THE SALIVARY GLANDS AND THEIR SECRETIONS IN CONNECTIVE TISSUE DISEASE

by

Derrick MacKenzie Chisholm

B.D.S. (Glas.)

T H E S I S

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
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V O L U M E I

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P R E F A C E

The work upon which this thesis is based was carried out during the past four years at the University of Glasgow Dental Hospital and School, the Centre for Rheumatic Diseases, Baird Street, Glasgow, and the University of Glasgow Departments of Medicine, Western Infirmary, and Pathology, Royal Infirmary. Some of the data contained in this thesis has already been published, accepted for publication or read at scientific meetings.

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1. Salivary duct antibodies in a variety of arthritides (1967)
   J. dent. Res. 46: 6, 1291.
   (with W.W. Buchanan and R.M. McSween).

2. Labial salivary gland biopsy in Sjögren's disease (1968)
   J. Clin. Path. 21: 5, 656.
   (with D.K. Mason).

3. Lymphocytic sialadenitis in the buccal mucosa in Sjögren's syndrome, rheumatoid arthritis and other arthritides: a clinical and laboratory study (1968)
   Acta. rheum. scand. 14: 4, 289.
   (with K. Whaley, W. Downie, C. Dick and J. Williamson)

4. Post-mortem prevalence of lymphocytic adenitis of the submandibular and labial minor salivary glands (1968)
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(with J.P. Waterhouse).

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6. A comparison between the gastric and salivary concentration of iodide, pertechnetate and bromide in man (1969)
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9. Parotid salivary flow studies and sialography in patients with Sjögren's syndrome
J. dent. Res. (in press)
(with G.S. Blair)

10. Iso-electric focussing in polyacrylamide gels of parotid salivary proteins from patients with connective tissue disease
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11. Salivary duct antibodies in a variety of arthritides (1967)
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12. Post-mortem prevalence of lymphocytic adenitis of the submandibular and labial minor salivary glands (1968)
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The Dental School, Welsh National School of Medicine, Cardiff (April, 1968).

13. The minor salivary glands: a review (1968)
Second Meeting of the Oral Pathology Club,
University of Edinburgh, (September, 1968).

14. Minor salivary gland pathology in Sjögren's syndrome and rheumatoid arthritis (1969)
4th International Conference of the International Academy of Oral Pathology
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15. Clinico-Pathology aspects of lesions of the minor salivary glands (1970)
Glasgow Odontological Society (March, 1970)

16. Parotid salivary flow studies and sialography in patients with Sjögren's syndrome (1970)
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I N T R O D U C T I O N

In recent years, considerable attention has been focussed upon the connective tissue diseases and of particular dental interest has been the symptom complex of Sjögren's syndrome, first described by the Swedish ophthalmologist, Henrik Sjögren (Figure I, 1) in 1933. Increasing interest has been shown also in the clinical and immunological derangements in the connective tissue diseases in general. Among the most prominent have been systemic lupus erythematosus and Hashimoto's thyroiditis.

Sjögren's syndrome consists of the triad of xerostomia, keratoconjunctivitis sicca and rheumatoid arthritis. Salivary gland or lacrimal gland enlargement may or may not be present. The term "sicca syndrome" refers to the association of keratoconjunctivitis sicca and xerostomia. Two of the three major components are generally considered sufficient for making a diagnosis of Sjögren's syndrome but excluded from the diagnosis are specific diseases of the salivary or lacrimal glands, such as sarcoid, lymphoma and tuberculosis. From recent work, Bunim (1961) and Bloch et al (1965), it is apparent that another

connective tissue disease may be present instead of rheumatoid arthritis, and among these are systemic lupus erythematosus, progressive systemic sclerosis, dermatomyositis and polyarteritis nodosa. The salivary gland component of Sjögren's syndrome may cause considerable diagnostic problems. Though the diagnosis of keratoconjunctivitis sicca and rheumatoid arthritis are fairly readily made by well-defined criteria, no such criteria exists for the salivary gland component. The major features of this component are xerostomia and salivary gland enlargement. However, these features are not pathognomonic and may occur singly or together in a wide range of diseases quite distinct from Sjögren's syndrome. Xerostomia, for example, may occur as the side effect of drug therapy or be the result of anxiety, neurological complaint, or post-irradiation. Salivary gland swelling may occur through a wide range of disease processes from non-specific infection to neoplasia.

In recent years, special attention has been directed towards patients with sicca syndrome. These patients display some clinical and serologic features which tend to suggest that such patients may form a separate clinical entity. Indeed the onset of

malignant lymphoma has been described in 4% of these cases. Defining the salivary gland component in such patients becomes especially important. Furthermore, there have been physiological and pathological studies of patients with rheumatoid arthritis alone which have suggested that a sub-clinical salivary gland defect may exist in a proportion of these patients. Reliable methods of assessing salivary gland function become important to monitor a disease process which may progress to Sjögren's syndrome in such patients.

In the past four years, the author has had the opportunity to make a special study of salivary involvement in patients with connective tissue disease. Investigations were carried out at the Glasgow Dental Hospital, The Centre for Rheumatic Diseases, Baird Street, Glasgow, at the University of Glasgow Departments of Medicine, Western Infirmary, and of Pathology, Royal Infirmary, Glasgow.

The assessment of the degree of involvement of the salivary glands in Sjögren's syndrome and other connective tissue diseases has been approached from various different aspects including clinical, laboratory and histopathologic investigations of both major and minor glands

It is the purpose of the study to describe and report the results of a number of investigative techniques which may be used to assess the degree of salivary gland involvement in Sjögren's syndrome, rheumatoid arthritis alone and in various other arthritides and connective tissue diseases. Also included in this study are groups of patients with xerostomia due to causes other than Sjögren's syndrome. The methods employed are clinical studies, parotid salivary flow estimation, sialography, scanning, minor salivary gland iodide concentration studies, histopathology of the minor salivary glands and serology, especially the presence of the salivary duct autoantibody.

In addition, I have collaborated with Dr. J.A. Beeley in a study of parotid salivary protein separation using polyacrylamide gel electrophoresis in patients with connective tissue disorders. A microbiological examination of saliva and minor gland tissue for the presence of mycoplasma was carried out in collaboration with Dr. A.M. Gordon.

In this thesis, the results are presented in five parts. Part I is concerned with a review of the

main clinical, aetiology and pathological aspects of the connective tissue diseases. Part II is devoted to the investigation of the major salivary gland involvement in the connective tissue diseases, with emphasis placed on salivary flow studies, sialography and scanning. The histopathologic aspects of minor salivary gland tissue in the connective tissue diseases form the framework of Part III. In Part IV, the serological, biochemical and microbiological aspects of salivary involvement are considered employing the techniques of indirect immunofluorescence for the detection of specific autoantibodies, mycoplasma culture, iso-electric focussing of salivary proteins in polyacrylamide gels and finally the concentration of iodide in saliva and salivary gland tissue. Part V is concerned with salivary gland function studies in patients with symptomatic xerostomia and the allied disorders of Mikulicz's disease and syndrome.

It is to be noted that in this thesis, the term "connective tissue disease" is employed in its original sense as a descriptive morphological term (See Introduction, Part I). It is used in preference to "collagen disease", with no aetiological or pathogenetic implication.

P A R T I

REVIEW OF THE CONNECTIVE TISSUE

DISEASES STUDIED

INTRODUCTION

PART I

INTRODUCTION

The connective tissues, first described by Johannes Müller in 1834 as the 'Bindegewebe' (Robb - Smith, 1954) are the most widely distributed and most abundant of all the tissue elements in the body and are involved in most of its functional activities. Although extra-cellular, they form a continuous inter-cellular matrix throughout the body, which includes tendons, fasciae, ligaments, joint capsules, septa and capsules of practically all organs, loose interstitial tissue, cartilage and bone, adipose and mucous tissues. The blood cells are derived from stem cells in the bone marrow and the cellular components of the reticulo-endothelial system arise from primitive mesenchymal cells. The cavities of joints, eyeballs, inner ear, ovarian follicles and umbilical cord (Wharton's jelly) contain fluids which are very similar to the ground substance of loose connective tissue, (Fitton Jackson, 1964).

All these tissues are derived from the primitive mesoderm, which in the adult persists to some extent in the loose connective tissues and in newly formed granulation tissue following injury.

Despite the fact that the connective tissues form such a large proportion of the body tissues, surprisingly little is as yet known of their function. Mechanical support and protection are among the most important functions and have been long recognised. The smooth movement of the body on its parts is facilitated by lubrication of ground substance components. Energy stores of fat and protein are released from the connective tissues for metabolic use in response to hormonal influence. Since connective tissue is interposed between blood vessels and epithelial structures, it necessarily has an important function as an organ of transport of both essential nutrients and metabolic waste products. Antibody formation, inflammation and reparative potential establish the connective tissues as one of the most important defence systems in the body.

The "collagen diseases", or what are now more appropriately termed the "connective tissue diseases", since collagen is but one component of connective tissue, comprise a group of clinical disorders of unknown aetiology, which have been classified together because of their pathological similarities. The strongest link binding together the various members

of the connective tissue group of diseases is the common pathological features: oedema of the inter-fibrillary ground substance, proliferation of fibro-blasts, inflammatory reaction characterised by the presence of mononuclear infiltration, and necrosis associated with "fibrinoid" degeneration. These changes are in no way specific for the connective tissue diseases and merely represent the limited number of ways in which connective tissue can respond to injury. The term "collagen disease", originated by Klemperer and his associates in 1942, was designed to include principally systemic lupus erythematosus, systemic sclerosis, rheumatoid fever, rheumatoid arthritis, polyarteritis nodosa and dermatomyositis. In 1950, Klemperer wrote "I believe today that even this cautious synthesis was premature because it resulted in an indiscriminate acceptance of a term with a diagnostic and pathogenetic import not originally intended when it was conceived. A peculiar worship of diagnostic terms has led to an exaggerated popularity of the diagnosis collagen disease". Klemperer and associates (1950, 1952) have described with special clarity the pathological changes in connective tissue encountered in these diseases although they were not the first to describe them or to recognise the significance of a systemic disease of a tissue.

Klinge (1929) in particular described such changes in rheumatic fever and rheumatoid arthritis. The concept of a tissue as a functioning unit, subject to specific diseases goes back to Bichet (1800 ; 1802).

The view that collagen diseases are allergic in origin was first advanced prominently by Klinge (1929 ; 1933) who emphasized the close similarity of the fibrinoid degenerations in rheumatic fever to lesions produced in rabbits by hypersensitive reactions to foreign protein. Rich (1947) reviewed the connective tissue diseases in the light of his views on hypersensitivity. The favourable response of most cases of these diseases to the administration of cortisone or corticotrophin was at first regarded as strong evidence of a close relationship if not a common aetiology, particularly an allergic aetiology. It became evident, however, that the therapeutic effect of these hormones was limited, that their action was not specific and that they merely controlled and to a large extent masked the response of the tissues to the various pathogenic agents (Duthie, 1954). Systemic lupus erythematosus originally considered the key "collagen disease", has become the classic

prototype of the autoimmune disease and is characterised by a multiplicity of autoantibodies against numerous components of the body. "Collagen disease" as already stressed refers to the principle site of involvement while the term autoimmune refers to a pathogenic mechanism. There is good evidence for an autoimmune basis for systemic lupus erythematosus and rheumatoid arthritis; but scleroderma, dermatomyositis and polyarteritis nodosa are less firmly established as being autoimmune in nature, (Bunim, 1961).

The problem of the aetiology and pathogenesis of the collagen diseases is far from solved. In all probability the causation is multiple. Genetic, infective, immunological and "stress" components may all play a part (Burnet, 1959).

As pointed out by Robb-Smith (1954), although there has been misuse of the term collagen disease as indicating a diagnostic entity, the conception of the connective tissue as a functioning unit is sound. The term is useful in indicating a relationship between these diseases if used in its original sense as a descriptive morphological term. Robb-Smith concludes his excellent review with a quotation

from Whitehead "Without generalization there is no meaning, and without concreteness there is no significance".

CHAPTER 1

SJÖGREN'S SYNDROME

In 1933, Henrik Sjögren, the Swedish ophthalmologist, published a monograph in which he described in detail the clinical and histological components of the syndrome that later came to bear his name. The syndrome consisted of kerato-conjunctivitis sicca very often combined with rheumatoid arthritis and xerostomia.

Forty-five years earlier, at a meeting of the Clinical Society in London, a patient with symptoms similar to those of Sjögren's syndrome, had been reported by Hadden (1888). The patient was a 63 year old widow who complained of "dry mouth" and deficiency of lacrimal secretion. Hadden described the patient's tongue as "red, devoid of epithelium" and cracked "like a crocodile's skin, and absolutely dry". The mucous membranes were "shiny and pale"; there was no history of salivary gland enlargement. Lacrimal secretion could not be stimulated by smelling strong ammonia solution.

Other reports describing some components of the syndrome were published by Fuchs (1891 ; 1919) and Gougerot, (1926). The relationship between fila-

mentary keratitis and arthritis was first stressed by Houwer in 1927; six of ten patients having arthritis. In the same year, Albrich described a dense, lymphocytic infiltrate in the lacrimal gland of a patient with filamentary keratitis; this patient also had bilateral swelling of parotid and sublingual glands.

In the years between 1928 and 1933, several papers were published in which the associations of xerostomia and xerophthalmia, and filamentary keratitis and arthritis were described (Isakowitz, 1928; Chamberlain, 1930; Hauer, 1931; Wisemann, 1932; Critchley and Meadows, 1933).

That rheumatoid arthritis was the joint disease in relation to this syndrome, became apparent in 1947 (Stenstam).

In Sjögren's initial series of 19 women of whom 13 had arthritis, the majority were post-menopausal. From the widespread nature of the pathological involvement, Sjögren concluded that the disease could be considered one of a generalised nature.

Of great interest has been the relationship

between Sjögren's syndrome and the disease reported by Mikulicz in 1888. Mikulicz described a patient with bilateral symmetrical enlargement of the lacrimal and parotid glands. The submandibular, sublingual buccal and palatal glands were also enlarged, but there was no evidence of reduction of lacrimal or salivary secretion. Massive round cell infiltration and atrophy of acinar tissue were noted in biopsy material from the lacrimal and submandibular glands. The patient died of peritonitis six months later and Mikulicz concluded that the condition was one of low grade infection.

Following the work of Schaffer and Jacobsen (1927) we recognise Mikulicz's disease proper of unknown aetiology and benign course; and Mikulicz's syndrome, in which salivary and lacrimal gland enlargement is attributable to a recognised disease such as tuberculosis, sarcoidosis, leukaemia or lymphosarcoma. Morgan and Castleman, on the basis of pathological appearances, suggested that Mikulicz's disease may be a variant of a larger symptom complex Sjögren's syndrome (1953, 1954).

Initially, patients with Sjögren's syndrome

were usually described by ophthalmologists, but in recent years the syndrome has been of great interest to immunologists and rheumatologists. Much of our recent knowledge stems from the work of Bunim and his colleagues at the National Institute of Health, Bethesda, U.S.A.

The clinical, serological and pathological characteristics of 62 patients with varying degrees of severity of Sjögren's syndrome were described by Bloch et al in 1965. Histological changes in the major salivary glands varied from acinar atrophy and sparselymphocytic infiltration to massive replacement of acinar tissue by lymphoid tissue. Hypergammaglobulinaemia and rheumatoid factors were demonstrated in nearly all patients in the series. Antibodies directed specifically against lacrimal and salivary constituents could not be demonstrated. Reticulum cell sarcoma developed in three patients.

Because of the wide range of serologic reactivity, the lymphocytic infiltrates and the relation to connective tissue diseases, it has been suggested that Sjögren's syndrome may be an autoimmune disease. However, several attempts to produce the salivary

gland lesions in experimental animals by immunisation with salivary or lacrimal gland homogenates and Freund's adjuvant, have been unsuccessful (Bloch et al, 1965; Waterhouse, 1963; Chan, 1964). A series of abnormalities occurring spontaneously in NZB and NZBxNZWF, mice, resembling the abnormalities found in Sjögren's syndrome have been reported by Kessler (1968).

Bernier and Bhaskar (1958) have suggested that the underlying pathology in Sjögren's syndrome is a hyperplasia of the intra-parotid lymphnodes. Several factors, they suggest, including rheumatoid arthritis might predispose to this hyperplasia.

Rheumatoid arthritis and keratoconjunctivitis sicca are diagnoses readily made by well-defined criteria. The position is less clear regarding xerostomia and the degree of salivary gland involvement in the diagnosis of Sjögren's syndrome. Some authors accept the history and appearance of dry mouth as proof of this component of the sicca complex, (Allington, 1950), while others require sialographic evidence or the results of salivary flow studies (Bloch et al, 1965) the factors involved in the aetiology of xerostomia have been well described by Mason and Glen (1968) and Bertram (1967).

The close association of Sjögren's syndrome with other connective tissue diseases has been emphasized in a number of reports. In an extensive study, 39 of 62 patients showed evidence of an associated collagen disease (Bloch et al, 1965). Thirty had definite rheumatoid arthritis, three had scleroderma and four had myopathy. It is unusual that no instance of systemic lupus erythematosus was noted in this group since this disease has been noted in association with Sjögren's syndrome (Morgan and Castleman, 1953; Morgan, 1954; Heaton, 1959; Bain, 1960; Bencze and Lakatos, 1963; Gahagan, 1965). Of interest, was the observation that serologic aberrations, including the presence of rheumatoid factor, anti-nuclear and anticytoplasmic antibodies, and hypergammaglobulinaemia were more pronounced in those patients with kerato-conjunctivitis sicca alone than in those with associated connective tissue diseases (Bloch et al, 1964). Also it was within the group of kerato-conjunctivitis sicca alone that three patients developed recticulum cell sarcoma and a fourth had findings which suggested Waldenström's macroglobulinaemia. These observations suggest that some patients with Sjögren's syndrome may be prone to develop lymphoma. Talal et al (1967) described eight patients with Sjögren's

syndrome and who had extrasalivary lymphoid abnormalities. Two had primary macroglobulinaemia, one had reticulum cell sarcoma and five had a syndrome designated as pseudolymphoma. The patients with pseudolymphoma were not diagnosed as primary macroglobulinaemia or lymphoma since they had lesser amounts of lgM which contained both types of L chains and because the histologic changes were consistent with reactive hyperplasia of inflammatory or other origin. Talal et al (1967) conclude that these patients with Sjögren's syndrome, macroglobulinaemia and pseudolymphoma have a disorder that lies between hyperplasia and neoplasia. Other lymphoid abnormalities in Sjögren's syndrome have been described by Talal (1966). Studies in in vitro lymphocyte transformation in Sjögren's syndrome have demonstrated that peripheral blood lymphocytes show a depressed response when stimulated with phytohaemagglutinin or streptolysin-O. This has further suggested that lymphocytes originating from intra-parotid lymphoid tissue may circulate in the blood of patients with Sjögren's syndrome and are responsible for the decreased transformation response and for the predisposition to extrasalivary lymphomas and pseudolymphoma.

Serologically several findings have suggested to

some workers an autoimmune aetiology to Sjögren's syndrome. Hypergammaglobulinaemia is commonly found in the sera from patients with Sjögren's syndrome, antinuclear factor is common, specific thyroid antibodies frequently found and Rose-Waaler's Test and R.A. Test are often positive. Jones (1958) and Anderson et al (1961) found a precipitin against extracts of lacrimal tissue in sera from patients with Sjögren's syndrome, but the phenomenon was not specific since extracts of other organs could give similar reactions (Bertram and Halberg, 1964) described a specific antibody against the epithelium of the salivary ducts and sera from 11 of 19 patients with Sjögren's syndrome. MacSween et al, 1967, showed the antibody to be present in 15% of patients with sicca syndrome, 65% of R.A. and Sjögren's syndrome and in 26% of R.A. alone. In none of a large group of controls was the antibody present. They suggest that the antibody shows some organ specificity. The reason why patients with rheumatoid arthritis who have Sjögren's syndrome develop this antibody is not known. Recently, the neuropathy of Sjögren's syndrome has been described (Kaltreider and Talal, 1969). They described 10 patients with Sjögren's syndrome who had peripheral

or trigeminal neuropathy or both. The peripheral neuropathy was predominantly sensory and characteristically mild, distal and symmetrical. Spinal fluid examinations were normal. The neuropathy was indistinguishable from that seen in rheumatoid arthritis and corticoid dosage was not related to the onset of neuropathy. A sensory trigeminal neuropathy, characterised by numbness hyperaesthesia and tingling paraesthesia was noted in four of these ten patients. Furthermore, Kaltreider and Talal suggested that the divisional distribution of the sensory deficit favoured a peripheral rather than a central lesion. Interestingly, the presence of neuropathy correlated strongly with vasculitis in the nerves and muscles and they suggested that the vascular inflammation was causally related to the pathogenesis of the neuropathy. It is of interest that neurological manifestations have been reported in other related diseases such as systemic lupus erythematosus (Johnston and Richardson, 1968; Lewis, 1965; Bailey, Sayre and Clark, 1956) rheumatoid arthritis (Sokoloff and Bunim, 1957) and scleroderma (Beighton et al, 1968).

An unusual feature of Sjögren's syndrome is a renal concentrating defect, which on rare occasions may take the form of nephrogenic diabetes insipidus (Kahn et al, 1962). Other instances of renal tubular dysfunction include aminoaciduria, phosphaturia and renal tubular acidosis (Shearn and Tu, 1965). Histologically, kidneys from patients with Sjögren's syndrome often demonstrate parenchymal lymphocytic infiltration (Bunim et al, 1964; Bloch et al, 1965). A study of pancreatic function in Sjögren's syndrome was prompted by the occurrence of acute pancreatitis in two patients (Fenster et al, 1964). Secretion tests in 11 cases showed diminished volume in 3. Six of the 11 had increased levels of serum amylase. Oesophageal disturbances may be severe. Circular folds with web-like structures in the upper part of the oesophagus were noted in one fourth of the patients in one series (Lenoch et al, 1964). In addition, Lenoch and his associates (1964) noted that gastric achlorhydria is common in patients with Sjögren's syndrome. However, they also noted a high incidence of gastric hyposecretion in patients with rheumatoid arthritis alone. In a study of six patients (Buchanan et al, 1966) noted histologically proven gastritis in five, autoantibodies to gastric parietal

cells in three and achlorhydria in four. Using mitochondrial antibody as a marker, evidence of liver disease in Sjögren's syndrome has been reported (Whaley et al, 1970). An overt rheumatoid type of vasculitis, detected by muscle biopsy, may occur in association with Sjögren's syndrome (Bloch et al, 1965). The occurrence of microcysts in the muscle fibres of 12 of 23 patients with the sicca syndrome has been reported by Denko and Old (1969).

In 1966, Waterhouse and Doniach in an autopsy study of over 500 necropsis in which strict criteria was applied showed focal lymphocytic adenitis in 23% of females and 9% of males. They suggest that the lesion represents a focal manifestation of the lesion in Sjögren's syndrome. Their finding of focal sialadenitis in all 12 females and four of five males with rheumatoid arthritis provides strong evidence of the association between the two conditions. Dental decay of rapid onset has been reported in Sjögren's syndrome (Bloch et al, 1965; Jacobson, 1966; Bertram, 1967). Involvement of the minor salivary glands, showing extensive lymphocytic infiltration, is now recognised (Calman and Reifman, 1966; Cifarelli et al, 1966; Cahn, 1967; Bertram, 1967; Chisholm and Mason, 1968). Fischer et al

(1968) reported differences in the electrophoretic pattern of parotid saliva in patients with Sjögren's syndrome compared to normal controls. An increase in a Beta migrating glycoprotein was observed in diseased samples. In one of these patients, radiation therapy resulted in a normal electrophoretic pattern being restored (Weisberger et al, 1968). Recently, Talal et al (1969) described the immunoglobulin synthetic ability of lower lip biopsies in 22 patients with Sjögren's syndrome. It was noted that this ability differed from a control group in that a significantly greater amount of lgG and lgM was produced compared to the normal dominance of lgA production. They suggested that the cells synthesizing lgG and lgM probably arose from an extrasalivary source. Skin dryness accompanied by a generalised pruritus has been reported in Sjögren's syndrome (Feuerman, 1968). The rare occurrence of Sjögren's syndrome in a 10 year old child has been reported (Duncan et al, 1969).

C H A P T E R 2

RHEUMATOID ARTHRITIS AND ALLIED DISORDERS

Rheumatoid Arthritis

Rheumatoid arthritis is a slowly progressive chronic inflammatory disease of connective tissue which affects principally the joints and may lead to a permanent deformity. It is a disease of world-wide distribution, affecting many races, but apparently it is more common among those living in temperate climate. At first there is an acute polysynovitis of the smaller diarthrodial joints but, with time, the disease leads to increasing destruction of articular cartilage and to fibrous ankylosis. It is a disease which is three times more frequent in females than in males (Laurence, 1961). Rheumatoid arthritis may start at any age, but more commonly in the third and fourth decades. In addition to poly-arthritis, some patients develop systemic changes affecting many different organs and tissues, so that some workers have preferred the term "rheumatoid disease" (Ellman and Ball, 1948). Though crippling, rheumatoid arthritis is only indirectly a cause of death. The onset of the disease

is often insidious. Vague symptoms of fatique are combined with loss of weight, anorexia, and muscular stiffness. They are accompanied by a raised erythrocyte sedimentation rate and anaemia (Duthie, 1964). Occasionally, the onset is more abrupt, perhaps precipitated by physical or mental disturbance, and in these cases there is good prospect of controlling the disease by early, active treatment. An acute exacerbation of the local disease may result from synovial rupture (Dickson and Grant, 1964).

The articular lesion in rheumatoid arthritis is a hypertrophic, villus synovitis. The synovial tissue becomes thickened and shows projecting villae which result from proliferation of vascular fibrous tissue in which chronic inflammatory cells, mostly lymphocytes and plasma cells are noted infiltrating throughout the tissue. As the disease progresses, erosion and replacement of articular cartilage by granulation tissue with involvement of the underlying bone becomes apparent. Adherence of the granulation tissue between opposing joint surfaces may lead to limitation of movement, while the destructive changes may progress to gross disorganisation

of the joint with later fibrous or bony ankylosis occurring. The pattern of hypertrophic villus synovitis is typical of rheumatoid arthritis though it is not pathognomonic of the disease and may be seen in other forms of chronic arthritis such as psoriatic arthritis, Reiter's disease and ankylosing spondylitis. Subcutaneous nodules of rheumatoid arthritis have a higher degree of histologic specificity although they are present in only 15% to 20% of patients. Inflammation of various small arteries has been noted as an occasional finding in patients with the disease (Cruickshank, 1954; Sokoloff (1963). Changes similar to those in the subcutaneous nodules and synovial membrane are occasionally observed in other organs and tissues such as the heart, lung, pleural membrane, eye and splenic capsule (Bunim, Sokoloff, William and Black 1955). Focal infiltrations of lymphocytes occur in the skeletal muscle in the majority of patients with rheumatoid arthritis and may be associated with muscle fibre degeneration. Enlargement of lymph nodes occurs especially in relation to affected joints but may be generalised. Splenomegaly occurs in

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approximately 3% to 5% of patients with rheumatoid arthritis and, when associated with lymphadenopathy and leukoplakia is referred to as Felty's syndrome, (Felty, 1924; Gardner and Roy, 1961). Secondary amyloidosis is a common complication of rheumatoid arthritis and may be found at necropsy in as many as 20% of severe cases (Calkins and Cohen, 1960; Arapakis and Tribe, 1963).

Despite intensive efforts to identify infectious agents, endocrine disturbances, nutritional or metabolic abnormalities, psychosomatic factors and hypersensitivity mechanisms, the aetiology of rheumatoid arthritis remains obscure (Dresner, 1955). Some evidence favouring a genetic factor has been advanced, although no definite genetic pattern has emerged (Laurence and Ball, 1958). A polyarthritis resembling rheumatoid arthritis has been produced in rats after a single parenteral infection of water in oil adjuvant (Pearson, 1963) and by intra-articular injection of fibrin in rabbits immunised to autologous or heterologous fibrin (Dumonde and Glynn, 1962), but the full clinical picture of rheumatoid arthritis remains peculiar to man.

In rheumatoid arthritis, the serum rheumatoid factor comprises a number of separate immunoglobulins some of which behave as autoantibodies. It has been shown by immunofluorescence antibody techniques that rheumatoid factor is produced in plasma cells in the affected synovia, subcutaneous nodules and lymph nodes, and in germinal centre cells in lymph nodes, (Mellors et al, 1951, 1961 and McCormick, 1963). Although rheumatoid factor is found especially in patients with rheumatoid arthritis, it is by no means specific for the disease and is found, albeit usually in low titre in a variety of diseases especially other connective tissue diseases, chronic infections and a small proportion of apparently healthy individuals.

Osteoarthritis

Osteoarthritis is a chronic form of arthropathy characterised by extensive destruction of the articular cartilage, leading to painful, limited motion in the affecting joint and sometimes to severe disabling joint damage.

It is found to a greater or lesser degree in virtually all persons over the age of 50, males and

females are affected equally often (Heine, 1926; Kellgren, 1961). This condition is such a consistent accompaniment of old age that it is considered by some to represent the inevitable consequence of wear and tear on the joints over the course of years. It may occur as a mono-articular or polyarticular involvement. The large joints of the body and the spine are principally affected. The principal changes are degeneration of the cartilage rather than inflammation of the synovia. No true pannus develops.

It is an insidious disease that is usually first noticed as a slight stiffness or decreased mobility in the affected joints. There is often a history of trauma and in this connection it is generally believed that faulty posture, obesity and occupational stress all predispose to the development of the condition. There are no constitutional signs of an inflammatory disease and the joints rarely have local evidence of inflammation. One special form of osteoarthritis exists, namely the formation of so-called Heberden's nodes about the bases of terminal phalanges of the fingers, (Heberden, 1802). The

tendency to develop Heberden's nodes is inherited as a single autosomal characteristic (Stecher, 1955). In rare cases, a generalised, multiple form of degenerative joint disease occurs accompanied by fever, a raised erythrocyte sedimentation rate and anaemia, (Kellgren, 1961; Kellgren et al, 1963). A potent factor leading to osteoarthritis is the previous or continuing presence of arthritis. This is often noted in rheumatoid arthritis where a moderate or severe inflammatory reaction with pannus formation and cartilaginous replacement fibrosis, accelerates normal cartilage age change and leads to premature and progressive osteoarthritis. There is no satisfactory treatment for this condition and the disorder is usually slowly progressive over the remaining years of life.

Ankylosing Spondylitis

Ankylosing Spondylitis is an uncommon form of arthritis attacking in particular young men, though its occurrence in women is now well recognised. The male incidence has been given as 6:1000 (Sharp, 1962), whilst estimates of the sex ratio of its occurrence vary from 4.4:1 (Wilkinson and Bywaters, 1958) to 9:1 (Turney, 1952). In 70% of patients, the disease

is said to commence between the ages of 20 to 40 though a possible prepubertal onset has been stressed, a diagnostic feature separating it from Reiter's Disease.

The aetiology of the disease is still unknown, though Hersch et al (1950) confirmed its familial occurrence and concluded inheritance to be due to an autosomal dominant factor with a 70% penetrance in males and 10% in females. The disease resembles rheumatoid arthritis by occasionally causing similar lesions in limb joints but is distinguished by the characteristic age and sex distribution, anatomical sites, by the absence of systemic disease and by negative tests for rheumatoid and antinuclear factors.

The initial pathological lesion is an acute osteitis affecting the fibro-cartilagenous joints of the axial skeleton. It may be accompanied by an acute synovitis in which plasma cells and lymphocytes are common. This is followed by articular cartilage replacement by granulation tissue, leading to fibrosis and bony ankylosis. Severe deformity results with immobilisation of the thoracic cage and of the spine. Four main complications of ankylosing spondylitis are

recognised, namely iritis, atlanto axial subluxation, aortitis and amyloid disease, (Williams, 1965). Of these, amyloid disease is the most rare and occurs only in the most severe cases. Apart from these complications, an association also exists between ankylosing spondylitis and pulmonary tuberculosis, intestinal disease and peptic ulceration. Mason et al (1958) have shown that 83% of patients with ankylosing spondylitis have a prostatitis.

Primary Gout

This is an hereditary disease in which the predisposing metabolic disorder, probably enzymic, is transmitted by a dominant autosomal gene with greater penetrance for males than females. The result of this inherited disorder is a raised blood uric acid level. Hyperuricaemia is thought to be due to the excess production of uric acid but an additional fault in renal tubular excretion of urates is suspected. In a proportion of patients, crystalline sodium urate is deposited in connective tissues and articular cartilage, probably because of local changes in mucopolysaccharide structure. The aggregates of crystals elicit the formation of granulomata called tophi and a progressive

and chronic arthritis. Together, the tophi and arthritic changes constitute the clinical syndrome of gout.

Gout is a disease of great antiquity and its features were noted in the Hippocratic writings. A classic monograph on the clinical aspects of the disease was written by Sydenham (1683). The history of gout has been reviewed by Copeman (1964) and its treatment by Rodnan and Benedek (1963).

Gout is recognised by the sudden, unexpected onset of intense local pain with swelling and redness of a joint such as that of the first metatarsal phalyngeal junction. The local signs of inflammation mimic an acute purulent infection and are often accompanied by a systemic disorder which includes fever and a polymorphonuclear leukocytosis. The disease is episodic in clinical presentation and in the period between acute attacks, the so-called inter-critical period, the diagnosis is made by the recognition of hyperuricaemia. Although gout was once believed to have been born of the seduction of Aphrodite by Dionysus, considerable evidence points to a more terrestial aetiology and pathogenesis. Seegmuiller and Howell

(1962) have produced acute gouty arthritis in dogs and humans by intra-articular injection of uric acid crystals. These findings led Seegmiller and his colleagues (1963) to propose a pathogenetic mechanism for acute gouty arthritis. Uric acid crystals precipitate in and around the joint and phagocytosis of these crystals by polymorphonuclear leukocytes induces an acute inflammatory reaction. This reaction, once started is self-perpetuating, the release of lactic acid during the process of inflammation resulting in a fallen pH which favours further precipitation of uric acid.

Phenylbutazone in full therapeutic dose is still endorsed universally in the treatment of acute gouty arthritis. The treatment of chronic tophaceous gout is often difficult with patients having renal impairment due to deposition of uric acid in the renal parenchyma. In this situation, conventional therapy is of little value. Recently the introduction of a new compound allopurinol has transformed the possibilities of lowering serum uric acid levels in the face of severe renal failure (Rundles et al, 1963; Yü and Gutman, 1964).

Psoriatic Arthritis

Psoriasis and rheumatoid arthritis are both common diseases. Although they may therefore be expected to occur together by chance, the simultaneous existence of the two conditions and the development of arthritis in patients with psoriasis have pointed to the possibility of a specific form of arthritis which has been termed "psoriatic arthropathy" (Vilanova and Pinol, 1951; Rosenberg, 1958). Usually, arthritis begins some years after the skin lesions. The distal joints of hands and feet are particularly involved. Radiologically, marginal destruction of phalyngeal bone causes erosions, an appearance described as "gnawing away" and the outcome of this process is extensive bone and joint destruction so severe that it may come to resemble the gross disorganisation encountered in neurogenic arthropathy.

The histological changes in the diarthrodal joints in psoriatic arthropathy have been described by Bauer et al (1941) and by Sherman (1952). The microscopic changes are not specific and in fact closely resemble those encountered in classical rheumatoid arthritis. Nevertheless Sherman (1952) regards psoriatic arthropathy

as an entity distinct from rheumatoid arthritis.

Reiter's syndrome

In Reiter's syndrome, (Reiter, 1916), there is acute polyarthritis, urethritis and conjunctivitis in young adult males. A venereal infection with pleuropneumonia-like organisms (P.P.L.O.) is suspected but has not been satisfactorily substantiated. Attempts at sub-cultures of these organisms having failed, and complement fixation tests having been negative, (Claus et al, 1964), Gonococcal infection is coincidental. It remains possible that this syndrome may result from more than one cause, thus a very similar sequence may follow bacillary dysentery. The arthritis may become chronic and disabling.

In a long-term study of 16 patients (Weinberger et al, 1962), three clinical groups were recognised. Firstly, those with a typical onset followed by remission and subsequent period of good health extending up to 8 years. Secondly, those with a characteristic onset, followed by episodic recurrences over a period of months or years. Thirdly, a group, with episodes confined to one system for one or more years, but subsequently involving all three systems of the triad.

A close association between Reiter's syndrome and psoriasis has been described (Wright and Reed, 1964; Perry and Mayne, 1965), and psoriatic arthritis (Kahn and Hall, 1965).

The histopathologic features of the syndrome have been described by Weinberger et al (1962) and Mori Zak (1960). Early pustular lesions showed a spongiform pustule in the upper epidermis together with parakeratosis and elongation of the rete ridges. The cutaneous lesions keratitis blennorrhagica were found most often on the soles of the feet, the palms and the circumcised glans penis. They resembled closely those of pustular psoriasis. Indeed, a similarity between the cutaneous involvement in Reiter's syndrome and that seen in psoriatic arthritis has been demonstrated both clinically and histologically (Wright and Reed, 1964). The lesions of the mucous membranes resemble the changes of cutaneous lesions but lack evidence of keratosis. Papules or plaques are noted in the buccal mucosa, urethra and bladder. In the joints, there is a subacute polyarthritis, which may die down but may become persistent and chronic.

Behcet's Syndrome

Behcet's syndrome (Behcet, 1937), is a rare disorder characterised by uveitis and recurrent ulceration of the oral mucous membranes and external genitalia. The syndrome may also include arthritis, erythema nodosum, thrombophlebitis and a variety of neurological, cardiac and gastrointestinal abnormalities, (Mason and Barnes, 1969). A viral aetiology is suspected (Behcet, 1937; Evans et al, 1957) and recently an autoimmune aetiology has been postulated (Shimizu et al, 1965; Nally, 1968). It is particularly interesting that this curious syndrome should be associated with anaemia, leukocytosis and hypergammaglobulinaemia.

Histological study of the joint (France et al, 1951) has shown an inflammatory reaction with the perivascular aggregation of histiocytes and lymphocytes but not of plasma cells. The aphthae and genital ulcers show a non-specific inflammatory reaction with ulceration. The disease tends to pursue a low-grade remittent course but the development of central nervous lesions with perivascular infiltration and thrombosis of small vessels may lead to fatal meningoencephalitis (Schottland et al, 1963).

Dermatomyositis

This is an inflammatory and degenerative disease of skin, muscles (polymositis) and occasionally other organs. The disease may occur at any age although it is most commonly found in middle life. It is slightly more common in females. Weakness and tenderness of affected muscle, fever, cutaneous eruptions, oedema, dysphagia and progressive wasting are the characteristic symptoms. The prognosis generally is poor and death usually results from respiratory or cardiac failure or superimposed infection.

The aetiology of dermatomyositis is obscure. However, of interest is the association in adults but not in children with a malignant tumour in approximately 15% of cases (Mills, 1963). Pathologically, the muscles show focal necrosis, infiltration with chronic inflammatory cells, often in the perimysium, near blood vessels, attempts at regeneration and variation in the size of muscle fibres (Pagel et al, 1949). The cutaneous lesions have no specific histologic pattern. There have been few studies in dermatomyositis reporting the prevalence of serum autoantibodies. Rheumatoid factor has been reported in 20% to 30% of patients with

dermatomyosisitis (Singer, 1961), and positive L.E. cell tests have occasionally been noted. The familial occurrence of dermatomyositis has been reported (Christianson et al, 1956; Lambie and Duff, 1963; Winkler, 1956). In addition, rheumatoid arthritis and scleroderma, hypergammaglobulinaemia and positive tests for rheumatoid factor and nuclear antibodies have been recorded in relatives of patients with dermatomyositis (Lambie and Duff, 1963).

Progressive Systemic Sclerosis

This is a chronic disease of unknown aetiology characterised by diffuse sclerosis of the skin (scleroderma) gastrointestinal tract, heart, striated muscle, lungs and kidneys. The disease occurs more often in women usually between the ages of 20 and 50 years. The onset and course of progressive systemic sclerosis are usually insidious. Skin changes include induration, hyperpigmentation, patches of depigmentation and telangiectasis. Raynaud's phenomenon (digital arterial insufficiency provoked by cold) is common. Calcification of subcutaneous tissue is a late manifestation, and polyarthritis develops in a high proportion of patients and may

clinically resemble rheumatoid arthritis. Intestinal involvement may lead to abdominal pain and swelling and intestinal malabsorption may occur leading to marked wasting. Pulmonary involvement results in breathlessness and pulmonary insufficiency. Uraemia and hypertension may develop as a result of renal involvement. Histopathologically, progressive systemic sclerosis is characterised by marked hyperplasia of collagen fibres in skin and viscera. Inflammation is usually absent in lesions of well-advanced cases, but mild perivascular chronic inflammatory cell infiltration may be present in the early lesion. A lymphocytic and plasma cell synovitis associated with the polyarthritis closely resembles that of rheumatoid arthritis but there is less pannus formation and cartilage destruction (Rodnan, 1962). The renal changes of progressive systemic sclerosis consist of intimal thickening of the interlobular arteries and fibrinoid necrosis of small vessels with ischaemic changes in the cortex (Rodnan et al, 1957).

Evidence for an immunological abnormality in the pathogenesis of progressive systemic sclerosis rests mainly on the finding of hypergammaglobulinaemia (Rodnan, 1963). Various autoantibodies in a proportion of

patients with the disease has been noted. Rheumatoid factor has been reported in approximately 40% of patients (Kellgren and Ball, 1959). Antinuclear factor has been found in as many as 80% of patients (Alexander et al, 1960; Beck, 1963; Hall et al, 1960). Progressive systemic sclerosis has been reported in association with agammaglobulinaemia (Rodnan, 1963).

Systemic lupus erythematosus

Systemic lupus erythematosus is a serious constitutional disorder of unknown aetiology with protean manifestations which may affect skin mucosae, serous membranes, small blood vessels, endocardium, kidneys, brain and blood. Young females between the ages of 10 and 40 years are particularly prone to the disease. About 80% to 90% of reported cases at any age have been in females. The course of the disease is varied and occasionally may extend over a period of years. Renal involvement (Harvey et al, 1954) frequently results in death.

The pathologic changes are those of cellular infiltration and fibrinoid necrosis. The characteristic lesions include verrucose endocarditis

of Libman and Sacks (1924) thickening of individual capillary loops in the renal glomeruli - the "wire-loop" lesions, onion skin lesions of the splenic arterioles and the presence of haemotoxophil bodies.

Although clinically the skin lesions in chronic discoid lupus erythematosus are usually distinct from those of systemic lupus erythematosus, there is increasing evidence that they may represent variants of the same pathological process (Schrank and Donniach, 1963; Shulman, 1963).

Interest in the immunologic aspects of systemic lupus erythematosus was first aroused by the discovery of the lupus erythematosus cell phenomenon (L.E. cell) (Figure II, 22) by Hargreaves et al (1948) and by the identification of the active principle by Miescher and Fauconnet (1954) as antibody to a component of cell nuclei. Nuclear antibodies form a heterogenous group and react with at least five distinct nuclear antigens as shown by cross absorption experiments (Holman, 1960) and their identification has been greatly facilitated by the morphologic patterns of their immunofluorescent staining reaction with nuclei. The L.E. cell test has been reported as being positive in 40% to 100% of patients

with systemic lupus erythematosus, (Wilkinson and Rees, 1958). This wide variation is due to differences in diagnostic criteria, and since there is no diagnostic test by which systemic lupus erythematosus can invariably be recognised, there is no means of establishing the reliability of the L.E. cell test. However, there is no doubt that some patients with unequivocal systemic lupus erythematosus cannot be shown to form L.E. cells despite repeated testing using the best available techniques. False negative L.E. cell tests have been shown in some instances to be due to low levels of serum complement (Formijne and van Soeren, 1958). There is some degree of correlation between the frequency and strength of the L.E. cell test and the activity and severity of the disease, (Rothfield and Pace, 1962).

The demonstration of a disease resembling systemic lupus erythematosus which occurs spontaneously in specially inbred strains of New Zealand mice provides indirect evidence in support of the role of a genetic factor in the pathogenesis of systemic lupus erythematosus in humans (Helyer and Howie, 1961).

Administration of various drugs can produce a

clinical syndrome indistinguishable from systemic lupus erythematosus. Among these drugs, the best known is hydralazine (Hildrith et al, 1960; Holly, 1961; Shulman and Harvey, 1960). Other drugs include various anticonvulsants, procainamide, penicillin, phenylbutazone and anti-tuberculosis drugs. Usually symptoms abate promptly when the drug is discontinued. Whether the drugs act as haptens, as proposed by Rallison et al (1961), or whether they unmask latent systemic lupus erythematosus is not known.

Three features appear to distinguish drug induced lupus from the spontaneously occurring clinical disorder. The sex ratio of patients with the drug induced syndrome is more nearly equal, nephritis is not a feature, and when the offending drug is withdrawn, the symptom complex usually abates and the responses to tests for L.E. factor, antinuclear factor and other laboratory manifestations return to normal.

P A R T II

MAJOR SALIVARY GLAND INVOLVEMENT IN THE CONNECTIVE TISSUE DISEASES

INTRODUCTION

Chapter 3 CLINICAL STUDIES

Chapter 4 PAROTID SALIVARY FLOW STUDIES IN CONTROL SUBJECTS

Chapter 5 PAROTID SALIVARY FLOW STUDIES IN THE CONNECTIVE TISSUE DISEASES

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P A R T II

I N T R O D U C T I O N

Before describing the salivary gland involvement in the connective tissue diseases, it is of importance to mention briefly the methods of diagnosing in such patients the arthritic and ocular components.

In the present study, the diagnoses of rheumatoid arthritis (Figures II, 1 - II, 2) and allied disorders were made by Dr. W. Watson Buchanan and his colleagues at the Centre for Rheumatic Diseases, Glasgow. The ophthalmological examinations were performed by Dr. John Williamson who, at the times of examination, was unaware of the clinical diagnosis.

Rheumatoid arthritis was diagnosed using the criteria of the American Rheumatism Association (Ropes et al, 1958). All the patients had "definite" or "classical" disease.

The method outlined by Williamson et al (1967)

was employed for the examination for keratoconjunctivitis sicca (Figures II, 3 - II, 5)

C H A P T E R 3

CLINICAL STUDIES

Materials and Methods

Patients studied

In all, 273 patients with various connective tissue diseases were examined for evidence of oral and salivary gland involvement. The clinical diagnosis, age and sex distribution are shown in Tables II, 1 - II, 2.

History Taking

Each patient was carefully questioned regarding a history and duration of xerostomia and of associated oral and pharyngeal symptoms of Sjögren's syndrome. Each patient was questioned about the following symptoms: duration and nature of xerostomia, difficulty in swallowing or mastication, increased fluid intake either during meals or in general, abnormalities of taste sensation, oral soreness, denture problems, oral ulceration and fissuring or ulceration of the lips. The patients were then examined for any signs of xerostomia, lingual changes, dental caries and denture status, angular cheilitis and ulceration (Figures II,6 - II,7).

In addition, each patient was questioned regarding a history of major salivary gland enlargement.

Visual examination together with palpation of the major salivary glands were carried out in each patient.

Lingual Changes

In each patient, the tongue was examined for evidence of reddening, fissuring, papillaru atrophy and lobulation. The lingual changes were graded according to the following standard (Bertram, 1967):

Grade I Slight reddening and mild fissuring with some atrophy of the filiform papillae on the tip of the tongue.

Grade II Moderate reddening, fissuring, sporadic papillary atrophy and incipient lobulation.

Grade III Pronounced reddening, total papillary atrophy and severe lobulation or deep fissuring.

Examples of Grades I, II and III are shown in Figures II, 8 - II, 14). Details of the clinical examinations in patients with Sjogren's syndrome are shown in Tables II, 3 - II, 5.

RESULTS

Sicca Syndrome

All thirty-six patients complained of xerostomia, twenty (56%) reporting the dryness to be intermittent in character whilst the remaining sixteen (44%) reported persistent oral dryness. All had clinical evidence of dry mouth. The mean duration of xerostomia prior to the initial examination was 4.4 years (range three months to 20 years.) All patients complained of dryness of the eyes and lack of tear secretion. Definite keratoconjunctivitis sicca was diagnosed in all thirty-six patients. The mean duration of dryness of the eyes prior to initial examination was 3.8 years (range six months to 17 years.) In general, patients were rather vague concerning whether the symptoms of xerostomia preceded those of keratoconjunctivitis sicca or vice versa. The majority of patients reported that the onset of the sicca symptoms in both the oral cavity and the eyes occurred about the same time.

A history of salivary gland enlargement was given by thirteen (36%) of patients with the sicca syndrome. However, the presence of salivary gland enlargement on examination was noted in only three (8%) of patients.

Both from the history and clinical observation, the parotid gland was most commonly involved.

An increased fluid intake was reported in twenty-eight (76%) of patients. The increase was confined to mealtimes in twelve (33%) patients and a general increase including mealtimes in sixteen (43%) patients. Difficulty in swallowing was experienced by four (11%) of the patients. None of the patients reported peculiarities of taste sensation. Twenty-four (67%) patients complained of oral soreness and eleven (31%) complained of oral ulceration. Fifteen of the thirty-three patients who wore dentures complained of difficulty with retention though in the majority of cases this problem could be related to the ill-fitting dentures rather than to the lack of saliva. Of the three patients with standing teeth, one reported that a large number of silicate fillings had broken down since the onset of xerostomia (Figures II, 15 - II, 16). Grade I lingual changes were observed in fifteen (42%) patients and Grades II and III in twelve (33%) and four (11%) patients respectively. Lingual changes were absent in only five (14%) of these patients with sicca syndrome.

Sjögren's syndrome with rheumatoid arthritis

Forty-eight (89%) patients complained of xerostomia of which thirty-one (57%) and seventeen (32%) were considered to be intermittent and persistent respectively. However, only forty (74%) were considered to have evidence of xerostomia on clinical examination. All but one patient complained of dryness of the eyes and fifty (93%) had definite keratoconjunctivitis sicca on ophthalmological examination.

An increased fluid intake in general was reported by sixteen (30%) patients whilst an increase related to meals only was reported in a further twenty (37%) patients. Difficulty in swallowing was experienced by six (11%) patients. No abnormalities of taste sensation were noted in any of these patients with Sjögren's syndrome complicated by rheumatoid arthritis. Oral soreness and oral ulceration were the complaints of forty-one (76%) and twenty-one (39%) patients respectively. Denture problems were reported by twenty-six of fifty-three (49%) of patients who wore dentures. The one female patient with standing

teeth had severe chronic gingivitis and was in need of conservative dental treatment (Figure II, 17).

A history of major salivary gland enlargement was noted in eleven (20%) patients, though clinical evidence of enlargement was observed in only three (6%) patients. The parotid gland was the most commonly involved (Table II, 4 and Figure II, 18).

Grade I lingual changes were noted in twenty-five (46%) whilst Grades II and III were observed in nine (17%) and five (9%) patients respectively. Lingual changes were absent in fifteen (28%) patients. It was a most interesting feature that two years to 48 years with a mean value for the group of 14.6 years in only two cases did the sicca component precede the onset of arthritic symptoms.

Rheumatoid arthritis alone

It was of interest that of eighty patients with rheumatoid arthritis alone, eleven (5o%) males and nineteen (33%) females complained of dryness of the mouth. However, in only four patients was a dry mouth confirmed on clinical examination. Of these

four patients, one suffered from Bell's palsy which had followed rupture of a parotid abscess, one had the Brown Kelly Paterson syndrome (see Chapter 16) and one was examined initially a few days following an arthroplasty. In this latter case, subsequent examination revealed a reasonably moist oral cavity. In the fourth patient, no cause of the xerostomia could be determined though drug abuse was suspected.

Two patients described an increased fluid intake especially at night. A history of salivary gland enlargement was given by three patients. Two patients complained of dryness of the eyes. About one-third of the patients complained of oral soreness and ulceration but this could be related to the presence of ill-fitting dentures. Grade I lingual changes were noted in twelve (7%) patients with rheumatoid arthritis. More severe grades were absent.

Osteoarthritis

Eight (33%) patients complained of xerostomia though a clinically dry mouth was observed in only one of these patients. Four females gave histories of salivary gland enlargement, unilateral parotid in two cases, bilateral parotid in one and bilateral sub-

mandibular in the other. However, in none of the patients was there evidence of gland enlargement on clinical examination. Lingual changes of Grade I severity were noted in the one patient with a clinically dry mouth. Oral soreness and ulceration were observed in a few cases only.

Psoriatic arthritis

Five patients complained of mild dryness of the mouth although in no instance was this confirmed clinically. None complained nor was there clinical evidence of salivary gland enlargement. Again the few cases of oral ulceration could be related to ill-fitting dentures. One male patient with a history of chronic alcoholism had gross dental caries due to neglect.

Akylosing spondylitis

Two patients complained of mild xerostomia, though on clinical examination oral and salivary gland changes were unremarkable.

Reiter's syndrome

Two patients complained of xerostomia. However, on clinical examination none had evidence of oral dryness. Only three of the twenty patients examined had oral lesions associated with the triad of non-gonococcal arthritis, bilateral conjunctivitis and oral ulceration (Figures II, 19 - II, 20). Keratosis blennorrhagica was observed in a few cases though arthritis was a common manifestation.

Gout

None of the three patients with gout complained of nor had clinical evidence of oral or salivary gland disease.

Behcet's syndrome

Both females with Behcet's syndrome complained of xerostomia and one had clinical evidence of dry mouth. Muco-cutaneous ulceration was observed at various times in both patients.

Systemic lupus erythematosus

Six patients of the ten female patients complained

of xerostomia and in four, there was clinical evidence of dryness of the mouth. Bilateral parotid enlargement was noted in three patients with clinically observed xerostomia, and two of these patients had Grade II lingual changes.

Progressive systemic sclerosis

Two of the seven females complained of xerostomia, though clinical evidence was detectable in only one of these patients. None gave a history of salivary gland enlargement. Two patients complained of a 'tightness' of the lips (Figure II, 21).

Dermatomyositis

The one young sixteen year old girl with dermatomyositis had no oral or salivary gland complaint nor was there clinical evidence of such involvement.

DISCUSSION

In his original publication, Sjögren (1933) states that nine of nineteen patients examined were suffering from xerostomia. However, the frequency of oral changes have been shown to be of the order of 80 - 95% in recent studies (Denko and Bergenstal, 1960; Bloch et al, 1965; Mason, 1966; Bertram, 1967. Apart from the studies mentioned above, recent reports of the oral and salivary gland symptoms of Sjögren syndrome have tended to be confined to a few or single case reports (Thonard, 1956; Ehrlich, 1965; Sussman and Mandel, 1965; Otano and Turo, 1965; Schwartz and Mandel, 1965; Jacobson, 1966; Cahn, 1967; Abramson et al, 1968; Sood, 1968; Feuerman, 1968).

In the present study, clinically observed xerostomia was noted in 74% of patients with Sjögren's syndrome with rheumatoid arthritis and in all patients with the sicca syndrome alone. These findings are in broad agreement with the major studies of Bloch and associates (1965), Mason (1966) and Bertram (1967). Oral dryness, decreased salivation, difficulty with mastication, increased fluid intake with meals or in general, fissuring and ulceration of mouth and lips

oral soreness and difficulty with denture retention were all common findings in patients with Sjögren's syndrome in this study. These features, especially persistent dry mouth and increased fluid intake were more severe in the sicca syndrome patients. It is also of interest that lingual changes were more common in sicca syndrome patients than those with Sjögren's syndrome complicated with rheumatoid arthritis. In a recent investigation, Bertram (1967) reported lingual changes, varying from slight reddening with mild fissuring to pronounced reddening with severe lobulation and deep fissuring, to be present in twenty-eight of thirty-four (82%) patients with Sjögren's syndrome. A disappointing feature of the present investigation was that an increased dental caries rate could not be assessed directly. As Mason (1966) has observed the large proportion of patients with Sjögren's syndrome are in the older age group and tend to be edentulous at the time of examination. However, it is to be noted that several workers have reported an increase in the frequency of dental caries and recurring breakdown of dental restorations have also been observed (Bloch et al, 1965; Jacobon, 1966; Bertram, 1967). Abnormalities of taste sensation were noted in only a few of thirty patients studied by Mason (1966).

However, in the present investigation, none of the patients reported this symptom.

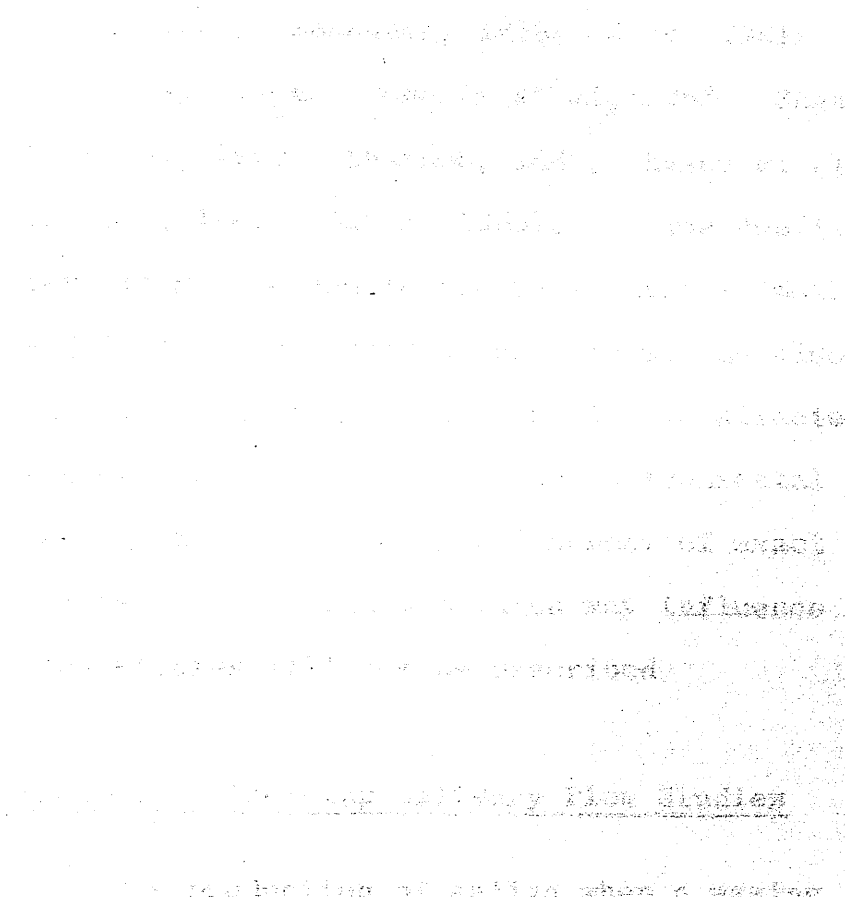
A most interesting finding was that despite approximately one-third of patients with Sjögren's syndrome giving a history of salivary gland enlargement, only six of the ninety patients with or without rheumatoid arthritis had clinical evidence of such involvement. Enlargement of the parotid salivary glands in about one-third of cases has been reported by Rauch (1959). However, Bloch et al (1965) reported a 50% involvement of the salivary glands as evidenced by glandular swelling. They comment that the parotid gland is more frequently involved than the submandibular glands. Salivary gland swelling was present in only four of the thirty patients studied by Mason (1966). Unilateral parotid swelling in four patients was reported by Bertram (1967) in his study of thirty-four cases of Sjögren's syndrome. Where salivary gland swelling occurred in the present study, the parotid gland was the most commonly affected.

With regard to the onset of the main component of the syndrome, it was of interest that only two

patients reported the sicca component preceding the onset of rheumatoid arthritis. Holm (1949) and Godwin (1952) have suggested that xerostomia occurs late in the disease and only in serious cases. It was of interest that thirty of eighty patients with rheumatoid arthritis alone complained of dryness of the mouth. However, in only a few was there clinical evidence of xerostomia. Nevertheless, the possibility of the existence in such patients of an early sub-clinical form of Sjögren's syndrome must be considered. It is to be noted, however, that MacSween et al (1967) reported a similar high percentage of symptomatic xerostomia in rheumatoid patients. In a proportion of these patients in which salivary flow studies were performed, the values were within the limits of a normal range.

The oral signs and symptoms of salivary involvement in the other connective tissue diseases, with the exception of systemic lupus erythematosus, were unremarkable. The association of systemic lupus erythematosus and Sjögren's syndrome has been reported (Heaton, 1958; Bencze and Lakatos, 1963). In this study, four of ten females had clinical evidence of xerostomia, strongly suggesting that in these patients with systemic lupus erythematosus, the disease

process was complicated by Sjögren's syndrome.



CHAPTER 4

PAROTID SALIVARY FLOW STUDIES IN CONTROL SUBJECTS

The quantitative production of parotid saliva has been the subject of many studies (Schneyer and Levin, 1955; Schneyer, 1956; Kerr, 1961; Curry and Patey, 1964; Sewards et al, 1966; Shannon and Chauncey, 1967; Shannon, 1967; Mason et al, 1967; Ericson, 1968; Dawes, 1969). The conflicting results may be due to the variations in methods used and in the experimental conditions, and also to the fact that the secretion of saliva is affected by a number of psychological and environmental factors making difficult the establishment of exact normal values. The factors which may influence salivary flow studies will now be described.

Factors influencing Salivary Flow Studies

The production of saliva when a waking person is at rest and unaffected by external stimuli may be termed resting secretion or spontaneous secretion (Babkin, 1950). Under essentially resting conditions, it has been shown (Schneyer and Levin, 1955), that the submaxillary glands contribute approximately 69%, the

parotid glands 26% and the sublinguals 59% of the total secretion derived from these three major gland pairs. Schneyer (1956) has further suggested that at rest the minor gland contribution is negligible. However, Östlund (1953) has shown that the palatal minor salivary glands are capable of continued spontaneous secretion after removal from the oral cavity and storage in Ringer's solution. Shannon (1962) has shown that with stimulated total salivary flow studies the percentage contribution by the parotid salivary glands rises to approximately 49%. Gore (1938) has suggested that in unstimulated collections the parotid contribution was slightly more than 50% of the whole saliva volume, and that paraffin stimulation resulted in a six fold increase in parotid flow while the mandibular saliva flow rate was slightly less than doubled. In two patients, Suhara et al (1959) found parotid flow to be 18.4 and 30.8 per cent of the total for the three pairs of major glands.

The extensive studies of total resting secretion (Becks and Wainwright, 1943; Gerke and Klemt, 1951; Mason, 1966) have shown that dispersion is very great so that groups which are small must not be used if the

secretions in different groups of persons are to be evaluated.

The dependence of resting secretions on sex has been studied by a number of workers. Brawley and Sedwick (1940) and Östlund (1953) found a significant sex difference in the amount of secretion per unit of time while others (Becks and Wainwright, 1943; Kerr, 1961) found no sex difference. However, a significantly higher secretion in men was shown by Bertram (1967). Secretion at relative rest has been shown to be lower in women by Ericson (1968).

