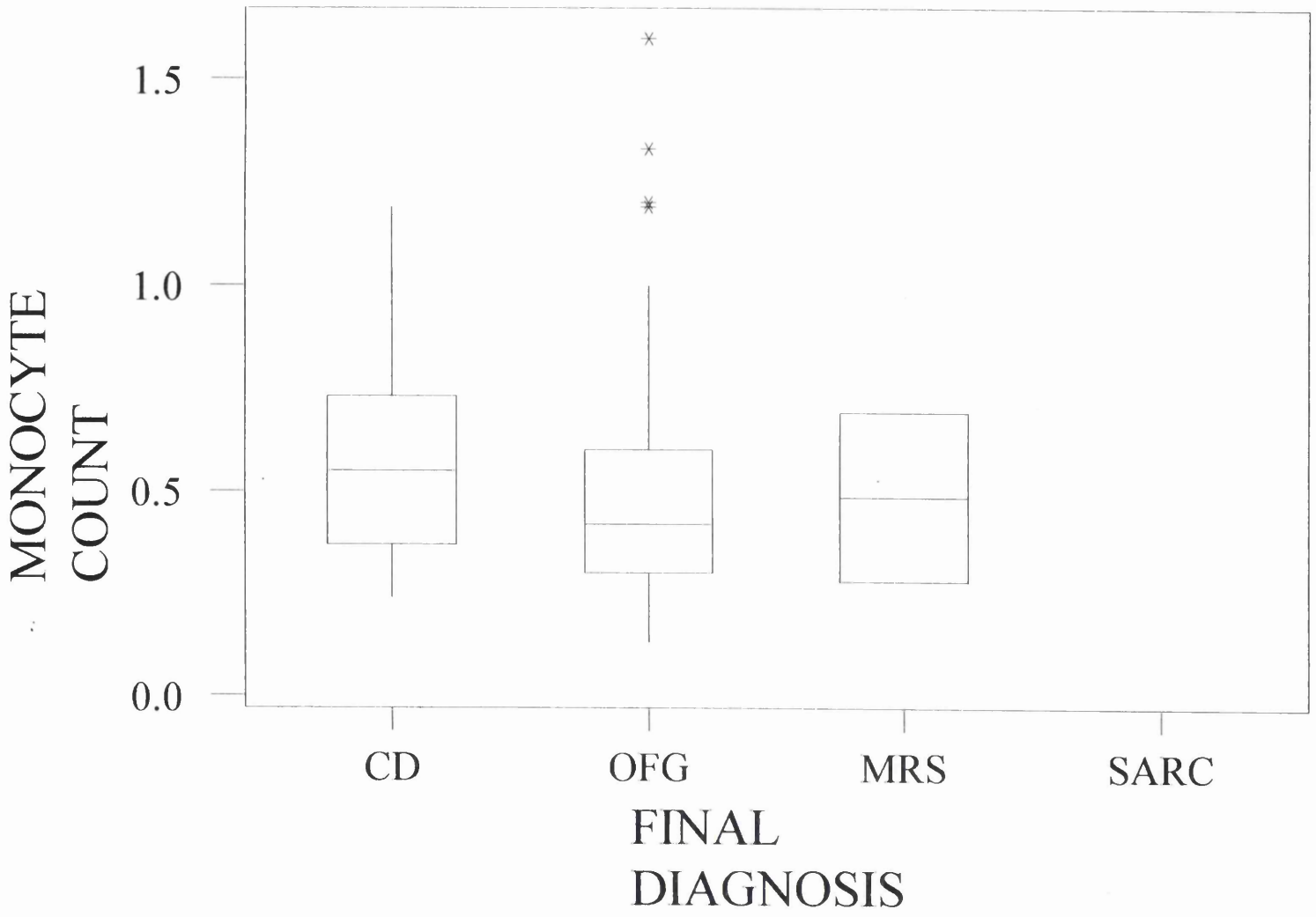


Figure 7.23 Eosinophil count, according to final diagnostic category

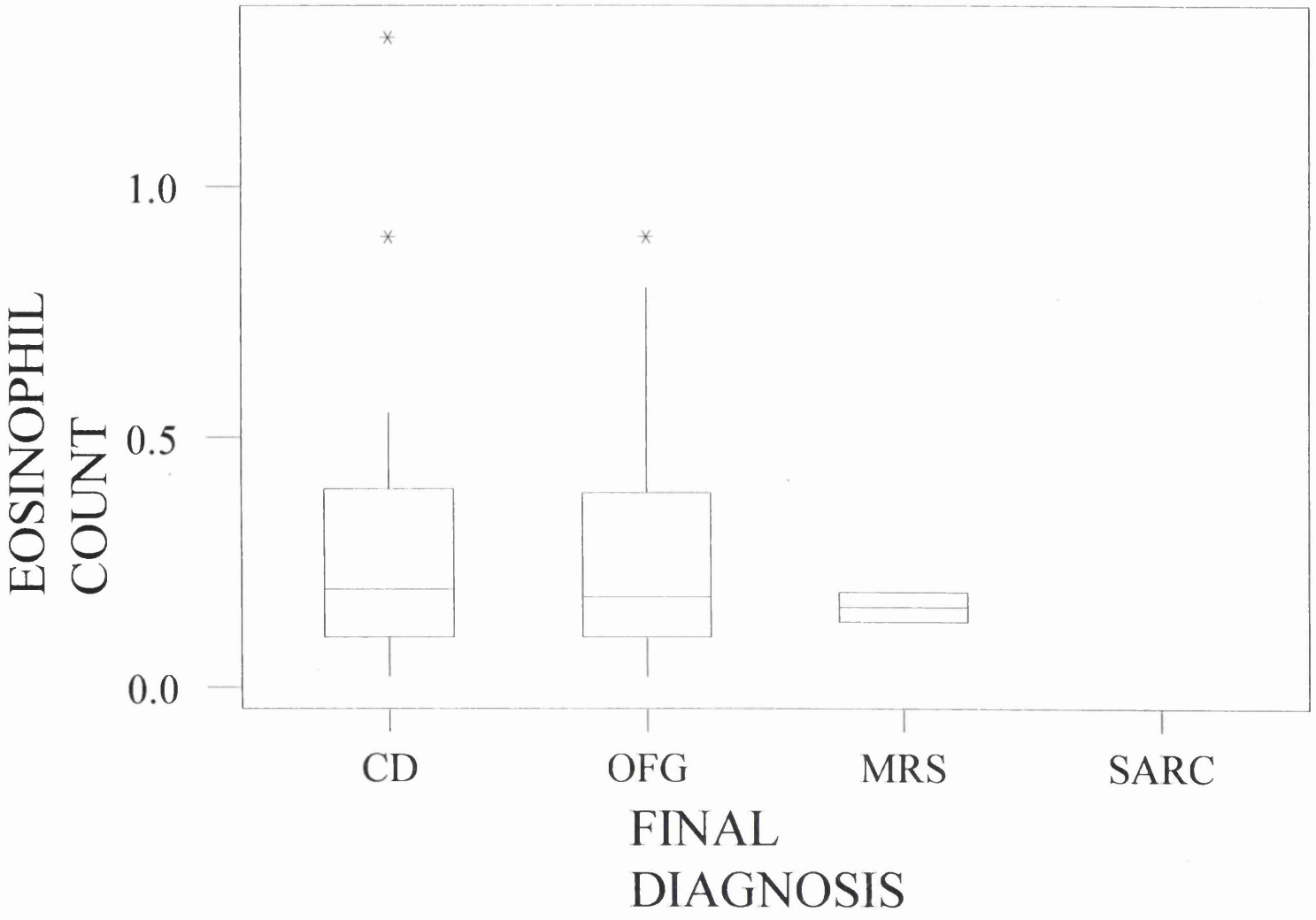


Figure 7.24 Basophil count, according to final diagnostic category

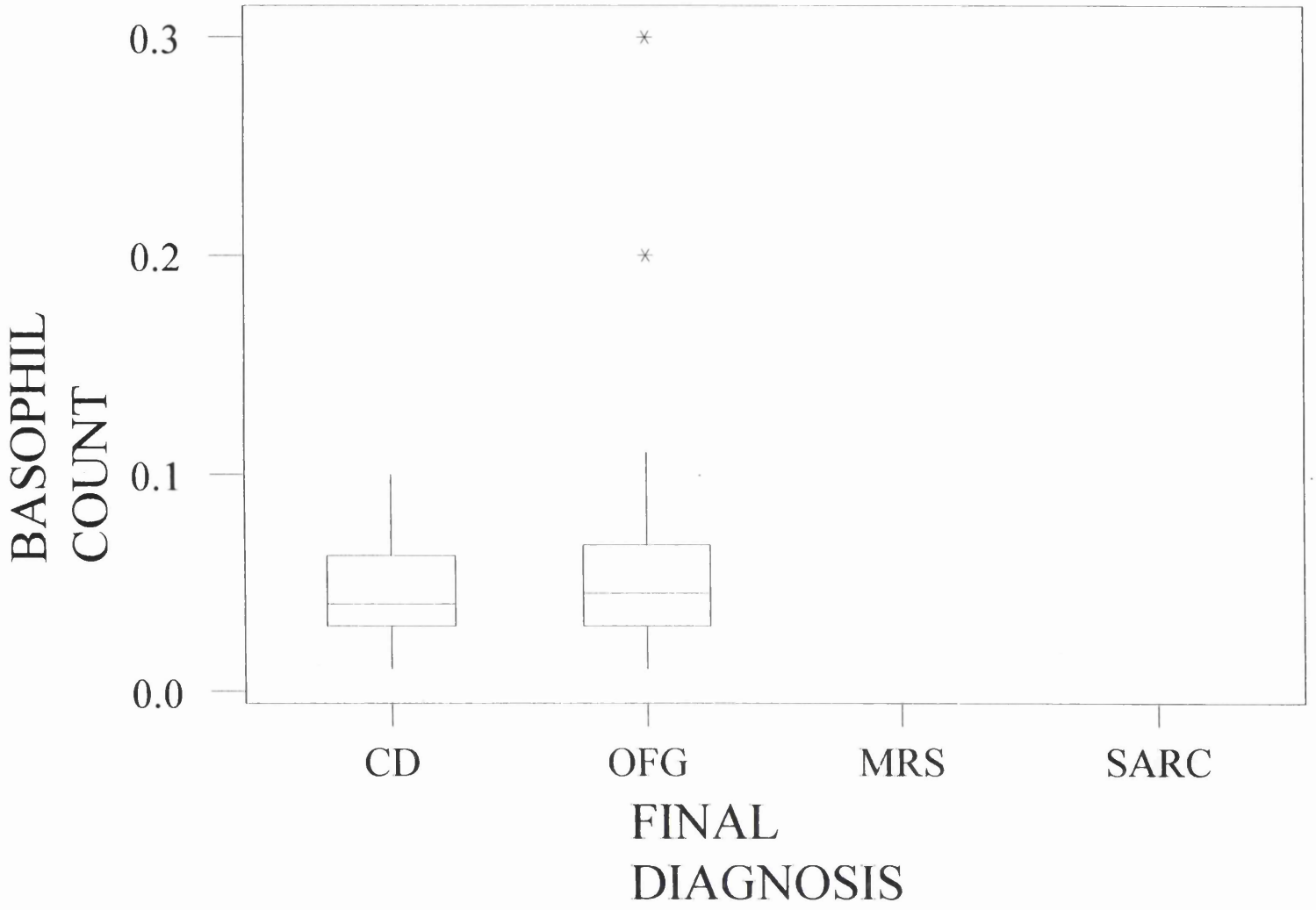


Figure 7.25 Red cell count, according to final diagnostic category

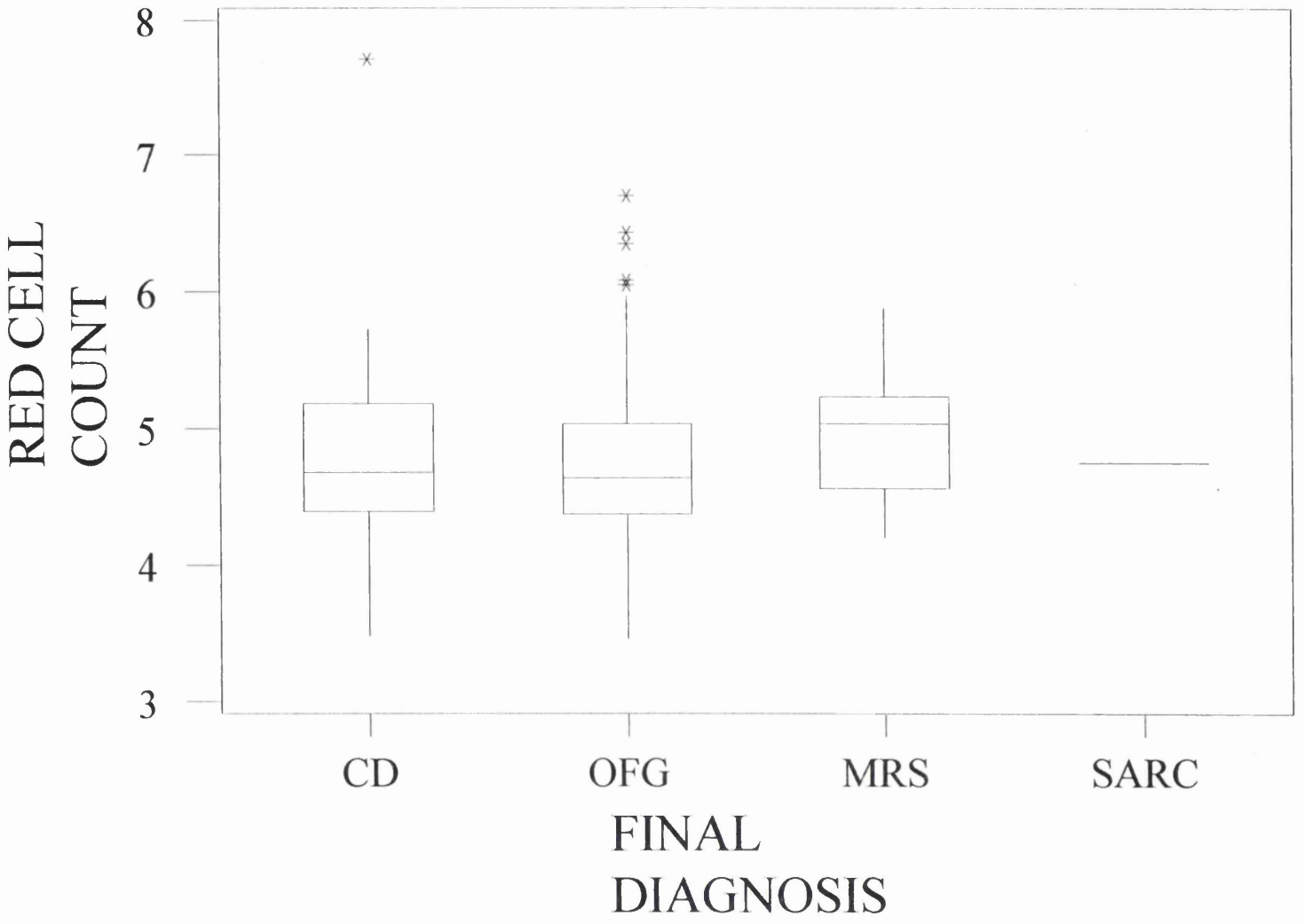
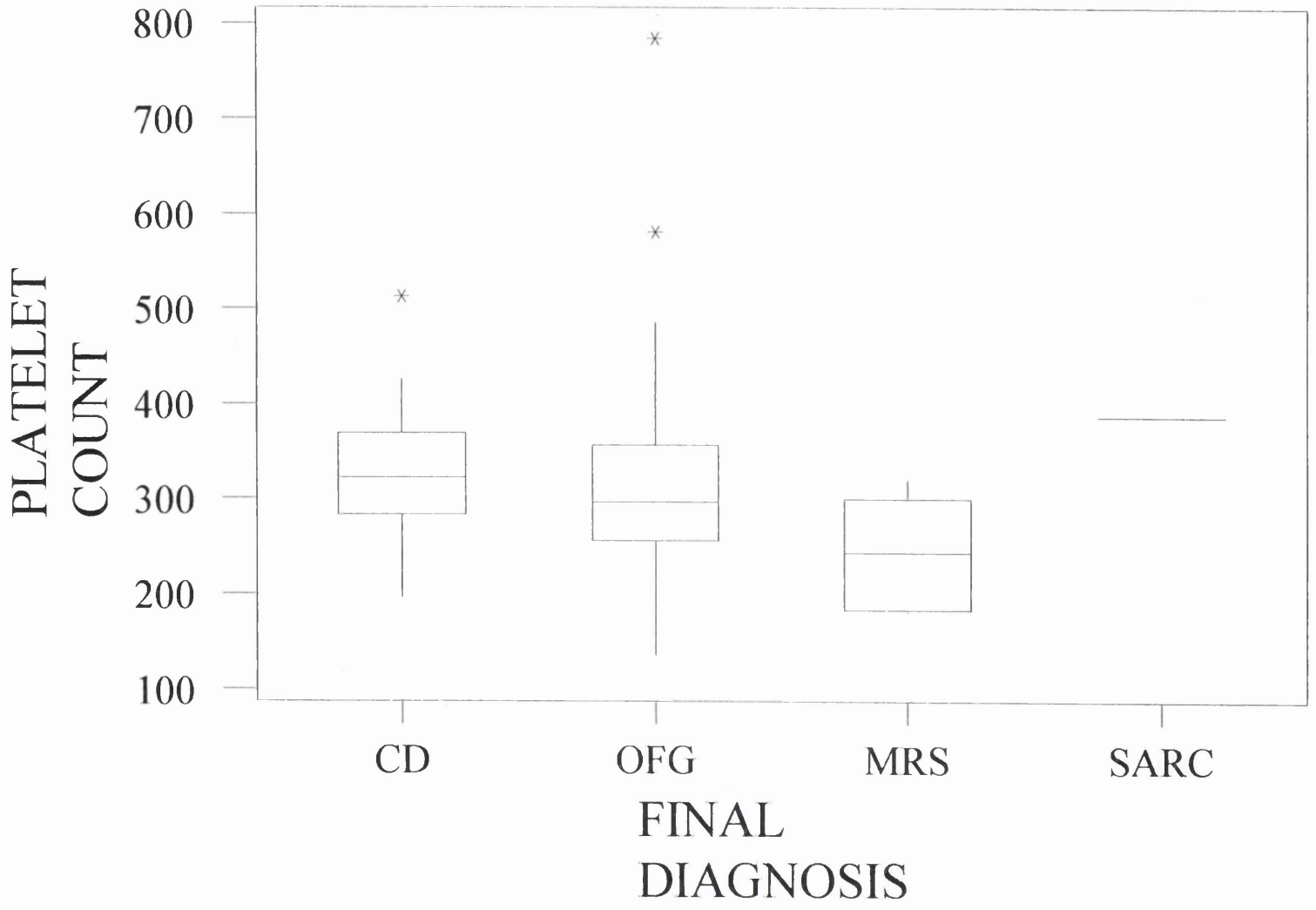


Figure 7.26 Platelet count, according to final diagnostic category



7.4.2 Erythrocyte sedimentation rate (ESR)

The values obtained for Erythrocyte Sedimentation Rates in each group were as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	95 (40.0)	27 (60.0)	8 (80.0)	1 (16.7)	131 (43.5)
Erythrocyte Sedimentation Rate (mm/hr)					
Mean	12.2	23.0	10.4	1.0	14.2
Minimum	1.0	1.0	2.0	1.0	1.0
Maximum	67.0	90.0	28.0	1.0	90.0

Analysis using the Kruskal-Wallis test revealed the following results:

$H = 5.29$; $DF = 1$; $p = 0.021$

Thus, there was a statistically significant difference between patients with OFG and CD in terms of their erythrocyte sedimentation rate (ESR), a non-specific marker of inflammation.

These data are presented graphically in Figures 7.27 and 7.28.

Figure 7.27 Erythrocyte sedimentation rate (mm/hr) (absolute values for each patient group)

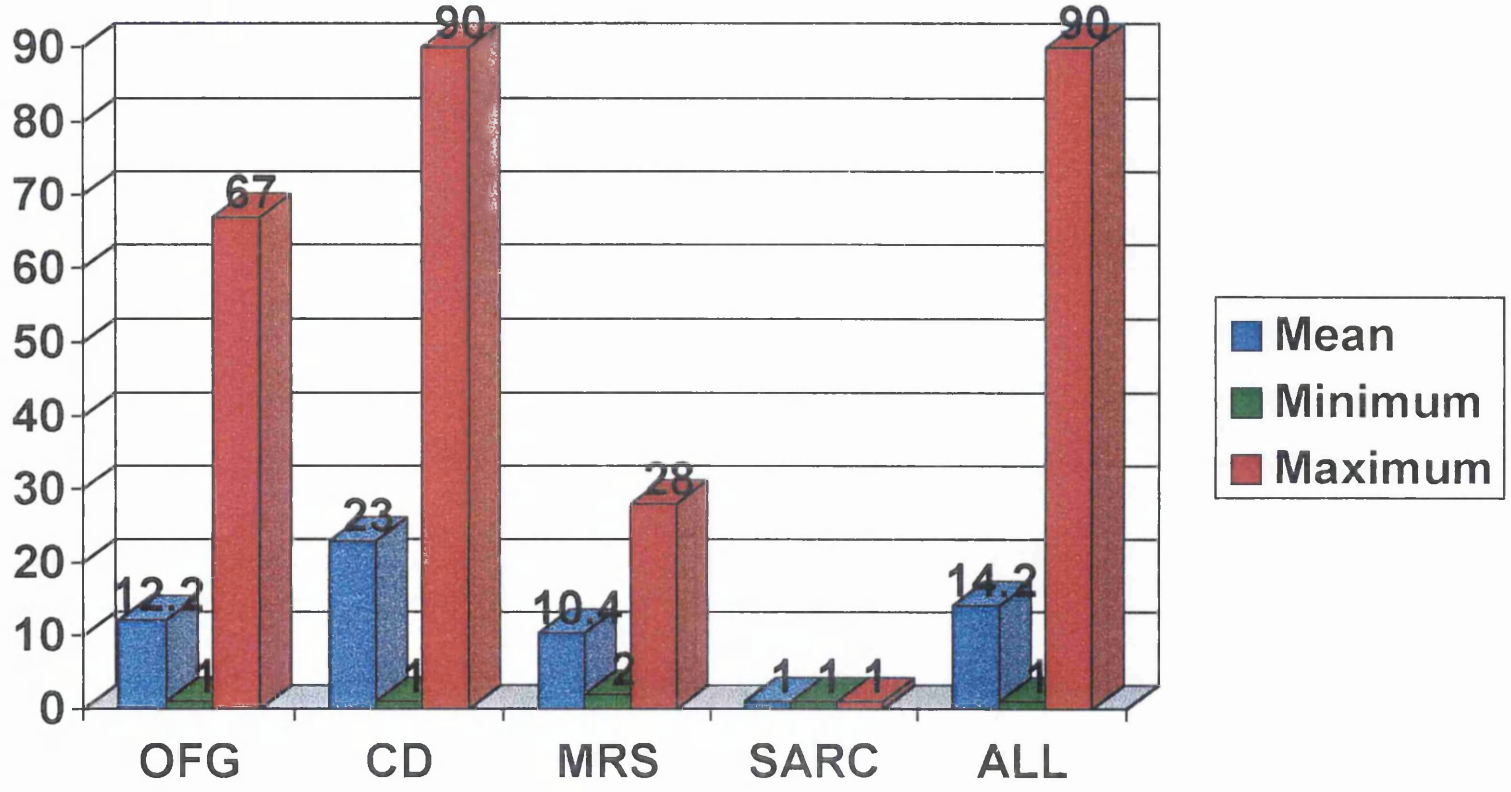
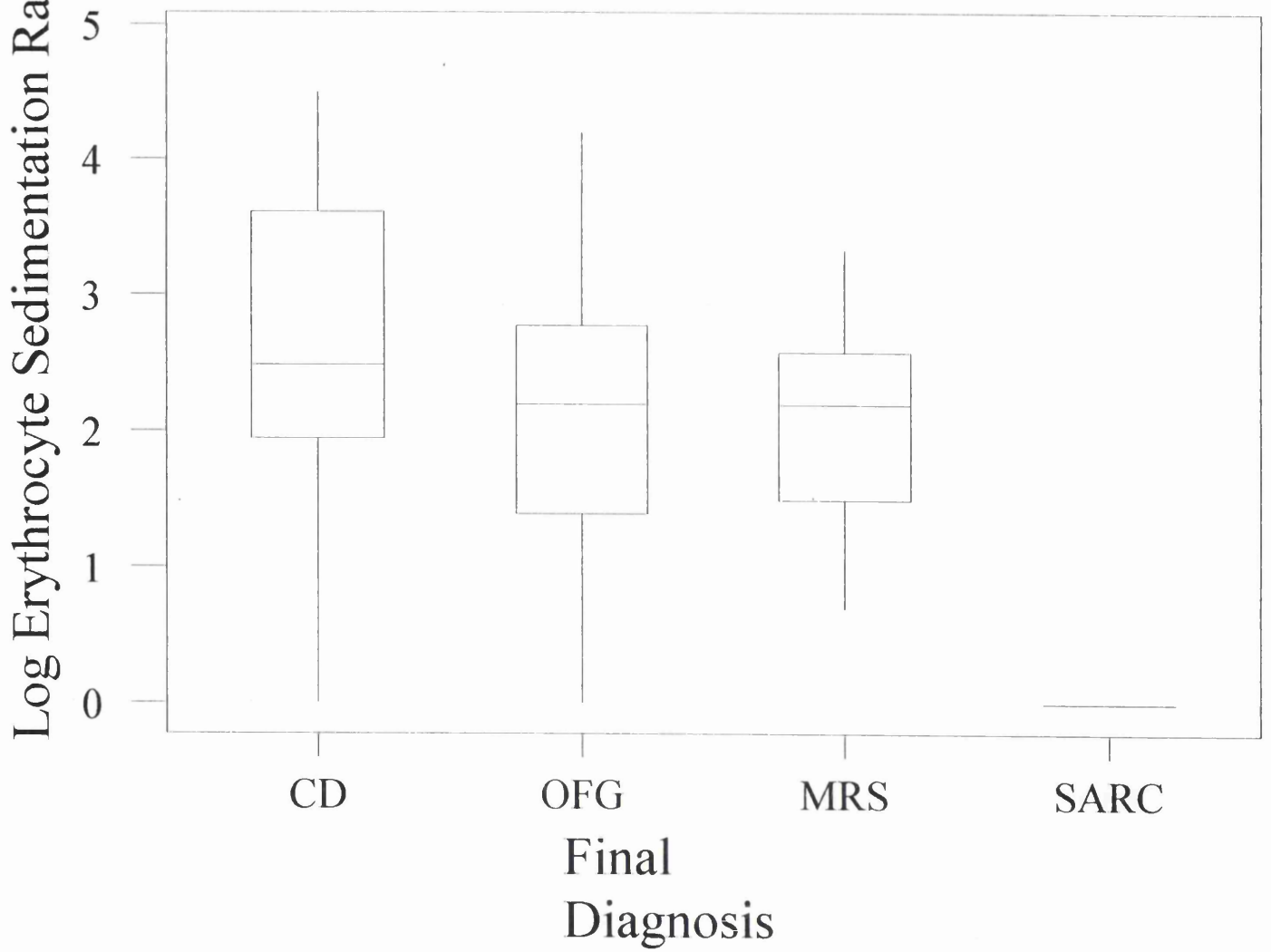


Figure 7.28 Log of erythrocyte sedimentation rate (mm/hr), according to final diagnostic category



7.4.3 Serum ferritin

The serum ferritin assays recorded for each patient group were as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	174 (72.5)	39 (86.7)	8 (80.0)	3 (50.0)	224 (74.4)
Serum ferritin (ng/ml)					
Mean	63.9	27.2	56.5	28.3	56.8
Minimum	1.0	1.0	11.0	25.0	1.0
Maximum	953.0	183.0	107.0	35.0	953.0

Analysis using the Kruskal-Wallis test revealed the following results:
$H = 31.95$; $DF = 1$; $p = 0.000$

Thus, there was a statistically significant difference between patients with OFG and CD in terms of their serum ferritin level prior to intervention.

These data are presented graphically in Figures 7.29 and 7.30.

Figure 7.29 Serum ferritin (ng/ml) (absolute values for each patient group)

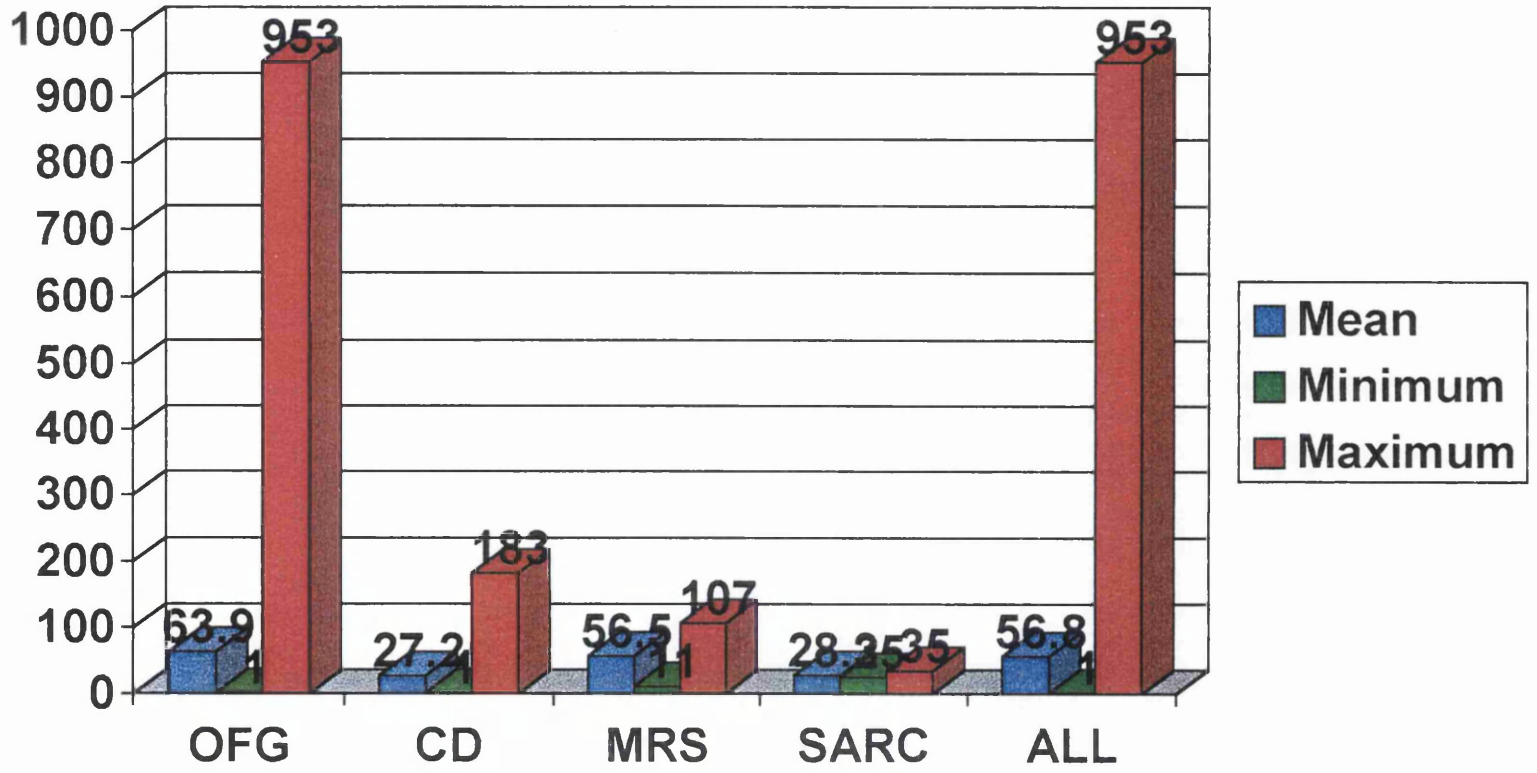
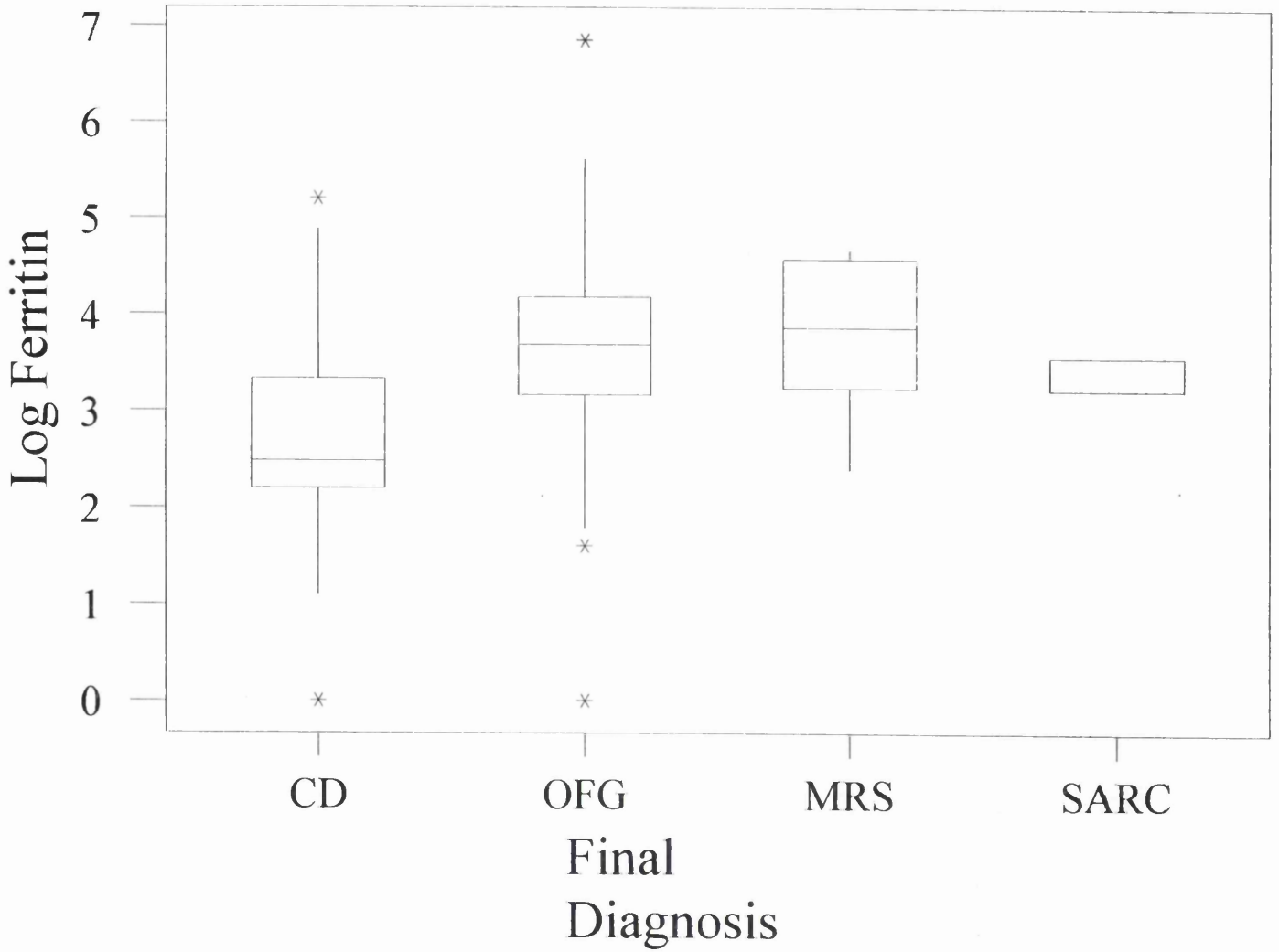


Figure 7.30 Log serum ferritin, according to final diagnostic category



7.4.4 Serum vitamin B_{12}

The serum vitamin B_{12} assays recorded for each patient group were as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	174 (72.5)	38 (84.4)	8 (80.0)	3 (50.0)	223 (74.1)
Serum vitamin B_{12} (pg/ml)					
Mean	477.2	547.3	387.8	375.3	484.6
Minimum	129.0	203.0	206.0	269.0	129.0
Maximum	>2000.0	>2000.0	610.0	511.0	>2000.0

Analysis using the Kruskal-Wallis test revealed the following results:

$H = 2.72$; $DF = 1$; $p = 0.099$

These data are presented graphically in Figures 7.31 and 7.32.

Figure 7.31 Serum vitamin B12 (pg/ml) (absolute values for each patient group)

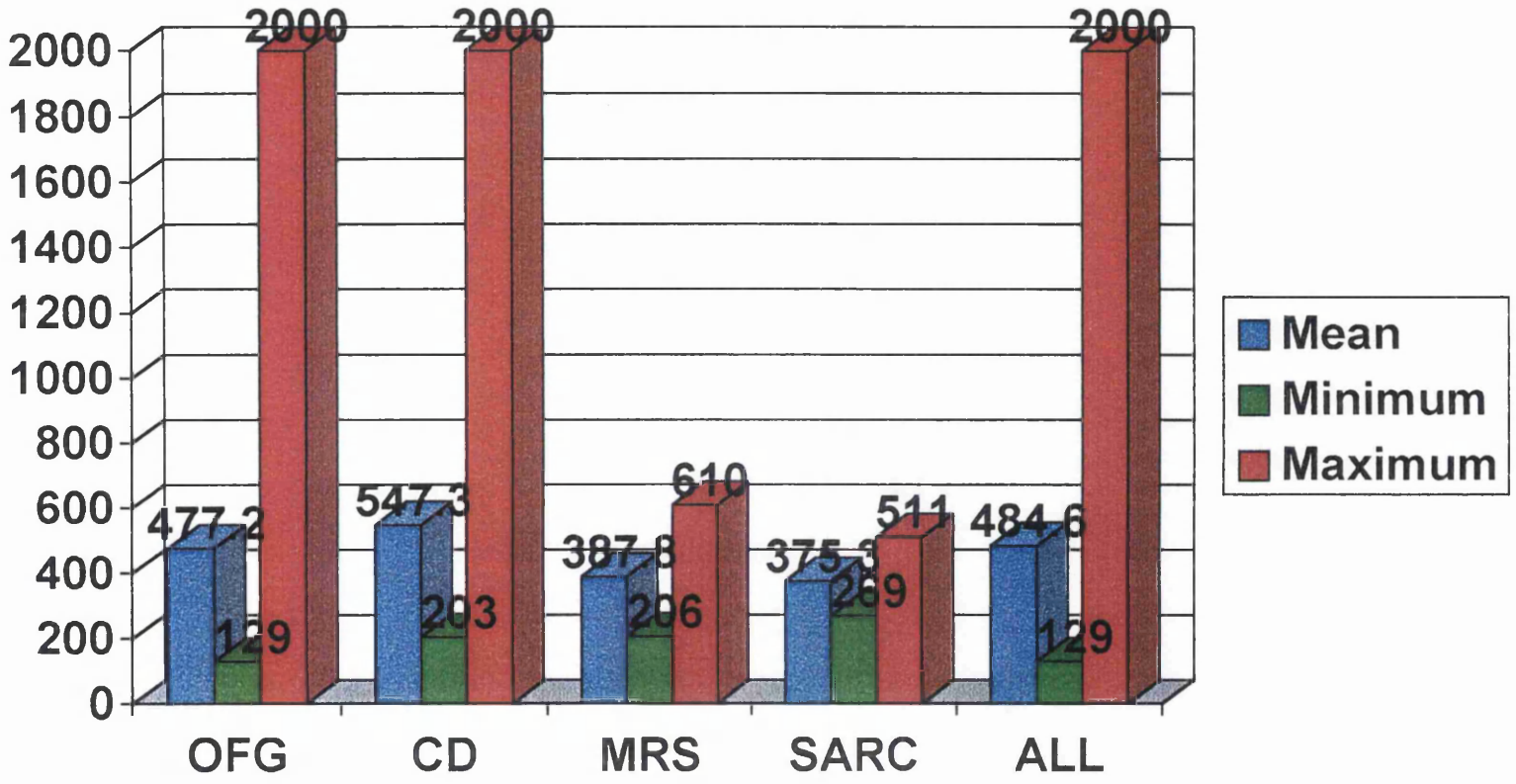
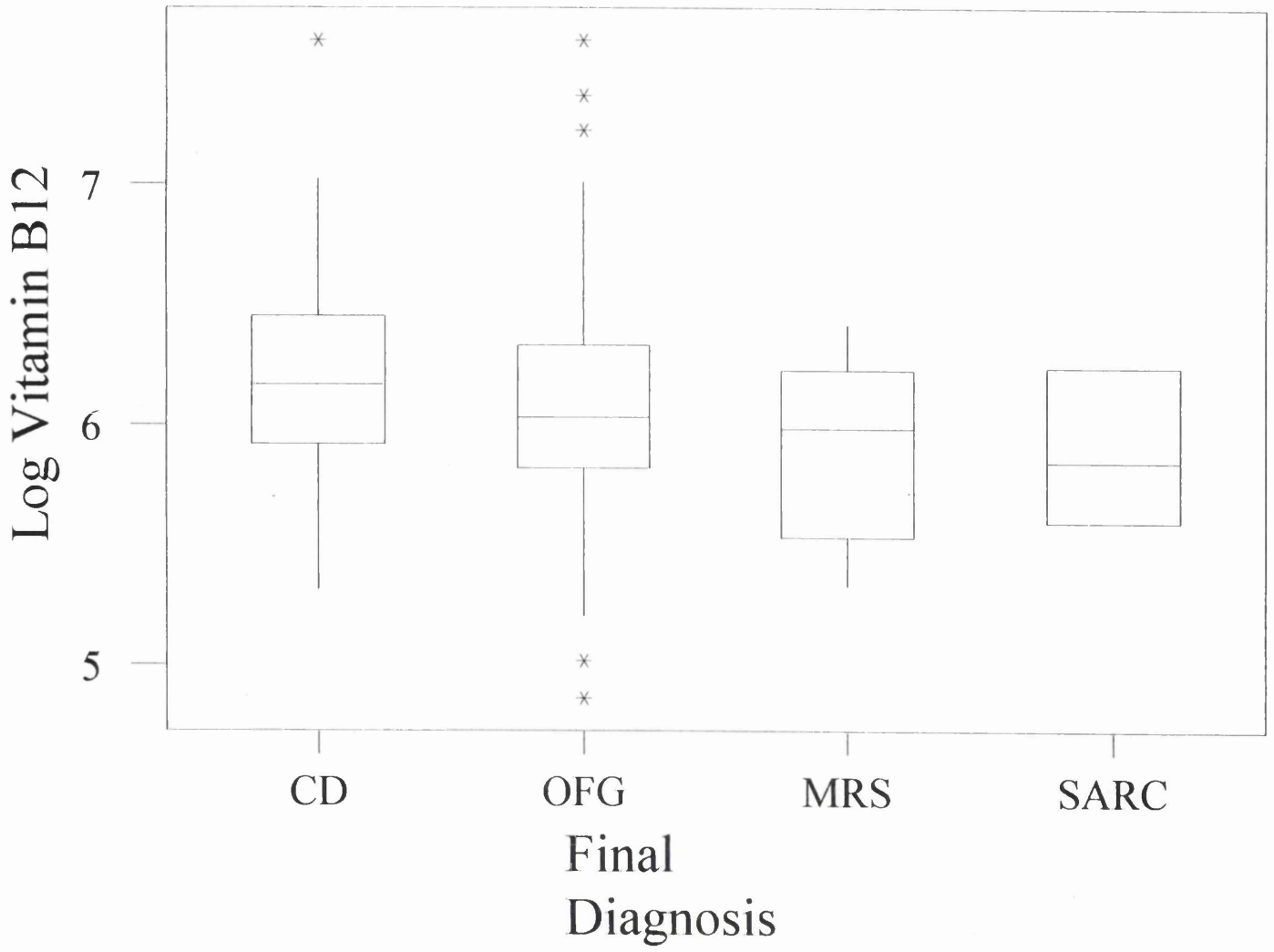


Figure 7.32 Log serum vitamin B12, according to final diagnostic category



7.4.5 Whole blood folate

The whole blood folate assays recorded for each patient group were as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	174 (72.5)	38 (84.4)	8 (80.0)	3 (50.0)	223 (74.1)
Whole blood folate (ng/ml)					
Mean	242.8	290.7	168.13	233.0	248.2
Minimum	2.0	95.0	101.0	146.0	2.0
Maximum	600.0	635.0	244.0	338.0	635.0

Analysis using the Kruskal-Wallis test revealed the following results:

$H = 2.20$; $DF = 1$; $p = 0.138$

These data are presented graphically in Figures 7.33 and 7.34.