Age of the subject is an important factor as has been shown by Becks and Wainwright, (1943). They found a lower mean value for salivary secretions in persons 50 - 95 years of age than in persons of 5 - 49 years. The difference, however, was not significant. Östlund (1953) found that the secretory capacity of the salivary glands decreases with age. Korting and Kleinschmidt (1953) showed that the resting secretions diminished with age but no difference was found by Brun and Domine (1958). Differences in division of the groups, however, may be of importance. Brun and Domine (1958) divided subjects into two groups at 50 years whilst Korting and Kleinschmidt (1953)

divided their groups at 65 years of age. Bertram (1967) divided his subjects at 65 years of age and from his material found that males had higher salivary secretion than females, that secretion diminished in old age and that persons in poor health produced less saliva. However, he concluded that xerostomia was not likely to arise as a result of the ageing process. Secretion diminished with age in the series reported by Ericson (1968). The method of collection is an important factor which influences the secretion of saliva (Kerr, 1961).

The methods used to measure total resting secretion are the draining method (Becks and Wainwright, 1943), the spitting method (Zaus and Fosdick, 1934) and the suction method (Fabian, 1938; Schneyer and Levin, 1954/55; Kerr, 1961; Bertram, 1967). Kerr (1961) carried out comparative tests of the three methods. The draining method gave the lowest secretion values, the spitting method higher values and the suction method gave the highest values. Other errors of method which may affect the values for resting secretion are collection time, fatigue leading to lowering of the flow rate (Zaus and Fosdick, 1934).

Hydration (Winsor, 1930; Kerr, 1961) and hyper-

hydration (Shannon et al, 1967) cause diminution in salivary secretion. Lower flow rates are observed in hospitalized patients (Bertram, 1967). Mental stress, similarly causes a reduction in salivation (Bates and Adams, 1968). Fatigue (Nekrasow and Ghranilowa, 1933), infection (Krasnotgorski, 1931) and temperature (Goldman et al, 1961), all affect salivary gland function. Cigarette smoking has been shown to cause increase in parotid salivary flow rates (Barylko-Pikielna et al, 1968). Recently, the affect of drugs, especially the tranquillizer and ganglion-blocking agents have been shown to cause marked oral dryness (Scopp and Heyman, 1966).

Diurnal variation of salivary flow is a further important factor to be considered during the assessment of salivary gland function. Salivary secretion has been shown to be lowest in the morning and highest in the afternoon (Hildes and Ferguson, 1958; Holmes, 1964). Environmental factors such as agitation, noise and room temperature can reduce the resting secretion of saliva (Goldman et al, 1961). Body weight does not appear to influence salivary secretion (Kerr, 1961; Bertram, 1967). As Kerr (1961) and Mason (1966) have shown, the muscle activity involved

in chewing on one side of the mouth leads to an increase in flow rate on that side.

The present chapter is concerned with the determination of the salivary flow rates of the parotid salivary glands in normal individuals. The following chapter deals with the parotid salivary flow rates in patients with various connective tissue disorders. A selective method for the collection of parotid saliva is required. Methods which have been described are Lashleys draining tube (1961) Krasnotgorski's draining chambers (1931) and Razran's absorbant cotton technique (1933). Recent studies by Mason (1966) established a reliable method of collecting parotid saliva by the use of a modified Carlsson-Crittenden cup (Carlsson and Crittenden, 191o). Ericson (1968) recorded parotid salivary flow rates using the method of Diamant, Diamant and Holmstedt (1957) which was developed and described in detail by Enfors (1962). Ericson described salivary volumes at rest and during stimulation with intra-venous acetyl-beta-methyl cholineiodide and intra-oral citric acid. The intra-venous injection of 5 mg. of pilocarpine and collection of parotid saliva over the following five minutes, forms the basis of a clinical test of parotid function described by Curry and Patey (1964). The

possible side effects to patients with cardiac lesions, however, cannot be overlooked and do not recommend this method for routine use.

In the present study, the method described by Mason (1966) was used to measure parotid salivary flow rates.

The control group consisted of 171 normal subjects, who were attending the Glasgow Dental Hospital and the Gardiner Institute, Department of Medicine, Western Infirmary, Glasgow, together with volunteer members of staff and students of the Dental Hospital. None of this control population had evidence of xerostomia, Sjögren's syndrome (Bloch et al, 1965), salivary gland disease, rheumatoid arthritis or other connective tissue disease. As far as possible, salivary collections were performed during the afternoon not less than one hour before or after a meal.

Sialometric Method

Parotid saliva was collected using a modified Carlsson-Crittenden cup with an internal chamber diameter of 10 mm., an external chamber diameter of 20 mm., and a depth of 4 mm. (Figure II, 23). The inner chamber was placed over the parotid duct

orifice and the cup maintained in position by air suction applied through the outer chamber. The source of air suction was a conventional water pump applied to a water tap. The pump had a pressure range of 0-760 mm. Hg. and the pressure applied was approximately 200-250 mm. Hg. For collections carried out in other hospitals, a rubber ball syringe was used to provide air suction allowing a portable saliva collection kit (Figure II, 24). The cup is constructed from nylon which is well tolerated by the oral mucosa. Saliva was collected from the parotid salivary gland under resting conditions and following fruit gum, lemon juice and occasionally paraffin wax stimulation. Following application of the cup, one drop of lemon juice was applied to the patient's tongue. This initial stimulus flushed out stagnant secretion and the saliva collected during the following 10 mins. was discarded to avoid "salivary rest transients" (Kestigus and Martin, 1937; Mason, 1966). The procedure therefore for routine parotid saliva collection was as follows:

1) The patient was seated comfortably in the dental chair and the procedure explained to him.

2) The collection cup was applied and a drop of lemon juice applied to the tongue. Saliva collected during the following 10 mins. was discarded.

3) Saliva was then collected under conditions of "rest" or minimal stimulation for 30 min., fruit gum stimulation for 5 min., and lemon juice for 2 min. These stimuli were applied for 2 min. before the collection of the sample commenced. Saliva was collected into sample bottles which were weighed accurately before and after collection of the sample. The maximum error involved in assuming the specific gravity of salivary secretion as 1.000 is 1% (Kerr, 1961).

RESULTS

Salivary Flow Rates in normal subjects

The salivary flow rates obtained in normal subjects are shown in Tables II, 6 - II, 7 and Figures II, 25 - II, 30. Tables II, 6 - II, 7 show the values in groups of similar age and sex, and values being expressed in ml/min. It can be seen that under resting conditions there is no significant difference in mean parotid salivary flow rates between males and females when each is compared with the corresponding age group. With regard to the effect of age on resting parotid salivary flow rates in males, the mean flow rate under 20 years is highly significantly lower, ($P < 0.0005$), compared

with the mean flow rate between 21 and 40 years. However, the resting parotid salivary flow rates in males between 41 and 60 years, and over 61 years of age, are not statistically significantly different from each other, or from the resting parotid salivary flow rates in males under 20 years or between 21 and 40 years of age.

With regard to the effect of age on the resting parotid salivary flow rate in female subjects, the mean flow rate in the 21 to 40 year age group is higher, but not significantly, than the mean flow rate in females under the age of 20 years. There is, however, a significant fall ($P < 0.05$) in the mean resting parotid salivary flow rates in the 41 - 60 year old females when compared with the females between 21 and 40 years of age. No difference was noted when resting salivary flow rates in the females over 61 years of age were compared with the resting flow rates in females between the age of 41 and 60 years. It is also of interest that the mean resting parotid salivary flow rates in females under the age of 20 years did not differ significantly from that in the 41 to 60 year old group of female subjects.

With moderate parotid salivary gland stimulation

with fruit gums, it was found that in only those control subjects under the age of 20 years was there a significant difference in parotid flow rates between males and females ($P < 0.05$). There was no significant decrease in parotid salivary flow rates with increasing age when fruit gum stimulation was employed. However, in the female controls, a definite tendency to reduction in parotid salivary flow rate was noted in the older age groups.

Under conditions of maximal parotid salivary secretion following lemon juice stimulation, definite changes in salivary flow were noted when male controls were compared with females. Under 20 years of age, the mean flow rate in males was highly significantly lower ($P. < 0.005$). In those patients between the ages of 21 and 40 years, salivary flow rates were almost identical in both sexes. However, in the 41 to 60 year old control subjects, females had significantly lower flow rates than males ($P < 0.05$). The same pattern was noted in the group of controls of 61 years of age or older ($P < 0.01$). From Tables II, 6 - II, 7, it can be seen clearly that the sex difference in parotid salivary flow rates under conditions of maximal stimulation with lemon juice is due to a marked decrease of salivary flow rates in the female controls in the older age groups.

Females between 21 and 40 years of age have a significantly lower mean flow rate than those under 20 years ($P < 0.005$).

The salivary flow rates in females between the ages of 41 and 60 years of age is significantly lower than the mean maximal parotid salivary flow rates in females between 21 and 40 years of age, ($P < 0.001$). Again, females over the age of 61 years show a further significant fall in mean maximal parotid salivary flow rate, ($P < 0.001$).

In male patients, maximal parotid salivary flow rates also tended to fall with age, but in no instance was the decrease statistically significant.

DISCUSSION

The aim of this part of the study was to investigate, in normal subjects, the effect of age and sex on parotid salivary flow rates at three levels of flow: resting, during moderate stimulation with fruit gum, and during maximal stimulation with lemon juice. Under resting conditions, no sex or age differences were observed, whereas under fruit gum stimulation, the mean parotid salivary flow rate decreased with age in the female subjects, although not significantly when lemon juice was used to provoke a maximal response of the salivary gland. The mean parotid salivary flow rates in females between the ages of 41 and 60 years, and over the age of 61 years were significantly lower than the corresponding flow rates in the male subjects. This is due to a marked decline in parotid salivary flow rates in females with increasing age. The exact reason for the decline in the ability of the parotid gland, in middle-aged/elderly females to produce saliva is unknown. It is unlikely to be due to females having smaller salivary glands than males, as the mean lemon juice stimulated parotid salivary flow rate in females between the ages of 21 and 40 years is similar to those flow rates recorded in males, and

under the age of 20 years it is significantly higher in the females. Waterhouse and Winter (1964) have provided evidence for a trend of replacement of functional secreting cells by fat and connective tissue in human submandibular glands through adult life. In a randomly chosen sample of thirty-six subjects (six from each decade from 25 to 84 years), they reported an average loss of about a quarter of the relative secretory cell volume between the end of active growth and old age, by which time the more extremely affected glands may have lost one-half. No account was taken, however, of the sex distribution. Waterhouse and Doniach (1966) have demonstrated that focal areas of lymphocytic infiltration could be found in the major salivary glands at autopsy, and these increased in frequency with increasing age. The prevalence of these foci was significantly increased in females, suggesting that the female patients may develop subclinical Sjögren's syndrome, manifested by diminished salivary flow rates. This situation may be analogous to that occurrence in the thyroid gland in which the thyroid microsomal autoantibody has been shown to be present in 8% of middle-aged women (Hymans et al, 1961). Furthermore, the autoantibody is associated with focal, or diffuse thyroiditis resembling classical Hashimoto's disease in minor form (Goudie et al,

1959). In females, at the age of 50 years, a sharp increase in the incidence of the antibody to 16% is observed and this has been shown to correspond to the increased frequency of focal thyroiditis and oxyphilic epithelium found in this age group (Lennox, 1948; Goudie et al, 1959; White et al, 1961).

A striking increase in the frequency of thyroglobulin antibodies has been demonstrated in females of 30 years of age; whereas in males, there is only a slight increase (Hackett et al, 1960; Hill, 1961). However, no studies have been reported correlating directly asymptomatic thyroiditis and thyroglobulin autoantibodies. Hill (1961) drew attention to the fact that the age incidence of thyroglobulin antibodies in apparently normal individuals is similar to the age of onset of primary myxoedema and Hashimoto's disease. The sex ratio of the antibodies is similar to that of primary myxoedema. However, there is apparently no decrease in the serum protein-bound iodine levels with increasing age, and the uptake of radioactive iodine into the thyroid gland in elderly females, (although slightly subnormal at six hours is normal at 24 hours), (Shock, 1968). The ability of the thyroid gland to respond to thyroid stimulating hormone as judged by the serum protein-bound iodine levels is

not impaired in elderly patients (Shock, 1968). Likewise, the gastric parietal cell autoantibody, which has been shown to be associated with the presence of chronic gastritis (Adams et al, 1964; Te Velde et al, 1964) shows a marked increase in incidence in females over the age of 40 years (Irvine and Davies, 1964; Anderson et al, 1967).

It is of interest that although the gastric parietal cell autoantibody shows an increased incidence in females, there is a reduction in gastric secretory function, at both a basal level and in response to histamine in both males and females over the age of 60 years (Grossman et al, 1963). The decrease in gastric secretion was more marked in males, but never became less than gastric secretion in females.

Sjögren's syndrome is associated with the presence of thyroglobulin autoantibodies (Bloch et al, 1960; Anderson et al, 1961; Bunim, 1961; Bloch et al, 1965) and occasionally with clinical evidence of chronic thyroiditis, for example Hashimoto's disease and primary myxoedema (Bloch et al, 1965). Also, there is a significant association between the presence of Sjögren's syndrome and gastric parietal cell auto-

antibodies (Anderson et al, 1965). Waterhouse and Doniach (1967) demonstrated clearly that focal sialadenitis was related to the presence of thyroiditis found at post-mortem examination in normal individuals. It is possible from this evidence that the decrease in salivary flow rates in females, might be due to a focal Sjögren's syndrome.

However, the decrease in mean lemon juice stimulated parotid salivary flow rates begins in the 41 - 60 year-old females and it may be that the decrease is related to ovarian failure with consequent oestrogen deficiency. There is no evidence to support this statement; but it is of interest that patients with cirrhosis of the liver, who allegedly cannot metabolise oestrogens and consequently gynaecomastia and testicular atrophy may develop in males as an occasional parotid gland enlargement may.

It is also of considerable interest that diethyl-stilboestrol when administered to euthyroid patients in a dose of 2 mgm. daily, increased the average uptake of a tracer dose of $^{I}131$ (Jensen, 1959). Apart from a direct effect on the thyroid gland, sex

hormones may possibly suppress thyroiditis as it has been convincingly shown that the titre of thyroglobulin autoantibodies decreases in pregnancy and it has been suggested that this may be a hormonal effect (Parker and Beierwaltes, 1961; Hjort and Pederson, 1962). The increase in the prevalence of thyroid and gastric parietal cell autoantibodies in middle-aged females, may also be due to a hormonal deficiency as only a slight rise in the prevalence of these autoantibodies occurs in male patients. From this discussion, it is obvious that if hormonal deficiency does play a part in the decline in salivary flow rates in ageing females, it may be either through a direct effect of gland function or, in some way, facilitating a chronic inflammatory process to occur within the salivary glands.

At a practical level, these findings suggest that when an assessment of parotid salivary function is being made, using flow rate as an index, it is essential to compare the result with the range of normal values obtained from control subjects of the same age and sex distribution.

Finally, the fact that a definite decrease in parotid salivary flow rates in females was obtained

only during lemon juice stimulation, it is suggestive that this is the most sensitive clinical index of salivary gland function, as the maximum capacity of salivary gland to produce saliva is being tested.

SUMMARY

Parotid salivary flow rates have been measured in 171 normal subjects under resting conditions, during moderate stimulation with fruit gum and during lemon juice stimulation. It has been shown that there is little effect of either age, or sex, on mean resting or fruit gum stimulated parotid salivary flow rate. However, in females, a significant reduction was noted in the mean lemon juice stimulated parotid salivary flow rate with increasing age. The possible explanations of this observation have been discussed. It is suggested that salivary flow rates should be compared to the normal range of an age and sex-matched control population and that the lemon juice stimulated salivary flow rate was the most sensitive index of salivary gland function.

CHAPTER 5

PAROTID SALIVARY FLOW STUDIES IN THE CONNECTIVE TISSUE DISEASES

Materials and Methods

Patients studied

The clinical diagnosis, age and sex distribution of the patients studied in this part of the investigation are shown in Table II, 8. Parotid salivary flow studies were performed as described in Chapter 4.

Results

The results of resting, fruit gum stimulated and lemon juice stimulated parotid flow rates in males and females with rheumatoid arthritis, rheumatoid arthritis and Sjögren's syndrome, and the sicca syndrome are shown in Figures II, 31 - II, 36, and Tables II, 9 - II, 14. The results are presented for patients between 21 and 40, 41 and 60 years and 61 years or over, as has been shown in the previous chapter, since parotid flow tends to decline with advancing years, especially in females.

Sicca syndrome

Two of the three male patients with the sicca syndrome had low normal parotid flow rates under resting conditions and during moderate stimulation with fruit gum, but all three had subnormal flow rates following stimulation with lemon juice (Figures II, 31 - II, 33).

The twenty female patients with the sicca syndrome had very low mean parotid flow rates under all three conditions of salivary collection when compared to control subjects. Patients aged between 41 and 60 years had a mean resting flow rate which is significantly lower ($P < 0.001$), than the control group. The mean fruit gum stimulated parotid flow rate was also significantly lower ($P < 0.001$), than the controls as was the mean lemon juice stimulated parotid salivary flow rate ($P < 0.001$). Female sicca syndrome patients over the age of 61 years had a mean resting parotid flow rate which was significantly lower ($P < 0.005$) than that of the control group. The mean flow rate following fruit gum stimulation was significantly lower ($P < 0.001$). Following lemon juice stimulation, the mean parotid flow rate was considerably lower ($P < 0.001$) than the mean value in normal females.

Sjögren's syndrome and rheumatoid arthritis

Mean parotid flow rates in male patients with rheumatoid arthritis and Sjögren's syndrome were lower than those in age and sex-matched control subjects. Those male patients between 41 and 60 years had a significantly lower ($P < 0.05$) mean resting flow rate than the controls. The mean fruit gum stimulated flow rate although lower than that of the controls is not significantly so. In contrast, the mean lemon juice stimulated flow rate is significantly less ($P < 0.001$), than the controls. Unfortunately, only four male patients with rheumatoid arthritis and Sjögren's syndrome over the age of 61 years we included in the study and hence statistical analysis of the results was impossible. During the lemon juice stimulation, all had subnormal salivary flow rates. However, under resting conditions and during fruit gum stimulation three patients had parotid flow rates within the normal range.

Female patients with Sjögren's syndrome and rheumatoid arthritis exhibited a similar pattern of mean parotid flow rates. In the group of female patients under the age of 40 years the mean parotid flow rate was significantly lower ($P < 0.005$)

than that in the female controls, as were the mean fruit gum stimulated ($P < 0.001$) and the lemon juice stimulated parotid salivary flow rates ($P < 0.001$). Female patients between 41 and 60 years again had significantly lower ($P < 0.001$) mean resting flow rates than controls. The mean fruit gum stimulated parotid flow rate was also significantly lower ($P < 0.001$), than the female control value as was the mean lemon juice stimulated parotid salivary flow rate ($P < 0.001$). The female patients over the age of 61 years had a significantly lower mean resting flow rate ($P < 0.01$), a significantly lower mean fruit gum stimulated flow rate ($P < 0.001$) and a mean lemon juice stimulated flow rate which is significantly lower ($P < 0.001$) than the control mean value.

Mean flow rates in the patients with Sjögren's syndrome and rheumatoid arthritis are all higher than those in patients with the sicca syndrome but only in the females aged between 41 and 60 years, and over 61 years were there sufficient numbers for statistical analysis. In females between 41 and 60 years, the resting flow rate, although lower in the sicca patients, is not significantly lower whereas with fruit gum stimulation ($P < 0.05$) and lemon juice

stimulation ($P < 0.05$) the differences are significant. In females over 61 years of age, the mean resting flow rate, although lower in patients with the sicca syndrome, is not significantly different but with fruit gum ($P < 0.005$) and with lemon juice stimulation ($P < 0.05$) the mean value is also significantly lower.

Rheumatoid arthritis

Mean parotid flow rates performed on male patients with rheumatoid arthritis did not differ significantly from age and sex-matched control values. Female patients with rheumatoid arthritis between 21 and 40 years had mean resting and fruit gum flow rates, which were not significantly different from controls. However, mean lemon juice stimulated flow rates were significantly lower ($P < 0.005$). Female rheumatoid patients between 41 and 60 years had a mean resting flow rate which was significantly lower ($P < 0.05$) than controls. The mean fruit gum stimulated flow rate ($P < 0.001$) and the mean lemon juice stimulated flow rate ($P < 0.05$) were also significantly lower than controls. The mean resting flow rate in rheumatoid females over 61 years was lower than in controls but the

difference is not significant. However, the mean fruit gum stimulated flow rate is significantly lower ($P < 0.001$) as is the mean lemon juice stimulated flow rate ($P < 0.05$). The mean parotid flow rates in patients with rheumatoid arthritis were also compared with mean flow rates in patients with Sjögren's syndrome and rheumatoid arthritis. The male patients with rheumatoid arthritis and Sjögren's syndrome had lower mean flow rates than male patients with rheumatoid arthritis alone. The mean resting ($P < 0.01$) and mean lemon juice stimulated flow rates ($P < 0.001$) were significantly different. There were only four males with rheumatoid arthritis and Sjögren's syndrome over 61 years, hence statistical analysis was not possible.

Female patients with rheumatoid arthritis alone also had higher mean flow rates than females with Sjögren's syndrome and rheumatoid arthritis. Patients between 21 and 40 years had a significantly higher mean lemon juice stimulated flow rate ($P < 0.001$). All three flow rates were significantly higher in rheumatoid patients between 41 and 60 years. The same pattern was observed in female patients over 61 years. All differences were significant at the level $P < 0.001$.

Osteoarthritis

There was only one female in the 21 - 40 year old age group and the mean parotid flow rates at rest and following fruit gum and lemon juice stimulation were 0.14, 0.76 and 1.40 ml/min. respectively. The mean flow rates for the five females in the 41 - 60 year old age group were 0.07, 0.56 and 1.70 ml/min. respectively. Three males were within this age group and the mean flow rates were 0.09, 0.49 and 1.41 ml/min. The six females over 6o years had mean flow rates of 0.14, 0.51 and 1.22 ml/min. respectively. The mean flow rates of the one male in this age group were 0.09, 0.53 and 1.72 ml/min.

One seventy-one year old female had a lemon juice stimulated flow rate of 0.40 ml/min. in the right parotid gland whilst the left parotid flow rate under similar stimulation was 0.52 ml/min.

In females in the 41 - 60 year old age group, lemon juice stimulated flow rates were significantly higher than in the control groups ($P < 0.05$). For females over 60 years of age, resting flow rates were significantly lower than the control group ($P < 0.05$).

Ankylosing spondylitis

The seven male patients in the 21 - 40 year old age group had mean parotid flow rates of 0.08, 0.34 and 1.54 ml/min. respectively. Only one female was included in this age group and her mean flow rates were 0.11, 0.55 and 1.90 ml/min. The remaining seven male patients were within the 41 - 60 year old age group and the mean flow rates were 0.11, 0.48 and 1.45 ml/min. respectively. The mean flow rates of the two females in this age group were 0.07, 0.35 and 0.99 ml/min. respectively. Lemon juice stimulated flow rates only were assessed in the remaining 73 year old female and the mean flow rate was 1.61 ml/min.

In male patients between 21 and 40 years the resting parotid flow rates were significantly reduced compared to the control subjects ($P < 0.05$). There were insufficient numbers of females for statistical analysis though at no flow measurement in any of the patients were the rates reduced compared to the control series.

Psoriatic arthritis

The mean parotid flow rates for the two females

in the 21 - 40 year old age group, were 0.04, 0.35 and 1.47 ml/min. respectively and for the one male, 0.09, 0.47 and 1.94 ml/min. respectively. Eight females were within the 41 - 60 year old age group, and the mean flow rates were 0.06, 0.36 and 1.41 ml/min. The mean flow rates for the two males in this age group were 0.09, 0.33 and 1.36 ml/min. The mean flow rates for the remaining male and female patients were 0.07, 0.19 and 0.88, and 0.03, 0.85 and 1.02 ml/min. respectively. Both these patients were over 61 years of age.

In females in the 21 - 4o year old age group lemon juice stimulated flow rates were significantly lower than control subjects ($P < 0.05$) and fruit gum stimulated flow rates in females in the 41 - 60 year old age group were also significantly lower than the control group ($P < 0.05$).

Reiter's disease

In the 21 - 40 year old age group the mean flow rates were 0.11, 0.51 and 1.67 ml/min. respectively. The mean flow rates in the 41 - 60 year old age group were 0.09, 0.49 and 1.41 ml/min. respectively. In no instance were flow rate values outwith the normal

range for three conditions collection.

Gout

Only three patients (two females and one male) with gout were examined. All patients were over 6o years of age. For the females, the mean flow rates were 0.04, 0.43 and 1.15 ml/min. The male patient had mean flow rates of o.13, o.65 and 1.73 ml/min.

Behcet's syndrome

One 27 year old female and one 42 year old female with Behcet's syndrome had salivary flow studies performed. The mean flow rates for the younger patient were 0.06, 0.31 and 1.39 ml/min. and for the older patient the values were 0.02, 0.25 and 0.87 ml/min. respectively.

Systemic lupus erythematosus

Four (40%) patients had lemon juice stimulated flow rate values that were well below the lower limits of the control subjects. All four patients complained of xerostomia and had clinical evidence of xerostomia on examination. Two patients had

a history of bilateral parotid gland swelling. The mean parotid flow rates for these four patients were 0.01, 0.14 and 0.44 ml/min. for resting, fruit gum and lemon juice stimulation respectively. Two patients were over 60 years of age and the mean values for these patients were 0.002, 0.09 and 0.26 ml/min. The other two female patients were within the 21 and 40 year age group and the mean values were 0.01, 0.18 and 0.62 ml/min.

Of the other six females with systemic lupus erythematosus, four were between 21 and 40 years of age. The mean flow rate values were 0.04, 0.35 and 1.22 ml/min. The remaining two subjects were in the 41 - 6o year old age group and their mean flow rate values were 0.02, 0.39 and 1.20 ml/min. Two of these six patients complained of xerostomia but none had clinical evidence of dryness of the oral cavity on examination. In each instance, the flow rate values were within the normal control range.

Progressive systemic sclerosis

There was one female patient aged seventeen, who gave a history of salivary gland enlargement, and her mean parotid flow rate values were 0.03,

0.31 and 1.00 ml/min. respectively. Two patients were within the 21 - 40 year age group and the mean flow rate values were 0.03, 0.42 and 1.25 ml/min. The remaining four patients were included in the 41 - 6o year old age group and the mean flow rate values were 0.02, 0.31 and 1.04 ml/min.

With the exception of the 17 year old female, whose resting and lemon juice stimulated flow rates were below the normal control range, all values were within normal limits.

Dermatomyositis

Parotid salivary flow measurements were undertaken for a sixteen year old Sikh girl with dermatomyositis. The mean flow rate values were 0.04, 0.41 and 2.10 ml/min. respectively.

DISCUSSION

Considerable attention has been devoted to the connection between keratoconjunctivitis sicca and rheumatoid arthritis (Holm, 1949; Henderson, 1950; Thompson and Eadie, 1956; Blatz, 1961), though the importance of xerostomia in Sjögren's syndrome is divided owing to the criteria for the investigation.

In the present study, patients with the sicca syndrome and patients with Sjögren's syndrome and rheumatoid arthritis had markedly reduced parotid flow rates when compared to the age and sex-matched controls. Patients with the sicca syndrome had greater reduction than patients with Sjögren's syndrome and rheumatoid arthritis, an observation which was also noted by Bloch et al (1965). This, however, is probably due to the diagnostic criteria employed (Bloch et al, 1965) since xerostomia need not be present for the diagnosis of Sjögren's syndrome in patients with rheumatoid arthritis. Mason et al (1967) showed that nine of fourteen patients with Sjögren's syndrome had reduced values for resting and stimulated salivary secretion. Of 35 patients with Sjögren's syndrome examined by

Bertram, 27 had xerostomia and a clear correlation between an increasing degree of lingual alteration and reduced salivary secretion was shown. When the patients with Sjögren's syndrome are considered as a group, 50 of 102 (49.2%) had normal parotid flow rates under resting conditions 51 of 105 (48.6%) following fruit gum stimulation and 35 of 116 (31%) following stimulation with lemon juice. The difference between the number of patients with Sjögren's syndrome and patients with normal parotid flow rates following lemon juice stimulation is significantly less than the number with normal parotid flow rates under either resting conditions ($P < 0.005$) or following fruit gum stimulation ($P < 0.005$). It would, therefore, appear that parotid salivary flow rates under maximal stimulation with lemon juice provides the best index of parotid gland hypofunction in Sjögren's syndrome. The function of the salivary glands in cases of rheumatoid arthritis alone have been studied by Günther (1959), Bremova et al (1962), Lenoch et al (1964) and Ericson (1968). Günther found a generally lower volume of saliva in 30 rheumatoid cases compared to 20 healthy individuals. Bremova et al considered xerostomia to be present in 14% of 25o patients with rheumatoid arthritis, whilst

Lenoch and his colleagues reported a tendency to lower salivary gland secretion. Ericson showed that the rate of parotid secretion was significantly lower in a rheumatoid group than in a control group, on stimulation with 1% and 6% citric acid. No significant differences were noted between the groups when resting secretions were recorded. A qualitative difference in xerostomia between cases with rheumatoid arthritis and controls has also been noted (Ericson and Jacobsson, 1968). Reduction in salivary secretion of the parotid gland in patients with rheumatoid arthritis uncomplicated by Sjögren's syndrome was noted by Ericson (1968) and has been confirmed in the present study especially in female patients. Of the 15 patients with subnormal salivary flow rates, nine (60%) had symptomatic xerostomia whereas it occurred in forty-five (33.8%) of 133 patients had normal flow rates. This difference is significant $X^2 = 5.05390$ $P < 0.05$. Possible keratoconjunctivitis sicca was not noted in any of the patients with diminished salivary flow rates. These findings suggest that the reduction in parotid flow rates in rheumatoid arthritis may be due to the presence of focal Sjögren's syndrome in the parotid glands. The situation is perhaps akin to focal lesions of chronic thyroiditis which are commonly found in

middle-aged and elderly females, and which are associated with thyroid auto-antibodies and a slight but significant lowering of the serum protein-bound iodine (Buchanan et al, 1965). It is possible, however, that other mechanisms may be operative, such as atrophy of the salivary glands as part of the general wasting process in rheumatoid arthritis. Furthermore, the effect of analgesic and corticosteroid drug therapy on salivary secretion is unknown. There have been no detailed studies of salivary flow rates in patients with connective tissue diseases other than rheumatoid arthritis. With the exception of systemic lupus erythematosus, the parotid salivary flow studies in the various other connective tissue diseases were within normal limits. The four female patients with systemic lupus erythematosus and markedly reduced flow rates, all had clinical evidence of xerostomia and two gave a history of bilateral parotid swelling. By definition (Bloch et al, 1965), these patients have Sjögren's syndrome.

SUMMARY

Parotid salivary flow rates at rest and following fruit gum and lemon juice stimulation have been measured in patients with connective tissue

disease. Compared to a control group, flow rates were significantly reduced in patients with the sicca syndrome and Sjögren's syndrome with rheumatoid arthritis. Flow rates were also lower in patients with rheumatoid arthritis, especially females, and suggest that these patients may have subclinical Sjögren's syndrome: other mechanisms, however, may be responsible for the reduction in salivary flow rates. With the exception of four patients with systemic lupus erythematosus, a salivary gland defect could not be detected in patients with various other connective tissue disorders. It has also been suggested that salivary flow rates performed during lemon juice stimulation provide the best measure of impaired salivary gland secretary capacity.

CHAPTER 6

SIALOGRAPHY

Sialography is the radiographic demonstration of the ductal system of the salivary glands, after the injection of contrast medium into the excretory ducts. The technique of introducing radio-opaque material into salivary glands was first described by Barsony in 1925. The method has proved a useful diagnostic aid in the clinical examination of patients with disease of the salivary glands and demonstrates the anatomical changes which may be present in a wide variety of conditions. Sialography has been used not only to evaluate duct architecture but also to assess salivary gland function (Rubin and Holt, 1957).

Two main methods of introducing contrast material into the excretory ducts have been described in the literature. These are the hand injection technique and the hydrostatic method. In the former, two principles are described. Firstly, a minimum volume of medium varying from 1.5 to 2.5 ml. is injected at a slow rate (Rose, 1950; Ollerenshaw and Rose, 1956; Carusi, 1964). In the second method of hand injection, the volume

introduced is adapted to the patients sensation of pain (Blady and Hocker, 1938; Rubin et al, 1955; Diamant and Forsberg, 1959). According to Rubin et al (1955) this method allows o.5 - 1 ml. to be introduced without the risk of overfilling and thus reducing the diagnostic value.

The method of introduction of contrast media by hydrostatic means was first described by Gullmo and Böök-Hederström (1957) and developed by Drevattne and Stiris (1964). Modifications of this technique have been described by Park and Mason (1966) and Park and Bahn (1969). Park and Mason (1966) devised a sialographic technique whereby a water-soluble contrast medium (Triosil '45') was introduced into the salivary duct system at a contrast pressure of 70 - 90 cms. of H_2O. The latter was achieved using a 20 cc. syringe and tubing set at a height of 70 - 90 cms. above the gland to be investigated. At this pressure, filling of the gland occurred in about 5 - 10 seconds and overfilling rarely occurred.

Materials and Methods

Equipment

The essential instrumentation for hydrostatic sialography is shown in Figure II, 37.

Preparation of Catheters

A polyethylene catheter is heated over an alcohol flame (Figure II, 38), till it softens and can be drawn out by application of gentle traction. A small blister usually forms and if the catheter is removed from the heat whilst traction is maintained, it will harden and retain its new shape. It may then be cut to the desired length through its narrowest portion.

Technique

As already described, the technique of sialography is an established and useful method of investigating salivary glands in disease, and in the present study the hydrostatic technique of Park and Mason (1966) was employed.

Plain radiographs are taken first to demonstrate sialolithiasis, calcification or gland enlargements.

This technique of hydrostatic sialography involves two phases -

1) passive filling

and

2) active emptying

Before commencing the examination, it is briefly explained to the patient, who is asked to indicate when discomfort or pain is felt by raising the right hand. With the apparatus assembled, the syringe barrel is filled with contrast material and set at a height of 70 cms. above the level of the patient's mouth. The medium is allowed to run through the catheter system freely to expel all air bubbles. The patient is placed on a skull table in the supine position. Under good illumination the parotid duct orifice is located with a lacrimal probe (Figure II, 39). The patient's cheek is gently everted with the thumb, thus stretching the mucosa and dilating the duct opening. In this way, the site of the duct orifice, usually on the apex of a small papilla, is made easier to locate. The tapered end of the catheter is then introduced 0.5 to 1 cm. into the duct (Figure II, 40). Once the catheter is inserted into the duct opening, the periductal tissues contract around it in an almost sphincteric action that prevents the catheter from becoming dislodged.

If difficulty is experienced due to insufficiently dilated duct openings, then dilation of increasing diameter may be introduced until the opening is adequate for acceptance of the catheter, and the patient is asked to grip the catheter gently with the lips and catheter loop taped with adhesive (Figure II, 41.), to the side of the forehead for added stability. At this point, the exposure is made at once while the contrast agent is flowing. The tap is then closed to prevent unnecessary distension of the gland. Antero-posterior and lateral oblique projections are usually adequate and the same procedure is repeated for each view (Figure II, 42 - II, 43). The patient is given a slice of lemon to suck immediately after the passive filling phase is completed and the catheter is removed from the mouth. Films may be taken to provide a simple and quick assessment of secretory function during the emptying phase. A normal gland actively expels the water - soluble contrast media as soon as the catheter is removed and complete emptying occurs within about half a minute. After a five minute interval further exposures are obtained, and this secretory phase film normally shows no evidence of residual contrast medium. Retension of the contrast agent within the gland is considered

abnormal when water - soluble contrast media are used (Park and Mason, 1966).

Criteria for Gland Filling

The following criteria for satisfactory filling and delineation of the duct system were adopted. Filling was considered satisfactory, if there was -

i) Complete definition of all ducts, including the fine terminal branches of the peripheral ducts.

ii) "Acinar clouding" - a hazy appearance in the sialogram characterises this phenomenon on which most authors regard as normal (Ollerenshaw and Rose, 1951, 1956; Samuel, 1950). Disease would be suspected if this sialo-acinar reflux is persistently absent (Park and Mason, 1966).

iii) Unless the gland is grossly distended before the examination starts, the degree of swelling provides good evidence of gland filling and will be visible and palpable.

Underfilling may occur, especially in the anxious or nervous patient who may stop the filling phase prematurely. In order to differentiate this from persistent pathological non-filling, antero-posterior, and lateral oblique views are taken in succession, so that complete filling is ensured with the third film. Overfilling is usually prevented by painful stimuli in provoked patients. Using the technique described, Park and Mason (1966) reported no cases of overfilling in their series of 108 patients.

Criteria of sialographic abnormality

The criteria used to evaluate each radiograph were based on those described by Blatt et al (1956) and Bloch et al (1965).

Blatt et al (1956) recognised four types of sialectasis: punctate, globular, cavitary and/or destructive. In addition to these findings, atrophy of the duct system has been mentioned by Ericson (1968). In this study, three degrees of sialectasis were recognised:

i) punctate - where the sialectatic defect is less than 1 mm. in diameter.

ii) globular - where the defect is 1 - 2 mm. in diameter, and

iii) cavitary - where the defect is more than 2 mm. in diameter,

Atrophy was defined as 'sparsity of the duct branches with diminution in their calibre'. Where it occurred, main duct dilatation was noted. To ensure accurate assessment of sialectasis, templates were constructed and superimposed on the films being examined. Examples of normal and abnormal sialograms are shown in Figures II, 44 - II, 48.

Patients studied

Sixty-four patients with Sjögren's syndrome were included in this study. Twenty-one had the sicca syndrome and forty-three had Sjögren's syndrome associated with rheumatoid arthritis. Nineteen of the patients with sicca syndrome were female and two were males, whereas thirty-eight of the patients with Sjögren's syndrome and rheumatoid arthritis were females, and five were males. All but two patients in each group had both parotid glands examined so that in all 124 patients' parotid

glands from this group were included in the series.

Unilateral parotid sialographic examination was performed in a further 121 patients with various other connective tissue disorders, including rheumatoid arthritis alone.

In addition, 45 subjects without clinical evidence of salivary gland disease or connective tissue disease were examined by unilateral parotid sialography and included as a control group.

RESULTS

Sjögren's syndrome and control group

The distribution of sialographic abnormality is shown in Table II, 15. Forty-five salivary glands were normal, eight (20%) with sicca syndrome, thirty-seven (44%) with Sjögren's syndrome complicated by rheumatoid arthritis. Fifty-nine showed sialectasis, of which twenty-nine were punctate, fifteen globular and fifteen cavitary. Atrophy of the duct system was noted in fourteen glands and a further six showed evidence of dilatation of the main duct, without evidence of sialectasis or atrophy.

Mean parotid salivary flow rates for these patients after lemon juice stimulation are also shown in Table II, 15. The mean lemon juice stimulated parotid salivary flow rates of those glands with normal sialograms was 0.85 ml/min. (S.E. 0.07 ml/min.), whereas with those with punctate sialectasis was 0.38 ml/min. (S.E. 0.04 ml/min.). This difference is significant ($P < 0.005$). The mean lemon juice stimulated parotid salivary flow rate for those glands showing globular sialectasis were slightly lower than those with punctate sialectasis (0.34 ml/min., S.E. 0.06 ml/min.); but this is not significant. Those salivary glands exhibiting cavitary sialectasis had a mean lemon juice stimulated parotid salivary flow rate of 0.13 ml/min. (S.E. 0.04 ml/min.). This difference is significantly lower than the value obtained in those glands with globular sialectasis ($P < 0.005$). The fourteen salivary glands showing the changes of atrophy on sialographic examination, had a mean parotid flow rate of 0.25 ml/min. (S.E. 0.05 ml/min.). When compared to the mean parotid flow rate of those glands with a normal sialographic appearance, the difference was again noted to be highly

significant ($P < 0.0005$). The flow rate is also significantly lower than the flow rate noted from those glands with punctate sialectasis ($P < 0.05$). However, no significant difference was noted between the mean flow rates of those glands showing atrophy or globular sialectasis. From Table II, 15, it can be seen that the mean parotid flow rate found in those glands showing cavitary sialectasis was markedly lower than those showing atrophic changes ($P < 0.05$). It is interesting to note that the mean parotid flow rates of those patients showing dilatation of the main duct, without sialectasis or atrophy, was 0.32 ml/min. (S.E. 0.07 ml/min.). This is lower than the mean flow rate noted in those patients with globular or punctate sialectasis but not significantly so. The reason for this is obscure, but it should be noted that whereas relatively large numbers of glands were included in the other groups, only six were in the group showing main duct dilatation. The parotid flow rate in this group is significantly lower, however, than the group with normal sialograms ($P < 0.005$), and is significantly greater than the mean parotid flow rate noted in the group with cavitary sialectasis ($P < 0.01$). Further, each of these groups showing

sialectasis was examined to see whether duct dilatation or retention of contrast media following the secretory phase was associated with a greater reduction in salivary flow rate than those glands not showing these changes. However, the numbers were small and could not be subjected to statistical analysis, but there was a tendency for flow rates to be slightly lower where duct dilatation or retention was noted, in addition to the predominant sialographic abnormality.

Rheumatoid arthritis alone and other connective tissue diseases

The results of sialographic examination of 131 patients with connective tissue diseases are shown in Table II, 16. The fifty-six patients with rheumatoid arthritis alone, included ten who had subnormal parotid flow rates, and of these patients, three showed sialographic evidence of duct dilatation. Atrophy of the duct system was noted in two and duct dilatation in one of the remaining forty-six patients with normal flow rates. One patient with ankylosing spondylitis and one patient with Reiter's disease showed changes of atrophy. Both had normal flow rates. Interestingly, five of the ten patients

with systemic lupus erythematosus showed sialographic abnormalities. Sialectasis was noted in two patients and atrophy in three. The patients with the changes of sialectasis and two of the patients with atrophic duct systems had subnormal parotid flow rates. The one patient with progressive systemic sclerosis accompanied by a reduced parotid flow rate had a punctate sialectasis.

Control group

All forty-five subjects in the control group had normal sialograms.

DISCUSSION

The purpose of this part of the study was to note the degree of parotid gland abnormality as assessed by hydrostatic sialography in patients with connective tissue disease, and further to correlate such changes with diminution of parotid gland function as assessed by salivary flow rates following lemon juice stimulation. It has been shown in this study that patients with Sjögren's syndrome who have sialographic abnormalities have lower parotid flow rates than those patients with Sjögren's syndrome who have normal sialograms. The mean parotid flow rate decreases with increasing degrees of sialectasis and extremely low flow rates were recorded in those patients with cavitary sialectasis. However, it is interesting that patients with globular sialectasis do not have a significantly lower parotid flow rate than those with punctate sialectasis although the value is slightly lower. This would suggest that the degree of parotid gland involvement is probably of equal severity in patients having punctate and globular sialectasis.

Atrophy of the duct system as shown by sialography is associated with considerable gland dysfunction

as shown by the reduced mean parotid salivary flow rate, which is significantly lower than that observed when punctate sialectasis is present ($P < 0.05$). This finding is not altogether unexpected as Ericson (1968) showed that patients with duct atrophy had severe histological changes on parotid gland biopsy. Although the number of patients exhibiting dilatation of the main duct in the absence of any other abnormality is small, it is of considerable interest that the mean parotid flow rate was 0.32 ml/min. This is significantly lower than the mean flow rate in those patients with Sjögren's syndrome having normal sialograms ($P < 0.005$). This suggests that even in the absence of sialographic evidence of damage to that part of the salivary gland concerned with saliva formation, a significant decrease in function may have occurred. We have obtained further evidence to support this suggestion by comparing the mean lemon juice stimulated parotid salivary flow rate in an age and sex-matched group of normal subjects, not having evidence ofSjögren's syndrome or other connective tissue disease, with the mean flow rate in the patients with Sjögren's syndrome who had normal sialograms (Table II, 15.). The mean flow rate in forty-five controls was 1.29 ml/

min. (S.E. 0.12 ml/min.) which is significantly higher ($P < 0.0025$) than the mean lemon juice stimulated parotid salivary flow rate in patients with Sjögren's syndrome having normal sialograms (mean = 0.85 ml/min.; S.E. 0.07 ml/min.).

Maynard (1965) attempted to correlate sialographic abnormality with salivary flow rates in seventy-three patients with recurrent parotid swelling of whom seven had Sjögren's syndrome. He noted that sialectasis alone or sialectasis associated with minor duct changes was associated with normal flow rate; whilst, sialectasis with main duct changes and main duct changes alone was associated with reduced flow rates. This would suggest that main duct changes are associated with considerable gland dysfunction. Blatt et al (1956) and Ericson (1968) have suggested that changes to the main duct are probably the results of infection secondary to diminished flow rate. It would, therefore, appear logical to expect a higher incidence of main duct dilatation associated with salivary glands showing severe grades of sialectasis. In this series, six of fifty-one glands (11.8%) without evidence of sialectasis or atrophy had main duct dilatation, whereas two

of twenty-nine (6.9%) with punctate sialectasis, five of fifteen (33.3%) with globular sialectasis and eight of fifteen (53.5%) with cavitary sialectasis had main duct dilatation as an associated feature. With the exception of the group of patients with punctate sialectasis the incidence of main duct dilatation increased with severer grades of sialectasis. However, on statistical analysis using the chi-squared test (with a Yate's correction for small numbers) only in those glands showing cavitary sialectasis was the prevalence of main duct dilatation significantly higher than those without evidence of sialectasis or atrophy (X^2 = 11.9841, $P < 0.001$). The prevalence of main duct dilatation in the group with cavitary sialectasis is not significantly different from the group with globular sialectasis. These findings support the view that main duct dilatation is associated with diminution in salivary flow rates. Main duct dilatation could be due to infection and in this respect it is of interest that main duct dilatation is also seen in patients who have recurrent chronic obstructive parotitis (Maynard, 1965).

With regard to patients with rheumatoid arthritis alone, it is of interest that six (10.7%) showed

sialographic abnormalities of duct dilatation or duct atrophy. However, sialectasis was not shown in any of these patients. Ericson (1967) reported punctate sialectasis in six (11%) of fifty-four patients with rheumatoid arthritis. However, of these, three complained of dryness of both mouth and eyes and one of dry eyes only and Ericson suggested that these sialographic changes were of the type seen in Sjögren's syndrome. In the present study, though sialographic abnormalities in rheumatoid arthritis were significantly higher than in a control group ($P < 0.001$), evidence of subclinical Sjögren's syndrome in the form of sialectasis was absent. The finding of punctate sialectasis in two patients with systemic lupus erythematosus and one patient with progressive systemic sclerosis, associated with the presence of sub-normal parotid flow rates, strongly suggests that these patients have Sjögren's syndrome.

It would thus appear from these results that the severity of sialographic abnormalities is to some extent paralleled by decrease in parotid gland function as shown by lemon juice stimulated salivary flow rates. However, this is by no means constant, as some patients with normal sialograms, except for

main duct changes, have a mean parotid salivary flow rate lower than patients with punctate or globular sialectasis. It would also be reasonable to conclude that the amount of salivary gland destruction between patients having punctate or globular sialectasis is not very marked as there is little difference between the mean parotid salivary flow rates of these two groups. These data support the view that salivary flow rates provide a more sensitive index of salivary gland disease than sialography and this has been confirmed by the observation that patients with Sjögren's syndrome with normal sialograms have significantly lower parotid flow rates than age and sex-matched control subjects.

SUMMARY

In this chapter, hydrostatic sialography was used to assess the severity of the salivary gland component on 195 patients with connective tissue disease. Varying degrees of sialectasis were noted in 80% of patients with the sicca syndrome and 58% of patients with Sjögren's syndrome complicated by rheumatoid arthritis. In forty-five control subjects, no sialographic abnormalities were observed.

Though duct dilatation was present in 11% of patients with rheumatoid arthritis alone, sialectatic changes were absent. However, sialectasis was noted in 20% of patients with systemic lupus erythematosus and in one of six patients with progressive systemic sclerosis. It was noted that the patients with Sjögren's syndrome with normal sialograms had a mean lemon juice stimulated parotid flow rate of 0.85 ml/min. whereas in those with punctate, globular and cavitary sialectasis, the flow rate values were 0.38, 0.34 and 0.13 ml/min. respectively. Patients with Sjögren's syndrome having normal sialograms have significantly lower flow rates than control subjects ($P < 0.0025$). It is concluded that salivary flow studies provide a more sensitive index of salivary dysfunction than sialography.

CHAPTER 7

SCANNING

Introduction

In recent years, the technique of scintillation scanning has been introduced and this enables visualisation of organ function following intravenous injection of a radioisotope. The salivary glands are known to selectively concentrate iodide and some other anions to many times the plasma level (Elmer, 1938; Schiff et al 1947; Honour, et al 1952; Harden et al 1966). One of these is ^{99m}Tc pertechnetate the peroxyanion of technetium, an artifical radio-active element close to iodine on the periodic table and of similar ionic size. Suitably labelled solutions may therefore be employed in the clinical assessment of salivary gland function. To be of use in organ scintillation scanning, an isotope should have the properties of a short half-life, both physical and biological, have no ß emissions, γ emissions of approximately 150 keV, and a low critical tissue dose. Pertechnetate ^{99m}Tc is the almost ideal scanning agent. Its short half life

of six hours, together with the near absence of particulate radiation, permits relatively large doses to be administered with little hazard to the patient and the 140 keV γ radiation is convenient for scanning. Well-defined scans can thus be quickly completed.

The usefulness of ^{99m}Tc pertechnetate as a scanning agent has been demonstrated in many situations (McCready 1967). Among the many organs displayed by the isotope, are the stomach (Harden et al 1967); the heart cavity (Witcofski and Bolliger 1965); the salivary glands (Harden et al 1967); the brain (Davis et al 1966); extra-cranial tumours (Whitley et al 1966) and the placenta (Larson and Nelp 1965). A detailed study of the iodide trapping mechanism of the salivary glands is possible using ^{99m}Tc pertechnetate and isotope scanning techniques, thus providing a test of salivary gland function.

Harden and Alexander (1967) have noted that the salivary iodide concentration may be low in patients with Sjögren's syndrome. However, no studies have been reported of the quantitative isotope uptake

measured over the salivary glands in salivary gland disease. The aim of the present study, therefore, is to investigate the uptake of ^{99m}Tc pertechnetate in normal subjects and in patients with connective tissue disease.

Materials and Methods

Patients studied

The following groups of patients were studied:

GROUP A - Twenty-five subjects with no evidence of thyroid or salivary gland disease.

GROUP B - Seventeen patients with rheumatoid arthritis alone.

GROUP C - Seven patients with Sjögren's syndrome without salivary gland involvement. These patients had rheumatoid arthritis together with kerato conjunctivitis sicca confirmed by the Schirmer tear test and the Rose-Bengal staining of the conjunctivae. Salivary flow rates and sialographic appearances were normal.

GROUP D - Twenty-four patients with Sjögren's syndrome with salivary gland involvement. Ten of these patients had the sicca syndrome.

Method

Each patient lay supine, between the detecting heads, on the examination table (Figure 11, 49). Both detecting heads were adjusted so that they were 15 cm. above and below the salivary glands to be scanned. The patient's head was held firmly between foam-rubber lined clamps and, by means of a spot-light on the upper detecting head, the points 1,2,3 and 4 corresponding to the areas of bridge of nose, lobe of ear, gonial angle and mid-point of chin were established on the scan paper (Figure 11,50 - 11, 51).

In each patient, 1 mCi of ^{99m}Tc pertechnetate was injected intravenously. Using a SELO DS 4/4 Superscanner, the subjects were scanned in the antero-posterior position over an area between the bridge of the nose and the cricoid cartilage which includes all the salivary glands. Scans were commenced at

1 min., 6 min., and 11 min., after the tracer dose, (each scan taking approximately 4 minutes). A line spacing of 4 mm and a scan speed of 20 mm per second were used. An aliquot of the dose solution was scanned under identical conditions. Slow scans, more suitable for visualisation were obtained by adjusting the speed to 10 mm per second and the line spacing to 2 mm. Such scans took approximately 20 minutes and were carried out at the termination of the third fast scan.

The γ emissions from the circulating isotope are received by both detecting heads of the machine and transferred through the unit to the two print-out units. Here the disintegrations are recorded either as single dots on the upper printing-table, or as coloured numbers on the lower tray (Figure 11, 52). The dot-counter is capable of recording a pre-set number of disintegrations per dot, while the colour head prints out a coded number for the counts occurring within a preselected time. Both pre-set values can be altered independently of each other to give optimum graphic conditions in spite of variations in isotope activity. This is an important feature, as gland concentration alters with both passage of time and with natural decay of the isotope. However, with the

1 mCi dose administered, a scaling factor (F) of 4 usually provided the optimum conditions for the upper dot print-out while a factor of 2 corresponded for the coloured print-out.

A colour map is formed by distributing four differently coloured figures on parallel lines (Figure 11, 53). These lines correspond to the to and fro scanning movement of the detecting heads. A different value (V) is ascribed to each printed mark according to the figure and colour (Figure 11, 54). From these data, the percentage dose of injected isotope taken up by any area in a known time (T) can be readily calculated using the formula $A(cps) = \frac{V \times F}{T}$. In this way, an assessment of the functional capacity of the region is determined and, by altering the scan-speed and scaling factor, a pictorial scan can be obtained. Paired rectangles of equal size were drawn on the scan around the parotid salivary glands and another set of squares around the submandibular glands. The dots enclosed were counted. To correct these counts for background radiation, the counts in an area of similar tissue density, but remote from the concentrating sites, was determined, and correction for background made in proportion to the areas of the

gland and the background rectangles (Andros et al 1955; Shimmins et al 1969). The net dot counts over the glands are expressed as a percentage of an injected dose at the mid-time of the scan. Inaccuracies may arise from a statistical counting error and from estimation of the background activity, however, the error is approximately $\pm$ 0.05% dose. (Harden et al 1967). A pictorial scan (from a normal subject) is shown in Figure II, 55.

Results

The mean values for the uptake of ^{99m}Tc pertechnetate by the salivary glands are summarised in Table II, 17. Uptake values in the parotid and submandibular glands increased with time, but the increase was less marked in the submandibular gland. At each time interval, in the normal subjects, the uptake over the parotid gland was higher than that in the submandibular gland ($P < 0.01$). In the patients with rheumatoid arthritis alone and those with rheumatoid arthritis and kerato conjunctivitis sicca, this difference was not apparent until 8 mins. The uptake in the parotid gland in patients with Sjögren's syndrome with salivary gland involvement was significantly

different from that in the submandibular gland only at 15 mins., and this difference was smaller than in the other groups. The best separation between the different groups was obtained at the mid-scan time of 8 mins., (Figure 11,57 - 11, 58). The uptakes over the parotid and submandibular glands in the patients with rheumatoid arthritis alone and rheumatoid arthritis and kerato conjunctivitis sicca are not significantly different from the control group. In Sjögren's syndrome, the uptake over both glands at each time interval studied, was significantly less than the uptake in the control subjects. (Figure II, 56). There was no significant difference in the uptakes of the patients with rheumatoid arthritis, between those with and those without kerato conjunctivitis sicca. Although the mean uptake in the group with rheumatoid arthritis and kerato conjunctivitis sicca was slightly less than the mean uptake in the control group, this difference was not significant. The mean uptake in the submandibular glands of the rheumatoid arthritis and kerato conjunctivitis sicca group was not significantly different from the mean uptake in the submandibular glands in the Sjögren's group.

All the normal patients had mean parotid gland uptakes greater than 0.16% dose at 8 min. In contrast, in those with Sjögren's syndrome, 14 of the 24 patients had values below 0.16% (Figure 11, 57). When the submandibular glands were considered, the control values were always greater than 0.07% of the administered dose, whereas seven of the twenty-four Sjögren's syndrome patients had lower values (Figure 11, 58). Nine of the patients with Sjögren's syndrome had uptake values at 8 min., within the normal range (0.16% dose for a parotid gland; 0.07 % dose for a submandibular gland. Of the remaining 15, the uptake in 5 subjects was below the lowest value for the normal range in all 4 glands. The uptake was low in both parotid glands in 12 patients, and in a single parotid gland in 3 of the 15 patients. In every case, where a patient had a submandibular value below this normal range, a low uptake in a parotid gland was also found. In 6 of the 8 patients where a low submandibular value occurred, both glands were involved.

In the patients with Sjögren's syndrome, there was a significant correlation ($P < 0.001$) between the mean parotid gland uptake and the mean submandibular gland uptake at 8 min., (Figure 11, 59).

DISCUSSION

Salivary gland scanning is being increasingly used to assess function of the salivary glands (Veronesi et al 1967; Sorsdahl et al 1969; Stebner et al 1968; Gates and Work 1967).

In Sjogren's syndrome, it has been suggested that the uptake of pertechnetate by the salivary glands may be decreased, but no standard quantitative measurement has been employed (Abramson et al 1968; Grove and Di Chiro 1968; Harden et al 1968). In the present study, the uptake of ^{99m}Tc pertechnetate has been measured as a percentage of the administered dose per gland. Values found in normal subjects have been compared with those in patients with rheumatoid arthritis alone, and those with Sjögren's syndrome with and without salivary gland involvement. Almost two-thirds of the patients with Sjögren's syndrome with salivary gland involvement, as demonstrated by abnormal salivary flow rates, had salivary gland uptake values below the lowest value recorded in the control group. An uptake value over a parotid gland of less than 0.16% dose at

a mid-scan time of 8 min., or a submandibular gland uptake of less than 0.07% dose at a mid-scan time of 8 min., indicates abnormal salivary gland function.

In this study, it has been possible to compare both the parotid and submandibular gland involvement in this disease.

Submandibular gland involvement did not occur without parotid gland involvement and parotid gland involvement was usually bilateral. Although in control subjects, the submandibular gland uptake is lower than in the parotid gland, in the group with Sjögren's syndrome there was no significant difference. Although the submandibular gland is less affected than the parotid, there is a correlation between the parotid and submandibular values of the group as a whole.

Of the patients with Sjögren's syndrome without salivary gland involvement, as demonstrated by salivary flow studies, one patient had reduced uptake over both parotid glands and one had reduced uptake over one gland. In neither case was the submandibular uptake reduced.

In 5 of the 17 patients with rheumatoid arthritis alone, one or both glands were involved and this may well represent early salivary gland involvement. As noted in the previous chapter, Ericson (1968) and Ericson and Sundmark (1970) have shown salivary gland changes as demonstrated by sialography in some patients with rheumatoid arthritis alone. It has been suggested that the site of iodide concentration is in the ducts of the salivary glands (Cohen et al 1955). Abnormal salivary gland uptake of ^{99m}Tc pertechnetate would therefore suggest salivary duct involvement. In the early stages of Sjögren's syndrome, there may only be acinar atrophy and lymphocytic infiltration without duct proliferation (Morgan 1954). This would explain the finding of an abnormally low salivary flow rate, but a normal salivary gland uptake of ^{99m}Tc pertechnetate. A normal salivary gland scan does not therefore exclude the presence of Sjögren's syndrome with salivary gland involvement. Salivary gland scanning is, however, a simple procedure, which inconveniences the patient little, and can be readily quantitated. It is therefore a useful

adjunct to the diagnosis of Sjögren's syndrome by salivary flow studies and sialography.

P A R T III

MINOR SALIVARY GLAND INVOLVEMENT IN THE CONNECTIVE TISSUE DISEASES

INTRODUCTION

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Chapter 9 POST-MORTEM STUDY

Chapter 10 LABIAL GLAND BIOPSY

Chapter 11 AMYLOIDOSIS AND RHEUMATOID ARTHRITIS

CHAPTER 8

REVIEW OF THE MINOR SALIVARY GLANDS

Development

During foetal life, each salivary gland is formed at a specific site in the oral cavity through the growth of a bud of primitive oral epithelium into the underlying developing connective tissue. The developmental pattern of the salivary glands appears to be the same for all irrespective of location and size. Only two differences exist, those of time and place of origin. The primordia of the parotid and submandibular glands appear during the sixth week, while the primordium of the sublingual gland appears during the seventh week of foetal life. The primordia of the minor salivary glands appear later (Patten, 1946). The minor sublingual gland appears a short time after the eighth week whilst the labial buccal and palatine glands begin their development in about the third month in utero (Provenza, 1964).

The glandular primordia stems from a proliferating bud of primitive oral epithelial cells.

As the cells continue to divide, the bud, which at this stage, is an epithelial cord, invades the underlying mesenchymal tissue (Figure III, 1). The epithelial cord develops into an extensively branched system which at first is solid, but gradually the older portions develop a lumen and become ducts (Figure III, 2). The secretory portions develop later than the duct system and arise from the bullous terminations of the finer ducts (Figure III, 2). Human parotid gland acini, for example, do not appear until the fourth month in utero (du Plessis, 1957). The labial salivary glands arise as aggregations of undifferentiated cells in the ninth week of foetal development but it is not until the twentieth week that typical acinar cells can be recognised (Goodman and Stern, 1967). The connective tissue component of the salivary gland plays an important part in the morphogenesis of the glandular epithelium (Provenza, 1964). It has been shown (Grobstein, 1953) that the isolated epithelial component of the mouse submandibular gland can undergo branching and form a duct system with terminal budding only if recombined with fragments of submandibular mesenchyme. Submandibular epithelial morphogenesis could not be induced by mesenchyme

derived from other sources.

The nervous system plays an important role in the development of the salivary glands (Wells, 1963). Wells has shown that extirpation of the superior cervical ganglion or the administration of an adrenergic blocking agent such as Dibenamine results in a significant reduction in gland weight. Levi-Montalcini (1964) has shown that on the other hand, the salivary glands are sites of concentration of a factor that affects the growth and differentiation of sympathetic ganglia.

Structure

The human salivary glands are compound merocrine glands, their ducts opening into the oral cavity. The minor salivary glands, which lack a definite capsule, are located subepithelially in almost all parts of the oral cavity except the gingiva and the anterior lateral and raphe regions of the hard palate (Provenza, 1964). They have been classified according to their location or secretory cell type (Table III, 1), being either pure mucous or predominately mucous, i.e. a mixture of serous and mucous acini. It is to be noted that the posterior

lingual glands (glands of Von Ebner) are pure serous in nature. The structural components of the minor salivary glands, irrespective of location, are fundamentally the same. Each gland consists of a parenchyma of functional cells of a secretory nature and a stroma of fine fibrous connective tissue which in areas is condensed into connective tissue septa or trabeculae and divide the parenchymal element into lobules. Contained within the connective tissue stroma are the vascular, lymphatic and neural elements. The salivary glands possess no hilus so that the afferent vascular supply enters the gland from many different points. The minor salivary gland duct system may be classified according to location with reference to gland lobules, hence intralobular or interlobular ducts. However, they may also be classified according to their function or structure so that three basic types are recognised, excretory, secretory (or striated) and intercalated ducts.

The cell types which may constitute an acinus, irrespective of its morphologic characteristics are mucous, serous and both mucous and serous (i.e. mixed). Mucous cells are relatively large pyramidal cells that stain pale blue with routine haematoxylin and eosin. The cells may be arranged to form an alveolus

or tubular terminal of the gland. In such a preparation the nucleus is angular in shape and is located at the base of the cell. Serous cells show intense cytoplasmic staining with haemotoxylin and eosin, giving them a darkly basophilic colour in contrast to the mucous cells. They are roughly pyramidal in shape and line a small lumen. The nucleus is more centrally placed. It must be noted, however, that the appearances of mucous and serous cells vary with the state of functional activity.

The excretory ducts which are those located more proximal to the oral cavity are lined by either pseudostratified or simple tall columnar cells, though the walls of these ducts in the oral epithelium are of stratified squamous variety. The striated ducts are lined by a single layer of tall columnar epithelial cells whilst the intercalated ducts are lined by a single layer of low cuboidal cells. In the terminal portions of the ducts of the minor salivary glands, two other cell types may be noted, - myoepithelial cells and oncocytes.

The myoepithelial cells form a delicate branched network and lie between the basement membrane and the

glandular or ductal epithelium. They are most likely epithelial in nature and are believed to be contractile. They are eosinophilic, bire-fringent and stain intensely with phosphotungstic acid haemotoxylin and iron haemotoxylin (Mylius, 1960). The presence of adenosine triphosphatase in the myo-epithelium of rat sublingual gland has been shown by Shear (1964). Scott and Pease (1959) demonstrated the fibrillar character of the cytoplasm of these cells which is similar to that of smooth muscle cells. Oncocytes are large cells having a small centrally-placed nucleus and abundant, strongly eosinophilic cytoplasm. These cells were first described in the minor salivary glands of man by Schaffer (1897). They were termed oncocytes by Hamperl (1931) and noted to be normally present in the salivary glands, especially in elderly subjects (Meza-Chavez, 1941). These cells have also been found in the bronchus (Stout, 1943) hard palate (Ahlbom, 1935) nose and larynx (Nohteri, 1946) as well as in the thyroid and para-thyroid, pancreas and hypophysis (Hamperl, 1936-7). Electron microscopic studies on the thyroid (Irvine and Muir, 1963) parathyroid (Munger and Roth, 1963) and parotid (Balogh and Roth, 1965) have

confirmed the light microscopic finding (Roth et al, 1962) that the cytoplasm of oncocytes in these organs is largely filled with mitochondria. The function of the oncocyte is unknown.

The histological appearance of normal minor labial salivary gland tissue is shown in Figure III, 1 - III,3).

Ultrastructure

Recently the fine structure of the human labial salivary glands has been studied by electron-microscopy (Tandler et al, 1969A and B) and Tandler and Ross (1969). In contra-distinction to previous reports (Nadler, 1897; Provenza, 1964; and Warwick et al, 1964) it was shown that these glands were pure mucous in nature and that no serous elements were present. All stages of maturation were noted in the acinar cells and an extensive and highly organised rough surfaced endoplasmic reticulum characterised immature cells. The Golgi apparatus was extremely prominent and was shown to consist of stacks of flattened cisternae and swarms of small vesicles. Mucous droplets were almost completely absent. Tandler et al (1969) further showed that as secretory activity progressed, the endoplasmic

reticulum involuted, while the Golgi cisternae became distended and formed many vacuoles. They noted also that in mature cells mucous droplets showing variation in density from cell to cell, were liberated from acinar cells by an apocrine process. These membrane bound droplets, released into the acinar lumen soon became lysed and their contents fused into the mucus stream. Further, Tandler et al (1969) noted that the occasional mucous cell failed to reconstitute its apical surface so that its entire contents were released into the acinar lumen.

Tandler and Denning (1966) and Tandler et al (1969B) have shown further that at a certain stage of the secretory cycle of the mucous cell, unusual intranuclear inclusions were present in many of the cells of half the donors studied. More than one inclusion was sometimes observed within a single nucleus. They measured about 1 μ in diameter; were eosinophilic; some stained with nile blue sulphate and some were P.A.S. positive though all were Feulgen-negative. It was noted that they were bounded by a single membrane and never in continuity with the nuclear envelope. Considerable morphological variation was a feature of note. Tandler et al (1969B) commented that these intranuclear inclusions

which apparently were non-viral in origin, were in some way related to the secretory cycle of the mucous cells, since they were found only in immature cells and never in cells in which the secretory products were abundant.

The existence of a motor innervation to the secretory and myoepithelial elements of salivary glands has been supported by physiological and pharmacological studies extending over a century (reviewed by Babkin, 1950; Emmelin, 1967). However, the exact morphological nature of this innervation at the acinar level is less well established. For rodent salivary glands, Scott and Pease (1959), demonstrated the presence of axons between parenchymal elements deep to the basement membrane. These early observations were confirmed by Shackleford and Schneyer (1964) for the rodent salivary gland, by Ruskell (1968) for the monkey lacrimal gland and by Watari (1968) for the exocrine pancreas of several different vertebrae. Tandler (1965) demonstrated a direct contact between an axon and a myoepithelial cell in the human submandibular gland. It has been shown in these studies that a space of 200-250Å exists between the neural membrane and the effector

cell membrane when they are in a 'direct' contact (Grillo, 1966). However, 'indirect' contacts with a space of o.1μ or more between the neural element and the acinar element, together with the interposition of the basement membrane have been described in the salivary glands of dogs (Fujita et al, 1964), rats (Tamarin, 1966), cats (Garrett, 1966) and man (Garrett, 1967). The observations of intra-acinar axons and axonal varicosities in human labial salivary glands by Tandler and Ross (1969) supported the concept of a direct-contact innervation of the secretory and contractile elements of these glands. However, as Ruskell (1968) has pointed out it may be that two different systems are involved, an interstitial and a parenchymal autonomic plexus, each with different functions.

Minor salivary gland saliva

Technical difficulties have so far precluded a detailed study of the secretions of the minor salivary glands in man (Figure III, 4). It is interesting to note that the flow of saliva in the minor glands is continuous whilst that of the major glands is intermittent (Babkin, 1950). Estimates of the volume of their resting secretion have been

made by comparing the volumes collected from major glands, with the volumes of the total mixed saliva collected under similar conditions and over a similar period of time. This method is subject to high error but in two estimates the total flows were 31 and 45% greater than the flow collected from the ducts of the major glands. These findings suggest that the minor glands play quite a large part in determining the environment of the mouth, (Jenkins, 1964).

However, Schneyer (1956) has suggested that this discrepancy is not due to contribution from the minor gland but was attributable to undue stimulation by the method selected initially for the collection of the total mixed saliva. The technique used by Schneyer (1956) consisted of a parotid cup modified to the extent of eliminating the collecting tube from the central chamber. The central chamber was then filled with a sheet of absorbent cellulose. After a measured amount of time, the cup was removed and the change in weight of the absorbent cellulose noted. Schneyer showed that although the initial period of collection yielded appreciative amount of saliva (0.002 - 0.008 gm. in 10 mins.),

subsequent collections on re-application of the device revealed no consistent increase in the amount of secretion during the lengthened period of time. So although the labial gland may secrete in response to the stimulation resulting from the application of the collecting device little or no secretion occurs without reflex stimulation. Schneyer's results confirmed the work of Montgomery and Stuart (1936) that the mucosal glands in dogs produce no appreciative volume of saliva under resting conditions,

However, as has been noted, Östlund (1953) showed that palatine salivary gland tissue is capable of continued spontaneous secretion after removal from the oral cavity and storage in Ringer's solution. Recently, the use of capillary tubes has been described for collecting minor salivary gland secretions (Kutscher et al, 1967). Each tube when filled holds precisely ($\pm$ 1%) a volume of 1, 5 or 10 lambda (micro-litres) of fluid respectively. Reflected labial mucosa is dried and tube placed adjacent to droplet of secretion. Fluid is collected by capillary action, the tube being moved from pool to pool over the distended mucosa.

Using this method, Wood and Dawes (1968) have shown that the concentration of sodium and chloride both rise (in minor gland saliva) in a similar fashion to parotid saliva in response to increase in flow rate. They report a slight fall in potassium concentration with increased flow rate. Tandler (1968) quoted the unpublished observation of Mandel, that the amylase concentration of labial saliva is minute.

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[illegible]. There is evidence that
secreting glands throughout the body
ted in Sjögren's syndrome (Bloch et
t is considered that intra-oral biop
salivary glands may be of value as
id in this condition.

CHAPTER 9

POST-MORTEM STUDY

Biopsy of the major salivary glands is usually not justified in patients with Sjögren's syndrome unless marked glandular enlargement is present and a diagnosis of neoplasia cannot be excluded. Furthermore, it may be difficult to perform as the diseased glands are often atrophic. The procedure too is inconvenient to patients and there is the possible complication of salivary fistula. Needle biopsy is unsatisfactory because of their small size and their relationship to important neurovascular anatomical structures. There is evidence that other mucus secreting glands throughout the body may be affected in Sjögren's syndrome (Bloch et al, 1965). It is considered that intra-oral biopsy of the minor salivary glands may be of value as a diagnostic aid in this condition.

In studies on children with fibrocystic disease, Warwick et al (1964) have reported the involvement of the labial mucus salivary glands. Furthermore, Calman and Reifman (1966) have reported involvement of the buccal glands in one patient

with Sjögren's syndrome, and Cifarelli et al (1966), Cahn (1967) and Bertram (1967) have described patients with this condition in which the minor palatal glands showed histopathologic changes characteristic of Sjögren's syndrome. The normal appearance of the minor salivary glands has not been well defined. Cifarelli (1966), in a post-mortem study of specimens taken from the palatal region of fifteen subjects, found no lymphocytic infiltration in fourteen and only a few lymphocytes in the remaining subject. Labial salivary gland material from eleven autopsies were examined by Mason (1966), ten being unremarkable histologically.

The aim of the present study was to investigate the prevalence and degree of lymphocytic sialadenitis in the submandibular and minor labial glands in a series of post-mortem subjects. Waterhouse (1963) has shown that the changes observed in the submandibular gland in the post-mortem subject reflect the degree of focal adenitis present in the parotid and lacrimal glands. Furthermore, Waterhouse and Doniach (1966) have provided strong evidence for the association of focal lymphocytic sialadenitis and rheumatoid arthritis and have

suggested that this lesion may represent a focal manifestation of the lesion in Sjögren's syndrome. In view of these findings, therefore, it is felt that it would be of value to observe and correlate the changes noted in the submandibular and labial salivary glands for each subject in a post-mortem series of over 100 subjects.

Materials and Methods

A submandibular salivary gland and an ellipse of oral mucosa and subjacent tissue down to the muscle layer of the lower lip were excised at autopsy. Tissue was obtained from autopsies at the Bernhard Baron Institute of Pathology, London Hospital, and the University Department of Pathology, Royal Infirmary, Glasgow, between March and June, 1967. They were taken from all autopsies on fixed days of the week excepting a few not obtainable for administrative reasons.

The autopsy material was fixed in 10% formalin and routine paraffin sections, stained with haematoxylin and eosin, were prepared. Each submandibular gland and each ellipse of oral mucosa was bisected so that a large representative histological

section would be obtained. The total number of cases examined was 129 and, of these, thirteen were excluded for the reasons given below leaving 116 cases admitted to the present series.

Criteria of exclusion

Before a gland was included in the series, the following criteria, designed to exclude infection and neoplasm as effective causes of pathological change, were applied. Gland lobules were acceptable if they were free from duct dilatation, indicating obstruction to flow of secretion, and were free from extra-vascular polymorphonuclear leucocytes. These pathological changes, when present, were not infrequently limited to isolated lobules. Glands were acceptable if a representative section of the gland (approximately 4 sq. cm. for the submandibular and 4 sq. mm. for the labial) remained after exclusion of abnormal lobules.

Cases were admitted to the series if they were free from neoplasm of lymphocyte-like cells, and had not received cytotoxic drugs within the last three months prior to death. Leukaemia

patients were excluded altogether.

The thirteen exclusions comprised seven with autolysis of gland parenchyma, and therefore not suitable, one with tumour material present in the ducts and five with extra-vascular polymorphonuclear leucocytes together with duct dilatation.

Criteria of focal lymphocytic adenitis

A 'focus' was defined as one consisting of an aggregate of 50 or more lymphocytes and histiocytes, usually with a few plasma cells placed peripherally, adjacent to and apparently replacing gland acini (Figure III, 5). Such foci are commonly found in relation to small veins and at the edge of intralobular ducts (Waterhouse and Doniach, 1966).

The grading standard used for the submandibular gland is shown in Table III, 2.).

In order to standardise the area examined and record the degree of histopathologic change, the grading standard shown in Table III,1 was employed for the labial salivary glands. All minor salivary gland tissue in the sections was examined and scanned

for the presence of lymphocytic foci and/or diffuse lymphocytic infiltration. The degree of lymphocytic infiltration and/or number of foci were expressed as a value per 4 sq. mm. of minor salivary tissue. The level of correlation between the grade of focal lymphocytic adenitis in the submandibular gland and the degree of lymphocytic infiltration in the labial glands of each subject was computed as the non-parametric Spearman rank correlation coefficient applying the correction for tied ranks, and a significance test was carried out (Siegel, 1956). The non-parametric correlation coefficient was used in order to avoid the assumption that the data were normally distributed.

RESULTS

Submandibular glands:

The figures for the prevalence of focal lymphocytic submandibular sialadenitis found in 56 female and 60 male subjects are given in Tables III, 3 - III, 9, and Figure III ,6-III,7. In this series, of 116 subjects, the frequency of involvement by sialadenitis is not significantly different in males or females. This is so, either when all

degrees of sialadenitis (X^2 - 0.003, P = o.5), or when moderate and severe degrees of sialadenitis (X^2 -.0.05, P = 0.5) are considered. Affected glands occur in the three age groups listed (Tables III, 5 - III, 6, and Figure III, 6. A tendency for more severe degrees to occur in females aged 45-64 appears in Table III, 5 and Figure III, 6 but numbers in the groups are too small for a formal test of significance to be carried out.

Labial glands:

The figures for the prevalence of lymphocytic infiltration of the labial salivary glands (the exclusions quoted above having been made) are given in Tables III, 7 and III, 8 and Figure III, 7 The frequency of involvement of the glands in male or females is not significantly different, either when all grades of severity (X^2 - 0.8, P = 0.05) or when moderate degrees only (X^2 - 0.2, P = 0.05) are considered. A fractionally higher proportion of slightly, and also of moderately involved glands is found in females between 45 and 64 years by contrast with other age groups in males or females (Tables III, 3 - III, 8 and Figure III, 7. The difference does not approach statistical significance (see, however, discussion below). In none of the labial glands were foci of lymphocytes observed.

Correlation between findings in submandibular and labial glands:

The gradings in the submandibular and labial glands for each subject (Table III, 9), positively correlated. The non-parametric Spearman rank correlation coefficient r_s has the value of 0.32 which, for a sample size of 116 is significant at the level P = 0.001 ($t_{114} = 3.6$). In view of the lower of P, there can be little doubt of the significance of the result, despite the modest number of grades of severity (0, 1 and 2) of adenitis of labial glands and the correspondingly high number of tied observations. The correction for tied observations was applied as stated above and the effect of them is not great (Siegel, 1956).

If the labial glands only of this group had been examined and graded in the manner described, of the 116 subjects for whom the material met the criteria for acceptance in the series, one with a grade 3 ('severe') submandibular gland would have been graded as 0 ('normal') on the grounds of normal labial gland (Table III, 9).

DISCUSSION

The autopsy results confirm the work of Waterhouse and Doniach (1966) in showing the high prevalence of focal lymphocytic adenitis in the submandibular gland. As they have suggested, it may be that these changes represent a potential rheumatoid state in these subjects, since they have shown strong evidence of an association between moderate focal adenitis of the submandibular salivary gland and rheumatoid arthritis in the post-mortem subject.

A fractionally higher frequency of slight (71%) or of moderate degrees (29%) of lymphocytic infiltration of labial glands in females between 45 and 64 years than in other groups of either sex was found (see results). The difference between these values and the values in other groups does not approach statistical significance. The finding is, however, of interest in view of the predilection for middle-aged female subjects of both focal lymphocytic submandibular sialadenitis and the comparable focal lymphocytic thyroiditis that was found in a larger earlier series (Waterhouse and Doniach, 1966). Both focal lymphocytic sialadenitis and focal lymphocytic thyroiditis may

be evidence of a general disturbance of the immunological system.

Lymphocytic foci were not demonstrated in this series in the labial salivary glands. This strongly suggests that lymphocytic foci do not normally occur in these glands.

The significant degree of correlation between lymphocytic infiltration in the labial salivary glands and focal lymphocytic submandibular sialadenitis and the presence of lymphocytic foci in labial glands in Sjögren's syndrome suggests that the minor glands in fact reflect salivary gland involvement as a whole and that lymphocytic infiltrations and lymphocytic foci are related to each other. This is of importance in the investigation of disease such as Sjögren's syndrome. The biopsy necessary to obtain direct histopathological evidence of minor labial as opposed to major salivary gland involvement involves only a trivial surgical procedure and avoids any danger of the complication of salivary fistula.

SUMMARY

In the present investigation, the prevalence of focal lymphocytic adenitis in the submandibular salivary gland was observed in a series of 116 post-mortem subjects, after suitable exclusions had been made. Focal lymphocytic adenitis could not be demonstrated in the labial salivary glands. The degree of lymphocytic infiltration in the labial salivary glands is positively correlated with the level of focal lymphocytic adenitis in the submandibular glands in the same subject. Lymphocytic foci and lymphocytic infiltrations found under these circumstances are probably related. This finding provides conceptual support for the examination, by biopsy, of the labial glands in patients suspected of Sjögren's syndrome.

C H A P T E R 10

LABIAL GLAND BIOPSY

INTRODUCTION

The histopathologic characters of the major salivary glands in Sjögren's syndrome include parenchymal and ductal alterations (Sjögren, 1933; Bloch et al, 1965; Morgan et al, 1953, Morgan, 1954; Cardell et al, 1954; Vanselow et al, 1953). There is a decrease or disappearance of acini, lymphocytic infiltration and hyperplasia of the lining cells of the intraglandular ducts. The formation of epimyoepithelial cell islands has also been described, Morgan et al (1953), and appears to be a late feature in the disease process. Furthermore, Morgan and Castleman (1953) have shown that the histopathological features of Sjögren's syndrome and Mikulicz's disease (See Chapter 17) appear to be similar, and subsequently these findings have been confirmed (Cardell et al, 1954; Seifert et al, 1957; Heaton, 1959; Bain, 1960; Bertram et al, 1964), and the term 'Mikulicz's/ Sjögren's syndrome' has been applied by some workers (Bain, 1960; Bertram et al, 1964; Shearn, 1961; Castleman et al, 1962). In the previous chapter, focal lymphocytic adenitis of the labial salivary gland could not be demonstrated in any of

the 116 post-mortem subjects. However, a positive correlation between the changes observed in the submandibular and labial salivary glands could be made. This suggested that the labial salivary glands reflect salivary gland involvement as a whole and provides conceptual support for the examination, by biopsy, of minor salivary glands in patients suspected of Sjögren's syndrome. The aim of the present part of the study was to investigate further the histopathological appearances of the labial salivary glands in Sjögren's syndrome, rheumatoid arthritis alone and various other connective tissue diseases.

Materials and Methods

Patients

214 patients were studied. The clinical diagnosis, sex distribution, mean age and age range are shown in Table III, 10). In addition, in 111 patients included in this part of the study, the following clinical features were recorded: duration of arthritis, presence of subcutaneous nodules, functional grade and x-ray classification. Also, laboratory investigations included full blood count, white cell count, erythrocyte sedimentation rate (Westergren) and determination of serum globulin.

These clinical and laboratory findings were correlated with the histopathologic changes observed. The age and sex distribution and clinical groups are shown in Table III, 10).

Labial Salivary Gland Biopsy Technique

The minor salivary glands of the lower lip were chosen for biopsy since they are easily accessible, lie above the muscle layer, are separated from the mucous membrane by a thin layer of fibrous connective tissue and the chance of excessive bleeding is minimal (Meskin et al, 1964). With the patient seated in the dental chair and after oral preparation with cetavlon, sterile draping and local anaesthetic (Xylocaine 1%) an ellipse of oral mucous membrane down to the muscle layer was removed. The biopsy wound was closed using 04 gauge black silk sutures. These were removed after four or five days and in all cases healing was satisfactory. None of the patients reported undue discomfort, either during or after the biopsy procedure. The biopsy material was fixed in 10% formaldehyde and standard paraffin preparations made. The tissue was serially sectioned at 6 μ setting and every ninth or tenth section taken for standard staining with haematoxylin and eosin.

The biopsy technique is shown in Figures III, 8 - III, 15. The grading standard shown in Table III,1 was employed to record the degree of histopathologic change. A 'focus' has been defined, (Chapter 8), as one consisting of an aggregate of 50 or more lymphocytes and histiocytes usually with a few plasma cells placed peripherally, adjacent to an apparently replacing gland acini.

RESULTS

The results for 214 patients are shown in Table III, 11. Focal lymphocytic adenitis of the labial salivary gland was found to be present in 61% of patients with the sicca complex, 70% of patients with Sjögren's syndrome complicated by rheumatoid arthritis, 19% of patients with rheumatoid arthritis alone, 3% of patients with pararheumatic disease and in 22% of patients with a connective tissue disease alone. Of the two patients with pararheumatic disease and the two patients with a connective tissue disease in which a positive labial gland biopsy was noted, one had psoriatic arthritis, one had osteoarthritis, one had systemic lupus erythematosus and the other had dermato-myositis. In the patient with psoriatic arthritis, the Schirmer

tear test (Williamson et al, 1967) suggested hypofunction of the lacrimal glands but no definite kerato conjunctivitis sicca was present. No other stigmata of Sjögren's syndrome were present in these patients.

Table III, 12 shows the clinical features of the patients with rheumatoid arthritis. The majority of patients with rheumatoid arthritis, included in this part of the study, had positive tests for rheumatoid factor and/or erosions on x-ray examination. All satisfied the criteria of "definite" or "classical" disease. It can be seen from Table III, 12 that there is no correlation between any of the clinical features and the presence of focal lymphocytic sialadenitis of the labial glands in patients with rheumatoid arthritis alone. This included the features of symptomatic xerostomia and possible kerato conjunctivitis sicca, which may represent an early, mild form of Sjögren's syndrome in rheumatoid arthritis. The laboratory features of these patients are shown in Table III, 13. Patients with focal lymphocytic labial sialadenitis had a significantly higher erythrocyte sedimentation rate, than those without focal lymphocytic involvement of these glands.

There was no significant difference in serum globulin levels, though those patients with positive biopsies had slightly higher values.

Examples of lymphocytic involvement of the minor labial salivary glands are shown in Figures III, 16 - III, 26.

DISCUSSION

The results of the labial salivary gland biopsy in the present study show a definite association between foci of lymphocytes in the labial glands in Sjögren's syndrome, with or without rheumatoid arthritis and in rheumatoid arthritis alone. The degree of involvement was considerably more severe in the Sjögren's group. The finding of focal lymphocytic sialadenitis in 19% of patients with rheumatoid arthritis alone and in two of nine patients with connective tissue disease alone, is of considerable interest. The post-mortem work of Waterhouse and Doniach (1966) showed the high prevalence of focal lymphocytic adenitis in the submandibular gland. As they have suggested, it may be that these changes represent a potential rheumatoid state in these subjects, since they have shown strong evidence of an association between moderate focal lymphocytic adenitis of the submandibular gland and rheumatoid arthritis in the post-mortem subject. Furthermore, a positive correlation between focal lymphocytic submandibular adenitis and labial lymphocytic infiltration in each instance in a post-mortem study has been shown (Chapter 8). This significant correlation, together with the presence of lymphocytic foci in the labial glands in Sjögren's syndrome, suggests that the minor glands

reflect salivary gland involvement as a whole and that lymphocytic infiltration and lymphocytic foci are related to each other. It may be suggested, therefore, that focal lymphocytic labial sialadenitis in patients with rheumatoid arthritis and connective tissue disease alone may reflect a subclinical form of the salivary gland component of Sjögren's syndrome

The lack of marked ductal change was a feature of the histopathological findings in the present study. The formation of epimyoepithelial cell islands was not found in any of the sections examined, though interstitial fibrosis and mild hyperplasia of the intraglandular duct system, where present, were more common in the sicca complex group. However, as has been observed by Sokoloff (Bunim et al, 1964) classical epimyoepithelial cell islands are not an invariable finding in the major salivary glands in Sjögren's syndrome. It is of interest that focal aggregates of lymphocytes in Sjögren's syndrome may be observed in sites other than the salivary glands, e.g., skin and lacrimal gland.

The role of the lymphocyte in connective tissue

disease is uncertain. There is, however, overwhelming evidence that lymphocytes possess immunologic activity and among the various functions ascribed to the lymphocyte are those of immunological carrier, tissue invader and haemopoietic stem cell (Porter and Cooper, 1962). Large and small lymphocytes have different functions as has been demonstrated by the classic work of Gowans (1959). Small lymphocytes form a recirculating pool whereas large lymphocytes are maintained by cell division. Furthermore, small lymphocytes pass from blood through endothelial cells to the lymph nodes, spleen and Peyer's patches, whilst in contrast, large lymphocytes apparently develop into plasma cells in the blood (Gowans and McGregor, 1965). The main immune functions of these cells, which have been demonstrated experimentally and clinically, include graft vs. host reaction (Stastny and Ziff, 1962), primary and secondary antibody response, potential proliferative activity, delayed hypersensitivity and antilymphocyte serum (Spector, 1967; Currey and Ziff, 1966).

It is of interest that similar lymphoid infiltrates to those in Sjögren's syndrome have been observed in the salivary glands of NZB and NZB/NZW F,

mice who develop an autoimmune disorder resembling systemic lupus erythematosus (Kessler, 1968). It is possible that the infiltrating lymphocytes in Sjögren's syndrome may be reacting immunologically against antigenic structures in the salivary glands. This concept is supported by the presence of auto-antibodies to salivary duct epithelium in patients with Sjögren's syndrome (Bertram and Halberg, 1965; MacSween et al, 1967). Recently, the synthesis of immunoglobulins by cells infiltrating the labial salivary glands in Sjögren's syndrome have been studied by Talal et al (1970). Greater synthesis of immunoglobulins in Sjögren's syndrome compared to rheumatoid arthritis was noted and the work established the immunologic competence of these cells, and suggested, too, an origin from an extra-salivary source.

Earlier, Talal and colleagues (1966) had demonstrated abnormal function of peripheral lymphocytes in Sjögren's syndrome with regard to in vitro transformation and failure to develop delayed sensitivity.

The presence of lymphoid tissue in the minor salivary glands tends to support the concept of Sjögren's syndrome having an auto-immune pathogenesis. The labial biopsy is a valuable investigative technique

in the diagnosis of Sjögren's syndrome and allied disorders and provides a readily obtainable source of tissue for further investigation in this fascinating symptom complex. In Chapter 15, the labial biopsy is used to provide tissue for the study of iodide concentration by the minor glands.

SUMMARY

In the present chapter, a labial salivary gland biopsy technique is described and was used to study 214 patients with Sjögren's syndrome, rheumatoid arthritis alone and various other connective tissue disorders. Focal lymphocytic adenitis of the labial salivary glands was found to be a consistent finding in patients with the sicca complex and Sjögren's syndrome. Approximately 20% of both patients with rheumatoid arthritis alone and patients with systemic lupus erythematosus had focal lymphocytic sialadenitis. In patients with rheumatoid arthritis alone, there was no correlation between the presence of foci of lymphocytes and clinical features suggestive of sub-clinical Sjögren's syndrome. The labial biopsy is shown to be a further valuable investigative procedure in the diagnosis of Sjögren's syndrome.

C H A P T E R 11

AMYLOIDOSIS AND RHEUMATOID ARTHRITIS

The name 'amyloidosis' is applied to a condition in which a fibrillar glycoprotein is deposited in slowly increasing quantities in the connective tissue ground substance, usually between capillary endothelial cells, their supporting reticulum fibres and underlying basement membranes, in the inter-cellular substance between the medial muscle cells of larger vessels and in aggregates within subcutaneous and intermuscular tissues. Most commonly, the deposits occur in the course of diseases such as rheumatoid arthritis and chronic infections where there has been prolonged hyperactivity of the reticulo-endothelial system with antibody production. In these circumstances, generalised, secondary amyloidosis is often accompanied by changes in plasma proteins and hyperglobulinaemia is characteristic.

With the relative elimination of chronic infections, rheumatoid arthritis has emerged as the commonest disease associated with this type of amyloidosis (Missen and Taylor, 1956; Gardner,

1962; Ennevaara and Oka, 1964). Amyloid has been observed in six of 115 rectal biopsies from patients with rheumatoid arthritis of more than three years duration (Arapakis and Tribe, 1963). Secondary amyloidosis is occasionally encountered in ankylosing spondylitis and in progressive systemic sclerosis. The consequence of amyloid deposition is to lead mechanically to functional disturbances of the affected organs and tissues. The effects are observed most commonly in the kidney, and lead to renal excretory failure. In a disease such as rheumatoid arthritis, renal failure is among the commonest causes of death. In other cases, the nephrotic syndrome develops, sometimes with renal vein thrombosis. Diagnosis of amyloidosis by biopsy is important since there is some evidence that adrenal corticosteroids may control or supress the deposition of amyloid (Parkins and Bywaters, 1959).

In the present study, an opportunity was taken to examine sections of labial mucosa and labial salivary gland tissue from patients with long-standing rheumatoid arthritis for the presence of amyloid.

Materials and Methods

Patients

Labial salivary gland biopsy specimens from fifty-two patients were examined for the presence of amyloid. They included a few specimens in which amyloid was suspected from the initial haematoxylin and eosin section. The remainder comprised patients with severe rheumatoid arthritis of long-standing duration. These were the criteria of inclusion in the series.

Laboratory Method

Conventional staining methods reveal amyloid in paraffin-embedded sections as a homogeneous, faintly eosinophilic material, with no identifiable structure. The dye Congo red is adsorbed physically by amyloid and glycoprotein; the resultant deep rose pink colour affords a useful permanent preparation and a means of confirming microscopic recognition (Pearse, 1960). In this series, in each case, the paraffin embedded sections were stained by the alkaline Congo red technique (Puchtler et al, 1962) and examined for birefringence using polarised light. However, the adsorption of Congo red is non-specific and when amyloid is

present in very small quantities, some confusion may occur with elastic laminae which tend to adsorp the dye. Amyloid retains the fluorescent dye Thioflavine-T and with ultra-violet light it is consequently possible to identify the location of small amyloid deposits. Therefore, in this study in addition to Congo red staining in each case the fluorescent thioflavine-T staining method of Vassar and Culling (1959) was employed.

RESULTS

Amyloid deposits were noted in only one case in the series of fifty-two patients with long-standing rheumatoid arthritis. The patient was a 39 year old male with a twelve year history of severe crippling arthritis. The material from the labial biopsy in this case demonstrated positive Congo red binding and showed ultra-violet fluorescence after staining with thioflavine-T (Figure III, 27-III, 28). Within the salivary glands, amyloid was deposited in a periductal and perivascular fashion, though distribution within the interstitial tissue was also noted. Perivascular deposits were observed in the lamina propria of the biopsy specimen.

DISCUSSION

The precise mechanism by which amyloid is deposited in human tissues is not fully understood. However, there is much evidence for believing that the formation of amyloid is related to an immune response. Hyperimmunisation is a satisfactory experimental means for producing the disease (Zenoni, 1902), which can be identified in horses used for prolonged periods as a source of diphtheria antitoxin. Furthermore, many human diseases which culminate in amyloidosis are accompanied both by hyperglobulinaemia and by cellular evidence of exaggerated antibody synthesis. Gammaglobulin, possibly of antibody origin has been identified in sites of amyloid deposition (Vazquez and Dixon, 1957) but the amount of gammaglobulin present is now thought to be less than had at first been supposed. That hyperglobulinaemia is not the full explanation for the syndrome is due to the recognition that amyloidosis may develop in the course of acquired agammaglobulinaemia (Teilum, 1964).

A variety of methods for producing amyloidosis

in experimental animals has been described and the initial observation was that of Kuczynski (1922) who fed mice on a diet rich in the sulphur-containing protein casein. Similar feeding experiments have been used successfully to produce amyloidosis in rats and rabbits (Eklund and Reimann, 1936; Teilum, 1956; Giles and Calkins, 1958; Cohen et al, 196o). Prolonged infection with organisms such as staphylococci and tubercle bacilli may be used to induce amyloidosis in mice, rabbits, and fowl. Senility (Dunn, 1944) and vitamin C deficiency (Pirani et al, 1948) have been described as factors in the experimental production of amyloid. Also, the injection of Freund's adjuvant is an effective way of producing the condition (Rothbard and Watson, 1954). Oral deposits of amyloid have been reported in numerous cases of primary and secondary disease and, for this reason, gingival biopsy has been recommended as a simple means of confirming diagnosis in some of these forms of human amyloidosis (Selikoff, 1946; Selikoff and Robitzek, 1947; Gorlin and Gottsegen, 1949; Selikoff and Herschfus, 1949; Meyer, 1950; Lighterman, 1951; Tillman, 1957 and Trieger et al, 1959). A study of the diagnosis of amyloidosis in patients with rheumatoid arthritis and ankylosing spondylitis by biopsy of gingival and rectal mucosa has been

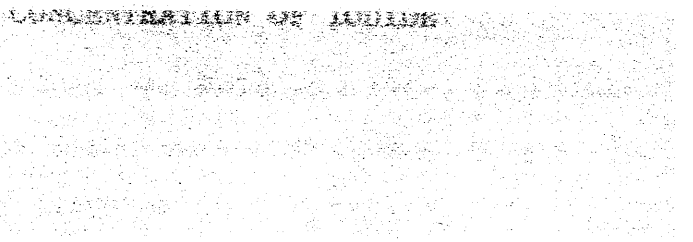
published recently (Maldyk et al, 1967). To my knowledge, there has been no report of minor salivary gland involvement by amyloid in man, though Miller and Clark (1968) have reported sub-maxillary gland deposits in experimental amyloidosis in mice.

In the present study, only one of fifty-two (1.9%) cases had a positive biopsy for amyloid. This compares unfavourably with the 4.3% incidence reported in gingival tissue for patients with rheumatoid arthritis and ankylosing spondylitis (Maldyk et al, 1967). However, in a study of gingival biopsies from 450 patients with pulmonary and/or osseous tuberculosis, Meyer (1950) found amyloid to be present in only seven cases (1.6%). The present study has shown a further oral site for the deposition of amyloid associated with a chronic disease process such as rheumatoid arthritis. Biopsy of the oral tissues remains a promising procedure. Technically, tissue to be studied is easily accessible and the operation for its removal is readily acceptable to the patient, being simple and safe. It is probable that gingiva remains the site of choice for oral biopsy for amyloid, since perivascular zones are the sites of predilection for deposition of this

material (Auerbach and Stemmermann, 1946). Further, Selikoff and Robitzck have shown that where amyloidosis has been firmly established, then gingival biopsies were 78% positive.

SUMMARY

Fifty-two labial biopsy specimens from patients with severe long-standing rheumatoid arthritis were studied for the presence of amyloid deposits. Using an alkaline Congo red and fluorescent thioflavine-T staining techniques, only one specimen showed amyloid deposits.



P A R T IV

SEROLOGICAL, BIOCHEMICAL AND MICROBIOLOGICAL STUDIES

INTRODUCTION

Chapter 12 IMMUNOPATHOLOGY

Chapter 13 MYCOPLASMA AND SJÖGREN'S SYNDROME

Chapter 14 ISO-ELECTRIC FOCUSSING OF SALIVARY PROTEINS

Chapter 15 CONCENTRATION OF IODIDE

C H A P T E R 12

IMMUNOPATHOLOGY

Introduction

An antibody to the cytoplasm of salivary duct epithelial cells has been demonstrated by an indirect immunofluorescence sandwich technique in patients with Sjögren's syndrome (Bertram and Halberg, 1964; Halberg, et al, 1965). These workers have reported the presence of antibody in eleven of nineteen patients with Sjögren's syndrome. In a recent study, (MacSween et al, 1967), the antibody was shown to be present in approximately 15% of patients with the sicca syndrome, 65% of patients with Sjögren's syndrome and rheumatoid arthritis and in 26% of patients with rheumatoid arthritis alone. Since sera containing the antibody did not give immunofluorescent staining of salivary gland acini or thyroid, Bertram and Halberg (1964) considered that the antibody might be specific for an antigen peculiar to salivary duct epithelium. MacSween et al (1967) suggest that the antibody shows some organ-specificity in not causing immunofluorescent staining of gastric, thyroid or prostatic epithelium.

The reason why patients with rheumatoid arthritis who have Sjögren's syndrome develop this antibody is not known. Autoantibodies may be defined concisely as antibodies formed by an animal to some constituent of its own body. However, some autoantibodies in the human are transient and follow common types of tissue damage (Waksman, 1962). The simple presence of autoantibodies in the serum does not in itself lead to progressive autoimmune disease (Doniach and Roitt, 1962). It has been suggested that the underlying abnormality or likelihood of developing these diseases is probably genetically determined, as evidenced by family studies.

In this part of the study, the incidence of the salivary duct antibody is investigated in groups of patients with the sicca syndrome, patients with Sjögren's syndrome complicated by rheumatoid arthritis, patients with rheumatoid arthritis alone and patients with various other arthritdes or connective tissue diseases.

MATERIALS AND METHODS

Patients Studied

The sera from 261 patients were examined for the

presence or absence of the salivary duct antibody. The clinical diagnosis, age and sex distribution are shown in Table IV, 1. In order to assess the persistence of the salivary duct antibody, fresh sera was collected from a selected group of patients at least one year following the initial serological examination and re-tested for the antibody. In addition, the sera was examined for the presence of anti-nuclear factor, rheumatoid factor, anti-thyroglobulin and non-tissue specific precipitins. Apart from the age, ophthalmological and oral examinations, the following laboratory data were also recorded: haemoglobin concentration, erythrocyte sedimentation rate (Westergren), white cell count and serum globulin level.

Salivary duct antibody

Human submandibular gland tissue, obtained at post-mortem within ten hours of death, was frozen to metal chucks with carbon dioxide snow, and 6μ sections cut in a cryostat. The patient's sera was applied undiluted to the unfixed section for 30 minutes at room temperature, then each section was washed in normal saline buffered with veronal (pH 7.2) for ten minutes followed by the application

of fluorescein-conjugated goat anti-human globulin serum of 30 minutes. Finally, the section was re-washed with buffered saline for 10 minutes, mounted in buffered glycerol, and examined under a Gillett and Sibert conference microscope using blue light. The fluorescein-conjugated anti-human globulin serum was absorbed twice with dried rat liver to reduce non-specific fluorescent staining.

In a number of cases, the test was repeated using the patients own minor salivary gland tissue, obtained from the labial biopsy specimen, as the substrate.

Antinuclear Factor (A.N.F.)

Beck's indirect immunofluorescence method (1961) was employed, with rat liver as the substrate. A dilution of 1 in 16 was initially used for testing, and positive sera were then titrated in quadrupling dilutions until an end point of nuclear staining was reached.

Anti-thyroglobulin

This was detected using the tanned red cell haemagglutination technique of Fulthorpe, Roitt, Doniach and Couchman (1961), thyroglobulin coated

formolised tanned sheep red cells (Burroughs Wellcome). Initially the sera were tested at a 1 in 16 dilution and positive sera were titrated in quadrupling dilutions.

Thyroid "Microsomal" Antibody

The indirect immunofluorescence technique of Holborow Brown, Roitt and Doniach (1959) using un-fixed thyrotoxic thyroid tissue as substrate was used. The test sera were applied at a 1 in 4 dilution.

Rheumatoid Factor

This was detected using the Hyland Latex (R.A.) test technique (Hyland Laboratories, California) and also by the sheep cell agglutination test, a titre of 1 in 32 or greater being considered positive.

Non-specific Tissue Precipitin Tests were performed, using the method of Anderson, Gray, Beck and Kinnear (1961) with human thyroid tissue as antigen. All sera were tested undiluted and at a 1 in 8 dilution.

RESULTS

Salivary Duct Antibody

Figures IV, 1 - IV, 3 show positive staining of the cytoplasm of salivary duct epithelial cells. Positive immunofluorescent staining varied in intensity but even with the brightest staining pattern it was found that the antibody was present in low titre, none exceeding 1 : 32.

Table IV,1 shows the prevalence of the salivary duct antibody in the clinical groups studied. The antibody was present in 19.4% patients with the sicca syndrome, in 67.9% of patients with Sjögren's syndrome complicated by rheumatoid arthritis and in 50% of patients with rheumatoid arthritis alone. Of the 104 patients with other arthritides or connective tissue diseases, only two had the salivary duct antibody present. These were a 63 year old female with definite ankylosing spondylitis and a 47 year old female with progressive systemic sclerosis both having a positive sheep cell agglutination test for rheumatoid factor at a titre of 1 : 256, but neither had clinical evidence of Sjögren's syndrome, or rheumatoid arthritis. The thirty four year old female with systemic lupus

erythematosus was subsequently proved to have Sjögren's syndrome.

Anti-nuclear Factor

Twenty-seven patients (50%) with Sjögren's syndrome complicated by rheumatoid arthritis and nine patients (25%) with sicca syndrome alone had anti-nuclear factor present in their sera. The pattern of nuclear fluorescence is shown in Figures IV, 4 - IV, 6 and in Table IV, 4. Homogenous nuclear staining was observed in 24 patients with Sjögren's syndrome with rheumatoid arthritis and in all nine patients with the sicca syndrome. The remaining three patients with Sjögren's syndrome with rheumatoid arthritis showed speckled nuclear staining in two cases and nucleolar staining in one case.

Of the 80 patients with rheumatoid arthritis alone, seventeen (21.3%) showed anti-nuclear factor to be present in their sera. In each case, a homogenous pattern of nuclear staining was observed. Patients with para-rheumatic or degenerative joint disease showed anti-nuclear factor in one case (4.1%) with osteoarthritis, one case (6.3%) with Reiter's disease and one case (5.9%) each with ankylosing

spondylitis and psoriatic arthritis.

Anti-nuclear factor was present in a high percentage of the patients with connective tissue disease. Nine patients (90%) with systemic lupus erythematosus had the antibody present. A homogenous pattern of nuclear fluorescence was noted in six patients. Speckled, nucleolar and membranous patterns of nuclear fluorescence were observed in one case each. Five of the six patients (83.3%) with progressive systemic sclerosis (scleroderma) had the antibody present and in each case a homogenous pattern of nuclear staining was noted. Anti-nuclear factor showing homogenous staining was present in the sera of the one patient with dermatomyositis.

The anti-nuclear factor was absent from the sera of patients with Behcet's syndrome and those with gout.

Rheumatoid Factor

In patients with Sjögren's syndrome complicated by rheumatoid arthritis and patients with rheumatoid arthritis alone, rheumatoid factor was present in

the sera of 44 (81.5%) and 70 (87.5%) patients, respectively. The factor was present in 10 (27.7%) patients with sicca syndrome alone. Two patients (12.5%) with Reiter's disease and four patients (23.5%) with psoriatic arthritis had positive tests for rheumatoid factor. Of the patients with connective tissue disease, 3 (30%) with systemic lupus erythematosus, 4 (66.6%) with progressive systemic sclerosis, and the one patient with dermatomyositis had the factor present in their sera. In none of the patients with osteoarthritis, ankylosing spondylitis, Behcet's syndrome or gout could the factor be detected. (Table IV 2).

Anti-thyroglobulin

In patients with Sjögren's syndrome with rheumatoid arthritis, rheumatoid arthritis alone and with sicca syndrome, anti-thyroglobulin was detected in 9 (16.6%) 12 (15%) and 8 (22.2%) patients, respectively. One patient (10%) with systemic lupus erythematosus, one (6.3%) with Reiter's disease and one (5.9%) with ankylosing spondylitis also had anti-thyroglobulin present in their sera. The antibody was absent from the sera of all other patients examined.

Non-specific Tissue Precipitins

Non-specific tissue precipitins were present in the sera of 13 (24.1%) 3 (3.8%) and 6 (16.6%) of patients with Sjögren's syndrome with rheumatoid arthritis, rheumatoid arthritis alone and sicca syndrome, respectively. In six other patients, two with osteoarthritis (8.3%) 2 with systemic lupus erythematosus (20%) and two with ankylosing spondylitis (11.8%) non-specific tissue precipitins were detected. In the sera from all other patients such precipitins were absent. (Table IV 3)

The results of the repeat salivary duct antibody test in a sample of 40 patients two years later, showed a different result in only three patients. In each case, a negative result was obtained in the latter test. One patient had Sjögren's syndrome complicated by rheumatoid arthritis, one had rheumatoid arthritis alone and one had systemic lupus erythematosus. These results suggest a persistence of the auto-antibody and confirm the consistency of the initial examination.

Correlation of Salivary Duct Antibody and Focal Lymphocytic Labial Sialadenitis

Table IV, 5 shows the prevalence of salivary duct autoantibody and focal lymphocytic sialadenitis in 130 patients studied.

Salivary duct autoantibody was present in 10% of patients with the sicca syndrome, in 70.4% of patients with Sjögren's syndrome and rheumatoid arthritis, and 44.6% of patients with rheumatoid arthritis uncomplicated by Sjögren's syndrome. The prevalence of salivary duct autoantibody in patients with the sicca syndrome and Sjögren's syndrome and rheumatoid arthritis is similar to that found in the larger series of patients studied but the prevalence of salivary duct autoantibody in patients with sicca syndrome is lower (10% of 18.4%).

Of the 46 patients with other arthritides and connective tissue diseases, only one had salivary duct autoantibody present. This was the 63 year old woman with a positive sheep cell agglutination test for rheumatoid factor at a titre of 1 : 256, but without clinical evidence of Sjögren's syndrome.

The prevalence of focal lymphocytic sialadenitis was the same in patients with sicca syndrome and Sjögren's syndrome and rheumatoid arthritis (60% and 63% respectively). The prevalence in rheumatoid arthritis alone was 27.7%.

Two patients with psoriatic arthropathy had focal lymphocytic sialadenitis but neither had salivary duct autoantibody. One of these patients had "possible" keratoconjunctivitis sicca.

Two patients with ankylosing spondylitis had focal lymphocytic sialadenitis, and one of these, mentioned above, had salivary duct autoantibody present. Neither of these two patients had any evidence of Sjögren's syndrome.

Of the four patients with progressive systemic sclerosis, one had focal lymphocytic sialadenitis but no evidence of Sjögren's syndrome

One patient with osteoarthritis had a positive buccal mucosal biopsy and had evidence of "possible" keratoconjunctivitis sicca. No salivary duct autoantibody was detected in this patient's serum.

The relationship of the salivary duct autoantibody and other autoantibodies and laboratory data in patients with the sicca syndrome, Sjögren's syndrome

and rheumatoid arthritis, and rheumatoid arthritis alone is shown in Tables IV, 6 - IV, 8.

It can be seen that in none of these three groups, did the salivary duct autoantibody correlate with focal lymphocytic sialadenitis. None of the other autoantibodies including rheumatoid and anti-nuclear factors, non-tissue specific precipitins, anti-thyroglobulin, anti-thyroid "microsomes" and gastric parietal autoantibodies correlated with focal lymphocytic sialadenitis in any of the three clinical groups, with the exception of the anti-nuclear factor in patients with rheumatoid arthritis (Table IV, 8, $P < 0.02$). None of the other laboratory features correlated with the finding of focal lymphocytic sialadenitis in patients with Sjögren's syndrome whether associated with rheumatoid arthritis or not. In patients with rheumatoid arthritis alone, the haemoglobin ($p < 0.001$) and white cell count ($p < 0.02$) were significantly lower in patients with focal lymphocytic sialadenitis and the E.S.R. was significantly higher ($p < 0.001$).

DISCUSSION

Bertram and Halberg (1964) first described the occurrence in Sjögren's syndrome of an antibody against salivary duct epithelium. Since sera containing the antibody did not give immunofluorescent staining of salivary gland acini or of thyroid, they considered that the antibody might be specific for an antigen peculiar to salivary duct epithelium. Feltkamp (1966) and Feltkamp and van Rossum (1968) have shown that the antibody could be absorbed from the serum with extracts of salivary gland, but extracts of a number of other tissues including pancreas, thyroid, liver, adrenal, muscle and kidney failed to do so. MacSween et al (1967) and Whaley et al (1968) have shown that the antibody reacts with the individual's own tissues (i.e. is an autoantibody) and also causes immunofluorescent staining of small lacrimal ducts but not of gastric, thyroid or prostatic epithelium. Furthermore, MacSween et al (1967) have shown that the mitochondrial antibody found in a high percentage of patients with primary biliary cirrhosis (Walker et al, 1965; Goudie et al, 1966) gave an immunofluorescent staining pattern with salivary gland similar to that seen with salivary duct antibody positive sera. They have shown that

the salivary duct antibody differs from the mitochondrial antibody in that only the latter can be absorbed from sera with rat liver mitochondria. From these studies, it would appear that the salivary duct antibody shows some organ-specificity.

In the present series, the incidence of the salivary duct antibody compares well with the findings of MacSween et al (1967). The antibody is present in 19.4% of sicca patients and 68% of patients with Sjögren's syndrome complicated by rheumatoid arthritis. In rheumatoid arthritis the incidence of the antibody is 50% which is, however, much higher than the 26% reported by MacSween et al (1967). The finding, however, is in agreement with the 44% reported by Whaley et al (1968). The differences probably reflect a selection bias.

From this discussion, it is apparent that the salivary duct antibody is not peculiar to Sjögren's syndrome. It is found most commonly in Sjögren's syndrome complicated by rheumatoid arthritis but is also present in approximately one quarter to one half of patients with rheumatoid arthritis alone (MacSween et al, 1967; Whaley et al, 1968; present series). These observations suggest that the

antibody is in some way related to the rheumatoid disease process, whether or not there be clinical evidence of salivary gland involvement. Support for this concept has been provided by MacSween et al (1967) who showed that among rheumatoid arthritis patients, salivary duct antibody was found significantly more frequently in patients who were older and those with more severe forms of the disease.

As has been shown in Chapter V, the significantly lower salivary flow rates in older females with rheumatoid arthritis compared to control groups, suggests that sub-clinical form of Sjögren's syndrome may exist in these patients. The histological support for this concept has been provided by Waterhouse and Doniach (1966). They found focal lymphocytic sialadenitis in all 12 females and four of five males with rheumatoid arthritis examined at autopsy. It is thus not surprising that in rheumatoid arthritis, without clinical evidence of salivary gland involvement, there should be a high prevalence of salivary duct antibodies.

The finding of a lower incidence of the salivary duct antibody as between those patients with sicca syndrome and Sjögren's syndrome with rheumatoid arthritis

is of considerable interest. The detailed studies of Bloch et al (1965), Beck et al (1965) and Bunim et al (1965) have shown differences between these two sub-groups of Sjögren's syndrome. Non-organ specific autoantibodies were found to be consistently more persistent in sicca syndrome patients. Furthermore, Leventhal et al (1967) found that lymphocytic transformation in response to phytohaemagglutinin and streptolysin was less markedly reduced in Sjögren's syndrome with rheumatoid arthritis, as compared with sicca syndrome. Together, these findings suggest that the sicca syndrome patients may form a separate entity when considered from both clinical and laboratory points of view.

In the 130 patients included in the study of the correlation, between the presence of the salivary duct antibody and focal labial sialadenitis, the salivary duct autoantibody was present only in one of ten patients with the sicca syndrome (Sjögren's syndrome uncomplicated by rheumatoid arthritis or other connective tissue disease), although six had focal lymphocytic sialadenitis on labial salivary gland biopsy. This low prevalence of salivary duct autoantibody in patients with the sicca syndrome confirms previous findings. In contrast, 19 of

the 27 patients (70.4%) with rheumatoid arthritis and Sjögren's syndrome had salivary duct autoantibody, although the prevalence of focal lymphocytic sialadenitis in this group was the same as in patients with the sicca syndrome. This is further evidence that salivary duct autoantibody may be a manifestation of Sjögren's syndrome associated with rheumatoid arthritis rather than a reflection of Sjögren's syndrome *per se.* This conclusion is supported by the finding of a very high prevalence of salivary duct autoantibody in patients with rheumatoid arthritis alone (21 of 47, 44.6%) and the comparatively low prevalence of focal lymphocytic sialadenitis in this group (13 of 47, 27.7%). Furthermore, there was a complete lack of correlation between salivary duct autoantibody and focal lymphocytic sialadenitis in patients with the sicca syndrome with rheumatoid arthritis, and rheumatoid arthritis alone. These findings are in agreement with those of Bertram (1967) who performed palatal biopsies on eight patients with Sjögren's syndrome, six (75%) of whom had heavy lymphocytic and plasma cell infiltrates. Of these six patients, only two had the salivary duct antibody present in their serum.

It may be argued that the lack of correlation between the salivary duct antibody and lymphocytic sialadenitis may have been due to a sampling error, the changes in the labial salivary glands being patchy rather than generalised. However, in twelve post-mortem specimens, identical changes were found when biopsies were taken from both sides of the lower lip. It is unlikely that major salivary gland changes may have shown a correlation, since the post-mortem studies reported in Chapter 9 showed a positive correlation between labial lymphocytic infiltration and focal lymphocytic submandibular adenitis. The salivary duct antibody reacts with the epithelial cytoplasm of the lacrimal and salivary glands and has been shown to have autoreactivity in the four cases tested. The apparent organ specificity of the antibody corresponds to the occurrence of inflammatory lesions in lacrimal and salivary gland tissue in Sjögren's syndrome. Nevertheless, from the present investigation, the salivary duct antibody appears to be an epiphenomenon associated with the pathological changes of Sjögren's syndrome complicating rheumatoid arthritis rather than with Sjögren's syndrome occurring alone.

SUMMARY

The occurrence of the salivary duct antibody has been reported in 264 patients with connective tissue disease. The antibody has been shown to be present in 19% of patients with the sicca syndrome, 68% of patients with Sjögren's syndrome complicated by rheumatoid arthritis and in 50% of patients with rheumatoid arthritis alone.

The antibody is shown to have both persistence and auto-reactivity.

The relationship between the occurrence of the salivary duct antibody and focal lymphocytic sialadenitis in the labial salivary gland has been investigated in ten patients with the sicca syndrome, 27 patients with Sjögren's syndrome and rheumatoid arthritis, and 47 patients with rheumatoid arthritis alone. No correlation between the variables was found in any of the groups. Post-mortem studies on the labial salivary gland biopsy show that the method of biopsy is reproducible, and so the lack of correlation is not due to sampling error when the biopsy is taken.

It is suggested that the salivary duct antibody

is an epiphenomenon of rheumatoid arthritis rather than a manifestation of Sjögren's syndrome per se.

CHAPTER 13

MYCOPLASMA AND SJÖGREN'S SYNDROME

Introduction

The possibility of an infective agent in the development of rheumatoid arthritis has been a familiar one for a number of years (Hench et al, 1935). Recently, renewed interest in the role of infective agents including mycoplasmas, bedsoniae and diphtheroids in association with rheumatoid arthritis, Reiter's disease, systemic lupus erythematosus and other connective tissue diseases, has been stimulated by the studies of Bartholomew (1965), Duthie et al (1967), Schachter (1967) and Stewart et al (1969). In a brief review, Decker and Ward (1966) comment on the predilection for the joints of a variety of strains of mycoplasma in animals and fowl, and in some instances, the lesions produced are similar to those of rheumatoid arthritis. The impressive clinical immunological association of Sjögren's syndrome with other connective tissue disorders in which mycoplasmas have been found and the clinical prominence of salivary gland involvement have prompted a search for oral mycoplasmas in the saliva and minor salivary

gland tissue from patients with this fascinating symptom complex. The methods involved in this part of the study together with the results and discussion will now be described.

Patients Studied

Twenty-six patients with Sjögren's syndrome were included in the present study. Twenty-one patients had Sjögren's syndrome complicated by rheumatoid arthritis and five had the sicca syndrome alone.

Salivary secretion and tissue collection

Parotid saliva was collected using a modified Carlsson-Crittenden cup (Carlsson and Crittenden, 1910) with an outer chamber diameter of 20 mm., and inner chamber diameter of 10 mm., and depth of 4 mm. Saliva was collected for five minutes under stimulation with lemon juice as described in Chapter 4.

In six patients, lobules of minor salivary gland tissue were obtained by dissection from labial minor salivary gland biopsy specimens obtained by the method described in Chapter 10. Samples of saliva and

gland tissue were distributed into bottles containing mycoplasma growth medium and were transmitted for laboratory examination.

Laboratory Investigations

a) Media for cultivation of mycoplasmas

Liquid mycoplasma growth medium comprised 70 ml. Difco "PPLO" broth, 20 ml. unheated Burroughs Wellcome horse serum No. 6, 10 ml. of 25% aqueous extract of dried yeast (Distillers Company Ltd.), 2 ml. of a 2.5% aqueous solution of thallium acetate (reduced to 1 ml. of T-strain culture medium) 1 ml. of a solution containing 100,000 units of Penicillin G, and 2 ml. of a 0.1% aqueous solution of phenol red. Aliquots of this basic medium were separately supplemented with glucose, arginine and urea to a final concentration of 0.1%, for the detection of glucose fermenting, arginine and urea-splitting mycoplasmas respectively. The glucose medium was adjusted to pH 7.8 and the arginine and urea media to pH 6.5. Solid medium was prepared by the addition of 1% agar (Oxoid Ionagar No. 2) to the "PPLO" broth before the addition of the other constituents.

b) Cultural methods

Parotid salivary specimens were inoculated in 1 ml. amounts into 10 ml. volumes of glucose, arginine and urea indicator broths. The bottles of medium were incubated at 35°C and observed daily for any colour change. The broths were sub-cultured to solid "PPLO" agar twice weekly for a period of three weeks, the agar plates being incubated anaerobically at 37°C and examined for mycoplasma colonies every 48 hours. Incubation was continued for two weeks before the cultures were discarded. Salivary gland biopsy specimens were homogenised in "PPLO" broth in a Tenbroeck grinder and the homogenate was then treated in the same manner as the saliva.

c) Identification of mycoplasmas

The method used was that of growth inhibition using the technique of Clyde (1964).

RESULTS

Apart from one isolate, a strain of mycoplasma orale, recovered from the salivary secretion of one patient, no oral mycoplasmas were recovered from the stimulated parotid saliva of any of the other patients or from any of the minor salivary gland biopsy specimens.

DISCUSSION

As has been discussed in the previous chapter, the pathological basis for the finding of the salivary duct antibody in patients with Sjögren's syndrome has not yet been elucidated. However, the possibility of an auto-antibody being induced by an infective agent must be considered. A number of infective agents, including mycoplasmas, have been sought and found in the various connective tissue diseases, especially in rheumatoid arthritis. The view that the latter might have an infective aetiology has been held for many years, and various micro-organisms have been demonstrated in the synovial fluid or membrane in rheumatoid arthritis cases. Within recent years, Schachter (1967) has recovered bedsoniae from the joints of some patients with established rheumatoid arthritis and from cases of Reiter's disease. Diphtheroid organisms have recently been isolated from synovial tissues of patients with rheumatoid arthritis (Duthie et al, 1967) the significance of these findings being very doubtful (Stewart et al, 1969) though it seems possible that they are contaminants (Glasener et al, 1969). Other workers have isolated mycoplasmas from the tissues in rheumatoid arthritis and some allied connective tissue diseases (Bartholomew and

Himes, 1964; Bartholomew, 1965; Williams, 1968). The strains which have been isolated belong to a variety of species including M. fermentans, M. hominis and M. hyorrhinis. T-strain mycoplasmas have also sometimes been recovered from joint fluids in cases of Reiter's disease (Jonsson, 1961; Bartholomew, 1965). The significance of such isolations remains highly speculative though they may merely indicate contaminants or at most, the presence of secondary 'opportunists'in the tissues of individuals with immunological derangements. It is of interest, however, that mycoplasmas, including M. agalactiae and M. arthritidis may produce various types of arthritis in Veterinary pathology including in some cases a chronic polyarticular disease with synovitis (Ahe et al, 1966; Hughes et al, 1966; Moore et al, 1966; Ross et al, 1968) while a syndrome including joint manifestations has been encountered during M. pneumoniae infection in man (Lambert, 1968).

In this study, the salivary mycoplasma flora of patients with Sjögren's syndrome with or without rheumatoid arthritis has been investigated. It is important to appreciate that the healthy human oropharynx is colonised by a variety of mycoplasms,

principally M. salivarium and M. orale type 1, and less frequently M. orale types 2 and 3, M. hominis, and T-strains (Kundsen et al, 1967; Hendley et al, 1968). With the exception of M. pneumoniae which is rarely recovered from the healthy oro-pharynx, (Kundsen et al, 1967), and M. hominis which has induced pharyngitis in some human volunteers under experimental conditions, (Mufson et al, 1965), there is no real evidence so far that any of the naturally occurring oral mycoplasmas are ever pathogenic. M. pneumoniae is in fact the only human species that is indisputably pathogenic. Subsequent to its identification as the cause of Eatons primary atypical pneumoniae (Chanock et al, 1962), M. pneumoniae has become widely recognised as a fairly common cause of acute upper and lower respiratory tract infections (Marmion, 1967). It has also been associated with otitis media, bullous myringitis, meningo-encephalitis (including the Guillain-Barre syndrome), polyarthritis, and various muco-cutaneous rashes (Marmion, 1967). It has been known for many years that M. pneumoniae infection of the respiratory tract is often associated with the development of cold agglutinins while antibodies with a specificity for lung tissue (Thomas, 1964) have been recognised in Eaton agent (M. pneumoniae)

atypical pneumonia. More recently, it has been found that the cold agglutinin is a red cell auto-antibody possessing anti-I specificity arising as a result of a surface alteration by the mycoplasma of I receptors on the red cell surface (Feizi et al, 1967). The ability of one human pathogenic mycoplasma to induce auto-antibody formation during infection has led to considerable speculation about a possible association between mycoplasma infections and other diseases considered to have an auto-immune basis, and the recovery of these organisms from the tissues in some of the connective tissue diseases has serviced to intensify research along such lines, but there is no evidence so far that any of the other currently recognised human mycoplasma species other than M. pneumoniae is capable of evoking auto-antibody formation.