Figure 7.33 Whole blood folate (ng/ml) (absolute values for each patient group)

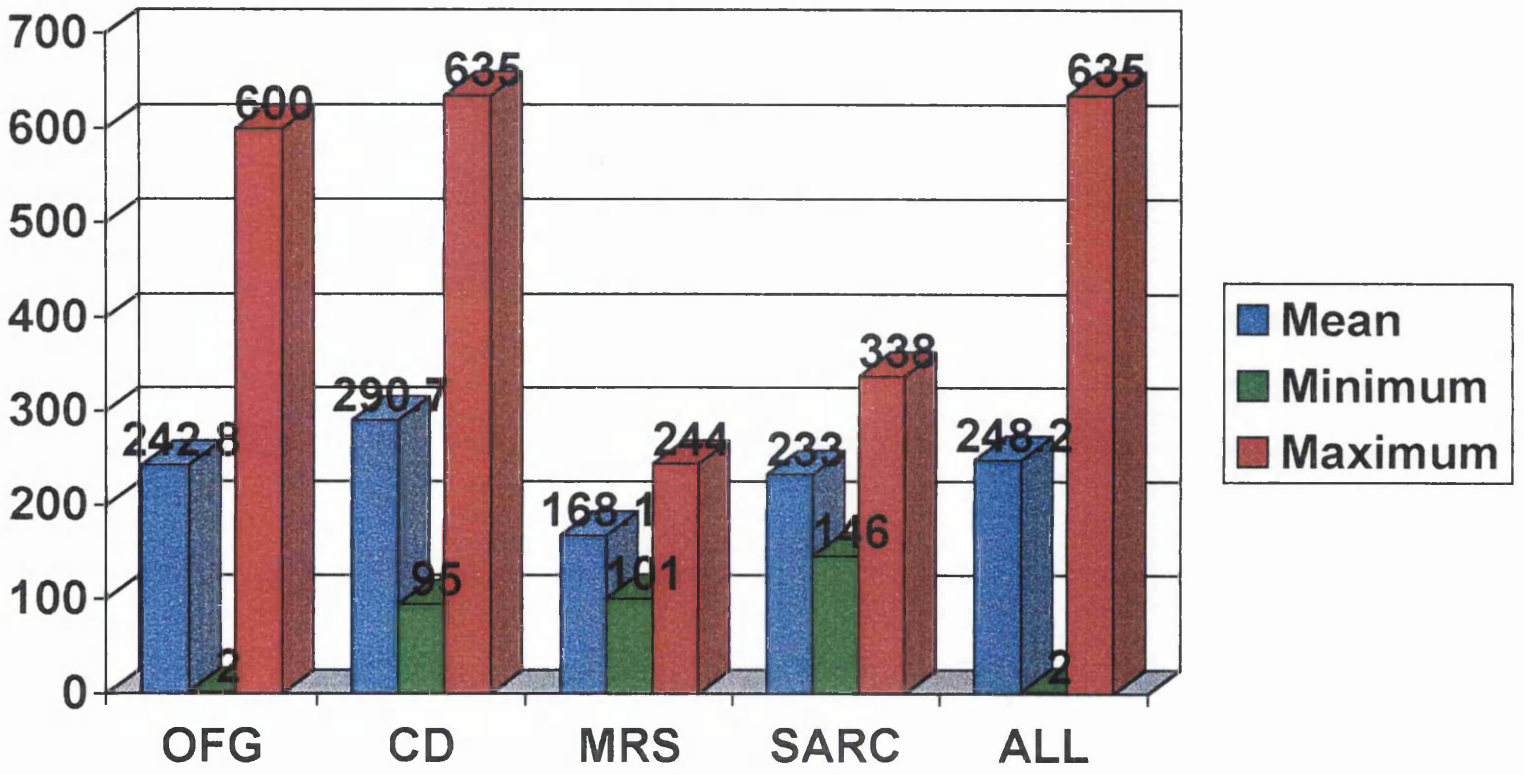
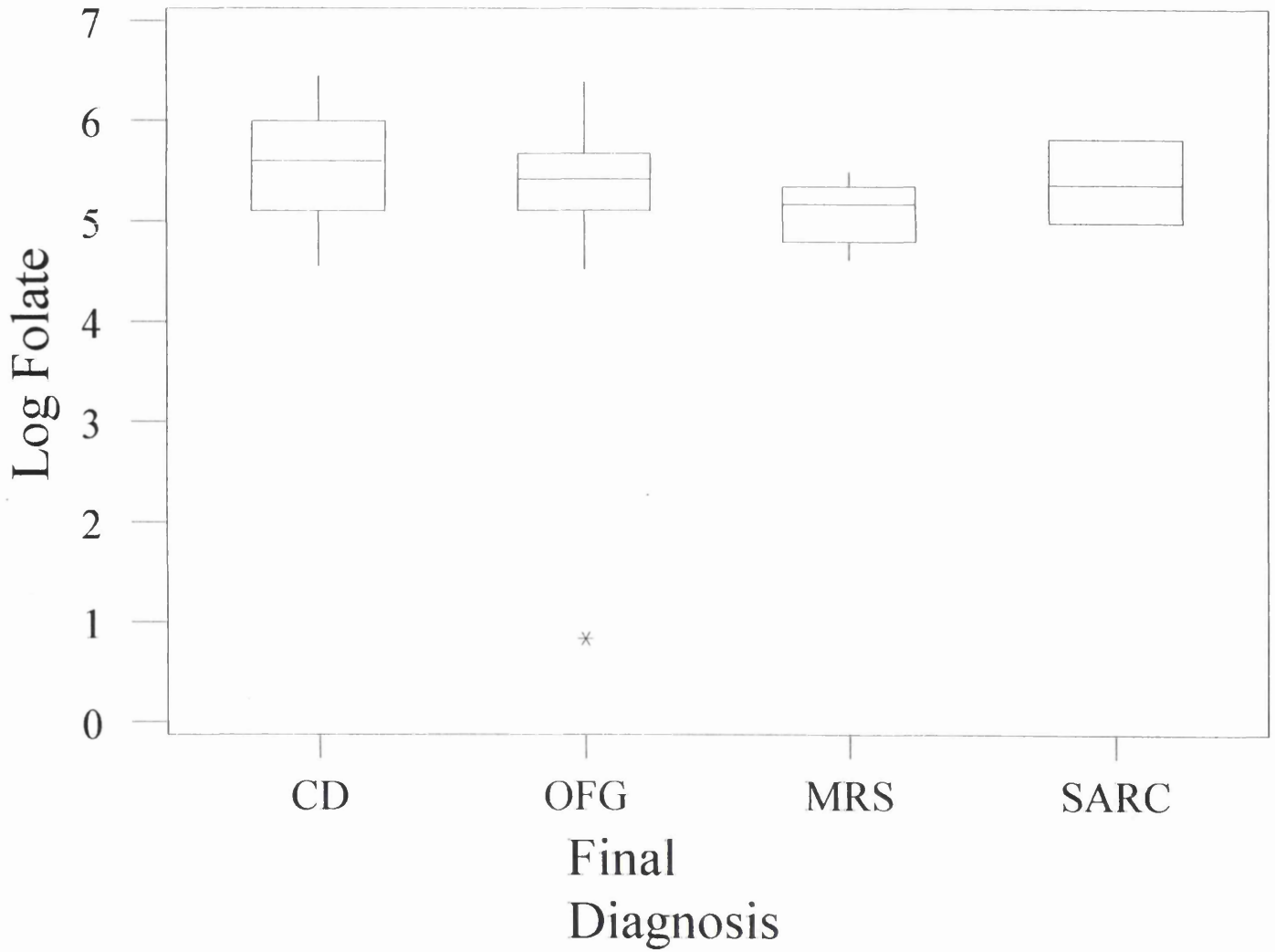


Figure 7.34 Log whole blood folate, according to final diagnostic category



7.5 Biochemical investigations

7.5.1 Urea and electrolytes

Abnormalities in the patients' urea and electrolyte profiles were evident as follows:

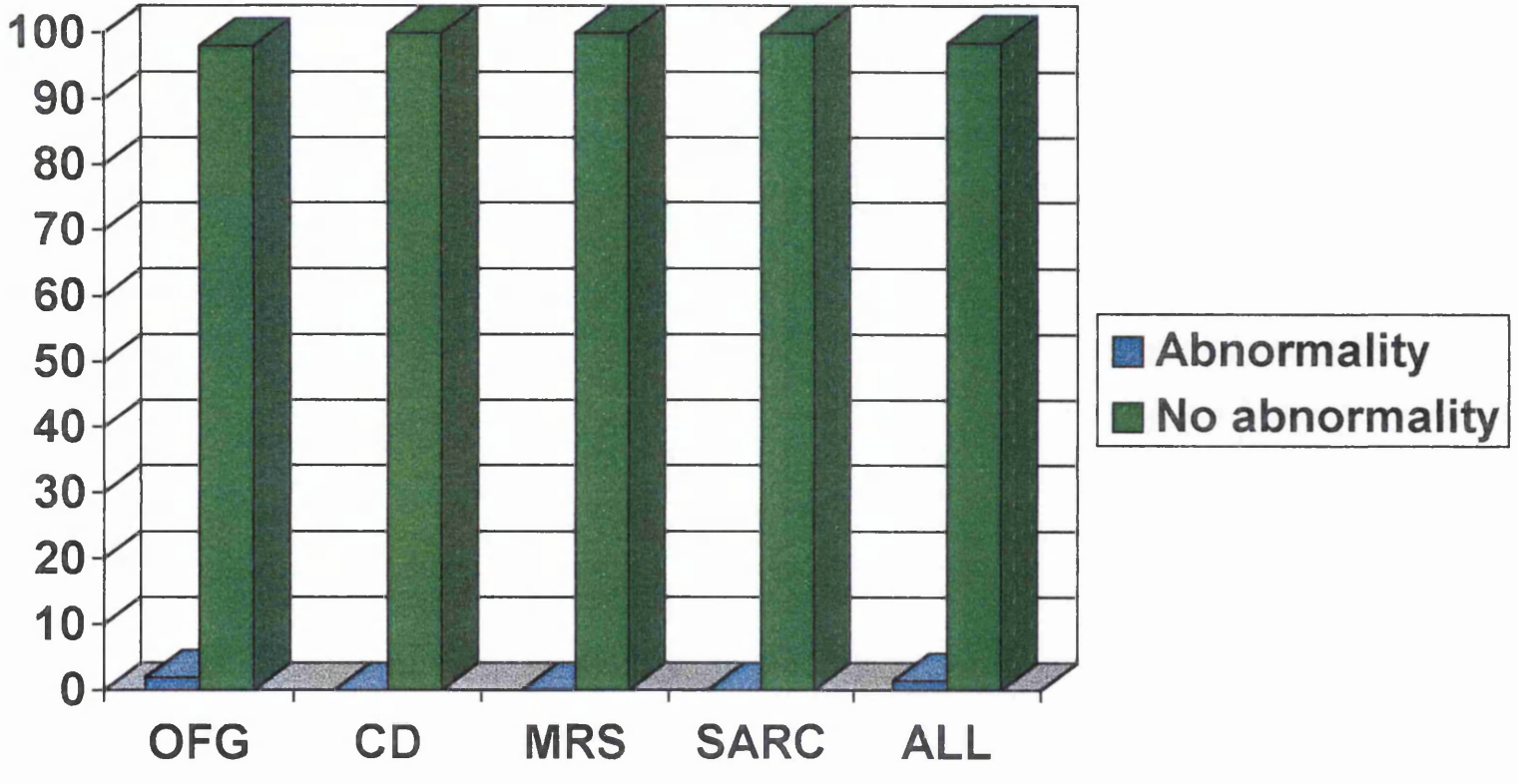
Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients tested (%)	108 (45.0)	29 (64.4)	6 (60.0)	3 (50.0)	146 (48.5)
Abnormality in urea and electrolyte profile (%)	2 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.4)

The specific abnormalities detected were noted as follows:

One patient with OFG had a marginally low albumin
One patient with OFG had a marginally high urea

These data are presented graphically in Figure 7.35.

Figure 7.35 Abnormalities in urea & electrolyte profiles (% of patients in each group)



7.5.2 Calcium, albumin and phosphate

Abnormalities in the patients' calcium, albumin and phosphate profiles were evident as follows:

Diagnosis	OFG	CD	MRS	SARC	ALL
Number of patients tested (%)	108 (45.0)	27 (60.0)	6 (60.0)	3 (50.0)	144 (47.8)
Abnormality in calcium, albumin and phosphate profile (%)	8 (7.4)	4 (14.8)	0 (0.0)	1 (33.3)	13 (9.0)

The specific abnormalities detected were noted in thirteen different patients as follows:

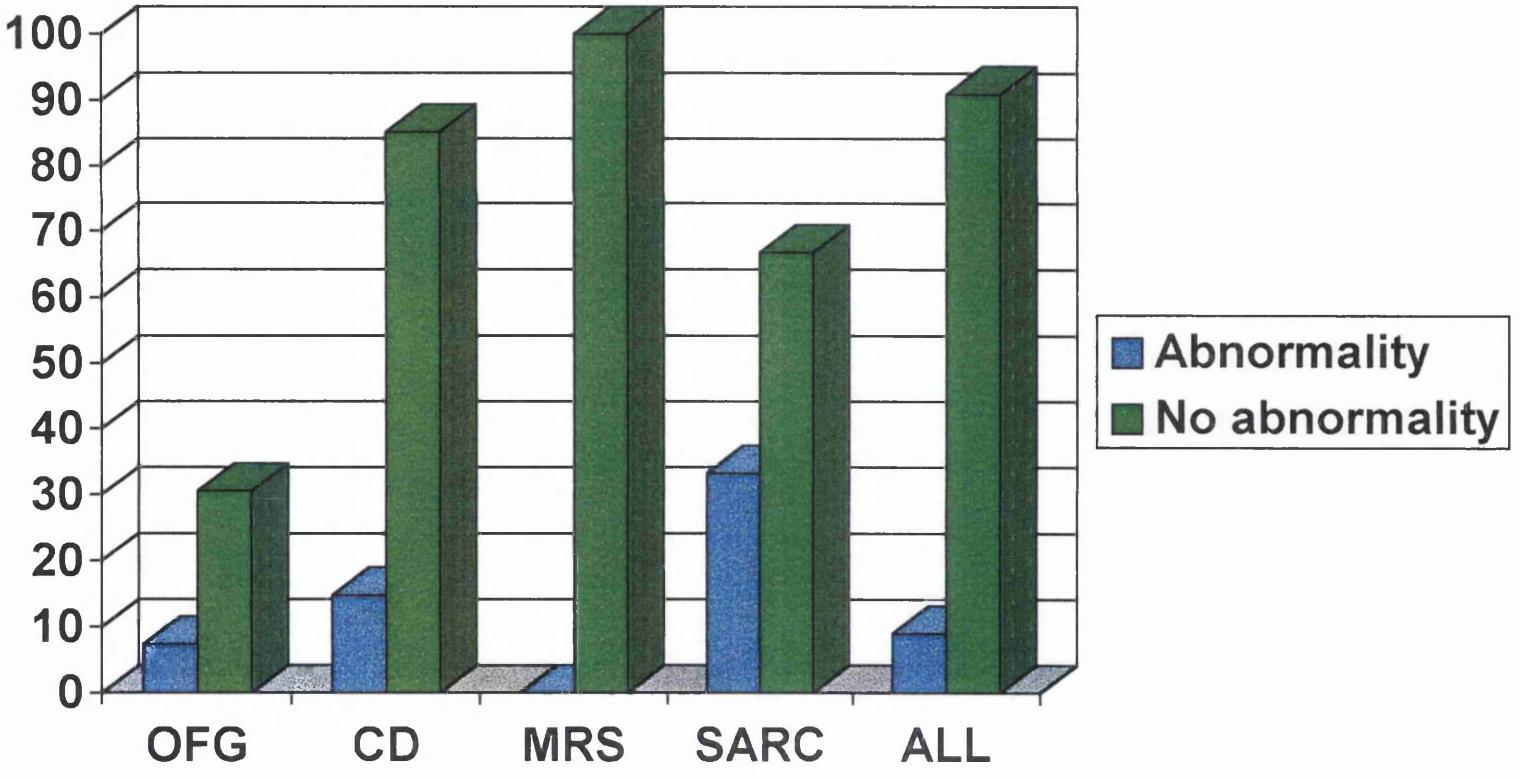
Two patients with OFG had high plasma protein levels (78 and 85g/L)
Three patients with OFG had low phosphate levels (0.51, 0.67 and 0.77mmmol/L)
One patient with OFG had low (corrected) serum calcium (2.16mmol/L)
One patient with OFG had a high phosphate level (1.48mmol/L)
One patient with OFG had a low serum albumin (33g/L)

One patient with CD had a high phosphate level (1.47mmol/L)
Three patients with CD had low (corrected) serum calcium levels (2.08, 2.16 and 2.18mmol/L)

One patient with SARC had a high (corrected) serum calcium (2.70mmol/L)

These data are presented graphically in Figure 7.36.

Figure 7.36 Abnormalities in calcium, albumin and phosphate profiles (% of patients in each group)



7.5.3 Liver function tests

Abnormalities in the patients' liver function tests were evident as follows:

Diagnosis	OFG	CD	MRS	SARC	ALL
Number of patients tested (%)	114 (47.5)	29 (64.4)	6 (60.0)	3 (50.0)	152 (50.5)
Abnormality in liver function tests (%)	25 (22.0)	9 (31.0)	2 (33.3)	0 (0.0)	36 (23.7)

The specific abnormalities detected were noted as follows:

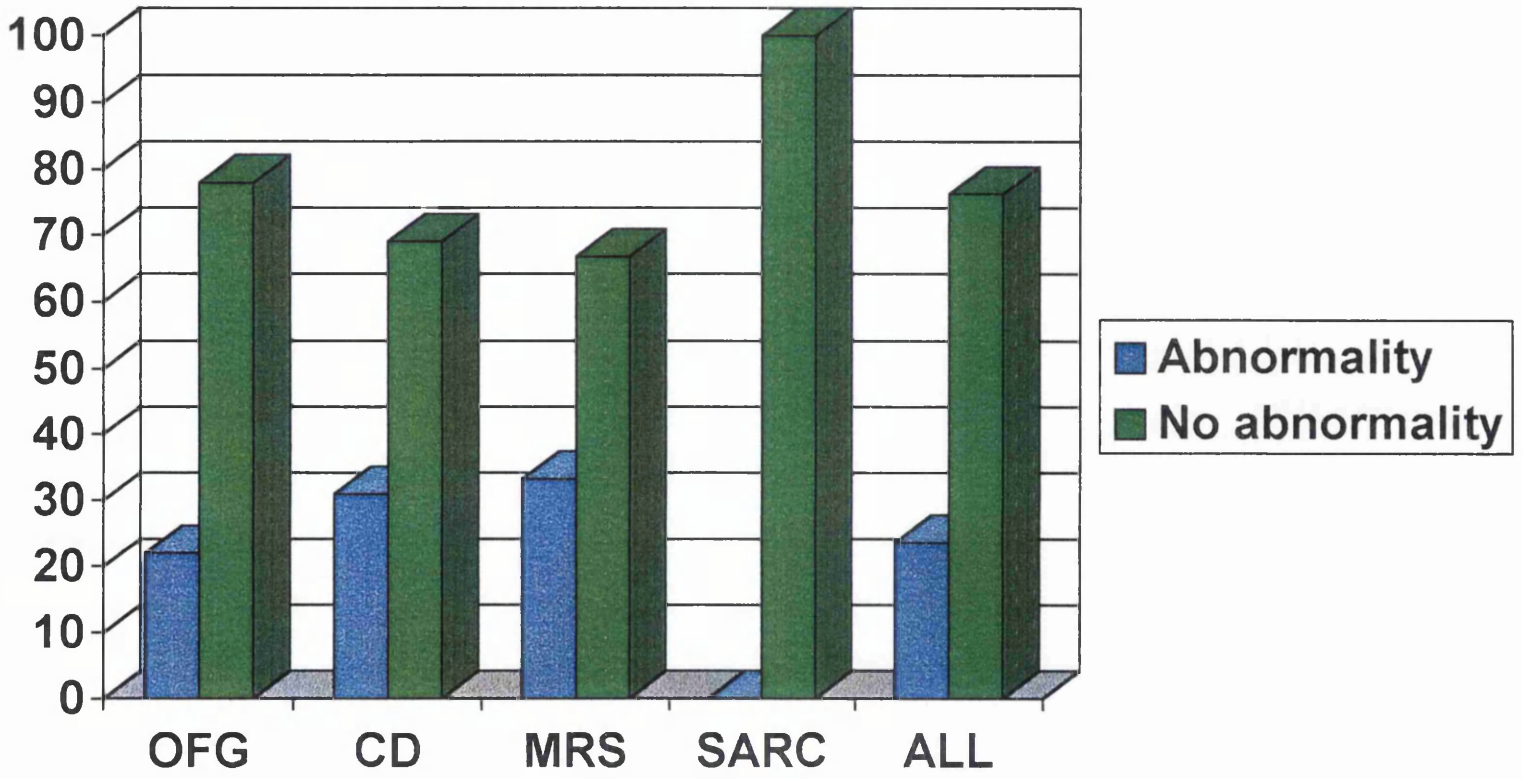
Fourteen patients with OFG had increased levels of Alkaline Phosphatase
Three patients with OFG had increased levels of AST
One patient with OFG had decreased levels of AST
Two patients with OFG had decreased levels of ALT
Two patients with OFG had increased levels of γGT
Two patients with OFG had increased levels of bilirubin
One patient with OFG had an unspecified abnormality in liver function tests

Two patients with CD had increased levels of Alkaline Phosphatase
One patient with CD had increased levels of AST
One patient with CD had decreased levels of AST
One patient with CD had decreased levels of ALT
Two patients with CD had increased levels of γGT
Two patients with CD had increased levels of bilirubin

One patient with MRS had increased levels of Alkaline Phosphatase
One patient with MRS had decreased levels of AST

These data are presented graphically in Figure 7.37.

Figure 7.37 Abnormalities in liver function test profiles (% of patients in each group)



7.5.4 Serum angiotensin converting enzyme

The patients' Serum angiotensin converting enzyme levels (U/L) were recorded for each group as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	99 (41.3)	23 (51.1)	6 (60.0)	2 (33.3)	130 (43.2)
Serum angiotensin converting enzyme (U/L)					
Mean	38.3	37.8	34.2	34.5	38.0
Minimum	0.0	12.0	24.0	26.0	0.0
Maximum	99.0	80.0	52.0	43.0	99.0

Analysis using the Kruskal-Wallis test revealed the following results:
$H = 0.000$; $DF = 1$; $p = 0.987$

These data are presented graphically in Figures 7.38 and 7.39.

It should be noted that the statistical analysis was between OFG and CD and does not comment on the serum angiotensin converting enzyme levels for patients with sarcoidosis.

The low levels of serum ACE in the patients with sarcoidosis may be due to small patient numbers (n=2) and that their pulmonary disease was relatively inactive at the

time of sampling. Both patients had substantially higher levels of serum ACE recorded at other times in the disease process.

Figure 7.38 Serum angiotensin converting enzyme levels (U/L)
(absolute values for each patient group)

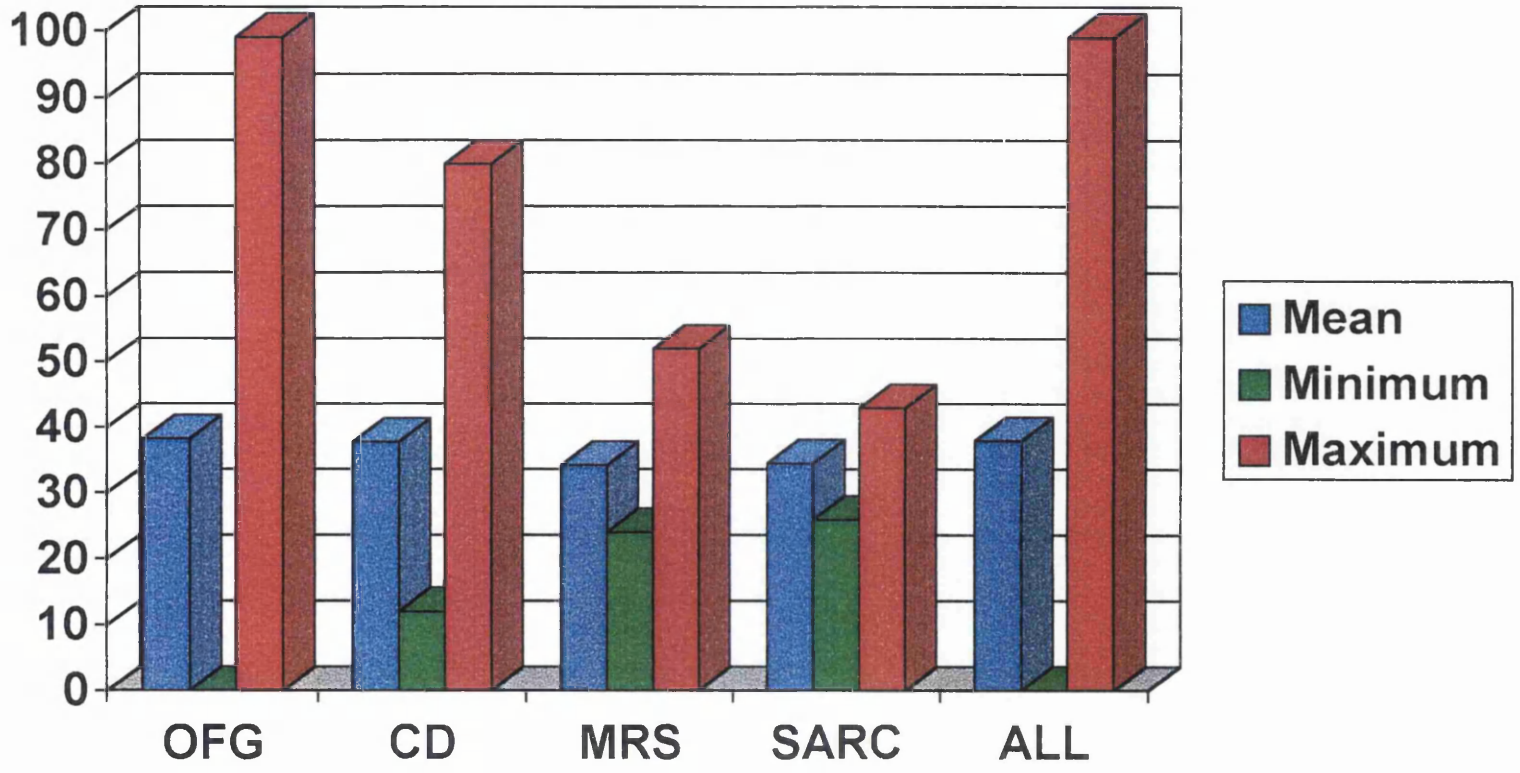
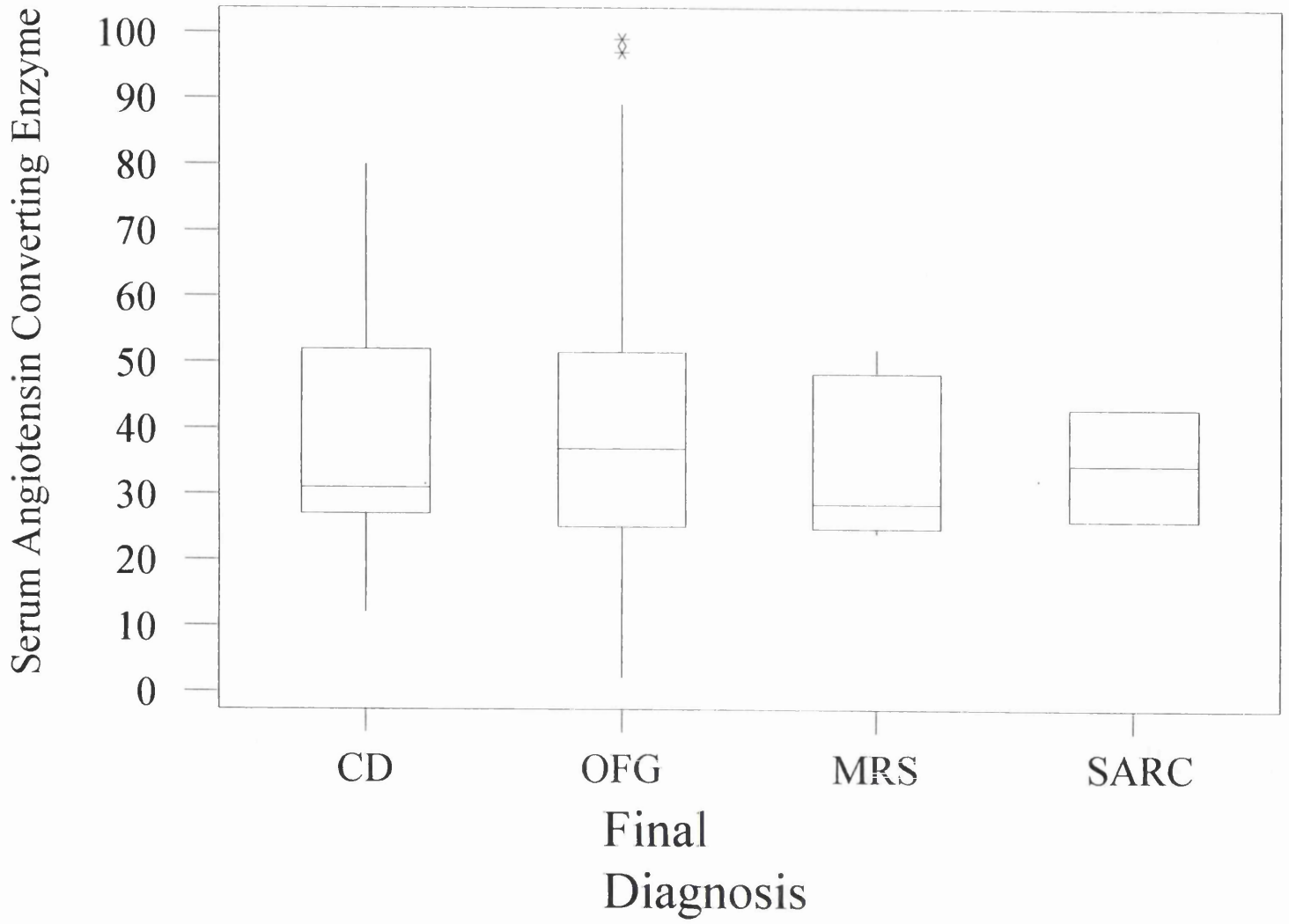


Figure 7.39 Serum angiotensin converting enzyme level (U/L), according to final diagnostic category



7.6 Immunological investigations

7.6.1 Immunoglobulin levels

7.6.1.1 IgA

The patients' Immunoglobulin A-levels (U/L) were recorded for each group as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	79 (32.9)	24 (53.3)	5 (50.0)	3 (50.0)	111 (36.9)
Immunoglobulin A-levels (U/L)					
Mean	2.30	2.88	1.74	2.97	2.42
Minimum	0.20	0.20	0.79	2.07	0.20
Maximum	5.20	7.20	2.40	4.19	7.20

These data are presented graphically in Figures 7.40 and 7.41.

Figure 7.40 IgA levels (U/L)
(absolute values for each patient group)

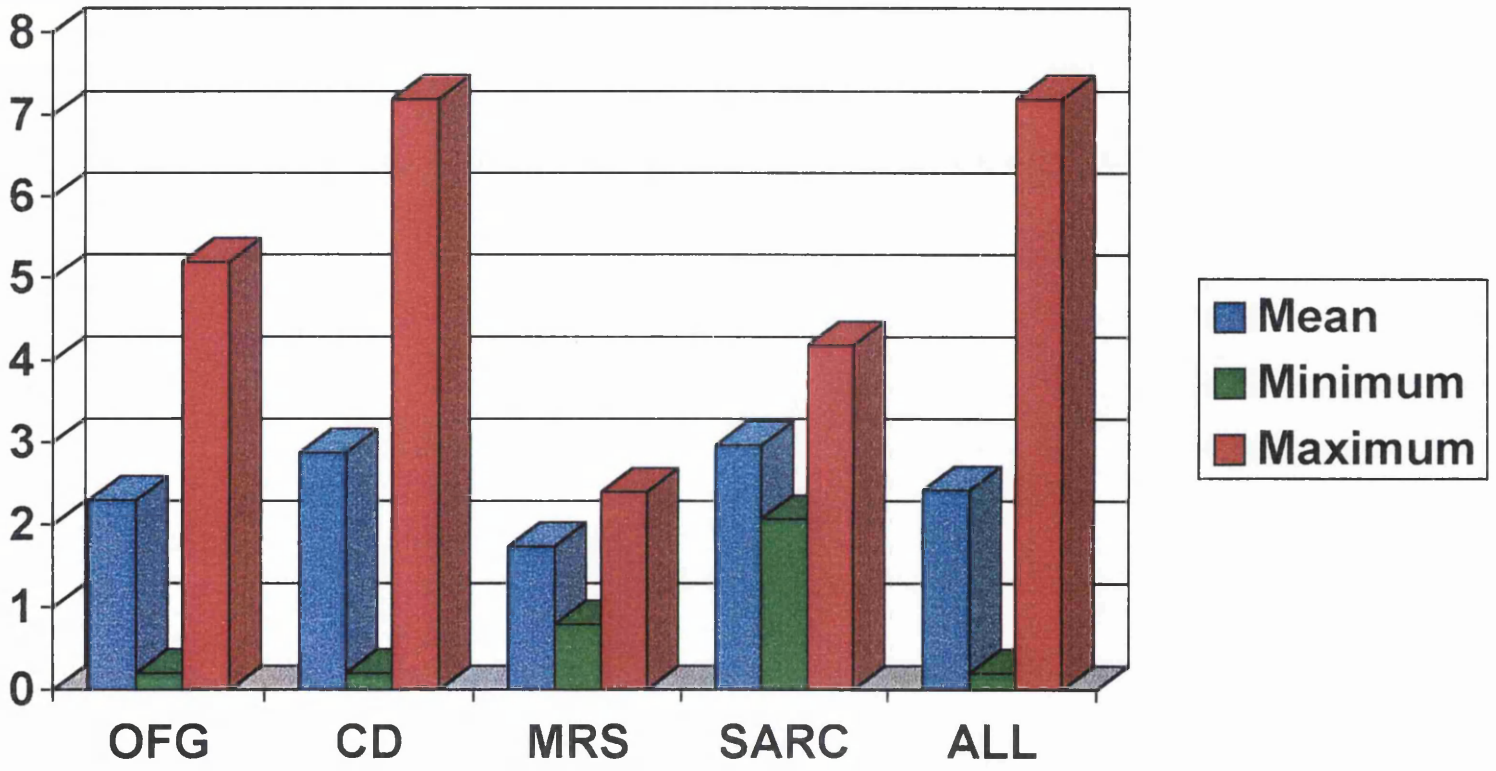
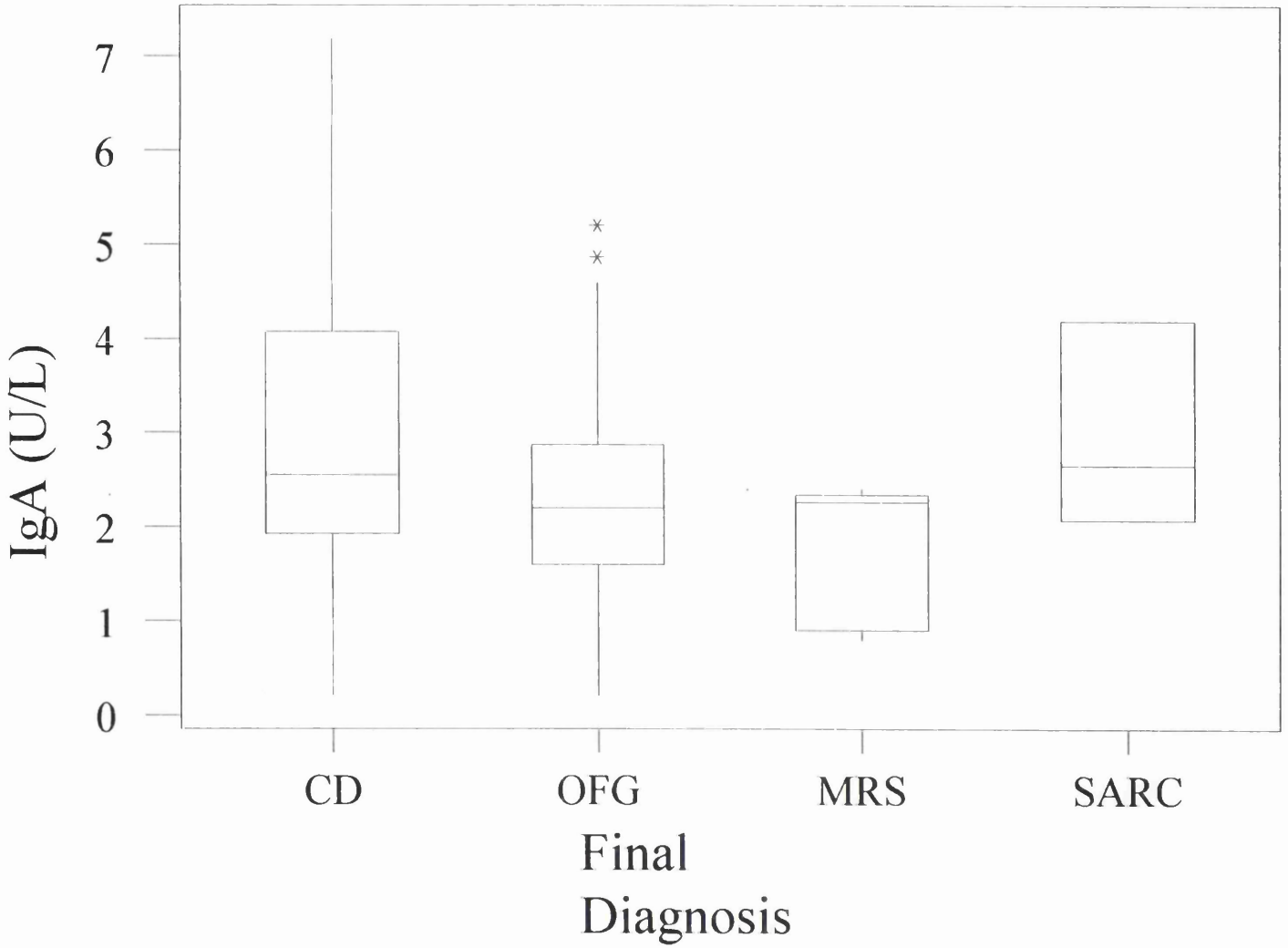


Figure 7.41 Immunoglobulin A levels (U/L), according to final diagnostic category



7.6.1.2 IgG

The patients' Immunoglobulin G-levels (U/L) were recorded for each group as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	80 (33.3)	24 (53.3)	5 (50.0)	3 (50.0)	112 (37.2)
Immunoglobulin G-levels (U/L)					
Mean	11.28	10.84	8.23	9.52	11.00
Minimum	7.28	1.10	5.79	9.15	1.10
Maximum	21.90	15.80	10.40	10.26	21.90

These data are presented graphically in Figures 7.42 and 7.43.

Figure 7.42 IgG levels (U/L) (absolute values for each patient group)

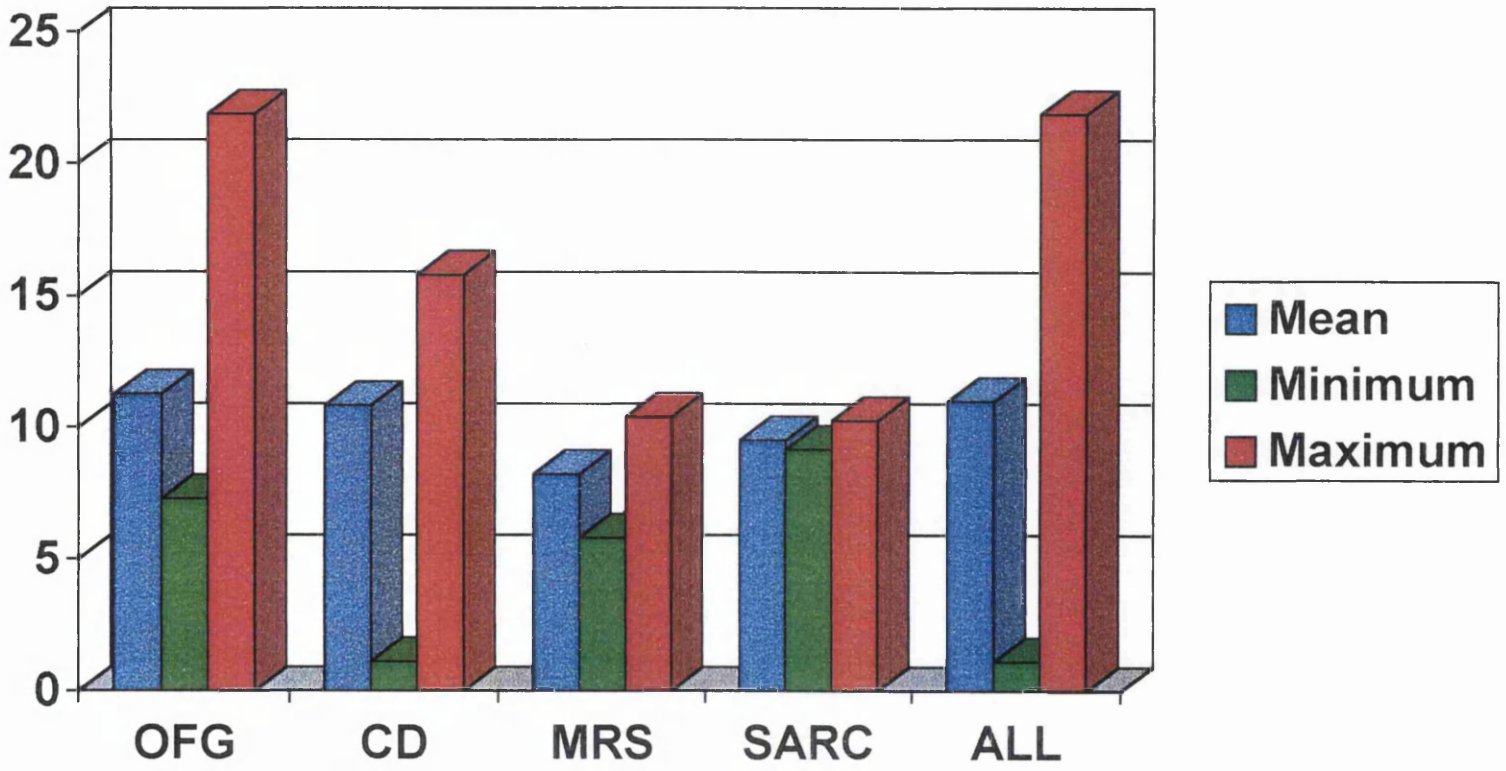
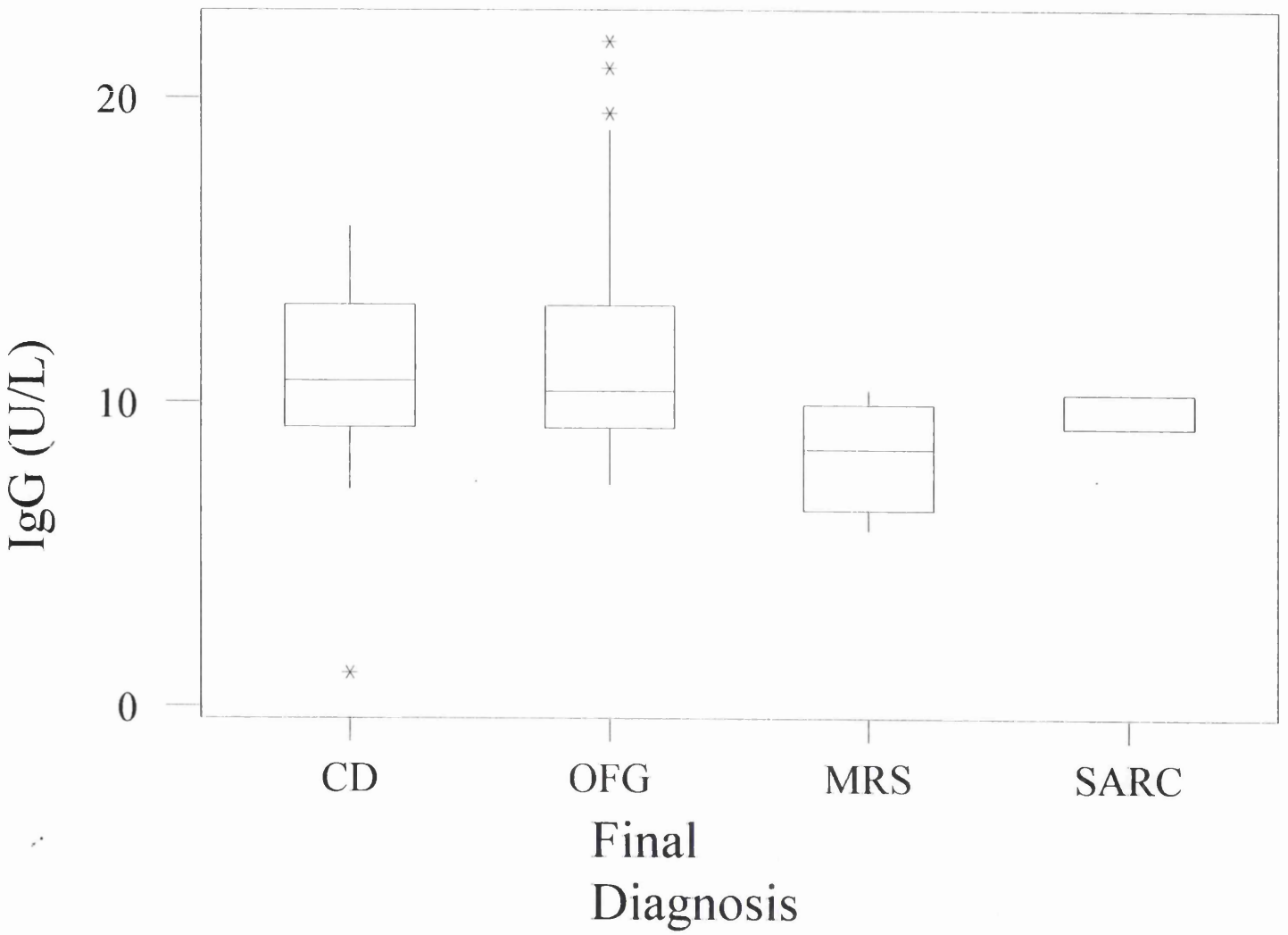


Figure 7.43 Immunoglobulin G levels (U/L), according to final diagnostic category



7.6.1.3 IgM

The patients' Immunoglobulin M-levels (U/L) were recorded for each group as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	79 (33.0)	24 (53.3)	5 (50.0)	3 (50.0)	111 (36.9)
Immunoglobulin M-levels (U/L)					
Mean	1.32	1.51	1.37	1.53	1.37
Minimum	0.30	0.50	0.87	1.46	0.30
Maximum	3.00	6.90	1.71	1.66	6.90

These data are presented graphically in Figures 7.44 and 7.45.

Figure 7.44 IgM levels (U/L)
(absolute values for each patient group)

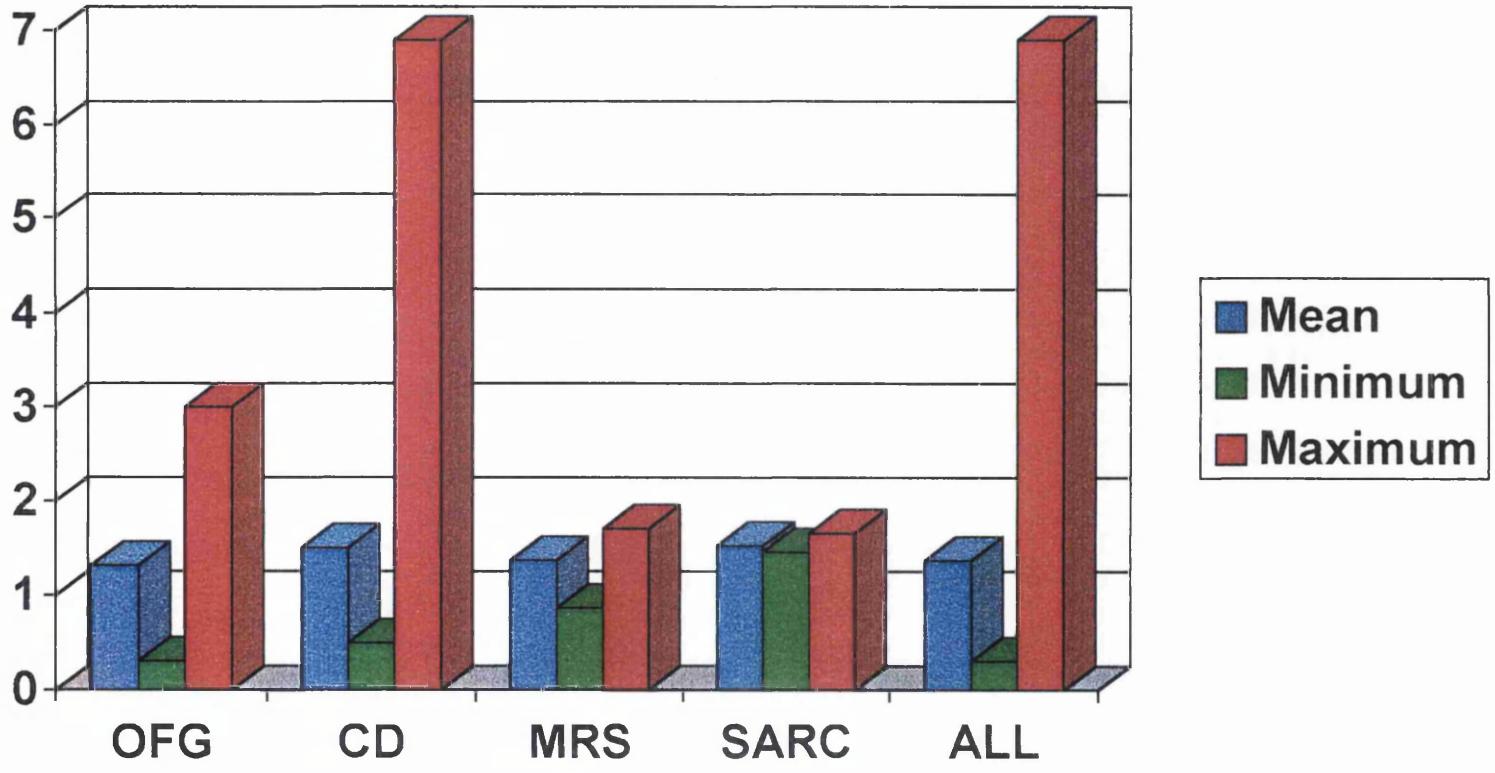
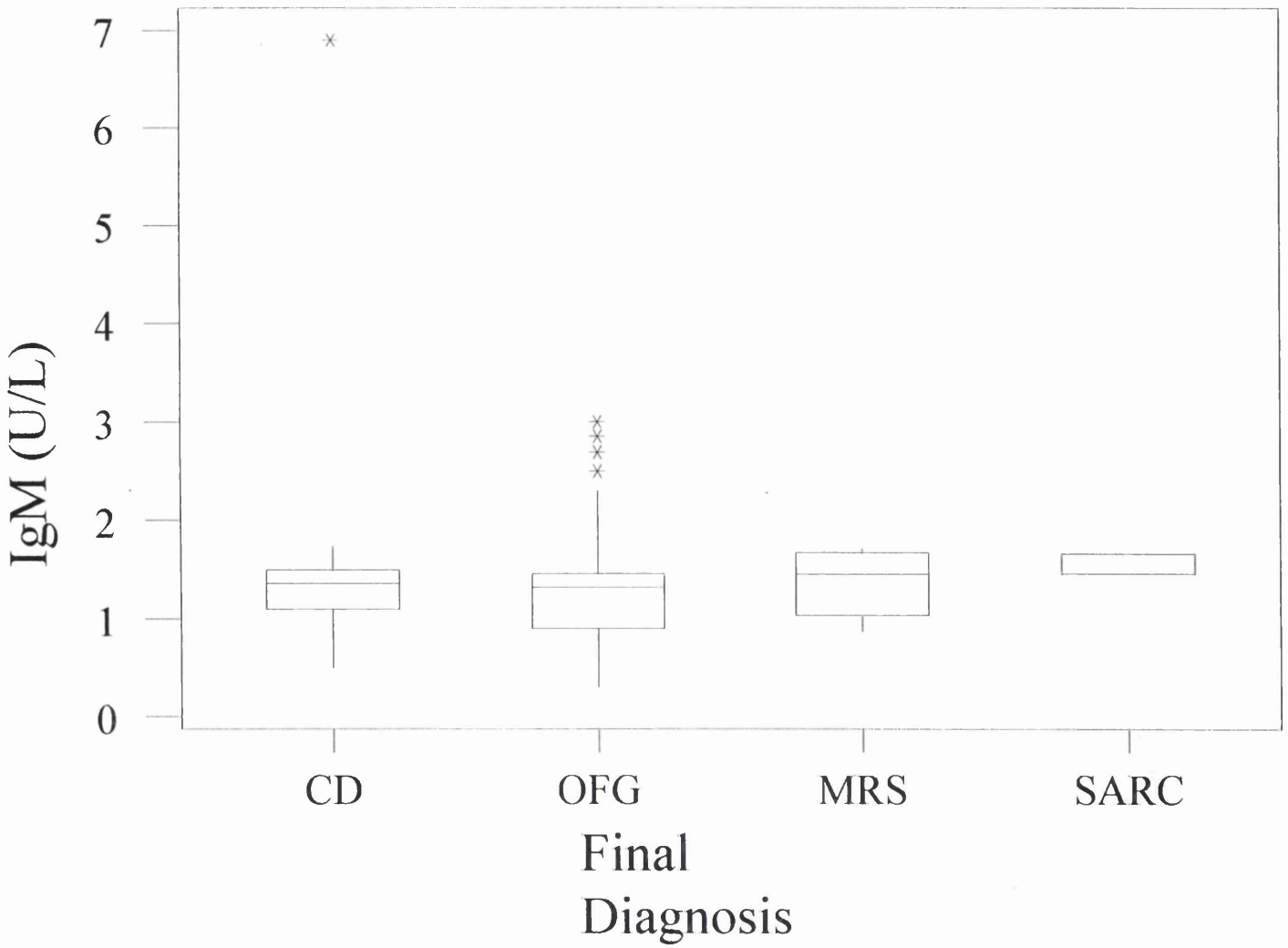


Figure 7.45 Immunoglobulin M levels (U/L), according to final diagnostic category



7.6.2 Radioallergosorbent (RAST) testing (IgE)

RAST (IgE) results are shown for each diagnostic category as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients tested (%)	136 (56.7)	29 (64.4)	10 (100.0)	5 (83.3)	180 (59.8)
RASTS (IgE) Very high					
House dust mite	4 (2.9)	1 (3.4)	0 (0.0)	0 (0.0)	5 (2.8)
Tree pollen	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Peanut	1 (0.7)	1 (3.4)	0 (0.0)	0 (0.0)	2 (1.1)
Grass pollen	1 (0.7)	1 (3.4)	0 (0.0)	0 (0.0)	2 (1.1)
Fish	0 (0.0)	1 (3.4)	0 (0.0)	0 (0.0)	1 (0.6)
Total (%)	7 (5.1)	4 (13.8)	0 (0.0)	0 (0.0)	11 (6.1)

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients tested (%)	136 (56.7)	29 (64.4)	10 (100.0)	5 (83.3)	180 (59.8)
RASTS (IgE) High					
House dust mite	29 (21.3)	4 (13.8)	1 (10.0)	0 (0.0)	34 (18.9)
Grass pollen	6 (4.4)	0 (0.0)	0 (0.0)	0 (0.0)	6 (3.3)
Poa pratensis	4 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.2)
Cat	5 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	5 (2.8)
Dog	4 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.2)
Tree pollen	3 (2.2)	1 (3.4)	0 (0.0)	0 (0.0)	4 (2.2)
Cow's milk	3 (2.2)	1 (3.4)	0 (0.0)	0 (0.0)	4 (2.2)
Peanut	2 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.1)
Wheat	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Soya	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Horse	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Feather	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Fish	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Total (%)	61 (44.9)	6 (20.7)	1 (10.0)	0 (0.0)	68 (37.8)

Result of RASTs (Continued)

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients tested (%)	136 (56.7)	29 (64.4)	10 (100.0)	5 (83.3)	180 (59.8)
RAST (IgE) Moderate					
House dust mite	6 (4.4)	0 (0.0)	2 (20.0)	1 (20.0)	9 (5.0)
Grass pollen	3 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.7)
Poa pratensis	2 (1.5)	0 (0.0)	1 (10.0)	0 (0.0)	3 (1.7)
Cat	3 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.7)
Dog	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Tree pollen	4 (2.9)	1 (3.4)	0 (0.0)	0 (0.0)	5 (2.8)
Cow's milk	2 (1.5)	1 (3.4)	0 (0.0)	0 (0.0)	3 (1.7)
Peanut	2 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.1)
Wheat	3 (2.2)	1 (3.4)	0 (0.0)	0 (0.0)	4 (2.2)
Soya	1 (0.7)	1 (3.4)	0 (0.0)	0 (0.0)	2 (1.1)
Egg white	0 (0.0)	1 (3.4)	0 (0.0)	0 (0.0)	1 (0.6)
Pork	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Potato	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Latex	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Almond	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Hazelnut	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Brazil nut	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Total (%)	33 (24.3)	5 (17.2)	3 (30.0)	1 (20.0)	42 (23.3)

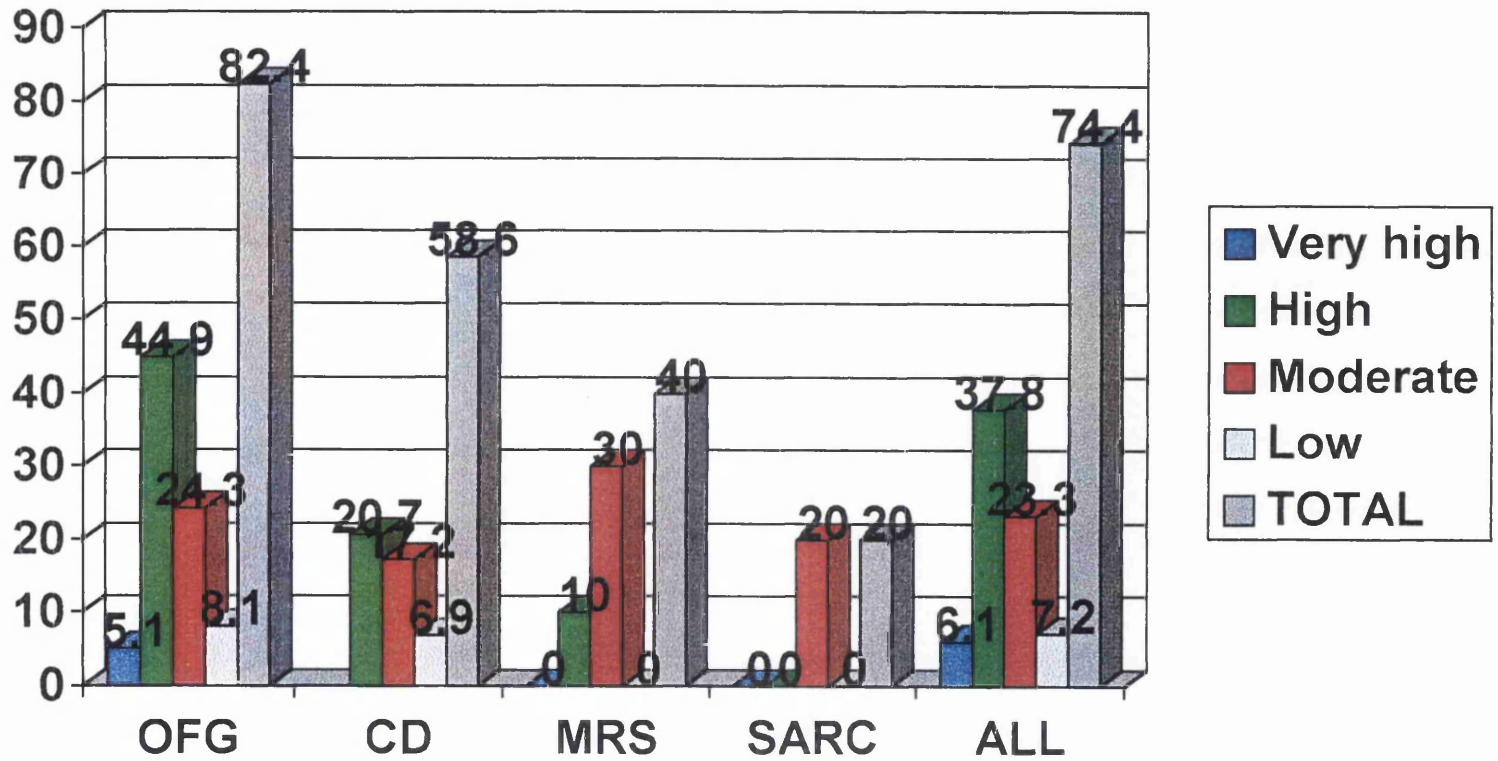
Diagnosis	OFG	CD	MRS	SARC	ALL
Number of patients tested (%)	136 (56.7)	29 (64.4)	10 (100.0)	5 (83.3)	180 (59.8)
RASTS (IgE) Low					
House dust mite	3 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.7)
Wheat	2 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.1)
Cow's milk	2 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.1)
Peanut	2 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.1)
Soya	0 (0.0)	1 (3.4)	0 (0.0)	0 (0.0)	1 (0.6)
Tree pollen	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Egg white	1 (0.7)	1 (3.4)	0 (0.0)	0 (0.0)	2 (1.1)
Total (%)	11 (8.1)	2 (6.9)	0 (0.0)	0 (0.0)	13 (7.2)
GRAND TOTAL (%)	112 (82.4)	17 (58.6)	4 (40.0)	1 (20.0)	134 (74.4)

Whilst statistical analysis was deemed inappropriate for individual allergens, overall analysis using the Kruskal-Wallis test revealed the following results:
$H = 0.000$; $DF = 1$, $p = 0.000$.

Although no difference was evident between the OFG and CD groups in terms of clinical atopy, the results of the RAST (IgE) are clearly statistically significant and add further credibility to the results elsewhere in this thesis suggesting an immunological (allergic) basis for OFG.

These results are presented graphically in Figure 7.46.

Figure 7.46 IgE levels (RASTs) (% of patients in each group)



7.6.3 Complement screen

The results for Complement screens in each of the patient groups is shown below:

(A) Complement C3

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	104 (43.3)	24 (53.3)	7 (70.0)	3 (50.0)	138 (45.8)
Complement C3 (g/L)					
Mean	1.29	1.35	1.26	1.11	1.30
Minimum	0.81	1.06	1.02	0.88	0.81
Maximum	1.94	2.15	1.54	1.22	2.15

These results are presented graphically in Figures 7.47 and 7.48.

(B) Complement C4

The results for Complement C4 levels are presented graphically in Figure 7.49.

Figure 7.47 C3 levels (g/L)
(absolute values for each patient group)

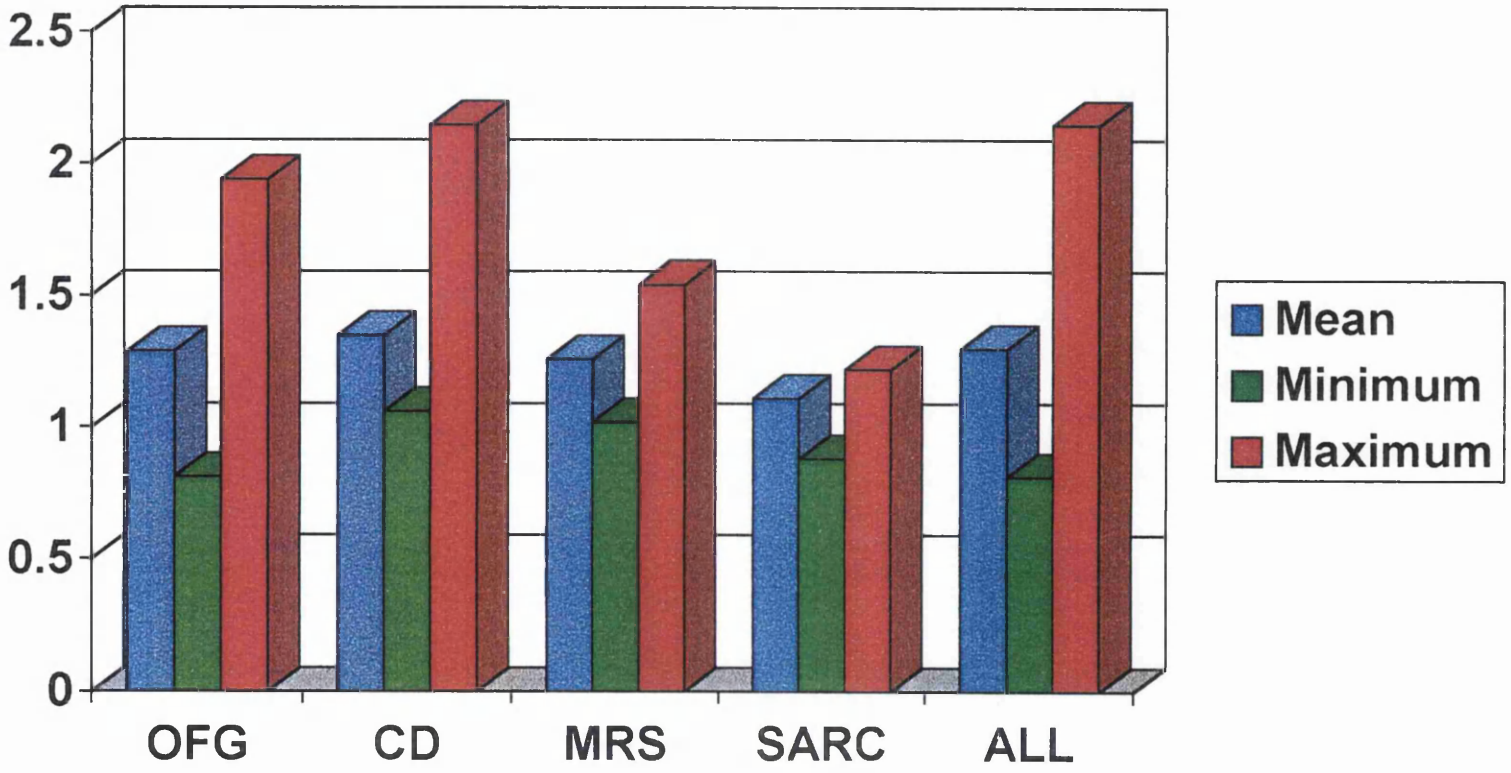


Figure 7.48 Complement C3 levels (g/L), according to final diagnostic category

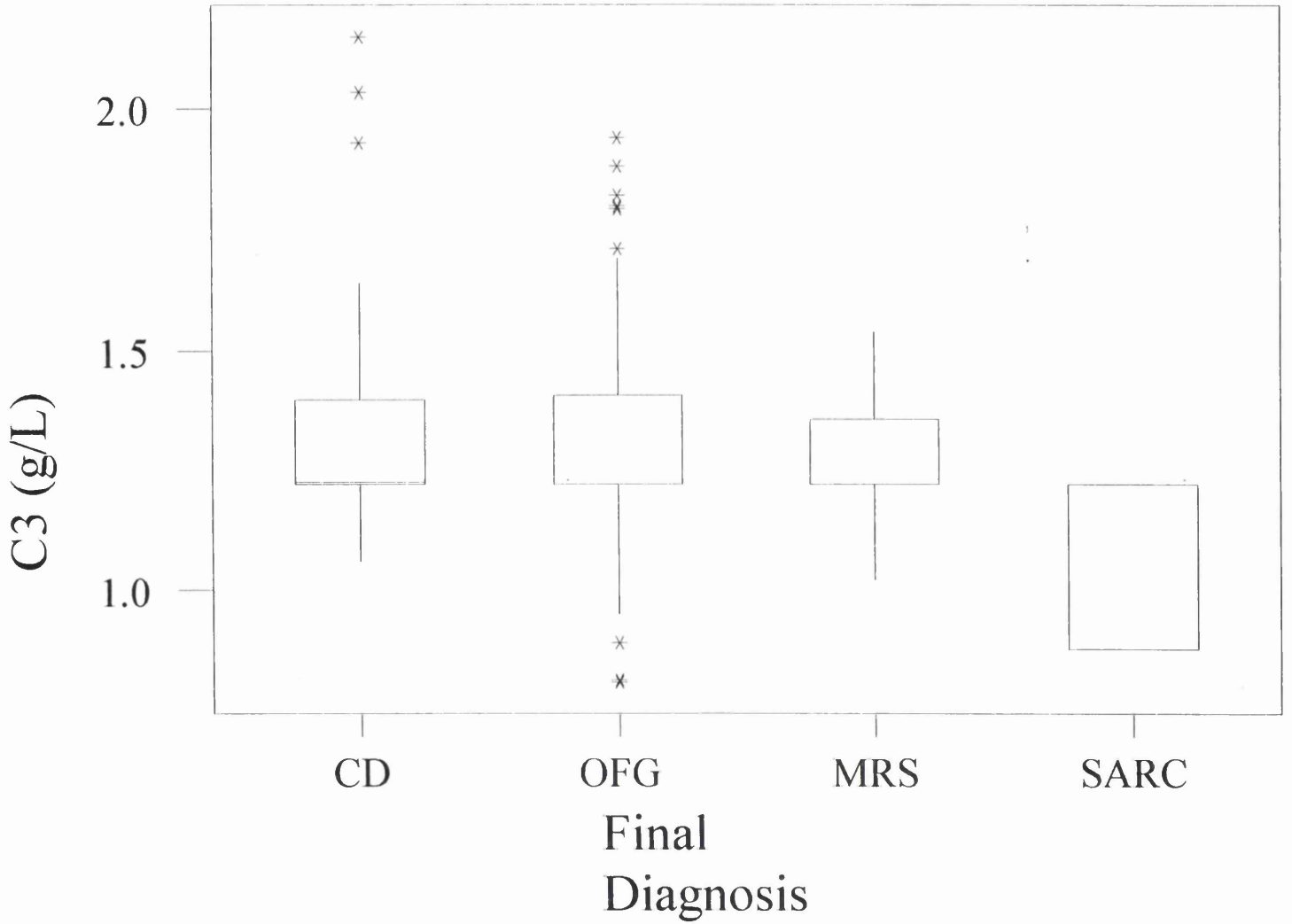
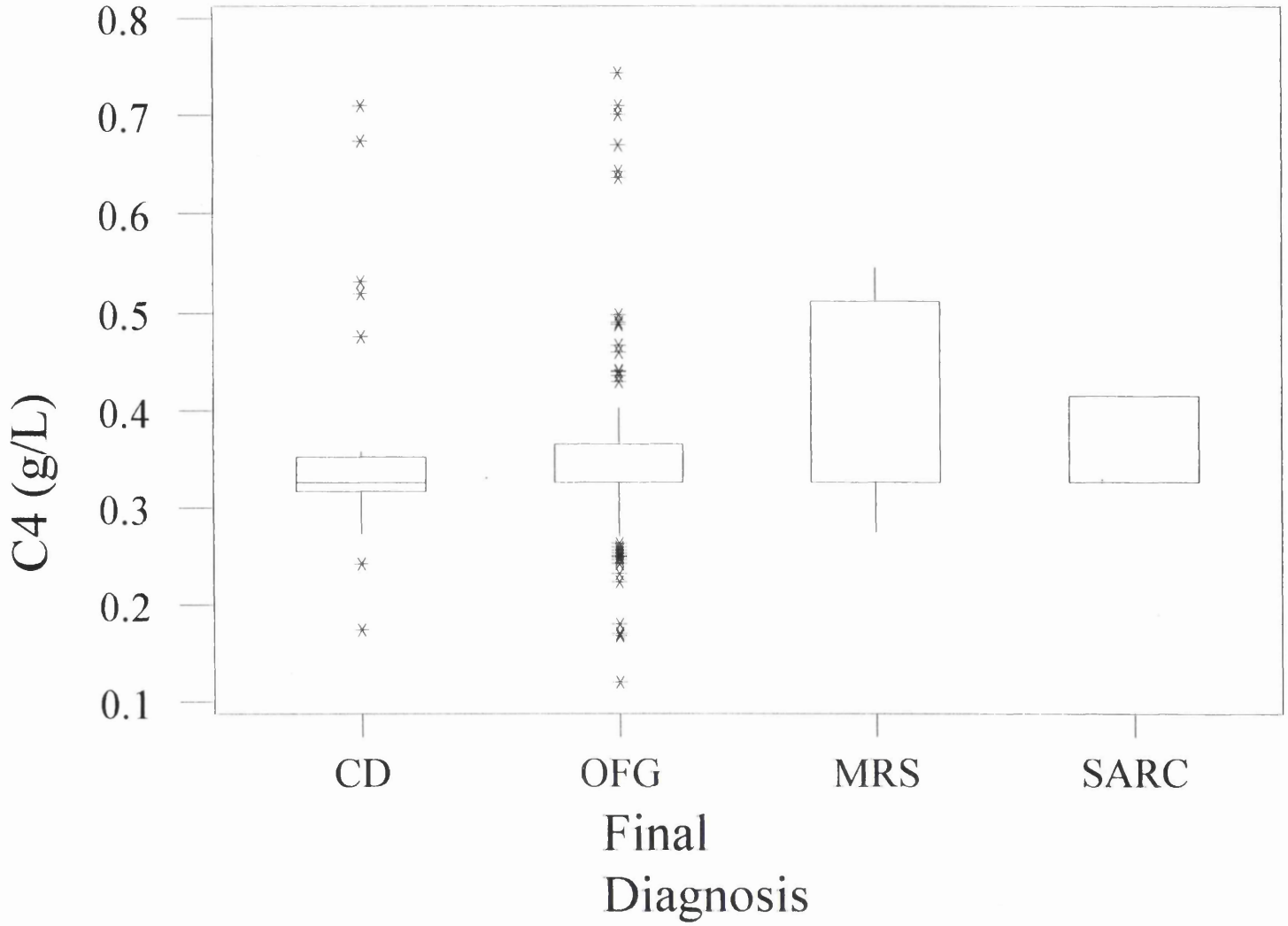


Figure 7.49 Complement C4 levels (g/L), according to final diagnostic category



(C) C1-esterase Inhibitor

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients (%)	102 (42.5)	24 (53.3)	6 (60.0)	3 (50.0)	135 (44.9)
C1-esterase inhibitor level (g/L)					
Mean	0.28	0.28	0.29	0.28	0.28
Minimum	0.17	0.22	0.23	0.27	0.17
Maximum	0.44	0.34	0.32	0.29	0.44

These data are presented graphically in Figures 7.50 and 7.51.

Figure 7.50 C1-esterase inhibitor levels (g/L) (absolute values for each patient group)

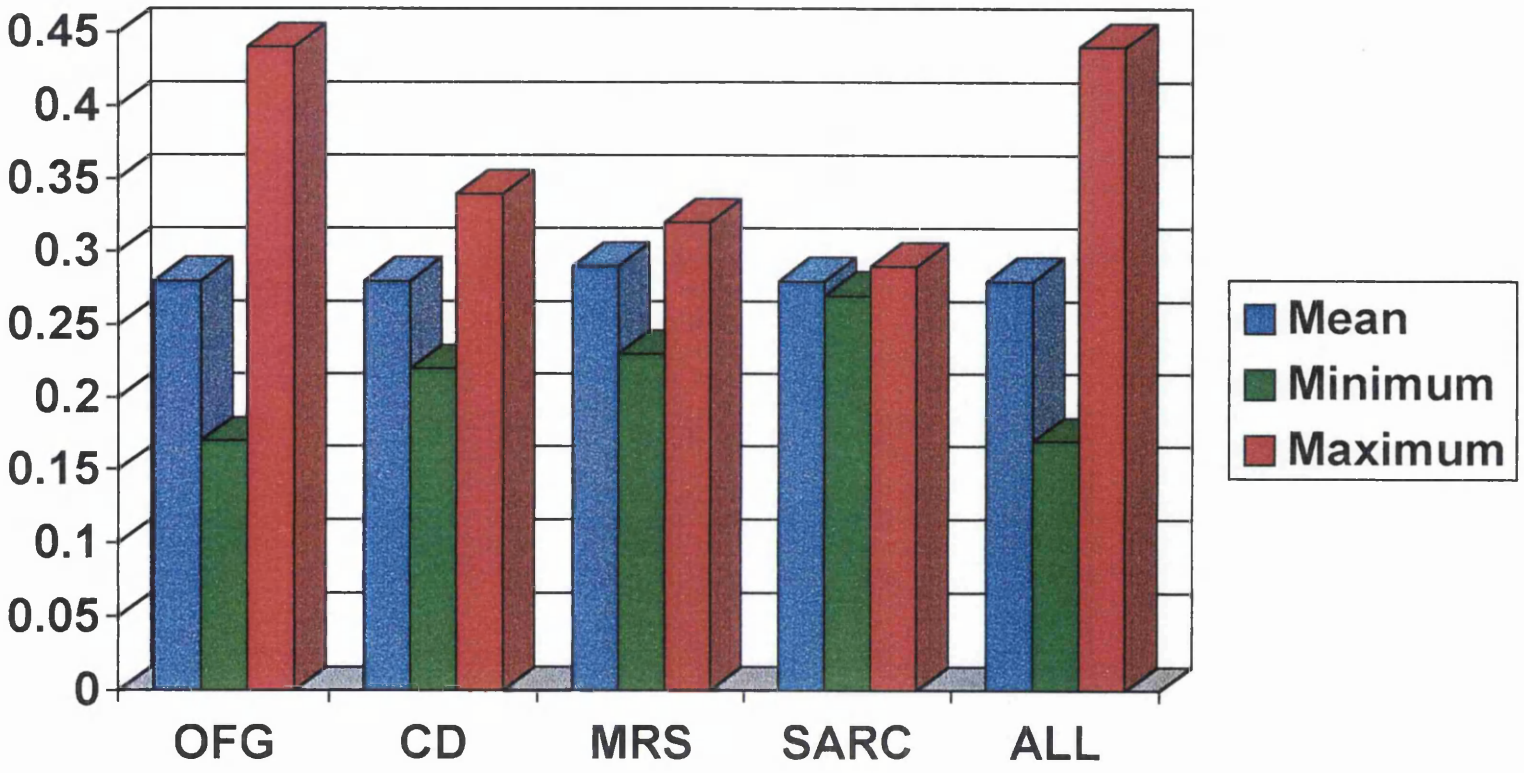
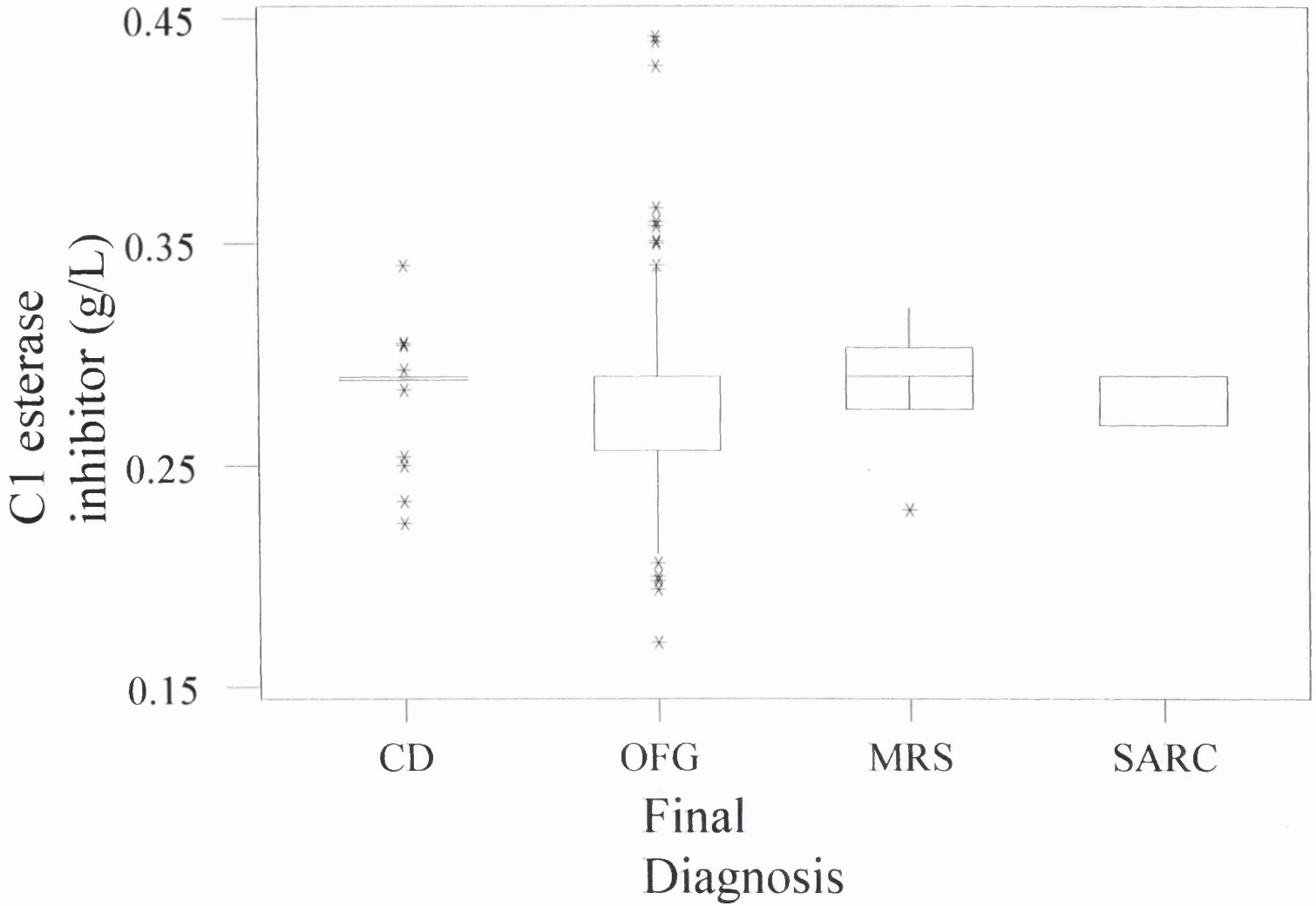


Figure 7.51 C1-esterase inhibitor levels (g/L), according to final diagnostic category



7.7 Oral mucosal biopsy

Oral mucosal biopsies were performed on 165 patients – 120 with orofacial granulomatosis, 30 with gastrointestinal Crohn's disease, 10 with Melkersson-Rosenthal syndrome, and 5 with sarcoidosis.

The histological assessment of these biopsies was as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients who underwent oral biopsy (%)	120 (50.0)	30 (66.7)	10 (100.0)	5 (83.3)	165 (54.8)
Granulomata present	83 (69.2)	24 (80.0)	5 (50.0)	3 (60.0)	115 (69.7)
Granulomata absent	37 (30.9)	(20.0)	5 (50.0)	2 (40.0)	50 (30.3)
Lymphoedema present	100 (83.3)	28 (93.3)	9 (90.0)	4 (80.0)	139 (84.2)
Lymphoedema absent	20 (16.7)	2 (6.7)	1 (10.0)	1 (20.0)	26 (15.8)

Chi-square analysis of OFG and CD revealed the following:

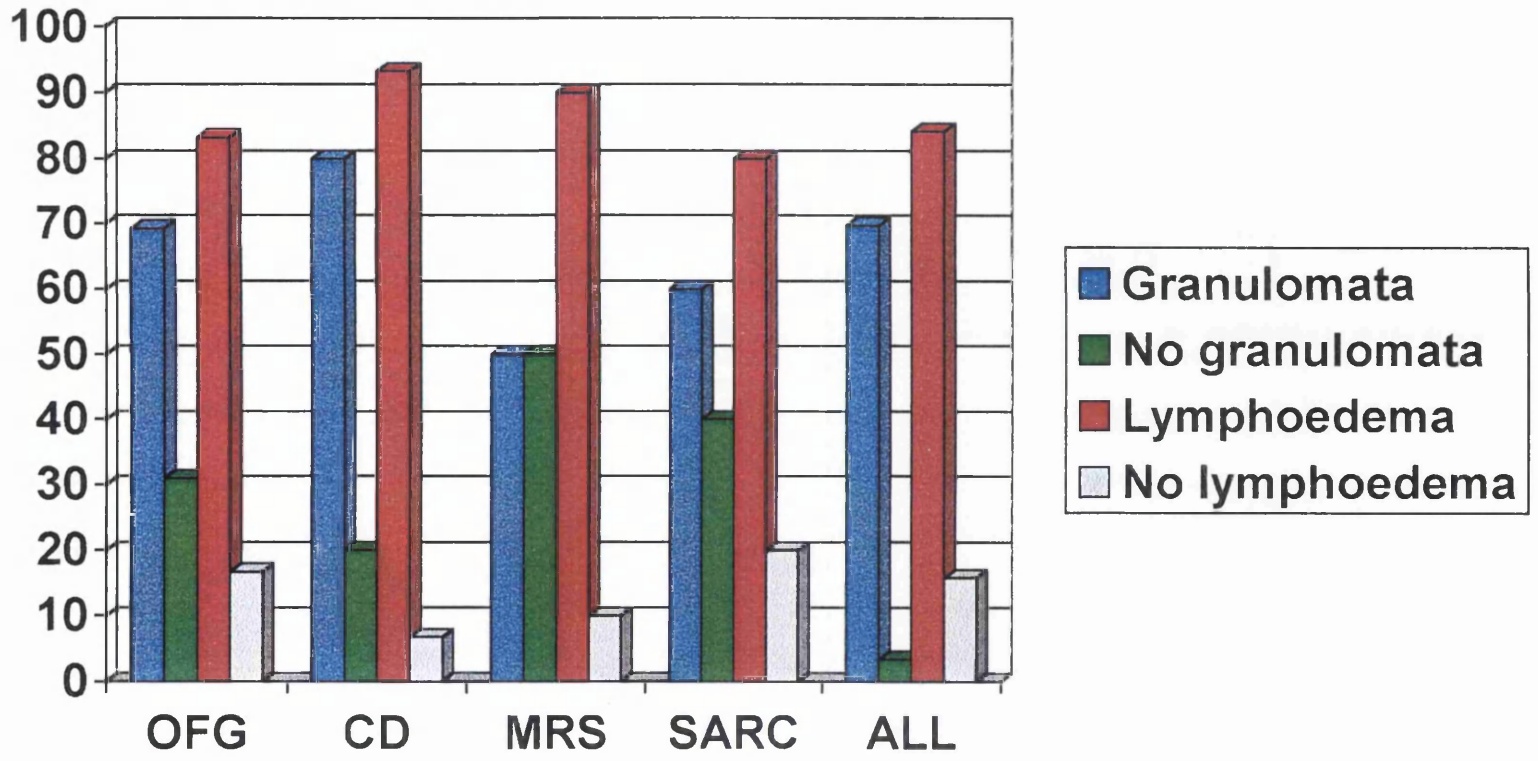
Presence of granulomas Chi-square = 6.310; DF = 1; p = 0.012

Presence of lymphoedema Chi-square = 8.136; GF = 1; p = 0.004

Thus, there was a statistically significant difference in the histological findings between OFG and CD - more likely to find both granulomas and lymphoedema, either singly or in combination, in the biopsies from patients with CD.