In this investigation, no evidence has been produced to implicate naturally occurring human oral mycoplasmas in Sjögren's syndrome with rheumatoid arthritis or in the sicca syndrome, but it is interesting to speculate on possible mechanisms of auto-antibody formation, locally or more generally, in this and allied disorders. Experimental work with M. pneumoniae has led to a greater understanding of the

mechanism of adsorption of mycoplasmas to cell surfaces. M. pneumoniae strongly adsorbs to red cells, spermatozoa, and to tracheal epithelial cells by smears of neuraminic acid receptors (Sobeslavsfy et al, 1968) and this affinity for respiratory tract epithelium provides an excellent opportunity for hydrogen peroxide secreted by the mycoplasma to damage the tissue cell membrane without being rapidly destroyed by catalase or peroxidase enzymes present in the extracellular fluids (Cohen et al, 1967). Some other human mycoplasmas also produce hydrogen peroxide but to a much lower degree than M. pneumoniae while they are not known to adsorb to epithelial cells to the same extent as M. pneumoniae (Cole et al, 1968). Nevertheless, by analogy with some virus-host cell relationships, prolonged contact of an oral mycoplasma with salivary epithelial cells might result in antigenic alteration on or with some cells with subsequent loss of 'self' recognition and the development of an auto-antibody. Alternatively, the occurrence of salivary duct antibody in Sjögren's syndrome might be explained by a sharing of antigenic determinents between components of salivary duct epithelium and mycoplasmal antigens. In this context, it is of interest that a study of M. mycoides pulmonary infection in cattle (Shifrine et al, 1969) has demonstrated

an antigenic relationship between a galactan of this mycoplasma and galactan-containing carbohydrates in bovine tissues which probably explains the occurrence of anti-lung antibodies in this infection.

SUMMARY

This study has been limited to a search for overt mycoplasma infection in salivary tissue and related secretions in Sjögren's syndrome, and the failure to find these organisms by conventional cultural methods does not exclude the possibility of the occurrence of hitherto uncultivable agents from the tissues or of 'latent' mycoplasmal infection in this or allied conditions. It will be logical to continue a search for mycoplasmas by utilisation of the techniques of electron microscopy or of immunofluorescence, but the defects of mycoplasma elementary bodies or of mycoplasma antigen in affected minor or major salivary tissue would not absolutely resolve the question of whether these agents are present in an aetiological role or merely as 'passengers' or 'opportunists', secondary to the pathological and immunological derangements associated with the syndrome.

C H A P T E R 14

ISO-ELECTRIC FOCUSSING OF SALIVARY PROTEINS

Introduction

Recently, Fischer et al (1968) observed that the electrophoretic pattern of parotid secretion was specific for small groups of patients with Sjögren's syndrome and rheumatoid arthritis. It was thought of value in this part of the study to separate parotid salivary proteins by iso-electric focussing in poly-acrylamide gels, a technique which gives significantly better resolution than previous methods (Beeley, 1969).

The technique of iso-electric focussing separates proteins as a function of their iso-electric point in a pH gradient, Svensson (1961) described the formation of a natural pH gradient by application of a potential across a mixture of large numbers of aliphatic aminocarbonylic acids; the system was set up in a sucrose gradient to prevent mixing of the solution after the pH gradient was formed. Proteins present in the mixture became focuss to form sharp bands at their iso-electric pH, (Vesterberg and Svensson, 1966). The method has high resolving power and will fractionate proteins which differ in their iso-electric points by only 0.02 units. Solutions are commercially available

as "Ampoline" electrolytes (LKB Produkter, A.B., Stockholm-Bromma, Sweden) which forms gradients of pH 3-10 or narrower ranges. Polyacrylamide gels have recently been used to stabilise these pH gradients, a method which uses much smaller amounts of protein and minimises the problems of convection, diffusion, etc., which occur in a liquid medium. A significant improvement in the resolution of serum proteins and lactate dehydrogenases (Dale and Latner, 1968), haemoglobin and myoglobin (Fawcett, 1968) and immunoglobulins (Awdeh et al, 1968), have been given using this technique.

MATERIALS AND METHODS

Collection and preparation

Parotid saliva was collected using a modified Carlsson-Crittenden cup as already described. Parotid secretion was obtained by lemon juice stimulation, and collected into an ice-cooled vessel. The fluid was then centrifuged at 7,000 **g.** at 2°C for ten minutes, concentrated and desalted immediately by means of an Amicon Ultra-filtration cell (Amicon Corporation, Lexington, Mass. 02173, U.S.A.) with a UM-1 membrane (10,000 mol. wt. cut off) and stored in the freezer. The protein concentration of the saliva was determined

according to Lowry et al (1951) using Bovine chymotrypsinogen, standardised by its extinction coefficient (Wilcox et al, 1957) as standard.

Iso-electric focussing was performed as described by Dale and Latner (1968) with the following modifications. Gels were made in 3 mm. internal diameter tubes. The ampholine/acrylamide monomer solution consisted of a mixture of the following aqueous solutions:

1 ml. 28% (w/v) acrylamide containing 0.735% (w/v) N, N' methylenebisacrylamide,

1 ml. 40% (w/v) sucrose,

0.1 ml. 40% ampholine of the required pH range,

0.25 ml. 1.6% (w/v) N, N, N', N' - tetramethylethyl enediamine and 0.5 ml. 0.004% (w/v) riboflavine;

0.3 of this ampholine/acrylamide monomer was mixed with the protein sample in 0.2 ml. of salt free solution. The sample containing monomer was layered over 40% (w/v) sucrose in the gel tubes and made up to 6 cm. columns with a mixture of 3 volumes of ampholine/ acrylamide monomer and 2 volumes of water. The gels were photopolymerized for one hour. A potential of 100 v was applied to the gels for 4-5

hours or overnight and the ampholine removed from the gels by stirring them in two changes of 5% (w/v) trichloracetic acid for 36 hours. The gels were stained for one hour in 1% (w/v) Lissamine Green SF in 7% (w/v) acetic acid and destained with several changes of 7% acetic acid. Lissamine Green SF gives less background colour with traces of ampholine remaining in the gel than Naphthalene Black 12B and unbound dye is more easily removed. Slight variations in band patterns were observed with different batches of ampholines. It is necessary to re-characterise each batch with control saliva. All results reported were obtained from the same batch of ampholine. Densitometric tracings of the gels were made using a Joyce-Loebl Chromoscan employing a magenta filter. The technique of iso-electric focussing is illustrated in Figures IV, 7 - IV, 10.

RESULTS

The separation of parotid salivary proteins at their iso-electric points on polyacrylamide gels using pH 3-10 ampholines is shown in Figure IV,11. The upper end of the gel is the anode and the lower,

the cathode. Towards the cathode end of the gel, the iso-enzymes of amylase are shown with a high degree of resolution. Above these an intermediate band pattern is noted and consists of more than five clearly defined bands. At the anode end of the band pattern, a very sharp characteristic band, which will be referred to as the 'X' band, was also frequently observed. Densitometric tracings of gel patterns from normal individuals are shown in Figures IV,12-IV,13. These findings were noted in each of the samples of saliva from all normal individuals, with the exception, however, of one case, in which two additional proteins were noted towards the anodal end of the gel.

Sjögren's syndrome complicated by rheumatoid arthritis

The band patterns obtained from parotid saliva from patients with Sjögren's syndrome complicated by rheumatoid arthritis are shown in Figure IV, 14. Distinct band patterns of the iso-enzymes of amylase, intermediate band patterns and X-band areas similar to those obtained in normal gels were observed in each of the subjects. However, differences were noted in bands which separated out towards the anodal end of the gel. Atlhough these proteins were not very intensly stained by Lissamine green, they pre-

cipitated out at their iso-electric pH and gave strongly absorbing peaks on a densitometric tracing. The characteristic feature of the densitometric tracings is a high peak obtained in the area beyond the X-band region. This peak could be resolved into three components - alpha, beta and gamma. This allowed the classification of samples from patients with Sjögren's syndrome complicated by rheumatoid arthritis into three groups, 'A', 'B' and 'C'. Group 'A' showed an alpha peak, group 'B' alpha and beta peaks, and group 'C' alpha, beta and gamma peaks. Densitometric tracings from a gel from each of these three groups of patients are shown in Figures IV,15 - IV, 17.

Rheumatoid arthritis alone

Figure IV,18 shows the band patterns of parotid saliva from patients with rheumatoid arthritis alone. As before, the iso-enzymes of amylase, an intermediate band pattern and an X-band region were observed. However, two distinct band patterns emerged due to additional bands beyond the X-band area. Densitometric tracings again showed strongly absorbing peaks to exist in these areas (Figures IV, 19-IV, 20). In this way, two groups of patients with rheumatoid arthritis, 'A'

and 'B' were determined, Group 'A' having alpha and beta peaks, whilst Group 'B' had alpha, beta and gamma peaks. In one gel, five abnormal bands were found in the X-band regions.

Sicca syndrome

The iso-enzymes of amylase, intermediate band patterns and rather ill-defined X-band areas were noted in gels from saliva of patients with sicca syndrome (Figure IV, 21.). However, in none of the samples from patients with this condition were bands noted beyond the X-band region. The appearances in fact were varied and more difficult to interpret. However, in the majority of cases the appearances were more similar to those from normal individuals than those with rheumatoid arthritis with or without Sjögren's syndrome.

Osteoarthritis

Figure IV,22 shows gels of saliva from patients with osteoarthritis. Iso-enzymes of amylase, intermediate band pattern and an X-band region similar to those noted in gels from normal individuals were observed. However, in all cases but one the distinctive feature was the separation of large amounts of protein towards the anodal end of the gel. This feature was noted

in only one other gel in one control subject who had normal salivary flow rates and no evidence of salivary gland disease.

Other arthritides

Gels from a small group of patients with other arthritides such as ankylosing spondylitis, psoriatic arthritis and Reiter's disease showed band patterns and densitometric tracings which were similar to those obtained from normal subjects.

DISCUSSION

The fractionation of salivary proteins by isoelectric focussing on polyacrylamide gels is a technique which gives better resolution of the proteins than previous methods (Beeley, 1969). In this part of the study, although only a small number of patients have been investigated, it has been shown that distinct differences exist in the band patterns obtained from patients with rheumatoid arthritis with or without the sicca component when compared with normal individuals. Interestingly, the band patterns obtained from saliva from patients with the sicca syndrome were more similar to those obtained from saliva from normal individuals. The distinct band patterns obtained would appear to be a feature of the rheumatoid process rather than the salivary gland component of the disease. As has already been discussed (Chapter 12), this concept has been suggested with regard to the presence of the salivary duct antibody. These results, therefore, provide further evidence that the sicca syndrome may differ from Sjögren's syndrome complicated by rheumatoid arthritis with respect to a number of clinical and laboratory findings. Of special interest was the difference noted in gels of saliva from patients with osteoarthritis, a disease in which salivary gland

involvement has not been reported. However, larger groups of patients require to be studied in order to assess the significance of these findings. Though no attempt has been made in the present study to identify the proteins such an investigation would lead to a fuller understanding of the changes reported.

SUMMARY

Separation of parotid salivary proteins from patients with connective tissue disease has been carried out by iso-electric focussing in polyacrylamide gels. Parotid saliva was collected using a modified Carlsson-Crittenden cup and following lemon juice stimulation. The secretions were desalted and concentrated in an Amicon ultra-filtration cell (UM/1 membrane) lyophilised and fractionated by iso-electric focussing at pH 3-10. Patients with Sjögren's syndrome and with rheumatoid arthritis alone showed two characteristic band patterns. However, patients with sicca syndrome showed band patterns similar to those in normal individuals. Patients with osteoarthritis had abnormal protein bands at the anodal end of the gel. The abnormalities reported did not occur

in the region of the amylase bands. The technique is considered a further valuable method of investigating salivary secretion in patients with connective tissue disease.

CHAPTER 15

CONCENTRATION OF IODIDE

Introduction

The concentrating ability of the major salivary glands for iodide has been extensively studied (Mason, et al, 1966A; Harden and Alexander, 1967). Iodide is concentrated by the parotid and submandibular glands to many times the plasma level and the concentration is inhibited by excess iodide and by other ions such as perchlorate. In some animals, iodide has been shown to be concentrated by the sub-maxillary but not parotid gland (Cohen and Myant, 1959). Little information is available about the chemical composition of saliva secreted by the minor salivary glands and no studies have been made of iodide secretion in man or in animals. The aim of the present part of the study was to investigate the concentration of iodide in the minor salivary glands, to study its relationship with flow rate and the effect on its concentration of potassium perchlorate. Further, the concentration of iodide by the minor glands is compared with that of the parotid gland. Finally, the results are compared with those obtained from patients with

connective tissue disease.

MATERIALS AND METHODS

Patients studied

Twenty-five normal subjects with no evidence of thyroid or salivary gland disease were studied. Twelve were females and thirteen were males and all volunteered for the investigations. Ages ranged from 26 - 69 years.

Fifty μCi/^{132}I was administered intravenously. Labial saliva was collected for a period of 5 to 8 min. commencing 20 min. after administration of the isotope using a technique similar to that described by Kutscher et al (1967). Ten microlitre capillary tubes (Figure IV, 23) were used to collect the secretion of the minor salivary glands of the lower lip as shown in Figures IV, 24- IV,25. The patients lower lip was held everted by an assistant, the mucosa gently dried with sterile gauze and a conventional dental aspirator placed in the floor of the patient's mouth. A few drops of citric acid (10%) was applied to the dorsum of the tongue. The saliva was collected by capillary action of the tubes. The collected saliva was expelled into weighed tubes which were then re-weighed.

Parotid saliva was collected using a modified Carlsson-Crittenden cup in 16 subjects at 30 min. after administration of the isotope using the same stimulant. The collection was continued until approximately 3 ml. saliva was obtained. This took an average 2 min. In 14 patients minor salivary gland tissue was obtained from the lower lip as described in Chapter 10.

In addition a labial biopsy was performed on six rheumatoid patients undergoing hand arthroplasty operations under general anaesthesia. This part of the study was designed to exclude the possibility of an effect on the minor glands from injection of local anaesthetic and also to provide an opportunity of studying patients with this disease.

Blood samples were obtained at 12 min, 25 min, and 35 min. The samples of saliva, plasma and gland tissue were counted in an automatic gamma scintillation counter (Nuclear Chicago) with pulse height analyser settings corresponding to an energy range of 520 to 760 KeV. A standard containing an aliquot of the dose was also counted. Sample counts were corrected for background and for radio-

active decay between counting of sample and standard. The saliva and plasma activities were expressed as percentage of dose per ml of sample and the gland activity as percentage of dose per gram. The plasma activity was plotted against time and the mean plasma activity for each saliva collection and tissue sample was calculated.

In three patients, immediately following the collection of parotid saliva, 500 mg potassium perchlorate crushed in water, was given orally. A second labial saliva collection was commenced 15 min later, and followed as before, by a parotid saliva sample. At the conclusion of this period, a further blood sample was obtained.

RESULTS

A) Comparison between iodide concentration in minor gland saliva and in parotid saliva

The minor gland flow rate ranged from 0.05 microlitres per min to 1.50 microlitres per min, mean 0.46 (S.E. 0.10). The saliva/plasma iodide ratio for minor saliva is shown in Figure IV, 26 plotted against salivary flow rate. As flow rate increased the saliva/plasma ratio fell from a value of 74 to less than 10 at high flow rates. The mean saliva/

plasma iodide ratio was 26.8 (S.E. 4.7). The saliva/plasma iodide ratio in parotid saliva, from the same patients and under the same conditions of stimulation, was 14.9 (S.E. 1.3). This was significantly lower than the ratio in labial saliva ($p < 0.05$).

B) Concentration of iodide in labial minor salivary glands

In eight patients, the labial gland/plasma ratio was measured and the results are shown in Table IV, 9. Values ranged from 0.47 to 3.08, the mean value being 1.26 (S.E. 0.31). The value in the six patients with rheumatoid arthritis and given a general anaesthetic was not significantly different (mean 1.56; S.E. 0.36). The gland/plasma ratio for the patients was significantly lower than the saliva plasma/ratio (mean 31.2; S.E. 7.4). No significant correlation was noted between the gland/plasma ratios and the saliva/plasma ratios. The mean parotid saliva/plasma ratio was 15.6 (S.E. 1.9).

C) Effects of perchlorate on labial and parotid saliva iodide concentration

The labial saliva/plasma ^{132}I ratio decreased after perchlorate administration. In the three patients studied, the mean value after perchlorate

was 16.1 and this represented a mean decrease of 47% of the control value. The decrease in the parotid saliva/plasma ratio after perchlorate was similar in one patient and greater in two patients (Table IV, 10).

D) Concentration of iodide in labial minor salivary glands in connective tissue disease

The labial gland/plasma ratios in twelve patients with connective tissue diseases are shown in Table IV, 11. Values for the six patients with rheumatoid arthritis alone ranged from 0.68 to 2.67 (mean 1.55; S.E. 0.36) and those for the Sjögren's syndrome group from 0.49 to 1.76 (mean 0.99; S.E. 0.27). These values were not significantly different. The relationship between the labial gland/plasma ratio and histopathologic grade in control subjects and patients with connective tissue disease is shown in Figure IV, 27).

DISCUSSION

The minor salivary glands like their major counterparts concentrate iodide. The concentration in labial saliva, as in parotid and submandibular saliva varies with flow rate being greatest at low flow rates (Mason et al, 1966). Variation of the concentration of other ions, notably sodium and chloride has been found with flow rate (Wood and Dawes, 1968). In minor saliva the concentration of phosphate is one-tenth the concentration in parotid or submandibular saliva while the concentration of chloride is similar or greater (Wood and Dawes, 1968). In the present study, it has been shown that iodide is concentrated to a greater extent in labial saliva than in parotid saliva collected under similar conditions of stimulation. In the stomach, iodide has been reported as being concentrated in the mucous cells of gastric mucosa (Logothetopoulos and Myant, 1956; Harden et al, 1969) and it is of interest that while the parotid is essentially a serous gland, the labial glands are pure mucous in nature (Tandler et al, 1969).

The labial salivary gland/plasma ^{132}I ratio is

significantly less than the concentration in the saliva. It is likely that in the minor glands as in the parotid gland where iodide is concentrated in the ductal cells, only a small proportion of cells are responsible for iodide concentration. In those cells not responsible for ^{132}I concentration the tissue/plasma ratio will be similar to that in surrounding tissues, and in animals has been shown to be approximately 0.25 (Papadopoulos et al, 1967). This would explain the lower overall gland/plasma iodide ratio. The lack of correlation between the labial gland/plasma ratio and saliva/plasma iodide ratio is not unexpected, as both are dependent on flow rate and the conditions of stimulation were not the same. Perchlorate is a competitive inhibitor of iodide concentration in the minor glands as it is in the major salivary glands. The inhibition, however, may be less complete in the minor gland tissue than in the parotid salivary gland.

Taking flow rate into consideration, previous studies (Mason et al, 1967; Harden et al, 1968) have shown that the parotid saliva/plasma ^{132}I ratio is low in some patients with Sjögren's syndrome.

In the present investigation, three patients with Sjögren's syndrome had low labial gland/plasma ^{132}I ratios, though in no instance was the value lower than the lowest value of the control group (Figures IV, 27). In the present study, there appears to be no relationship between labial gland/plasma ^{132}I ratio and the degree of lymphocytic infiltration of the minor glands (Figure IV, 27). It is of interest that although the uptake and secretion of iodide by the salivary glands has been extensively studied, its actual site within the gland is uncertain. However, considerable evidence has been provided that iodide is concentrated in the cells of the salivary ducts (Cohen et al, 1955; Burgess et al, 1959). As has been observed in Chapter 10, a feature of the histopathology of the labial glands in Sjögren's syndrome is the lack of duct hyperplasia leading to the formation of epimyoepithelial cell islands. As has been noted, this is a marked feature of major gland change. Though in this condition some of the patients in the present study showed focal lymphocytic labial sialadenitis, the absence of duct change may explain why the iodide concentrating mechanism is not markedly altered.

P A R T V

SALIVARY GLAND STUDIES IN SYMPTOMATIC XEROSTOMIA AND OTHER CONDITIONS

C H A P T E R 16

XEROSTOMIA IN CONDITIONS OTHER THAN SJÖGREN'S SYNDROME

During the course of the present study, numerous patients complaining of profound xerostomia were referred to the author for salivary gland investigation.

Twenty-one patients suffered from symptomatic or secondary xerostomia, one patient had sarcoid involvement of the salivary glands and five patients had Mikulicz's disease.

It is thought of interest and value to present the results of the investigations in this chapter. They serve to highlight the differences between these conditions, and Sjögren's syndrome and also underline the value of the methods of salivary gland investigation employed.

The results and discussion of each group are reported separately.

X E R O S T O M I A

Introduction

Bartley (1868) in a letter to the Editor of the Medical Times and Gazette gave the first description of a case of dryness of the mouth as the patient's only symptom. This case of 'suppressed salivary secretion' concerned a 77 year old female who had suffered a febrile illness some three weeks prior to the onset of dry mouth. Bartley described well the dry appearance of the oral mucosa and lingual mucosa.

The descriptive term xerostomia was used by Hutchinson (1888) and Hadden (1888) each of whom reported a case of dry mouth. Though the aetiological factors remained obscure, a number of case reports continued to be added to an expanding literature (Hutchinson, 1889; Frazer, 1893; Chappell, 1896; Fallenz, 1903). In a review of 36 cases from the literature of Fallenz (1903) drew attention to the fact that 80% of cases of xerostomia were women and that the mean age was about 49 years, tending to discount xerostomia

as a disease of old age. Simultaneous salivary, gastric parietal and pancreatic secretory insufficiency in a 28 year old male patient was described by Focke (1922). Vitamin deficiency became recognised as a cause of xerostomia and stomatitis (Glassheib, 1930; Chamberlain, 1930). In 1933, Sjögren described the condition which came to bear his name and in which xerostomia was a common finding.

Irradiation (Greenbaum and Tumen, 1936) and aplasia of the salivary glands (Ramsay, 1924; Core-Smith and Moncrieff, 1936; Sharp, 1937; de Silva, 1938; Zauz and Teuscher, 1940) were shown to give rise to xerostomia. The possibility of pernicious anaemia as an aetiological factor has been described (Beebe, 1936). Xerostomia became recognised as a symptom in a number of symptom complexes other than Sjögren's syndrome, e.g. Heerfordt's syndrome (Wiber and Schlüter, 1937) and sprue (Markoff, 1938).

A number of excellent reviews of the aetiological factors concerned in the development of xerostomia have been published (Faber, 1942, 1943, 1944; Allington, 1950; Bertram, 1967). Bertram

(1967) gives an excellent classification which he broadly divided into four main groups: aplasia; local (inflammatory, irradiation, degenerative changes); systemic (anaemia, diabetes mellitis, liver conditions, Sjögren's syndrome, hormonal, vitamin deficiencies, medicaments and neurological disorders); and idiopathic. He (Bertram) felt that the majority of cases could be explained by inflammatory and degenerative, hormonal, disturbances of innervation or degenerative processes alone.

Recently, interest has been shown in salivary gland swelling and xerostomia which may be a side-effect of drug therapy. Salivary gland swelling following phenylbutazone therapy has been described (Løkkegard, 1965; Banks, 1967). Dryness of the mouth occurs in 20% of patients taking phenylbutazone (Nassin and Pilkington, 1953). Xerostomia has been described in patients on medication with phenothiazine derivatives, all of which exert an atropine-like action (Ragheb, 1963) and Scopp and Heyman (1966) have drawn attention to dryness of the mouth as a side-effect of treatment with chlorpromazine hydrochloride.

Materials and Methods

Twenty-one patients with xerostomia due to causes other than Sjögren's syndrome were included in the present study. Clinical examination, salivary flow studies, hydrostatic sialography and serological tests for salivary duct antibody were carried out.

Results

The results of the various investigations are detailed in Tables V,1 - V,2 together with age range and sex distribution.

Clinical Examination

All patients complained of dryness of the mouth though clinical evidence of xerostomia was present in only nine of the 21 patients. Lingual changes were unremarkable, seven of the 21 patients having Grade I changes only. One patient (X 13) complained of difficulty in swallowing. Ulceration of the oral mucosa, where present, was related to ill-fitting dentures.

Salivary Flow Studies

Parotid salivary flow studies under lemon juice stimulation were performed in all patients. In only two patients (X 13 and X 18) was an individual gland flow rate below the normal range. In one

of these patients (X13) with diabetes mellitus, only pus could be obtained from the left parotid gland, In the other case (X18) a partial parotidectomy of the left gland had previously been performed for the removal of a benign mixed salivary tumour.

Hydrostatic sialography

Hydrostatic sialography was performed in 19 of the 21 patients. In the remaining two cases, (X2 and X18), plain films revealed salivary gland calculi and sialography was not performed. All sialograms were within the limits of normality.

Labial salivary gland biopsy

In sixteen of the 21 patients, a labial salivary gland biopsy was performed. In none of these were foci of lymphocytes observed. Six biopsy specimens showed no pathological abnormality (Grade 0), seven showed Grade 1 changes and three showed Grade 2 changes.

Salivary duct antibody

The sera from all 21 patients were tested for the presence or absence of the salivary duct antibody. Only one patient (X15) had a weakly positive test for

the antibody.

Other investigations

None of the patients complained of dryness of the eyes nor had keratoconjunctivitis sicca on examination. There was no evidence of rheumatoid arthritis or any other connective tissue disorder. Of the 21 patients, nine had a history of drug therapy for anxiety states or for the treatment of hypertension. These drugs included Promethazine hydrochloride and Chlorpromazine hydrochloride used in the treatment of anxiety states and chronic neurosis. The hypotensive drugs implicated in the drug-induced xerostomia encountered in the present series were mecamylamine hydrochloride and amitriptyline hydrochloride. One patient (X16) was taking a stomach powder containing belladonna.

Four patients suffered an anxiety state, though no history of appropriate drug therapy could be obtained. It was of interest that three patients with Fordyce spots (ectopic sebaceous glands in buccal mucosa) had a 'cancerphobia' with regard to these simple lesions. Three patients had haematological disorders including pernicious anaemia, iron deficiency anaemia and mekakaryocytic myelosis.

One patient had diabetes mellitus and two patients had low grade chronic sialadenitis. One patient, already referred to had had a partial parotidectomy. In one case the cause of xerostomia could not be determined.

DISCUSSION

The phenomenon of oral mucosal dryness presents a difficult diagnostic problem. Xerostomia is a logical effect of a drug such as chlorpromazine hydrochloride which has been shown to have a mild atropine-like inhibitory effect on gastric secretion (Mahrer, 1958). Among the primary or direct effects of this drug are the pharmacological extensions upon the autonomic nervous system. It is a moderately active adrenergic blocking agent and its blockage of effector organs of post-ganglionic nerves is demonstrable in the intestines, pupils, salivary glands and skin. Scopp and Heyman (1966) suggest that it is the cholinergic blocking action which produces the dryness of the mouth. However, it may be that the underlying anxiety state in itself may cause the sensation of oral dryness. To my knowledge no previous salivary flow studies have been reported in patients taking chlorpromazine hydrochloride. However, Dobkin and Paeko (1960) and Scopp and Heymen (1966) have reported the clinical observation that there is some reduction in salivary secretion. It is an interesting finding of this part of the study that parotid salivary flow rates in patients taking the drug,

were within the normal range. The possibility remains that there may be no marked reduction in flow, but rather an alteration in sensory perception in the oral mucosa secondary to anxiety or medication. It is interesting to note that Kitamuru and Okuda (1962) have reported that the decrease in salivary gland disease than those with psychogenic xerostomia. Patients taking hypotensive drugs, such as mecmyalmine hydrochloride and amitriptyline hydrochloride did show a more marked reduction in parotid salivary flow but the values were within the wide normal range. The finding of parotid flow rates within the low normal range in such patients was reflected further in the absence of definite oral signs of xerostomia. Furthermore, the minor salivary glands examined from such patients revealed a lack of histopathologic change. The absence of the salivary duct antibody in the sera of these patients, together with normal sialographic appearances were other features of note.

SUMMARY

Salivary gland function tests employed in this part of the investigation were within normal limits for those patients with symptomatic or secondary xerostomia with the predisposing factors of diabetes mellitus, blood disorders and chronic infection.

Thus, despite the profound symptoms of xerostomia reported by some of the patients in this series, the feature of the investigation was the lack of pathological change observed. These findings are in sharp contra-distinction to those noted in patients with true or idiopathic xerostomia associated with a condition such as Sjögren's syndrome, reported in previous chapters of this work.

Though the methods of investigation employed in this part of the study may lack the sensitivity to detect minor changes which may exist in symptomatic xerostomia, they nevertheless provide useful methods of distinguishing between the salivary gland components of symptomatic xerostomia and those of Sjögren's syndrome.

CHAPTER 17

MIKULICZ'S DISEASE AND SYNDROME

Introduction

In 1888, Johann von Mikulicz described at a meeting of the Society for Scientific Medicine in Königsberg, a case of benign, a symptomatic symmetric enlargement of the lacrimal and salivary glands. Histologically, excised lacrimal tissue showed lymphoid infiltration and acinar atrophy. The enlargement recurred and was again excised but the patient died two months later from peritonitis. Mikulicz's paper describing this original case was published in 1892, and on the basis of the benign course manifested by the patient without evident generalised lymphatic involvement and regression of the swellings, Mikulicz concluded that the condition was one of a chronic low grade infection. Following this original report, much confusion was caused by the fact that all types of cases with similar glandular enlargements were labelled as Mikulicz's disease (Howard, 1909). The first attempt to classify the syndrome on an aetiological basis was made by Thursfield (1914). This grouping was modified by Schaffer and Jacobsen (1927) into two main categories, Mikulicz's disease proper

of unknown aetiology, and Mikulicz's syndrome caused by leukaemia, lymphosarcoma, tuberculosis, syphilis or sarcoidosis. The diagnosis of Mikulicz's disease should then be reserved for those benign cases without any known cause (Schaffer and Jacobsen, 1927; Du Plessis, 1957). The first well documented clinicopathologic study of Mikulicz's disease was published in 1953 by Morgan and Castleman. In considering the clinical and pathologic aspects of 18 cases, they concluded that the disease was benign, chronic, occurred predominately in women in the fifth and sixth decades and may involve one or more salivary or lacrimal gland. Frequently, the disease was confined to one salivary gland. The lacrimal glands were less often involved than the salivary glands. Histologically, the disease was characterised by replacement of acinar parenchyma by lymphoid tissue together with a characteristic proliferation of ductal epithelium in the form of 'epimyoepithelial cell islands'. Morgan and Castleman suggested the appearance of the epithelial and myoepithelial component offered the most reliable means by which Mikulicz's disease could be distinguished from malignant lymphoma. They further suggested that on the basis of certain clinical and pathologic similarities, Mikulicz's

disease could be considered as a manifestation of the more generalised symptom complex of Sjögren's syndrome. The following year, Morgan (1954) published a comparative histologic study of the 18 previously reported cases of Mikulicz's disease and microscopic material from the original series of Sjögren's syndrome. The pathological appearances were found to be identical and a re-examination of the clinical records of cases of Mikulicz's disease showed that a number had other components of Sjögren's syndrome such as keratoconjunctivitis sicca, xerostomia and rheumatoid arthritis. Morgan concluded that Mikulicz's disease may be a less highly developed variant of Sjögren's syndrome.

Earlier, Godwin (1952) had reviewed 11 cases of parotid gland lesions which had been reported previously either lympho-epithelioma, lymphocytic tumour, chronic inflammation, Mikulicz's disease or adrenolymphoma. Histologically, the lesions consisted of masses of lymphoid tissue containing scattered foci of epithelial cells, tracable to ductal origin. Grossly, two types of lesions were noted, a well-circumscribed and a diffuse variety. Discomfort, occasional pain, xerostomia

and parotid enlargement either unilateral or bilateral were the presenting symptoms. The lesion was more common in middle-aged females and responded well to radio-therapy. In none of the cases were the lacrimal glands involved and the condition pursued a benign course. Godwin gave the condition the name 'benign lympho-epithelial lesion' and stated that it may arise either in the parotid lymph nodes which contain glandular tissue or may be a hyperplastic reactive manifestation unique to the parotid gland.

In a study of 186 cases of lympho-epithelial lesions of the salivary glands, Bernier and Bhaskar (1958) showed Mikulicz's disease or benign lympho-epithelial lesion of Godwin to be primarily a lesion of lymphoid tissue which because of its site, involved the salivary gland incidently. It represented a reactive hyperplasia of lymphatic tissue and a nodular and diffuse type was recognised. The reactive nature of the lesion was based on the fact that the parotid nodes drain a wide area of the face, some nodes are not encapsulated, that in some instances a history of regional infection was reported, that the lesion showed regression in some instances following removal of suspected local factor and finally that the infiltrating element was lymphocytic

rather than epithelial.

In an excellent review of the literature and a report of three cases (Du Plessis, 1958) takes the view that Mikulicz's disease is the result of chronic irritation and not a variant of Sjögren's syndrome. He pointed out that too often the diagnosis of Mikulicz's disease is made on histological grounds alone. He suggested that the application of the strict criteria of clinical picture, sialography and histology in considering the diagnosis will prevent confusion with Sjögren's syndrome. Du Plessis suggests the term idiopathic chronic parotitis.

In a collection of 11 cases (Cruickshank, 1965) showed the benign lympho-epithelial lesion to be an isolated one. Although the histopathological features were identical to those of Sjögren's syndrome the sicca component was absent in each case. Furthermore from long term follow-up studies there appeared no evidence for an eventual onset of symptoms of Sjögren's syndrome.

MATERIALS AND METHODS

Patients studied

Five patients with benign lympho-epithelial lesion (Mikulicz's disease) diagnosed on the basis of massive

bilateral parotid gland swelling of unknown aetiology were examined in this part of the study. Histopathologic evidence for the diagnosis was obtained from parotid gland biopsy in four of the patients. Two patients had received radio-therapy for the condition prior to being referred for salivary gland investigation. The results of salivary gland function tests are detailed in Tables V, 3 - V,4 and examples of parotid enlargement in Figures V,1 - V, 3.

Oral Examination

All five patients complained of dryness of the mouth, although clinical signs of xerostomia were present in only two of the patients. Lingual changes of Grade II severity were present in both these patients. Bilateral parotid swelling was present in all patients and bilateral submandibular swelling was observed in two patients.

Salivary flow studies

It was of interest that reduction of parotid salivary flow was noted in only two patients who had previously received radio-therapy.

Sialography

All five patients showed degrees of sialographic

abnormality when hydrostatic sialography was performed. Two patients showed atrophic changes in the parotid duct system, two patients showed cavitary sialectasis with gross duct dilatation and one showed globular sialectasis with marked duct changes.

Labial salivary gland biopsy

Focal lymphocytic sialadenitis affecting the labial minor glands was a feature of four of the patients. Moderate lymphocytic infiltration (Grade II) was noted in the other patient. Epi-myoepithelial cell islands were not observed in any of the biopsy specimens. Marked acinar atrophy and interstitial fibrosis were features of note.

Salivary duct antibody

The salivary duct antibody was present in only one patient with benign lympho-epithelial lesion. This patient also had positive antinuclear factor, rheumatoid factor and non-tissue specific precipitin test.

Pertechnetate scanning

Percentage uptake values of the isotope were

within normal limits in each case examined.

Other examinations

All five patients complained of dryness of the eyes, though keratoconjunctivitis sicca was present in only the two patients who had received radio-therapy. These ophthalmological changes were considered iatrogenic following irradiation.

Interestingly, none of the patients had clinical evidence of rheumatoid arthritis, though rheumatoid factor was noted in the sera of three of the five patients.

DISCUSSION

All five patients shared in common marked major salivary gland enlargement, sialographic abnormalities, focal lymphocytic involvement of the labial minor salivary glands and symptomatic ocular and oral dryness.

However, only two patients had definite kerato-conjunctivitis sicca and xerostomia, thus qualifying for the diagnosis of Sjögren's syndrome. Unfortunately, from a pathogenetic point of view these patients had received irradiation so that these symptoms may well have been iatrogenic following radio-therapy. A feature of the investigation was the relative paucity of serological abnormalities in these patients, only one patient having the salivary duct antibody present. Interestingly, however, three patients did have positive rheumatoid factor tests.

The salivary gland investigations in these patients showed findings consistent with the salivary component of Sjögren's syndrome. Further, symptomatic dryness of the eyes and the presence of rheumatoid factor in three of the

patients, suggests that at least a subclinical form of the condition was present. However, this investigation concerned only a small number of patients and was hampered by the fact that two patients had received radio-therapy making difficult the interpretation of the salivary gland findings.

The study has shown, however, that although the salivary gland involvement in the condition is severe, definite evidence of ocular and connective tissue component of Sjögren's syndrome is lacking. Though, four of the patients were female, the age range tended to be lower than that observed in classical Sjögren's syndrome. It may be that the condition represents an early manifestation of Sjögren's syndrome, since symptomatic evidence of the sicca components were present. The possibility, however, remains that the condition might well be the result of an unknown infective agent affecting principally salivary and lacrimal gland tissue. Obviously, a much larger series of patients of this rather rare condition must be examined before definite conclusions can be drawn.

MIKULICZ'S SYNDROME

Salivary gland involvement in sarcoidosis is well recognised but little is known about the changes in function of individual glands. Although a number of workers have reported some changes in salivary gland function including the biochemical composition of mixed or pooled saliva, there have been no studies of individual salivary gland function tests before and after treatment in this condition.

Patient studied

One patient, a 66 year old retired male, who had worked as a machine operator, is studied in this part of the investigation. He presented to his general medical practitioner complaining of bilateral parotid gland swelling together with dryness of the mouth and eyes. Loss of taste sensation, and bilateral temporomandibular joint pain were also reported. He was referred to an E.N.T. specialist by which time the swelling had reduced considerably. However, at the time of this examination, a patchy erythematous rash affecting the patient's arms, trunk and back

(Figure V, 4 - V, 5) was observed and the patient referred for dermatologic assessment. A skin biopsy confirmed the clinical diagnosis of Boeck's sarcoidosis. An ambiguous result, however, was obtained following a Kveim test, sarcoid deposits being present in the biopsy specimen, but not at the site of injection of the antigens. A Mantoux reaction was negative at 1 : 10. An ophthalmological examination revealed kerato-conjunctivitis sicca on slit-lamp examination. Respiratory function tests showed normal lung volumes and capacities but a diffusion defect was noted and was considered to be consistent with sarcoidosis. Salivary gland function tests were performed and these will now be described.

Clinical examination

The oral mucous membranes were dry, glazed and mildly inflamed. There was no evidence of major or minor salivary gland swelling at this stage. The appearance of the tongue was normal. The patient wore ill-fitting full upper and lower dentures, and mild denture-induced hyperplasia affected the soft tissue of the upper anterior alveolar ridge.

Salivary flow studies

Bilateral parotid salivary flow rates were measured using modified Carlsson-Crittenden cups as described in Chapter 4. The values following lemon juice stimulation for the right and left parotid glands were 0.3o ml/min and 0.25 ml/min respectively. These are remarkably low values compared to those obtained from normal individuals using the same technique (Chapter 4).

Sialography

Hydrostatic sialographic examination of the right parotid gland was performed. Slight hypertrophy of the gland was noted, but this was considered to be within normal limits.

Minor gland biopsy

A labial salivary gland biopsy showed marked replacement by discrete granulomas of epithelioid cells. Multinucleate giant cells of the Langhan's type were noted and towards the periphery of the granulomata, diffuse lymphocytic infiltration was observed (Figures V,4 - V,5). Special stains showed a fine network of reticulum fibres but failed to show pooling of mucus or the presence of asteroid bodies. Caseation necrosis

was absent. These features were similar to those noted in the skin biopsy.

Salivary duct antibody

Using the technique described in Chapter 12 a weak positive result was obtained for the presence of salivary duct antibody. Non-specific tissue precipitin, antinuclear factor, rheumatoid factor and antithyroid globulin tests were negative.

Scanning

A scan of the major salivary glands was performed using a tracer dose of 1 mCi ^{99m}Tc-pertechnetate was injected intravenously. A Selo DS4/4 superscanner was used to scan the patient in the antero-posterior position over an area between the bridge of nose and cricoid cartilage. The percentage uptakes of the isotope by both parotid and submandibular glands were within the normal range (Chapter 7).

Parotid amylase activity

Parotid saliva was collected and the amylase activity measured. Expressed as units/mg. protein, the results were 680.5 and 570 for the

left and right gland secretion respectively.

Treatment progress

Following these intensive investigations, systemic steroid therapy was prescribed and an excellent response to an initial dose of prednisolone (30 mg. daily) was noted. The skin manifestation of the disease resolved within the week and, interestingly, the parotid salivary flow rates returned to normal values. Three weeks following therapy, the flow rate values following lemon juice stimulation were 1.20 and 1.30 ml/min for the right and left parotid glands respectively. Parotid amylase activity at this stage, for both glands was 1125 units/mg. protein, values which are within the normal range. The patient reported the return of normal taste sensation. New full upper and lower dentures were constructed during the investigation and an improvement in the temporomandibular joint symptoms was noted.

DISCUSSION

In the report of an international symposium which met to discuss the aetiologic, pathologic, and immunologic aspects of sarcoidosis, the condition was defined as "a disease of unknown aetiology characterised pathologically by epithelial tubercles with inconspicuous or no necrosis, occurring in any organ or tissue and by the frequent presence of refractile or apparently calcified bodies in the giant cells of the tubercles" (Third International Conference in Sarcoidosis, 1964).

Among the many theories suggested concerning the aetiology of sarcoidosis are an atypical tuberculous response (Pinner, 1938; Kalkoff, 1963) allergy to pine pollen (Cummings et al, 1956) reaction to beryllium (Scadding, 1967) and reaction to zirconium (Rubin et al, 1956). Robbins (1967) states that many hold the view that sarcoidosis is some form of altered immunologic response, possibly to a variety of antigenic stimuli. Further support to this immunologic concept is offered by the recorded instances of sarcoidosis associated with polyarteritis nodosa and lupus erythematosus. Furthermore,

it has been shown that phytohaemagglutinin (PTA) stimulate peripheral lymphocyte transformation is significantly inhibited in cases of active sarcoidosis of the respiratory system (Langer et al, 1969). Recently, Elling and Wanstrup (1969) have reported an accumulation of IgD in sarcoid tissue.

Sarcoidosis is a systemic disease and a multi-organ involvement has been described in the literature (Maycock et al, 1963). It is of interest that the patient reported in this paper had histological evidence of sarcoid involvement of skin and minor salivary glands with probable further involvement of lacrimal glands and major salivary glands. Three types of cutaneous sarcoidosis have been recognised - Boeck's Sarcoid lupus pernio of Besnier and erythrodermic sarcoidosis (Lever, 1967).

Lacrimal gland involvement in sarcoidosis has been described (Wexler, 1939; Cullom and Goodpasture, 1941; Rosenbaum, 1941; Sniderman, 1941; Gruber, 1955). Uveoparotitis of Heerfordt (1909) is now known to be a manifestation of sarcoid (Waldenström, 1937; Michelson, 1939; Weber, 1955).

As in the case with present patient, salivary gland enlargement due to sarcoidosis gives rise to the clinical condition known as Mikulicz's syndrome. The majority of reports of oral manifestations of sarcoidosis have concerned soft tissue deposits in the mucous membranes (Covel, 1954; Kolas and Roche, 1960; Kerr, 1965), gingiva (Tillman, 1964). MacDonald et al (1969) have described a case in which the mandible was involved.

Minor palatal salivary gland involvement has been reported in 38% of cases with sarcoidosis, but no clinical evidence of palatal involvement (Cahn et al, 1964). Recently, Hobkirk (1969) reported a case in which the buccal salivary glands were involved clinically and histopathologically. It is believed that the present case is the third reporting minor salivary gland tissue involvement. It has been observed in a study of saliva from 24 patients with sarcoidosis that salivary flow volumes, enzyme content of amylase and of kallekrein were clearly reduced in many patients (Bhoola et al, 1969). In the present investigation, it was of interest that both the parotid salivary amylase activity and flow rate were markedly reduced during the

active phase of the disease and that a return to normal levels was noted in both cases following steroid therapy. Although the concentration of many constituents of saliva are dependent upon flow rate this does not appear to be the case with amylase in saliva collected from the ducts (Jenkins, 1966); though the activity may rise in mixed saliva with increased rates of flow. The wide diversity of effects of steroids in biological systems makes difficult an interpretation of the mode of action of the mechanism involved in the restoration of salivary function in the present case. Indeed, none of the known biochemical effects of steroids (Weissmann and Thomas, 1964) satisfactorily explain the excellent response to therapy. It seems unlikely that an indirect anti-inflammatory effect could account for such marked and rapid improvement. It is to be noted that although systemic corticosteroid therapy may cause a reduction in size of enlarged salivary gland lacrimal glands in Sjögren's syndrome, it does not increase secretion volumes (Bloch et al, 1965).

These findings, therefore, clearly indicate functional changes in the parotid glands in

patients with sarcoidosis.

It was of interest that an ambiguous result was noted in the Kveim test. Kveim (1941) described a technique in which an extract of known sarcoid tissue is injected intradermally. In a positive case, biopsy at the site of injection, four to six weeks later, will reveal characteristic sarcoid follicles. However, the test is not a fully reliable one. Hirsch et al (1961) report a 75% positive finding in subacute active sarcoidosis and 64% positive finding in chronic active sarcoidosis. False positives occur in 5% of persons with other disease processes. According to Danbolt (1962) the Kveim test represents a specific hypersensitivity to cellular protein present in human sarcoid tissue.

Arthritic manifestations of sarcoidosis occur in approximately 20% of patients and may precede other signs of the disease of many years (Engleman and Shearn, 1967). Recently, a history of arthritis in ten out of 64 cases of proven sarcoidosis have been reported (Spilberg et al, 1969). It is of interest that increased pre-

valence of positive tests for rheumatoid factor in a wide variety of infections and non-infective diseases, has been reported (Bartfield, 1960), and among these conditions sarcoidosis has been mentioned (Kunkel et al, 1958). In the case under review, the temporomandibular joint symptoms were dental in origin and in the absence of other joint symptoms, it is highly unlikely that arthritic complications of sarcoidosis were present in this patient.

The present case has shown the multi-organ involvement of sarcoidosis and highlighted the salivary gland manifestations. The salivary gland function tests described provide useful methods of diagnosing the disease process and monitoring the response to therapy.

S U M M A R Y

Salivary gland function in patients with various connective tissue diseases has been studied by a number of investigative techniques. The normal values for parotid salivary flow have been defined with respect to age and sex distribution. Patients with Sjögren's syndrome have been shown to have remarkably low parotid salivary flow rates. In a proportion of patients with rheumatoid arthritis alone, parotid flow rates are shown to be low, suggesting that a sub-clinical salivary gland lesion exists in such patients. Patients with osteoarthritis, ankylosing spondylitis, psoriatic arthritis, Reiter's disease and various other arthritides and connective tissue disorders had parotid flow rate values within the normal range. The value of hydrostatic sialography as a diagnostic aid was demonstrated though the technique was shown to lack the sensitivity of flow rate studies to detect early changes in salivary gland function. Scintiscanning of the salivary glands using ^{99m}Tc pertechnetate has been quantitatively assessed for the first time. Normal values have been

defined and a markedly reduced isotope uptake by the salivary glands in patients with Sjögren's syndrome has been demonstrated. A labial salivary gland biopsy technique together with a grading standard for degrees of lymphocytic infiltration are described. Conceptual support for the method is provided by a post-mortem study in which focal lymphocytic submandibular adenitis correlated with lymphocytic infiltration of the labial salivary glands for each subject in the series. Focal lymphocytic labial sialadenitis was not observed in any of the subjects in the post-mortem series. The majority of patients with Sjögren's syndrome and 20% of patients with rheumatoid arthritis alone were shown to have focal lymphocytic labial adenitis. The use of an indirect immunofluorescent antibody technique to detect a salivary duct autoantibody is described. The autoantibody is shown to be associated not only with patients with Sjögren's syndrome complicated by rheumatoid arthritis but also with 30% of patients with rheumatoid arthritis alone. The presence of the antibody did not correlate with degrees of histopathologic changes as detected by the labial biopsy. The con-

centration of iodide by the labial salivary glands in normal subjects is reported for the first time, and normal values are defined. Though patients with various connective tissue disorders had slight impairment of the iodide-trapping mechanism, the values reported were within the normal range. In a small group of patients, there was no correlation between labial gland/plasma ^{131}I ratios and labial gland histopathology. The association of Sjögren's syndrome and systemic lupus erythematosus was demonstrated by reduced parotid flow rates, sialectasis and focal lymphocytic labial sialadenitis.

The use of the technique of separating parotid salivary proteins using iso-electric focussing on polyacrylamide gels is reported for the first time for saliva from patients with Sjögren's syndrome, rheumatoid arthritis alone, osteoarthritis and other arthritides. Distinct protein band patterns were demonstrated in association with the rheumatoid process. Though the changes were difficult to determine, it is suggested that further work to isolate the protein fractions would yield further valuable information concerning the nature of the salivary gland defect which this test demonstrates. Though a search

for mycoplasma in the salivary secretions and labial gland tissue proved fruitless, the possibility of an infective aetiology in the pathogenesis of Sjögren's syndrome could not be discarded. It is suggested that further work utilizing electronmicroscopy and routine serological tests for mycoplasmal antibodies could provide additional useful information.

Salivary gland function is studied in patients with symptomatic xerostomia and the allied disorders of Mikulicz's disease and syndrome. Patients with symptomatic xerostomia are distinguished from those with Sjögren's syndrome by virtue of higher salivary flow rates and the lack of sialographic, serologic or histopathologic change. Patients with Mikulicz's disease are shown to have salivary gland involvement similar to those patients with the sicca syndrome rather than those with Sjögren's syndrome complicated by rheumatoid arthritis. The response to corticosteroid therapy in a patient with Mikulicz's syndrome due to sarcoidosis is shown using parotid flow rate and biochemical methods.

It is concluded that in the investigations of salivary gland function in patients with connective tissue disease, stimulated parotid salivary flow rates provide the most sensitive index. In addition where such facilities exist, hydrostatic sialography and quantitative scintiscanning are considered most useful methods of detecting salivary gland abnormalities in Sjögren's syndrome. The labial biopsy not only provides information concerning the degree and nature of the salivary gland defect in Sjögren's syndrome but also provides tissue for a number of laboratory research investigations.

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[illegible]SWITZER, W.P., [illegible]SIGHTOCK, R.W., [illegible]
[illegible]RISTIAN, C.[illegible]
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THE SALIVARY GLANDS AND THEIR SECRETIONS IN CONNECTIVE TISSUE DISEASE

by

Derrick MacKenzie Chisholm

B.D.S. (Glas.)

T H E S I S

Submitted for the degree of

Doctor of Philosophy

In the University of Glasgow

Faculty of Medicine

V O L U M E II

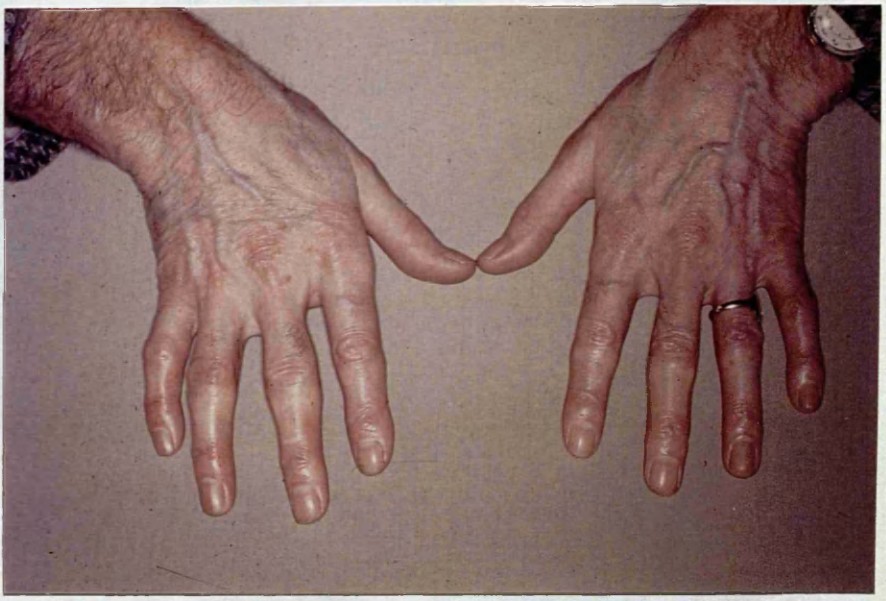
FIGURES AND TABLES

August 1970

FIGURE I, 1.

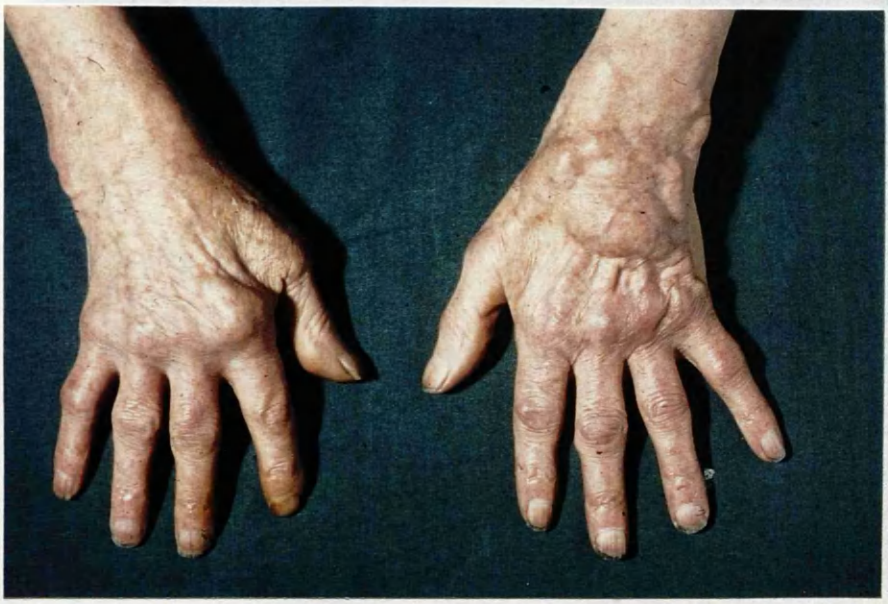
Henrik Sjögren

FIGURE II, 1.



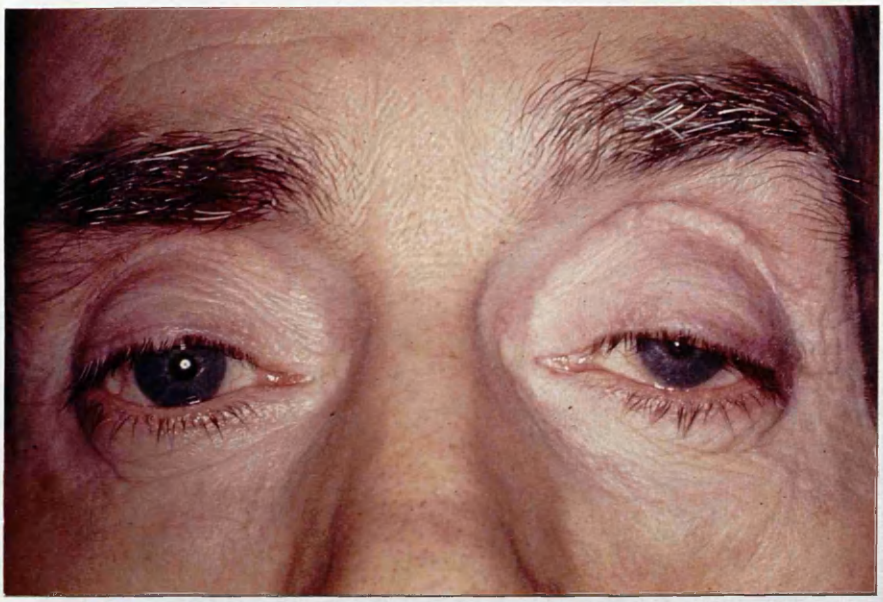
Early fusiform swelling of middle finger of right hand in a case of rheumatoid arthritis.

FIGURE II, 2.



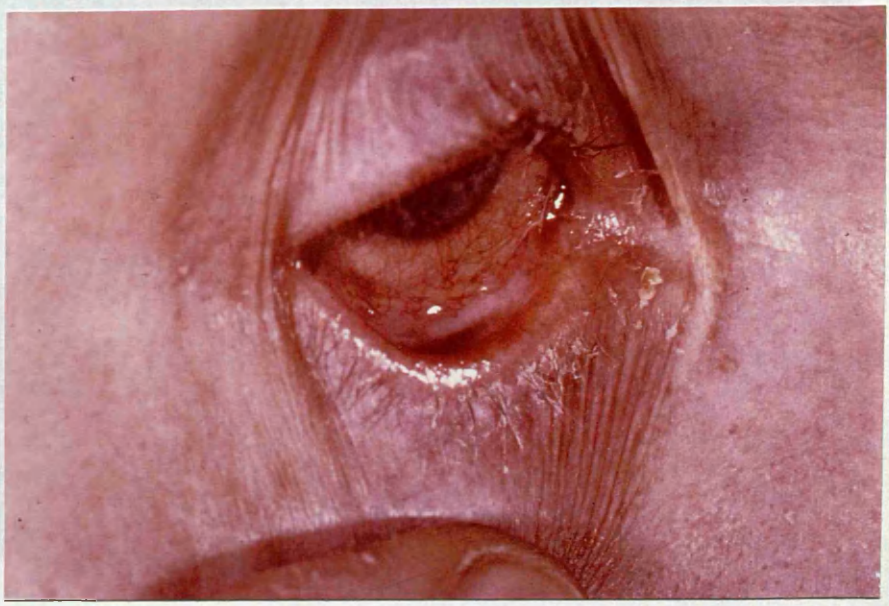
Later involvement of metacarpal phalyngeal joints in rheumatoid arthritis. Left hand shows synovial hypertrophy, involving the tendon sheath on dorsum of wrist. Right hand shows synovial hypertrophy together with muscle wasting.

FIGURE II, 3.



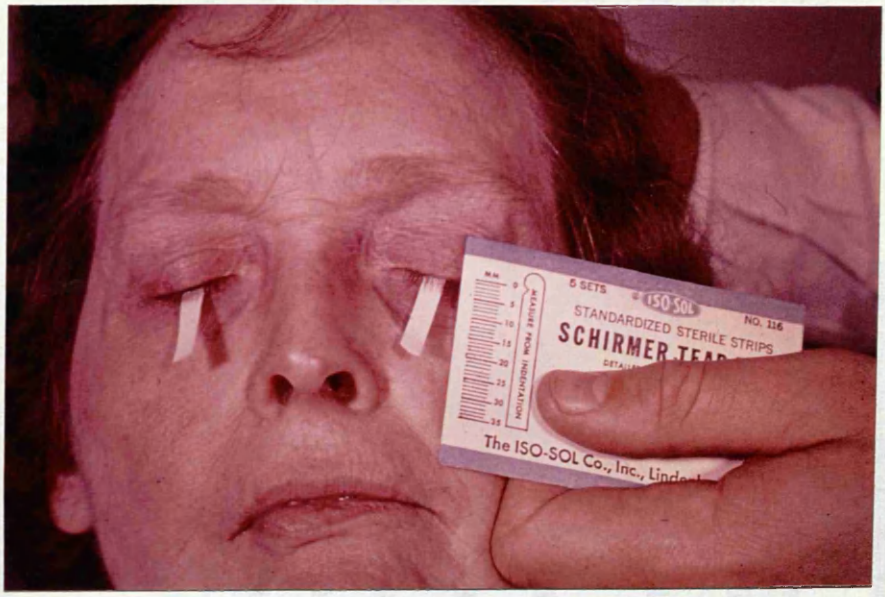
Kerato-conjunctivitis sicca in a 55 year-old male patient with Sjögren's syndrome. Moderate ptosis affects the left upper eyelid.

FIGURE II, 4.



Severe episcleritis which may be a complication of kerato-conjunctivitis sicca.

FIGURE II, 5.



SCHIRMER TEAR TEST

Use of standard sterile paper strips (35 x 5 mms) developed by Halberg and Berens. (Contactisol Inc. Linderhurst, New York, U.S.A.). Wetting of filter paper less than 15 mms following inhalation of ammonia (Schirmer II tear test) is considered abnormal. In such cases, the eye is then examined for punctate or filamentary keratitis using a Haag-Streit slit lamp, following instillation of a drop of 1% Rose-Bengal dye into each conjunctival sac.