These data are presented graphically in Figure 7.52.

Figure 7.52 Histological findings on biopsy (% of group totals)



7.8 Patch-testing and contact urticaria testing

For the purposes of analysis, patch-testing and contact urticaria results are recorded together.

The results of skin reactivity testing for each of the patient groups are as follows:

(A) Benzoic Acid

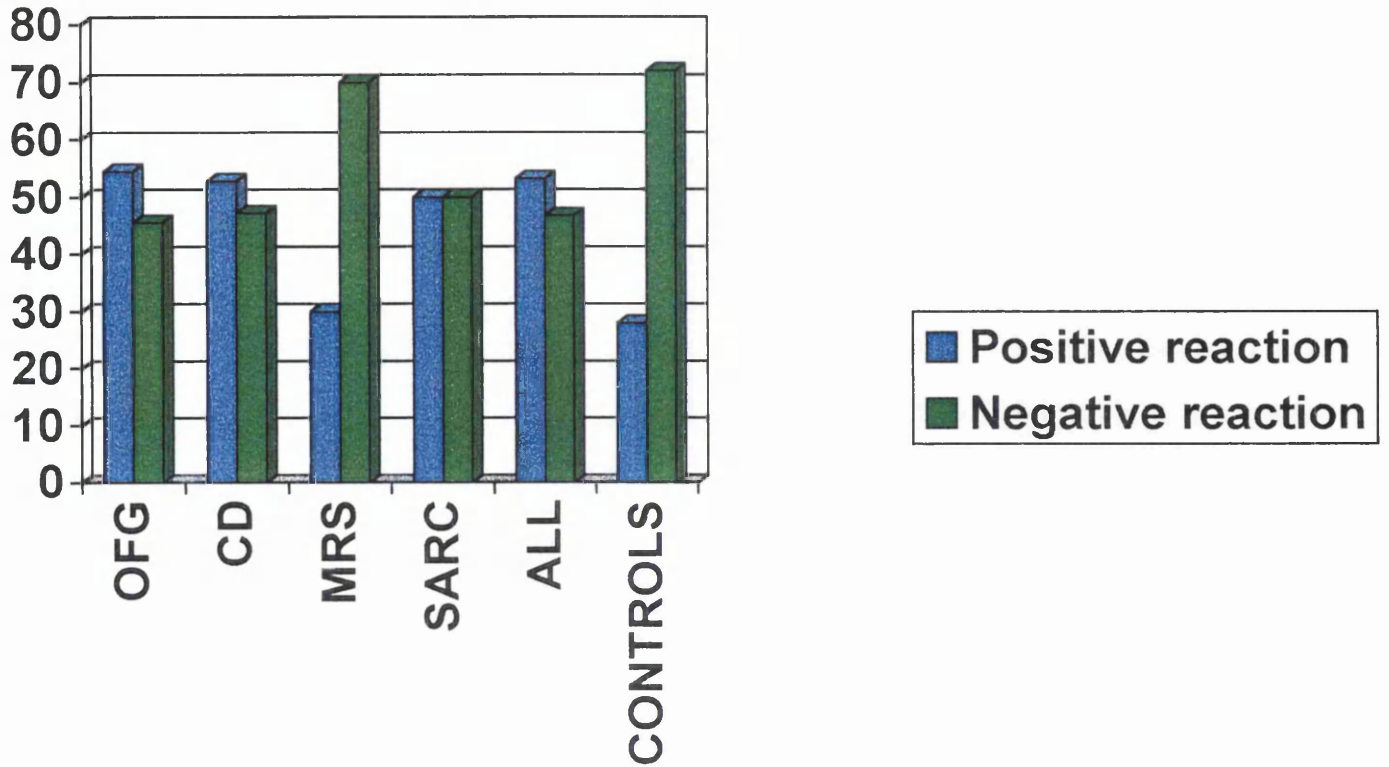
Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients who underwent patch-testing (%)	224 (93.3)	36 (80.0)	10 (100.0)	4 (66.7)	274 (91.0)
Positive reaction to Benzoic acid	122 (54.5)	19 (52.8)	3 (30.0)	2 (50.0)	146 (53.3)
Negative reaction to Benzoic acid	102 (45.5)	17 (47.2)	7 (70.0)	2 (50.0)	128 (46.7)

Chi-square analysis revealed the following results:

chi-square = 0.871; DF = 1; p = 0.351

These results are presented graphically in Figure 7.53.

Figure 7.53 Skin reactivity tests - benzoic acid (% of group totals)



(B) Cinnamonaldehyde

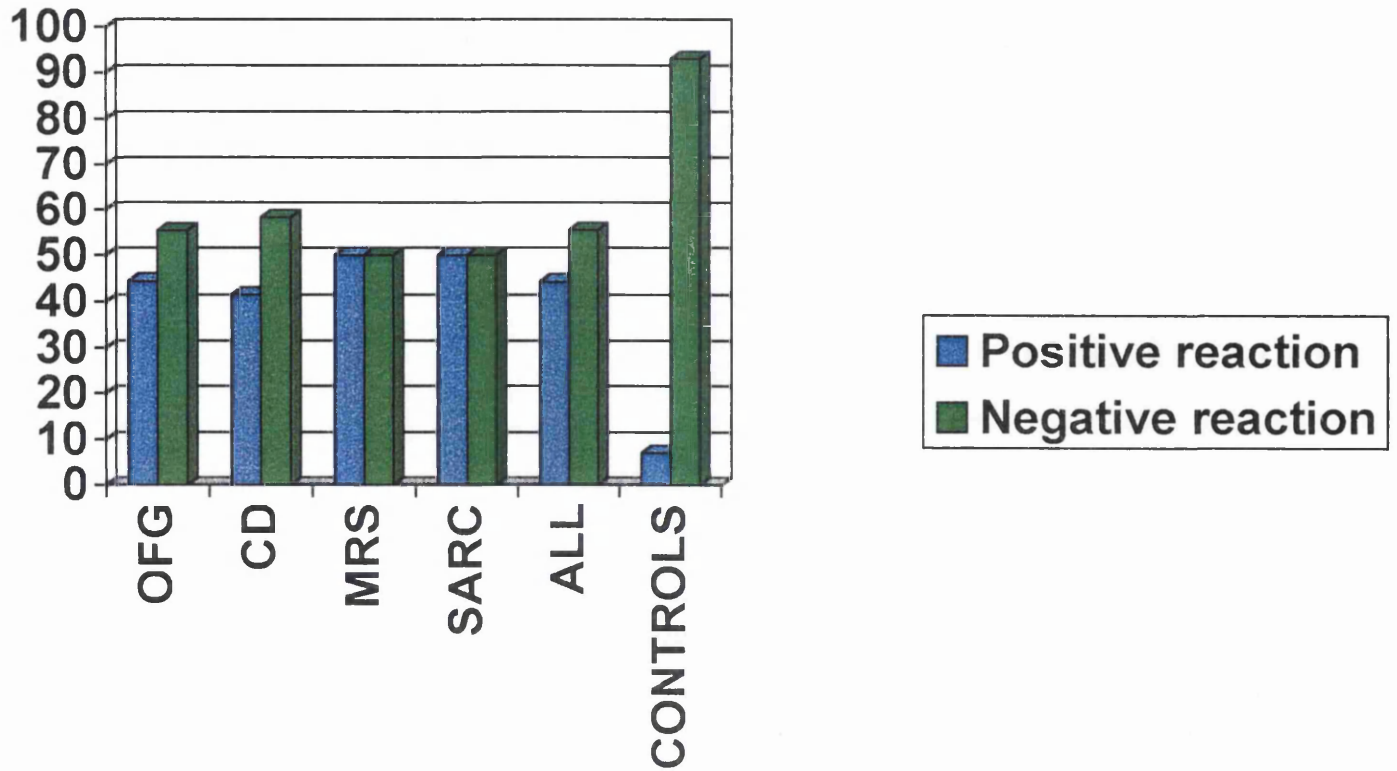
Diagnosis	OFG	CD	MRS	SARC	ALL
Number of patients who underwent patch-testing (%)	224 (93.3)	36 (80.0)	10 (100.0)	4 (66.7)	274 (91.0)
Positive reaction to Cinnamon	100 (44.6)	15 (41.7)	5 (50.0)	2 (50.0)	122 (44.5)
Negative reaction to Cinnamon	124 (55.4)	21 (58.3)	5 (50.0)	2 (50.0)	152 (55.5)

Chi-square analysis revealed the following results:

chi-square = 0.886; DF = 1; p = 0.347

These results are presented graphically in Figure 7.54.

Figure 7.54 Skin reactivity tests - cinnamonaldehyde (% of group totals)



(C) Others

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients who underwent patch-testing (%)	224 (93.3)	36 (80.0)	10 (100.0)	4 (66.7)	274 (91.0)
Positive reactions to (%)					
Chocolate	41 (18.3)	2 (5.6)	0 (0.0)	0 (0.0)	43 (15.7)
Nickel	27 (12.1)	5 (13.9)	4 (40.0)	1 (25.0)	38 (13.9)
Sorbic acid	21 (9.4)	4 (11.1)	0 (0.0)	0 (0.0)	25 (9.1)
Perfume/ Fragrance Mix	13 (5.8)	2 (5.6)	3 (30.0)	0 (0.0)	18 (6.6)
Rubber and accelerators	10 (4.5)	1 (2.8)	0 (0.0)	1 (25.0)	12 (4.4)
Balsams	8 (3.6)	0 (0.0)	0 (0.0)	0 (0.0)	8 (2.9)
Colophony	7 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	7 (2.6)
Parabens	5 (2.2)	3 (8.3)	1 (10.0)	0 (0.0)	9 (3.3)
Lanolin	5 (2.2)	2 (5.6)	0 (0.0)	0 (0.0)	7 (2.6)
Thiomersal	4 (1.8)	1 (2.8)	0 (0.0)	0 (0.0)	5 (1.8)
Cobalt	4 (1.8)	0 (0.0)	1 (10.0)	0 (0.0)	5 (1.8)
Toothpaste	4 (1.8)	2 (5.6)	2 (20.0)	0 (0.0)	8 (2.9)
Mercury and Compounds	3 (1.3)	1 (2.8)	0 (0.0)	0 (0.0)	4 (1.5)
Salicylic acid	3 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.1)
Formaldehyde	3 (1.3)	0 (0.0)	1 (10.0)	0 (0.0)	4 (1.5)
Propylene glycol	3 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.1)
Sodium metabisulphite	3 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.1)
Potassium dichromate	2 (0.9)	0 (0.0)	2 (20.0)	0 (0.0)	4 (1.5)

(C) Others (continued)

Menthol	2 (0.9)	0 (0.0)	1 (10.0)	0 (0.0)	3 (1.1)
Anethole	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)
Vanilla	2 (0.9)	0 (0.0)	1 (10.0)	0 (0.0)	3 (1.1)
Oak moss	2 (0.9)	1 (2.8)	0 (0.0)	0 (0.0)	3 (1.1)
Paraphenylene Diamine	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)
Epoxy resin	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)
Peppermint Oil	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)
Gold and compounds	1 (0.4)	2 (5.6)	1 (10.0)	0 (0.0)	4 (1.5)
Palladium	2 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.7)
Neomycin	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Eugenols	1 (0.4)	1 (2.8)	1 (10.0)	0 (0.0)	3 (1.1)
Quinoline	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Thiuram	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Tartrazine	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Butylated hydroxytoluene	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Wood tar	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Coal tar	0 (0.0)	1 (2.8)	0 (0.0)	0 (0.0)	1 (0.4)
Caine mix	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	1 (0.4)
Kathon	1 (0.4)	1 (2.8)	0 (0.0)	0 (0.0)	2 (0.7)
Organic dyes	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Dettol mix	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Glutamic acid	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Spruce/pine Essence	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Methylphenyl-Benzotriazole	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Butylated hydroxy-anisole (margarine)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Cigarette smoke	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	1 (0.4)
Germall	0 (0.0)	1 (2.8)	0 (0.0)	0 (0.0)	1 (0.4)
Ginger	0 (0.0)	1 (2.8)	0 (0.0)	0 (0.0)	1 (0.4)
Chilli powder	0 (0.0)	1 (2.8)	0 (0.0)	0 (0.0)	1 (0.4)
TOTAL	197	32	20	2	251

Twenty-two patients with OFG (9.8% of those tested) were noted to have a significant worsening of their orofacial condition (most notably lip swelling) during the test procedures.

Three patients with CD reacted to benzoic acid on patch-testing with worsening of lip swelling. These patients had been treated unsuccessfully with enteric-coated prednisolone; the enteric coating is known to contain benzoic acid (The Association of the British Pharmaceutical Industry, 1998).

CONTROL GROUP

Of the 100 volunteers who acted as a control population, results were obtained as follows:

(a) Food additives

33% gave positive reactions	-	11 were delayed hypersensitivity
		20 were contact urticaria
		2 were both

Benzoic acid	28%
Sodium metabisulphite	6%
Sorbic acid	3%

(b) Perfumes and Flavourings

7% gave positive reactions	-	1 was delayed hypersensitivity
		5 were contact urticaria
		1 was both

Cinnamonaldehyde 7% (i.e. all volunteers who reacted)

(c) Chocolate - 1 volunteer (1%) gave a positive reaction (delayed)

(d) Other substances

Substance	% of volunteers who reacted
Nickel	23
Colophony	5
Cobalt	3
Formaldehyde	-
Potassium dichromate	2
Paraphenylene diamine	1
Mercapto-mix	-
Neomycin	1
Parabens	-
Balsam of Peru	4
Thiuram-mix	1
PPD-mix	-
Fragrance-mix	6
Primin	-
Quaternium-15	-
Carba-mix	-
Wool alcohols	-
PTBP formaldehyde	-
Caine-mix	-
Epoxy resin	1
Quinoline	-
Ethylene diamine	-
Thiomersal	21
Control (PMF)	

In summary, 73% of the control subjects reacted to one or more substances and 27% showed no reaction.

These data are presented graphically (with those of the disease groups) in Figures 7.53 and 7.54.

7.9 HLA typing

HLA typing of the sixteen patients with OFG gave results as follows:

HLA Phenotype				
Class I				Class II
A	**B**	**C**	**Bw**	**DR**
3	7,15	-	6	4
3, 11	W62, 18	W3, 5	6	4, W6
1, 3	7, 8	-	6	2, 3
1, 2	7, 8	-	6	2
3, 11	7, 35	W4	6	4, 5
23	17, 44	W4	4	5, 7
3, 24	7, 18	-	6	2, 4
2	W62, 7	W3	6	2, 4
2	27, 44	W5	4	3, W6
2, 11	W62, 14	W3, 8	6	W6
1, 2	14, 44	W8	4, 6	2, W6
2, 11	7, 27	W2	4, 6	3, 5
3	7	-	6	2
1	8, 37	W6	4, 6	2, 3
1, 2	8, 40	W3	6	3, 4
2, 3	7, 44	-	4, 6	-

These results show that HLA Class I antigens were significantly expressed in patients with OFG as follows (West of Scotland serological genotype frequencies are shown in parenthesis; data from 516 patients):

A2	50.0%	(26.4%)
A3	43.8%	(17.0%)
B7	56.3%	(16.8%)
Bw6	87.5%	(now reclassified)

HLA Class II antigens (DR) were significantly expressed in patients with OFG as follows:

DR2	43.8%	(15.6%)
DR3	31.3%	(22.4%)
DR4	37.5%	(21.5%)

Using Chi-square analysis to compare the results of the OFG and control groups, the HLA alleles which show statistical significance are as follows:

A3 (odds ratio 3.800; p=0.0129)

B7 (odds ratio 6.6361; p=0.0005)

DR2 (odds ratio 4.216; p=0.0081)

It seems likely therefore, on the basis of this small sample size, that consistent HLA genotypes are evident in patients with OFG, in particular A2/3 B7 DR2/3/4. Such consistent percentages at significantly higher frequencies than the general West of Scotland population, would add credibility to the findings elsewhere in this thesis that an immunological mechanism underlies the clinical presentation of OFG. Importantly, genotypes A3, B7 and DR2 are known to occur in haplotypic association.

Furthermore, literature on HLA types in CD is somewhat confusing. Numerous studies have failed to prove consistent evidence of a significantly increased risk for the development of CD in any particular HLA phenotype of either Class I or Class II proteins. Even within families, CD does not follow HLA phenotypes (Lowes and Jewell, 1990).

The typing for this study was done by serological methodology. It has been demonstrated more recently, by molecular analysis, that the genes responsible for HLA expression (on the short arm of chromosome 6) display a high degree of allelic polymorphism, not shown by serological methods alone (Satsangi *et al.,* 1994).

Meta-analysis of published results of the pooled sera of 730 patients with CD has shown that HLA-A2 carries a comparative risk of 1.25, whereas HLA-A11 has a significant negative association (Satsangi *et al.,* 1994). 50% of the OFG patients tested had HLA-A2 and 25% had HLA-A11. Interestingly, two patients (12.5%) had both A2 and A11.

7.10 Lymphocyte sub-population studies

The results of the fluorescent activated cell sorting were available as percentage cell positivity for each of the subsets T_3, T_4, T_8, and HLA-DR. The total white blood cell count and total percentage of lymphocytes were also known for each sample. Thus, total numbers of lymphocytes in each of the categories could be calculated.

The results were as follows:

(A)

Group	**OFG**	**CONTROL**
T3 Monoclonal		
Number of samples	16	34
Mean count/ml	1392	1489
Standard deviation	369	435

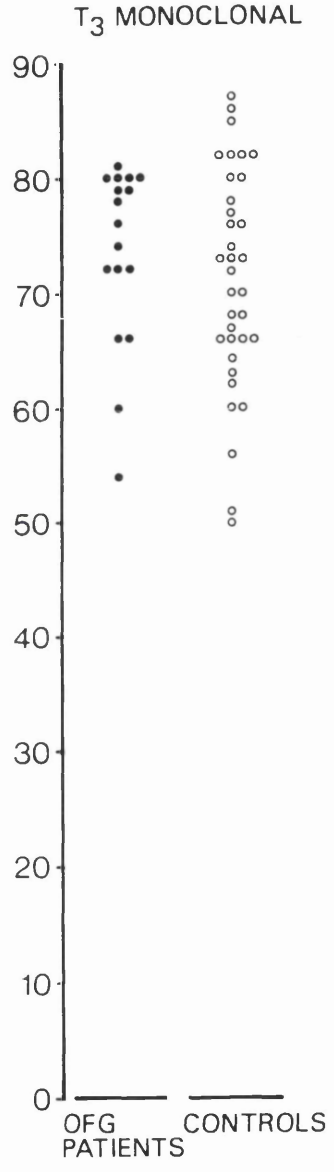
Student t-test was used to analyse the above data.

$t = 0.818$

$p > 0.05$ (not significant)

The percentage cells showing positive fluorescence is shown in Figure 7.55.

Figure 7.55 T_3 monoclonal (% lymphocyte subset)



(B)

Group	**OFG**	**CONTROL**
T4 Monoclonal		
Number of samples	16	33
Mean count/ml	920	891
Standard deviation	311	263

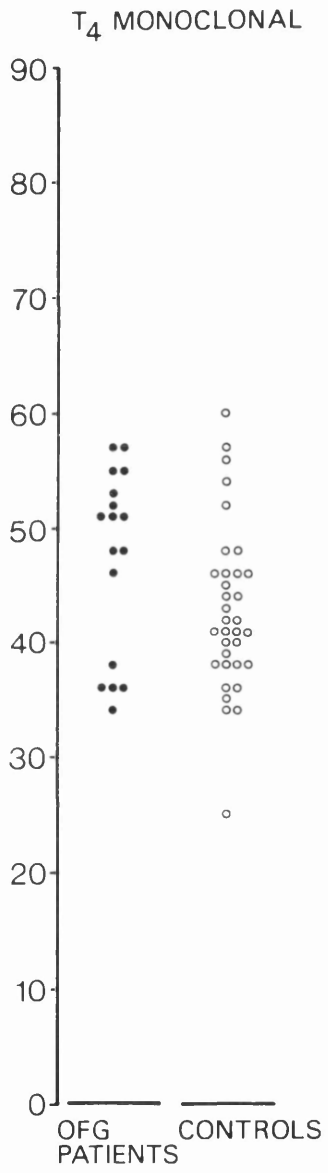
Student t-test was used to analyse the above data.

$t = 0.321$

$p > 0.05$ (not significant)

The percentage cells showing positive fluorescence is shown in Figure 7.56.

Figure 7.56 T_4 monoclonal (% lymphocyte subset)



(C)

Group	**OFG**	**CONTROL**
T8 Monoclonal		
Number of samples	16	31
Mean count/ml	484	665
Standard deviation	155	221

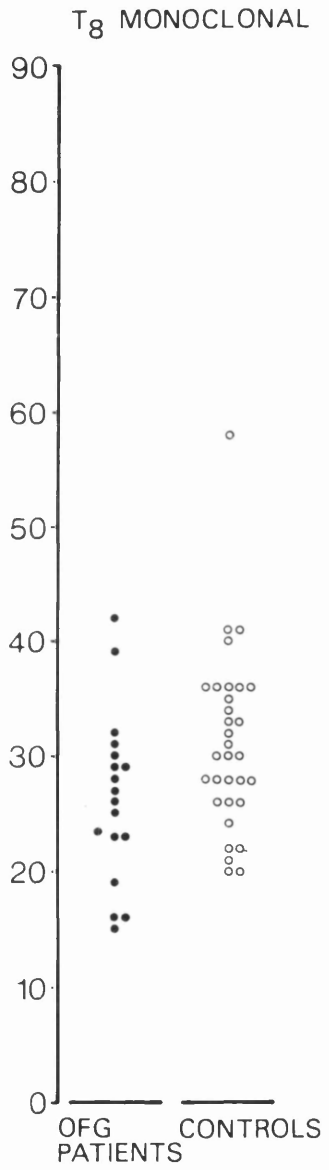
Student t-test was used to analyse the above data.

$t = 3.263$

$p < 0.01$ (statistically significant difference noted)

The percentage cells showing positive fluorescence is shown in Figure 7.57.

Figure 7.57 T_8 monoclonal (% lymphocyte subset)



(D)

Group	**OFG**	**CONTROL**
HLA-DR Monoclonal		
Number of samples	16	31
Mean count/ml	182	234
Standard deviation	102	15

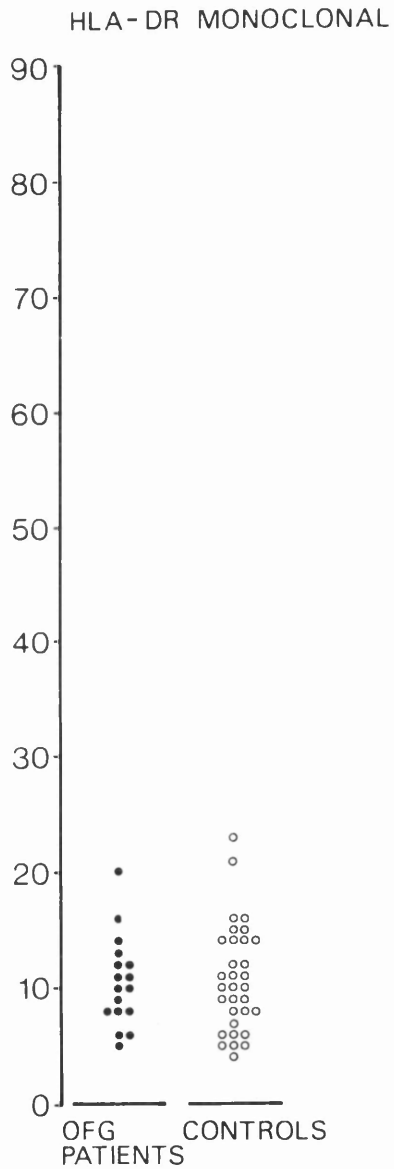
Student t-test was used to analyse the above data.

$t = 2.028$

$p < 0.05$ (statistically significant difference noted)

The percentage cells showing positive fluorescence is shown in Figure 7.58.

Figure 7.58 HLA-DR monoclonal (% lymphocyte subset)



It is therefore evident that the peripheral blood from patients with active OFG, as opposed to CD, exhibit no significant changes to a control population in terms of mean counts of T3 and T4 lymphocytes.

However, in terms of both T8 and HLA-DR expressed lymphocytes, patients with OFG exhibit a significant reduction of these cells in the peripheral blood at $p<0.01$ and $p<0.05$ respectively. It has been suggested recently that patients with active gut CD will exhibit significant decreases in circulating CD8 lymphocytes (Neil *et al.,* 1994). Further analysis of these T-lymphocyte sub-populations in the blood and, perhaps more importantly in affected tissues, might provide valuable insight into the immunological aberrations in inflammatory bowel diseases and may be of value in distinguishing OFG and CD.

7.11 Technetium-99m-HMPAO leucocyte labelling

Leucocyte labelling efficiencies of between 15 and 60% were calculated for all subjects studied, according to the formula given in Chapter 3.

Resultant radiographic images are shown in Figures 7.59 and 7.60.

Of the ten consecutive paediatric patients referred for investigation of orofacial granulomatosis who underwent Technetium-99m-HMPAO leucocyte labelling of the gastrointestinal tract, the results were as follows:

(a) Seven subjects (70%) showed no uptake at all in the gastrointestinal tract

(b) One subject (10%) showed Grade 2 intensity uptake in the nasopharynx only

(c) Two subjects (20%) showed Grade 2 or 3 intensity uptake in the gastrointestinal tract and both were subsequently confirmed as Crohn's disease histologically, affecting the colon and rectum.

Of the fifteen paediatric patients with objective evidence of inflammatory bowel disease (radiological +/- histological) who were used as positive controls, and who underwent Technetium-99m-HMPAO leucocyte labelling of the gastrointestinal tract, the results were as follows:

All 15 patients (100%) showed Grade 2 or 3 uptake in the small or large intestine.

Figure 7.59 Negative result, showing no tracer uptake in the gastrointestinal tract but normal appearance of liver and spleen.

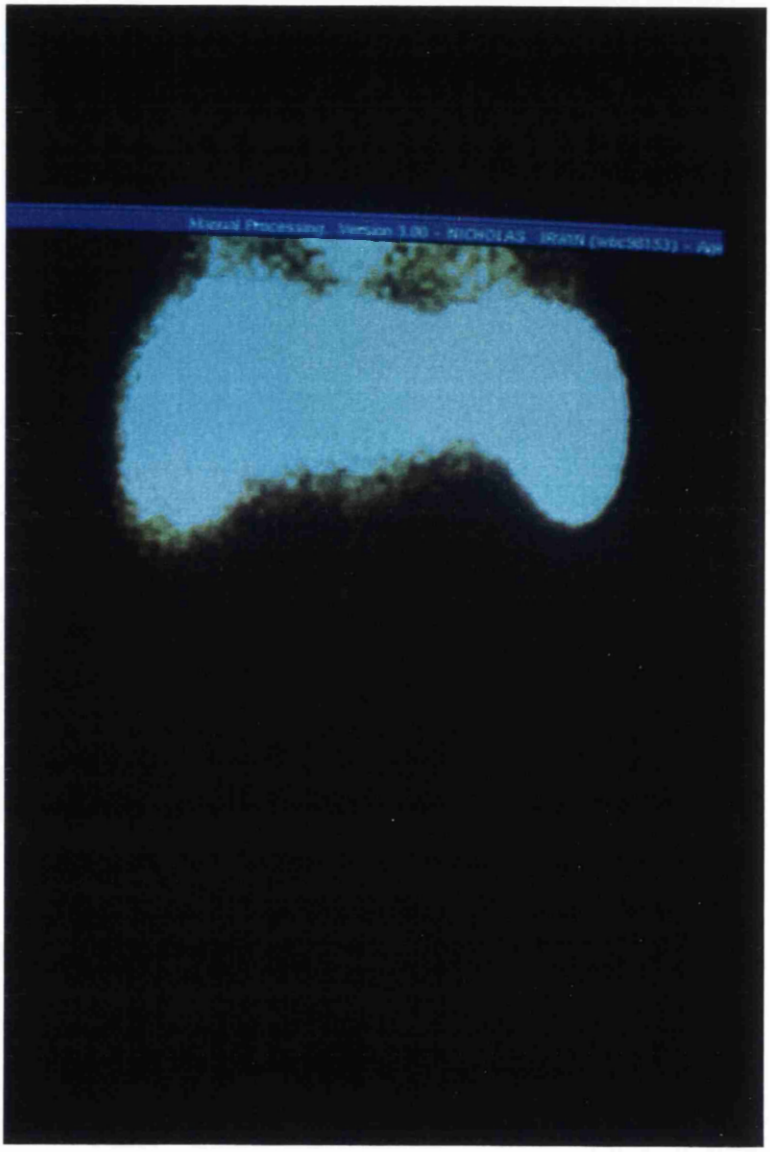
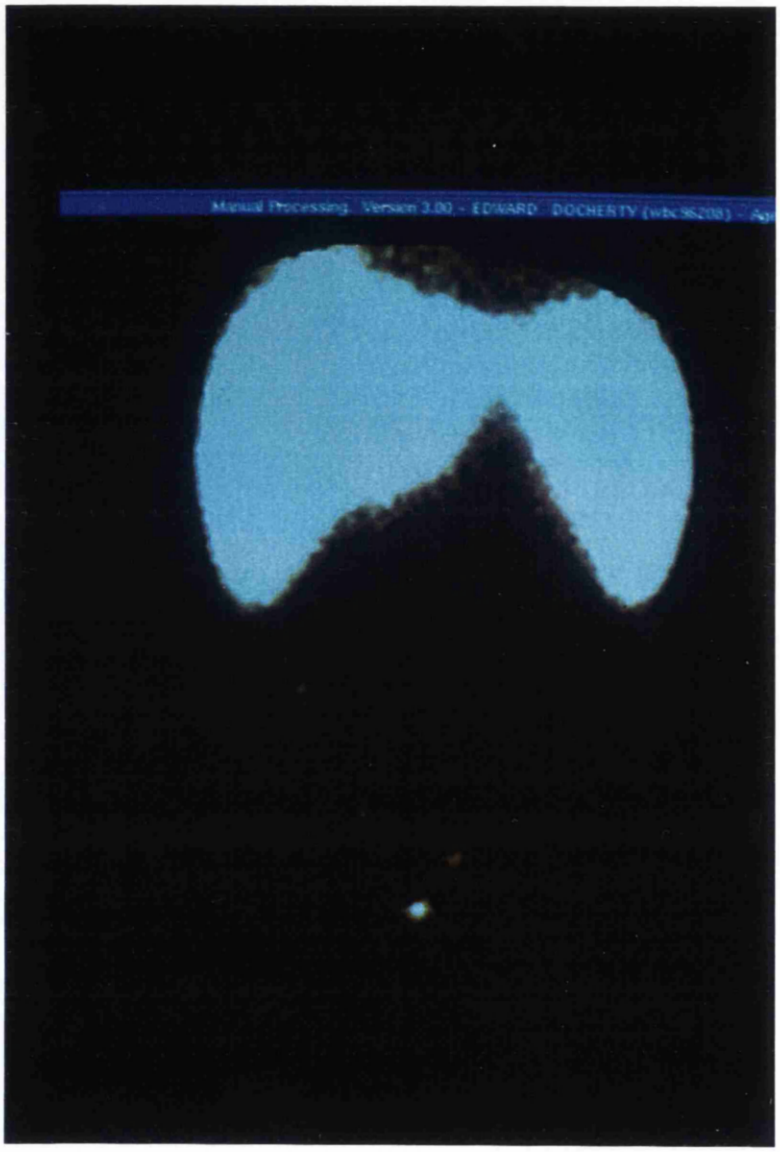


Figure 7.60 Positive result, showing tracer uptake in the gastrointestinal tract, with Grade 2 accumulation in the proximal ascending colon and Grade 3 accumulation in the terminal ileum.



7.12 Endoscopy and barium studies

A total of 57 patients (excluding those who entered the study with a pre-existing diagnosis of gastrointestinal Crohn's disease) underwent imaging of the gastrointestinal tract as follows:

Procedure	Number of patients
Barium studies alone	37
Barium studies plus endoscopic procedures	18
Endoscopic procedures alone	2

Of the 37 patients who underwent barium studies alone, putative abnormalities were detected as follows:

Abnormality detected	Number of patients
Oesophageal stricture (non-CD)	1
Old or active gastric ulceration	2
Old or active duodenal ulceration	2
Diverticular disease	5
Oesophageal CD	1*
Regional ileitis (CD)	2*
CD of colon (distal)	2*

*The five patients with CD all refused further imaging techniques. However, the clinical history and radiographic findings were consistent with CD and so they were labelled as such for the purposes of analysis.

Of the 18 patients who underwent barium studies and endoscopic procedures, abnormalities were detected as follows:

Abnormality detected	Number of patients
Features on barium consistent with CD; endoscopic features consistent with CD; histological features consistent with CD	13
Features on barium consistent with CD; no obvious endoscopic features; histological features consistent with CD	1
Features on barium consistent with CD; endoscopic features consistent with CD; histological features did not confirm nor refute CD	1
Features on barium non-specific; endoscopic features consistent with CD; histological features consistent with CD	3

Of the two patients who underwent endoscopic procedures alone, abnormalities were detected as follows:

Abnormality detected	Number of patients
Endoscopic features consistent with CD; histological features consistent with CD	2

Thus, overall, of the 57 patients who were subjected to imaging of the gastrointestinal tract, 25 (43.9%) were identified as having active Crohn's disease. The 57 patients, however, represented a biased population sample with symptoms and/or signs which could have represented active gastrointestinal disease. It will be recalled from Chapter 3 that it was not considered ethical to subject all patients to imaging of the gastrointestinal tract, given the morbidity and mortality associated with such procedures.

A total of 45 patients were thus identified with active gastrointestinal Crohn's disease: 25 from investigations inherent to the study and 20 enrolled with a pre-existing diagnosis of Crohn's disease.

7.13 Statistical Analysis

7.13.1 Logistic Regression

Log Transformations were taken when factors were non-normally distributed

7.13.1.1 Univariate Analyses

An obvious starting point in the modelling process was to ascertain which independent variables were useful indictors of outcome (i.e. development of gastrointestinal Crohn's disease). Each of the independent variables were modelled individually against Crohn's disease to establish whether the variable was significantly predictive of outcome.

7.13.1.2 Multivariate Analyses

Stepwise logistic regression operates in a sequential manner. It begins with NO terms in the predictive model for outcome. At this stage, the term which is the single most useful predictor of outcome is introduced into the predictive model for outcome. The significance of every other term adjusting for the most useful term is then calculated. If any of the remaining terms can add a significant amount of additional predictive information then the most significant of these terms is added into the predictive model at the next step. The process is repeated until none of the terms excluded from the model can add significantly to the predictive power of those terms included in the model. At this stage the process terminates and a set of independently useful predictors of outcome are obtained.

Given the sensitivity of the system for multivariate analysis, incomplete data sets cannot be used. The multivariate analysis was therefore only computed for factors with almost complete data.

Listed in Table 7.1 are all factors which had sufficiently complete data and were tested at the univariate and multivariate levels. Table 7.2 lists only those factors which were

tested on univariate analysis. A multivariate analysis was conducted on all factors presented in Table 7.1 in a stepwise fashion. The significant factors are presented in Table 7.3 in the order they entered the full model in the stepwise manner.

Table 7.1 Data sets for univariate analysis.

Factor	Univariate Analysis	
	Odds Ratio (Confidence Interval)	**P-Value**
Compliance (negative vs. good)	2.40 (1.08, 5.35)	0.0832
(poor vs. good)	1.27 (0.46, 3.51)	
(partial vs. good)	0.78 (0.32, 1.91)	
Allergy Blood Tests (abnorm vs. norm)	1.67 (0.71, 3.92)	0.2416
Mucosal Tags (yes vs. no)	1.67 (0.87, 3.21)	0.1226
Upper Lip Swelling (yes vs. no)	1.06 (0.56, 2.01)	0.8508
Lower Lip Swelling (yes vs. no)	1.09 (0.58, 2.08)	0.7839
Fissured Tongue (no vs. yes)	0.74 (0.21, 2.61)	0.6453
Facial Palsy (no vs. yes)	2.06 (0.88, 4.84)	0.0976
Atopic (no vs. Yes)	1.48 (0.77, 2.82)	0.2395
Right Angular Cheilitis (yes vs. no)	1.05 (0.52, 2.12)	0.8940
Left Angular Cheilitis (no vs. yes)	1.02 (0.50, 2.10)	0.9539
Bilateral Angular Cheilitis (yes vs. no)	1.00 (0.49, 2.05)	1.0000
Allergens: Cinnamon (no vs. yes)	1.38 (0.70, 2.71)	0.3480
Allergens: Benzoates (no vs. yes)	**1.36 (0.71, 2.60)**	**<0.0001**
Gum Inflammation (yes vs. no)	1.74 (0.91, 3.34)	0.0968
Altered Bowel Habits (yes vs. no)	**17.51 (8.05, 38.02)**	**<0.0001**
Weight Loss (yes vs. no)	**8.31 (3.21, 21.51)**	**<0.0001**
Non-Aphthoid Ulceration (yes vs. no)	**25.97 (9.51, 70.92**	**<0.0001**
Aphthoid Ulceration (yes vs. no)	**4.04 (2.04, 8.01)**	**0.0001**
stools (abnormal vs. normal)	**24.27 (10.64, 55.25)**	**<0.0001**
Rectal Bleeding (yes vs. no)	**11.15 (3.81, 32.57)**	**<0.0001**
Sex (male vs. Female)	1.39 (0.72, 2.69)	0.3335
Abdominal Pain (yes vs. no)	**34.36 (11.83,100)**	**<0.0001**
Mucosal Oedema (yes vs. no)	**3.42 (1.68, 6.94)**	**0.0007**
Papillary Hyperplasia (yes vs. no)	**4.11 (1.57, 10.73)**	**0.0039**
Smoker (no vs. Yes)	3.31 (0.76, 14.31)	0.1098
Age	0.99 (0.96, 1.01)	0.2068
Initial Sign Score	**1.28 (1.13, 1.46)**	**0.0001**
Initial Symptom Score	1.13 (0.97, 1.34)	0.1216

Table 7.2 Univariate Analysis - Factors not included in Multivariate Analysis due to the presence of missing data.

Factor	Univariate Analysis	
	Odds Ratio (Confidence Interval)	**P-Value**
Haemoglobin Level g/dl	0.82 (0.64, 1.04)	0.1122
Mean Corpuscular Volume fl	**0.905 (0.85, 0.97)**	**0.0028**
White Cell Count	1.16 (0.98, 1.37)	0.0874
Red Cell Count	1.21 (0.63, 2.20)	0.5519
Absolute number of Neutrophils	1.27 (0.99, 1.64)	0.0638
Absolute number of Lymphocytes	0.92 (0.56, 1.43)	0.7130
Absolute number of Monocytes	3.15 (0.60, 16.46)	0.1654
Absolute number of Eosinophils	1.57 (0.21, 10.16)	0.6394
Absolute number of Basophils	0.007 (0.0001, 999.0)	0.4919
Absolute number of Platelets	1.002 (0.998, 1.007)	0.2642
Erythrocyte Sedimentation Rate	**1.04 (1.02, 1.08)**	**0.0033**
Ferritin	**0.98 (0.96, 0.99)**	**0.0024**
Folate	**1.003 (1.000, 1.006)**	**0.0219**
Vitamin B12	1.001 (1.000, 1.002)	0.1316
Serum Angiotensin Converting Enzyme	1.00 (0.97, 1.02)	0.7775
Immunoglobulin A	**1.46 (1.01, 2.16)**	**0.0470**
Immunoglobulin G	0.95 (0.81, 1.11)	0.5351
Immunoglobulin M	1.35 (0.75, 2.59)	0.2974
Complement Factor 3	2.95 (0.46, 17.39)	0.2360
Complement Factor 4	2.73 (0.05, 98.18)	0.5962
Esterase Inhibitor	3.77 (0.001, 999.0)	0.8006

Table 7.3 Multivariate Analysis

Factor	Multivariate Analysis	
	Odds Ratio (Confidence Interval)	**P-Value**
Abdominal Pain (yes vs. no)	**9.37 (1.94, 45.25)**	**0.0054**
Non-Aphthoid Ulceration (yes vs. no)	**13.33 (3.23, 54.95)**	**0.0003**
Stools (abnormal vs. normal)	**15.31 (4.59, 51.28)**	**<0.0001**
Allergens: Cinnamon (no vs. yes)	**3.94 (1.20, 12.94)**	**0.0238**
Aphthoid Ulceration (yes vs. no)	**3.14 (1.09, 9.04)**	**0.0338**

7.13.2 Conclusions

7.13.2.1 Descriptive Statistics

Crosstabulations are presented principally to give an indication of the patient characteristics with respect to all four types of disease studied. Evidently there was only a small number of patients with the diseases Melkersson-Rosenthal Syndrome and Sarcoidosis. Hence, it was not possible to compute formal statistical tests for each factor by disease type.

7.13.2.2 Formal Analysis: Logistic Regression

Univariate logistic regression analysis was performed at the 5 % level of significance for all factors. Odds ratios and their respective confidence intervals were computed, and are presented against a reference range in the positive direction. For example, if a patient has rectal bleeding then the patient has 11.15 times greater odds of presenting for Crohn's Disease than if no rectal bleeding is experienced. Sixteen factors were significant on univariate analysis at the 5% level.

Multivariate logistic regression analysis was conducted at the 5 % level of significance in a stepwise manner. Five factors were significantly predictive of outcome: abnormal stools, non-aphthoid ulceration of the mouth, the presence of abdominal pain, not allergic to cinnamon and aphthoid ulceration of the mouth. These factors are tabulated in the results section and in the same vein as the univariate analysis are presented against a reference range in the positive direction.

The final model had sensitivity and specificity values of 86% and 88% respectively.

CHAPTER 8

CLINICAL RESULTS II :
TREATMENT AND MANAGEMENT

8.1 Exclusion of allergens

8.1.1 Compliance scores

The compliance with dietary and environmental exclusion advice was recorded as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Compliance score	**Number of patients (%)**				
0	37 (15.4)	14 (31.1)	1 (10.0)	2 (33.3)	54 (18.0)
1	30 (12.5)	6 (13.3)	1 (10.0)	0 (0.0)	37 (12.3)
2	65 (27.1)	8 (17.8)	2 (20.0)	2 (33.3)	77 (25.6)
3	108 (45.0)	17 (37.8)	6 (60.0)	2 (33.3)	133 (44.2)
Total	240 (100.0)	45 (100.0)	10 (100.0)	6 (99.9)	301 (100.1)

Analysis of this data using the Kruskal-Wallis test revealed the following results: $H = 2.95$; $DF = 1$; $p = 0.069$.

These date are presented graphically in Figure 8.1.

The mean compliance scores were as follows:

OFG	2.1
CD	1.7
MRS	2.3
SARC	1.2
ALL	1.9

These data are presented graphically in Figure 8.2.

Figure 8.1 Compliance scores (% of patients in each group)

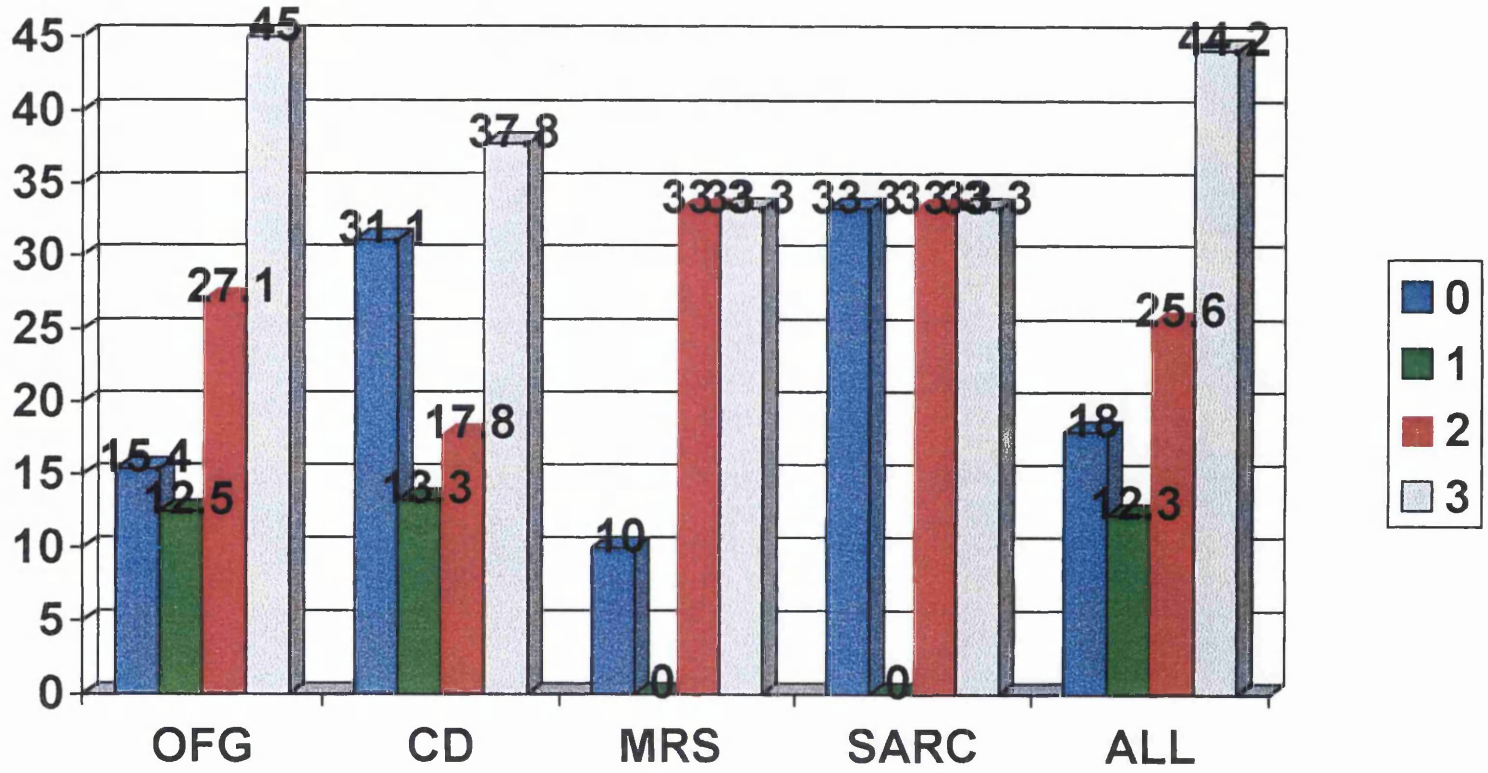
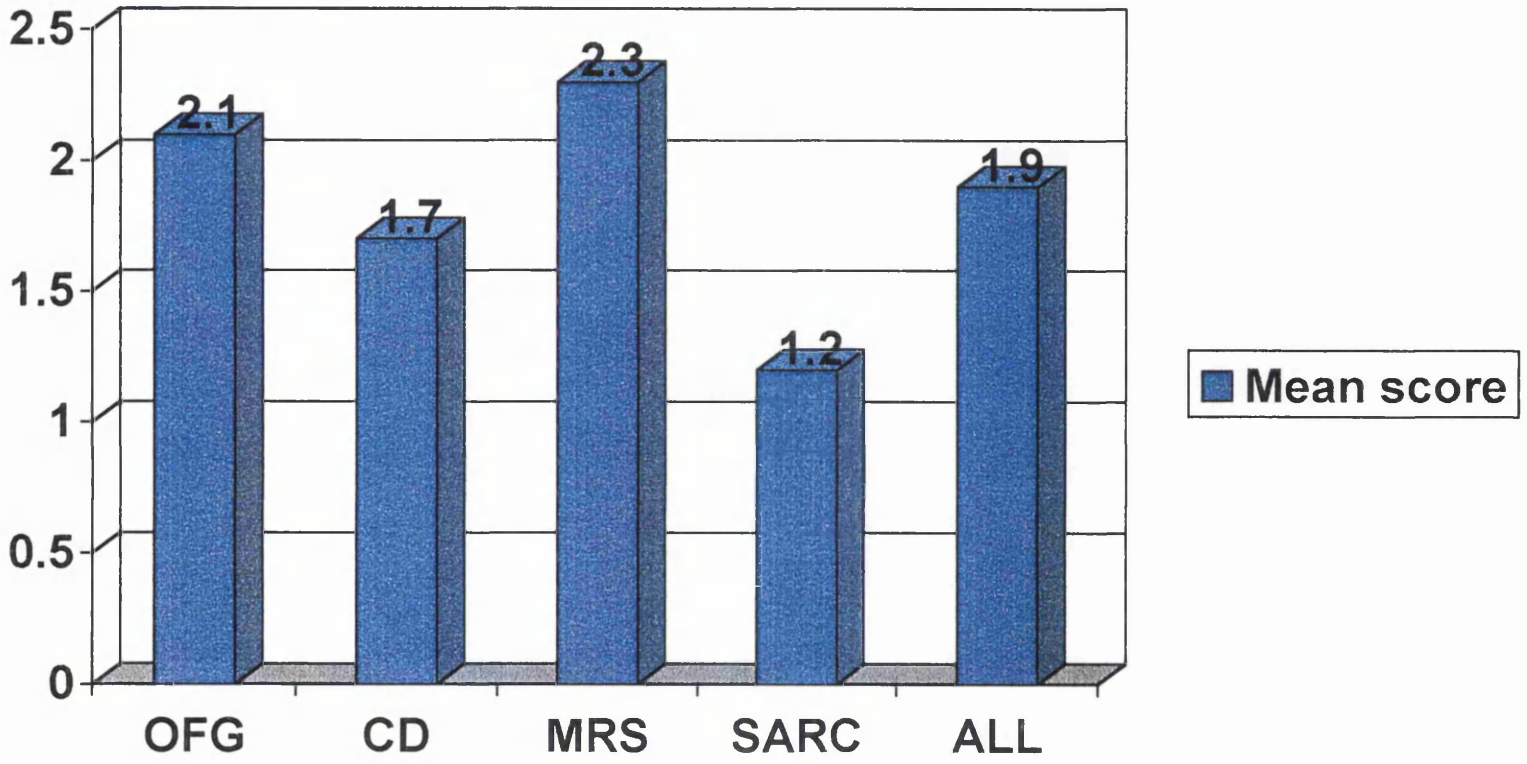


Figure 8.2 Mean compliance scores (absolute values for each patient group)



8.2 Follow-up time

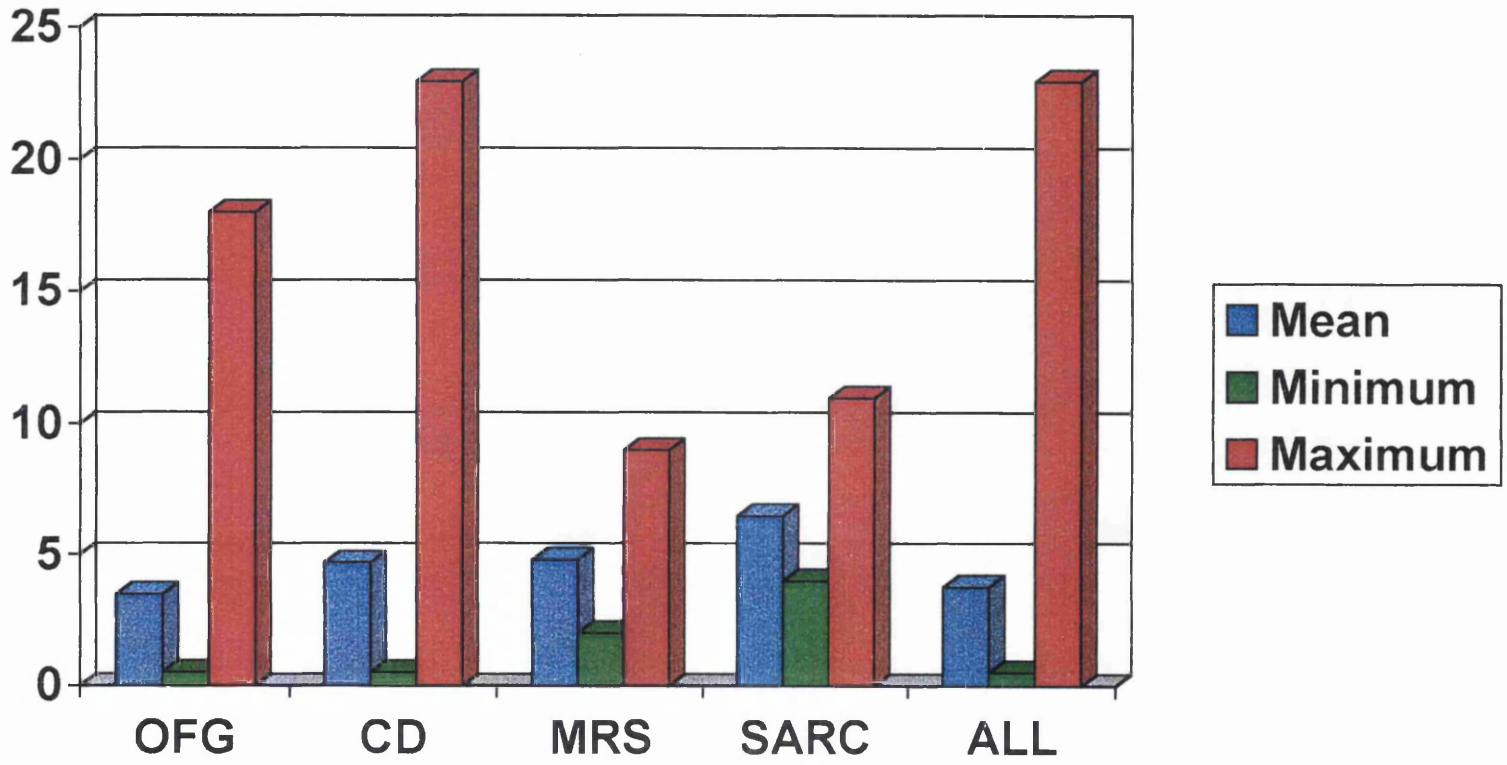
Follow-up times for each group were as follows:

Diagnosis / **Follow-up time (years)**	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients	240	45	10	6	301
Mean	3.5	4.7	4.8	6.5	3.8
Minimum	0.5	0.5	2.0	4.0	0.5
Maximum	18.0	23.0	9.0	11.0	23.0

Analysis of this data using the Kruskal-Wallis test revealed the following results: $H = 2.43$; $DF = 1$; $p = 0.116$.

These data are presented graphically in Figure 8.3.

Figure 8.3 Follow-up time (years) (absolute values for each patient group)



8.3 Assessment of response

8.3.1 Symptom scores (final)

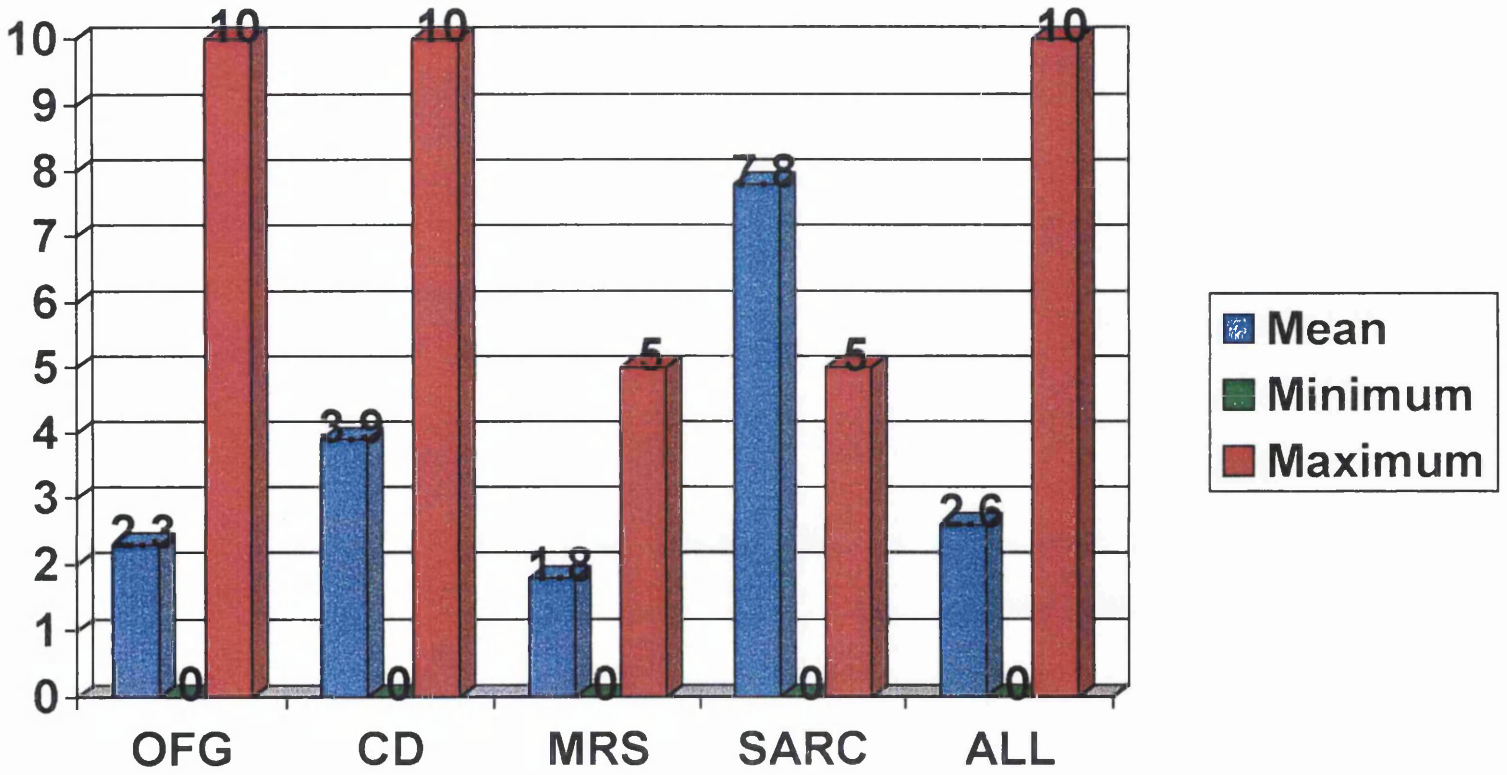
The final symptom scores for each group were recorded as follows:

Diagnosis / **Final symptom scores**	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients	240	45	10	6	301
Mean	2.3	3.9	1.8	7.8	2.6
Minimum	0.0	0.0	0.0	0.0	0.0
Maximum	10.0	10.0	5.0	5.0	10.0

Analysis of this data using the Kruskal-Wallis test revealed the following results:
$H = 6.18$; $DF = 1$; $p = 0.013$, indicating statistical significance in Final Symptom Scores between the OFG and CD groups, the mean OFG symptom scores being lower.

These data are presented graphically in Figure 8.4.

Figure 8.4 Final symptom scores (absolute values for each patient group)

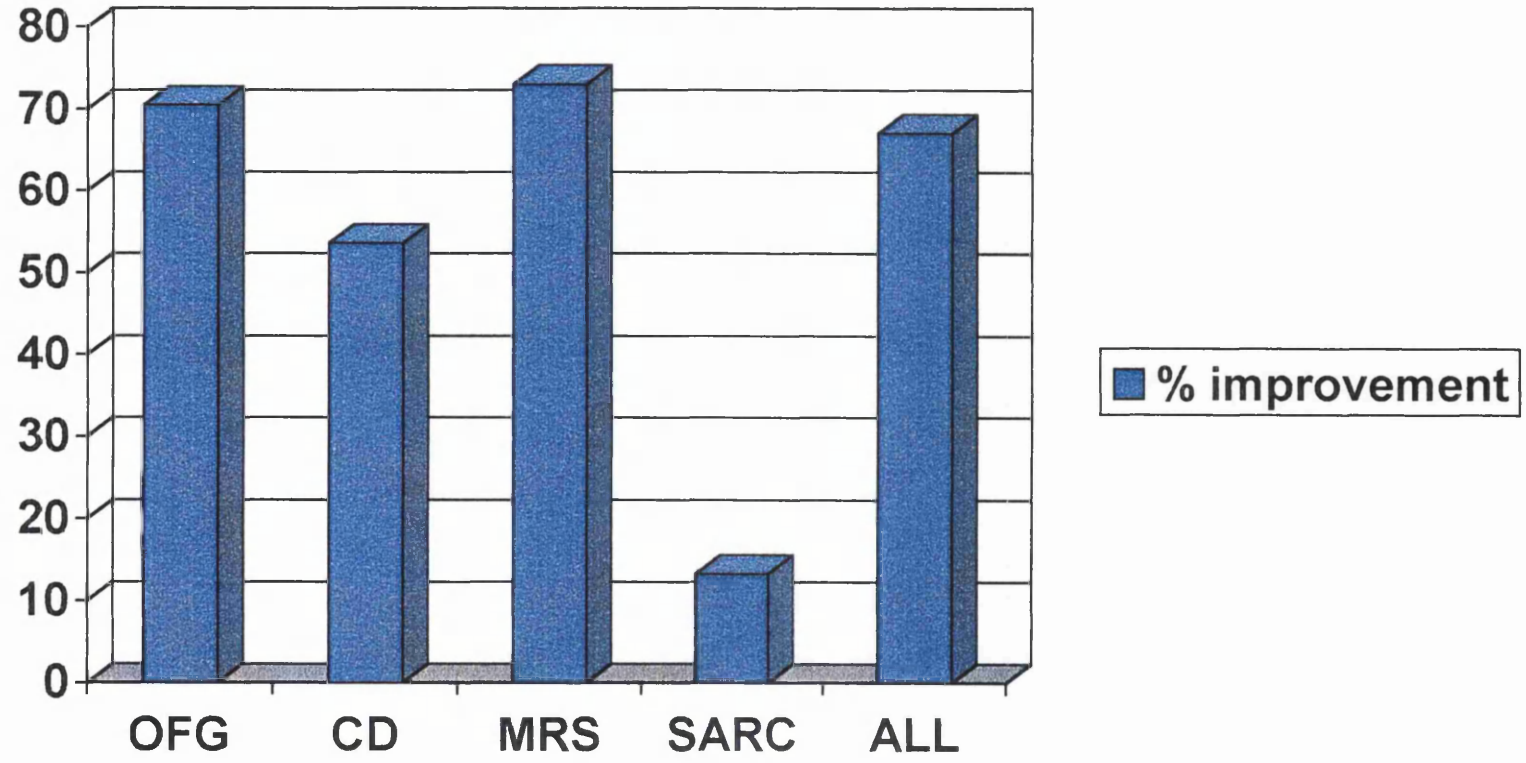


A comparison was then made between original and final symptom scores as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients	240	45	10	6	301
Mean original symptom score	7.8	8.4	6.7	8.7	7.9
Mean final symptom score	2.3	3.9	1.8	7.8	2.6
% improvement	70.5	53.6	73.1	13.3	67.1

These data are presented graphically in Figure 8.5.

Figure 8.5 Improvement in symptom scores (% change in each group)



8.3.2 Sign scores (final)

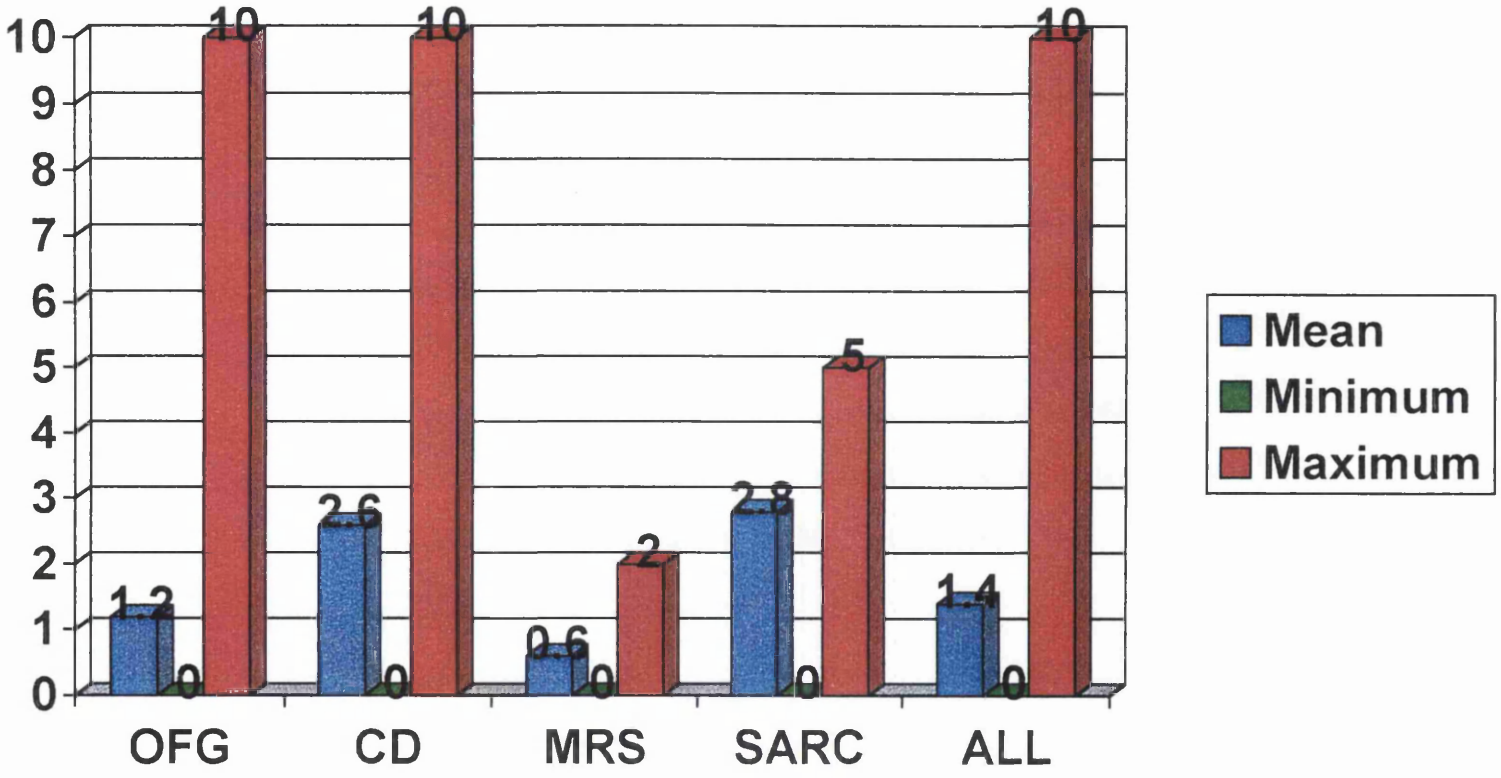
The final sign scores for each group were recorded as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Final sign scores					
Number of patients	240	45	10	6	301
Mean	1.2	2.6	0.6	2.8	1.4
Minimum	0.0	0.0	0.0	0.0	0.0
Maximum	10.0	10.0	2.0	5.0	10.0

Analysis of this data using the Kruskal-Wallis test revealed the following results:
$H = 12.58$; $DF = 1$; $p = 0.000$, indicating statistical significance in Final Sign Scores between the OFG and CD groups, the mean OFG sign scores being lower.

These data are presented graphically in Figure 8.6.

Figure 8.6 Final sign scores (absolute values for each patient group)

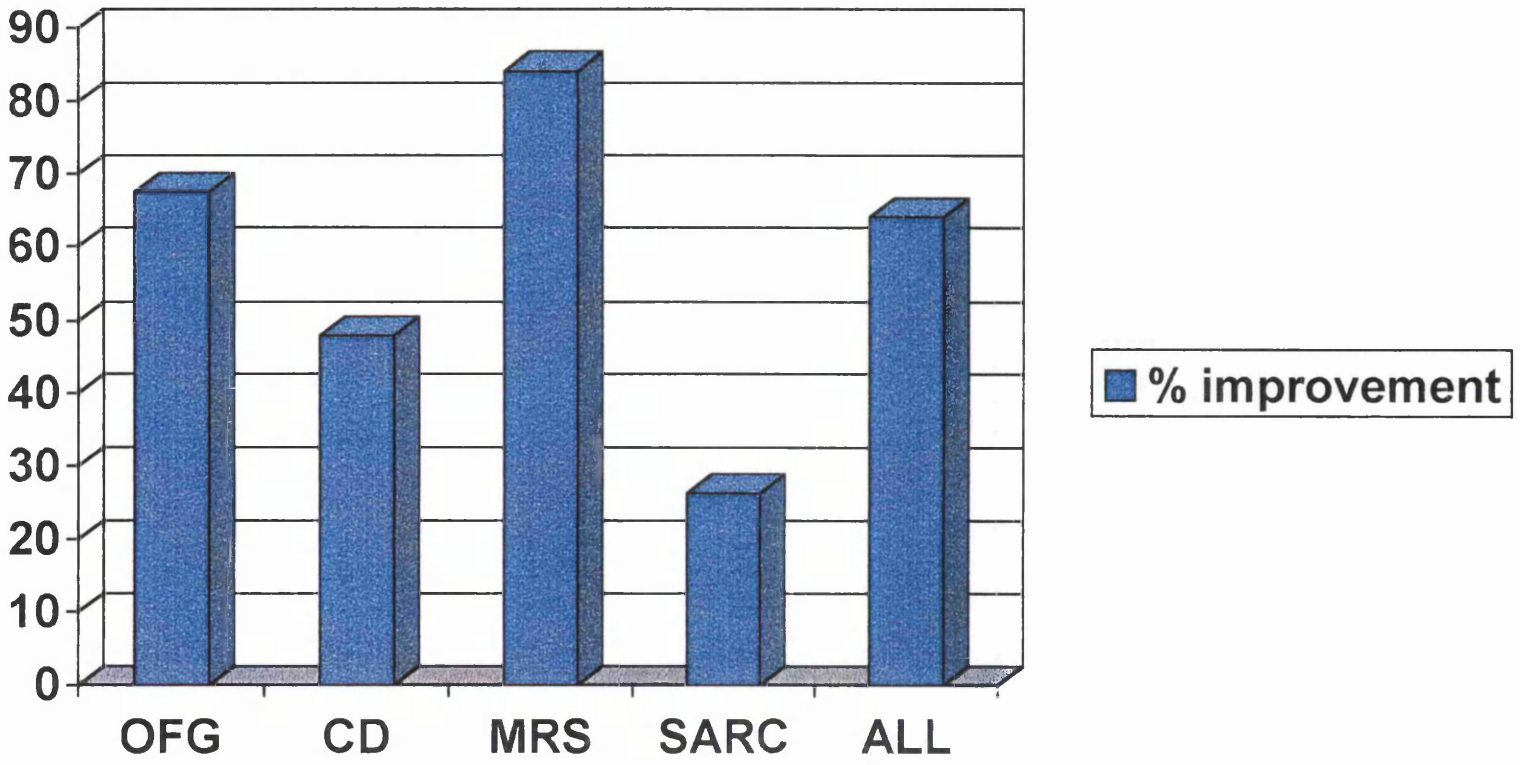


A comparison was then made between original and final sign scores as follows:

Diagnosis	**OFG**	**CD**	**MRS**	**SARC**	**ALL**
Number of patients	240	45	10	6	301
Mean original sign score	3.7	5.0	3.8	3.8	3.9
Mean final sign score	1.2	2.6	0.6	2.8	1.4
Percentage improvement	67.6	48.0	84.2	26.3	64.1

These data are presented graphically in Figure 8.7.

Figure 8.7 Improvement in sign scores (% change in each group)



CHAPTER 9

LABORATORY RESULTS

9.1 Detection of anti-gliadin antibodies

9.1.1 SDS Polyacrylamide Gel Electrophoresis

Isoelectric focusing (IEF) of crude gliadin proved unsuccessful. The preparation was virtually insoluble in water and non-ionic detergent, and required 8M urea for complete dissolution. However, IEF in 8M urea-containing agarose and polyacrylamide gels produced no visible protein bands after fixation and staining. Even strong fixatives (10% trichloracetic acid; 4.4% sulphosalicylic acid) failed to produce acceptable bands.

SDS (sodium dodecyl sulphate) electrophoresis of crude gliadin showed a highly heterogeneous pattern of proteins (Figure 9.1) with components ranging in molecular weight from about 14kD to greater than 200kD with most of the Coomassie Blue (CBB) staining proteins in the region 20-30kD. Below 30kD, only one major protein band was visible – at about 15-18kD. SDS/DTT (sodium dodecyl sulphate/dithiothreitol)-solubilised crude gliadin contained no highly aggregated protein; no CBB staining was visible in the stacking gel.

Kasarda *et al* (1974) found that α-gliadins were a major constituent in wheat proteins subjected to conventional electrophoresis at pH 3.2. Bernardin *et al* (1967) estimated the molecular weight of α-gliadin as 50-55kD by gel filtration and light scattering. Examination of Figure 9.1 suggests that this α-gliadin component was not present in significant amounts in the SDS-treated material in this experiment. However, Kasarda *et al* (1974) claimed that the molecular weight of purified α-gliadin estimated by SDS electrophoresis was 32 or 36kD, and Figure 9.1 does indeed show intense staining in this region. These authors did not account for the missing 20kD fragment and claimed to obtain only a single band. In Figure 9.1, there is a prominent component of molecular weight approximately 20,000 which could be the α-gliadin subunit released by DTT reduction of disulphide linkages in native α-gliadin.

Attempts were made to demonstrate the presence of antibodies to crude gliadin components in the sera of subjects with coeliac disease and orofacial granulomatosis, and also normal subjects, by immunodiffusion. No precipitin lines were observed, but these experiments were considered inconclusive due to the difficulties experienced in

dissolving the crude gliadin preparation. Antibody-antigen reactions would, of course, not proceed at low pH or in the presence of strong dissociating agents such as urea.

9.1.2 Western Blotting

See Figure 9.2a-d

Western blotting of crude gliadin using pooled sera from patients with coeliac disease (7), orofacial granulomatosis (4) and normal controls (11) produced interesting results. It is evident that IgG in pooled coeliac sera cross-reacts with all components in crude gliadin, with the exception of proteins in the molecular weight range 48-57kD (Figure 9.2b). This latter region could simply represent the "missing" native α-gliadin (molecular weight about 50kD) dissociated to its subunits by DTT reduction of disulphide bonds.

Pooled normal sera were shown to contain IgG antibodies to all the major protein components (Figure 9.2c) except those in the range 48-57kD. Staining, however, was markedly less intense compared to the coeliac tracks.

Comparing the coeliac sera with the normal sera (Figure 9.2b and 9.2c), it is evident that

1. Antibody levels were much higher in the sera of patients with coeliac disease.
2. The coeliac sera contained additional immunoglobulins recognising minor gliadin components in the molecular range 14-30kD. It is suggested that this may represent anti-endomysial antibodies although this would require further clarification.

Whereas the 32-36kD α-gliadin may be raised in coeliac sera in a previously documented way (O'Farrelly *et al.*, 1983) as indicated by increased intensity of immunostaining in this region, the more striking and novel observation is that the coeliac sera contain these additional bands. It is noteworthy that considering staining in the 17-20kD range, corresponding to the putative smaller α-gliadin fragment, there was no increased immunostaining with coeliac sera.

Gliadin tracks probed with pooled sera from patients with OFG showed highly intense staining compared with normal sera, but with a very similar pattern above 30kD (Figure 9.2a). Again, the putative 18kD fragment of α-gliadin showed no increased staining compared with normal sera.

However, OFG sera contained IgG recognising two additional components in the lower molecular weight range, one of which (arrowed Figure 9.2a) was not immunostained with normal or coeliac sera.

It would appear, therefore, that patients with OFG have circulating levels of IgG in excess of the normal population. The antigenic stimulus for this remains unidentified but is unlikely to be gliadin. Further work is required on a larger patient and control sample.

Figure 9.1 SDS Electrophoresis of Crude Gliadin; Coomassie Blue stained gel.

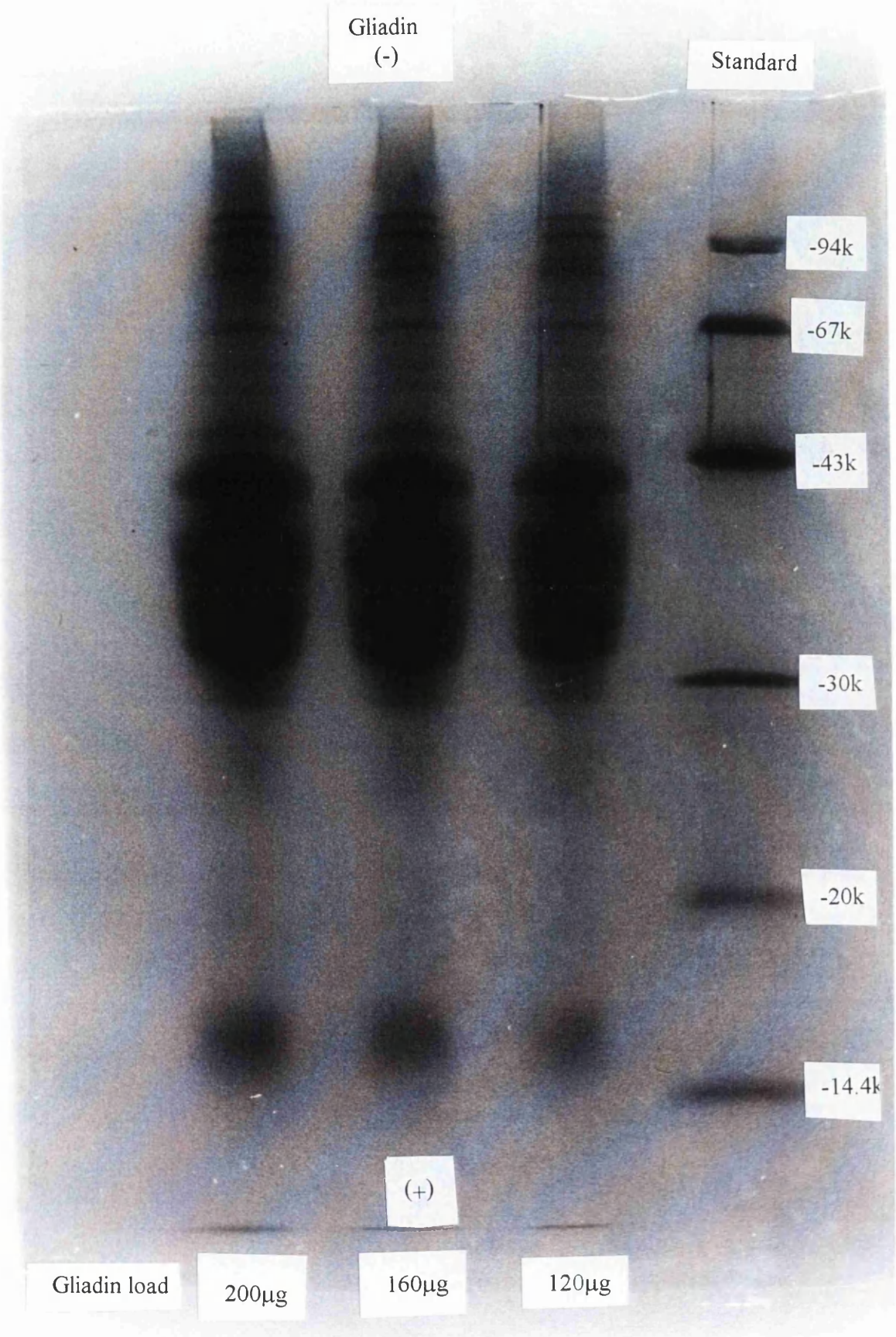


Figure 9.2 Western Blotting of Crude Gliadin using pooled sera from (a) OFG patients; (b) Coeliac Disease patients; (c) Normal control subjects; (d) All 3 patient groups.

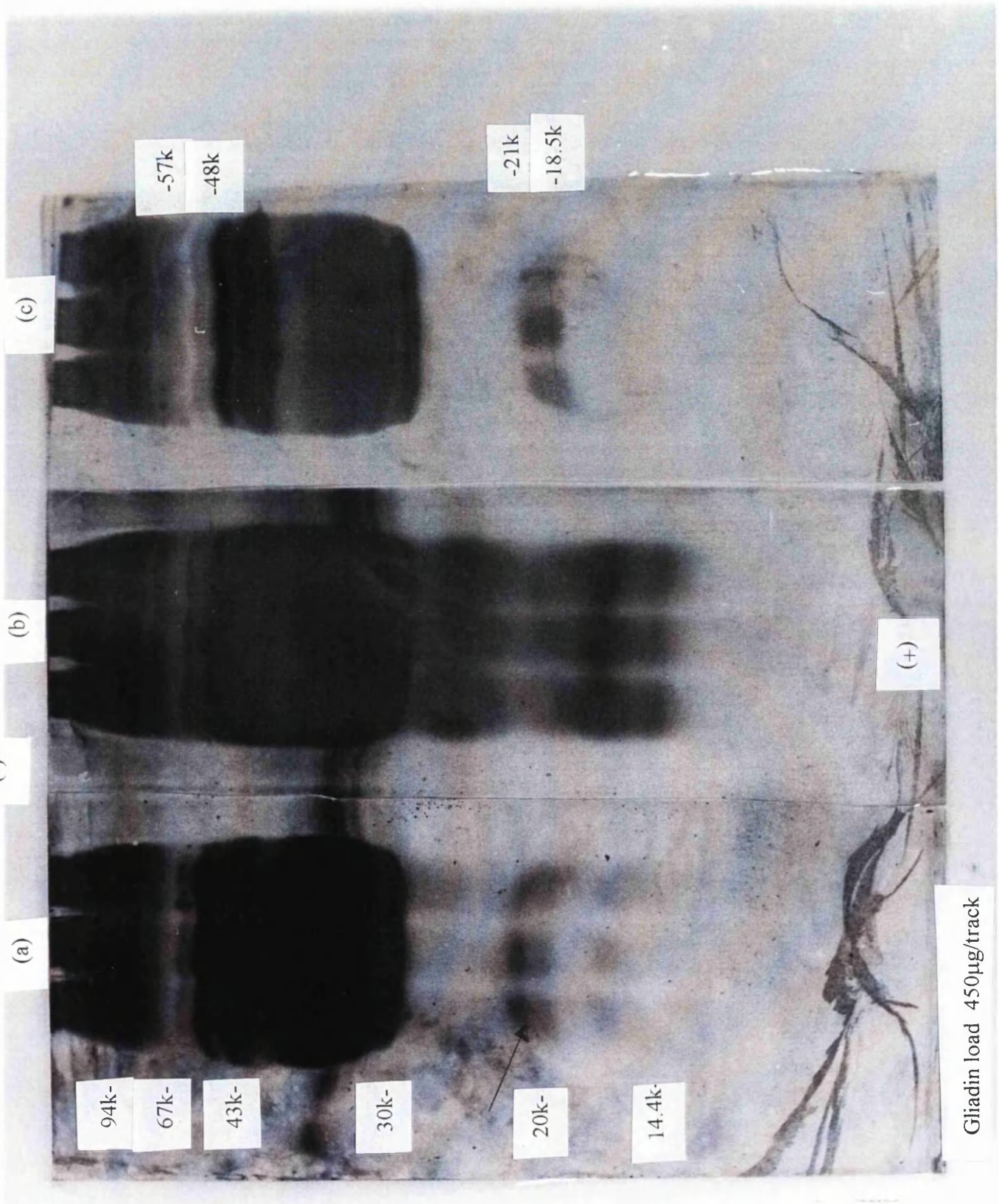
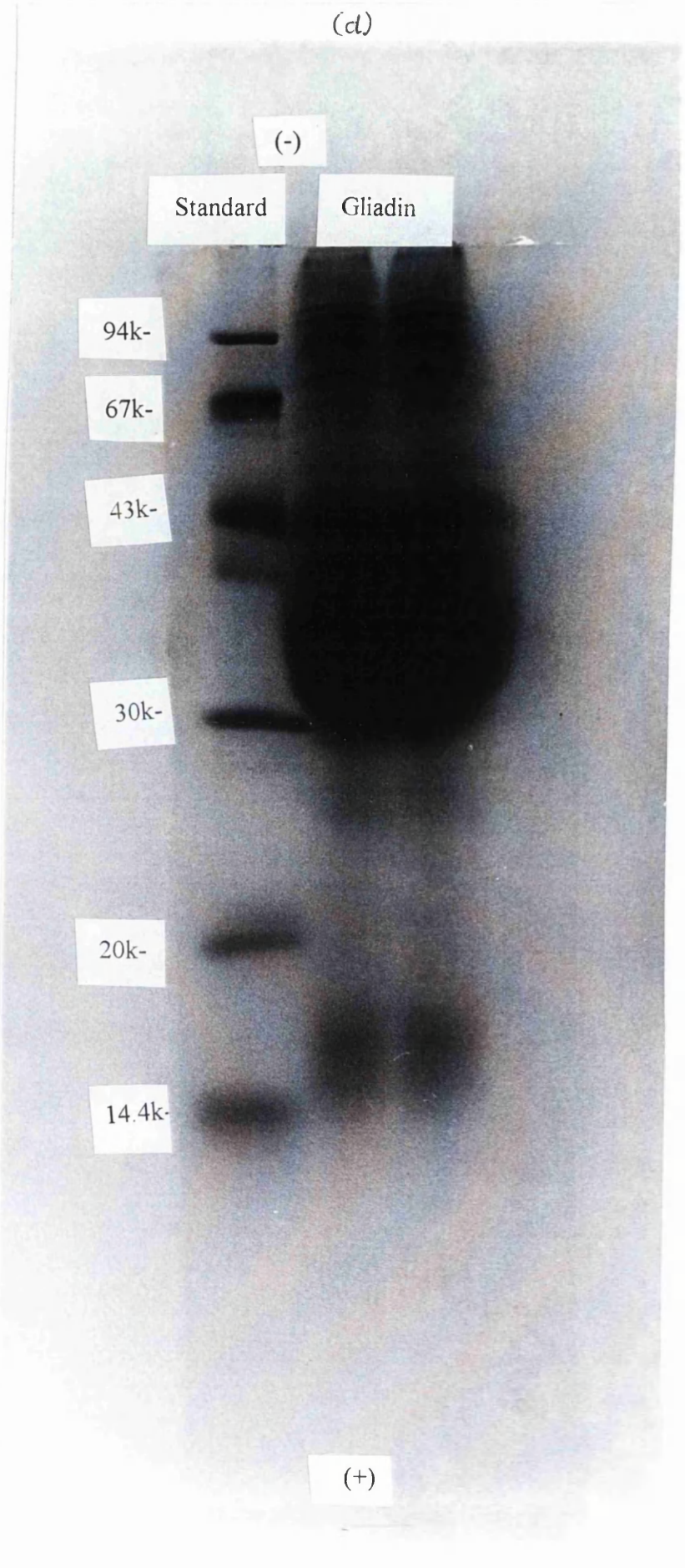


Figure 9.2 (cont)



9.2 Molecular biological studies – the polymerase chain reaction

The purpose of this part of the study was to investigate the possibility of mycobacterial involvement in OFG and oral Crohn's disease tissue samples. *M. paratuberculosis* is a slow growing organism which has been shown to be the causative agent of Johne's disease, a chronic enteritis of ruminants (Chiodini *et al.*, 1984a; Vary *et al.*, 1990). Due to the extreme difficulties encountered in attempting to isolate the organism by culture, many investigators have utilised the PCR technique for its detection in diseased tissue. Since *M. paratuberculosis* DNA has previously been demonstrated in the intestinal tissue of up to 72% of patients affected by Crohn's disease by PCR in several studies (Sanderson *et al.*, 1992; Dell'Isola *et al.*, 1994; Lisby *et al.*, 1994; Fidler *et al.*, 1994), it seemed prudent to investigate the possible presence of this mycobacterial species in OFG and oral Crohn's disease tissue. The primers used for PCR in this study targeted the same 5' region of the IS900 DNA insertion element of *M. paratuberculosis* as primers previously described (Sanderson *et al.*, 1992; Millar *et al.*, 1995).