TABLE II, 1

CLINICAL STUDIES: DIAGNOSIS, AGE and SEX DISTRIBUTION

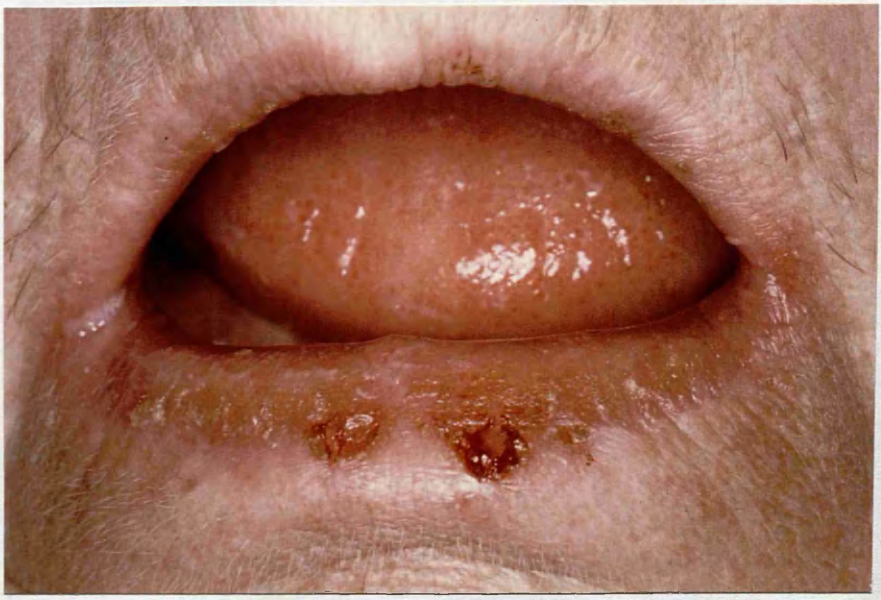
CLINICAL DIAGNOSIS	TOTAL No. of PATIENTS	FEMALES		MALES	
		No.	Mean age/range (yrs)	No.	Mean age/range (yrs)
SICCA SYNDROME	36	34	62.6 (26 - 83)	2	58.5 (55 - 62)
SJÖGREN'S SYNDROME WITH R.A.	54	45	56.9 (22 - 82)	9	58.7 (48 - 66)
RHEUMATOID ARTHRITIS	80	58	54.3 (21 - 74)	22	54.9 (36 - 74)
OSTEOARTHRITIS	24	20	61.1 (24 - 75)	4	55.8 (43 - 66)
PSORIATIC ARTHRITIS	18	14	47.8 (32 - 79)	4	44.6 (20 - 53)
ANKYLOSING SPONDYLITIS	18	14	51.2 (26 - 73)	4	41.6 (23 - 60)

TABLE II, 2

CLINICAL STUDIES: DIAGNOSIS, AGE and SEX DISTRIBUTION

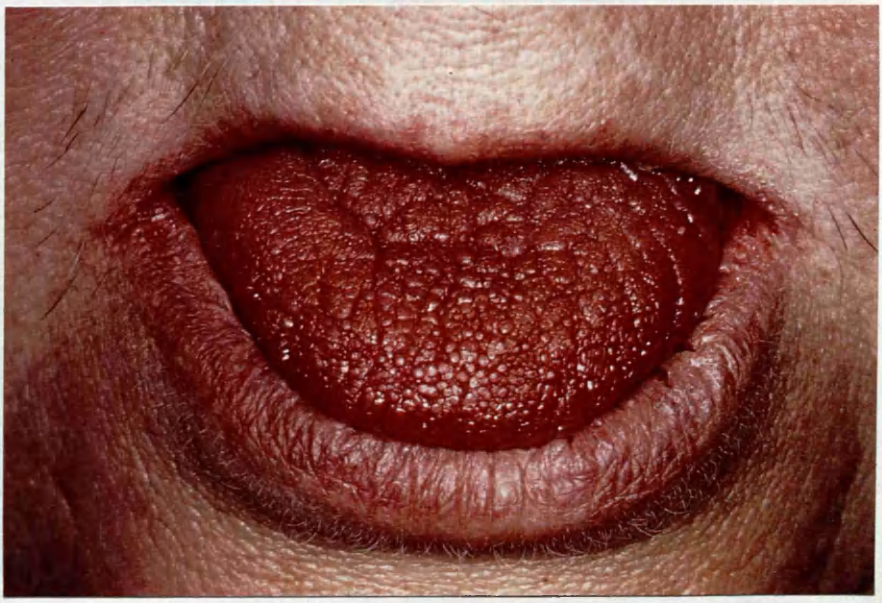
CLINICAL DIAGNOSIS	TOTAL No. of PATIENTS	FEMALES			MALES		
		No.	Mean age/range (yrs)		No.	Mean age/range (yrs)	
REITER'S SYNDROME	20	-	-	-	20	32.7	(20 - 54)
BECHET'S SYNDROME	2	2	38.5	(27/42)	-	-	-
SYSTEMIC LUPUS ERYTHEMATOSUS	10	10	38.3	(22-65)	-	-	-
PROGRESSIVE SYSTEMIC SCLEROSIS	7	7	40.7	(17-54)	-	-	-
DERMATOMYOSITIS	1	1	16	-	-	-	-
GOUT	3	2	62.5	(60/65)	1	62	-

FIGURE II, 6.



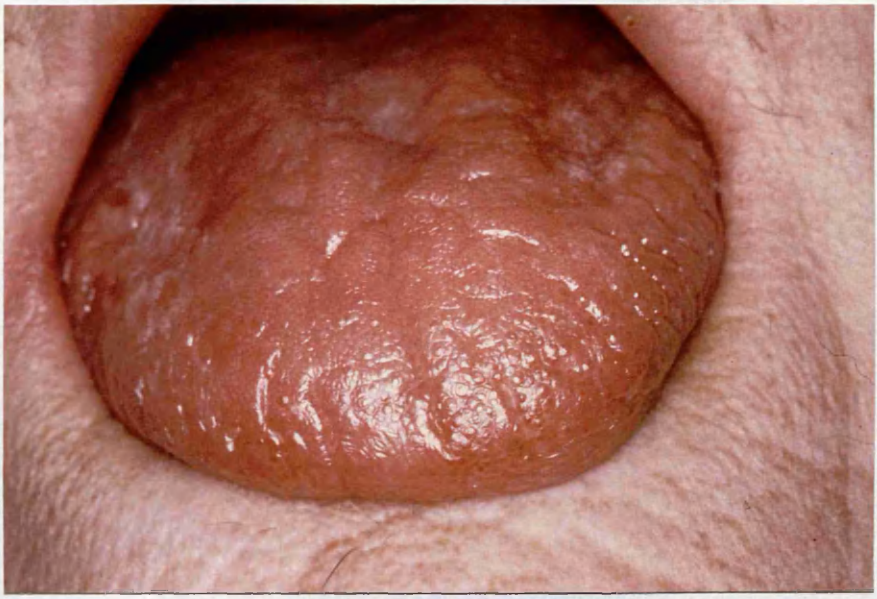
Angular cheilitis and ulceration
of lips.

FIGURE II, 7.



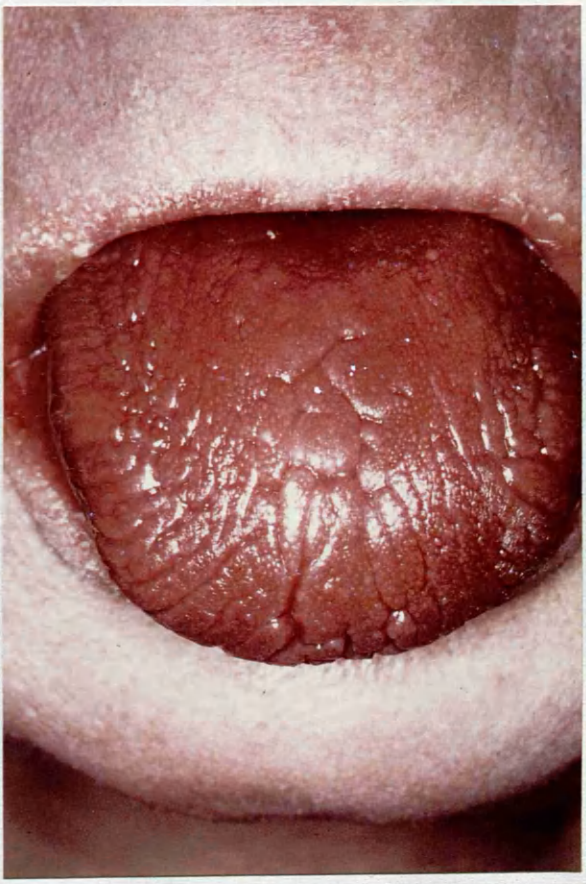
Angular cheilitis and glossitis.

FIGURE II, 8.



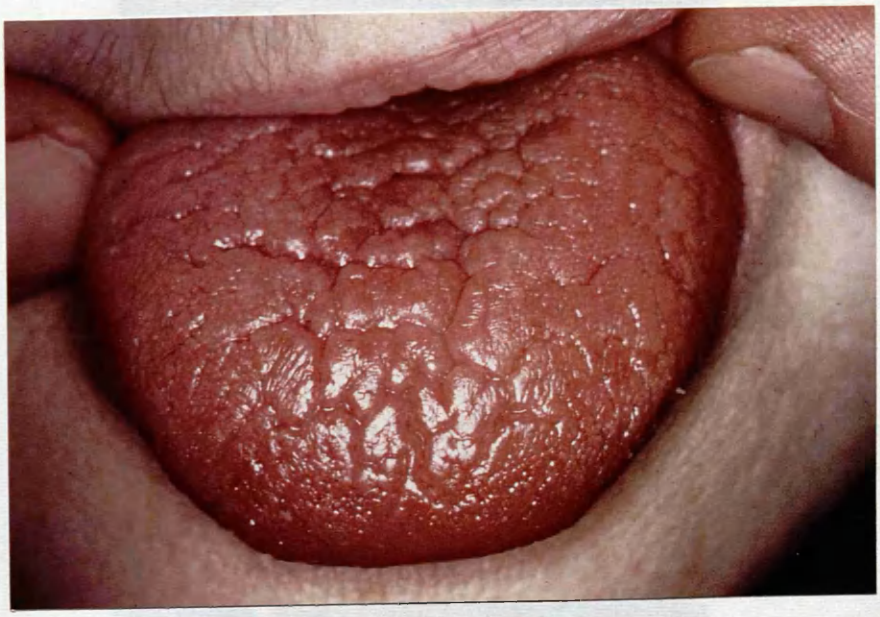
Grade I lingual changes in patient with Sjögren's syndrome

FIGURE II, 9



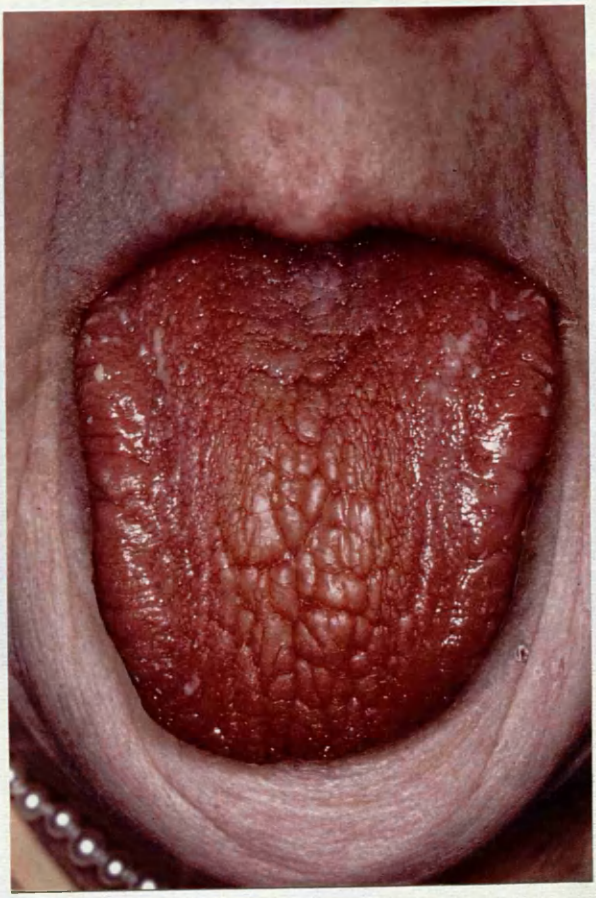
Grade I lingual changes

FIGURE II, 10.



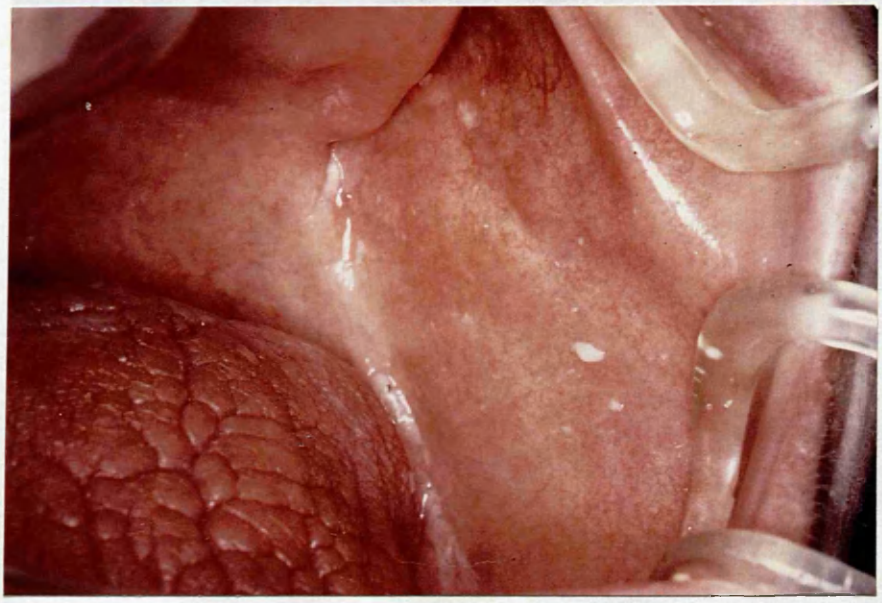
Grade II lingual changes

FIGURE II, 11.



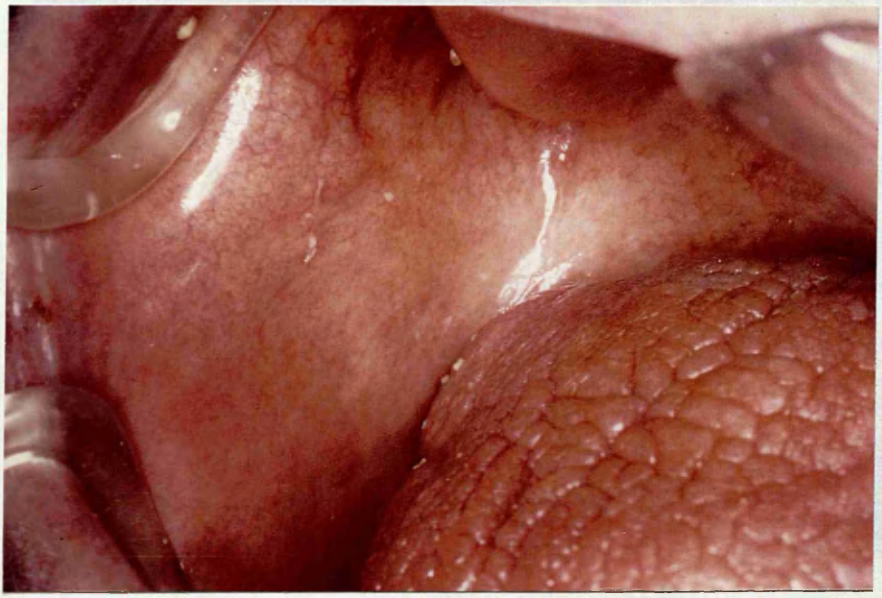
Grade III lingual changes

FIGURE II, 12.



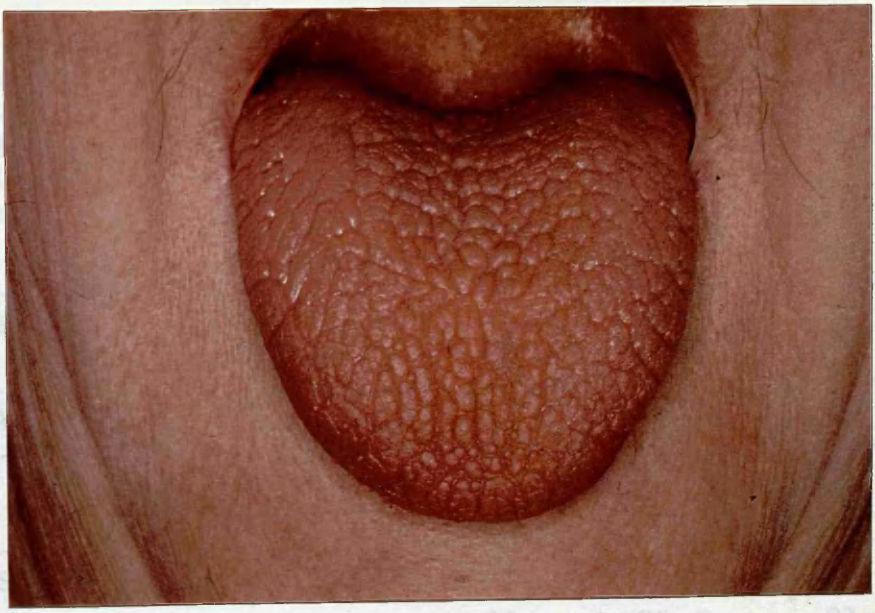
Grade III lingual changes

FIGURE II, 13.



Grade III lingual changes

FIGURE II, 14.



Grade III lingual change

TABLE II, 3

ORAL SIGNS AND SYMPTOMS IN SJÖGREN'S SYNDROME

	A*		B**	
	No.	%	No.	%
PATIENTS	36	100	54	100
XEROSTOMIA				
complaining of	36	100	48	89
on examination	36	100	40	74
mean duration(yrs)	4.4		2.8	
NATURE intermittent	20	55.6	31	57.5
persistant	16	44.4	17	31.5
INCREASED FLUID INTAKE	28	77.7	36	66.7
related to meals only	12	33.3	20	37.0
persistant	16	44.4	16	29.6
ORAL SORENESS	24	66.7	41	76.0
ORAL ULCERATION	11	30.6	21	39.0
TASTE ABNORMALITY	0	0	0	0
DIFFICULTY IN SWALLOWING	4	11.1	6	11.1
LINGUAL CHANGES	31	86	39	72
Grade 1	15	42	25	46
Grade 11	12	33	9	17
Grade 111	4	11	5	9
KERATO-CONJUNCTIVITIS SICCA	36	100	50	93
mean duration (yrs)	3.8		3.8	
ARTHRITIS				
mean duration (yrs)	0		14.6	

* SICCA SYNDROME

** SJÖGREN'S SYNDROME WITH RHEUMATOID ARTHRITIS

TABLE II, 4

SALIVARY GLAND ENLARGEMENT IN SJÖGREN'S SYNDROME

	A*		B**	
	No.	%	No.	%
HISTORY OF GLAND ENLARGEMENT	13	36	11	20
PAROTID ONLY				
Bilateral	8		6	
Single	0		2	
SUBMANDIBULAR ONLY				
Bilateral	2		1	
Single	0		1	
PAROTID AND SUBMANDIBULAR (Bilateral)	3		1	
GLAND ENLARGEMENT ON EXAMINATION	3	8	3	6
PAROTID ONLY				
Bilateral	2		1	
Single	0		1	
PAROTID AND SUBMANDIBULAR (Bilateral)	1		1	

* SICCA SYNDROME

** SJÖGREN'S SYNDROME WITH RHEUMATOID ARTHRITIS

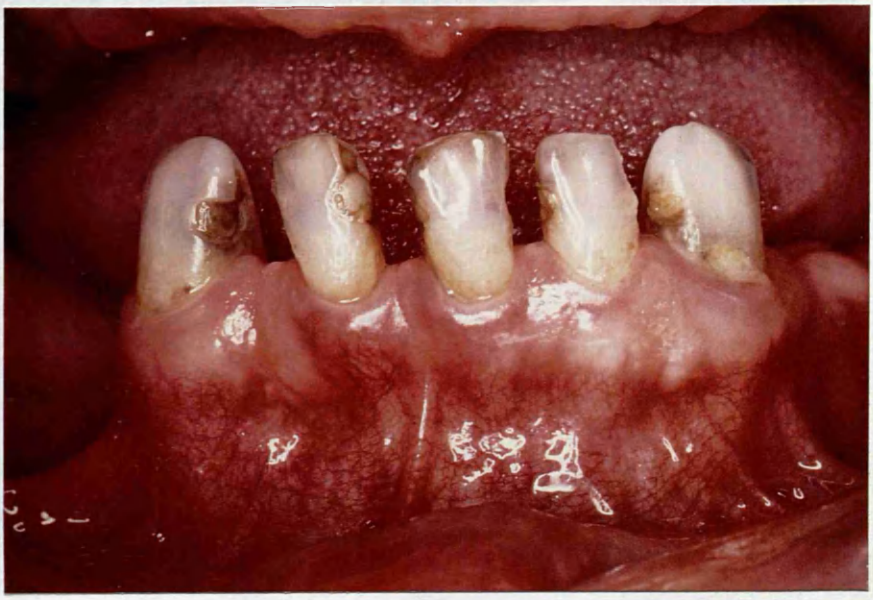
TABLE II, 5

ONSET OF SYMPTOMS IN SJÖGREN'S SYNDROME

SICCA SYNDROME (36 patients)	No.
Xerostomia initially	10
Kerato-conjunctivitis sicca initially	13
Sicca symptoms together	13

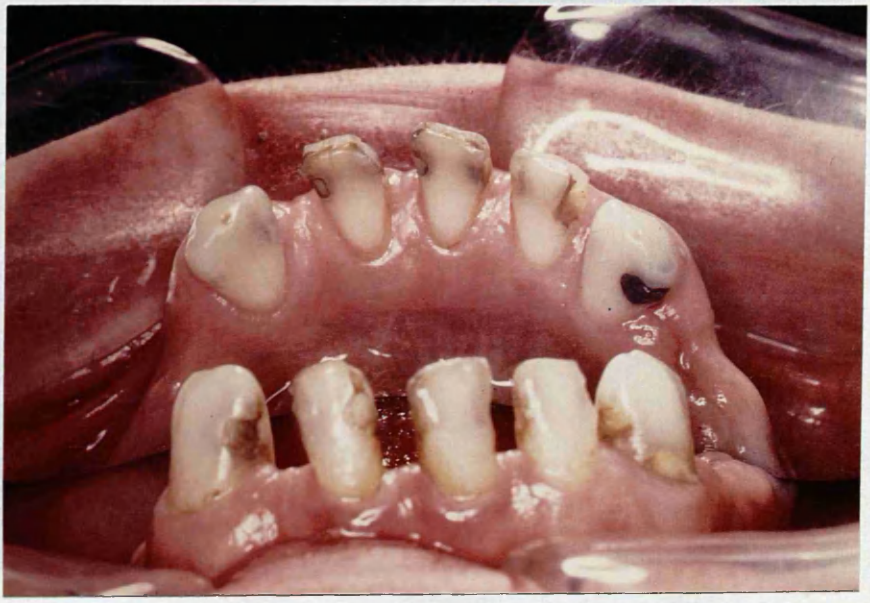
SJOGREN'S SYNDROME WITH RHEUMATOID ARTHRITIS (54 patients)	
Sicca symptom initially	2
Rheumatoid arthritis initially	52
Initial Sicca Symptom	
Xerostomia	9
Kerato-conjunctivitis sicca	25
Together	19

FIGURE II, 15



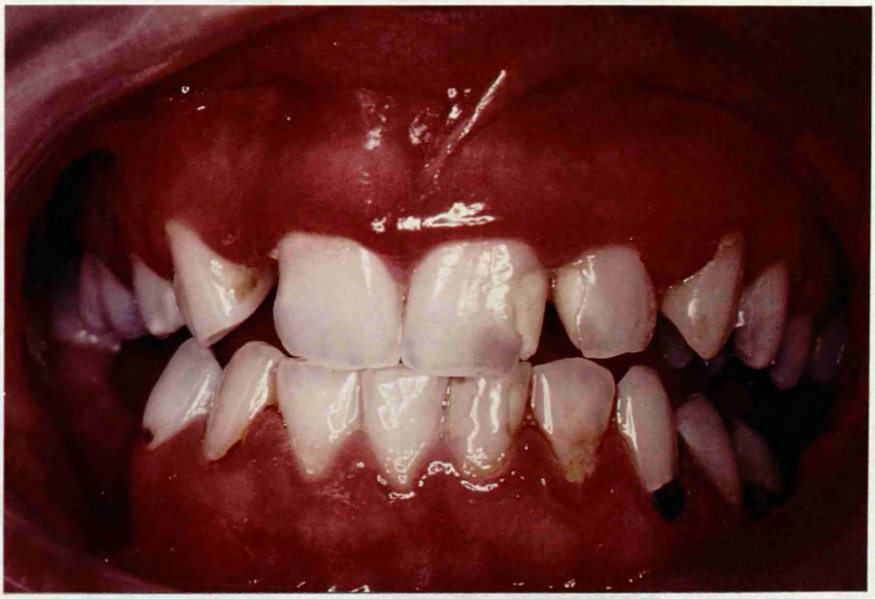
Rapid dental caries and breakdown of silicate restorations in a patient with Sjögren's syndrome.

FIGURE II, 16.



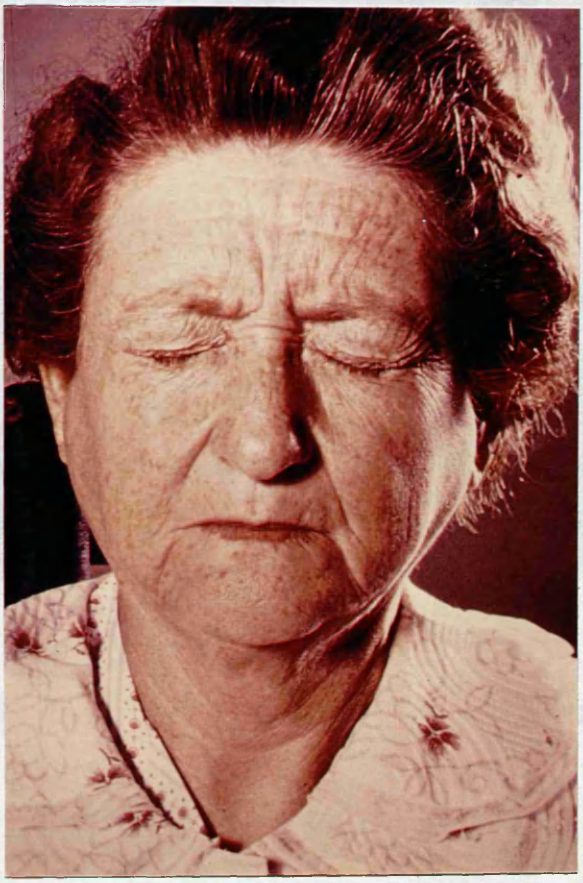
Same case as Figure II, 15, showing both labial and lingual aspects of lower incisors.

FIGURE II, 17.



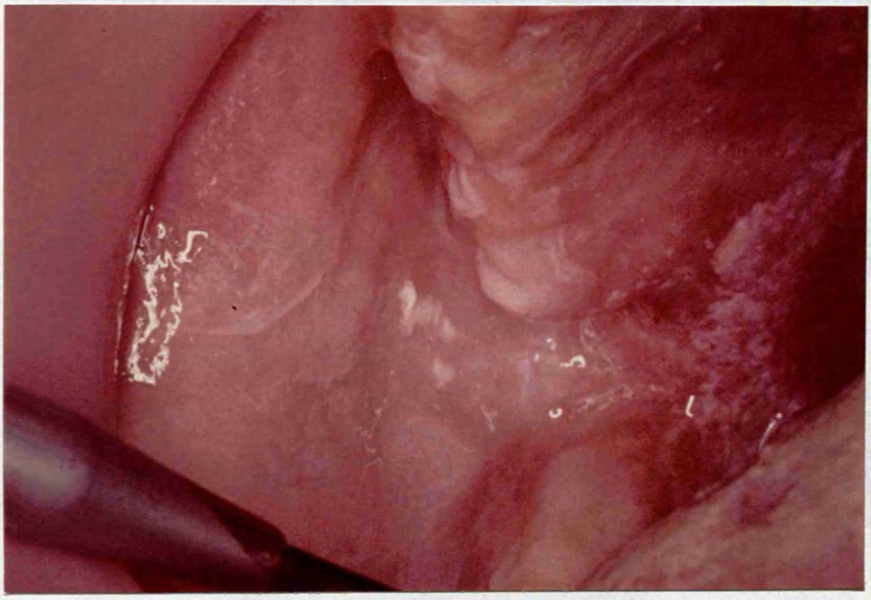
Severe chronic gingivitis accompanied by rapid onset of dental caries in a 22 year-old female with Sjögren's syndrome

FIGURE II, 18.



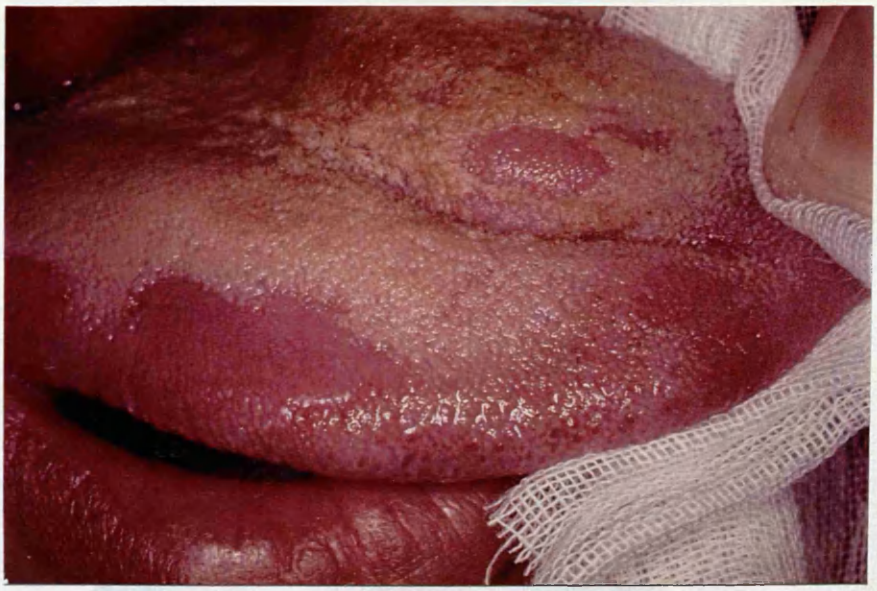
Bilateral parotid gland enlargement in a middle-aged female with Sjögren's syndrome.

FIGURE II, 19.



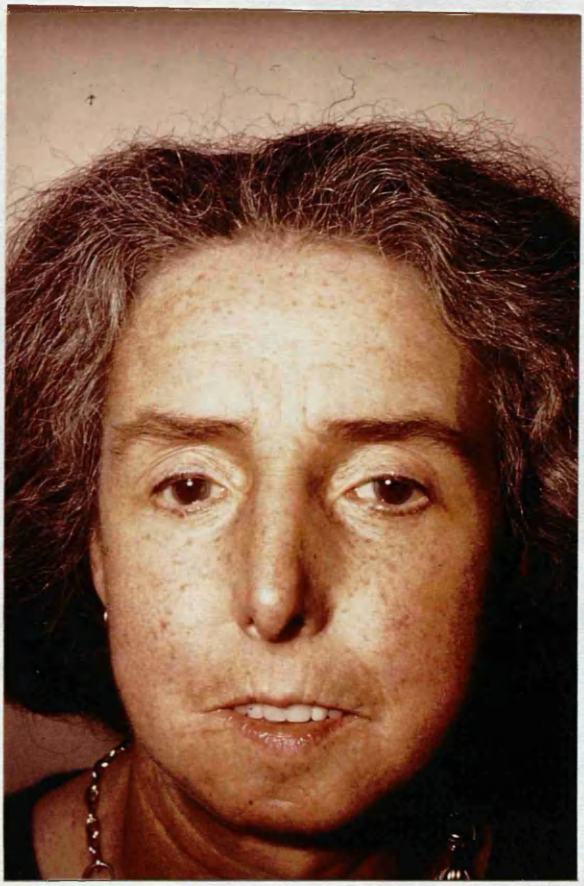
Oral lesions in a male patient with Reiter's syndrome.

FIGURE II, 20.



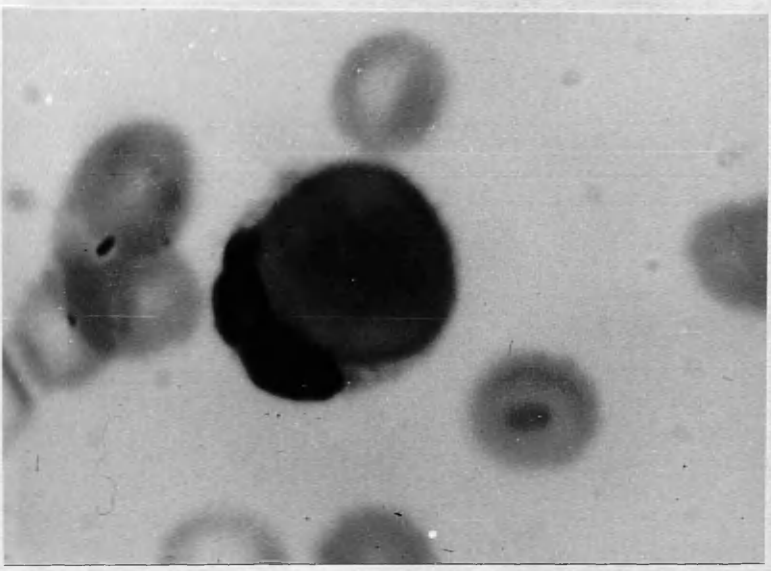
Lingual lesions in a male patient
with Reiter's syndrome

FIGURE II, 21.



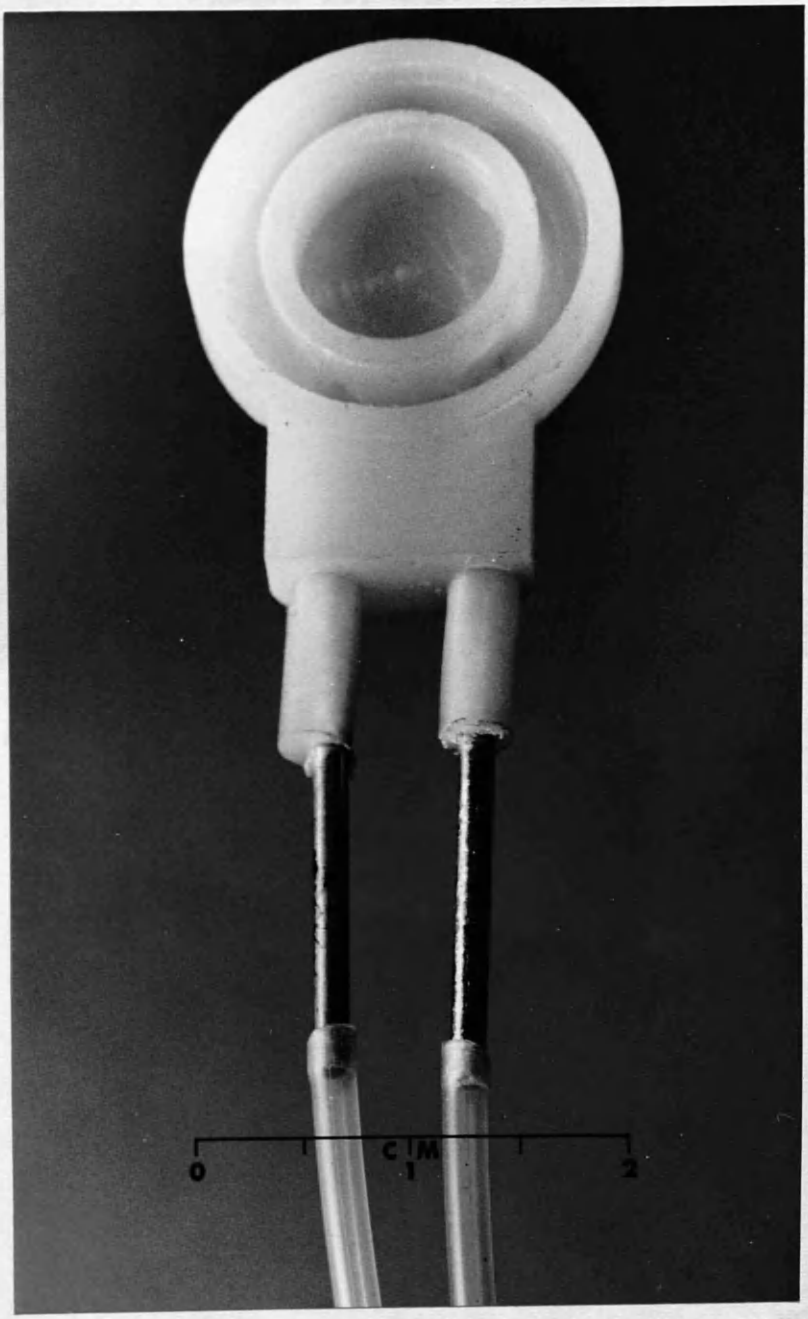
Female patient with progressive systemic sclerosis. Face assumes an expressionless, masklike appearance. Rigidity of lips in this case led to difficulty in mastication and opening and closing of mouth.

FIGURE II, 22.



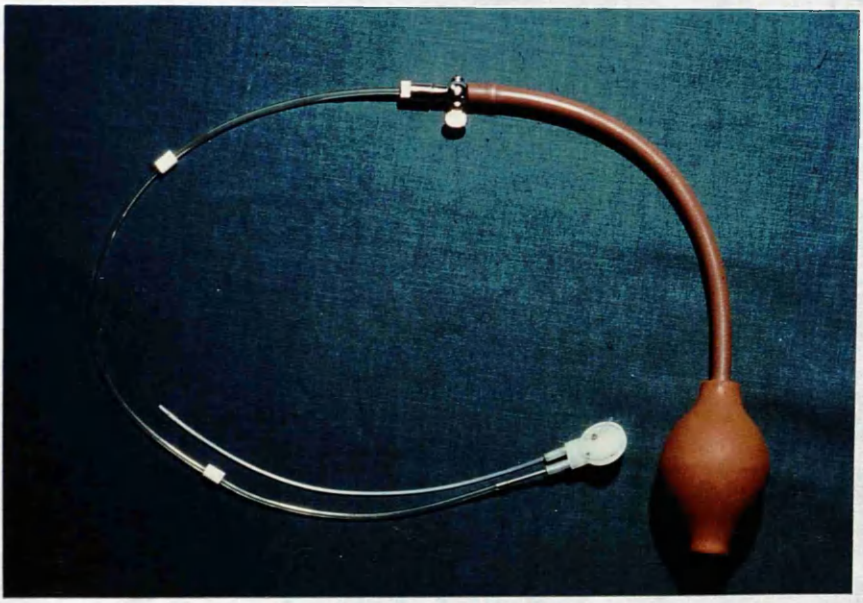
An L.E. cell which consists of an L.E. body phagocytosed by a neutrophil polymorph (x 1350).

FIGURE II, 23.



Modified Carlsson-Crittenden cup.

FIGURE II, 24.



Portable parotid saliva collection device for hospitalized patients

TABLE II, 6

MEAN PAROTID SALIVARY FLOW RATES IN NORMAL SUBJECTS (ml/min.)

AGE RANGE (yrs)		RESTING		FRUIT GUM		LEMON JUICE	
		Male	Female	Male	Female	Male	Female
20	18M	0.061±0.007	0.083±0.02	0.66±0.06	0.46±0.07	1.49±0.09	1.99±0.14
	8F	(0.02 -0.12)	(0.04 -0.16)	(0.36-1.07)	(0.27-0.80)	(0.60-2.10)	(1.25-2.50)
21-40	20 M	0.104±0.008	0.09 ±0.01	0.47±0.03	0.50±0.05	1.73±0.09	1.76±0.09
	20 F	(0.06 -0.20)	(0.01 -0.19)	(0.32-0.85)	(0.09-1.04)	(0.85-2.99)	(1.03-2.70)

Mean ± Standard error of the mean (S.E.M.)

Ranges indicated in parenthesis.

TABLE II, 7

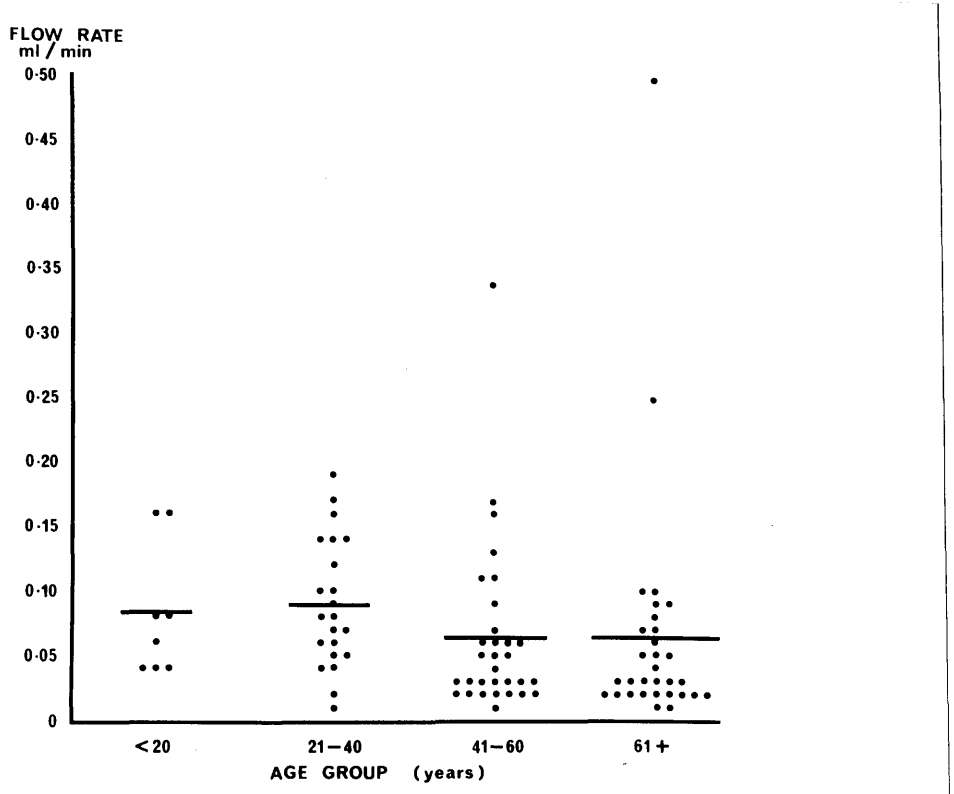
MEAN PAROTID SALIVARY FLOW RATES IN NORMAL SUBJECTS (ml/min.)

AGE RANGE (yrs)		RESTING		FRUIT GUM		LEMON JUICE	
		Male	Female	Male	Female	Male	Female
41-60	27M 31F	0.078±0.02 (0.01 -0.31)	0.064±0.01 (0.01 -0.34)	0.59±0.08 (0.15±1.76)	0.52±0.05 (0.13-1.15)	1.68±0.11 (0.85-3.20)	1.36±0.12 (0.50-3.02)
61	14M 31F	0.084±0.02 (0.03 -0.20)	0.06± 0.02 (0.01- 0.5)	0.50±0.08 (0.15-1.26)	0.43±0.03 (0.14-0.83)	1.58±0.16 (0.90-2.76)	1.15±0.08 (0.47-2.20)

Mean ± Standard error of the mean (S.E.M.)

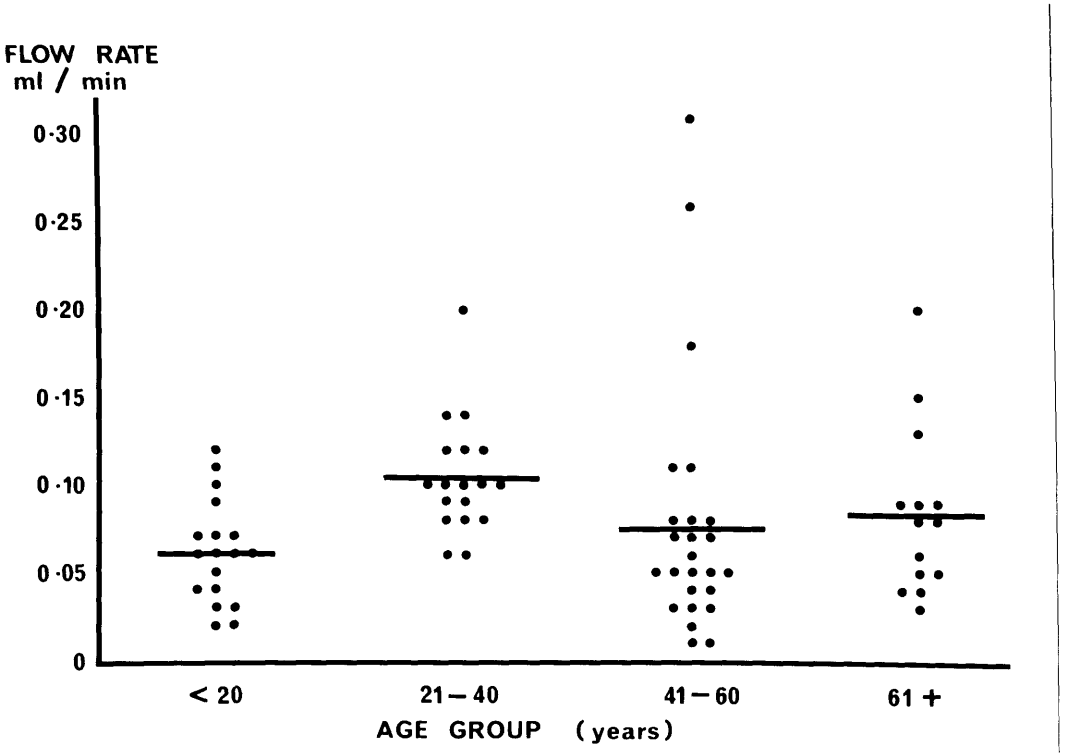
Ranges indicated in parenthesis.

FIGURE II, 25.



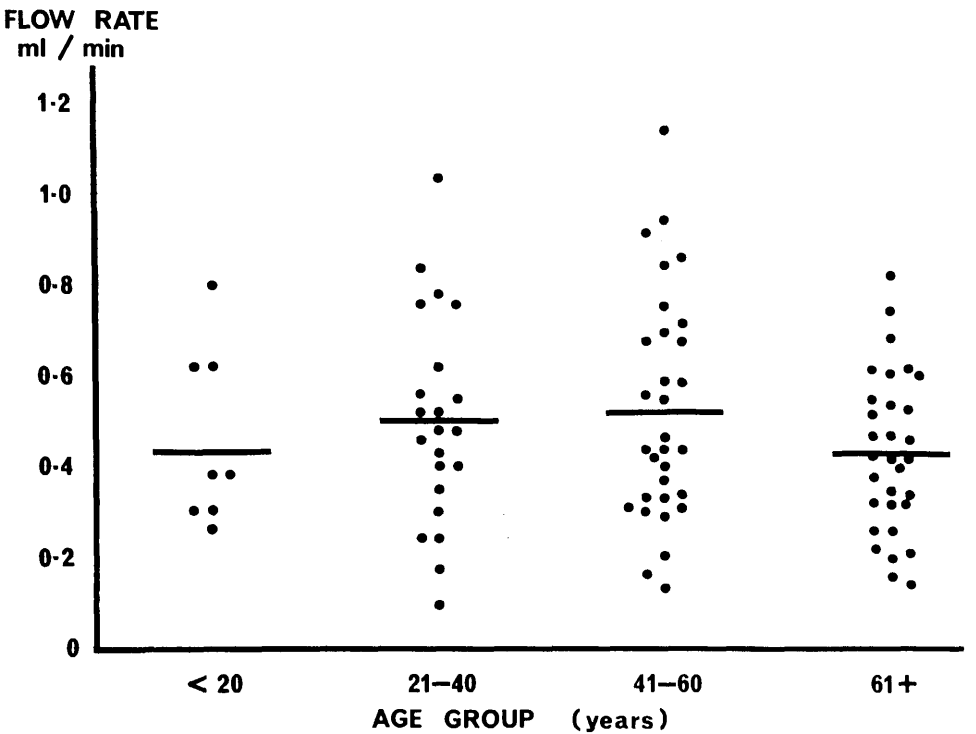
'Resting' parotid flow rates in 90 normal female patients.

FIGURE II, 26.



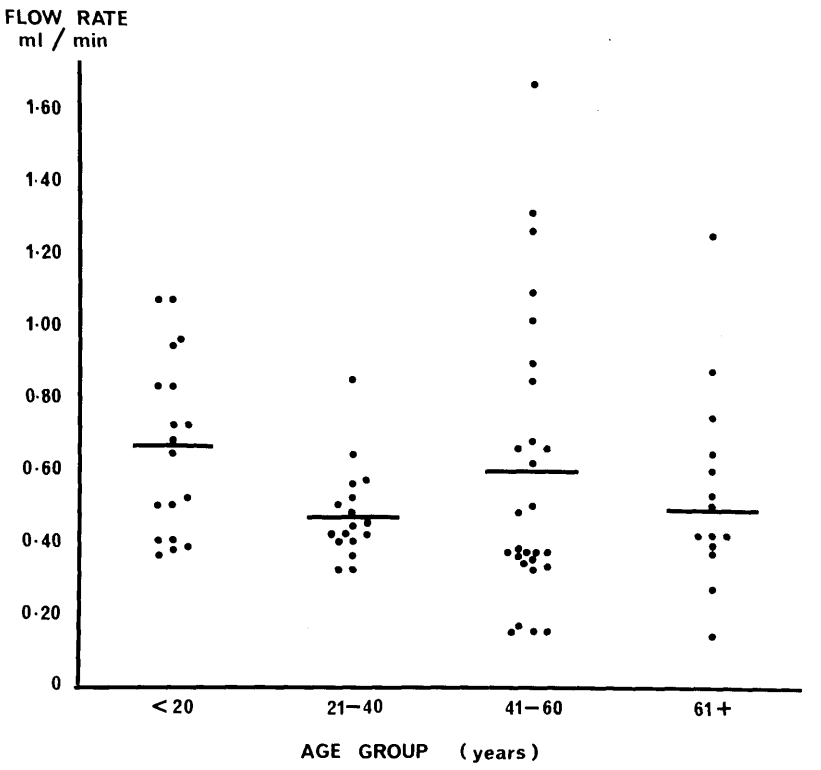
'Resting' parotid flow rate values (ml/min) in normal male subjects.

FIGURE II, 27.



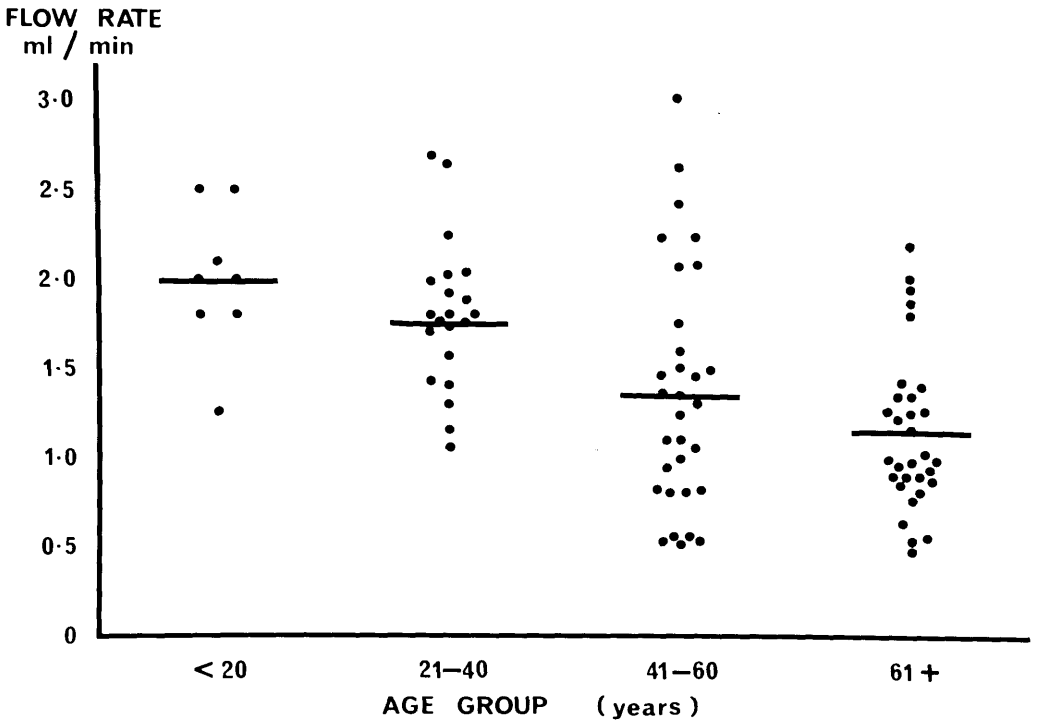
Fruit gum stimulated parotid flow rate values in normal female subjects.

FIGURE II, 28.



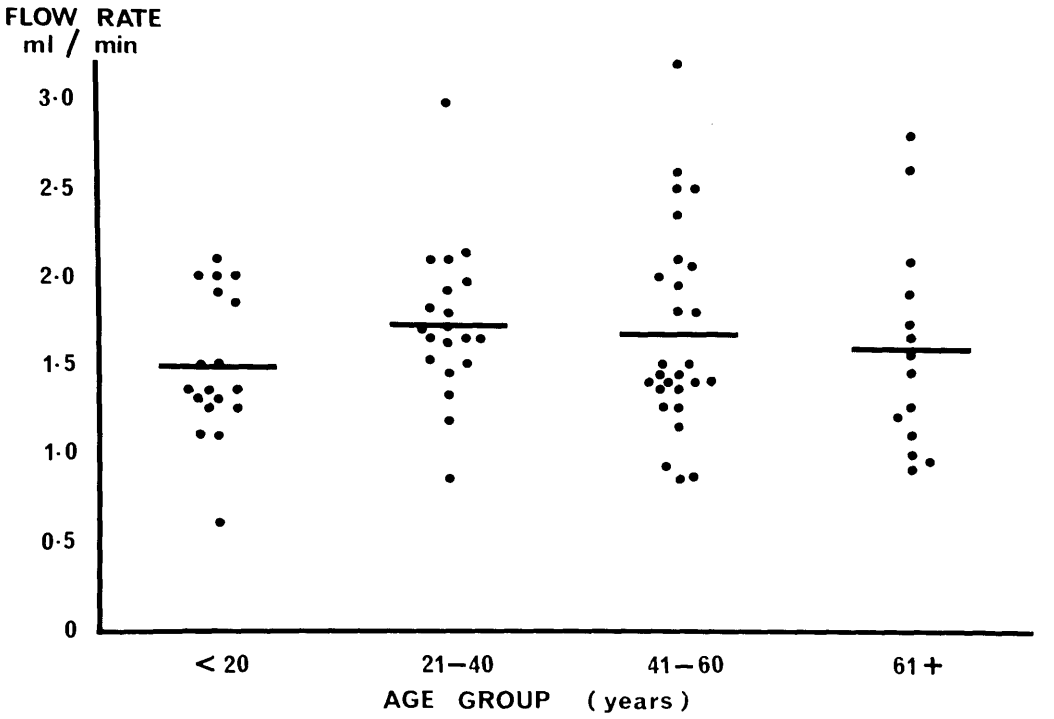
Fruit gum stimulated parotid flow rate values in normal male subjects.

FIGURE II, 29.



Lemon juice stimulated parotid flow rate values in normal female subjects

FIGURE II, 30.



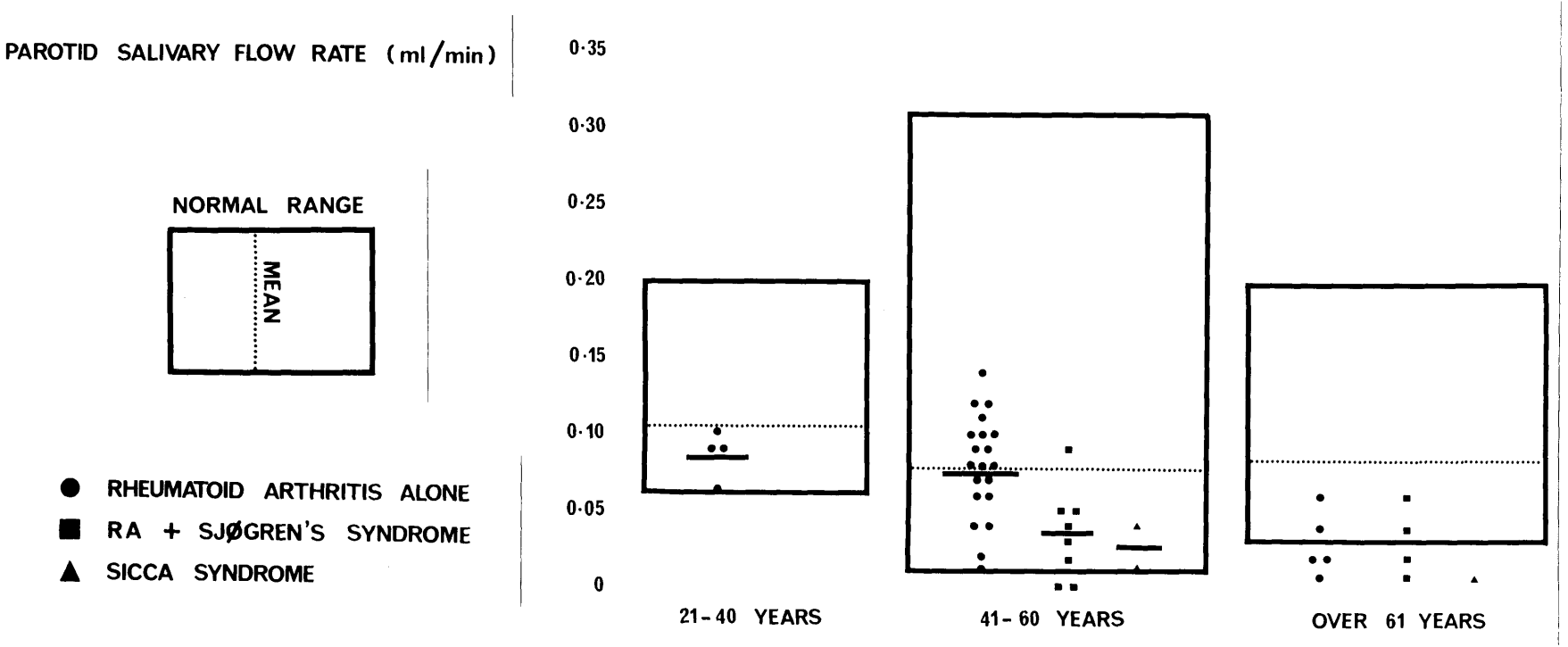
Lemon juice stimulated parotid flow rate values in normal male subjects.

TABLE II, 8

PAROTID FLOW STUDIES:
PATIENTS EXAMINED

CLINICAL DIAGNOSIS	No.	Sex Distribution F.	M.
SICCA SYNDROME	32	29	3
SJÖGREN'S SYNDROME	86	72	14
RHEUMATOID ARTHRITIS	152	112	40
OSTEOARTHRITIS	20	16	4
ANKYLOSING SPONDYLITIS	18	14	4
PSORIATIC ARTHRITIS	15	11	4
REITER'S SYNDROME	20	-	20
GOUT	3	2	1
BEÇHET'S SYNDROME	2	2	-
SYSTEMIC LUPUS ERYTHEMATOSUS	10	10	-
PROGRESSIVE SYSTEMIC SCLEROSIS	7	7	-
DERMATOMYOSITIS	1	1	-

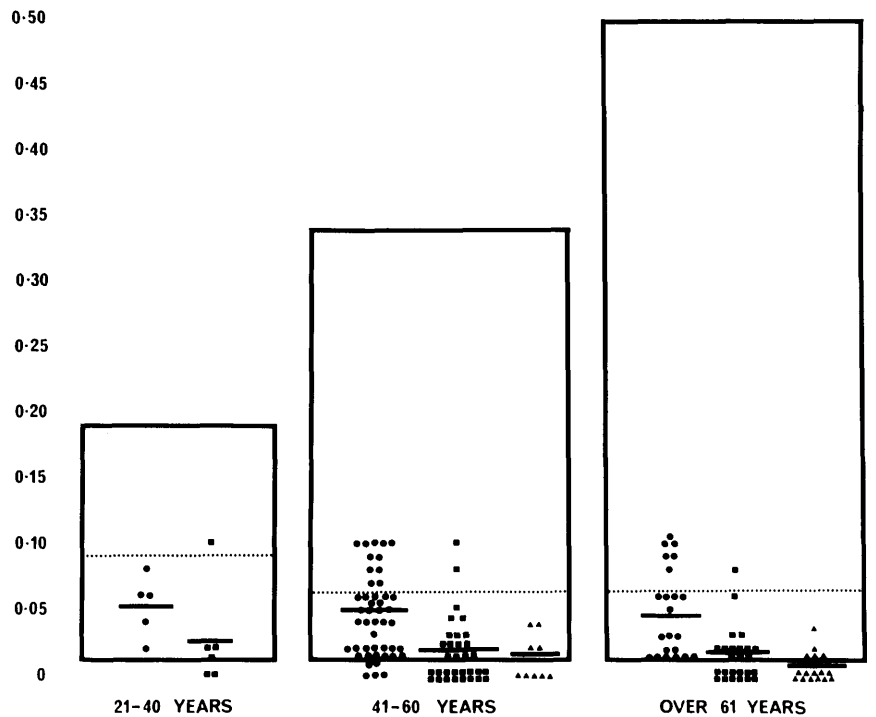
FIGURE II, 31



(KEY TO FIGURES
II,31 - II, 36)

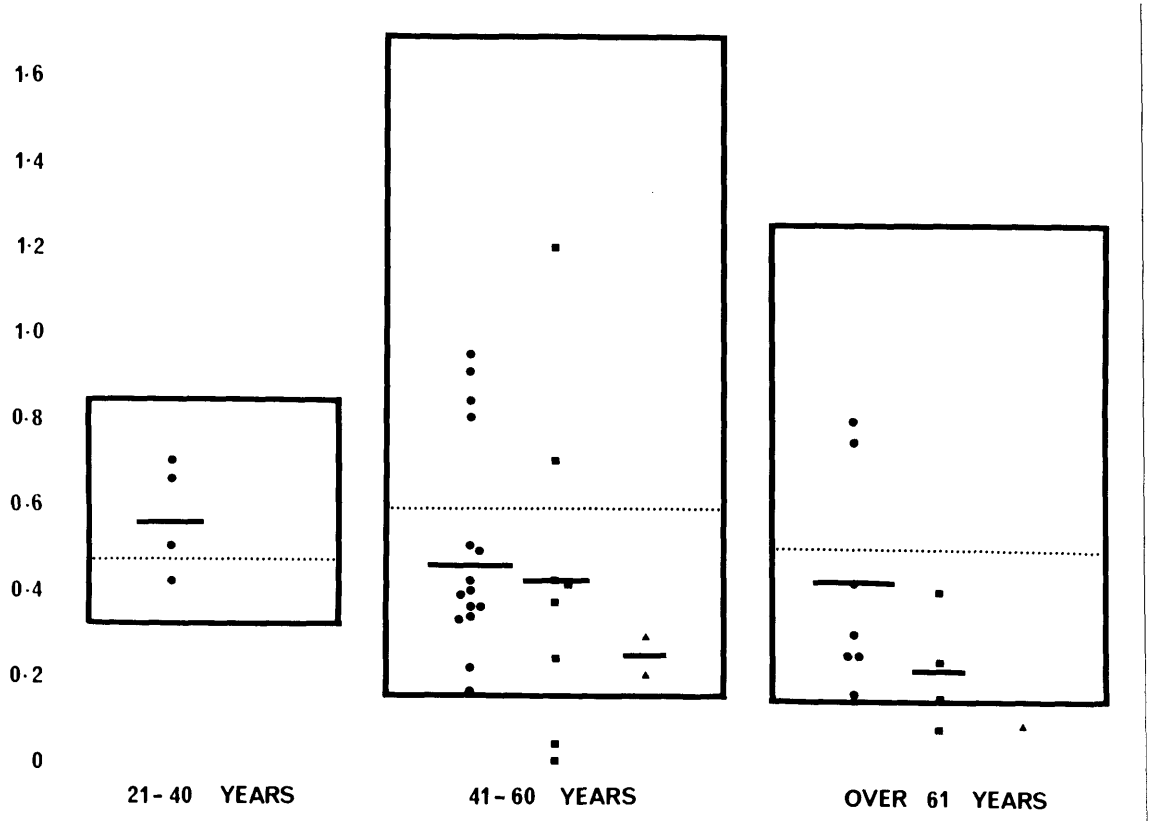
Resting parotid flow rate values in male patients with Sjögren's syndrome and rheumatoid arthritis.