Duplicate sets of all the samples analysed demonstrated PCR positivity for the ß-haemoglobin gene, as indicated by the amplification of a 165 bp product, after two rounds of PCR using nested primer pairs. This indicated that DNA extraction was successful for each tissue sample being analysed and that the extracted DNA was of sufficient purity and free of PCR inhibitors, thus rendering it suitable for use in subsequent PCR analysis.

The sensitivity of the PCR assay following two rounds of amplification was such that 10 fg of *M. paratuberculosis* DNA was detectable by agarose gel electrophoresis, which is the equivalent of two mycobacterial genomes.

M. paratuberculosis IS900 PCR was performed on duplicate sets of samples. Following a single round of 40 cycles of PCR using the *M. paratuberculosis* IS900 P90+/P91+ primer pair, all of the samples were negative for the presence of *M. paratuberculosis* DNA both by agarose gel electrophoresis and Southern blot hybridisation, with only the positive control producing a product of 413 bp.

In order to increase the sensitivity of the assay, a second round of PCR using identical conditions to the first round but with 5 µl of first round product as template was performed. A single OFG sample gave a PCR product, which was slightly smaller in size to that expected for *M. paratuberculosis* positivity (Figure 9.3). However, this product did not hybridise to the 229 bp IS900 probe in Southern blot hybridisation (Figure 9.4). No other samples were positive by gel electrophoresis and no samples previously negative by agarose gel electrophoresis following two rounds of PCR demonstrated positivity following Southern blot hybridisation.

For each batch of tissue samples being analysed, the *M paratuberculosis* positive controls were always positive and the negative controls always negative, both by agarose gel electrophoresis and Southern blot hybridisation.

Figure 9.3 2% agarose gel electrophoresis of selected PCR products (20µl) obtained from tissue DNA samples following two rounds of 40 cycles of amplification with *M. paratuberculosis* IS900 primers P90+ and P91+. Lane1 shows the 100bp DNA ladder; lanes 2-8 show the OFG samples; lanes 9-11 show the Crohn's disease samples; lanes 12-14 show the normal samples; lane 15 shows the negative PCR control and lane 16 shows the positive PCR control. The single PCR product obtained from the sample analysed, which is slightly smaller in size than the positive control PCR product, is shown in lane 6 and is an OFG sample.

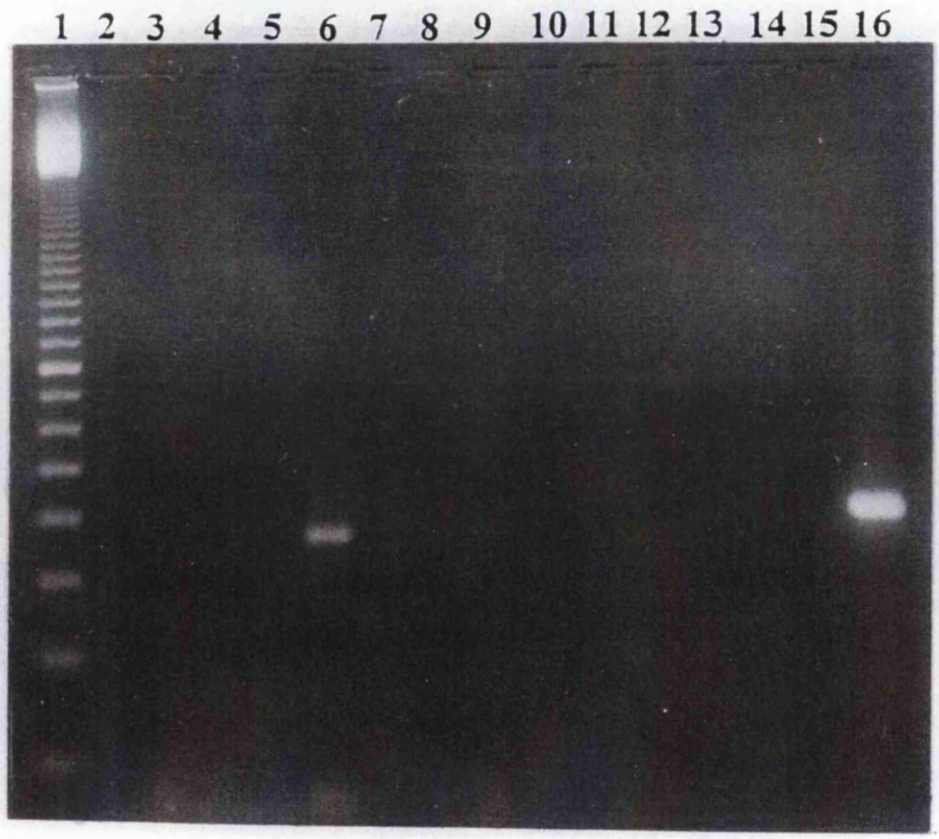
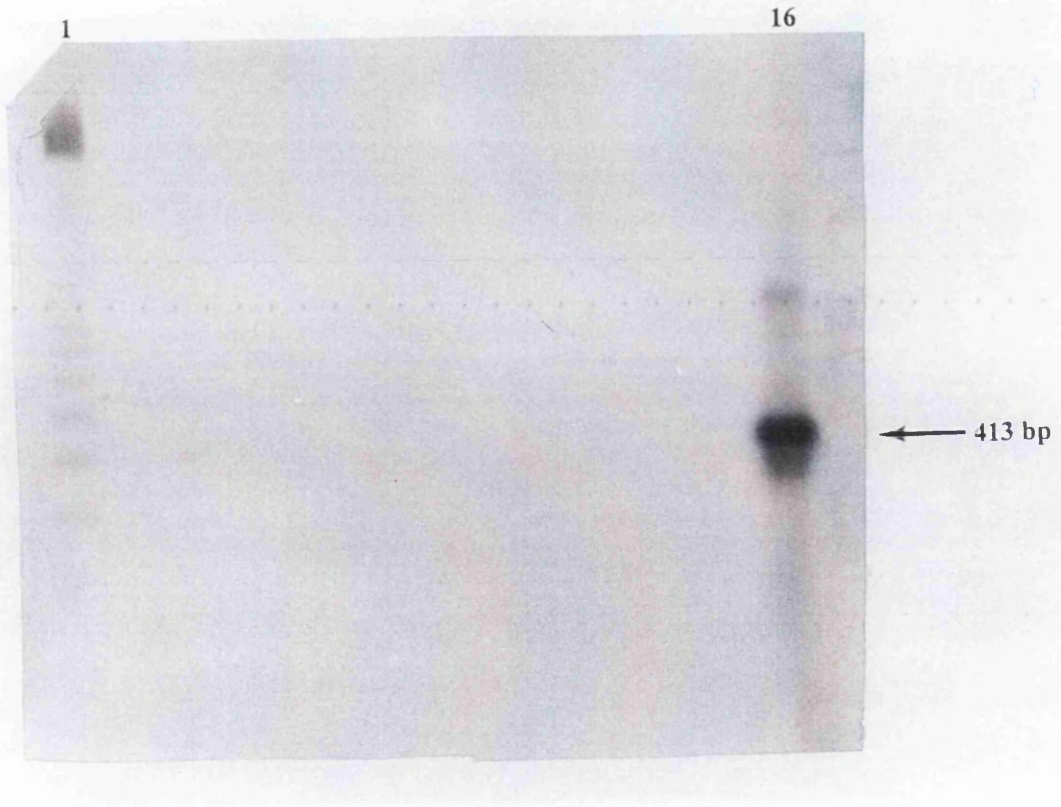


Figure 9.4 Corresponding Southern blot hybridisation to that shown in Figure 9.3. For orientation of the membrane, lanes 1 and 16 correspond to the 100bp DNA ladder and PCR positive control lanes respectively. The PCR product in lane 6 (OFG) did not hybridise to the probe.



The results suggest that in this patient group, *M. paratuberculosis* does not appear to be associated with OFG or the oral lesions of Crohn's disease. After two rounds of 40 cycles of PCR followed by Southern blot hybridisation, all tissue samples were found to be negative. The IS900 PCR assay used in this study was similar to that previously described by Sanderson *et al* (Sanderson *et al.,* 1992) who reported that as little as 5 fg of *M. paratuberculosis* DNA could be detected, which is equivalent to a single mycobacterial genome.

In view of the fact that the PCR assay used is as sensitive as can reasonably be expected by carrying out two rounds of PCR and Southern blot hybridisation, and no tissue sample was positive for *M. paratuberculosis* DNA it is clear that, at least in the group of patients used in this study, *M. paratuberculosis* DNA is rarely found in OFG and oral Crohn's disease tissue.

However, an important consideration is the possibility that some areas of infection within the tissue may be excluded when using paraffin-embedded tissue sections for DNA extraction and analysis as opposed to homogenates of whole fresh tissue. In other studies, when using paraffin-embedded tissue sections as a tissue DNA source only 7 to 13% of samples demonstrated PCR positivity for *M. paratuberculosis* (Lisby *et al.,* 1994; Fidler *et al.,* 1994), which is in sharp contrast to positivity rates of 46 to 72% obtained when using fresh tissue for analysis (Sanderson *et al.,* 1992; Dell'Isola *et al.,* 1994; Lisby *et al.,* 1994). It is clearly more difficult to detect low-abundance *M. paratuberculosis* DNA reliably in paraffin-embedded samples than when using fresh tissue, since the use of fresh tissue permits the extraction of DNA from a much larger volume of tissue and consequently increases the probability of sampling a discrete focus of infection. It would be most important and helpful to extend this aspect of the study by analysing both paraffin-embedded and, where possible, fresh tissue from patients with OFG or oral Crohn's disease lesions in several geographical locations in the UK in order to investigate whether there is an altered distribution of the organism in different patient groups.

The potential involvement of *M. paratuberculosis* in Crohn's disease is a controversial issue. Although some studies have demonstrated the presence of *M. paratuberculosis* DNA in Crohn's disease tissue (Sanderson *et al.,* 1992; Dell'Isola *et al.,* 1994; Lisby *et al.,* 1994; Fidler *et al.,* 1994), PCR negativity for *M. paratuberculosis* DNA has been

reported in other studies (Frank and Cook, 1996; Rowbotham *et al.*, 1995; Dumonceau *et al.*, 1996). Frank and Cook (1996) failed to detect *M. paratuberculosis* DNA in any of 27 Crohn's disease tissue samples examined using nested PCR primers, whilst the use of a fluorescent PCR method demonstrated negativity in all of 68 Crohn's disease tissue samples analysed (Rowbotham *et al.*, 1995). In a further study, PCR demonstrated the presence of mycobacteria with a similar frequency in the intestinal tissues of patients with Crohn's disease and of normal controls, although no *M. paratuberculosis* DNA was detected in any sample (Dumonceau *et al.*, 1996). PCR negativity for *M. paratuberculosis* DNA has also been obtained in tissue samples of sarcoidosis (Lisby *et al.*, 1993), which is a generalised granulomatous disease involving multiple organs and which resembles mycobacterial infection histologically. However, the involvement of other mycobacterial species could not be excluded, particularly in view of the fact that *Mycobacterium tuberculosis* DNA was found in the bronchoalveolar lavage fluid (Saboor *et al.*, 1992) and spleens (Mitchell *et al.*, 1992) of sarcoidosis patients in other studies. The potential involvement of other mycobacterial species in OFG and oral lesions of Crohn's disease would undoubtedly be worthy of investigation.

The standard PCR protocols which have previously been used could be further refined by the use of a solid-phase hybridisation capture technique, which has recently been developed and applied to PCR detection of *M. paratuberculosis* and *M. avium* subsp. *silvaticum* (Millar *et al.*, 1995). Solid-phase hybridisation capture of mycobacterial DNA from tissue DNA extracts prior to PCR increases sensitivity and substantially eliminates false positives arising due to amplicon contamination. This method should prove valuable in detecting low abundance target DNA sequences in tissue samples, and its application in attempting to identify *M. paratuberculosis* DNA in oral tissue may further clarify the possibility of any aetiological role for this organism in OFG and oral lesions of Crohn's disease.

CHAPTER 10

DISCUSSION

10.1 Discussion

Orofacial granulomatosis is assuming increasing importance in the practice of Oral Medicine and in the medical and dental literature (Anonymous, 1991b). This is particularly evident in the west of Scotland where over 400 patients have been identified with the condition over a 10-year period. This study has analysed the clinical and immunological parameters in 301 patients with orofacial granulomatosis.

The main aim of this study was to identify clinical and/or biochemical parameters which could be used to determine whether or not there were various granulomatous disorders under the umbrella term of OFG, or if patients with OFG had a homogeneous disease process. Small numbers of patients with sarcoidosis (2.0%) and MRS (3.3%) excluded these conditions on the whole from statistical analysis. The major comparison was therefore made between patients with OFG and CD.

During the study, 240 patients were identified with OFG, 45 patients with gastrointestinal Crohn's disease (20 having a pre-existing diagnosis; 25 having the diagnosis established during the study), ten patients with MRS and six patients with sarcoidosis.

The demographic details of patients in this study were in keeping with other published data. In particular, the overall mean age at presentation was 24.0 years with the category breakdown as follows: OFG 24.0 years, CD 20.8 years, MRS 38.5 years and sarcoidosis 27.2 years.

This study's results on smoking and alcohol consumption were inconclusive, given the age profile of the patient population, biased towards the younger age group. It is known, however, that smoking is implicated in worsening gastrointestinal Crohn's disease (Bozdech and Farmer, 1990).

Working definitions of gastrointestinal Crohn's disease have been used by large multi-centre study groups to ensure homogeneity of patients and to provide a starting point for disease definition (Winship *et al.*, 1979). It is, however, generally expressed in the

literature that these definitions and criteria are imperfect (Bozdech and Farmer, 1990) with the diagnosis being an art rather than a purely scientific process.

The finding of non-caseating granulomata on biopsy or surgical specimens helps to confirm the clinical suspicion of intestinal Crohn's disease; however, the rate of detection depends on the criteria used to define "granuloma", and the vigour used in searching for such lesions. Therefore to assume that a histological diagnosis of Crohn's disease, or indeed the absence of "classical" histological features, is beyond reproach is naïve. Granulomata are actually seen in the minority of patients (Bozdech and Farmer, 1990) and so their absence is of no utility. Therefore, the salient features of histology are chronic patchy inflammation, the predominance of submucosal involvement, and the long-term clinical sequelae of stricturing and fistula formation (Bozdech and Farmer, 1990).

Intestinal Crohn's disease is seen in four main clinical patterns: ileocolonic is the commonest presentation, seen in about 40% of patients; isolated small intestinal and isolated colonic involvement occurs in less than 30% of patients; and isolated anorectal involvement is seen in about 3% of cases. Although Crohn's disease is quoted as exhibiting "skip lesions" and therefore may involve the stomach, oesophagus and even the skin as isolated processes, this is rare and accounts for less than 10% of all Crohn's disease cases (Farmer *et al.,* 1975; Farmer *et al.,* 1985; Mekjian *et al.,* 1979).

Seventy to eighty-five percent of patients with CD will have some degree of small intestinal involvement (Farmer *et al.,* 1975; Mekjian *et al.,* 1979; Lind *et al.,* 1985a). The triad of abdominal pain, diarrhoea and weight loss is seen in approximately 90% of patients with small intestinal involvement (Bozdech and Farmer, 1990).

Colonic involvement by CD is seen in approximately 60% of all patients with CD (Farmer *et al.,* 1975; Mekjian *et al.,* 1979; Lind *et al.,* 1985a). Diarrhoea, abdominal pain and weight loss are again seen in the majority of patients (Bozdech and Farmer, 1990). Rectal-sparing of the disease process is seen in only 20-25% of patients with colonic CD (Bozdech and Farmer, 1990). It is known that peripheral manifestations of CD are commoner in patients with colonic involvement – arthritis, perianal fistulae and skin lesions. This has been demonstrated in the current study with orofacial

manifestations being seen more commonly in patients with anal and peri-anal disease (peri-anal skin tags were identified in 57.5% of patients with CD). This could be referred to as "top-and-tail" Crohn's disease – a new clinical entity.

A further new clinical entity was identified in four patients: staphylococcal mucositis. Two patients with this condition had CD and were taking oral prednisolone; two patients had OFG and were taking no medication.

Gastrointestinal symptoms were found to be of practical value in identifying patients who may have gastrointestinal Crohn's disease. The following symptoms were all statistically significant in differentiating OFG from CD:

- weight loss (CD 24.4%; OFG 3.8%)
- altered bowel habit (CD 55.6%; OFG 6.7%)
- number of stools passed daily (higher in CD)
- rectal bleeding (CD 22.2%; OFG 2.5%)
- abdominal pain (CD 42.2%; 2.1%)

Similarly, systemic examination of patients yielded findings which aided differentiation of CD and OFG. Pain was identified on abdominal palpation in 30.0% of CD patients examined and only 6.6% of OFG patients. Peri-anal skin tags were present in 57.5% of CD patients and only 5.5% of OFG patients.

Two important features were evident in patients with sarcoidosis: erythema nodosum (16.7%) and lymphadenopathy (33.3%).

A landmark Scandinavian paper (Lind *et al.,* 1985b) has reported that radiography was very sensitive (91-100%) for the identification of small intestinal or small intestinal and large intestinal (combined) CD. However, the sensitivity of barium enema for colonic CD was only 48%. Colonoscopy had a sensitivity of approximately 85% for the diagnosis of colonic CD, but was less sensitive for the diagnosis of small intestinal CD or combined small intestinal/colonic CD. These findings pertained to both the adult and paediatric populations. This study suggests that technetium-labelled leucocyte scanning of the gastrointestinal tract is a useful screening tool in differentiating OFG and CD.

This has the advantage of being non-invasive, cheap, readily accessible, and delivering levels of radiation substantially less than conventional barium studies (Charron, 1997). Its diagnostic accuracy in inflammatory bowel disease is well established (Charron, 1997). However, the data from this study, from a largely paediatric population, requires confirmation in an adult population with inflammatory bowel disease.

The importance in differentiating OFG and intestinal CD lies in future management options for the individual patient.

An immunological (allergic) basis for OFG has been postulated in this study. Despite similar compliance scores and follow-up periods between the two groups (CD and OFG), dietary and environmental exclusion advice for substances identified on positive skin testing led to statistically significant differences in changes of both final symptom and sign scores. This finding was on the background of no significant differences in atopy rates between the OFG (49.6%) and CD (40.0%) groups, the overall rate being 48.5% versus 15% of the general population. However, very strict criteria were applied in this study to establish a diagnosis of atopy.

Furthermore, 54.5% of the OFG group and 52.8% of the CD group were reactors to benzoic acid versus 28% of the control group; 44.6% of the OFG group and 41.7% of the CD group were reactors to cinnamonaldehyde versus only 7% of the control group.

Further weight to the allergic basis for OFG was added with the overall levels of IgE demonstrated by RAST testing being significantly higher in the OFG group (82.4%) over the CD group (58.6%). In addition, levels of IgG directed against unidentified proteins in the sera of the OFG group, comparable to coeliac disease and significantly higher than control groups, further strengthen the likelihood of immunological reactivity in the OFG group.

The consistent HLA haplotypes (A2/3 B7 DR2/3/4) and results of lymphocyte studies suggest an immunological mechanism to the clinical presentation in OFG patients. Recent work on T cell receptor ß variability in a patient with OFG (Lim *et al.*, 1997) has suggested that TCRVß gene usage by lesional T-lymphocytes is notably restricted. In addition, a recurrent transcript in the Vß6 lesional lymphocytes was identified,

suggesting a clonal T cell expansion in the vicinity of the lesion. The biological role of these T cells remains to be determined. However, it is likely that the restricted T cell repertoire and clonal T cells are involved in a delayed hypersensitivity reaction to an antigen.

The results of this study would suggest that the antigen may be dietary in origin. However, the negative findings in searching for *Mycobacterium paratuberculosis* require further analysis; the use of fresh tissue from orofacial biopsies instead of paraffin-processed sections would be a most appropriate next step using PCR technology (Millar *et al.,* 1995).

Laboratory findings were also of practical value in differentiating the disease categories. Whereas haemoglobin concentrations and serum vitamin B12 levels were not statistically different between the groups, the mean corpuscular volume (lower in CD), ESR (higher in CD), whole blood folate, and serum ferritin (mean lower in CD) were statistically significant in highlighting differences between CD and OFG.

Biochemical parameters were largely redundant, with urea and electrolytes and calcium, albumin and phosphate levels revealing no real differences between the diagnostic groups. Liver function test abnormalities were recorded in 23.7% of patients overall (OFG 22.0%; CD 31.0%). Such levels of abnormality are similar to those recorded in CD studies previously (Mekjian *et al.,* 1979). The appropriateness of serum angiotensin converting enzyme (SACE) as a screening test for sarcoidosis in patients with granulomatous disorders is not proven from this study. Only two patients with sarcoidosis had SACE levels measured in this study, and these were within the normal reference range.

Analysis of histological data revealed that patients with CD were much more likely to have lymphoedema ($p=0.004$) and/or granulomata ($p=0.012$), alone or in combination, on mucosal biopsy than patients with OFG. However, 20.0% of CD biopsies and 30.9% of OFG biopsies had no granulomas present; but only 6.7% of CD biopsies and 16.7% of OFG biopsies had no lymphoedema present. It therefore seems inappropriate to persist with the title Orofacial Granulomatosis, a histopathological term used to describe a clinical entity, and the alternative of Orofacial Lymphoedema is suggested on the basis of clinical findings alone.

Initial sign scores showed statistically significant differences between the disease categories at p=0.000 (overall mean 3.9; CD 5.0; OFG 3.7; MRS 5.8; sarcoidosis 3.8).

The clinical findings in patients with OFG would lend themselves to the development of clinical criteria for diagnosis. The commonest findings were (in decreasing rank order) lower lip swelling (52.2%), upper lip swelling (51.2%), mucosal oedema and cobblestoning (48.8%), aphthous ulceration (39.2%), mucosal tags (31.2%), full-thickness gingivitis (30.2%), facial swelling (27.0%) and angular cheilitis (26.2%).

This leads to the helpful concept of Major and Minor diagnostic criteria for Orofacial Lymphoedema, with Major criteria being present in >30% of patients and Minor criteria being present in 7-29% of patients as follows:

Major:	Chronic lower lip swelling	52.2%
	Chronic upper lip swelling	51.2%
	Mucosal oedema and cobblestoning	48.8%
	Aphthous ulceration	39.2%
	Mucosal tags	31.2%
	Full-thickness gingivitis	30.2%
Minor:	Chronic facial swelling	27.0%
	Angular cheilitis	26.2%
	Fissured tongue	11.3%
	Non-aphthous ulceration	8.0%
	Papillary hyperplasia	7.3%

The diagnosis would be based on 2 major criteria (i.e. present in at least 60.4% of patients) or 1 major plus 3 minor criteria (i.e. present in at least 56.8% of patients). As shown in the univariate logistic regression analysis, the need for further investigation for systemic CD would be based on the presence of weight loss, altered bowel habit, stool frequency, rectal bleeding, and abdominal pain. The presence of peri-anal skin tags and a high initial orofacial sign score are good markers of CD, indicating a requirement to exclude gastrointestinal Crohn's disease.

Univariate logistic regression highlighted a low MCV, high ESR, and a low serum ferritin as good markers of gastrointestinal Crohn's disease.

Patch-testing to identify dietary and/or environmental allergens is shown by this study to constitute an essential aspect to the investigation and management of patients with Orofacial Lymphoedema.

This study demonstrated statistical significance in the percentage improvement in both symptom and sign scores following dietary and environmental allergen identification and avoidance advice. Compliance scores and follow-up times were similar across all patient groups. Final symptom scores were statistically significantly different between OFG and CD ($p=0.013$) as were final sign scores ($p=0.000$). Percentage improvements were noted in symptom and sign scores as follows: overall, 67.1% and 64.1%; OFG 70.5% and 67.6%; CD 53.6% and 48.0%; MRS 73.1% and 84.2%; sarcoidosis 13.3% and 26.3% respectively.

Comparable results (above the mean) were obtained with OFG and MRS patients with respect to dietary and environmental exclusion, suggesting that MRS may have more of an allergic basis than reported in the literature to date (Greene and Rogers, 1989).

Results for comparison of the MRS and sarcoidosis groups make the hitherto held belief that MRS represents a variant of sarcoidosis (James, 1994b) most unlikely.

The genetic background to the development of such clinical entities and the exact nature of the antigenic stimulus and the nature of the immunological response in individual patients require elucidation.

On the basis of this study, OFG, sarcoidosis and Crohn's disease are distinct clinical entities. OFG, or more appropriately Orofacial Lymphoedema, OFL, would appear to be primarily allergic in its pathogenesis (Type IV or delayed hypersensitivity), as would Melkersson-Rosenthal Syndrome.

Overall, 45 out of 301 patients in this study population had, or developed, evidence of gastrointestinal Crohn's disease - some 15% over a 10-year period. This is an important

advisory figure for new patients presenting with orofacial granulomatous disorders.

In summary, this study has demonstrated that patients with orofacial lymphoedema have evidence of an antigen-driven disorder. A number of possibilities should be considered. Firstly, the antigen may be a single entity, derived from dietary or environmental exposure. The commonest antigen identified was benzoic acid and response to dietary modification was statistically significant. This would seem highly probable given the chronic exposure to benzoic acid as a preservative molecule in many modern foods, particularly in the diets of the patients in this study population. In these circumstances, the granulomatous inflammation may simply be a cellular, delayed hypersensitivity (type IV) reaction.

Secondly, the antigen may be derived from *Mycobacterium paratuberculosis* or some other mycobacterial species. This would certainly explain the granulomatous inflammatory infiltrate evident on some biopsy specimens. However, the negative findings on PCR would require repetition with fresh tissue specimens.

Thirdly, there may be a combination antigen-driven response with granulomatous inflammation evident as a result of *Mycobacterium paratuberculosis* and the more prevalent lymphoedematous response due to subsequent exposure to other dietary or environmental antigen or antigens.

The identification of the true antigen and response will require further elucidation. Regardless, this study has established the likelihood of an allergic basis to the disorder orofacial lymphoedema.

10.2 Management recommendations

Ideally, patients presenting with the symptoms and signs of Orofacial Lymphoedema should have the following management protocol. This would be in keeping with evidence-based good clinical practice.

- Full history, to include weight loss, altered bowel habit, stool frequency, rectal bleeding, and abdominal pain
- Full systematic examination, to include abdomen and perineum
- Haematological parameters, specifically full blood count, serum ferritin, and ESR
- Technetium-labelled HMPAO white cell scanning of the gastrointestinal tract
- Oral microbiological sampling where oral symptoms are present
- Patch tests and contact urticaria tests, with appropriate skilled professional advice on dietary and environmental exclusion, where allergens are identified

APPENDICES

APPENDIX I

STUDY PROFORMA

OFG - Proforma

Name ..... Age .... years. Male/Female

Address .....

Telephone, Home ..... Work..... GDH No. .....

Occupation ..... Date of 1st attendance ../../..

PMH Crohn's / Sarcoid / TB / Facial palsy

Details .....

Atopy Eczema / Asthma / Hay fever

Weight loss Y / N ..... Bowel habit Normal/Altered

Smoker Y / N ..... Alcohol Y / N .....

Clinical complaint:

Swelling		Ulceration		A. cheilitis Y/N
Upper lip	[ ]	Upper lip	[ ]	Other .....
Lower lip	[ ]	Lower lip	[ ]	.....
Buccal mucosa	[ ]	Buccal mucosa	[ ]	.....
Tongue	[ ]	Tongue	[ ]	.....
Face	[ ]			

Clinical finding: Extra-oral .....

Swelling Y / N .....

Ulceration Y / N .....

Gingivae Y / N .....

Mucosal tags Y / N .....

Fissured tongue Y / N .....

Papillary hyperplasia Y /N .....

Mucosal oedema Y / N .....

Investigations: Biopsy Y / N Site ..... Path. No. .....

Stage of disease .....

Findings; Granuloma Y / N Lymphoedema Y / N Not diagnostic [ ]

Haematology Ferritin .... ng/ml B_{12} .... pg/ml Folate .... ng/ml

Glucose .... mmol/l Other .....

Immunology RAST Y / N .....

Immunoglobulins Y / N .....

FACS Y / N ..... Complement Y / N .....

Patch test No. .....

Allergens: Benzoate / Cinnamon / other .....

Comment.....

.....

Follow up

Treatment .....

.....

Dietary advice Y / N .....

Response;

Date .....

.....

Total / Partial / Nil score ... / 10

Next review .....

Date .....

.....

Total / Partial / Nil score ... / 10

Next review .....

Date .....

.....

Total / Partial / Nil score ... / 10

Next review .....

Date .....

.....

Total / Partial / Nil score ... / 10

Next review .....

Exacerbations .....

.....

.....

Comment .....

Duration of follow up ..... years ..... months

APPENDIX II

DIETARY AVOIDANCE INFORMATION SHEETS FOR PATIENTS

Glasgow Royal Infirmary

Dietetic Department

Benzoate Free Diet

As most of the Benzoates in the diet are added to foods as a PRESERVATIVE it is very important that you read the labels of any manufactured or prepared foods you consume. If Benzoates are added to the food, the manufacturer may name the particular Benzoate a serial number known as an E number, avoid those from E210 - E219 inclusive. If you are unsure whether a food / drink may contain Benzoate it is best to avoid it.

Avoid any food which just lists the word "Preservative" or "Permitted Preservative" as an ingredient.

The Benzoates used in foods are :

E210 - Benzoic acid.
E211 - Sodium Benzoate.
E212 - Potassium Benzoate.
E213 - Calcium Benzoate.
E214 - Ethyl 4-hydroxybenzoate.
E215 - Ethyl 4-hydroxybenzoate sodium salt.
E216 - Propyl 4-hydroxybenzoate.
E217 - Propyl 4-hydroxybenzoate sodium salt.
E218 - Methyl 4-hydroxybenzoate.
E219 - Methyl 4-hydroxybenzoate sodium salt.

AVOID ANY ITEM CONTAINING THESE

In general - **AVOID**

Commercially prepared meat and fish dishes of which the exact composition is not known - lasagne, pizza, curry, Chinese foods, flans, quiches. Keep to fresh or home cooked food wherever possible.

Check Labels on

1. Squashes, cordials and diluting drinks
2. Fizzy drinks
3. Glucose drinks e.g. Lucozade
4. Non alcoholic grape juice drinks, e.g. Schloer
5. Slush puppies, ice poles, ice lollies, Tip Top
6. Bottles shandy. Avoid Tennants 'L.A.' on draught
7. Chocolate drinks
8. Liquid coffee and chicory drinks e.g. Camp coffee
9. Flavoured milk drinks and milk shake syrup
10. Yoghurt
11. Colourings and flavourings used in home baking
12. Jams, marmalades, chocolate spreads and also diabetic jam
13. Liquid artificial sweetener, e.g. Sweetex liquid
14. Pickled products e.g. herring, onions, beetroot, pickles. Canned fish in tomato sauce, pickled herring.
15. Also check sauces - horseradish, brown sauce, tomato sauce, mustard and vinegar
16. Mayonnaise, salad cream and salad dressings. Coleslaw and any salads in delicatessen counters. Potato salad.
17. Dried fruit. Tins or jars of fruit or fruit puree. Avoid ice cream with fruit/fruit sauce in it.
18. White bread. Avoid bead made with bleached flour - check labels.
19. Fruit sauces, toppings on cheesecakes, gateaux etc.
    Fruit pie fillings. Dessert sauces. Bought dessert cakes and gateaux.
    Fruit pies.
20. Crystallised or glace fruit. **ANY** preserved fruit.

The recommended toothpastes are:

1. Kingfisher.
2. Colgate Regular, Colgate Junior, Colgate Tartar Control;, Ultrabite, Colgate Blue Minty Gel.
3. Crest - all types.
4. MacLeans Original, MacLeans Sensitive, MacLeans Milk Teeth, Aquafresh

Any queries please contact your Dietitian:

Name:

Hospital:

Tel.No.:

KAM.Dec.1995.

Glasgow Royal Infirmary University NHS Trust

Department of Nutrition & Dietetics

Non- Alcoholic Drinks -Free From Benzoates

Carbonated Drinks

Asda
Soda Water

Barr Cola
In cans & bottles

Britvic
Citrus Spring Cans

Ribena
Sparkling Blackcurrant Flavour Drinks in Cans, all varieties.
Spring Sparkling Blackcurrant Juice Drink with Spring Water in bottles.

Seven-Up
Diet / ordinary - cans only.

Schweppes
American Ginger Ale
Canaada Dry Ginger Ale
Dry ginger ale
Ginger beer - in bottles
Indian tonic water
Schizan Sparkling drink with fruit and plant extracts
Slimline Indian tonic water - low calorie
Soda water

St Clements
All sparkling fruit drinks - in cans only

Chocolate and Malt Drinks

Asda
Cocoa powder
Drinking chocolate - 250g and 500g.
Farm Stores instant hot chocolate.
Fat reduced drinking chocolate granules - 500g
Instant hot chocolate - 400g

Cadburys
Bournvita
Bournvita Break Instant malted chocolate drink
Choc-a-shake
Chocolate Break Milk chocolate beverage
Cocoa
Drinking chocolate
High Lights Low calorie chocolate beverage - coffee, mint creme, chocolate, hazelnut

Cadburys
High Lights Low Calorie chocolate beverage - dairy. fudge, tangerine.
Milk Drink

Galaxy
Galaxy drink

Horlicks
Low fat instant - All varieties in Cekacans / Sachets
Malted food drink in glass jars / tins

Jubilee
Hot chocolate mix

Mars
Drink

Maxpax
Chocolate drink mix

Chocolate and Malt Drinks (Contd.)

Maxpax

Malted milk drink mix

Nestle

Chococino hot chocolate drink - standard & light, all types.

Suchard

Chocolate flavour drink mix

Coffee

Asda

Ground coffee - all varieties
Instant coffee - all varieties
Unsweetened capaccino

Maxpax

Kenco Smooth blend white instant coffee
White instant coffee

Maxwell House

Cappuccino Instant cappuccino

Nestle

Espresso
Nescafe Instant Cappuccino - original, and unsweetened
Nescafe Nescafe Instant Coffee - all varieties

Fruit Drinks (including squashes and concentrates)

Asda

Apple C drink - UHT
Grapefruit C drink - UHT
Hi juice orange crush 3 x 250ml
Orange & apricot juice drink (no added sugar) - 3 x 250ml
Orange C drink - UHT
Orange juice drink (no added sugar) - UHT - 3 x 250ml
Ready to drink lemon juice drink 3 x 250ml

Bird's

Apeel Orange drink mix (foodservice only)

Maxpax

Blackcurrant flavour drink mix
Lemon flavour drink mix
Orange flavour drink mix

Ribena

Ready-to-drink - all Tetrapak varieties

C-Vit

Ready-to-drink multi vitamin fruit drinks - all varieties in TetraPak

Kia-Ora

Ready to Drink Lemon Drink
Ready to Drink Mixed fruit drink
Ready to Drink Orange & pineapple drink
Ready to Drink Orange drink
Ready to Drink Pear and blackcurrant drink
Ready to Drink Summer Fruits Drink
Ready to Drink Tropical Orange

Robinsons

Special R Tropical fruit drink RTD in Tetrapak
Whole orange drink RTD in Tetrapak

Rose's

Diabetic Fruit squashes
Lime juice cordial

Fruit Drinks (including squashes and concentrates)

Robinsons

Apple and blackcurrant drink - in Tetrapak RTD
Pineapple juice drink RTD in Tetrapak
Special R Apple and blackcurrant drink - RTD in Tetrapak
Special R Orange drink RTD in Tetrapak

Schweppes

Blackcurrant flavour cordial
Lime flavour cordial
Oasis Still fruit drinks with spring water
Peppermint cordial

Fruit Juices

Asda

All chilled and UHT pure fruit juices (including Farm Stores)
Apple & blackcurrant fruit drink (no added sugar) - 3 x 250ml

Britvic

Fruit juices - bottles, cans, Tetrapaks
Orange juice - draught

Cawston Vale

Pure Fruit Juices - various blends

Copella

Chilled Freshly pressed chilled juice, vaarious blends - bottles
First Press Freshly pressed juice, various blends - bottles

Schweppes

Dispensed orange juice
Fruit juices - in bottles

Copella

Freshly pressed English apple juice - bottles and cartons
Freshly pressed English juice, various blends - bottles and cartons

Libby

"C" fruit juice drinks - all varieties
Fruit juices, unsweetened
Tomato juice - canned, glass or carton
Um Bongo mixed fruit juice drink

Meridian

Fruit juice concentrates

Rowntree

Rowntree fruit juice drink, all varieties

St Ivel

Real Fruit juices

Mineral Waters

Asda

Val blanc French natural mountain spring water

Schweppes

Malvern Still and sparkling mineral water

Other Drinks (including yeast extracts)

Bovril

Beef and vegetable extracts
Beef flavoured drink (vending only)
Chiucken

Marmite

Yeast extract

Maxpax

Bovril Beef extract drink mix

Sports Drinks

Schweppes

Energade Isotonic sports drink

Tea

Asda

Tea loose and bags - all varieties

London Herb & Spice

Fruit and herbal teas - all varieties

Maxpax

Lemon tea
White tea

Typhoo

All black teas
QT Instant white tea

Appletise
Grapetise
Orangina (in cans)

GLASGOW ROYAL INFIRMARY UNIVERSITY NHS TRUST

DEPARTMENT OF NUTRITION & DIETETICS

CINNAMON FREE DIET

You have been found to be allergic to cinnamon. This is a natural substance which does not require to be stated on food labels. Therefore, always look for the term 'spices' on food labels which are not included in the Manufactured Products List here.

Foods to be avoided

Soup: Tinned or packet soup with tomato or spices on the label

Meat: Canned meat in spicy sauce. Cold meat containing spices. Avoid spice for beef ham.

Fish: Canned fish in spicy sauce. Avoid made up meat or fish dishes containing spices e.g. pies, bridies, fish pie, lasagne, pizza.

AVOID curries and chinese food. Curry pastes, curry sauces.

Spices: Mixed spice, cinnamon, curry powder, Allspice. Check labels on any other spice mixes.

Baked beans in tomato sauce, ravioli, spaghetti in tomato sauce. "Invaders". Pork sauces and beans. Spaghetti Hoops.

Manufactured cakes and biscuits on list overleaf - and those containing spice. Check label. Gingerbread. Coffee Buns. Rich Fruit Cake. Keep to home-baking whenever possible.

Pickles and ketchup - see list.

Nuts - Dry Roasted type, Bombay Mix, Spicy Nut Mixes.

Crisps + Snacks - avoid spices. Check labels and Products List.

Mincemeat and Christmas pudding. Mincemeat pies and Christmas cake. Apple Strudel. Apple cake/tart with cinnamon or spices.

Soft drinks: Coca Cola, Red cola, Vimto, Pepsi Cola, Dr. Peppers.

Alcoholic drinks: Red wine, gin, red and white Martini and Cinzano. Avoid the perfumed type of alcoholic drinks, e.g. Dubonnet, Malibu, Dark Rum, Tia Maria etc. Mulled wine.

Toothpaste: All others apart from on Allowed List.. No "Tartar Controlled" toothpaste. **AVOID MOUTHWASHES ALSO,** unless otherwise stated on Manufactured Products List.

Cola sweets: Cola cubes, Cola Chewits, cinnamon sticks, cola chews, etc.

Also see list of manufactured products containing cinnamon.

Foods Allowed

Soup: Homemade or tinned or packet soup **not** containing spices or tomato.

Meat: All fresh or frozen.

Fish: All fresh or frozen.

Eggs: All types.

Dairy Produce: Milk - all types. Yoghurt. Butter, cheese, cream - all types. Ice cream.

Fats & Oils: Margarine, cooking oils, lard.

Fruit: All types.

Vegetables: All types including potatoes, salad vegetables.

Bread: All types.

Rice, pasta, pulses (peas, beans, barley, lentils), nuts (**avoid** dry roasted type).

Cereals: All cereals. Breakfast cereals, porridge. Also flour, tapioca, sago, custard powder, cornflour.

Cakes & Biscuits: Homemade. Shopbought cakes and biscuits avoiding spices on label.

Drinks: Tea, coffee, Oxo, Bovril, Marmite, Bournvita, Cocoa, Drinking Chocolate, Horlicks. Squashes, lemonade and any other fizzy drinks apart from those on the 'Avoid List'. Soda water, Appletise, fresh fruit juices. Spring waters.

Alcoholic Drinks: White wine, cider, lager, beer, whisky, vodka.