FIGURE II, 32.



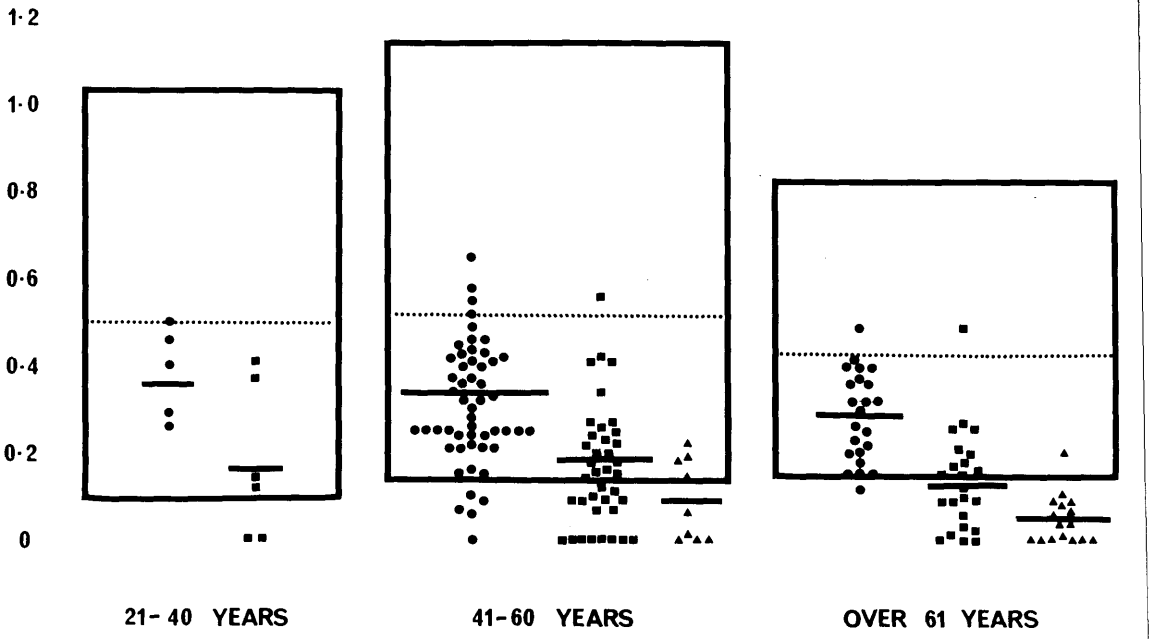
Resting parotid flow rate values in female patients with Sjögren's syndrome and rheumatoid arthritis alone.

FIGURE II, 33.



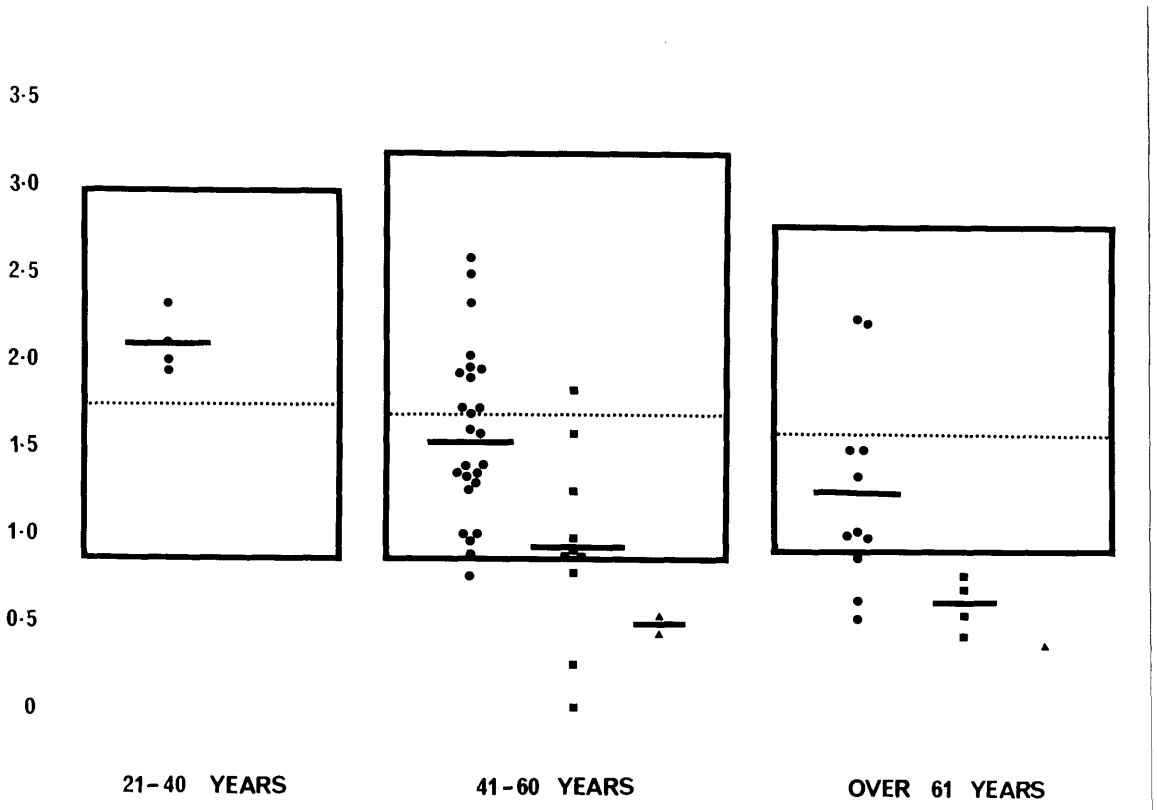
Fruit gum stimulated parotid flow rate values in male patients with Sjögren's syndrome and rheumatoid arthritis alone.

FIGURE II, 34.



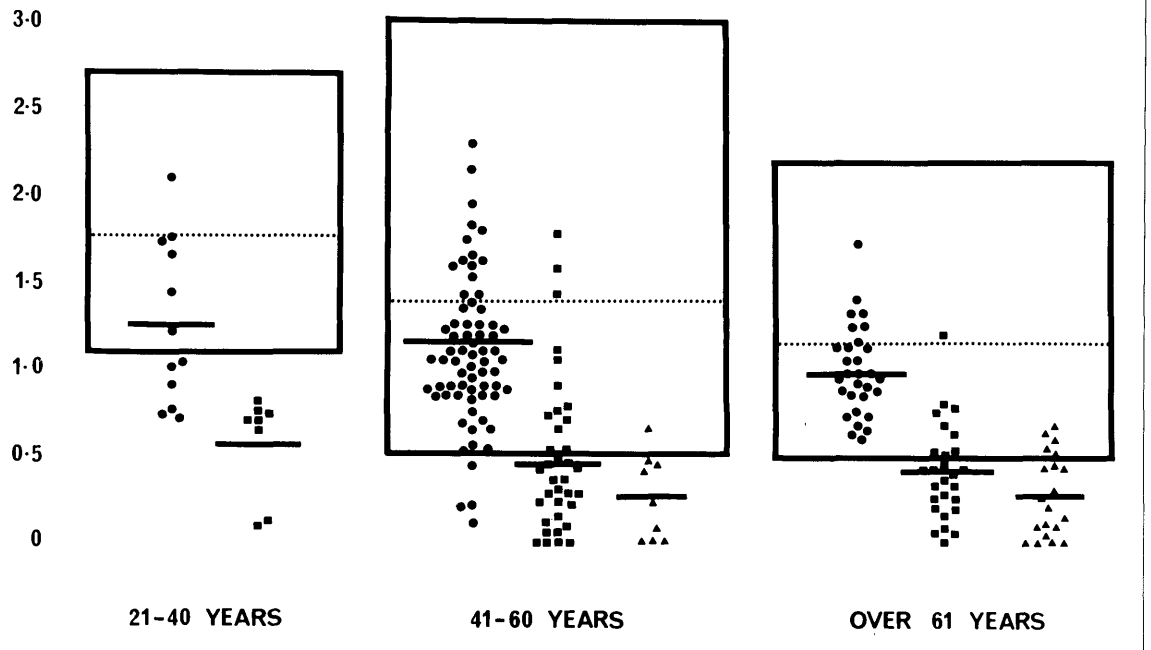
Fruit gum stimulated parotid flow rate values in female patients with Sjögren's syndrome and rheumatoid arthritis alone.

FIGURE II, 35.



Lemon juice stimulated parotid flow rate values in male patients with Sjögren's syndrome and rheumatoid arthritis alone

FIGURE II, 36.



Lemon juice stimulated parotid flow rate values in female patients with Sjögren's syndrome and rheumatoid arthritis alone.

TABLE 11, 9.

MEAN PAROTID SALIVARY FLOW RATES (ml/min)

SICCA SYNDROME (MALES)

AGE GROUP (YRS)	RESTING	FRUIT GUM	LEMON JUICE
20-40	-	-	-
41-60	0.03	0.25	0.47
61 +	0.01	0.09	0.38

TABLE 11, 10.

MEAN PAROTID SALIVARY FLOW RATES (ml/min)

SICCA SYNDROME (FEMALES)

AGE GROUP (YRS)	RESTING	FRUIT GUM	LEMON JUICE
20-40	-	-	-
41-60	0.01 (0.01)	0.09 (0.03)	0.24 (0.08)
61 +	0.01 (0.01)	0.05 (0.01)	0.27 (0.05)

S.E.M. indicated in parenthesis.

TABLE 11, 11.

MEAN PAROTID SALIVARY FLOW RATES (ml/min)

SJOGREN'S SYNDROME (MALES)

AGE GROUP (YRS)	RESTING	FRUIT GUM	LEMON JUICE
20-40	-	-	-
41-60	0.35 (0.01)	0.42 (0.14)	0.93 (0.17)
61 +	0.01 (0.01)	0.01 (0.01)	0.42 (0.04)

S.E.M. indicated in parenthesis.

TABLE 11, 12

MEAN PAROTID SALIVARY FLOW RATES (ml/min)

SJÖGREN'S SYNDROME (FEMALES)

AGE GROUP (YRS)	RESTING	FRUIT GUM	LEMON JUICE
20-40	0.03 (0.06)	0.16 (0.06)	0.56 (0.05)
41-60	0.02 (0.01)	0.18 (0.02)	0.47 (0.08)
61 +	0.01 (0.01)	0.13 (0.02)	0.43 (0.03)

S.E.M. indicated in parenthesis.

TABLE 11, 13.

MEAN PAROTID SALIVARY FLOW RATES (ml/min)

RHEUMATOID ARTHRITIS (MALES)

AGE GROUP (YRS)	RESTING	FRUIT GUM	LEMON JUICE
20-40	0.09 (0.04)	0.56 (0.12)	2.04 (0.13)
41-60	0.08 (0.03)	0.46 (0.06)	1.52 (0.10)
61 +	0.03 (0.01)	0.42 (0.10)	1.25 (0.17)

S.E.M. indicated in parenthesis.

TABLE 11, 14.

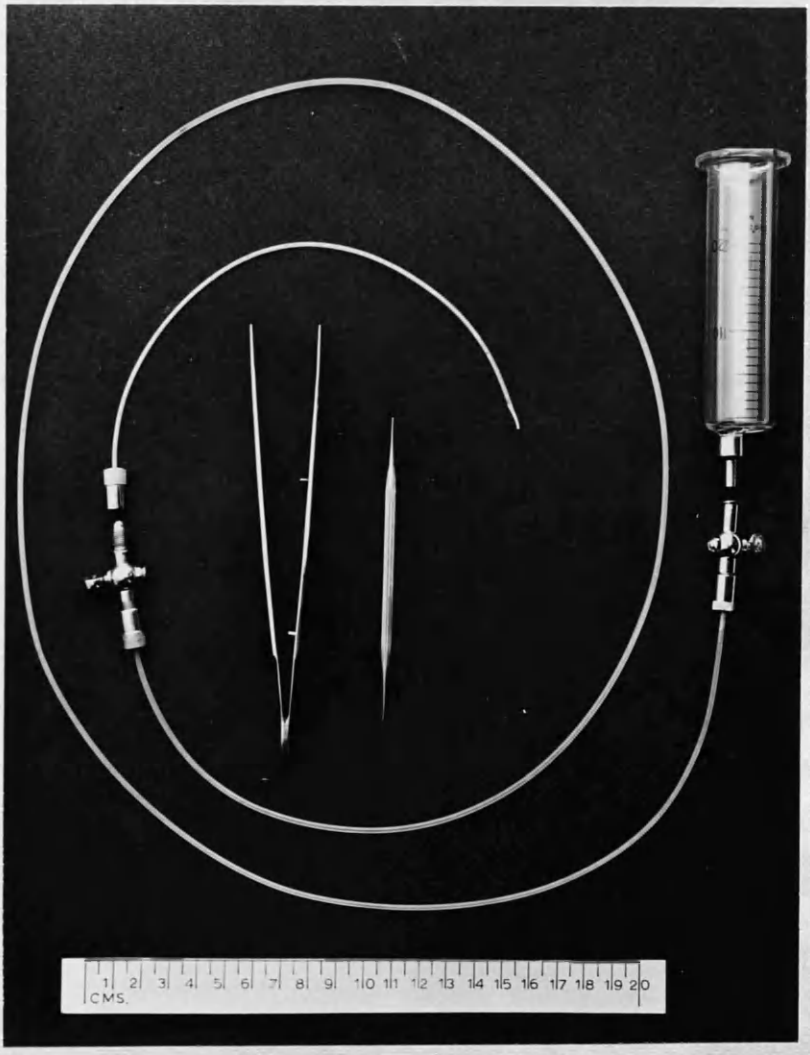
MEAN PAROTID SALIVARY FLOW RATES (ml/min)

RHEUMATOID ARTHRITIS (FEMALES)

AGE GROUP (YRS)	RESTING	FRUIT GUM	LEMON JUICE
20-40	0.05 (0.02)	0.36 (0.02)	1.24 (0.13)
41-60	0.05 (0.01)	0.34 (0.02)	1.14 (0.05)
61 +	0.04 (0.01)	0.29 (0.02)	0.98 (0.05)

S.E.M. indicated in parenthesis.

FIGURE II, 37.

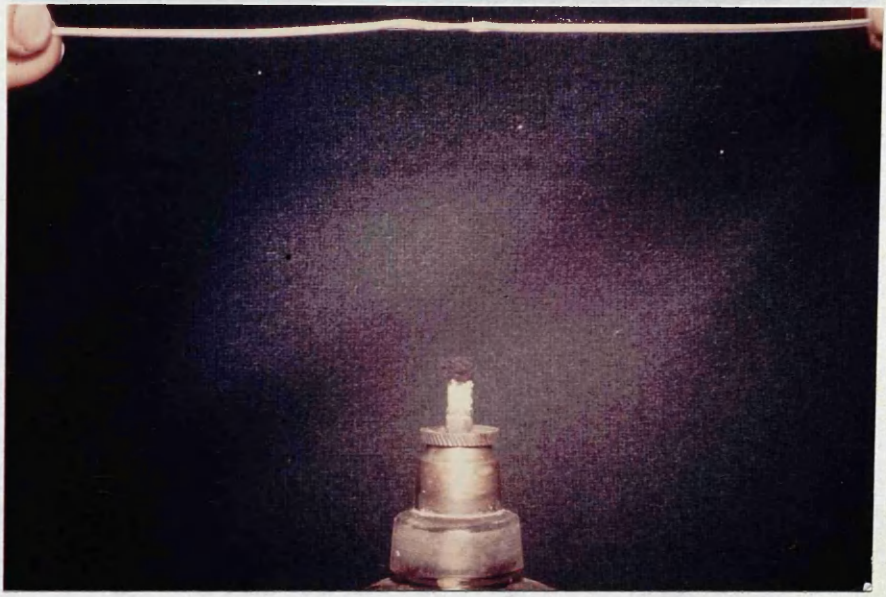


Instrumentation for hydrostatic sialgraphy:

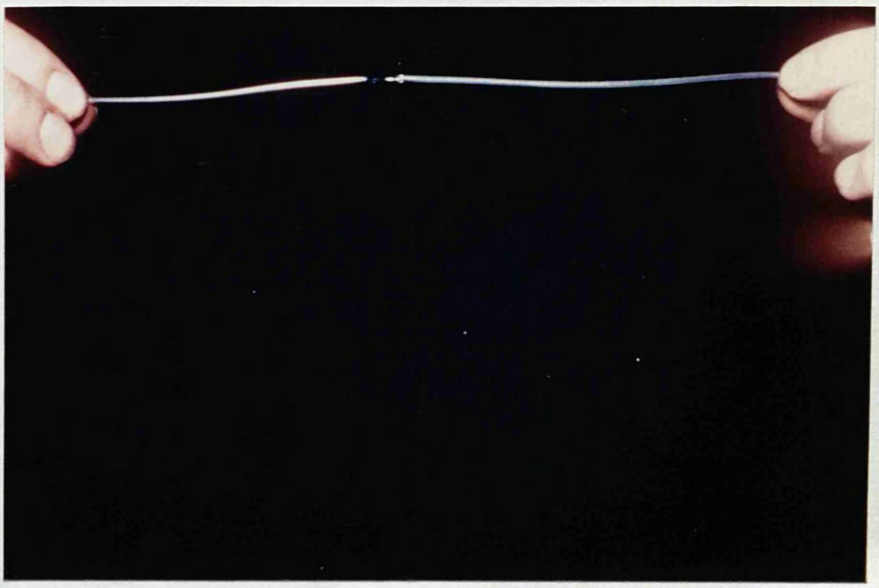
20 c.c. Glass syringe barrel
Adaptor and cap
Polythene tubing P.E. 205
Cap Adaptor Cap
Polythene tubing P.E. 160
Tissue forceps non-toothed
Lacrimal probe dilator.

FIGURE II, 38.

PREPARATION OF CATHETERS



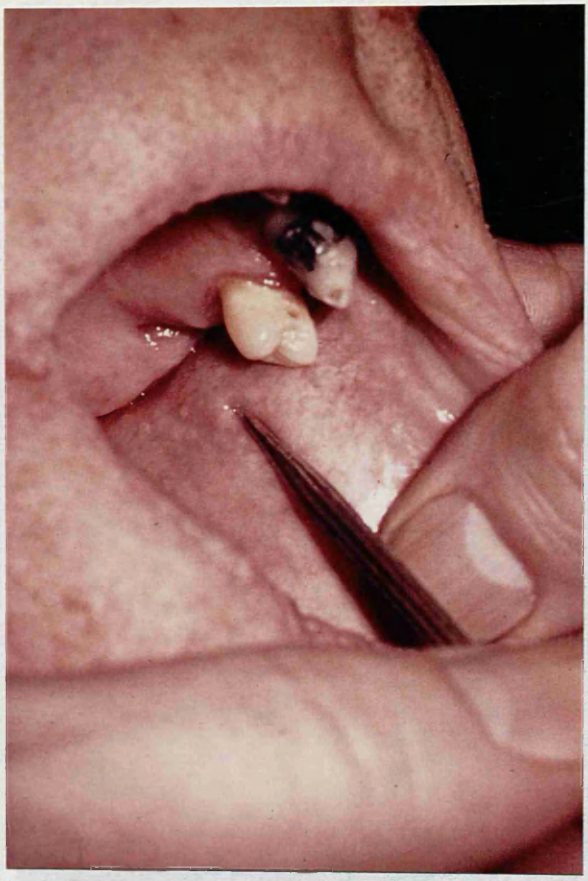
A) Softening of P.E. 160 catheter over an alcohol flame and the drawing out of the catheter by gentle traction.



B) Removal of catheter from heat source, gentle traction being maintained.

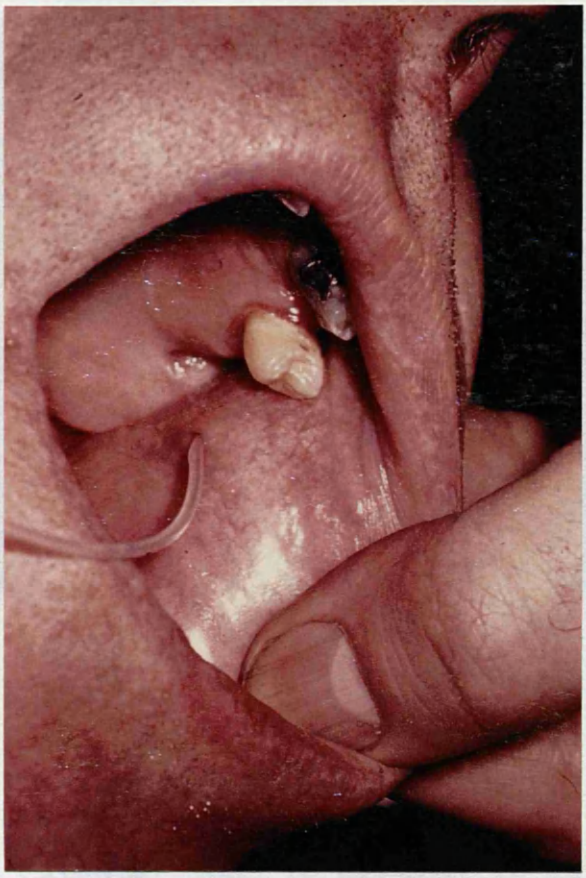
C) Fine catheter tip suitable for introduction to duct orifice.

FIGURE II, 39.



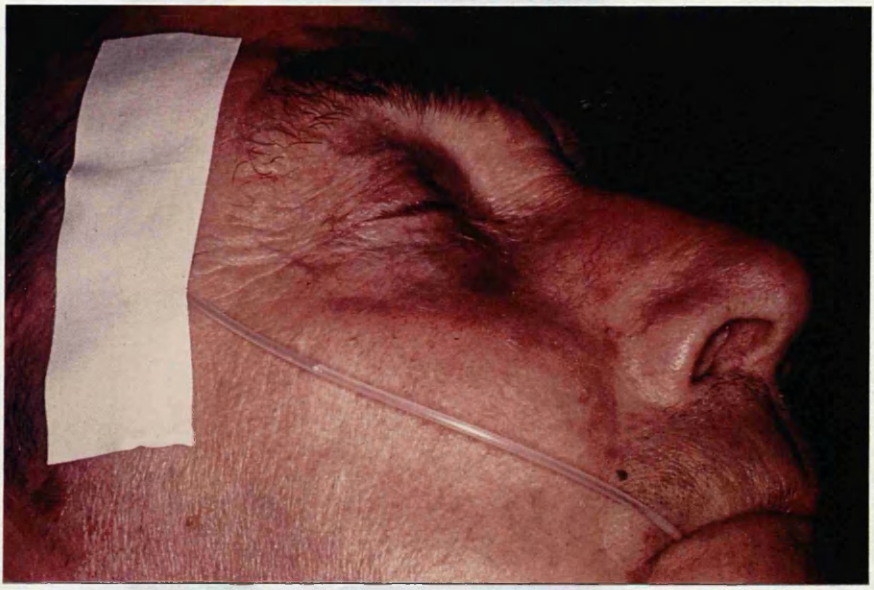
Location of left parotid
duct orifice using a
lacrimal probe dilator

FIGURE II, 40.



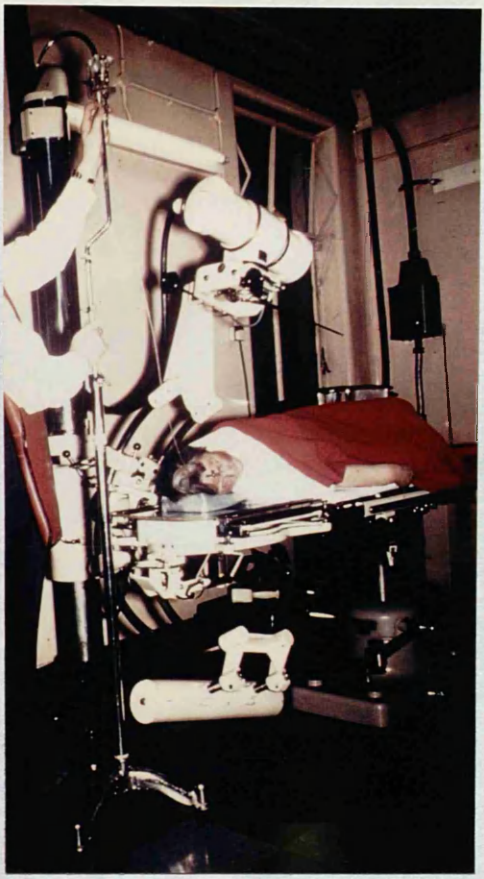
Introduction of prepared catheter with the left parotid duct orifice

FIGURE II, 41.



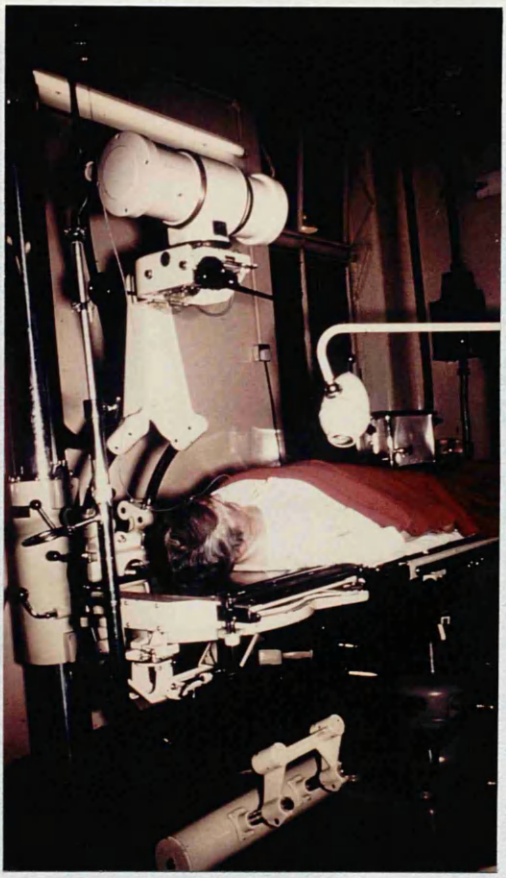
Catheter loop is gripped by patient's lips and taped to the forehead for stability.

FIGURE II, 42.



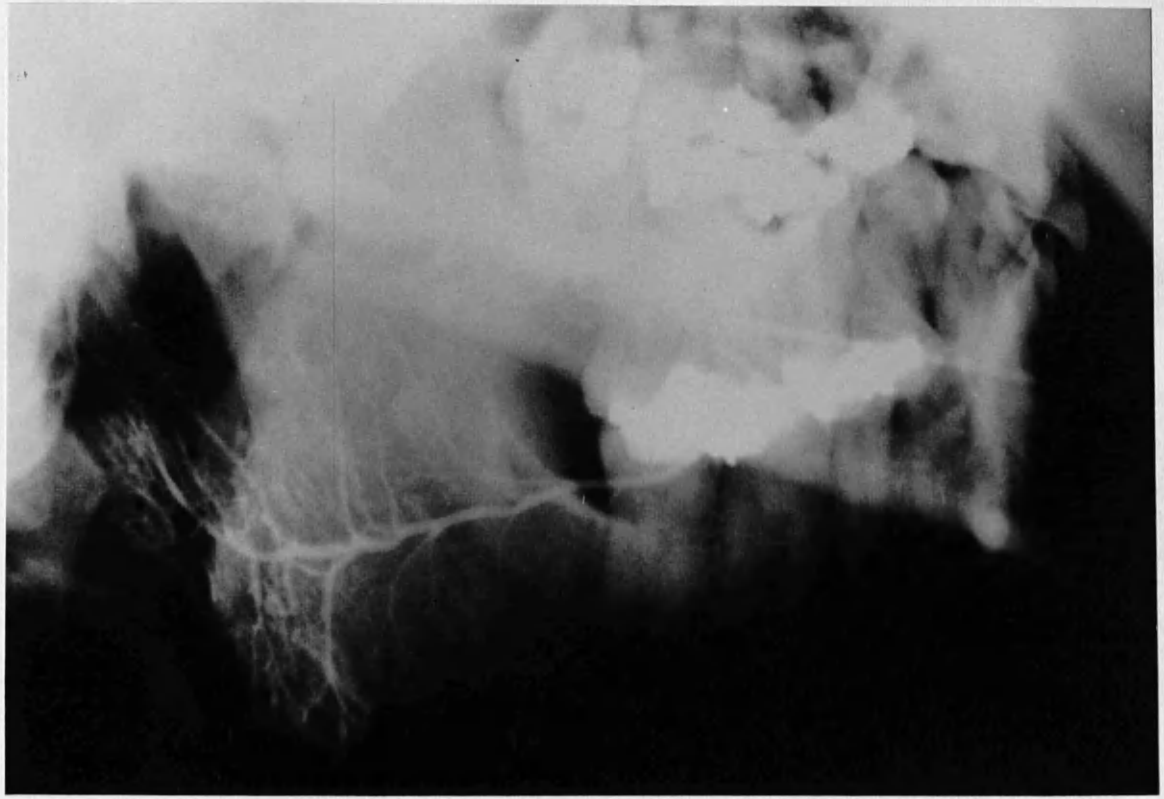
The syringe barrel is adjusted to a height of 70 cms above the patient's head. The patient lies horizontally on an Elema-Schonander skull table and is positioned for a lateral oblique projection.

FIGURE II, 43.



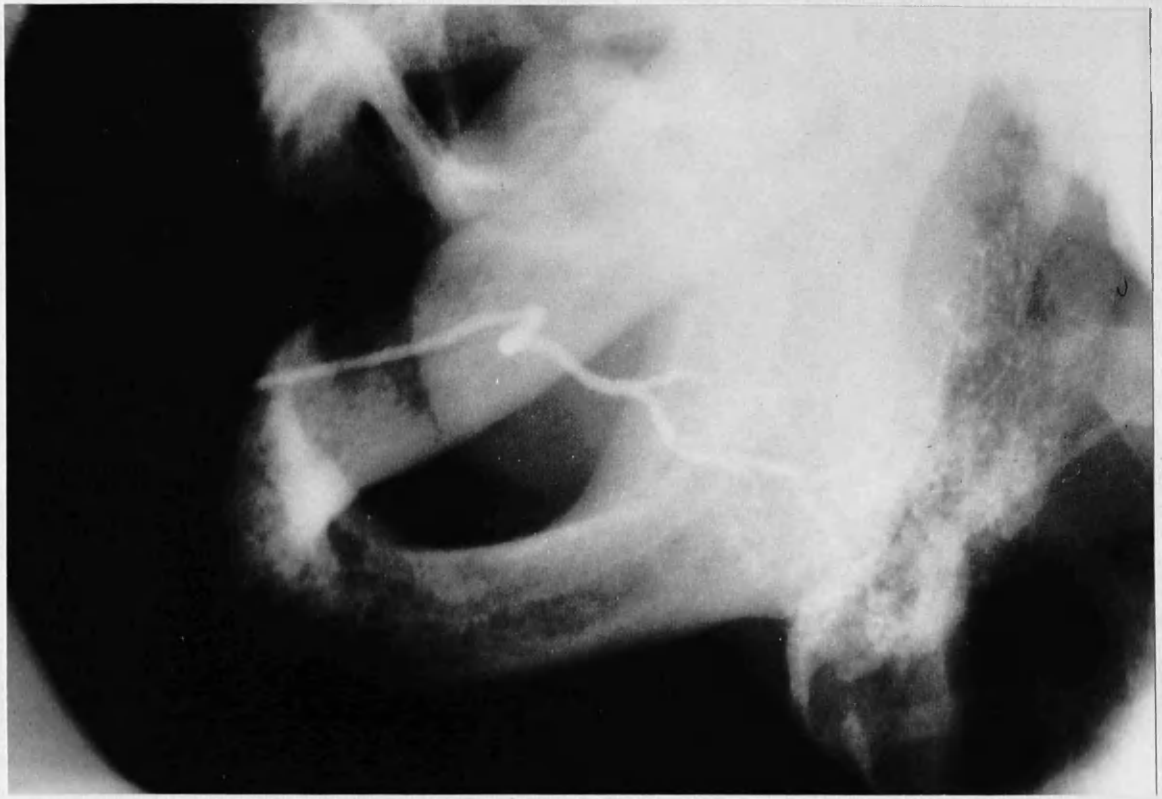
Patient positioned for an
antero-posterior projection.

FIGURE II, 44.



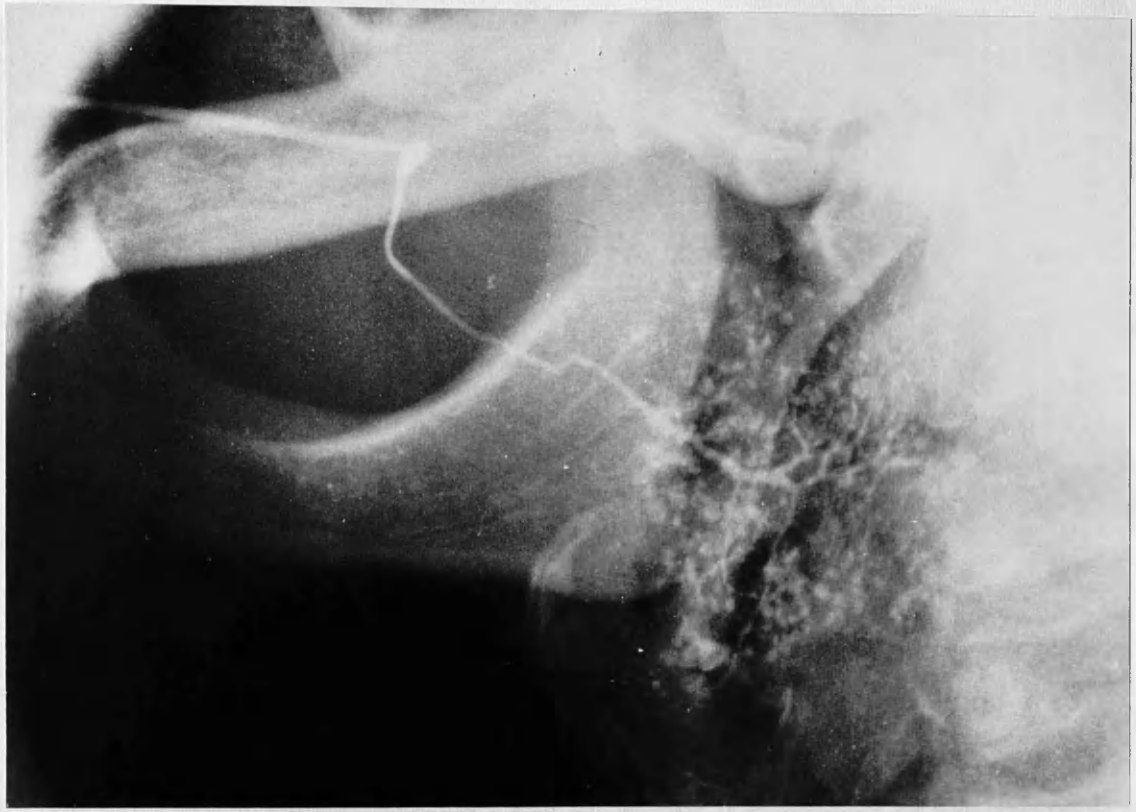
Normal 15° lateral oblique jaw sialogram

FIGURE II, 45.



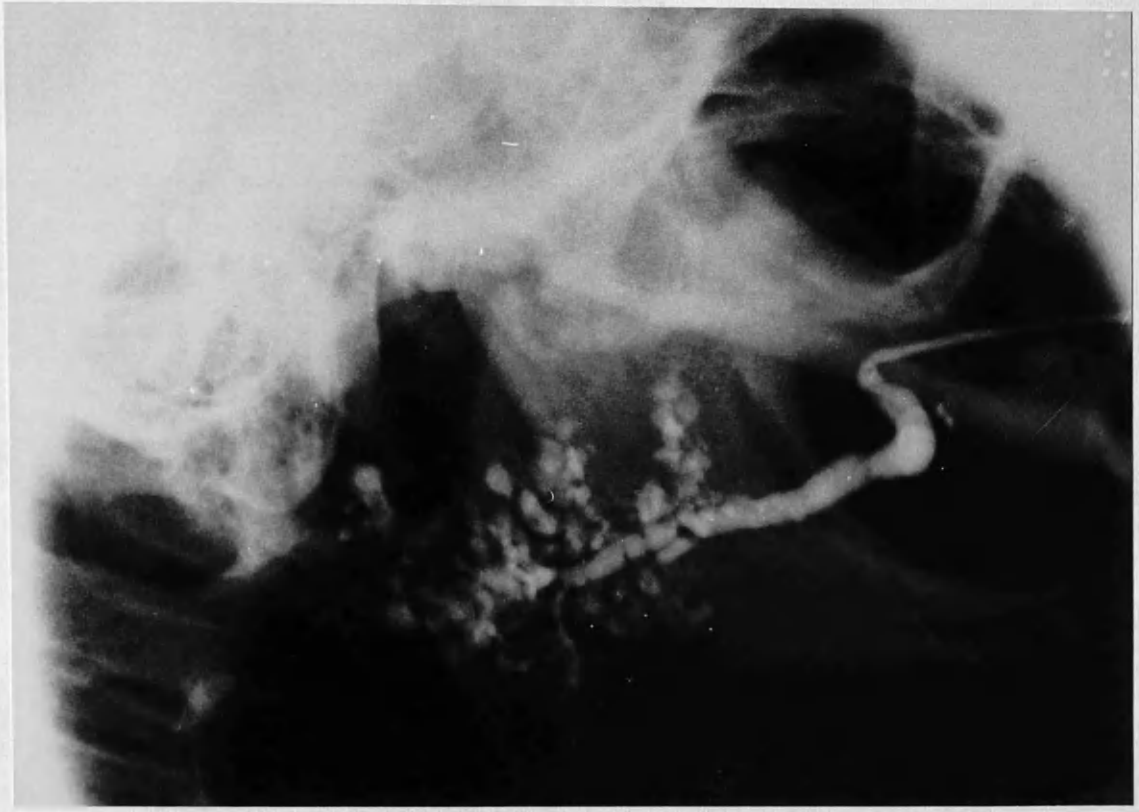
Punctate sialectasis.

FIGURE II, 46.



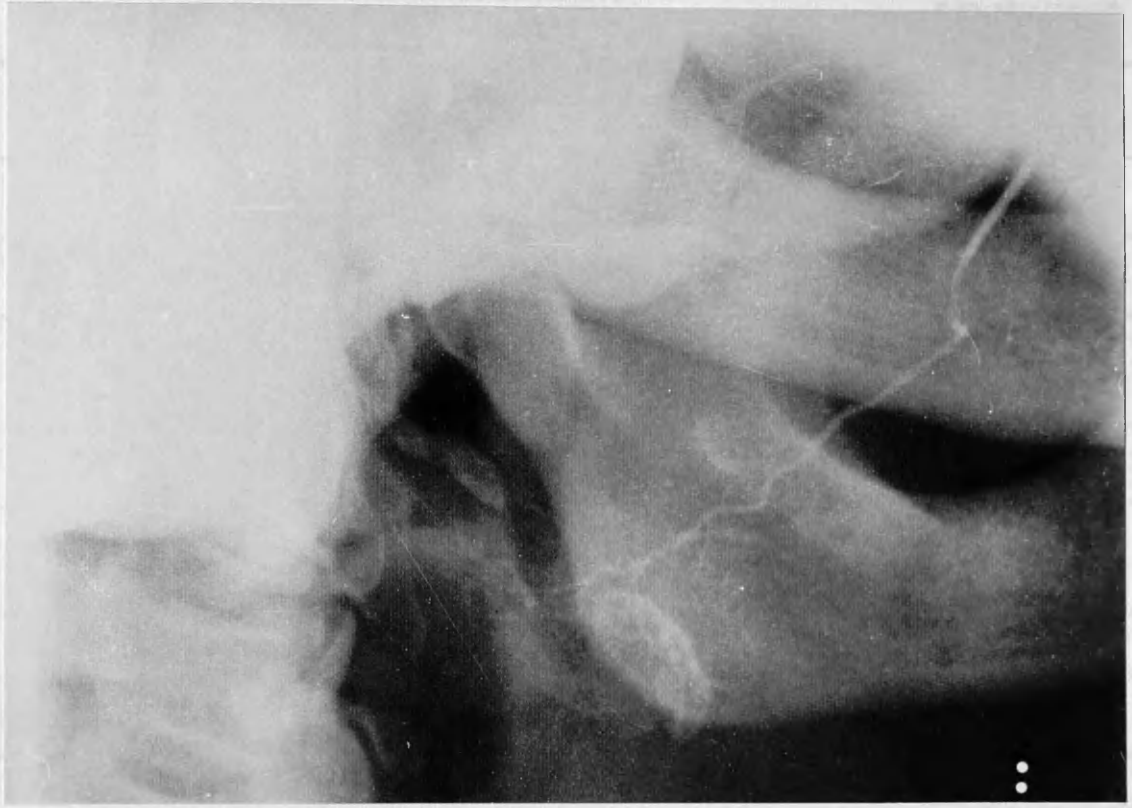
Globular sialectasis in patient with sicca syndrome

FIGURE II, 47.



Cavitary sialectasis with main duct dilatation

FIGURE II, 48.



Atrophy of the complete duct
system in a 31 year-old female

TABLE II, 15

SIALOGRAPHIC APPEARANCES IN SJÖGREN'S SYNDROME

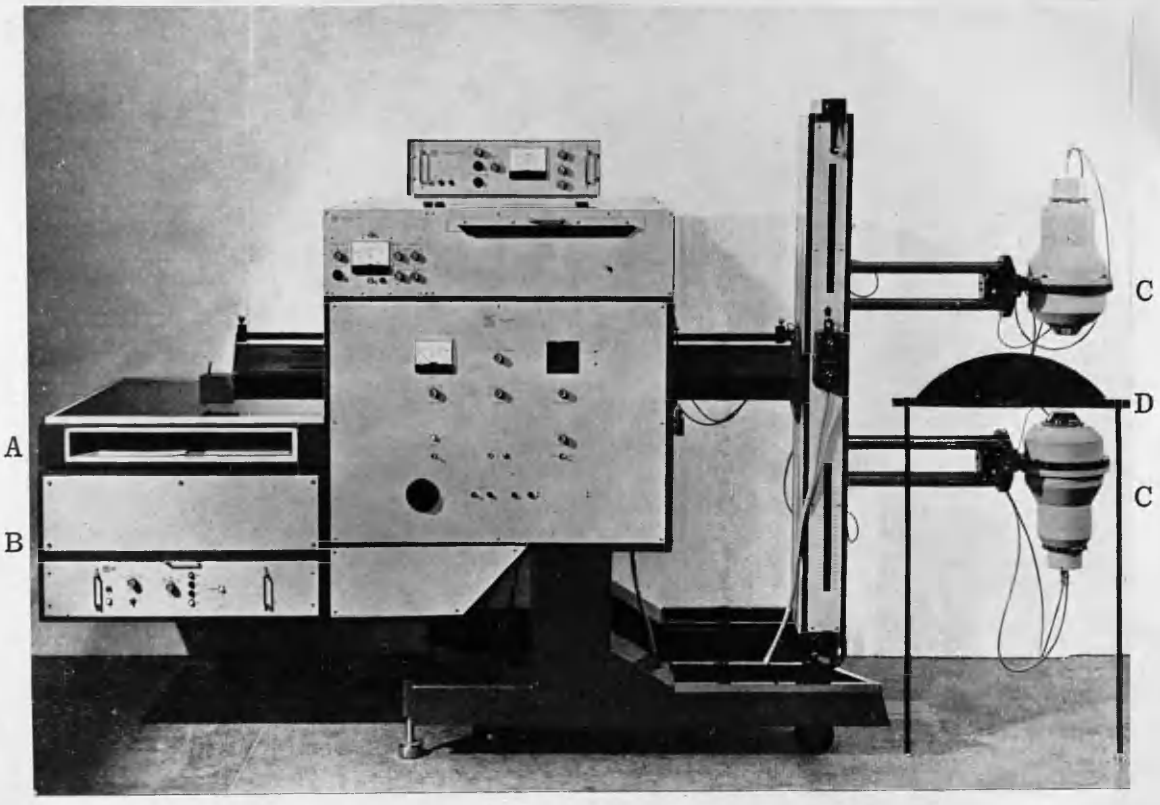
	NORMAL	SIALECTASIS			ATROPHY	MAIN DUCT DILATATION	CONTROL GROUP
		Punctate	Globular	Cavitary			
Total number of glands	45	29	15	15	14	6	45
Number with sicca syndrome	8	10	5	11	4	2	-
Sex distribution	8F OM	10F OM	2F 3M	11F OM	4F OM	2F OM	-
Number with Sjögren's syndrome & R.A.	37	19	10	4	10	4	-
Sex distribution	29F 8M	17F 2M	10F OM	4F OM	10F OM	4F OM	-
Mean parotid flow rate ml/min	0.85	0.38	0.34	0.13	0.25	0.32	1.29
S.E.M.	0.07	0.04	0.06	0.04	0.05	0.07	0.81
Range ml/min	0.26-2.00	0.0-0.80	0.0-1.00	0.00-0.42	0.01-0.50	0.08 - 0.48	0.47 - 2.70

TABLE II, 16.

SIALOGRAPHIC APPEARANCES IN CONNECTIVE TISSUE DISEASE

DIAGNOSIS	NUMBER	NORMAL	SIALECTASIS	OTHER ABNORMALITY
Rheumatoid arthritis	56	54	-	6
Osteoarthritis	12	12	-	-
Ankylosing spondylitis	14	12	-	1
Psoriatic arthritis	12	12	-	-
Reiter's disease	8	7	-	1
Gout	3	3	-	-
Systemic lupus erythematosus	10	5	2	3
Progressive systemic sclerosis	6	5	1	-
TOTAL:	131	120	3	11

FIGURE II, 49



SELO DS 4/4 SUPERSCANNER

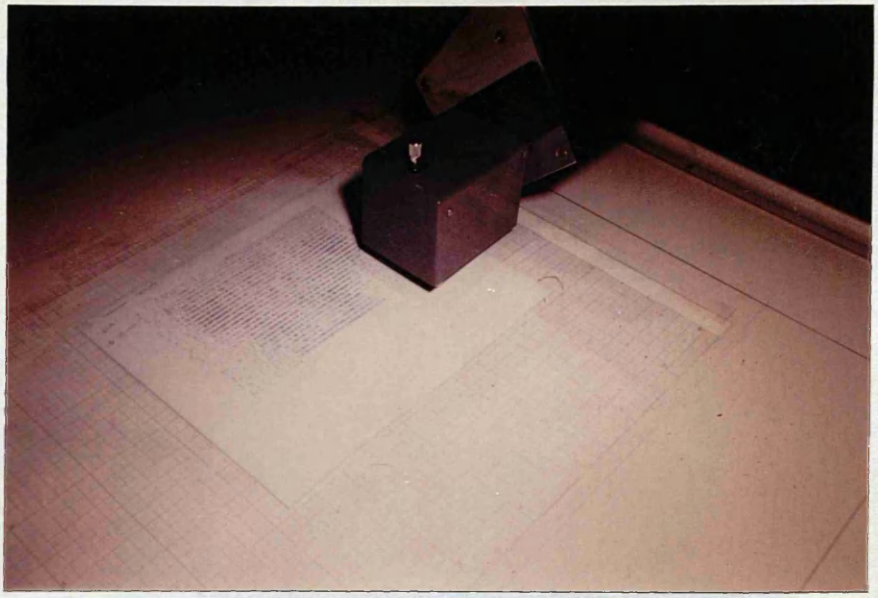
A. Upper print-out tray (black and white)

B. Lower print-out tray (colour)

C. Detecting heads

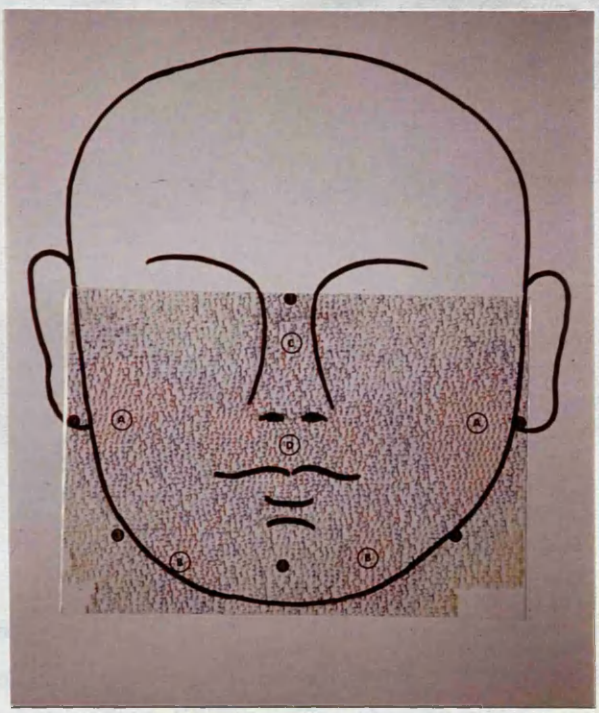
D. Diagramatic representation of subject on scanning table.

FIGURE II, 50



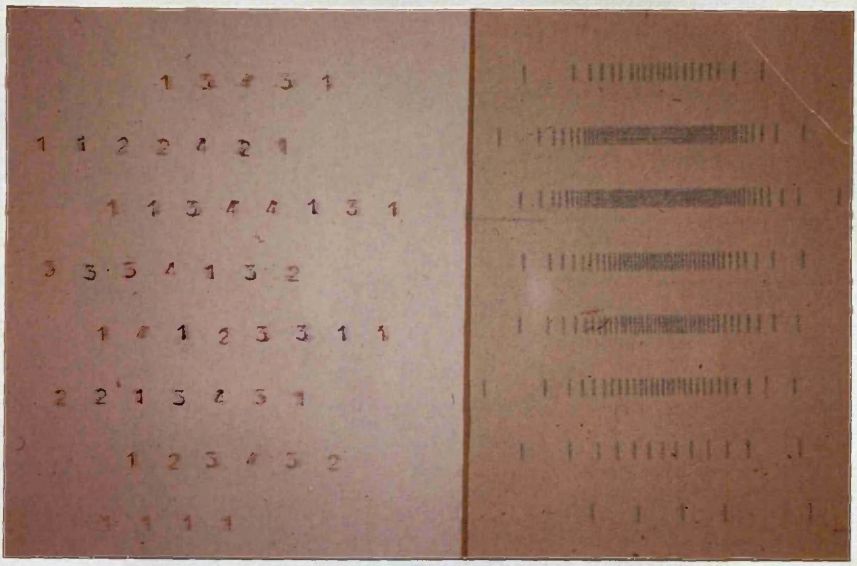
Upper print-out tray, showing dot scan.

FIGURE II, 51



Normal antero-posterior salivary scan, following administration of 1 m Ci ^{99m}Tc pertechnetate.

FIGURE II, 52

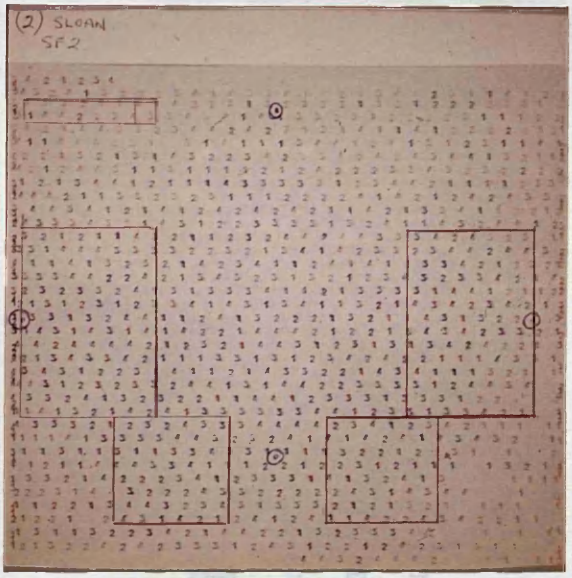


A B

Composite photograph showing comparable prints-out for (A) colour scan and, (B) dot scan of a ^{99m}Tc standard dose

B

A

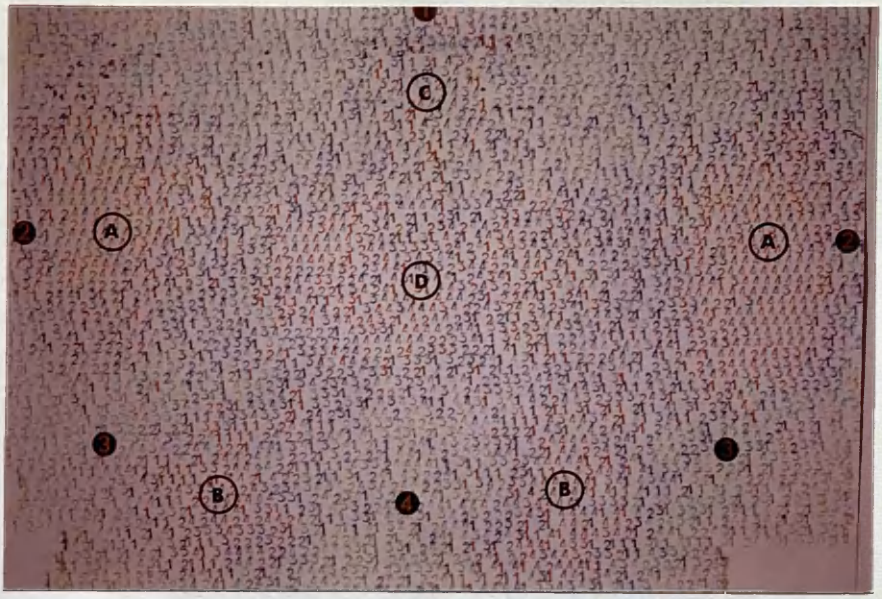


Fast colour scan showing boxes used for quantitative assessment of salivary (A) and background (B) regions.

FIGURE II, 54

THE RELATIONSHIP BETWEEN THE DIFFERENT COLOUR-CODES AND THE MATHEMATICAL VALUES EMPLOYED IN QUANTITATIVE ASSESSMENT IS NOTED BELOW:

COLOUR :				
SYMBOL :	1, 2, 3, 4	1, 2, 3, 4	1, 2, 3, 4	1, 2, 3, 4
VALUE :	1, 2, 3, 4	5, 6, 7, 8	9, 10, 11, 12	13, 14, 15, 16

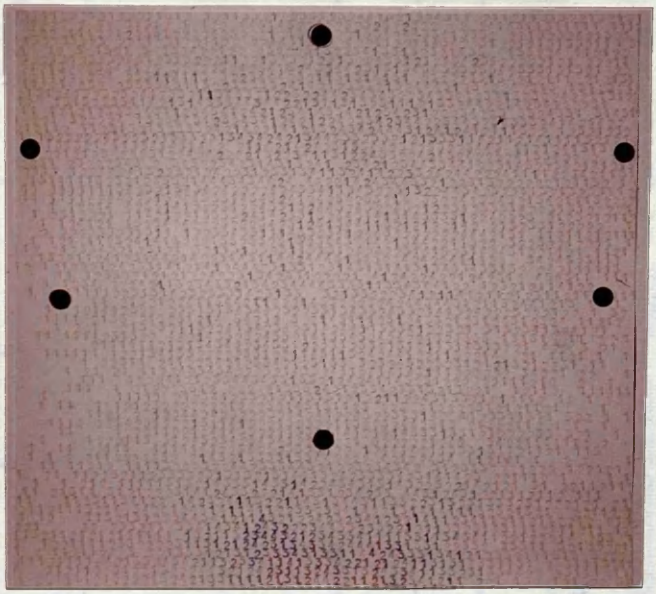


Enlarged photograph of scan shown in Figure II, 51.

A. Parotid gland
B. Submandibular gland
C. Nasal region
D. "Mouth" region

1. Bridge of nose
2. Lobe of ear
3. Gonial angle
4. Mid-point of chin

FIGURE II, 56



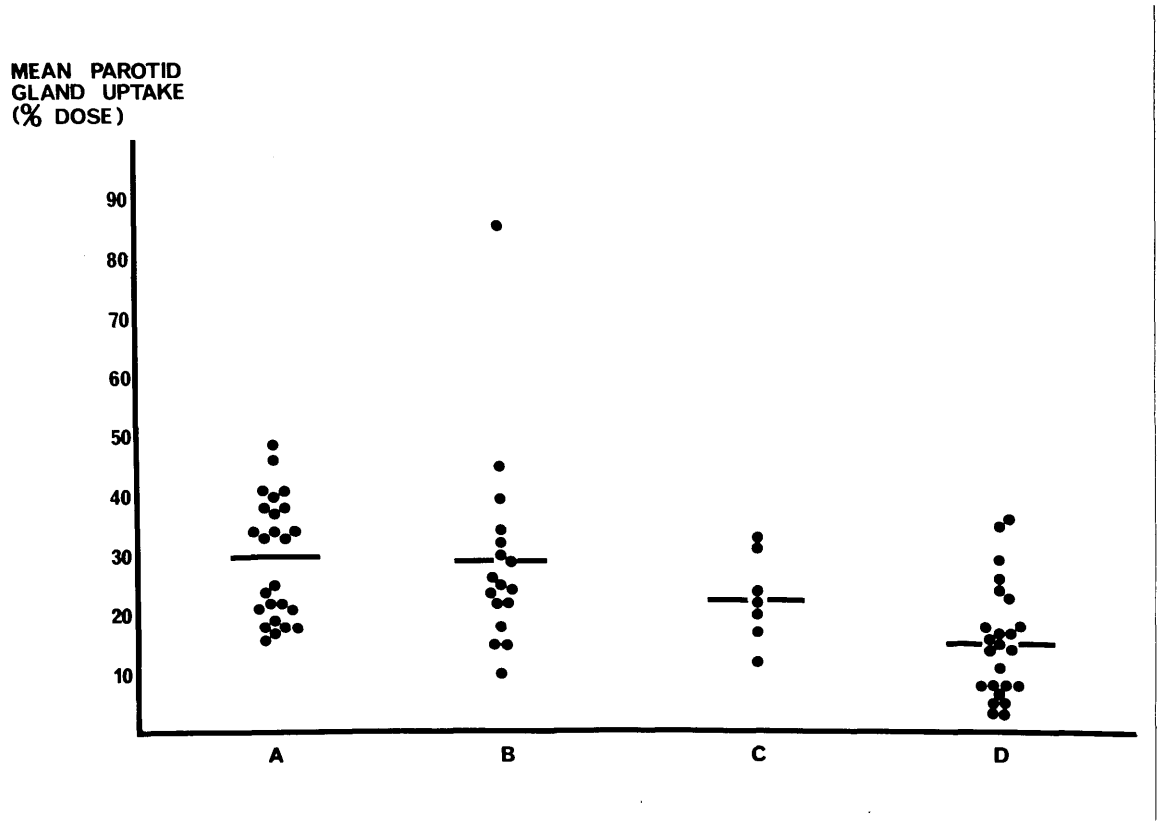
Scan of patient with Sjögren's syndrome. Little evidence of major or minor salivary gland activity, or of oral uptake. Normal thyroid function is evident.

TABLE II, 17.

UPTAKE OF ^{99m}Tc-PERTECHNETATE BY PAROTID AND SUBMANDIBULAR SALIVARY GLANDS AT MID-SCAN TIMES OF 3, 8 and 13 min. VALUES IN GROUP D ARE SIGNIFICANTLY LOWER THAN IN NORMAL SUBJECTS (GROUP A) BY WILCOXON'S ONE-TAILED SIGN-RANKED TEST.

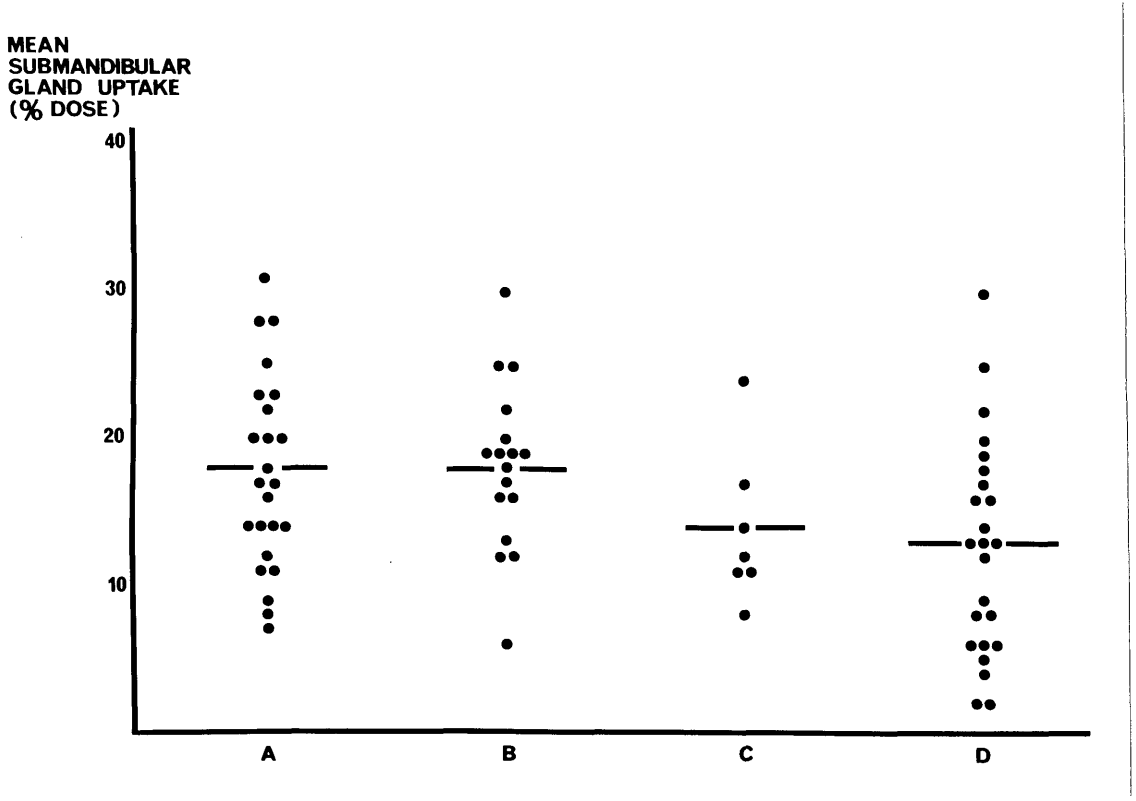
		GROUP A		GROUP B			GROUP C			GROUP D		
GLAND	TIME (MIN.)	Mean	S.E.	Mean	S.E.	P	Mean	S.E.	P	Mean	S.E.	P
Parotid	3	0.21	0.02	0.19	0.03	NS	0.18	0.04	NS	0.10	0.01	0.005
	8	0.30	0.02	0.29	0.04	NS	0.23	0.03	NS	0.15	0.02	0.005
	13	0.31	0.02	0.36	0.06	NS	0.28	0.05	NS	0.17	0.02	0.005
Submandibular	3	0.17	0.03	0.15	0.02	NS	0.16	0.03	NS	0.12	0.01	0.025
	8	0.18	0.01	0.18	0.01	NS	0.14	0.02	NS	0.13	0.02	0.01
	13	0.18	0.02	0.20	0.02	NS	0.17	0.02	NS	0.13	0.01	0.025

FIGURE II, 57



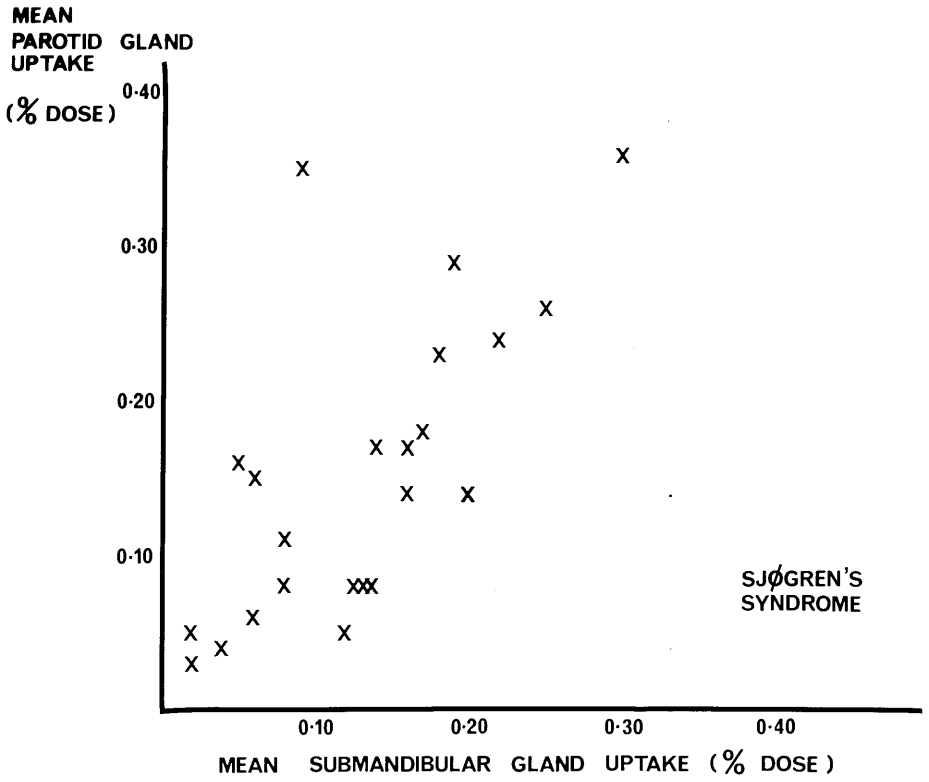
Mean uptake of ^{99m}Tc over the parotid glands at 8 min., in normal subjects (A), patients with rheumatoid arthritis (B), patients with rheumatoid arthritis and kerato conjunctivitis sicca (C), and patients with Sjögren's syndrome (D).

FIGURE II, 58



Mean uptake of ^{99m}Tc over the submandibular glands at 8 min., in normal subjects (A), patients with rheumatoid (B), patients with rheumatoid arthritis and kerato conjunctivitis sicca (C), and patients with Sjögren's syndrome (D).

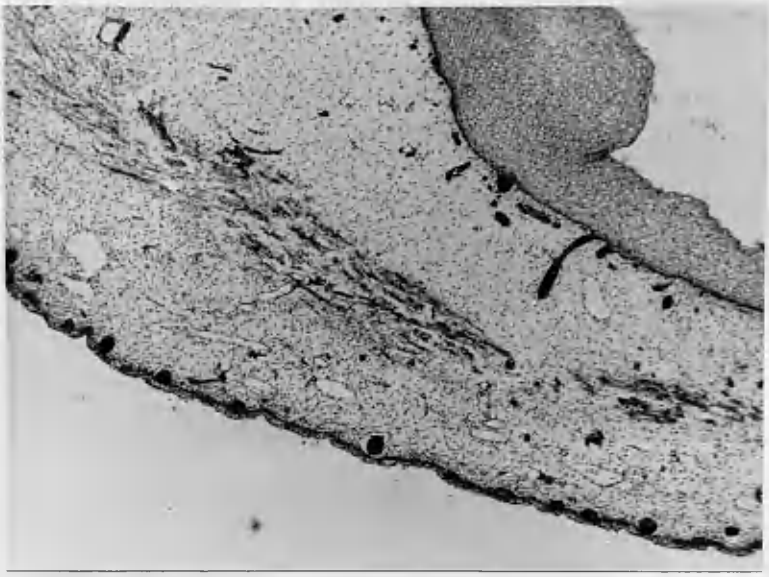
FIGURE II, 59



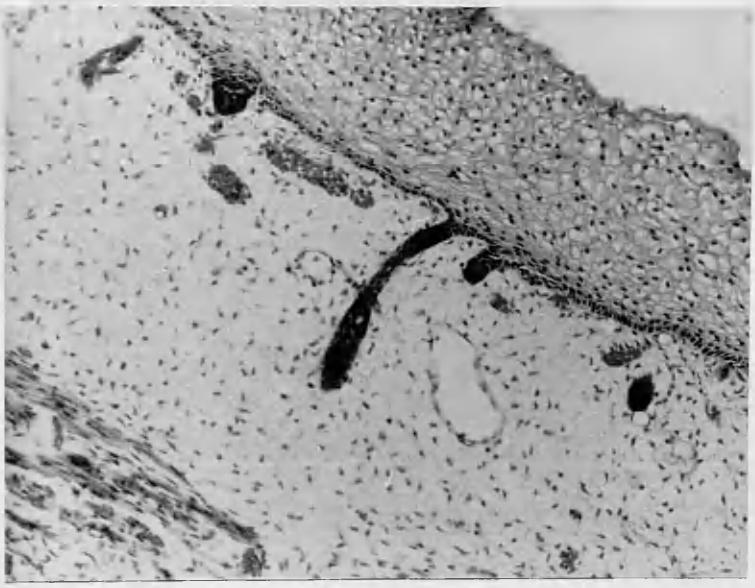
Relationship between parotid and submandibular uptake of ^{99m}Tc, 8 min., after administration of 1 m Ci ^{99m}Tc pertechnetate.
(r = 0.66; P 0.001)

FIGURE III, 1.

a)



b)

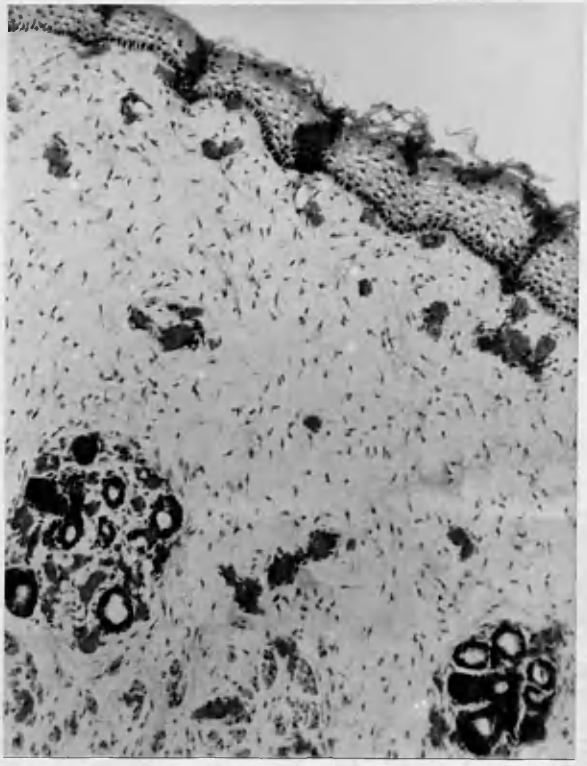


Early development of labial salivary glands
(60 mm C.R.)

a) Full thickness section through lower lip. Note budding and proliferation of primitive oral epithelium. Skin appendages are developing in relation to primitive dermis (x 25)

b) Budding of oral epithelium (x 75).

FIGURE III, 2.



Oral
Epithelium

Duct
Differentiation

Development of labial salivary glands (135 mm C.R.)

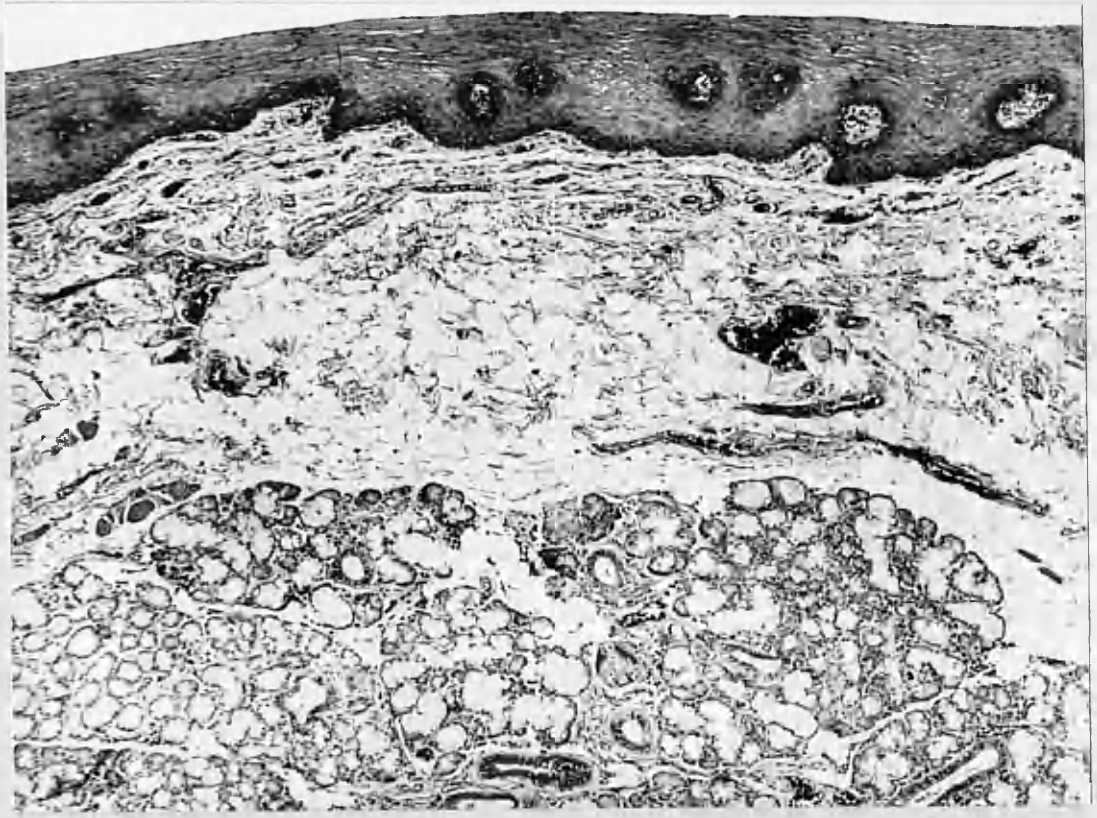
Hollowing out of epithelial buds to form early duct structures (x 75).

TABLE III, 1

GRADING STANDARD FOR LABIAL GLANDS

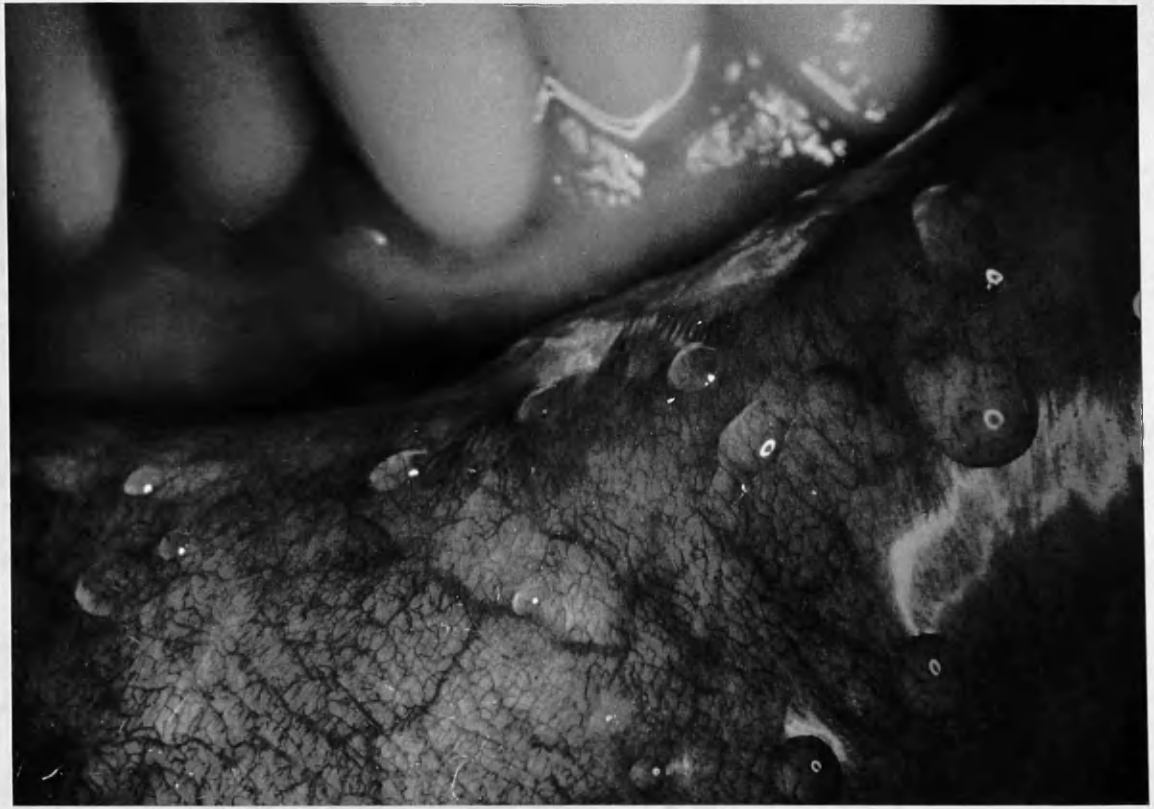
GRADE	LYMPHOCYTIC INFILTRATION OR FOCI PER 4 sq.mm. OF SALIVARY TISSUE
0	Absent
1	Slight infiltrate
2	Moderate infiltrate
3	One focus
4	More than one focus

FIGURE III, 3.



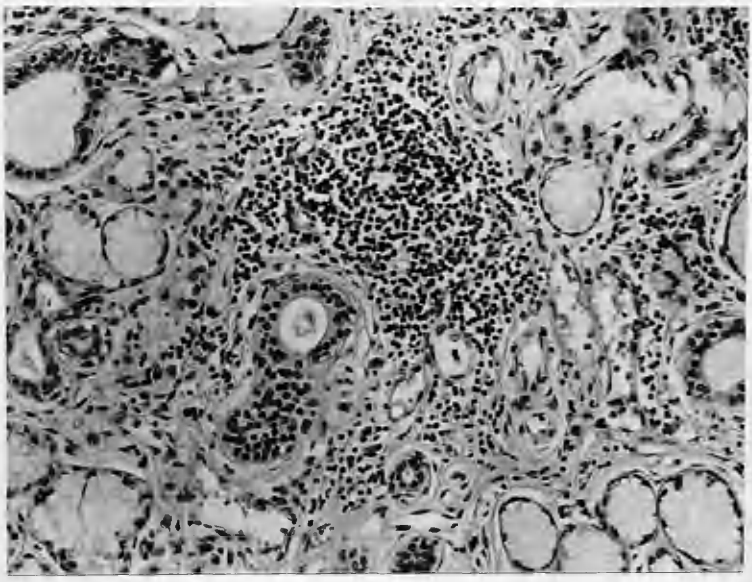
Normal minor labial salivary gland tissue. Note relationship to oral mucosa. Lobules of gland tissue lie above the muscle layer of lip (x 75).

FIGURE III, 4.



Beads of minor labial gland saliva secreted in response to application of a drop of lemon juice to the dorsum of the patient's tongue.

FIGURE III, 5.



A periductal focus of lymphocytes affecting minor labial salivary gland tissue (x 75).

TABLE III, 2

GRADING STANDARD FOR SUBMANDIBULAR GLAND

GRADE		LYMPHOCYTIC FOCI PER 4 sq.cm. OF SALIVARY TISSUE
0	None	0 - 1
1	'Slight'	2 - 8
2	'Moderate'	9 - 40
3	'Severe'	40+
4	'Very severe'	more than half gland parenchyma replaced

TABLE III, 3

PREVALENCE OF FOCAL LYMPHOCYTIC ADENITIS

(Submandibular Gland)

ALL CASES 116	Females 56	(All Positives)	23 (41%)
		(Grades 2,3 & 4 only)	17 (30%)
	Males 60	(All Positives)	26 (43%)
		(Grades 2,3 & 4 only)	16 (27%)

TABLE III, 4

PREVALENCE OF SLIGHT OR MODERATE

LYMPHOCYTIC INFILTRATION OF LABIAL GLAND

ALL CASES 116	(Females	(All Positives)	31 (55%)
	(56	(Grade 2)	14 (25%)
	(Males	(All Positives)	39 (65%)
	(60	(Grade 2)	12 (20%)

TABLE III, 5

NUMBER OF FEMALES WITH SUBMANDIBULAR LYMPHOCYTIC SIALADENITIS

Age in years	Number with grade of severity				Total	per cent positive
	0	1	2	3		
0 - 44	7	2	1	0	10	30
45 - 64	12	1	5	3	21	43
65+	14	3	6	2	25	44
T O T A L :	33	6	12	5	56	41

TABLE III, 6

NUMBERS OF MALES WITH SUBMANDIBULAR LYMPHOCYTIC SIALADENITIS

Age in years	Number with grade of severity				Total	per cent positive
	0	1	2	3		
0 - 44	6	3	2	0	11	45
45 - 64	18	4	6	3	31	42
65+	9	4	5	0	18	50
T O T A L :	33	11	13	3	60	43

TABLE III, 7

NUMBERS OF FEMALES WITH LYMPHOCYTIC INFILTRATION OF THE LABIAL GLANDS

Age in years	Number with grade of severity			Total	per cent positive
	0	1	2		
0 - 44	8	1	1	10	20
45 - 64	6	9	6	21	71
65+	11	7	7	25	56
T O T A L:	25	17	14	56	55

TABLE III, 8

NUMBERS OF MALES WITH LYMPHOCYTIC INFILTRATION OF THE LABIAL GLANDS

Age in years	Number with grade of severity			Total	per cent positive
	0	1	2		
0 - 44	5	3	3	11	54
45 - 64	10	17	4	31	68
65 +	6	7	5	18	67
TOTAL:	21	27	12	60	65

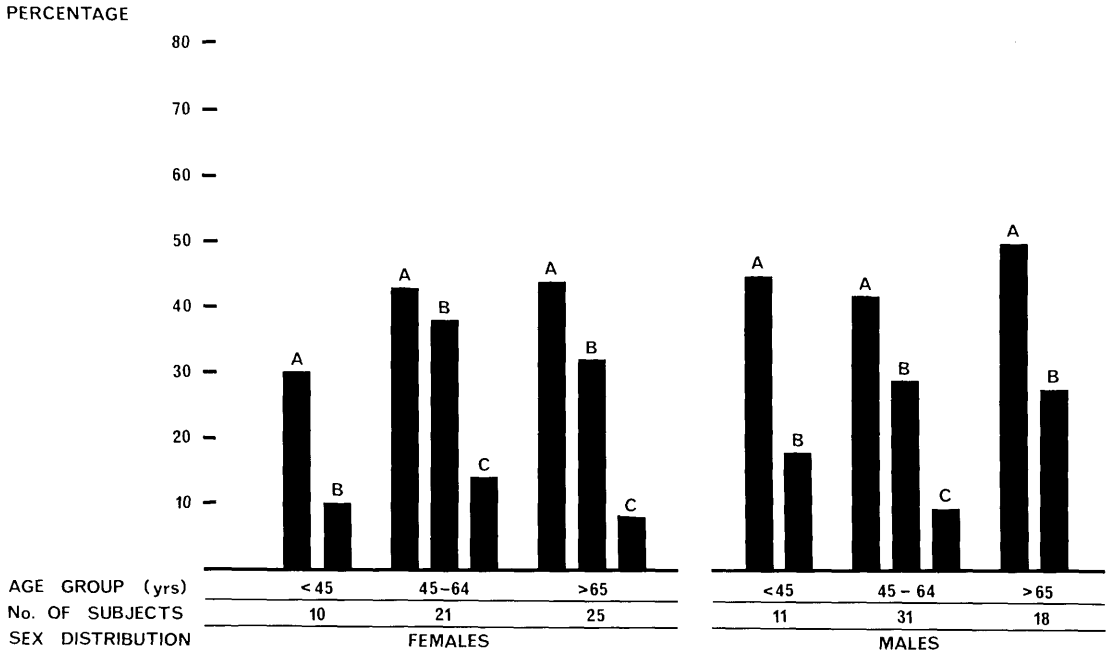
TABLE III, 9

POST-MORTEM SERIES - 116 SUBJECTS

- Numbers of subjects showing various combinations of submandibular and labial gland lesions -

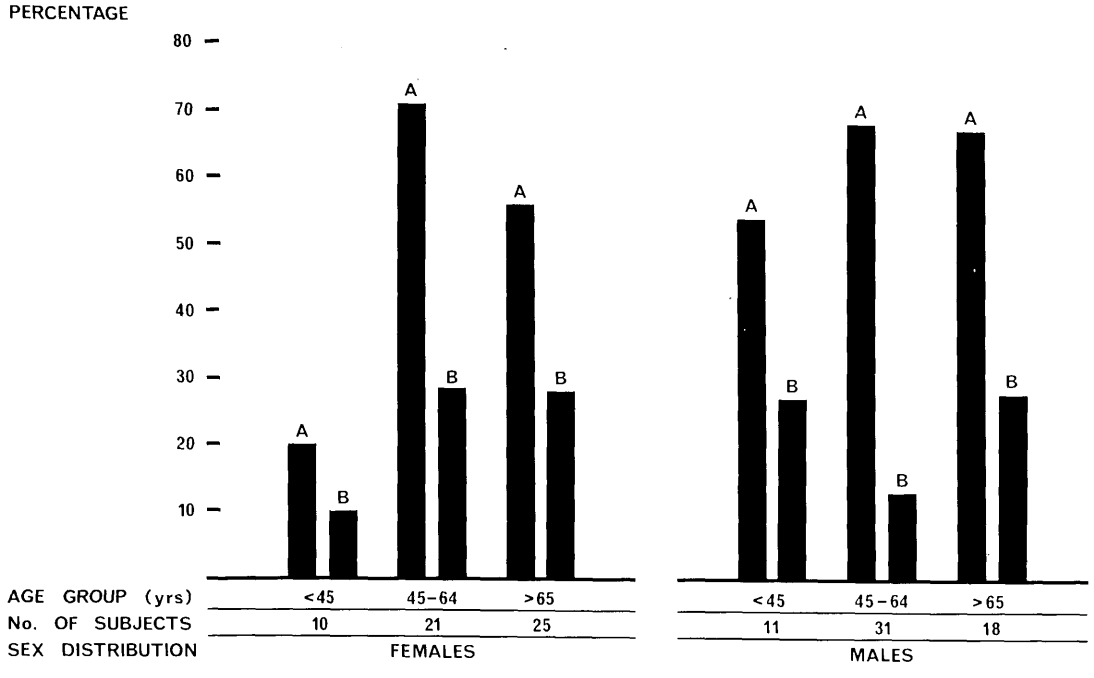
	GRADE	SUBMANDIBULAR 0	1	2	3	4	TOTAL
LABIAL	0	31	5	9	1	0	46
	1	31	5	6	2	0	44
	2	5	6	10	4	1	26
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	Total:	67	16	25	7	1	116

FIGURE III, 6.



The prevalence of lymphocytic submandibular sialadenitis in males and females
A = Grade I or worse; B = Grade II or worse; C = Grade III or worse.

FIGURE III, 7.



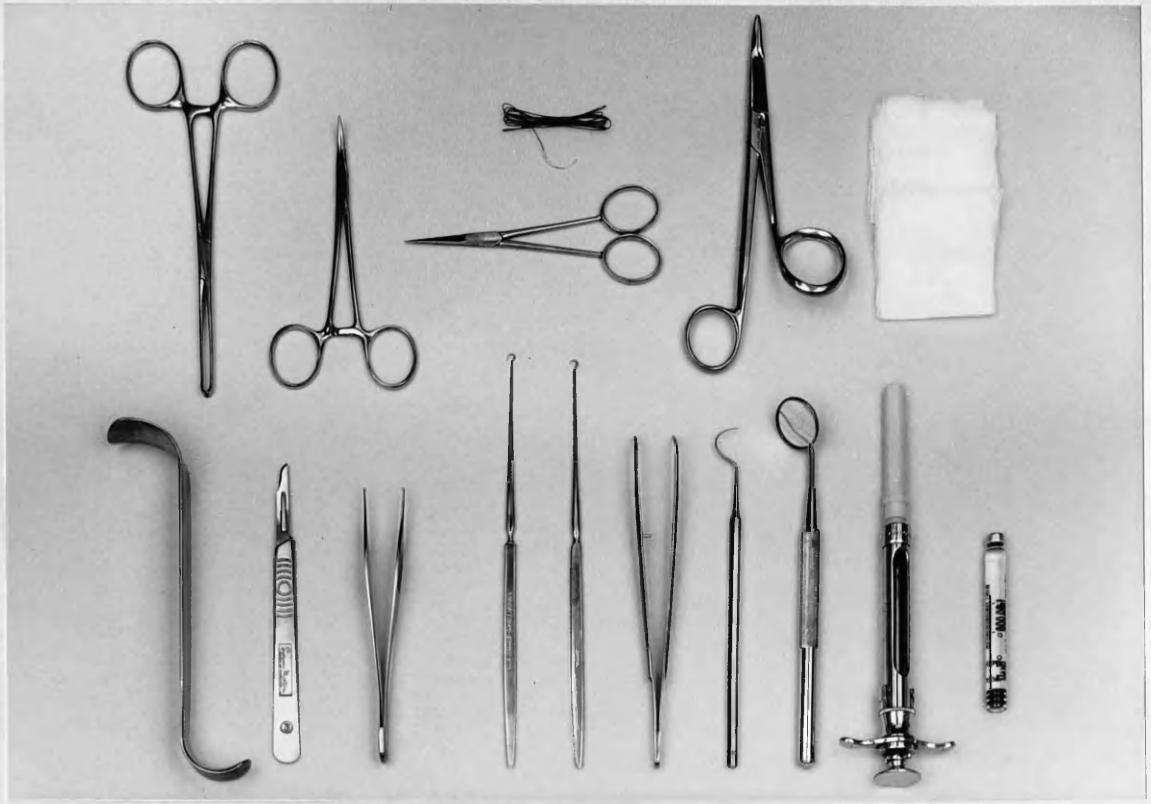
The prevalence of lymphocytic labial sialadenitis in males and females
A = Grades I and II; B = Grades II only

TABLE III, 10

LABIAL GLAND BIOPSY : PATIENTS STUDIED

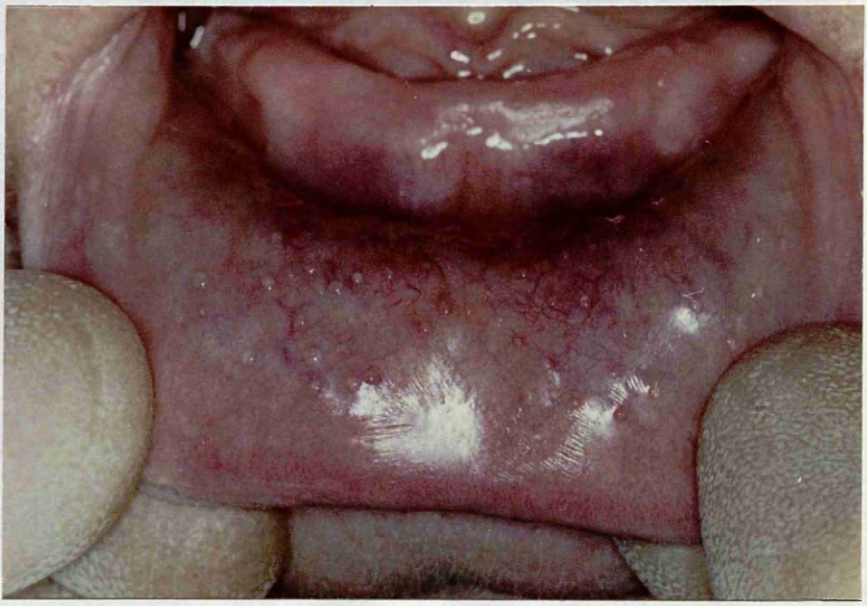
CLINICAL DIAGNOSIS	NO.
SICCA SYNDROME	21
SJÖGREN'S SYNDROME	50
RHEUMATOID ARTHRITIS	73
PARA-RHEUMATIC DISEASE:	
PSORIATIC ARTHRITIS	16
OSTEOARTHRITIS	19
ANKYLOSING SPONDYLITIS	12
REITER'S DISEASE	12
GOUT	2
CONNECTIVE TISSUE DISEASE:	
SYSTEMIC LUPUS ERYTHEMATOSUS	5
PROGRESSIVE SYSTEMIC SCLEROSIS	3
DERMATOMYOSITIS	1

FIGURE III, 8.



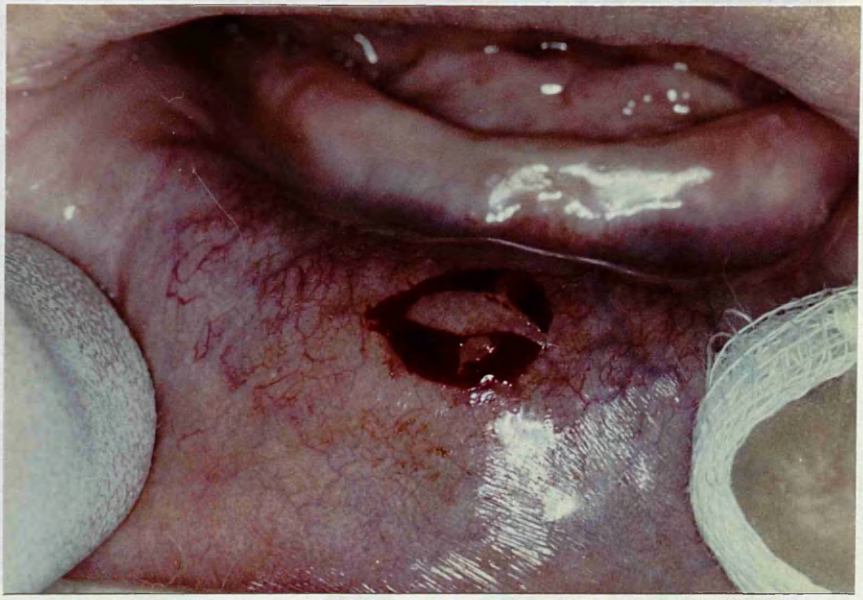
Instrumentation for labial salivary gland biopsy:
Allis forceps; artery forceps; suture material, scissors and needle holder; check retracter; scalpal and toothed tissue forceps; skin hooks; tweezers, probe and mirror; syringe and anaesthetic cartridge.

FIGURE III, 9.



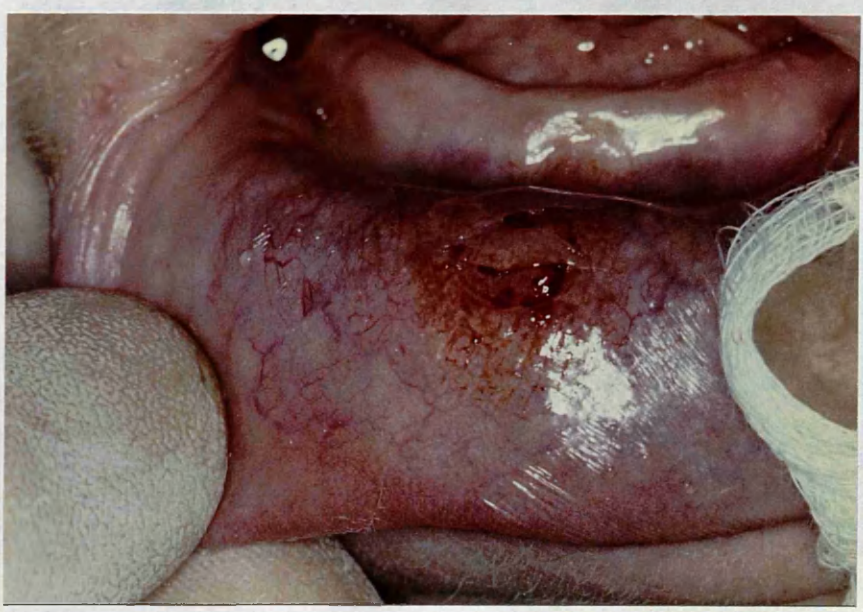
Retraction of lower lip, prior to biopsy

FIGURE III, 10.



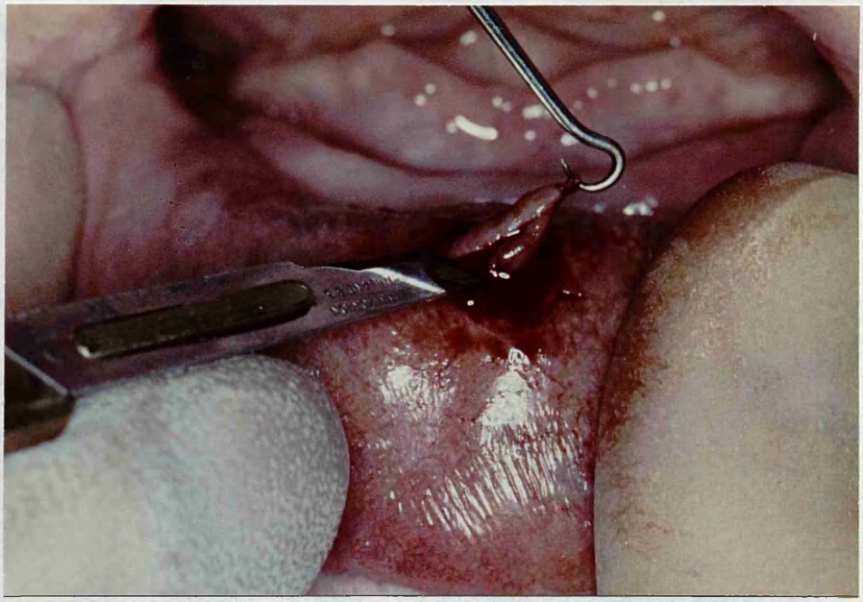
Initial elliptical incision approximately 0.8 x 0.4 cms.

FIGURE III, 11.



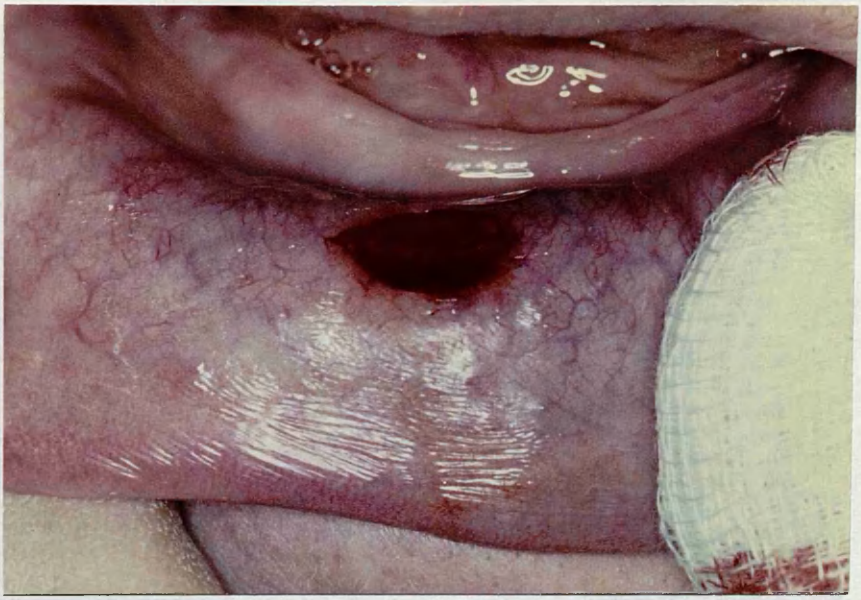
Initial incision after control of slight early haemorrhage.

FIGURE III, 12.



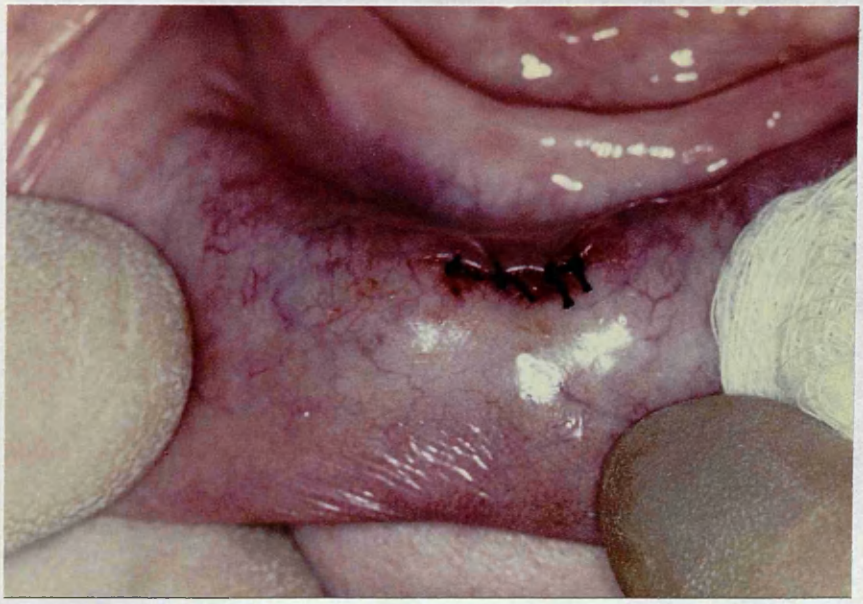
Dissection of labial biopsy specimen. Skin hook is applied through edge of biopsy specimen. Minor gland tissue can be observed on under-surface.

FIGURE III, 13.



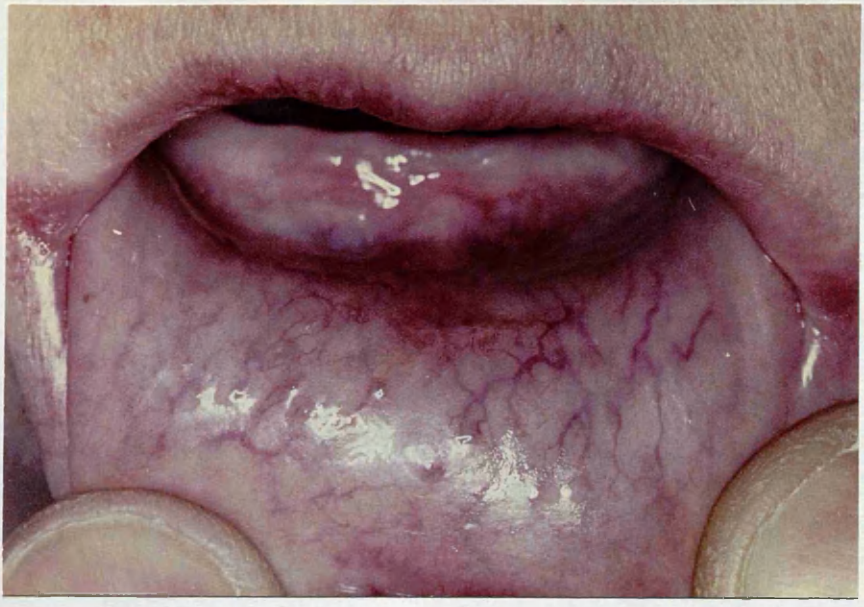
Biopsy wound.

FIGURE III, 14.



Biopsy wound closed with 04 gauge black silk sutures.

FIGURE III, 15.



Biopsy site with satisfactory healing one week later.

TABLE III, 11

LYMPHOCYTIC ADENITIS OF LABIAL SALIVARY GLANDS

CLINICAL DIAGNOSIS	No.	GRADE 0	1	2	3	4	% with foci present
SICCA SYNDROME	21	1	3	4	5	8	61
SJOGREN'S SYNDROME	50	1	10	4	15	20	70
RHEUMATOID ARTHRITIS	73	30	20	9	12	2	19
*PARA-RHEUMATIC DIS.	61	40	15	4	2	0	3
*CONNECTIVE TISSUE DISEASE	9	3	3	1	0	2	22

*See Table III, 10.

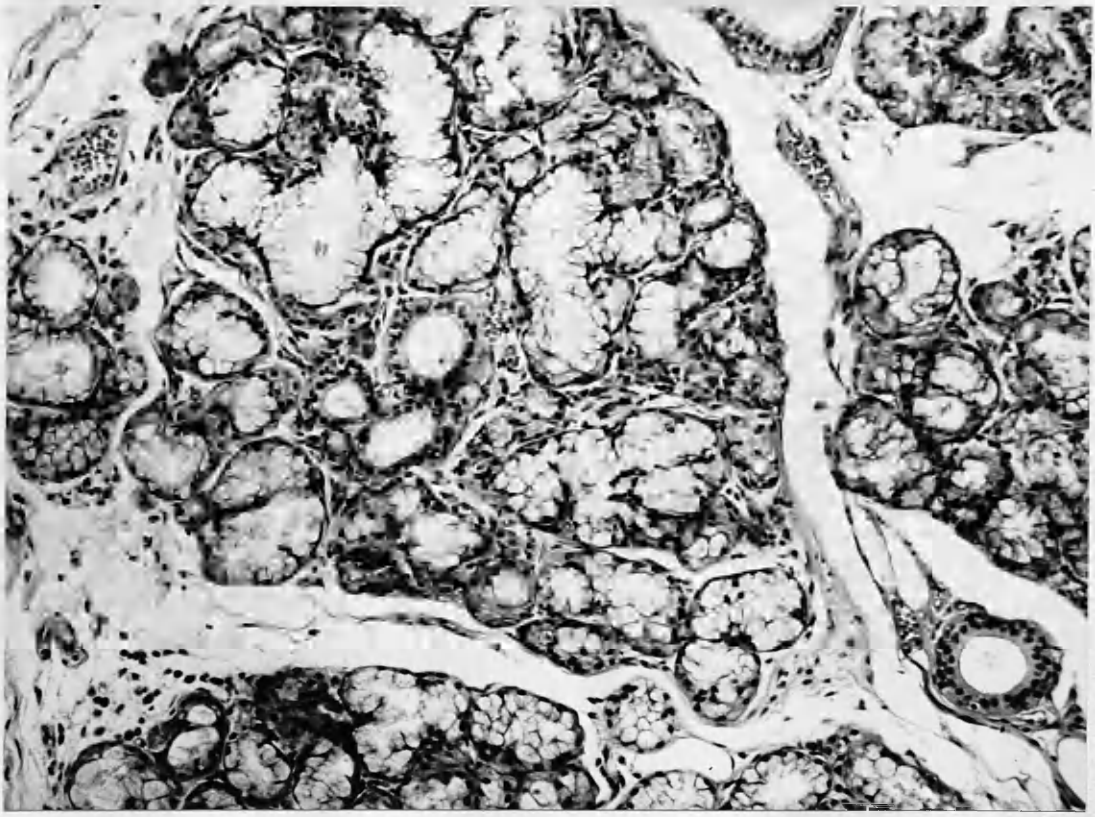
TABLE III, 12 THE RELATIONSHIP OF FOCAL LYMPHOCYTIC SIALADENITIS IN THE LABIAL GLANDS AND THE CLINICAL FEATURES OF PATIENTS WITH RHEUMATOID ARTHRITIS ALONE.

			PRESENT	ABSENT
FOCAL LYMPHOCYTIC SIALADENITIS:			11	29
Age (yrs)		Mean	51.5	55.6
		Range	20-73	39-73
Sex			10F. 1 M.	20F. 9 M.
Positive Kerato-conjunctivitis sicca			1 (9.1%)	4 (13.8%)
Symptomatic Xerostomia			1 (9.1%)	8 (29.4%)
Abnormal Sialogram			-	1 (3.4%)
RHEUMATOID ARTHRITIS	Duration	Mean	12.9	10.3
		Range	3/12 - 47	6/12-41
	Functional Grade	I & II	2 (18.2%)	5 (17.2%)
		III & IV	9 (81.8%)	24 (82.8%)
	X-ray Stage	I & II	2 (18.2%)	5 (17.2%)
		III & IV	9 (81.8%)	24 (82.8%)
	Nodules		2 (18.2%)	8 (27.6%)

TABLE III, 13 THE RELATIONSHIP OF FOCAL LYMPHOCYTIC SIALADENITIS IN THE LABIAL GLANDS AND THE LABORATORY FEATURES OF PATIENTS WITH RHEUMATOID ARTHRITIS ALONE

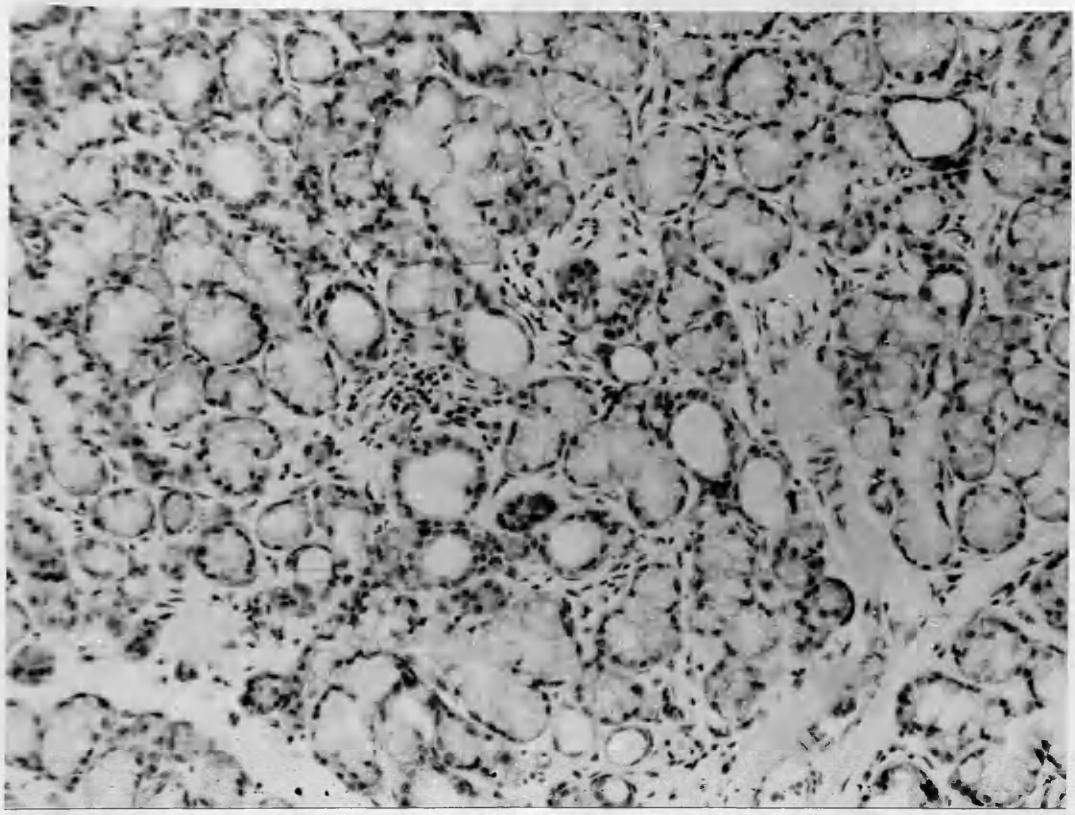
FOCAL LYMPHOCYTIC SIALADENITIS:		PRESENT	ABSENT	SIGNIFICANCE
		11	29	
Haemoglobin (G./100 ml)	Mean	11.4± 1.7	13.1±1.65	$p < 0.001$
	Range	8.7-14.4	7.1-15.3	
White cell count	Mean	6,052±2,462	8,127±3,009	$p < 0.02$
	Range	2,000-11,600	5,000-15,900	
Erythrocyte Sedimentation Rate(mm./hr.-Westergren)	Mean	78.9±19.3	41.6±26.1	$p < 0.001$
	Range	36 - 107	5-106	
Serum Globulin	Mean	3.9± 0.65	3.5± 0.68	-
	Range	3.2- 5.5	2.6- 5.2	

FIGURE III, 16



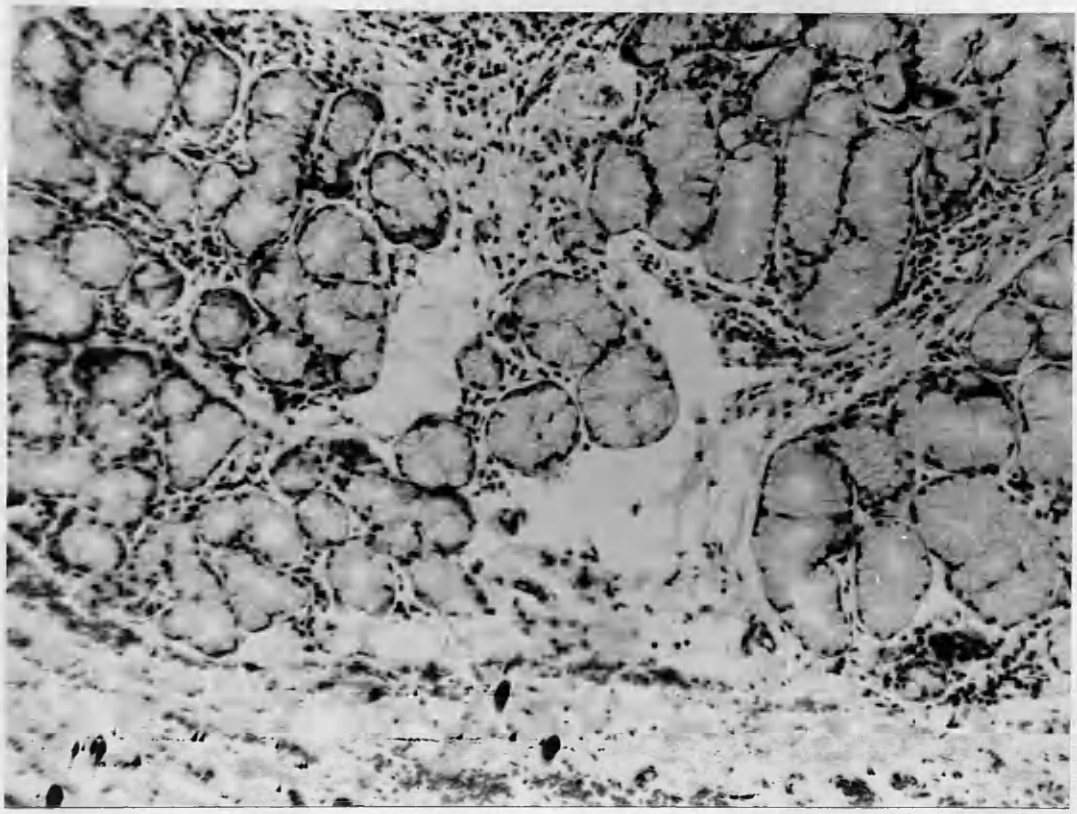
Normal minor labial salivary gland tissue (x 190).

FIGURE III, 17



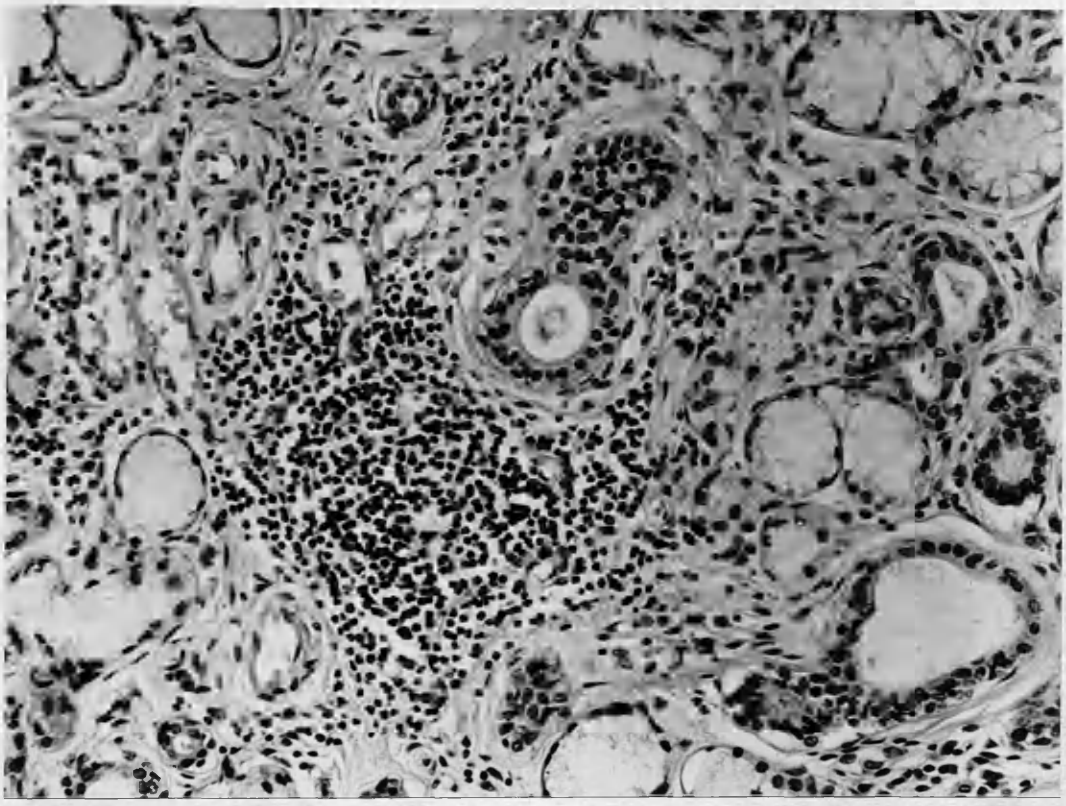
Grade 1 lymphocytic infiltration of labial salivary gland tissue (x 75).

FIGURE III, 18



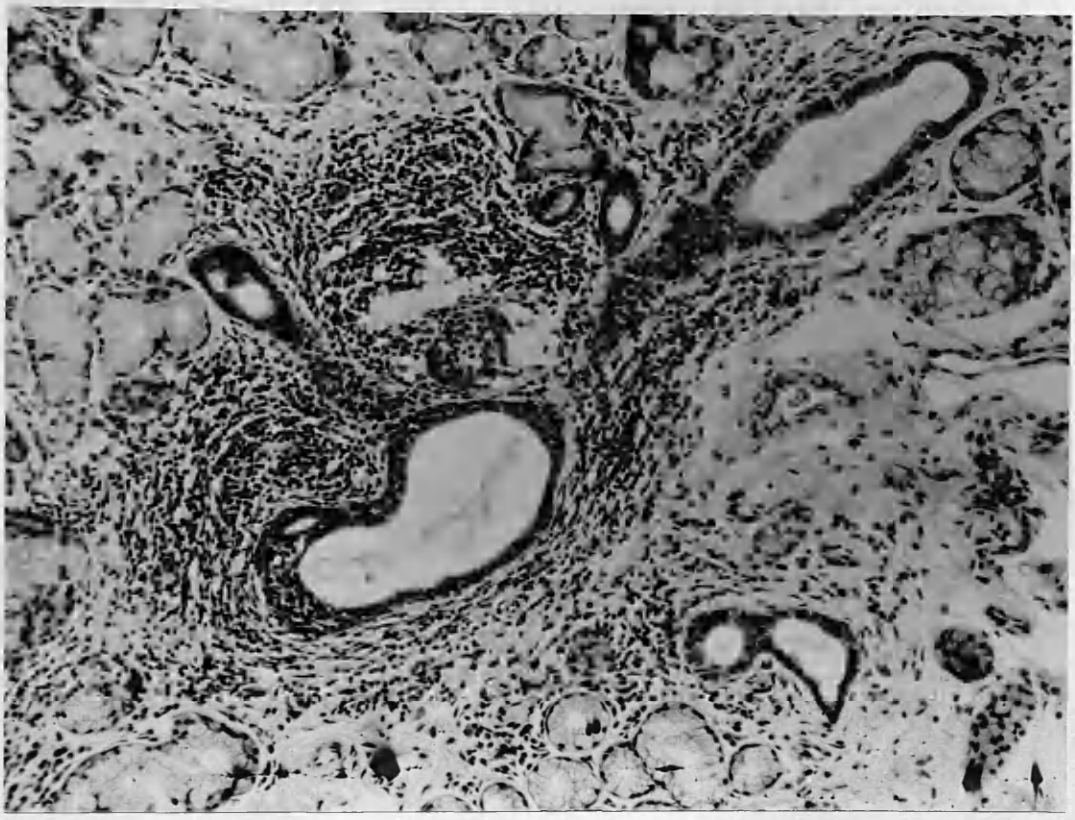
Grade 2 lymphocytic infiltration by labial salivary gland tissue (x 90).

FIGURE III, 19



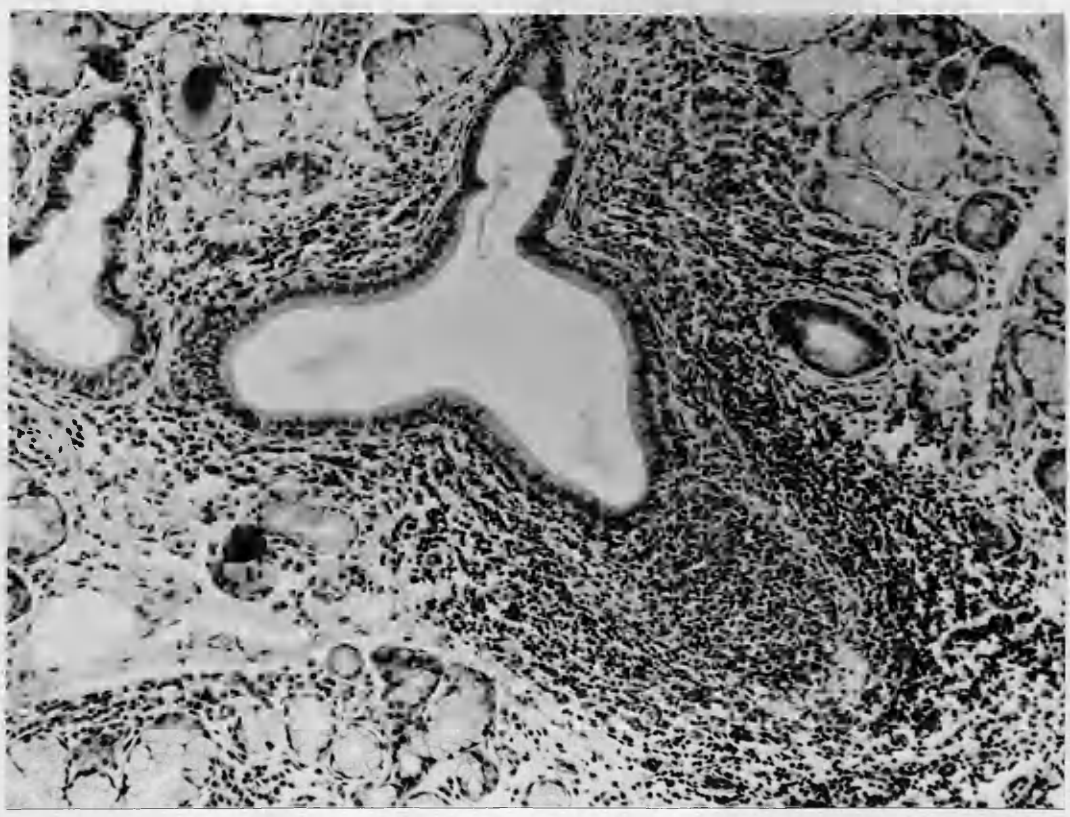
A periductal focus of lymphocytes affecting labial salivary gland tissue from a patient with Sjögren's syndrome (x 200).

FIGURE III, 20



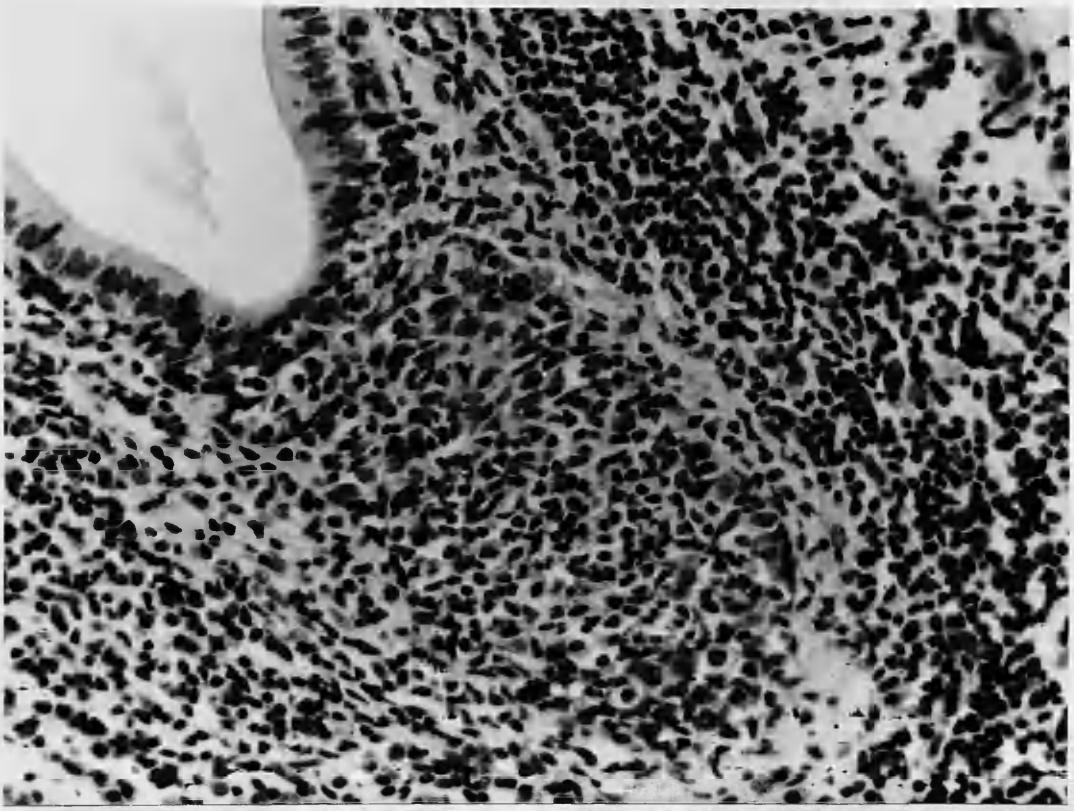
Marked periductal lymphocytic involvement of the labial salivary glands. Mild acinar atrophy and interstitial fibrosis are associated features of note (x 190).