Miscellaneous: Sugar, salt, pepper, herbs, spices not mentioned on 'Avoid List', e.g. nutmeg, chilli powder, ginger etc. Jams,marmalade, honey, lemon curd, peanut butter, saccharin and other artificial sweeteners.

KAM/RS
12.12.95.

MANUFACTURED PRODUCTS INFORMATION FOR CINNAMON

ASDA; No information available.

BARR; Strike Cola only product containing cinnamon.

BATCHELORS; No information available.

BERNARD MATTHEWS; Turkey Tikka only product containing cinnamon.

CADBURY;(INCL. SWEPPES) Chocolate Cream, Old Jamaica only products containing cinnamon.

CAMPBELLS; Avoid Tomato Soup only

COLMANS; **Products containing cinnamon**:

OK Fruit Sauce, Wholegrain Mustard, French Mustard, Dijon Mustard, Tikka Masala Dry Sauce Mix. Traditional Herb Mustard.
ANY Curry Sauce or Products.

CO-OP PRODUCTS CONTAINING CINNAMON:

Frozen; American Style Double Cream Apple Pie Ice Cream
Apple Strudel
Summer Fruit Strudel

Canned Traditional Rice Pudding
Apple, Sultana & Cinnamon Custard Style Yoghurt
Apple & Cinnamon Fruit Filled Biscuits
Plain Chocolate Ginger Rings
Milk Chocolate Ginger Rings
Ground Mixed Spice
Ground Cinnamon

It is also recommended to avoid any product with **SPICES** in the ingredients.

DAIRY CREST; No products containing cinnamon

FARLEY'S; No products containing cinnamon

GOLDEN WONDER; **Products free from Cinnamon;**

Crisps	Ready Salted		Spring Onion
	Salt & Vinegar		Steak & Onion
	Cheese & Onion		Pickled Onion
	Smokey Bacon		Roast Chicken
Ringos	Salt & Vinegar		Cheese & Onion
Wotsits	Bacon	Cheese	Barbequed Beef
Golden Lights	Lightly Salted		Grilled Chicken
Pot Noodles	Chicken & Mushroom		Chicken Curry
	Bolognaise		Spicy Chicken
	Vegetable Korma		

HALLS;

Fruit Pudding)
Economy Black Pudding) **ALL CONTAIN**
Puritan Black Pudding) **CINNAMON**
Marks & Spencer Black Pudding)

HEINZ; See attached list for products free from cinnamon.

H.P. FOODS; See attached list for products free from cinnamon

JACOBS; Fruit & Nut Crunch only product containing cinnamon

KELLOGS; Apple Pop Tarts and also Chocos contain cinnamon

KRAFT; Tomato Ketchup contains cinnamon

LYONS TETLEY; No products containing cinnamon

MARS; Honey flavoured Tunes only product containing cinnamon

MARKS & SPENCER; No information available.

MR. KIPLING; **Products containing cinnamon;**

Mr. Kipling Country Fruit Lemon Tart
" " Country Slices
" " Mince Pies
" " Luxury Mince Pies
" " Glazed Mince Tartlets
" " Mince + Brandy Sauce Pies
" " Country Fruit Cake
" " Christmas Slices
" " Christmas Cake
" " Stolen Slices

NESTLE; **Ice Cream Division**; No products containing cinnamon

Food Division: Creamola Foam - Raspberry flavour
Pan Yan Sandwich Piccalilli
Pan Yan Original Pickle

NESTLE(incl. ROWNTREE CONFECTIONERY): Rowntree's Fruit Gums only product containing cinnamon

ROWATS OR ROTHWELL; **Products containing cinnamon:**

Tomato Ketchup
Family Sauce
Pickles Silverskin & Cocktail Onions
Pickled Chip Onions
Pickled Red Cabbage
Piccalilli
Hamburger Relish

SAFEWAY; No information available at present

SAINSBURY; **Products containing cinnamon**:

Soft Drinks: All products free from cinnamon

Confectionery: Milk chocolate covered almonds dusted with cinnamon

SAINSBURY - Cont'd.

Cakes & Biscuits:
1) Chocolate hearts - spicy jam filled Lebkuchen
2) Lebkuchen
3) Lebkuchen Gingerbread Men
4) Milk/Plain Chocolate Continental biscuit assortment
5) Pfeffernusse
6) Plum Pudding
7) Alcohol-free Christmas Pudding
8) Raisin & Walnut Malt Loaf.

ST. IVEL; No products containing cinnamon

TESCO; Follow general guidelines - avoid coke, baked beans, sauces, curried and spicy foods. Fresh Chicken Korma (in chilled cabinet) and Barbeque Sauce are free from cinnamon.

TREBOR BASSETT; No products containing cinnamon

THORNTONS; No products containing cinnamon

THOMAS TUNNOCK; Chocolate Perkins and Perkins contain cinnamon.

WALLS; 'Country Fair' Vermont Apple Cinnamon Pie contains cinnamon

FOX'S BISCUITS; Fruit Shrewsbury are only products containing cinnamon

WRIGLEY; Big Red and Juicy Fruit are only products containing cinnamon

TOOTHPASTE; **Colgate/Palmolive - Products containing cinnamon**

Colgate Blue Minty Gel
Colgate Plax
Colgate Actibrush

Stafford Miller - Search Dental Rinse contains cinnamon. All other products suitable for use.

Smith Kline Beecham - All products free from cinnamon

Procter & Gamble - All products free from cinnamon i.e. Crest.

The following Heinz products are free from Cinnamon

WEIGHT WATCHERS FROM HEINZ PRODUCTS

SAUCES, SALADS & PICKLES

Cooking Sauce, French White Wine & Dill
Cooking Sauce, Indian Korma
Cooking Sauce, Italian Tomato & Onion
Cooking Sauce, Oriental Sweet & Sour
Low Fat Dressing
Low Fat Dressing, Mild Mustard
Low Fat Dressing, Thousand Island
Low Fat Dressing, Yoghurt & Herb
Mayonnaise, Reduced Calorie

PASTA

Italiana, Bolognese Shells
Italiana, Pasta Tubes in Cheese Sauce with Bacon
Italiana, Tortellini
Italiana, Tuna Twists
Italiana, Vegetable Ravioli with Tomato Sauce
Spaghetti in Tomato Sauce, No Added Sugar

DAIRY PRODUCTS

Cheese Slices, Reduced Fat
Dairy Spread with Cheese & Ham, Reduced Fat
Dairy Spread with Cheese, Reduced Fat
Dairy Spread ,Cheese, Onions & Chives, Reduced Fat
Hard Cheese, Reduced Fat

FRENCH BREAD PIZZAS

French Bread Pizza, Cheese & Tomato
French Bread Pizza, Ham & Mushroom
French Bread Pizza, Ham & Pineapple
French Bread Pizza, Pepperoni

FROZEN READY MEALS

Beef Lasagne
Beef Oriental with Rice
Big Deal, Beef Goulash with Parsley Rice
Big Deal, Cajun Chicken
Big Deal, Chilli Con Carne with Rice
Big Deal, Keema Curry with Cumin Rice
Cannelloni Filled with Vegetables
Cauliflower Cheese
Chicken & Broccoli Pasta Bake
Chicken Chasseur with Rice
Chicken Curry with Rice
Chicken Marengo with Rice
Chicken Supreme
Fish Provincale with Noodles
Mediterranean Chicken
Pasta Bolognese
Pasta Shells with Vegetables & Prawns
Salmon & Prawn Fricasse
Salmon Mornay
Seafood Bake with Broccoli
Smoked Fish with Pasta Bows
Spaghetti Bolognese
Sweet & Sour Chicken with Rice
Sweet & Sour Vegetables
Tagliatelle Carbonara
Vegetable au Gratin
Vegetable Chilli with Rice
Vegetable Hotpot
Vegetable Lasagne
Vegetable Moussaka

DESSERTS

Cheesecake, Blackcurrant
Cheesecake, Strawberry
Dairy Ice Cream, Chocolate Ripple
Dairy Ice Cream, Neapolitan
Dairy Ice Cream, Strawberry
Dairy Ice Cream, Vanilla
Dessert Bombes, Chocolate with Orange Liqueur
Dessert Bombes, Lemon
Dessert Bombes, Mint Chocolate
Dessert Bombes, Tropical Fruit
Premium Ice Cream, Lemon Meringue
Premium Ice Cream, Strawberries & Cream
Premium Ice Cream, Triple Chocolate Fudge
Premium Ice Cream, Triple Toffee Fudge
Rice Pudding, No Added Sugar, Low Fat
Torte, Orange & Lemon
Torte, Peach & Apricot
Torte, Raspberry

JAMS & MARMALADES

Apricot Jam, Reduced Sugar
Blackcurrant Jam, Reduced Sugar
Fruits of the Forest Jam, Reduced Sugar
Marmalade, Reduced Sugar
Morello Cherry Jam, Reduced Sugar
Raspberry Jam, Reduced Sugar
Strawberry Jam, Reduced Sugar

BREAD & ROLLS

Brown Bread
Danish Brown Bread
Danish Malted Softgrain Bread
Danish White Bread
Oat Danish Bread
Soft Brown Rolls
Soft White Rolls
White Bread

CANNED SOUPS

Chicken & Ham with Rice Soup
Chicken Noodle Soup
Chicken Soup
Country Vegetable & Beef Soup
Country Vegetable Soup
Lentil & Carrot Soup
Mushroom Soup
Vegetable Soup
Wholesome Soup, Lentil & Chicken
Wholesome Soup, Winter Vegetable

INSTANT SOUPS

Asparagus & Leek Soup, 40kcal
Chicken & Sweetcorn Soup, 60kcal
Chicken & Vegetable Soup with Noodles, 60kcal
Chicken & Vegetable Soup, 40kcals
Chicken Soup, 40kcal
Minestrone Soup, 60kcal
Mushroom Soup, 40kcal
Mushroom Soup, 60kcal
Tomato Soup, 40kcal
Tomato Soup, 60kcal
Vegetable Soup with Croutons, 60kcal
Vegetable Soup, 40kcal

BISCUITS & SNACKS

Cookies, Dark Treacle
Cookies, Real Chocolate Chip
Cookies, Stem Ginger

The following Heinz products are free from Cinnamon

CORE PRODUCTS

BAKED BEANS

Barbecue Beans
Curried Beans with Sultanas

LUNCH BOWLS

Beef Curry with Rice
Beef Goulash with Noodles
Chicken Curry with Rice
Chilli Con Carne with Rice
Lamb and Vegetable Casserole

PASTA

Chef's Specials, Chicken Ravioli in Tomato Sauce
Chef's Specials, Macaroni Cheese
Chef's Specials, Pasta Pipes with Tuna & Bacon
Chef's Specials, Ravioli in Spicy Sauce
Chef's Specials, Sausage Hotpot with Pasta
Chef's Specials, Spicy Beef Pasta Twists
Dinosaurs with Mini Meat Boulders
Dinosaurs, Pasta Shapes in Tomato Sauce
Magic Roundabout, Spaghetti Shapes in Tomato Sauce
Noodle Doodles, Spaghetti Shapes in Tomato Sauce
Spaghetti Hoops in Tomato Sauce
Spaghetti in Tomato Sauce
Spaghetti with Sausages
Super Mario, Spaghetti Shapes in Tomato Sauce
Thomas the Tank Engine, Spaghetti in Tomato Sauce

SAUCES, SALADS & PICKLES

Apple Sauce
Coleslaw Salad
Coronation Sauce
Mayonnaise
Mixed Bean Salad
Pasta Salad
Ploughman's Piccalilli
Ploughman's Pickle
Ploughman's Tangy Sandwich Pickle

HEINZ CORE PRODUCTS Cont'd.

SAUCES, SALADS & PICKLES Cont'd.

Ploughman's Tomato Pickle
Potato Salad
Salad Cream
Salad Cream, Spoonable
Salsa, Medium-Hot
Salsa, Milk-Medium
Sandwich Spread, Cucumber
Sandwich Spread, Spicy
Sandwich Spread, Sweetcorn & Red Pepper
Silverskin Onions
Vegetable Salad

SPONGE PUDDINGS

Sponge Pudding, Banana with Toffee Sauce
Sponge Pudding, Chocolate with Chocolate Sauce
Sponge Pudding, Lemon
Sponge Pudding, Mixed Fruit
Sponge Pudding, Strawberry Jam
Sponge Pudding, Treacle

READY TO SERVE SOUPS

Beef Soup
Big Soup, Beef & Bacon Hotpot
Big Soup, Beef & Vegetable
Big Soup, Beef Broth
Big Soup, Chicken & Ham
Big Soup, Chicken & Vegetable
Big Soup, Chicken, Leek & Potato
Big Soup, Giant Minestrone
Big Soup, Spicy Tomato with Beef Pasta Parcels
Big Soup, Thick Country Vegetable with Ham
Chicken & Mushroom Soup
Cream of Asparagus Soup
Cream of Celery Soup
Cream of Chicken Soup
Cream of Mushroom Soup
Farmhouse Beef & Vegetable Soup
Farmhouse Beef Broth

CINNAMON FREE PRODUCTS Cont'd.

READY TO SERVE SOUPS Cont'd.

Farmhouse Chicken & Vegetable Soup
Farmhouse Potato & Leek Soup
Farmhouse Scotch Broth
Mulligatawny Soup
Oxtail Soup
Pasta Soup, Beef & Tomato Bolognese
Pasta Soup, Chicken Pastini
Pasta Soup, Minestrone
Pasta Soup, Mushroom Carbonara
Pasta Soup, Tomato Napoli
Premium Soup, Beef, Potato & Red Pepper
Premium Soup, Carrot, Potato & Coriander
Premium Soup, Chicken Sweet Pepper & Dill
Premium Soup, Chicken, Sweetcorn & Asparagus
Premium Soup, Country Bean with Mushroom
Premium Soup, Seven Vegetable
Scottish Vegetable Soup with Lentils
Spicy Soup, Chilli Bean & Beef
Spicy Soup, Curried Chicken with Rice
Spring Vegetable Soup
Vegetable Soup
Wholesoup, Farmhouse Vegetable
Wholesoup, Ham & Butter Bean
Wholesoup, Lentil
Wholesoup, Pea & Ham
Wholesoup, Winter Vegetable

TOPPERS

Toast Toppers, Chicken & Mushroom
Toast Toppers, Ham & Cheese
Toast Toppers, Mushroom & Bacon

CANNED TUNA

Tuna Chunks, Canned in Brine
Tuna Chunks, Canned in Vegetable Oil
Tuna Steak, Canned in Brine
Tuna Steak, Canned in Vegetable Oil

HP FOODS LTD. NUTRITION INFORMATION **21.3.94.**
CINNAMON CONTENT

	PRESENT	ABSENT
HP SAUCE		√
HP FRUITY SAUCE		√
HP TOMATO KETCHUP	√	
HP SPICY TOMATO SAUCE		√
HP CHILLI SAUCE	√	
HP CURRY SAUCE		√
HP FRUITY BARBECUE SAUCE	√	
HP SWEET & SOUR SAUCE		√
HP ORIGINAL BARBECUE SAUCE	√	
HP MEXICAN SPICY BARBECUE SAUCE		√
HP RICH JAMAICAN BARBECUE SAUCE		√
HP MINT SAUCE		√
HP MAYONNAISE (BULK)		√
HP MALT VINEGAR		√
HP BEANS IN TOMATO SAUCE	√	
HP HEALTHY BEANS	√	
HP BAKED BEANS IN TOMATO SAUCE (CATERING)	√	
HP HEALTHY BEANS IN TOMATO SAUCE (CATERING)	√	
HP BEANS AND BEEFBURGERS	√	
HP BEANS AND SAUSAGE	√	
HP SAUCY BEANS	√	
HP BIG BREAKFAST BIG BEANS IN TOMATO SAUCE		√
HP BIG BREAKFAST BIG BEANS & JUMBO SAUSAGES		√
HP BATMAN BOLOGNESE		√
HP PASTA SHAPES IN TOMATO SAUCE		√
HP SPAGHETTI IN TOMATO SAUCE		√
HP GLADIATORS IN TOMATO SAUCE (REDUCED SUGAR)		√
HP GLADIATORS IN SMOKEY BACON SAUCE		√
HP GLADIATORS IN PIZZA SAUCE WITH PEPPERONI		√
HP SONIC AND SAUSAGES IN TOMATO SAUCE		√
HP SONIC RAVIOLI SHAPES IN TOMATO SAUCE		√
HP CHICKEN KORMA BISTRO BREAK	√	

HP SWEET & SOUR BISTRO BREAK		√
HP CANNELLONI BISTRO BREAK		√
HP LASAGNE BOLOGNESE BISTRO BREAK		√
DADDIES TOMATO KETCHUP		√
DADDIES BROWN SAUCE		√
DADDIES BURGER RELISH		√
DADDIES SALAD CREAM		√
FLETCHERS TIGER SAUCE		√
FLETCHERS TITBITS SAUCE		√
FLETCHERS BROWN SAUCE		√
FLETCHERS TOMATO KETCHUP	√	
FLETCHERS BAKED BEANS IN TOMATO SAUCE	√	
FLETCHERS SHORT CUP SPAGHETTI IN TOMATO SAUCE		√
L & P WORCESTERSHIRE SAUCE		√
L & P CHILLI & GARLIC SAUCE		√
L & P GINGER & ORANGE SAUCE		√
L & P GARLIC & SPRING ONION SAUCE		√
L & P HOT PEPPER SAUCE		√
L & P MUSTARD & PEPPERCORN SAUCE	√	
L & P GARLIC SAUCE		√
L & P FRUIT SAUCE		√
L & P WORCESTER KETCHUP		√
L & P TOMATO KETCHUP WITH MILK CURRY SPICES	√	
L & P SOY & GARLIC		√
L & P HOT PEPPER & LIME		√
L & P SOY & FIVE SPICE	√	
L & P CURRY CONCENTRATE		√
L & P ITALIAN VINAIGRETTE MAKER		√
L & P WHITE WINE & GARLIC VINAIGRETTE MAKER		√
L & P CLASSIC FRENCH VINAIGRETTE MAKER		√
L & P PICKLE WITH WORCESTER SAUCE		√
L & P SWEET PEPPERS WITH CHILLI SAUCE		√

L &B P GINGER SAUCE	√
L & P GREEN JALAPENO SAUCE	√

Home Made Tomato Soup

4 Pints Vegetable Stock
1/2 lb Onions
tsp. Tomato Puree
One small tin evaporated milk

1 1/2 oz Plain Cornflour } make to a fluid with a little water
2 oz granulated sugar }

Salt & Pepper

Mince onions finely.

Add to stock with tomato puree. Bring to boil, then simmer.
Add cornflour and sugar. Simmer for about 1/2 hour.
Strain, re-heat, add evaporated milk and salt & pepper.

Glasgow Royal Infirmary University NHS Trust

Department of Nutrition & Dietetics

Benzoate & Cinnamon Free Diet

You have been found to be allergic to the above **preservative** - Benzoate, and the **spice** - Cinnamon.

Benzoate - As most of the benzoates in the diet are added to foods as a preservative it is very important that you read the labels of any manufactured or prepared foods you consume. If benzoates **are** added to the food the manufacturer may name the particular benzoate **or** use a serial number known as an **E** number. Avoid those containing E210 - E219 inclusive.

The E numbers and the benzoate names are listed below :

E210	-	Benzoic Acid
E211	-	Sodium Benzoate
E212	-	Potassium Benzoate
E213	-	Calcium Benzoate
E214	-	Ethyl 4-hydroxybenzoate
E215	-	Ethyl 4- hydroxybenzoate sodium salt
E216	-	Propyl 4-hydroxybenzoate
E217	-	Propyl 4-hydroxybenzoate sodium salt
E218	-	Methyl 4-hydroxybenzoate
E219	-	Methyl 4-hydroxybenzoate sodium salt

Avoid any food or drink which just lists the word **Preservative** or **Permitted Preservative** as an ingredient.

Cinnamon This is a natural substance which does not require to be stated on food labels. This diet sheet and accompanying manufactured product list will give you information on the foods to be avoided. Also, always look for the term **"spices"** on food labels which are not included in the manufacturer's product list and avoid these.

Avoid Any Item Containing These

If you have any queries regarding the dietary information please contact :

Dietitian :

Hospital :

Telephone Number :

Benzoate and Cinnamon Avoidance

Foods Allowed	Foods to Check Labels for E210 - E219	Foods to be Avoided
Meat - Cook from fresh whenever possible. Fresh & frozen meats.		Canned meat in a spicy sauce. Cold meats containing spices. Commercially made meat dishes containing spices, e.g. lasagne, pizza. Pizza, Curries, Chinese Meals. Avoid 'spice' on beef ham. Some sausages, pies, bridies, etc. MAY contain spices - check labels.
Fish - Cook from fresh whenever possible. All types of fresh, rozen, tinned fish (not in sauce), shell fish allowed.		Commercially made fish dishes containing spices. Canned fish in spicy sauce. Prawn cocktail. Prawn Marie Rose.
Spices - Nutmeg, ginger, cumin, coriander, chilli powder, tumeric and all other spices apart from those on AVOID LIST		Spices - Cinnamon, mixed spice, allspice, curry powder. Check labels on any other spice mixes.
Herbs - All herbs including garlic. Salt, pepper, black pepper.		Gelatin capsules.
Soup - Most soups - homemade, tinned packet - apart from those on AVOID LIST	Soup concentrates.	Tinned or packet soup containing tomato, spices, tomato puree.
Sugar - Tablet artificial sweetener	Liquid artificial sweetener.	

Benzoate and Cinnamon Avoidance

Foods Allowed	Foods to Check Labels for E210 - E219	Foods to be Avoided
Crisps - check products list for those free from cinnamon. In general keep to salt & vinegar, ready salted, cheese & onion.		Crisps and snacks which contain spices, e.g. prawn cocktail, tomato, barbeque beef flavours.
Nuts - most types.		Dry roasted nuts. Bombay mix. Spicy nut mixes.
Vegetables - all types including potatoes, chips, salad vegetables, fresh and tinned tomatoes.	Tomato Puree. Check labels on any vegetables in a sauce.	Commercially made foods containing tomato puree if not marked " preservative free", e.g. pizza, lasagna, pasta dishes.
Bread - Use brown breads, wholemeal bread, brown or wheaten rolls. White bread made with **unbleached** white flour (check label on loaf).		White rolls } French bread } May cause a problem only. White bread made with **bleached** white flour. Check labels on loaves for type of flour used.
Fruit : Fresh fruit - all types	Any preserved fruit in any form, e.g. Fruit based dessert sauces. Fruit toppings for ice cream. Gateaux containing fruit. Apple pies - fruit pies in general. Fruit pie fillings. Toppings for cheese cakes. Crystalised or glace fruit. Dried fruit. Tins of fruit or fruit puree. Packet cake topping. Prepacked cake topping. Prepacked cake mixes.	Preserved fruit in any form if it is not known to be free from forbidden preservatives. Dried apricots. Candied peel.

Benzoate & Cinnamon Avoidance

Foods Allowed	Foods to Check Labels for E210 - E219	Foods to be Avoided
Milk, cream, butter, cheese, margarine.	Yoghurt Flavoured milk drinks, milk shake syrup. Artificial cream.	Ice cream with fruit or fruit syrup in it e.g. Raspberry ripple, Tutti frutti. "Raspberry" sauce for ice cream.
Cooking oils and lard. Salad oils.		
Eggs		
Pasta - all types		Commercially made pasta dishes with spices onthe label. All pasta tinned in tomato sauce.
	Made up pasta dishes.	
Rice - all types		Commercially made rice dishes with spices on the label.
Breakfast cereals - all types including porridge, bran.		
Peas, beans, lentils.		Baked beans in tomato sauce, spaghetti in tomato sauce, ravioli in tomato sauce. Any similar products tinned in tomato sauce.
Custard powder, custard, rice, semolina, tapioca, sago, cremola, cornflour, arrowroot. Tinned milk puddings.		

Benzoate & Cinnamon Avoidance

Foods Allowed	Foods to Check Labels for E210 - E219	Foods to be Avoided
Alcohol : White wine, whisky, vodka, white rum, sherry, brandy, cider, lager, beer, Tennant's "LA" in cans.	Be careful that any mixers are preservative free. Check labels on all Low Alcohol wines.	Red wine, gin, dark rum, Tia Maria, Martini**, Cinzano, Dubonnet and similar products. Tennant's "LA" on draught.
Pickles, sauces, chutneys **NOT** on Manufactured Products list and **NOT** containing forbidden preservatives.	All pickles, sauces, chutneys, pickled foods (e.g. herring, onions, beetroot), vinegar, mustard, tomato sauce, brown sauce. Mayonnaise, salad cream, salad dressings. **Remember** to check Manufactured Products list to see if they contain cinnamon.	Tomato sauce. **ALL** pickles, etc. On Manufactured Products list, i.e. containing cinnamon, or if containing forbidden preservatives.
Hot drinks : Tea, coffee, hot chocolate, cocoa, Ovaltine, Horlicks, Bournvita, Oxo, Marmite, Bovril.	"Camp" liquid coffee. Chocolate drinks.	
Jams and marmalade : Home made jam and marmalade, honey, syrup, treacle, peanut butter.	Lemon curd. Diabetic jam.	Preserves containing E210 - E219.
Home baking : Use **unbleached** flour for baking. Check labels on packet for this.	Dried fruit, mixed peel, glace cherries. Pie fillings.	Cinnamon, allspice, mixed spice, mincemeat.

** **NB** Many liqueurs may contain spices - be aware of this if any reaction occurs.

Benzoate & Cinnamon Avoidance

Foods Allowed	Foods to Check Labels for E210 - E219	Foods to be Avoided
Cakes and biscuits : Keep to home baked goods as much as possible.		Mincemeat pies and tarts. Bought fruit pies, Danish pastries, coffee buns, gingerbread. Rich fruit cake, Christmas cake / pudding, black bun, & biscuits containing large quantities of dried fruit.
Soft drinks : 7 Up (not diet), Appletise, Kiri, Orangina (cans only), Perrier water and other natural spring water. St. Clements canned drinks - not Coke. Sunquick squash, Robinsons High Juice Squashes & Barley Squashes. Robinsons "R" reduced sugar drinks.	Check labels on **ALL** squashes, fizzy drinks, soft drinks, bottled shandy, non-alcoholic grape juice drinks, e.g. Schloer. Ice lollies, ice poles, "Tip Tops", Slush Puppies, Soda waters.	Any soft drinks containing E210 - E219. Also avoid Coca Cola, Vimto, Dr. Peppers, Pepsi Cola.
Fudge, toffee, tablet, chocolate, liquorice, boiled sweets (no cola sweets).		Cinnamon sticks, cola cubes, cola sweets, cola chews etc. Irn Bru chews.
Mouthwash & Toothpaste - Colgate regular, Crest regular, Kingfisher. Mac Leans - Freshmint, Milk Teeth, Sensitive, Mouthguard.		All other mouthwashes & toothpastes. **NO** tartar controlled toothpastes. Colgate Plax, Colgate Actibrush, Colgate Blue Minty Gel, Aquafresh - Fresh & Minty, and Mild & Minty. MacLeans Mild Mint.
See also manufactured food products list.		

MANUFACTURED PRODUCTS INFORMATION FOR CINNAMON

ASDA; No information available.

BARR; Strike Cola only product containing cinnamon.

BATCHELORS; No information available.

BERNARD MATTHEWS; Turkey Tikka only product containing cinnamon.

CADBURY;(INCL. SWEPPES) Chocolate Cream, Old Jamaica only products containing cinnamon.

CAMPBELLS; Avoid Tomato Soup only

COLMANS; **Products containing cinnamon**:

OK Fruit Sauce, Wholegrain Mustard, French Mustard, Dijon Mustard, Tikka Masala Dry Sauce Mix. Traditional Herb Mustard.
ANY Curry Sauce or Products.

CO-OP PRODUCTS CONTAINING CINNAMON:

Frozen;
American Style Double Cream Apple Pie Ice Cream
Apple Strudel
Summer Fruit Strudel

Canned Traditional Rice Pudding
Apple, Sultana & Cinnamon Custard Style Yoghurt
Apple & Cinnamon Fruit Filled Biscuits
Plain Chocolate Ginger Rings
Milk Chocolate Ginger Rings
Ground Mixed Spice
Ground Cinnamon

It is also recommended to avoid any product with **SPICES** in the ingredients.

DAIRY CREST; No products containing cinnamon

FARLEY'S; No products containing cinnamon

GOLDEN WONDER; **Products free from Cinnamon;**

Crisps	Ready Salted		Spring Onion
	Salt & Vinegar		Steak & Onion
	Cheese & Onion		Pickled Onion
	Smokey Bacon		Roast Chicken
Ringos	Salt & Vinegar		Cheese & Onion
Wotsits	Bacon	Cheese	Barbecued Beef
Golden Lights	Lightly Salted		Grilled Chicken
Pot Noodles	Chicken & Mushroom		Chicken Curry
	Bolognaise		Spicy Chicken
	Vegetable Korma		

HALLS;

Fruit Pudding)
Economy Black Pudding) **ALL CONTAIN**
Puritan Black Pudding) **CINNAMON**
Marks & Spencer Black Pudding)

HEINZ; See attached list for products free from cinnamon.

H.P. FOODS; See attached list for products free from cinnamon

JACOBS; Fruit & Nut Crunch only product containing cinnamon

KELLOGS; Apple Pop Tarts and also Chocos contain cinnamon

KRAFT; Tomato Ketchup contains cinnamon

LYONS TETLEY; No products containing cinnamon

MARS; Honey flavoured Tunes only product containing cinnamon

MARKS & SPENCER; No information available.

MR. KIPLING; **Products containing cinnamon;**

Mr. Kipling Country Fruit Lemon Tart
" " Country Slices
" " Mince Pies
" " Luxury Mince Pies
" " Glazed Mince Tartlets
" " Mince + Brandy Sauce Pies
" " Country Fruit Cake
" " Christmas Slices
" " Christmas Cake
" " Stolen Slices

NESTLE; **Ice Cream Division;** No products containing cinnamon

Food Division: Creamola Foam - Raspberry flavour
Pan Yan Sandwich Piccalilli
Pan Yan Original Pickle

NESTLE(incl. ROWNTREE CONFECTIONERY): Rowntree's Fruit Gums only product containing cinnamon

ROWATS OR ROTHWELL; **Products containing cinnamon:**

Tomato Ketchup
Family Sauce
Pickles Silverskin & Cocktail Onions
Pickled Chip Onions
Pickled Red Cabbage
Piccalilli
Hamburger Relish

SAFEWAY; No information available at present

SAINSBURY; **Products containing cinnamon:**

Soft Drinks: All products free from cinnamon

Confectionery: Milk chocolate covered almonds dusted with cinnamon

SAINSBURY - Cont'd.

Cakes & Biscuits:
1) Chocolate hearts - spicy jam filled Lebkuchen
2) Lebkuchen
3) Lebkuchen Gingerbread Men
4) Milk/Plain Chocolate Continental biscuit assortment
5) Pfeffernusse
6) Plum Pudding
7) Alcohol-free Christmas Pudding
8) Raisin & Walnut Malt Loaf

ST. IVEL; No products containing cinnamon

TESCO; Follow general guidelines - avoid coke, baked beans, sauces, curried and spicy foods. Fresh Chicken Korma (in chilled cabinet) and Barbeque Sauce are free from cinnamon.

TREBOR BASSETT; No products containing cinnamon

THORNTONS; No products containing cinnamon

THOMAS TUNNOCK; Chocolate Perkins and Perkins contain cinnamon.

WALLS; 'Country Fair' Vermont Apple Cinnamon Pie contains cinnamon

FOX'S BISCUITS; Fruit Shrewsbury are only products containing cinnamon

WRIGLEY; Big Red and Juicy Fruit are only products containing cinnamon

TOOTHPASTE; **Colgate/Palmolive - Products containing cinnamon**

Colgate Blue Minty Gel
Colgate Plax
Colgate Actibrush

Stafford Miller - Search Dental Rinse contains cinnamon. All other products suitable for use.

Smith Kline Beecham - All products free from cinnamon

Procter & Gamble - All products free from cinnamon i.e. Crest.

The following Heinz products are free from Cinnamon

WEIGHT WATCHERS FROM HEINZ PRODUCTS

SAUCES, SALADS & PICKLES

Cooking Sauce, French White Wine & Dill
Cooking Sauce, Indian Korma
Cooking Sauce, Italian Tomato & Onion
Cooking Sauce, Oriental Sweet & Sour
Low Fat Dressing
Low Fat Dressing, Mild Mustard
Low Fat Dressing, Thousand Island
Low Fat Dressing, Yoghurt & Herb
Mayonnaise, Reduced Calorie

PASTA

Italiana, Bolognese Shells
Italiana, Pasta Tubes in Cheese Sauce with Bacon
Italiana, Tortellini
Italiana, Tuna Twists
Italiana, Vegetable Ravioli with Tomato Sauce
Spaghetti in Tomato Sauce, No Added Sugar

DAIRY PRODUCTS

Cheese Slices, Reduced Fat
Dairy Spread with Cheese & Ham, Reduced Fat
Dairy Spread with Cheese, Reduced Fat
Dairy Spread ,Cheese, Onions & Chives, Reduced Fat
Hard Cheese, Reduced Fat

FRENCH BREAD PIZZAS

French Bread Pizza, Cheese & Tomato
French Bread Pizza, Ham & Mushroom
French Bread Pizza, Ham & Pineapple
French Bread Pizza, Pepperoni

WEIGHT WATCHERS FROM HEINZ PRODUCTS Cont'd.

FROZEN READY MEALS

Beef Lasagne
Beef Oriental with Rice
Big Deal, Beef Goulash with Parsley Rice
Big Deal, Cajun Chicken
Big Deal, Chilli Con Carne with Rice
Big Deal, Keema Curry with Cumin Rice
Cannelloni Filled with Vegetables
Cauliflower Cheese
Chicken & Broccoli Pasta Bake
Chicken Chasseur with Rice
Chicken Curry with Rice
Chicken Marengo with Rice
Chicken Supreme
Fish Provincale with Noodles
Mediterranean Chicken
Pasta Bolognese
Pasta Shells with Vegetables & Prawns
Salmon & Prawn Fricasse
Salmon Mornay
Seafood Bake with Broccoli
Smoked Fish with Pasta Bows
Spaghetti Bolognese
Sweet & Sour Chicken with Rice
Sweet & Sour Vegetables
Tagliatelle Carbonara
Vegetable au Gratin
Vegetable Chilli with Rice
Vegetable Hotpot
Vegetable Lasagne
Vegetable Moussaka

DESSERTS

Cheesecake, Blackcurrant
Cheesecake, Strawberry
Dairy Ice Cream, Chocolate Ripple
Dairy Ice Cream, Neapolitan
Dairy Ice Cream, Strawberry
Dairy Ice Cream, Vanilla
Dessert Bombes, Chocolate with Orange Liqueur
Dessert Bombes, Lemon
Dessert Bombes, Mint Chocolate
Dessert Bombes, Tropical Fruit
Premium Ice Cream, Lemon Meringue
Premium Ice Cream, Strawberries & Cream
Premium Ice Cream, Triple Chocolate Fudge
Premium Ice Cream, Triple Toffee Fudge
Rice Pudding, No Added Sugar, Low Fat
Torte, Orange & Lemon
Torte, Peach & Apricot
Torte, Raspberry

JAMS & MARMALADES

Apricot Jam, Reduced Sugar
Blackcurrant Jam, Reduced Sugar
Fruits of the Forest Jam, Reduced Sugar
Marmalade, Reduced Sugar
Morello Cherry Jam, Reduced Sugar
Raspberry Jam, Reduced Sugar
Strawberry Jam, Reduced Sugar

BREAD & ROLLS

Brown Bread
Danish Brown Bread
Danish Malted Softgrain Bread
Danish White Bread
Oat Danish Bread
Soft Brown Rolls
Soft White Rolls
White Bread

CANNED SOUPS

Chicken & Ham with Rice Soup
Chicken Noodle Soup
Chicken Soup
Country Vegetable & Beef Soup
Country Vegetable Soup
Lentil & Carrot Soup
Mushroom Soup
Vegetable Soup
Wholesome Soup, Lentil & Chicken
Wholesome Soup, Winter Vegetable

INSTANT SOUPS

Asparagus & Leek Soup, 40kcal
Chicken & Sweetcorn Soup, 60kcal
Chicken & Vegetable Soup with Noodles, 60kcal
Chicken & Vegetable Soup, 40kcals
Chicken Soup, 40kcal
Minestrone Soup, 60kcal
Mushroom Soup, 40kcal
Mushroom Soup, 60kcal
Tomato Soup, 40kcal
Tomato Soup, 60kcal
Vegetable Soup with Croutons, 60kcal
Vegetable Soup, 40kcal

BISCUITS & SNACKS

Cookies, Dark Treacle
Cookies, Real Chocolate Chip
Cookies, Stem Ginger

The following Heinz products are free from Cinnamon

CORE PRODUCTS

BAKED BEANS

Barbecue Beans
Curried Beans with Sultanas

LUNCH BOWLS

Beef Curry with Rice
Beef Goulash with Noodles
Chicken Curry with Rice
Chilli Con Carne with Rice
Lamb and Vegetable Casserole

PASTA

Chef's Specials, Chicken Ravioli in Tomato Sauce
Chef's Specials, Macaroni Cheese
Chef's Specials, Pasta Pipes with Tuna & Bacon
Chef's Specials, Ravioli in Spicy Sauce
Chef's Specials, Sausage Hotpot with Pasta
Chef's Specials, Spicy Beef Pasta Twists
Dinosaurs with Mini Meat Boulders
Dinosaurs, Pasta Shapes in Tomato Sauce
Magic Roundabout, Spaghetti Shapes in Tomato Sauce
Noodle Doodles, Spaghetti Shapes in Tomato Sauce
Spaghetti Hoops in Tomato Sauce
Spaghetti in Tomato Sauce
Spaghetti with Sausages
Super Mario, Spaghetti Shapes in Tomato Sauce
Thomas the Tank Engine, Spaghetti in Tomato Sauce

SAUCES, SALADS & PICKLES

Apple Sauce
Coleslaw Salad
Coronation Sauce
Mayonnaise
Mixed Bean Salad
Pasta Salad
Ploughman's Piccalilli
Ploughman's Pickle
Ploughman's Tangy Sandwich Pickle

CINNAMON FREE PRODUCTS

HEINZ CORE PRODUCTS Cont'd.

SAUCES, SALADS & PICKLES Cont'd.

Ploughman's Tomato Pickle
Potato Salad
Salad Cream
Salad Cream, Spoonable
Salsa, Medium-Hot
Salsa, Milk-Medium
Sandwich Spread, Cucumber
Sandwich Spread, Spicy
Sandwich Spread, Sweetcorn & Red Pepper
Silverskin Onions
Vegetable Salad

SPONGE PUDDINGS

Sponge Pudding, Banana with Toffee Sauce
Sponge Pudding, Chocolate with Chocolate Sauce
Sponge Pudding, Lemon
Sponge Pudding, Mixed Fruit
Sponge Pudding, Strawberry Jam
Sponge Pudding, Treacle

READY TO SERVE SOUPS

Beef Soup
Big Soup, Beef & Bacon Hotpot
Big Soup, Beef & Vegetable
Big Soup, Beef Broth
Big Soup, Chicken & Ham
Big Soup, Chicken & Vegetable
Big Soup, Chicken, Leek & Potato
Big Soup, Giant Minestrone
Big Soup, Spicy Tomato with Beef Pasta Parcels
Big Soup, Thick Country Vegetable with Ham
Chicken & Mushroom Soup
Cream of Asparagus Soup
Cream of Celery Soup
Cream of Chicken Soup
Cream of Mushroom Soup
Farmhouse Beef & Vegetable Soup
Farmhouse Beef Broth

CINNAMON FREE PRODUCTS Cont'd.

READY TO SERVE SOUPS Cont'd.

Farmhouse Chicken & Vegetable Soup
Farmhouse Potato & Leek Soup
Farmhouse Scotch Broth
Mulligatawny Soup
Oxtail Soup
Pasta Soup, Beef & Tomato Bolognese
Pasta Soup, Chicken Pastini
Pasta Soup, Minestrone
Pasta Soup, Mushroom Carbonara
Pasta Soup, Tomato Napoli
Premium Soup, Beef, Potato & Red Pepper
Premium Soup, Carrot, Potato & Coriander
Premium Soup, Chicken Sweet Pepper & Dill
Premium Soup, Chicken, Sweetcorn & Asparagus
Premium Soup, Country Bean with Mushroom
Premium Soup, Seven Vegetable
Scottish Vegetable Soup with Lentils
Spicy Soup, Chilli Bean & Beef
Spicy Soup, Curried Chicken with Rice
Spring Vegetable Soup
Vegetable Soup
Wholesoup, Farmhouse Vegetable
Wholesoup, Ham & Butter Bean
Wholesoup, Lentil
Wholesoup, Pea & Ham
Wholesoup, Winter Vegetable

TOPPERS

Toast Toppers, Chicken & Mushroom
Toast Toppers, Ham & Cheese
Toast Toppers, Mushroom & Bacon

CANNED TUNA

Tuna Chunks, Canned in Brine
Tuna Chunks, Canned in Vegetable Oil
Tuna Steak, Canned in Brine
Tuna Steak, Canned in Vegetable Oil

HP FOODS LTD. NUTRITION INFORMATION **21.3.94.**
CINNAMON CONTENT

	PRESENT	ABSENT
HP SAUCE		√
HP FRUITY SAUCE		√
HP TOMATO KETCHUP	√	
HP SPICY TOMATO SAUCE		√
HP CHILLI SAUCE	√	
HP CURRY SAUCE		√
HP FRUITY BARBECUE SAUCE	√	
HP SWEET & SOUR SAUCE		√
HP ORIGINAL BARBECUE SAUCE	√	
HP MEXICAN SPICY BARBECUE SAUCE		√
HP RICH JAMAICAN BARBECUE SAUCE		√
HP MINT SAUCE		√
HP MAYONNAISE (BULK)		√
HP MALT VINEGAR		√
HP BEANS IN TOMATO SAUCE	√	
HP HEALTHY BEANS	√	
HP BAKED BEANS IN TOMATO SAUCE (CATERING)	√	
HP HEALTHY BEANS IN TOMATO SAUCE (CATERING)	√	
HP BEANS AND BEEFBURGERS	√	
HP BEANS AND SAUSAGE	√	
HP SAUCY BEANS	√	
HP BIG BREAKFAST BIG BEANS IN TOMATO SAUCE		√
HP BIG BREAKFAST BIG BEANS & JUMBO SAUSAGES		√
HP BATMAN BOLOGNESE		√
HP PASTA SHAPES IN TOMATO SAUCE		√
HP SPAGHETTI IN TOMATO SAUCE		√
HP GLADIATORS IN TOMATO SAUCE (REDUCED SUGAR)		√
HP GLADIATORS IN SMOKEY BACON SAUCE		√
HP GLADIATORS IN PIZZA SAUCE WITH PEPPERONI		√
HP SONIC AND SAUSAGES IN TOMATO SAUCE		√

HP SONIC RAVIOLI SHAPES IN TOMATO SAUCE		√
HP CHICKEN KORMA BISTRO BREAK	√	
HP SWEET & SOUR BISTRO BREAK		√
HP CANNELLONI BISTRO BREAK		√
HP LASAGNE BOLOGNESE BISTRO BREAK		√
DADDIES TOMATO KETCHUP		√
DADDIES BROWN SAUCE		√
DADDIES BURGER RELISH		√
DADDIES SALAD CREAM		√
FLETCHERS TIGER SAUCE		√
FLETCHERS TITBITS SAUCE		√
FLETCHERS BROWN SAUCE		√
FLETCHERS TOMATO KETCHUP	√	
FLETCHERS BAKED BEANS IN TOMATO SAUCE	√	
FLETCHERS SHORT CUP SPAGHETTI IN TOMATO SAUCE		√
L & P WORCESTERSHIRE SAUCE		√
L & P CHILLI & GARLIC SAUCE		√
L & P GINGER & ORANGE SAUCE		√
L & P GARLIC & SPRING ONION SAUCE		√
L & P HOT PEPPER SAUCE		√
L & P MUSTARD & PEPPERCORN SAUCE	√	
L & P GARLIC SAUCE		√
L & P FRUIT SAUCE		√
L & P WORCESTER KETCHUP		√
L & P TOMATO KETCHUP WITH MILK CURRY SPICES	√	
L & P SOY & GARLIC		√
L & P HOT PEPPER & LIME		√
L & P SOY & FIVE SPICE	√	
L & P CURRY CONCENTRATE		√

L & P ITALIAN VINAIGRETTE MAKER	√
L & P WHITE WINE & GARLIC VINAIGRETTE MAKER	√
L & P CLASSIC FRENCH VINAIGRETTE MAKER	√
L & P PICKLE WITH WORCESTER SAUCE	√
L & P SWEET PEPPERS WITH CHILLI SAUCE	√
L &B P GINGER SAUCE	√
L & P GREEN JALAPENO SAUCE	√

Home Made Tomato Soup

4 Pints Vegetable Stock
1/2 lb Onions
tsp. Tomato Puree
One small tin evaporated milk

1 1/2 oz Plain Cornflour } make to a fluid with a little water
2 oz granulated sugar }

Salt & Pepper

Mince onions finely.

Add to stock with tomato puree. Bring to boil, then simmer.
Add cornflour and sugar. Simmer for about 1/2 hour.
Strain, re-heat, add evaporated milk and salt & pepper.

Glasgow Royal Infirmary University NHS Trust

Department of Nutrition & Dietetics

Benzoate, Sorbic Acid and Cinnamon Free Diet

You have been found to be allergic to the above preservatives - Benzoate, Sorbic Acid and the spice - Cinnamon.

Benzoate - As most of the benzoates in the diet are added to foods as a preservative it is very important that you read the labels of any manufactured or prepared foods you consume. If benzoates are added to the food the manufacturer may name the particular benzoate or use a serial number known as an E number. Avoid those containing E210 - E219 inclusive.

Sorbic Acid is also added to foods as a preservative. It has 4 E numbers, E200, E201, E202 and E203. If a food contains this preservative it will be stated on the labels. The E numbers and the benzoate names are listed below:

E200	-	Sorbic Acid
E201	-	Sodium Sorbate
E202	-	Potassium Sorbate
E203	-	Calcium Sorbate
E210	-	Benzoate Acid
E211	-	Sodium Benzoate
E212	-	Potassium Benzoate
E213	-	Calcium Benzoate
E214	-	Ethyl 4-hydroxybenzoate
E215	-	Ethyl 4-hydroxybenzoate sodium salt
E216	-	Propyl 4-hydroxybenzoate
E217	-	Propyl 4-hydroxybenzoate sodium salt
E218	-	Methyl 4-hydroxybenzoate
E219	-	Methyl 4-hydroxybenzoate sodium salt

Avoid any food or drink which justs lists the word **preservative** or **permitted preservative** as an ingredient.

Cinnamon - This is a natural substance which does not require to be stated on food labels. This diet sheet and accompanying manufactured product list will give you information on foods to be avoided. Also, always look for the term "spices" on food labels which are not included in the manufacturer's product list and avoid these.

Avoid any item containing these.

Benzoate, Sorbate and Cinnamon Avoidance

Foods Allowed	Foods to Check Labels for E200 - E203 & E210 - E219	Foods to be Avoided
Meat - Cook from fresh whenever possible. Fresh & frozen meats.		Canned meat in a spicy sauce. Cold meats containing spices. Commercially made meat dishes containing spices, e.g. lasagne, pizza. Pizza, Curries, Chinese Meals. Avoid 'spice' on beef ham. Some sausages, pies, bridies, etc. MAY contain spices - check labels.
Fish - Cook from fresh whenever possible. All types of fresh, rozen, tinned fish (not in sauce), shell fish allowed.		Commercially made fish dishes containing spices. Canned fish in spicy sauce. Prawn cocktail. Prawn Marie Rose.
Spices - Nutmeg, ginger, cumin, coriander, chilli powder, tumeric and all other spices apart from those on AVOID LIST		Spices - Cinnamon, mixed spice, allspice, curry powder. Check labels on any other spice mixes.
Herbs - All herbs including garlic. Salt, pepper, black pepper.		Gelatin capsules.
Soup - Most soups - homemade, tinned packet - apart from those on AVOID LIST	Soup concentrates.	Tinned or packet soup containing tomato, spices, tomato puree.
Sugar - Tablet artificial sweetener	Liquid artificial sweetener.	

Benzoate, Sorbate and Cinnamon Avoidance

Foods Allowed	Foods to Check Labels for E200 - E203 & E210 - E219	Foods to be Avoided
Crisps - check products list for those free from cinnamon. In general keep to salt & vinegar, ready salted, cheese & onion.		Crisps and snacks which contain spices, e.g. prawn cocktail, tomato, barbeque beef flavours.
Nuts - most types.		Dry roasted nuts. Bombay mix. Spicy nut mixes.
Vegetables - all types including potatoes, chips, salad vegetables, fresh and tinned tomatoes.	Tomato Puree. Check labels on any vegetables in a sauce.	Commercially made foods containing tomato puree if not marked " preservative free", e.g. pizza, lasagna, pasta dishes.
Bread - Use brown breads, wholemeal bread, brown or wheaten rolls. White bread made with **unbleached** white flour (check label on loaf).		White rolls. French bread. White bread made with **bleached** white flour. Check labels on loaves for type of flour used.
Fruit : Fresh fruit - all types	Any preserved fruit in any form, e.g. Fruit based dessert sauces. Fruit toppings for ice cream. Gateaux containing fruit. Apple pies - fruit pies in general. Fruit pie fillings. Toppings for cheese cakes. Crystalised or glace fruit. Dried fruit. Tins of fruit or fruit puree. Packet cake topping. Prepacked cake topping. Prepacked cake mixes.	Preserved fruit in any form if it is not known to be free from forbidden preservatives. Dried apricots. Candied peel.

Benzoate, Sorbate & Cinnamon Avoidance

Foods Allowed	Foods to Check Labels for E200 - E203 & E210 - E219	Foods to be Avoided
Milk, cream, butter, cheese, margarine.	Yoghurt Flavoured milk drinks, milk shake syrup. Artificial cream. Low fat spreads, cheese slices, cheese spreads, margarines (see product info. Sheet for Sorbic Acid).	Ice cream with fruit or fruit syrup in it e.g. Raspberry ripple, Tutti frutti. "Raspberry" sauce for ice cream.
Cooking oils and lard. Salad oils.		
Eggs		
Pasta - all types	Made up pasta dishes.	Commercially made pasta dishes with spices onthe label. All pasta tinned in tomato sauce.
Rice - all types		Commercially made rice dishes with spices on the label.
Breakfast cereals - all types including porridge, bran.		
Peas, beans, lentils.		Baked beans in tomato sauce, spaghetti in tomato sauce, ravioli in tomato sauce. Any similar products tinned in tomato sauce.
Custard powder, custard, rice, semolina, tapioca, sago, cremola, cornflour, arrowroot. Tinned milk puddings.		

Benzoate, Sorbate & Cinnamon Avoidance

Foods Allowed	Foods to Check Labels for E200 - E203 & E210 - E219	Foods to be Avoided
Alcohol : White wine, whisky, vodka, white rum, sherry, brandy, cider, lager, beer, Tennant's "LA" in cans.	Be careful that any mixers are preservative free. Check labels on all Low Alcohol wines.	Red wine, gin, dark rum, Tia Maria, Martini**, Cinzano, Dubonnet and similar products. Tennant's "LA" on draught.
Pickles, sauces, chutneys **NOT** on Manufactured Products list and **NOT** containing forbidden preservatives.	All pickles, sauces, chutneys, pickled foods (e.g. herring, onions, beetroot), vinegar, mustard, tomato sauce, brown sauce. Mayonnaise, salad cream, salad dressings. **Remember** to check Manufactured Products list to see if they contain cinnamon.	Tomato sauce. **ALL** pickles, etc. On Manufactured Products list, i.e. containing cinnamon, or if containing forbidden preservatives.
Hot drinks : Tea, coffee, hot chocolate, cocoa, Ovaltine, Horlicks, Bournvita, Oxo, Marmite, Bovril.	"Camp" liquid coffee. Chocolate drinks.	
Jams and marmalade : Home made jam and marmalade, honey, syrup, treacle, peanut butter.	Lemon curd. Diabetic jam.	Preserves containing E210 - E219.
Home baking : Use **unbleached** flour for baking. Check labels on packet for this.	Dried fruit, mixed peel, glace cherries. Pie fillings.	Cinnamon, allspice, mixed spice, mincemeat.

** **NB** Many liqueurs may contain spices - be aware of this if any reaction occurs.

Benzoate, Sorbate & Cinnamon Avoidance

Foods Allowed	Foods to Check Labels for E200 - E203 & E210 - E219	Foods to be Avoided
Cakes and biscuits : Keep to home baked goods as much as possible.		Mincemeat pies and tarts. Bought fruit pies, Danish pastries, coffee buns, gingerbread. Rich fruit cake, Christmas cake / pudding, black bun, & biscuits containing large quantities of dried fruit.
Fresh fruit juice.	Check labels on all bottled fruit juices.	
Soft drinks : 7 Up (not diet), Appletise, Kiri, Orangina (cans only), Perrier water and other natural spring water. Home made fruit squash.	Check labels on **ALL** squashes, fizzy drinks, soft drinks, bottled shandy, non-alcoholic grape juice drinks, e.g. Schloer. Ice lollies, ice poles, "Tip Tops", Slush Puppies, Soda waters.	Any soft drinks containing E200 - E203 and E210 - E219. Also avoid Coca Cola, Vimto, Dr. Peppers, Pepsi Cola.
Fudge, toffee, tablet, chocolate, liquorice, boiled sweets (no cola sweets).		Cinnamon sticks, cola cubes, cola sweets, cola chews etc. Irn Bru chews.
Mouthwash & Toothpaste - Colgate regular, Crest regular, Kingfisher. Mac Leans - Freshmint, Milk Teeth, Sensitive, Mouthguard.		All other mouthwashes & toothpastes. **NO** tartar controlled toothpastes. Colgate Plax, Colgate Actibrush, Colgate Blue Minty Gel, Aquafresh - Fresh & Minty, and Mild & Minty. MacLeans Mild Mint.

GLASGOW ROYAL INFIRMARY UNIVERSITY NHS TRUST
DEPARTMENT OF NUTRITION & DIETETICS
LOW SALICYLATE DIET

Your condition is aggravated by **SALICYLATE** which is a substance found in aspirin, and also in some foods which we eat. It is recommended that you omit the foods listed in the **FOODS TO AVOID** column from your diet.

FOODS ALLOWED	FOODS TO AVOID
All meats,fish,eggs,milk,butter,cheese,cream, yoghurt,margarine.	-
Soups - most types.	Tomato soup.
Cereals - all types.	
Breakfast cereals not containing dried fruit.	Breakfast cereals containing dried fruit, e.g. muesli.
Pasta - all types.	Pasta dishes containing forbidden spices (see over)
Rice - all types.	
All nuts apart from those on 'AVOID' list.	Almonds,water chestnuts,peanuts,Brazil nuts.walnuts.pistachio nuts.
	Dried fruit - sultanas,raisins,currants,prunes, dates.apricots.dried figs.
Plain cakes.biscuits.scones and other baking not containing dried fruit or forbidden spices.	Foods containing the nuts and dried fruit Mentioned above i.e. Christmas pudding, fruit cake, Garibaldi biscuits, Fruit & Nut chocolate.fruit scones,muesli,fruit loaves, fruit jams and marmalade.
Chocolate caramels.toffee,boiled sweets not containing dried fruit or nuts.	Liquorice,peppermints,cinnamon balls, Chocolate caramels and toffees containing dried fruit or nuts.
Lemons,mangoes.bananas,lychees,pears, plums,pomegranate.rhubarb,fresh figs, passion fruit. Varieties of apple not on 'AVOID' list, i.e.Golden Delicious, Cox's Pippin. No more than half a pint of fresh orange, apple juice per day. Tomato Juice.	Apricots.raspberries,strawberries, loganberries.melon,blackcurrants, boysenberries,guava,pineapple,oranges, cherries.Canteloupe melons,cranberries, gooseberries,grapefruit,redcurrants,grapes, mandarin oranges.nectarines,peaches, watermelon.Granny Smith's apples,Jonathon apples. grapefruit.
Jam,jelly,marmalade etc.containing 'ALLOWED' fruits.	Foods which contain the above fruits e.g.jam Jelly.marmalade.fruit pie fillings, fruit toppings.

FOODS ALLOWED	FOODS TO AVOID
Lemon curd,syrup,maple syrup,sugar.	Honey.
Fizzy drinks e.g.Coca Cola,lemonade. Orange, lemon or grapefruit drinks.	Apple drinks, blackcurrant drinks, grape Juice.
Coffee (no chicory),cocoa,herbal teas, Ovaltine,drinking chocolate,Oxo,Bovril, Marmite etc. Carob drinks.	Coffee with chicory. Tea -only 1-2 cups per day (depending on reaction)
Spirits,lager,white rum. Dark rum) Claret) only allowed occasionally Vermouth) i.e. once a week; Cointreau) amounts i.e. one measure Tia Maria) (quarter gill) Sherry)	Beer,wine,champagne,port,cider. Drambuie. Benedictine liqueurs.
All vegetables except those on 'AVOID' list	Avocado,aubergine,broad beans,broccoli carrot,cucumber,canned tomato,tomato paste, tomato puree,olives,okra,parsnips, spinach,mushrooms,endive,sweetcorn, gherkins,yams (sweet potato).
Salt, pepper.	
Herbs and spices except those mentioned on the 'AVOID' list.	Aniseed,basil powder,caraway,cardamom, Celery powder,cumin powder,curry powder, cinammon,dill powder,garam masala,thyme, mixed herbs,oregano,paprika,rosemary, turmeric. Avoid any foods which may contain any of these spices - including curry and curried foods.
Mayonnaise.	Vinegar,bottled sauces containing vinegar and spices,tomato sauce,brown sauce, Worcestershire sauce,pickles,chutneys, salad cream,French dressing,mustard.
	Aspirin and medicines containing aspirin. Check with your GP or local pharmacist.

Remember! Any queries regarding your diet, phone the Dietitian who explained it to you.

Name: Telephone No.:
Hospital: Extension No.:

• Glasgow Royal Infirmary •
• Glasgow Royal Maternity Hospital •
• Canniesburn Hospital •
• Lightburn Hospital • Belvidere Hospital •

Royal Infirmary
84 Castle Street
Glasgow G4 0SF

Switchboard 0141 211 4000
Direct Dial 0141 211 4318
Fax Number

Department of Nutrition & Dietetics

1997

REF : KM / EB

Dear

I have received correspondence from Dr. Forsyth regards your allergy.

She has found you to be sensitive to a substance called Butylated Hydroxytoluene (B.H.T.). This has an "E" number - E321 and may be found in the following types of food :-

Packet convenience foods	Margarine
Packet breakfast cereals	Sachet marinade
Vegetable oil	Dehydrated mashed potato
Shortening	Crisps
Packet cake mix	Salted peanuts
Gravy granules	Potato rings
Chewing gum	
Inner packaging of breakfast cereals	

Please check the ingredient labels on the above types of foods for E321 and if found avoid same. When eating out where you are unsure whether a food may or may not contain this please err on the side of caution and avoid whenever possible. Please contact me if you have any queries regarding this.

Yours sincerely,

K.A. Milligan
Senior Dietitian (Mon. - Wed.)

Glasgow Royal Infirmary University NHS Trust

Department of Nutrition & Dietetics

E127

You have been found to be sensitive to a red dye called Erytriosine (E127) which is found in the following foods in particular :-

Glace cherries

Cocktail cherries

Tinned red cherries, strawberries and rhubarb

Scotch eggs

Packet trifle mix

Quick custard mix

Biscuits

Prepacked Swiss Roll

Stuffed olives

Chocolates

Dressed crab and salmon spread and pate

Garlic sausage

Luncheon meat

Danish salami

GLASGOW ROYAL INFIRMARY UNIVERSITY NHS TRUST
DEPARTMENT OF NUTRITION & DIETETICS
SORBIC ACID FREE DIET
E.200 - SORBIC ACID E.201 - SODIUM SORBATE E.202 - POTASSIUM SORBATE
E.203 - CALCIUM SORBATE

You have been found to be allergic to a PRESERVATIVE called SORBIC ACID. Sorbic Acid has 4 E numbers - E200, E201, E202 E203. If a food contains this preservative it will be stated on the label; so it is important to read the labels of any manufactured or prepared foods you consume.
Avoid any food which just lists the 'preservative' or 'permitted preservative' as an ingredient.

FOODS ALLOWED	FOODS TO CHECK LABELS OF
Milk, butter	Low fat spreads e.g. Gold, Outline
	Cheese spreads
	Cheese slices
	Margarine
	Hard cheeses
Eggs	
Meat - all fresh or frozen	Made-up meat dishes
Poultry - meat and poultry	
Fish - all fresh or frozen fish	Made-up fish dishes
Fruit - all fresh or tinned fruit	Fruit topping and fillings
Dried Fruit - all types except apricots, peel	Dried apricots. Candied peel
Vegetables - all fresh and frozen vegetables	Tinned vegetables in sauce
Cereals } Nuts } Pasta } All types Rice } Pulses - peas, beans and lentils}	Soya sauce Sauces, pickles, pickled products, chutneys
Preservative free jams, marmalade, pickles, chutneys and sauces	Preserves; jams, marmalade etc.
	Gelatine capsules
Alcoholic drinks; apart from cider	Cider
Soft drinks which are preservative free	Check labels on fizzy and soft drinks as this is the main area in which these preservatives will be found + non and low alcoholic wines
All fresh fruit juices	
Cakes, scones, biscuits	Packet cake topping; prepacked cake mixes. Dessert sauces
Soup; Fresh, tinned or packet soup	Soup concentrates

APPENDIX III

DIETARY AVOIDANCE INFORMATION SHEETS FOR CUSTOMERS (COMMERCIAL)

SAFEWAY

Safeway Stores plc
6 Millington Road
Hayes
Middlesex UB3 4AY

Telephone 0181 848 8744
Facsimile 0181 573 1865

SAFEWAY PRODUCTS FREE FROM THE ADDITIVES DETAILED ON THE ATTACHED LIST

As far as we are aware, the following products do not contain any of the additives identified on the attached list. Our manufacturers have been asked to declare all additives used in the preparation of Safeway products and also to identify any additive which is contained in the raw ingredients.

We will update this list as frequently as possible to allow you the full benefit of enjoying those Safeway products which are being developed to meet your important requirements.

SAFEWAY NUTRITION ADVICE SERVICE

(LIST001)

SAFEWAY PRODUCTS FREE FROM THE ADDITIVES

DETAILED ON THE ATTACHED LIST

BEVERAGES

Drinking Chocolate
Cocoa
Malted Food Drink
All Tea/Tea Bags
All Coffee

FRUIT JUICE

All Fruit Juice - Chilled and Long Life

BISCUITS

Wholemeal Thins
Poppy & Sesame Thins
Savoury Wheat Crackers
Rough Oat Cakes
Cream Crackers
Sesame Crackers
Water Biscuits
Cheese Savourys
Cheese & Chive Sandwich
Low Fat Cream Cracker
Clown Shortbread
Chocolate Chip & Nut Digestive
Duo Choc Chip Cookie
Half Coated Choc Chip Rings
Chocolate Chip Cookie
Chocolate Crunch Creams
Cashew Cookie
Malted Milk
Milk Chocolate Malted Milk
Milk Chocolate Nice
Nice
Highland Shorties
All Butter Biscuits
Almond Biscuits
Chocolate Sundaes
All Butter Fruit Biscuits
Butter Crinkle Crisp Biscuits
Chocolate Scrunchies
Peanut Scrunchies
Jaffa Cakes
Blackcurrant Jaffa Cakes
Shortcake
Milk Chocolate Digestives
Plain Chocolate Digestives
Party Rings
Lemon Puffs
Lemon Crisp
Coconut Rings
Syrup Crunch
Oaten Crunch
Ginger Nuts
Country Crunch
Milk Chocolate Country Crunch
Plain Chocolate Country Crunch
Duo Chocolate Chip & Nut Cookie
Chocolate Chip and Nut Cookie
Coconut Cookie
Milk Chocolate Wafers
Plain Chocolate Wafers
Milk Chocolate Orange Wafers
Vanilla Wafers
Take Two Bar
Tea Cakes
Mini Snowballs
Plain Chocolate Ginger Crunch
Plain Chocolate Orange Crunch
Milk Chocolate Coconut Crunch
Chocolate Bars x 6
Mint Chocolate Biscuit Bar
Vanilla Chocolate Biscuit Bar
Chocolate Bars x 7 (Bytes)
Chocolate Shortcake
Chocolate Cream Sandwich
Chocolate Orange Sandwich
Chocolate Mint Sandwich
Chocolate Caramel
Bourbon Fingers
Bourbon Creams
Lemon Finger Creams
Orange Finger Creams
Coconut Crumble Creams
Orange Custard Crumble Creams
Orange Crumble Creams
Custard Creams
Oaty Creams
Ginger Crunch Creams
Malted Milk Creams
Jam Sandwich Creams
Golden Crunch Creams
Mocha Finger Creams
Chocolate Crunch Creams

Stem Ginger Cookies
Chocolate Chunk and Pecan Cookies
Half Coated Hazelnut Cookies
Half Coated Brazil Cookies
Half Coated Chocolate Shortbread Triangles
Milk Chocolate Orange Covered Sandwich (x 14)
Chocolate Coated Malted Milk Creams (x 6)

CAKES

Country Cake
Ginger Cake
Raisin & Orange Cake
Cherry Bakewells
Trifle Sponges
Custard & Apple Mini Pies
Viennese Whirls
Coconut Square
Lemon Square
Luxury Swiss Rolls:
- Red Cherry Conserve
- Raspberry Conserve
- Strawberry Conserve
- Chocolate

Sponge Sandwiches:
- Raspberry Jam
- Apricot Jam & Butter Cream
- Raspberry Jam & Butter Cream

Frangipanes
Almond Fingers
Mince Pies
Meringue Nests
All Butter Wheatmeal Fruit Cake
All Butter Coconut Cake
All Butter Madeira Cake
Dundee Cake
Cherry Genoa Cake
Cherry Madeira Cake
Cherry Genoa Bar
Sticky Toffee Pudding
Oriental Ginger Pudding
Mini Sponge Puddings x 3
Milk Chocolate Yule Log
All Butter Luxury Dundee Cake with Whiskey
Luxury Rich Xmas Cake with Brandy

CRISPS/SNACKS/NUTS

Potato Sticks - Ready Salted
Crunchy Sticks - Ready Salted
Crinkle Cut Crisps - Ready Salted
Cheese Curls
Potato Rings - Ready Salted
Pizza Slices
Salt & Pepper Pipes
Tortilla Chips
Garlic Mini Breads
Salted Cashew Nuts
Salted Pistachio Nuts
Honey Roast Peanuts
Monkey Nuts
Natural Roast Peanuts
Mixed Nuts & Fruit
Peanuts & Raisins
Salted Roasted Mixed Nuts
Salted Roasted Peanuts
Peanuts, Raisins & Chocolate Chips
Trail Mix

CHILLED DESSERTS

'Pudding Club Puddings'
Sticky Toffee
Spotted Dick Syrup
Fresh Cream Strawberry Trifle
Fresh Cream Raspberry Trifle
Chocolate Mousse
Savers Chocolate Mousse
Lemon Mousse
White Chocolate Mousse
Chocolate Surprise Dessert
Apricot Fool
Raspberry Fool
Strawberry Fool
Gooseberry Fool
Blackcurrant Cheesecake
Strawberry Cheesecake

YOGHURTS

Yoghurts

All Flavours

French Set Yoghurts

All Flavours

Very Low Fat Yoghurts

All Flavours

Rich and Creamy Yoghurts

All Flavours

Very Low Fat Yoghurts

All Flavours

Greek Yoghurt

Natural
Honey
Apricot

Bio Yoghurt

Peach & Passion Fruit
Strawberry
Plum
Black Cherry
Natural

Fromage Frais

Fromage Frais
Fromage Frais with Fruit
Very Low Fat Fromage Frais with Fruit

ICE CREAM

Vanilla & Mint Choc Ice
White Chocolate Choc Ice
Traditional Vanilla Ice Cream
Country Strawberry Ice Cream
Toffee
Clotted Cream
Chocolate
Vanilla, Lemon & Lime Swirl
Soft Scoop: Honey & Almond
Chocolate
Strawberry
Fruits of the Forest
Peach & Raspberry
Toffee Fudge Ripple
Vanilla
Chocolate and Caramel

Sorbet: Mango
Peach Melba
Lemon
Biscuit & Caramel Ice Cream Bar

FROZEN DESSERTS

Mousse - Chocolate and Mint Swirl
- Raspberry
- Raspberry Ripple
- Strawberry

Bramley Apple Pie
Blackberry & Apple Pie
Deep Apple Pie
Banoffee Pie
Apple Pie
Bakewell Tart
Rhubarb Crumble
Apple Crumble
Bombe au Chocolate
Luxury Raspberry Pavlova
Gateau St. Honore Bar
Black Forest Gateau
Strawberry Gateau
Strawberry Gateau - Party Size
Chocolate Meringue Gateau
Mousse au Chocolat Gateau
Chocolate Brownie Dessert
Bread & Butter Pudding
Profiteroles
Profiteroles with Grand Marnier
Cheesecakes: Toffee & Pecan
Apricot & Orange
Strawberry
Blackcurrant
Raspberry
Fruits of the Forest
Chocolate
Blueberry

Chocolate Roulade
Summer Fruit Pavlova
Raspberry Pavlova
Strawberry Pavlova
Chocolate Pavlova
Dairy Cream Sponge
Chocolate Dairy Cream Sponge
Strawberry Cream Cake
Black Forest Cream Cake
Frozen Yoghurt: Natural
Strawberry
Blackcherry

OTHER DESSERTS

Creamed Rice Pudding
Traditional Creamed Rice

BREAKFAST CEREALS

Frosted Flakes
Coco Crunchies
Square Malt Bites
Wheat Honeys
Cornflakes
Bran Flakes
Rice Crunchies
Fibre Bran
Crunchy Cereal
Swiss Style Cereal
Wholewheat Biscuits
Hot Oat Cereal
Quick Cooking Oats
Wholewheat Flakes

BREAD

All Packaged Bread Range
Garlic Bread

BUNS AND MORNING GOODS

Potato Cakes	
Cheese Potato Cakes	
French Croissants	(4s)
Raisin & Lemon Pancakes	(6s)
Scotch Pancakes	x 8
Fruit & Spice Pancakes	(6s)
Crumpets	(8s)
Muffins	(6s)
Cheese Muffins	(6s)
Wholemeal Muffins	
Derby Scones	x 6
Sultana Scones	x 4
Fruit Scones (Scottish)	x 4
Plain Scones (Devon)	x 4
Currant Buns	
Fruit Teacakes	x 4
Spiced Fruit Buns	
Hot Cross Buns (White, Wholemeal, Extra Spicy)	

PRESERVES & SPREADS

All Honey
All Conserves: Strawberry
Raspberry
Blackcurrant

Ginger Preserve
Fresh Fruit Orange Marmalade
Peanut Butter - Smooth
Peanut Butter - Crunchy
Chocolate Hazelnut Spread
Chocolate Coconut Spread
Sandwich Spread

SOUPS

Cup Soup: Minestrone
Chicken & Leek
Chicken
Asparagus

Cup Soup with Croutons:

Creamed Tomato
Chicken & Leek

Slim Soups: Tomato Slim Soup
Vegetable Slim Soup
Tomato & Beef Slim Soup
Chicken Slim Soup
Minestrone Slim Soup

Dried: Golden Vegetable
Cream of Chicken

Canned: Cream of Celery
Chicken & Sweetcorn
Cream of Smoked Trout
Carrot & Orange
Cream of Tomato
Country Vegetable Soup
Cream of Mushroom

Chilled Soups: Brocolli and Cheddar
Italian Tomato
Mushroom
Cream of Pepper & Ginger
Sweetcorn Chowder
Butter Bean and Blue Chees
Carrot, Parsnip & Apple
Country Vegetable

SAUCES

Pesto Sauce
Pasta Sauce
Pasta Sauce with Peppers
Pasta Sauce with Mushrooms
Cranberry Sauce
Tartar Sauce
Brown Sauce
Fruity Sauce
Creamed Horseradish
French Mustard
English Mustard
Wholegrain Mustard with Honey
Wholegrain Mustard with Whiskey
Traditional English Beer Mustard
Chilli Sauce
Tomato Ketchup
Tomato Puree

Canned Cooking Sauces:

Tomato & Onion
Red Wine Sauce
Sweet & Sour Sauce
Chilli Sauce
White Wine Sauce
Curry Sauce

PICKLES & RELISHES

Red Wine Vinegar
White Wine Vinegar
Cider Vinegar
Distilled Vinegar
Curried Fruit Chutney
Peach Chutney
Mango chutney
Tomato Chutney
Almond Stuffed Olives
Stuffed Green Olives
Green Olives
Black Olives

SALAD DRESSINGS

Seafood Dressing
Reduced Calorie Vinegar & Oil Dressing
Thousand Island Dressing
Salad Cream

All Mayonnaises: Real
Lemon
Mustard
Garlic
Low Calorie

French Dressing
Italian Dressing
Garlic Dressing
Mustard Dressing

DRIED PRODUCTS & BAKING AIDS

Sage & Onion Stuffing Mix
Mushroom & Celery Stuffing Mix
Cornflour
Custard Powder
All Herbs & Spices
All Varieties of Pasta & Egg Pasta
All Dried Pulses (Beans, Lentils, Peas)
Seed Pearl Tapioca
Pearl Barley
Brown and White Rice (including Flaked & Ground)
Seeded Raisins
Currants
Sultanas
Desiccated Coconut
All Nuts
All Flour (Plain, Self-Raising and Strong)
Salt
Sugar - All Varieties
Puff Pastry
Shortcrust Pastry (Frozen)
Mincemeat
Almond/Peppermint/Vanilla Essence

CANNED FRUIT

Peach Slices & Halves in Syrup/Juice
Pear Quarters & Halves in Syrup/Juice
Peaches & Pears in Syrup
Pineapple Rings, Slices, Pieces & Crushed in Syrup/Juice
Mandarins Syrup/Juice
Prunes in Syrup/Natural Juice
Grapefruit Segments Syrup/Juice
Blackberries in Juice
Blackcurrants in Juice
Lychees in Syrup
Mango Slices in Syrup

Tropical Fruit Cocktail in Fruit Juice

VEGETABLES

Canned

Petit Pois
Petit Pois & Baby Carrots
Sliced Mushrooms
Whole Button Mushrooms
Jersey Royal Potatoes
Small New Potatoes
Whole Carrots
Whole Baby Carrots
Sliced Carrots
Crinkle Cut Carrots
Ratatouille
Mixed Vegetables
Mixed Summer Vegetables
Baked Beans
Reduced Sugar/Salt Baked Beans
Barbeque Baked Beans
Curried Baked Beans
Baked Beans and Beefburgers
Baked Beans and Sausages
Spaghetti in Tomato Sauce (shortcut, rings, letters)
Wholewheat Spaghetti in Tomato Sauce
Cut Green Beans
Whole Green Beans
Butter Beans
Chick Peas
Borlotti Beans
Haricot Beans
Canellini Beans
Black Eye Beans
Flageolet Beans
Red Kidney Beans
Chilli Beans
Mixed Bean Salad
Sweetcorn
Sweetcorn & Peppers
Whole Tomatoes
Tomatoes
Chopped Tomatoes
Chopped Tomatoes with Herbs
Chopped Tomatoes with Hot Chilli
Passata

Frozen

All Frozen Vegetables
Flame Roasted Vegetables (Plain)
Southern Fry Potato Wedges
Southern Fry Griddles
Saute Potatoes
Roast Potatoes
Hash Browns
Pommes Noisettes
Breaded Mushrooms
Cous Cous

CHILLED SALADS

Coleslaw
Reduced Calorie Coleslaw
Coleslaw with Cheese
Garlic & Herb Coleslaw
Prawn Coleslaw
Vegetable
Greek Style
Celery, Nut & Sultana
Carrot & Nut
Crispy Vegatable
Potato
Pasta & Pesto

CHILLED DIPS

Prawn
Satay
Garlic & Herb
Tzatziki
Taramasalata

SANDWICHES

Coronation Chicken
Tuna & Cucumber
Chicken Tikka
Spicy Jamaican Chicken
Exotic Chicken Triple
Country Salad
Ploughmans
Tuna Italienne
Roast Beef
Prawn Mayonnaise
Cream Cheese, Fruit and Walnut
Egg Roll
Ploughmans Roll

FATS & OILS

All Margarines & Spreads
All Butter
All Cooking Oils

MILK & MILK PRODUCTS

All Milk (Fresh Pasteurised, Sterilised & UHT)
All Cream
Instant Dried Milk
Evaporated Milk
Soya Milk (Sweetened, Unsweetened)
Trimrite Evaporated Low Fat Milk

CHEESE

All varieties of Cheese (hard, soft, cottage) except Edam/Gouda
Processed Cheese Slices
Processed Cheddar Slices
Full Fat Soft Cheese

FISH & FISH PRODUCTS

Frozen

All Plain Fish e.g. Cod Fillets
Cod Fillet in Breadcrumbs (Boxed)
Haddock Fillet in Breadcrumbs (Boxed)
Plaice Fillet in Breadcrumbs (Boxed)
Cod in Batter (Boxed)
Haddock in Batter (Boxed)
Fish Fingers
Prime Cod Fish Fingers
Breaded Scampi
Hoki Kiev

Fresh

All Fresh Chilled Fish
Breaded Cod
Breaded Plaice
Breaded Haddock
Kippers
Smoked Cod
Smoked haddock

CANNED FISH

Tuna in Oil/Brine
Sardines in Vegetable Oil
Sardines in Brine
Anchovies in Olive Oil
Anchovies with Capers in Olive Oil
Pink Salmon
Red Salmon
Skinless, Boneless Pink Salmon
Salmon & Shrimp Paste
Crab Paste
Sardine & Tomato Spread
Tuna & Mayonnaise Spread

READY MEALS & MEAT PRODUCTS

Frozen

Grill Steaks
Economy Burgers
Quarter Pounder Beefburgers
Beefburgers
Cornish Pasties
Premium Cornish Pasties
Cheese & Onion Flans
Minced Steak Pie (with red wine)
Individual Minced Steak Pie (with red wine)
Steak & Kidney Pie
Individual Steak & Kidney Pie
Beef Stew & Dumplings
Cannelloni
Chilli Con Carne with Rice
Ocean Pie
Seafood Lasagne
Fish Crumble
Cod Crumble with Broccoli and Prawns
Cauliflower and Broccoli Mornay
Moussaka
Bean & Cheese Enchilladas
VegetableChilli
Vegetable Rolls
Cauliflower Cheese
Macaroni Cheese
Spaghetti Bolognese
Canelloni
Vegetable Lasagne
Potato & Cheese Gratin
Spicy Chicken
Potato Topped Pie
Chicken Casserole
Chicken Curry

Beef Curry
Vegetable Curry
Chicken Tikka Masala with Pilau Rice

FRESH/CHILLED MEAT PRODUCTS

All Fresh Meat and Poultry
Chicken Kiev
Cooked Turkey
Cooked Topside of Beef
Cooked Leg of Pork
Chargrill Whole Chicken
Chargrill Chicken Drumsticks
Cooked Roast Chicken - Whole
- Portions
Chinese Style Chicken
Tandoori Style Chicken
Melton Pork Pie (Medium, Large, Individual)
Cottage Pie
Snack Pork and Mushroom Pies

Pies Individual

Deep Filled Vegetable Pie
Steak & Kidney Pudding
Cornish Pastries x 4
Individual Cornish Pastry
Chicken Cibouette
Chicken & Mushroom Slice
Beef & Onion Slice
Minced Beef & Onion Pasties

Pies/Large/Family

Lamb & Red Wine Pie
Steak & Vegetable Pie
Steak & Ale Pie
Salmon & Brocolli Pie
Pork & Vegetable Pie with Cider
Pork & Vegetable Cobbler
Beef & Vegetable Cobbler

READY MEALS & CONVENIENCE SNACKS

Canned:

Ravioli
Irish Stew
Beef Madras
Chicken Madras
Chilli Con Carne

Chilled

Basmati Rice
Gobi Aloo Saag
Vegetable Curry
Chicken Passanda
Chicken Curry with Pilau Rice
Chicken Korma
Chicken Dhansak
Chicken Saag
Chicken Biryani
Chicken Madras
Moussaka
Chicken Tikka Masala
Chicken Chow Mein
Special Fried Rice
Lasagne
Vegetable Lasagne
Canelloni
Tagliatelle with Tuna & Sweetcorn
Cauliflower Cheese
Liver & Bacon
Snack Meals
Chilli with Rice
Sweet & Sour Pork with Rice
Cauliflower Cheese
Macaroni Cheese

FRESH CHILLED PASTA

Tagliatelle Bianche
Tagliatelle Verdi
Tagliate (Garlic & Herbs)
Spaghetti
Paglia e Fieno
Agnolotti (Mushroom)
Agnolotti (Tuna & Onion)
Pappardelle (Herbs & Tomato)
Tortellini (Cheese & Tomato)
Tortellini (Chicken & Asparagus)
Tortellini (Garlic & Herb)
Capaletti (Blue Cheese & Chives)

CHILLED VEGETARIAN PRODUCTS

Cheese & Tomato Grill

Mushroom & Rice Escalope
Creamy tomato Escalope
Nut Cutlet
Vegetable Cutlet
Mushroom Goujons with Garlic & Chive Dip
Vegetable Goujons with Tomato Dip
Vegetable Satay
Potato Vol au Vents (Brocolli & Garlic)
Potato Vol au Vents (Creamy Mushroom)

Microwave Ready Meals

Chicken Korma with Rice
Chilli Con Carne and Rice
Beef Madras with Rice
Sweet and Sour Chicken with Rice
Lasagne
Pasta Bolognese

PIZZAS

Cheese & Tomato
Vegetable Chilli
Chilli Beef & Spring Onion
Spicy Pork & Cashew Nut
Mozzarella
Chinese Pork & Cashew Nut
Cheese Tomato & Mixed Peppers
Tuna & Anchovy
9" Pizza Breads
7" Pizza Breads

FROZEN PIZZAS

Luxury Prawn and Tuna

Stoneoven
Chicken & Vegetable
Chicken & Pepperoni
Cheese & Tomato Mini Pizzas
Cheese & Onion Mini Pizzas

THE FOLLOWING PERMITTED FOOD ADDITIVES ARE NOT INCLUDED IN THE FORMULATION OF THE PRODUCTS LISTED.

COLOURS

E102 Tartrazine
E104 Quinoline Yellow
E110 Sunset Yellow FCF
E120 Cochineal or Carmine Acid
E122 Carmoisine or Azorubine
E123 Amaranth
E124 Ponceau 4R or Cochineal
E127 Erthyrosine BS
128 Red 2G
E131 Patent Blue V
E132 Indigo Carmine or Indigotine
E133 Brilliant Blue FCF
E142 Green S (Acid Brilliant Green)
E150 Caramel
E151 Black PN (Brilliant Black BN)
E153 Carbon Black (Vegetable Carbon)
154 Brown FK (Kipper Brown)
155 Brown HT
E180 Pigment Rubine (Lithol Rubine BK)

FLAVOUR ENHANCERS

Monosodium Glutamate/Sodium Glutamate

621 Sodium Hydrogen L-Glutamate
622 Potassium Hydrogen L-Glutamate
623 Calcium Dihydrogen di-L-Glutamate
627 Guanosine 5 - (disodium phosphate)
631 Inosine 5 - (disodium phosphate)
635 Sodium 5 - Robonucleotide

ANTIOXIDANTS

E320 Butylated Hydroxyanisole (BHA)
E321 Butylated Toluene (BHT)
E310 Propyl Gallate
E311 Octly Gallate
E312 Dodecyl Gallate

PRESERVATIVES

E210 Benzoic Acid
E211 Sodium Benzoate
E220 Sulphur Dioxide
E250 Sodium Nitrite
E251 Sodium Nitrate
E221 Sodium Sulphite
E222 Sodium Hydrogen Sulphite
E223 Sodium Metabishulphite
E224 Potassium Metabishlphite
E226 Calcium Sulphite
E227 Calcium Bisulphite
E212 Potassium Benzoate
E213 Calcium Benzoate
E214 Ethyl 4 Hydroxybenzoate
E215 Ethyl 4 Hydroxybenzoate Sodium Salt
E216 Propyl 4 Hydroxybenzoate
E217 Propyl 4 Hydroxybenzoate Sodium SaltE
218 Methyl 4 Hydroxybenzoate
E219 Methyl 4 Hydroxybenzoate Sodium Salt

APPENDIX IV

PUBLICATIONS

Publications

The following publications have been generated from the work carried out for this thesis:

Investigation of the potential involvement of *Mycobacterium paratuberculosis* in oral Crohn's disease and orofacial granulomatosis by polymerase chain reaction.
Riggio MP, Gibson J, Lennon A, Wray D, and MacDonald DG.
Gut 1997; **41**: 646-650

Angioedema and swellings of the orofacial region.
Rees SR, and Gibson J.
Oral Diseases 1997; **3**: 39-41

Thiomersal sensitivity in Health Care Workers (letter).
Rees S, Gibson J, Forsyth A, and Wray D.
Brit Dent J 1997; **183**: 395

Identification of *Mycobacterium paratuberculosis* by PCR in Orofacial Granulomatosis.
Gibson J, Riggio MP, MacDonald DG, and Wray D.
J Dent Res 1995; **74**: 843 (abstract)

Orofacial Granulomatosis - the role of Patch Testing.
Gibson J, Forsyth A, and Milligan KA.
Brit J Dermatol 1995; **133**: 25 (abstract)

Dietary and Environmental Allergens in Patients with Orofacial Granulomatosis.
Gibson J, Forsyth A, and Milligan KA.
J Dent Res 1996; **75**: 334 (abstract)

Search for *Mycobacterium paratuberculosis* in OFG and oral Crohn's disease.
Riggio MP, Gibson J, Lennon A, Wray D, and MacDonald DG.
J Dent Res 1997; **76**: 405 (abstract)

Prevalence of Food and Environmental Allergy in Oral Mucosal Disease.
Rees S, Gibson J, Forsyth A, and Wray D.
J Dent Res 1998; **77**: 895 (abstract)

Technetium-99m-HMPAO leucocyte labelling in OFG and intestinal Crohn's disease.
Gibson J, Wray A, Neilly B, Evans J, MacKenzie R, and McKillop J.
J Dent Res 1998; **77**: 895 (abstract)

Pre- and post-treatment outcome measures in patients with orofacial granulomatosis.
Gibson J, Forsyth A, and Wray D.
J Dent Res 1998; **77**: 1009 (abstract)

Identifying markers of systemic disease in patients with orofacial granulomatosis.
Gibson J, Smith G, Wray D, and Forsyth A.
J Dent Res 1998; **77**: 1009 (abstract)

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