FIGURE III, 21



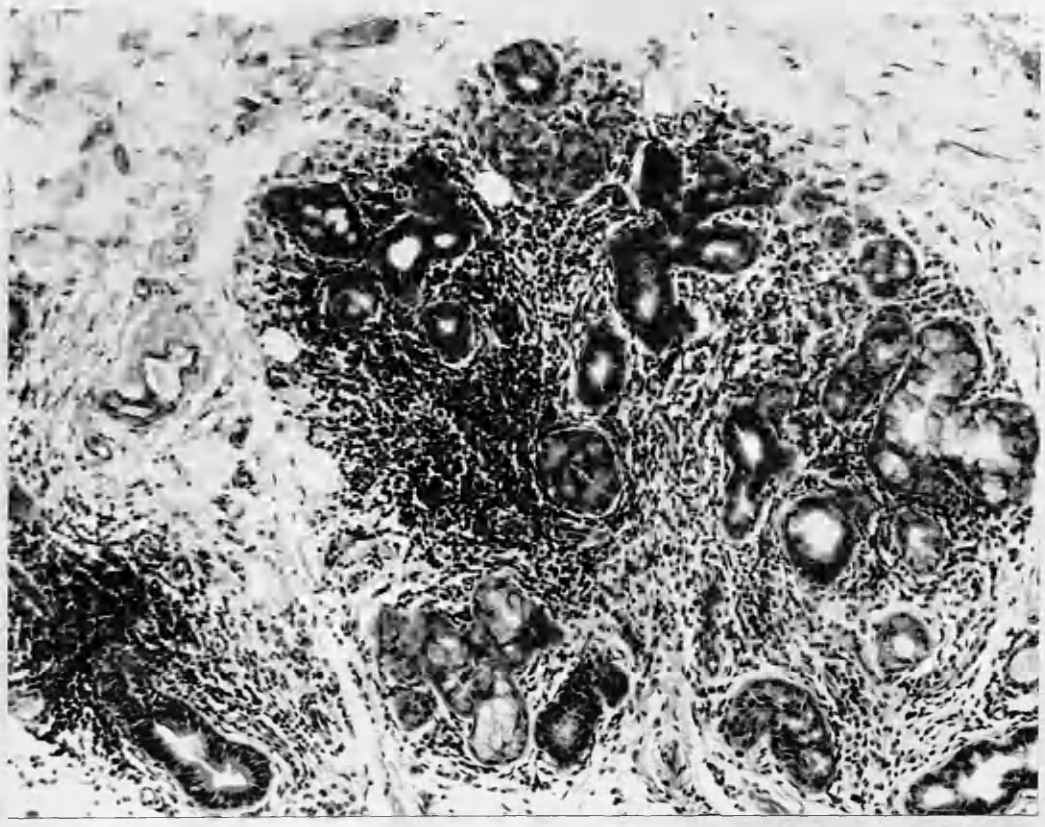
Marked focal lymphocytic labial sialadenitis. Acinar atrophy, mild interstitial fibrosis together with mild duct dilatation and diffuse lymphocytic infiltration are associated features (x 190).

FIGURE III, 22



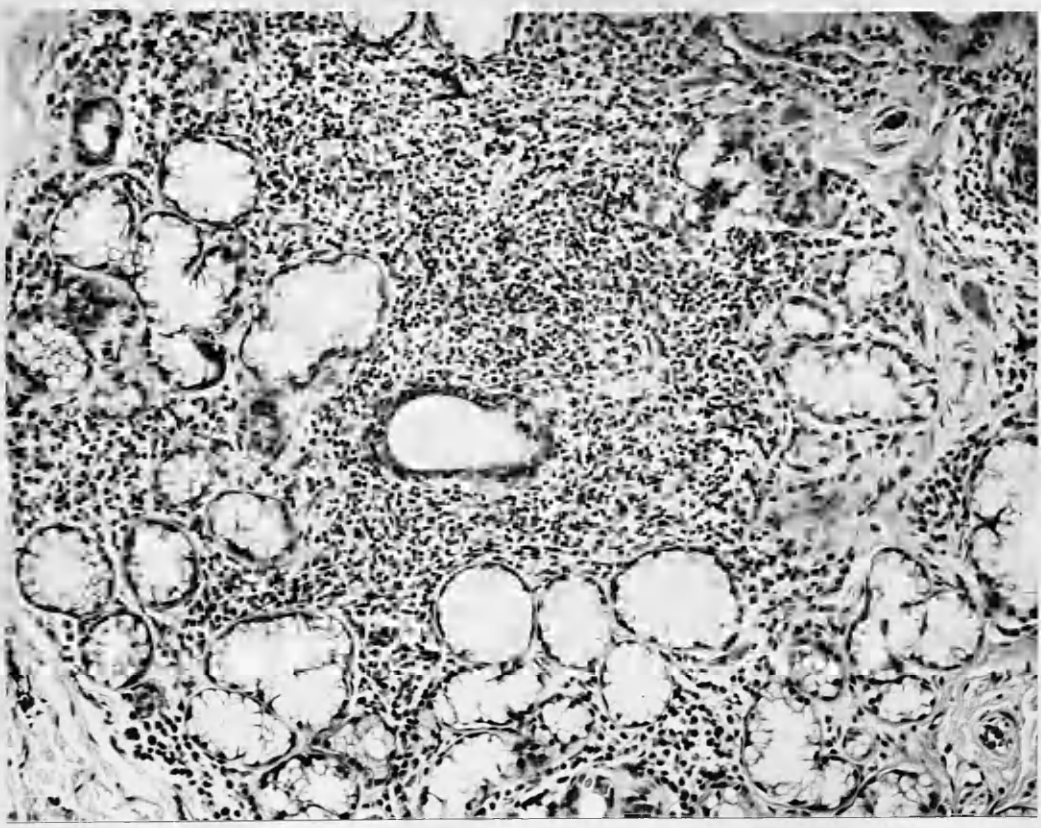
Higher magnification of focal lymphocytic labial sialadenitis shown in previous photomicrograph (x 250).

FIGURE III, 23



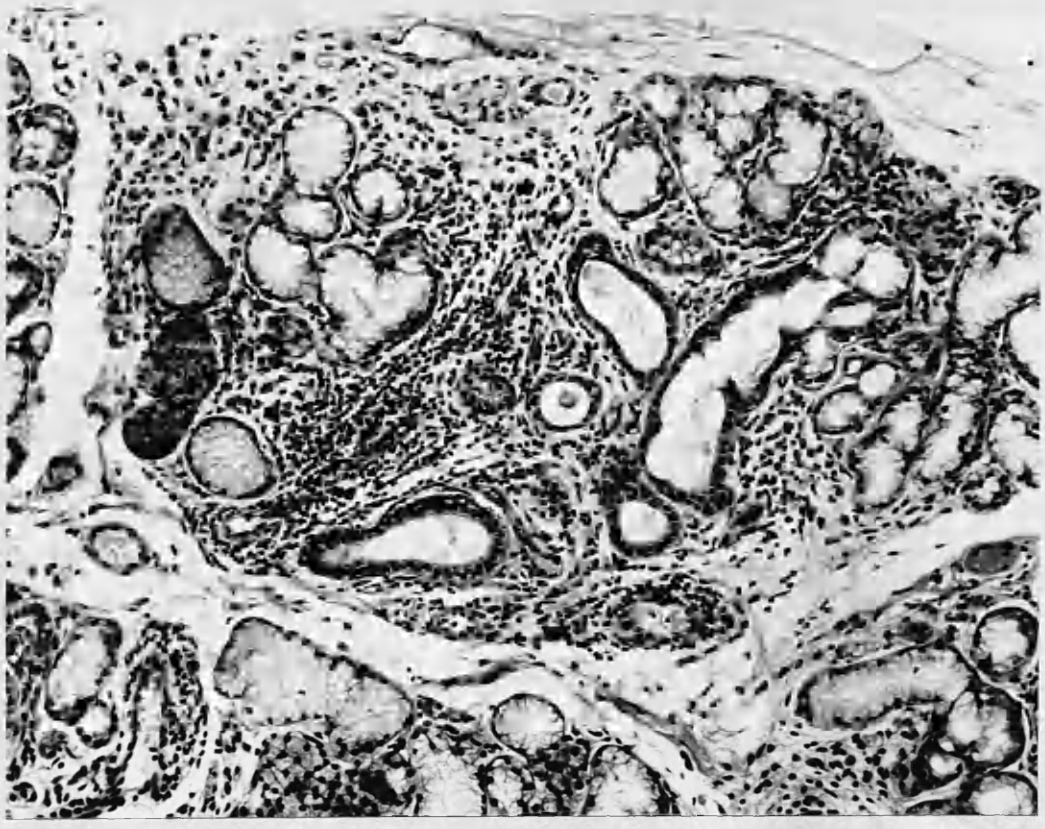
Massive lymphocytic replacement of labial salivary tissue (Grade 4) in a patient with Sjögren's syndrome (x 190).

FIGURE III, 24



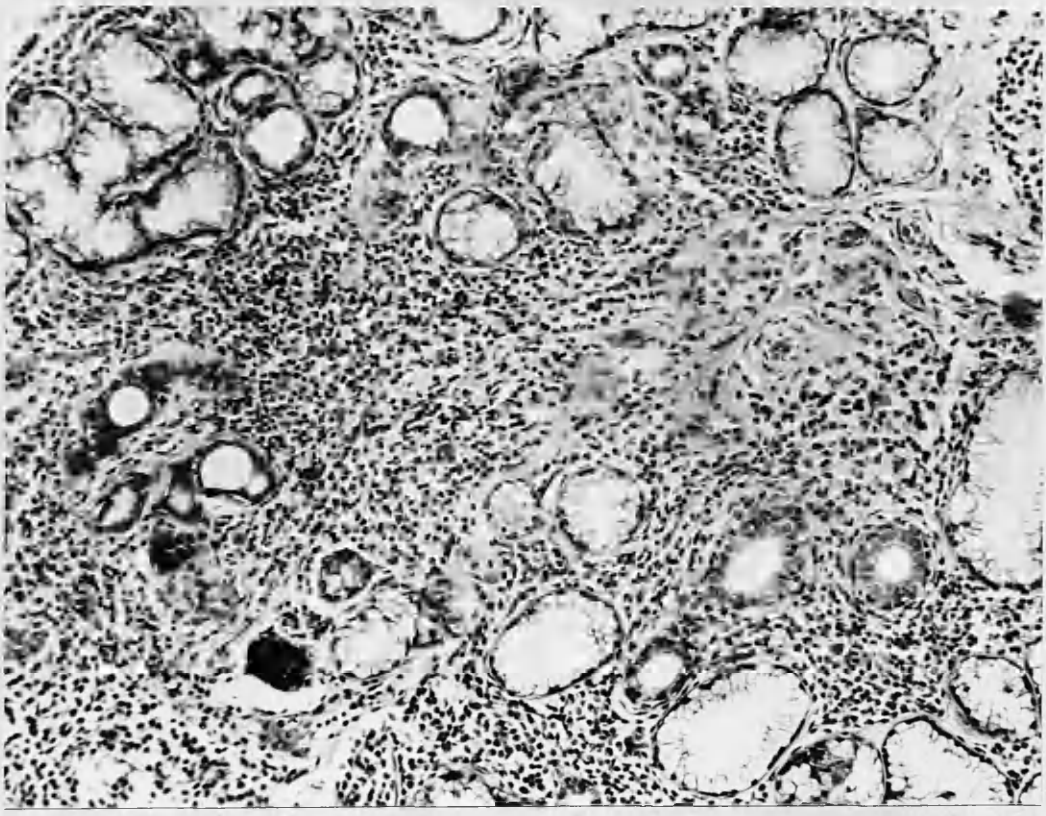
Marked lymphocytic replacement of acinar tissue with moderate interstitial fibrosis. (x 190)

FIGURE III, 25



Mild duct proliferation associated with marked lymphocytic infiltration which is both focal and diffuse in character (x 190).

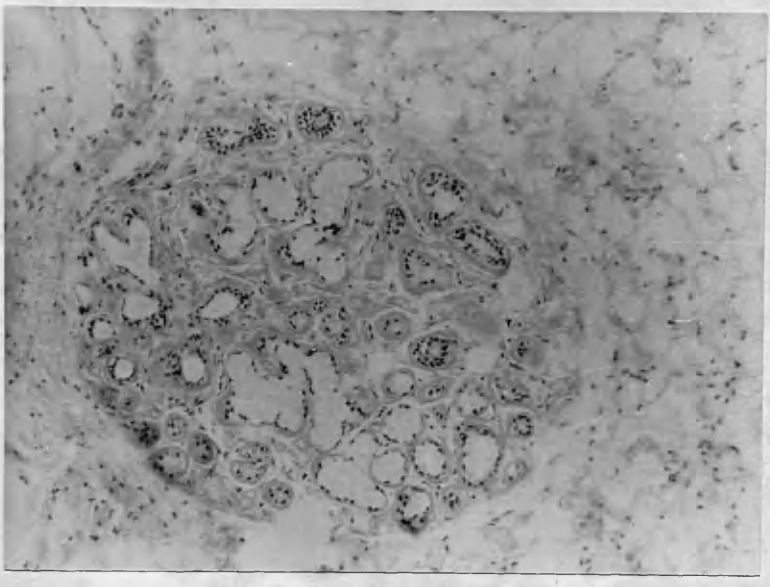
FIGURE III, 26



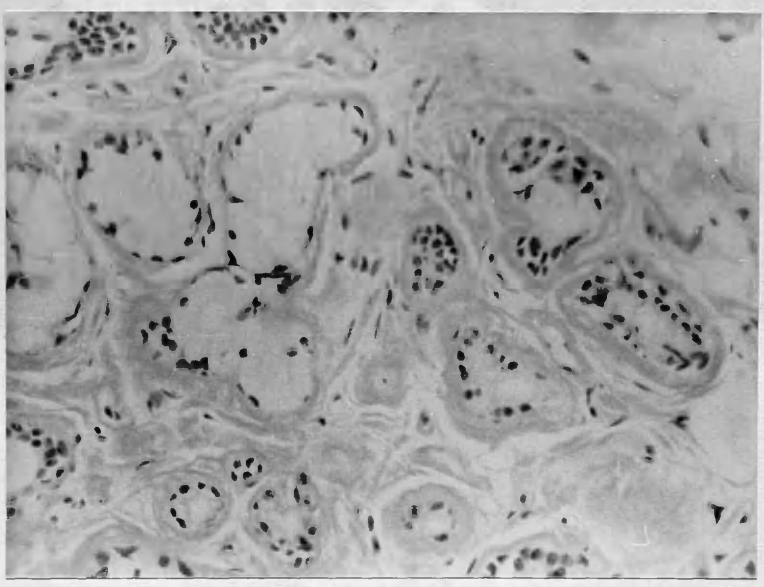
Interstitial fibrosis, acinar atrophy and associated lymphocytic replacement of labial salivary tissue (x 190).

FIGURE III,27

A

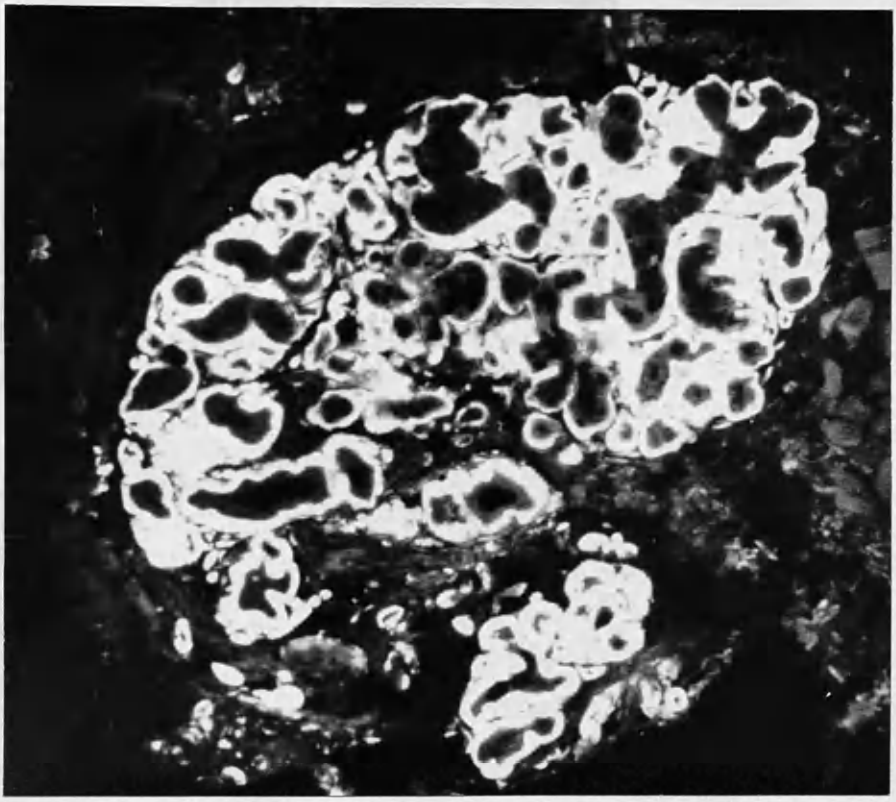


B



Periductal, perivascular and interstitial deposits of amyloid in the minor labial salivary glands of a male patient with severe rheumatoid arthritis.
Congo Red. A x 25 B x 75

FIGURE III, 28



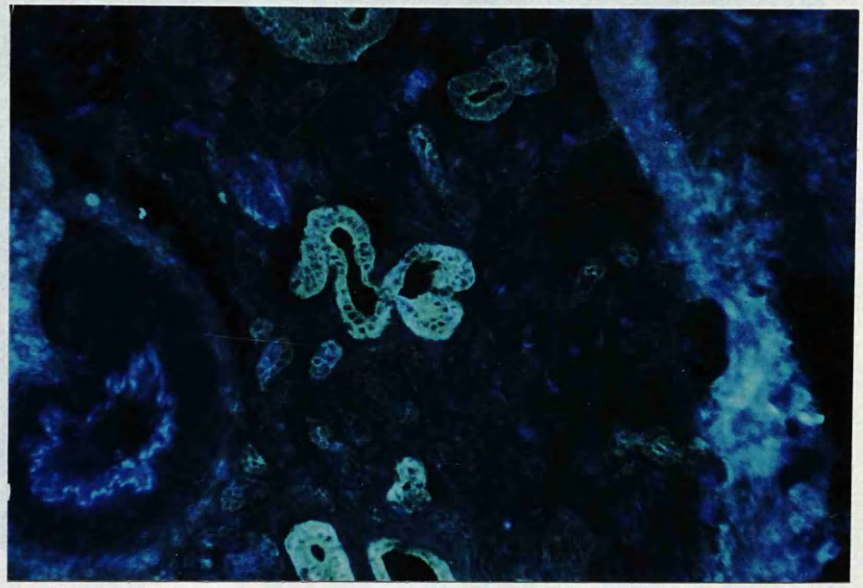
Minor salivary gland fluorescence
Thioflavine T and ultra violet light.

TABLE IV, 1

INCIDENCE OF SALIVARY DUCT AUTOANTIBODY IN THE CLINICAL GROUPS STUDIED

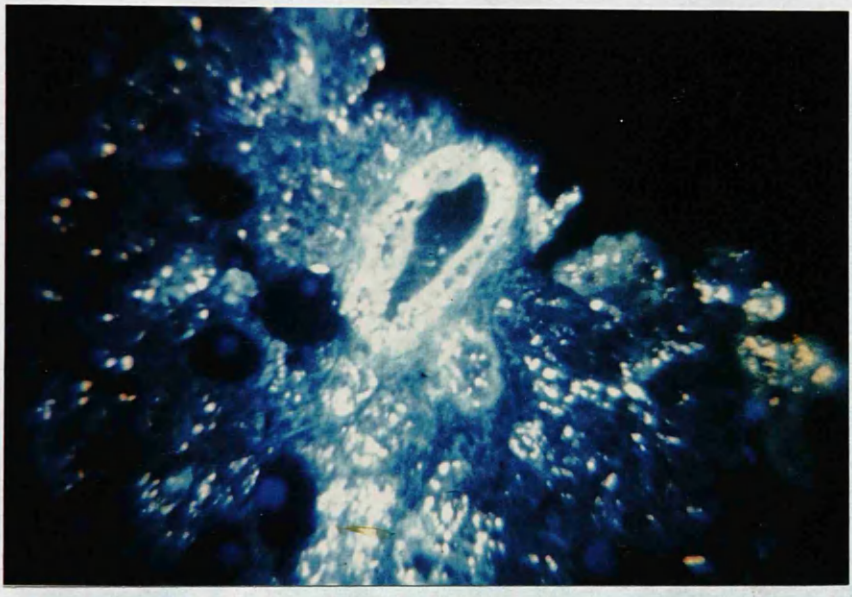
DIAGNOSIS	No.	Male	Female	No. S.D.A.+ve	%+ve
SICCA SYNDROME	31	2	29	6	19.4
SJÖGREN'S SYNDROME	53	9	44	36	67.9
RHEUMATOID ARTHRITIS	70	20	50	35	50.0
OSTEOARTHRITIS	17	3	14	0	-
ANKYLOSING SPONDYLITIS	17	14	3	1	5.9
REITER'S DISEASE	14	14	0	0	-
PSORIATIC ARTHRITIS	16	5	11	0	-
GOUT	3	1	2	0	-
SYSTEMIC LUPUS ERYTHEMATOSUS	9	0	9	0	-
SCLERODERMA	6	0	6	1	16.6
DERMATOMYOSITIS	1	0	1	0	-
STILL'S DISEASE	1	0	1	0	-
RAYNAUD'S PHENOMENON	1	1	0	0	-
BEHÇET'S SYNDROME	2	0	2	0	-

FIGURE IV, 1.



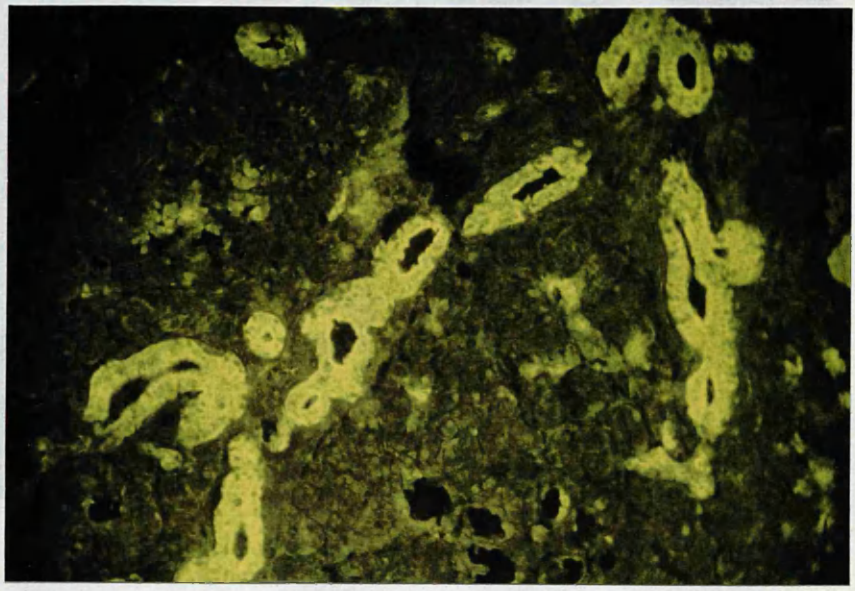
Indirect immunofluorescent staining of the cytoplasm of salivary duct epithelial cells. Serum from patient with Sjögren's syndrome

FIGURE IV, 2.



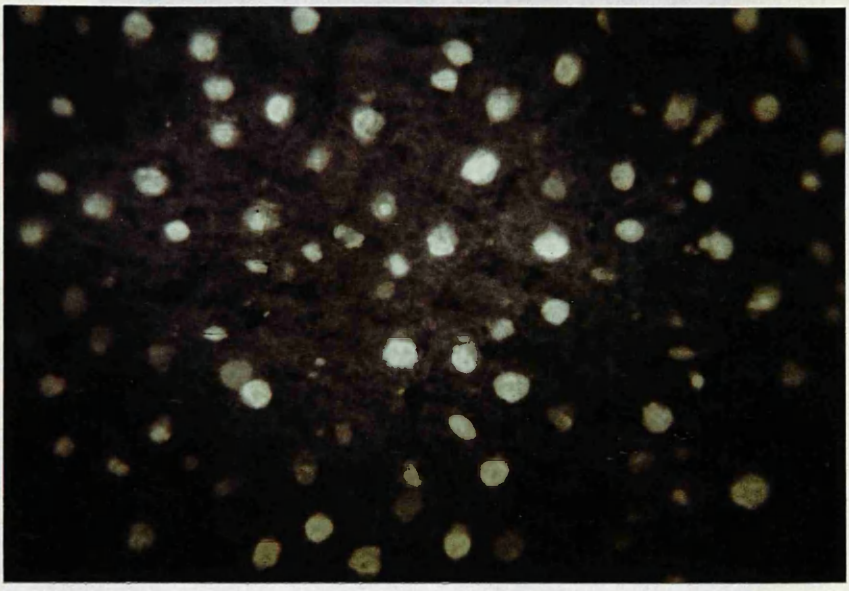
Indirect immunofluorescent staining of cytoplasm and salivary duct epithelial cells. Serum from patient with Sjögren's syndrome.

FIGURE IV, 3.



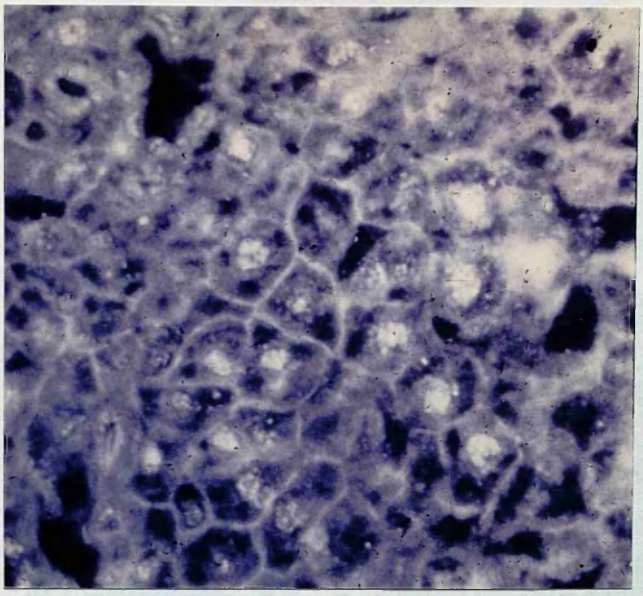
Indirect immunofluorescent staining of lining epithelium of salivary gland ducts. Serum from patients with Sjögren's syndrome.

FIGURE IV, 4.



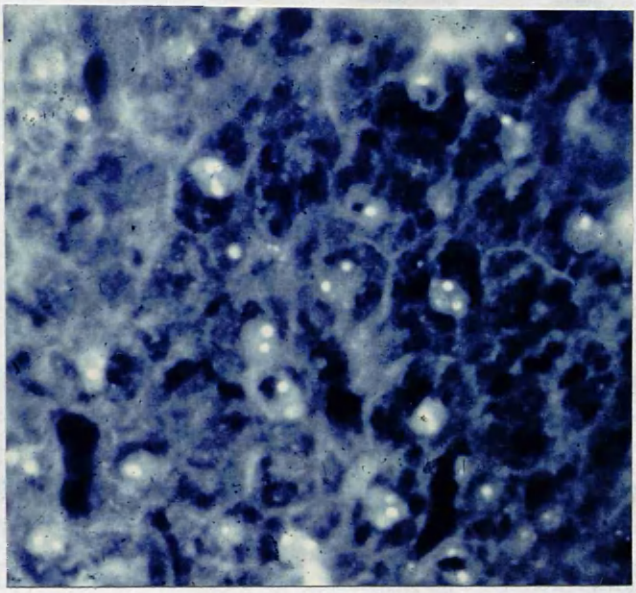
"Membranous" staining of rat liver nuclei
(x 650)

FIGURE IV, 5.



"Speckled' fluorescent staining of rat liver nuclei, using an indirect immunofluorescent, sandwich technique (x 775).

FIGURE IV, 6.



Indirect fluorescent staining of nucleoli of rat liver cells (x 790)

TABLE IV, 2

SEROLOGICAL FINDINGS IN CLINICAL GROUPS STUDIED

CLINICAL DIAGNOSIS	number tested	*ANF	%	**RF	%
SJÖGREN'S SYNDROME WITH R.A.	54	27	(50)	44	(81.5)
SICCA SYNDROME	36	9	(25)	10	(27.7)
RHEUMATOID ARTHRITIS	80	17	(21.3)	70	(87.5)
OSTEOARTHRITIS	24	1	(4.1)	0	(-)
SYSTEMIC LUPUS ERYTHEMATOSUS	10	9	(90)	3	(30)
REITER'S DISEASE	16	1	(6.3)	2	(12.5)
ANKYLOSING SPONDYLITIS	17	1	(5.9)	0	(-)
PSORIATIC ARTHRITIS	17	1	(5.9)	4	(23.5)
DERMATOMYOSITIS	1	1	(100)	1	(100)
SCLERODERMA	6	5	(83.3)	4	(66.6)
BEHÇET'S SYNDROME	2	0	(-)	0	(-)
GOUT	3	0	(-)	0	(-)

*ANF: anti nuclear factor

**RF : rheumatoid factor

TABLE IV, 3

SEROLOGICAL FINDINGS IN CLINICAL GROUPS STUDIED

CLINICAL DIAGNOSIS	number tested	*TTRC	%	**NSTP	%
SJOGREN'S SYNDROME WITH R.A.	54	9	(16.6)	13	(24.1)
SICCA SYNDROME	36	8	(22.2)	6	(16.6)
RHEUMATOID ARTHRITIS	80	12	(15)	3	(3.8)
OSTEOARTHRITIS	24	0	(-)	2	(8.3)
SYSTEMIC LUPUS ERYTHEMATOSUS	10	1	(10)	2	(20)
REITER'S DISEASE	16	1	(6.3)	0	(-)
ANKYLOSING SPONDYLITIS	17	1	(5.9)	2	(11.8)
PSORIATIC ARTHRITIS	17	0	(-)	0	(-)
DERMATOMYOSITIS	1	0	(-)	0	(-)
SCLERODERMA	6	0	(-)	0	(-)
BEHCET'S SYNDROME	2	0	(-)	0	(-)
GOUT	3	0	(-)	0	(-)

* TTRC: antithyroglobulin

** NSTP: non-specific tissue precipitin

TABLE IV, 4

	PATTERN OF NUCLEAR FLUORESCENCE			
	HOMOGENOUS	SPECKLED	NUCLEOLAR	MEMBRANOUS
SJÖGREN'S SYNDROME	24	2	1	0
SICCA SYNDROME	9	0	0	0
SYSTEMIC LUPUS ERYTHEMATOSUS	6	1	1	1

TABLE IV, 5 LABIAL LYMPHOCYTIC SIALADENITIS AND SALIVARY DUCT ANTIBODY IN THE CLINICAL GROUPS STUDIED

DIAGNOSIS	Number of patients studied	sex		age (yrs)		With lymphocytic sialadenitis		With sal. duct antibody	
		Male	Female	Mean	Range	No.	per cent	No.	per cent
SICCA SYNDROME	10	2	8	55.9	27-66	6	60.0	1	10.0
RHEUMATOID ARTHRITIS & SJÖGREN'S SYNDROME	27	6	21	60.3	48-78	17	63.0	19	70.4
RHEUMATOID ARTHRITIS ALONE	47	13	34	53.2	20-73	13	27.7	21	44.6
PSORIATIC ARTHRITIS	11	4	7	49.3	19-88	2	18.2	-	-
ANKYLOSING SPONDYLITIS	10	5	5	47.8	23-73	2	20.0	1	10.0
REITER'S SYNDROME	6	6	-	34.5	18-52	-	-	-	-
STILL'S DISEASE	1	-	1	29.0	-	-	-	-	-
SYSTEMIC LUPUS ERYTHEMATOSUS	1	-	1	21.0	-	-	-	-	-
PROGRESSIVE SYSTEMIC SCLEROSIS	4	-	4	32.0	16-40	1	20.0	-	-
GOUT	2	1	1	61.0	60-62	-	-	-	-
OSTEOARTHRITIS	11	3	8	64.9	53-74	1	9.1	-	-

TABLE IV, 6

LABORATORY AND IMMUNOLOGICAL FEATURES OF PATIENTS WITH SICCA SYNDROME

		Present	Absent
FOCAL LYMPHOCYTIC SIALADENITIS		6	4
Haemoglobin (G.%)	Mean)	12.9	13.4
	Range)	12.0-13.8	11.3-13.7
White cell count (per c.mm.)	Mean)	5,850	6,150
	Range)	3,300- 7,800	5,00-9,700
Erythrocyte sedimentation rate (mm./1st hr-Westergren)	Mean)	24	15
	Range)	14-38	3-32
Serum Globulin (G.%)	Mean)	3.3	3.5
	Range)	2.1-4.2	2.7-4.1
Salivary duct antibody		1(15%)	0
Rheumatoid Factor		5(83%)	3(75%)
Antinuclear Factor		2(33%)	1(25%)
Non-tissue specific precipitating autoantibody		-	-
Thyroglobulin autoantibody		1(13%)	2(50%)
Thyroid microsomal autoantibody		1 (17%)	1(25%)
Gastric Parietal Cell autoantibody		-	-

TABLE IV, 7

LABORATORY AND IMMUNOLOGICAL FEATURES OF PATIENTS WITH SJÖGREN'S SYNDROME AND RHEUMATOID ARTHRITIS

		Present	Absent
FOCAL LYMPHOCYTIC SIALADENITIS		17	10
Haemoglobin (G.%)	Mean)	12.2	12.9
	Range)	11.9-15.4	5.0-16.1
White cell count	Mean)	6,946	6,297
(per c.mm.)	Range)	3,400-14,600	1,260-10,500
Erythrocyte sedimentation rate (mm./1st hr.-Westergren	Mean)	42±26.9	65± 42.9
	Range)	12-121	8-125
Serum Globulin (G.%)	Mean)	3.6	3.8
	Range)	1.8-4.5	2.9-5.2
Salivary duct antibody		12(61%)	7(70%)
Rheumatoid Factor		16 (94%)	7(70%)
Antinuclear Factor		8 (47%)	3(30%)
Non-tissue specific precipitating autoantibody		4 (23.5%)	-
Thyroglobulin autoantibody		3 (17.6%)	1(10%)
Thyroid microsomal autoantibody		5 (29.4%)	3(30%)
Gastric parietal cell autoantibody		4 (23.5%)	-

TABLE IV, 8

LABORATORY AND IMMUNOLOGICAL FEATURES OF PATIENTS WITH RHEUMATOID ARTHRITIS

		Present	Absent	
FOCAL LYMPHOCYTIC SIALADENITIS		13	34	
Haemoglobin (G.%)	Mean)	11.3±1.7	13.0±1.65	[1]
	Range)	8.7-14.4	7.1-15.3	
White cell count (per c.mm.)	Mean)	6,108±2,471	8,201±3,023	[2]
	Range)	2,000-11,600	5,000-15,900	
Erythrocyte sedimentation rate (mm./1st hr. Westergren)	Mean)	78±19.3	41±26.1	[3]
	Range)	36-107	5-106	
Serum Globulin G.%)	Mean)	4.0±0.65	3.6±0.68	
	Range)	3.2-5.5	2.6-5.2	
Salivary duct antibody		5(38.5%)	16(47.1%)	
Rheumatoid Factor		13(100%)	29(85.3%)	
Antinuclear Factor		7(53.8%)	6(17.6%)	[4]
Non-tissue specific precipitating autoantibody		3(23.1%)	2(5.9%)	
Thyroglobulin autoantibody		5(38.5%)	6(17.6%)	
Thyroid microsomal autoantibody		4(30.8%)	7(20.6%)	
Gastric parietal cell autoantibody		2(15.4%)	9(26.5%)	

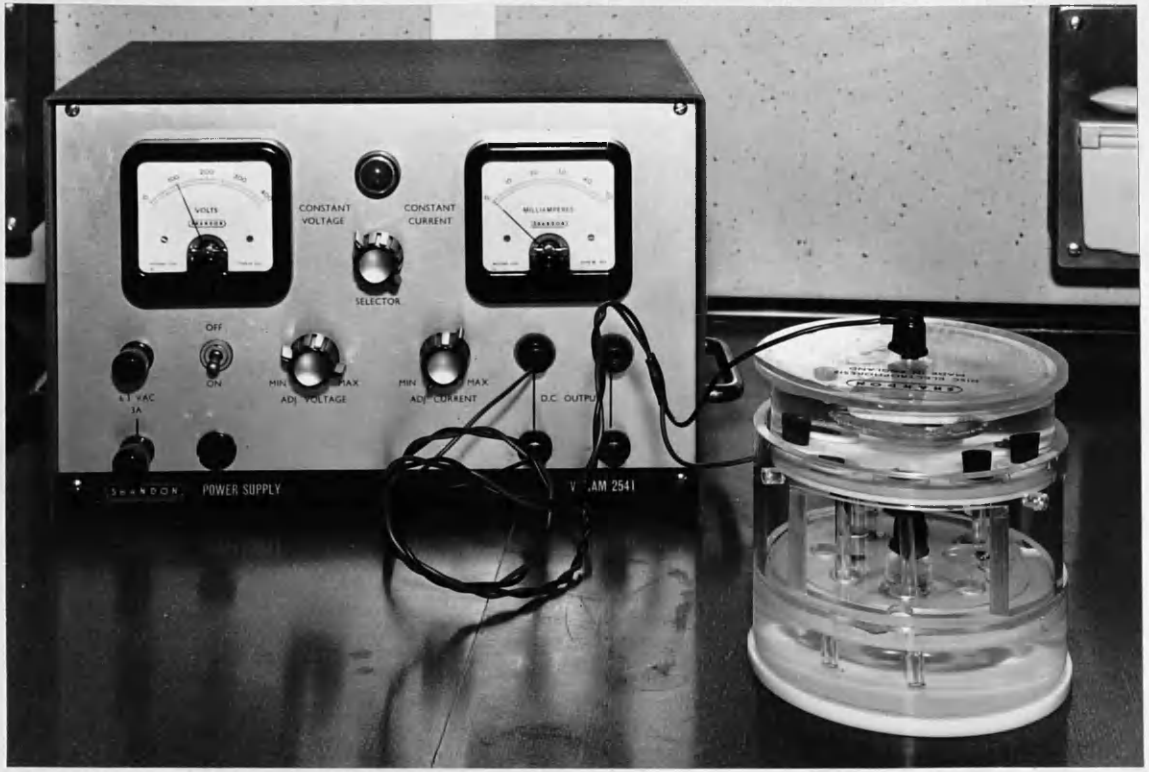
[1] $P < 0.001$

[2] $P < 0.02$

[3] $P < 0.001$

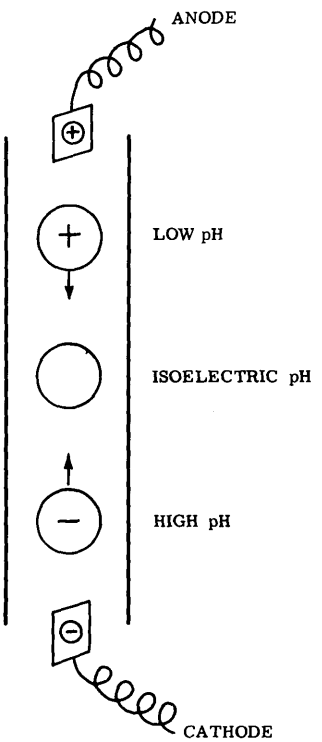
[4] $P < 0.02$

FIGURE IV, 7.



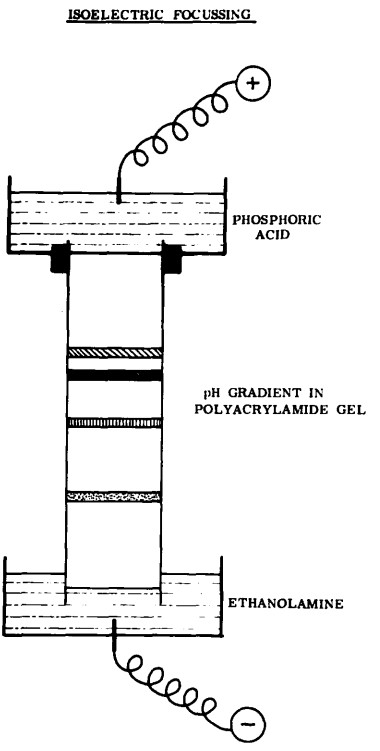
Shandon Disc Electrophoresis apparatus used for iso-electric focussing, together with a Shandon Vokam-power supply.

FIGURE IV, 8.



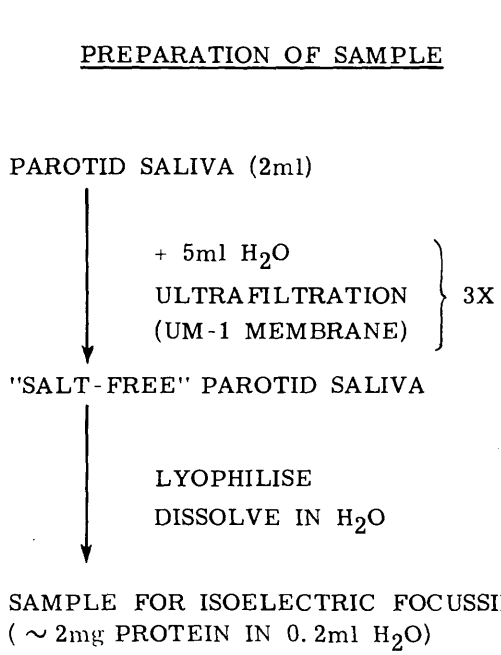
Principle of iso-electric focussing.
Circles represent protein molecules
in a pH gradient.

FIGURE IV, 9.



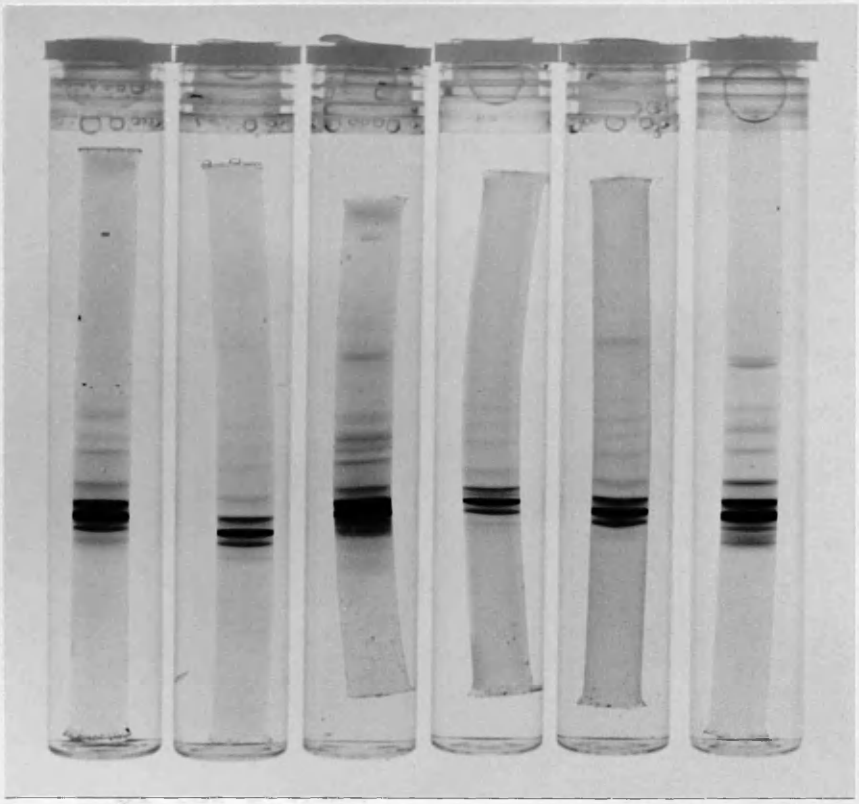
Diagrammatic representation of the apparatus used in iso-electric focussing.

FIGURE IV, 10.



Preparation of sample for analysis by iso-electric focussing.

FIGURE IV, 11.

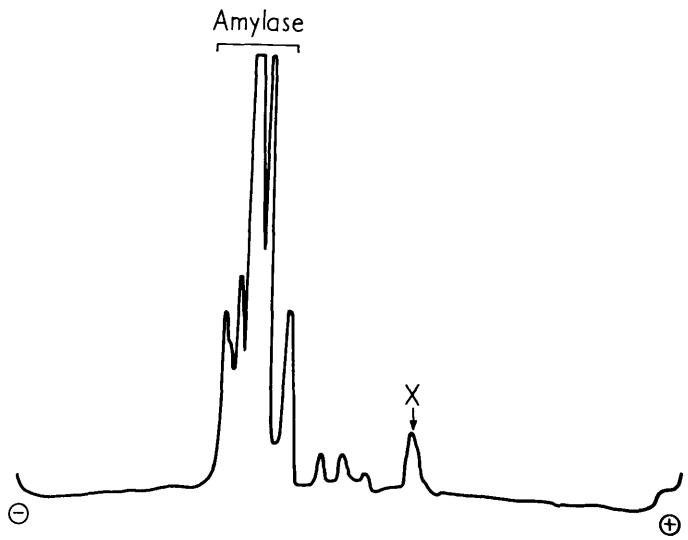


Band patterns in control subjects

A = anode B = cathode

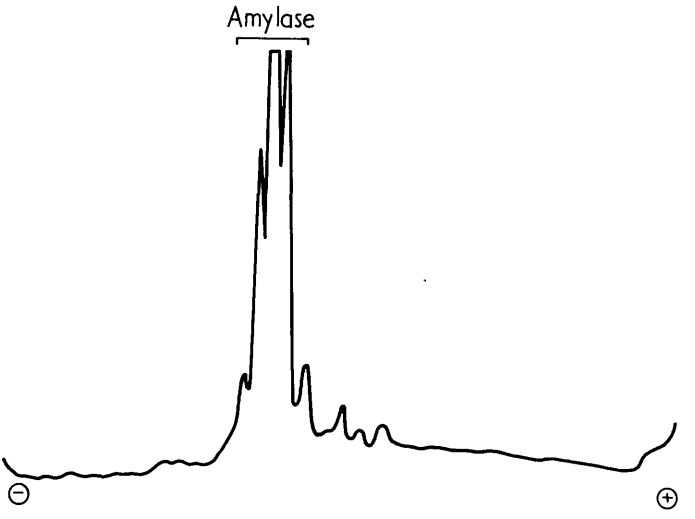
x - x-band
xx - intermediate band
xxx - iso-enzymes of amylase.

FIGURE IV, 12



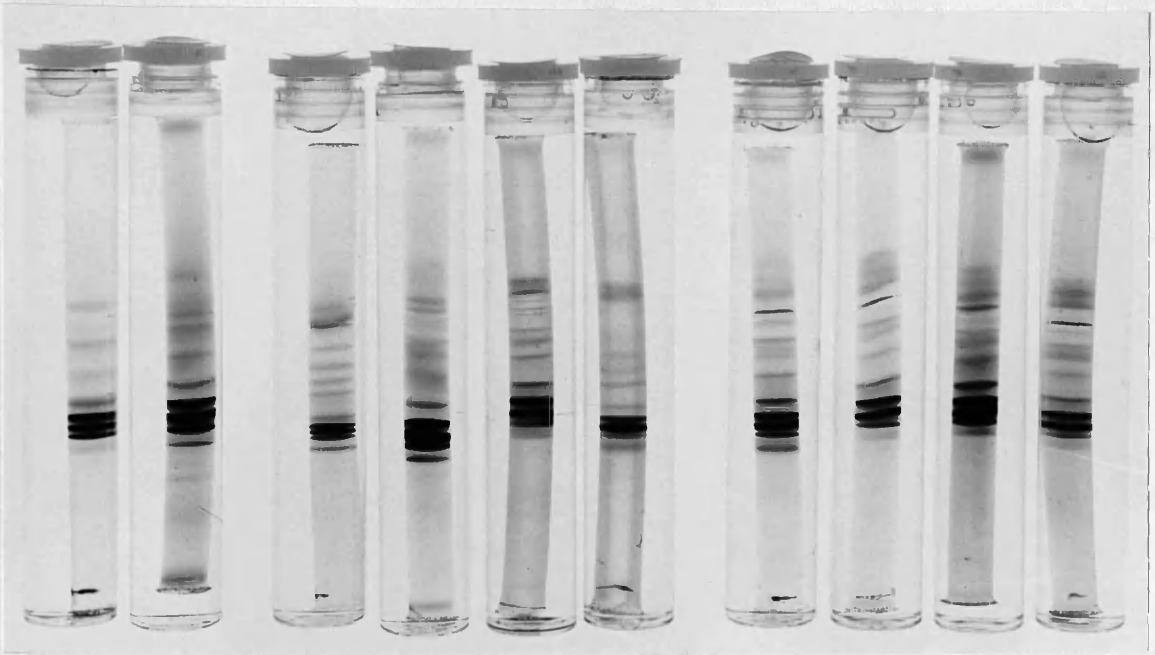
Densitometric tracing from normal subject showing the prominent peak of the x-band.

FIGURE IV, 13.



Densitometric tracing of gel from normal subject.

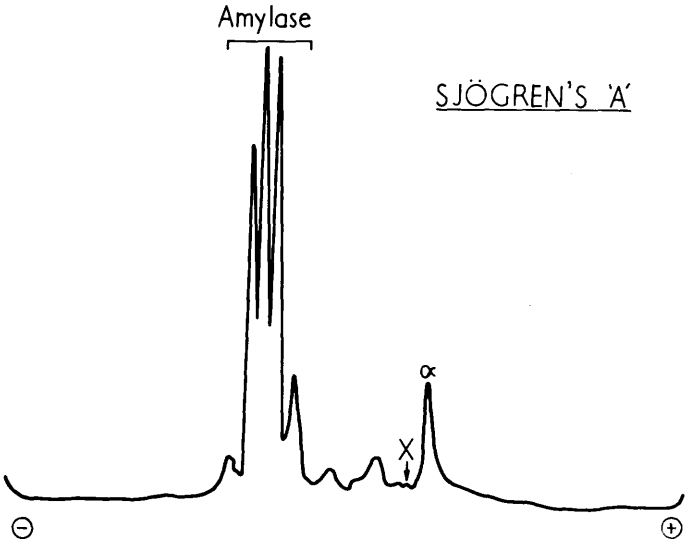
FIGURE IV, 14.



(Group A) (Group B) (Group C)

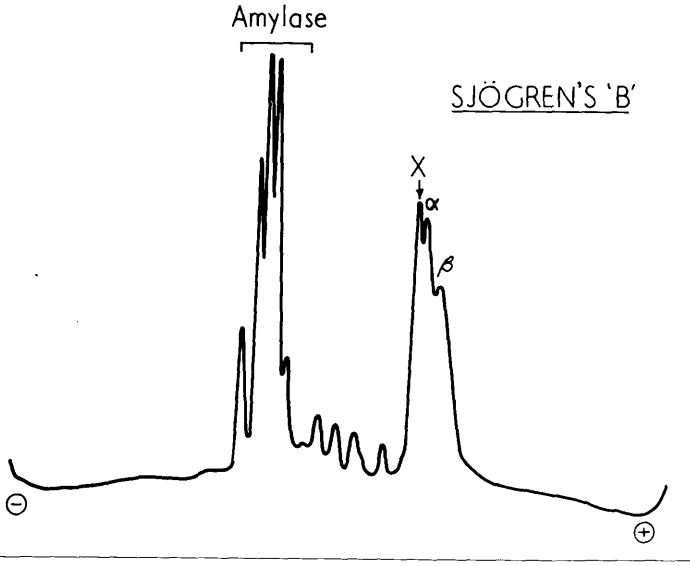
Band patterns from patients with Sjögren's syndrome, complicated by rheumatoid arthritis. Gels from groups 'A', 'B' and 'C' are shown.

FIGURE IV, 15.



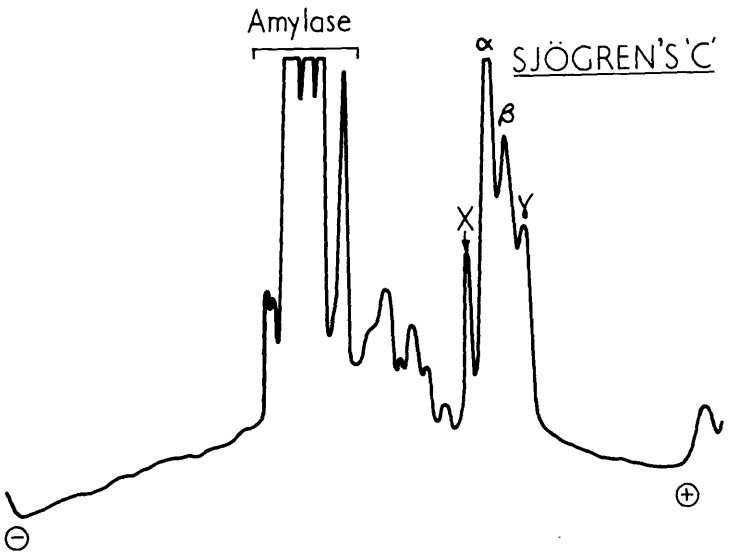
Densitometric tracing of gel from a patient with Sjögren's syndrome in Group 'A'.

FIGURE IV, 16.



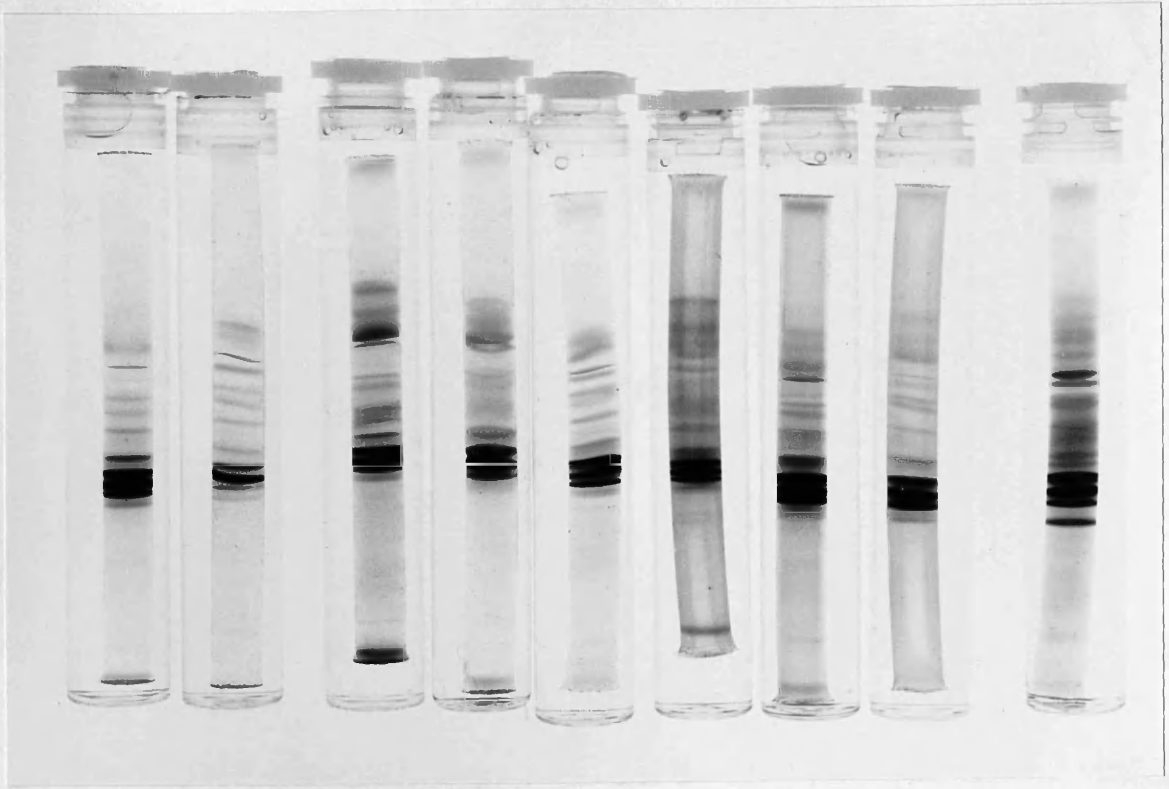
Densitometric tracing of gel from a patient with Sjögren's syndrome in Group 'B'.

FIGURE IV, 17.



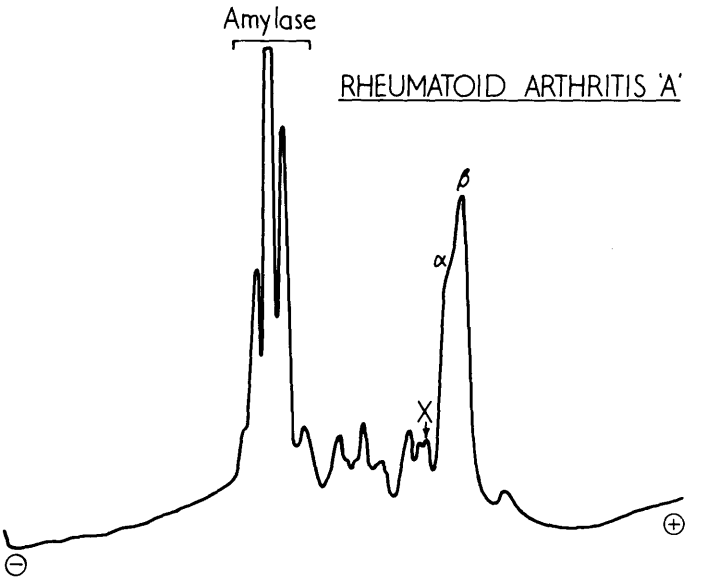
Densitometric tracing of gel from a patient with Sjögren's syndrome in Group 'C'.

FIGURE IV, 18.



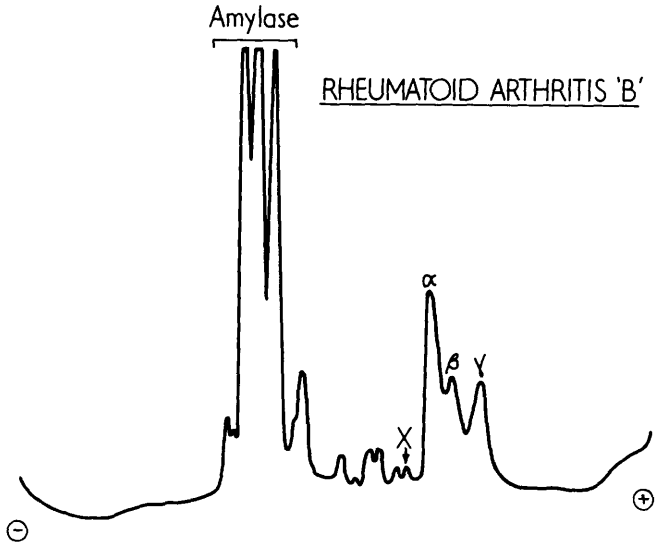
(Group A) (Group B)
Band patterns from patients with rheumatoid arthritis alone. Samples from Groups 'A' and 'B' are shown.

FIGURE IV, 19.



Densitometric tracing from Group 'A' patient with rheumatoid arthritis. Alpha and beta peaks are noted beyond the x-band peak.

FIGURE IV, 20.



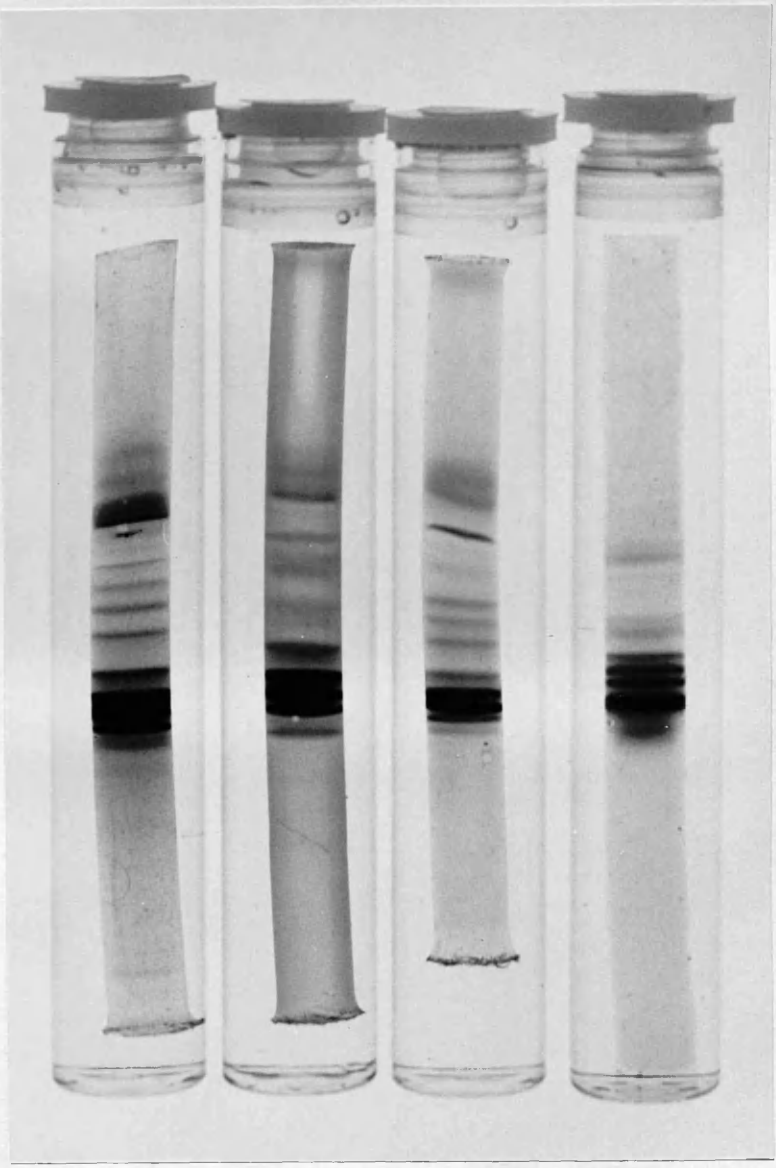
Densitometric tracing from Group 'B' patient with rheumatoid arthritis. Alpha, beta and gamma peaks are noted beyond the x-band peak.

FIGURE IV, 21.

Band pattern from four patients with the sicca syndrome.

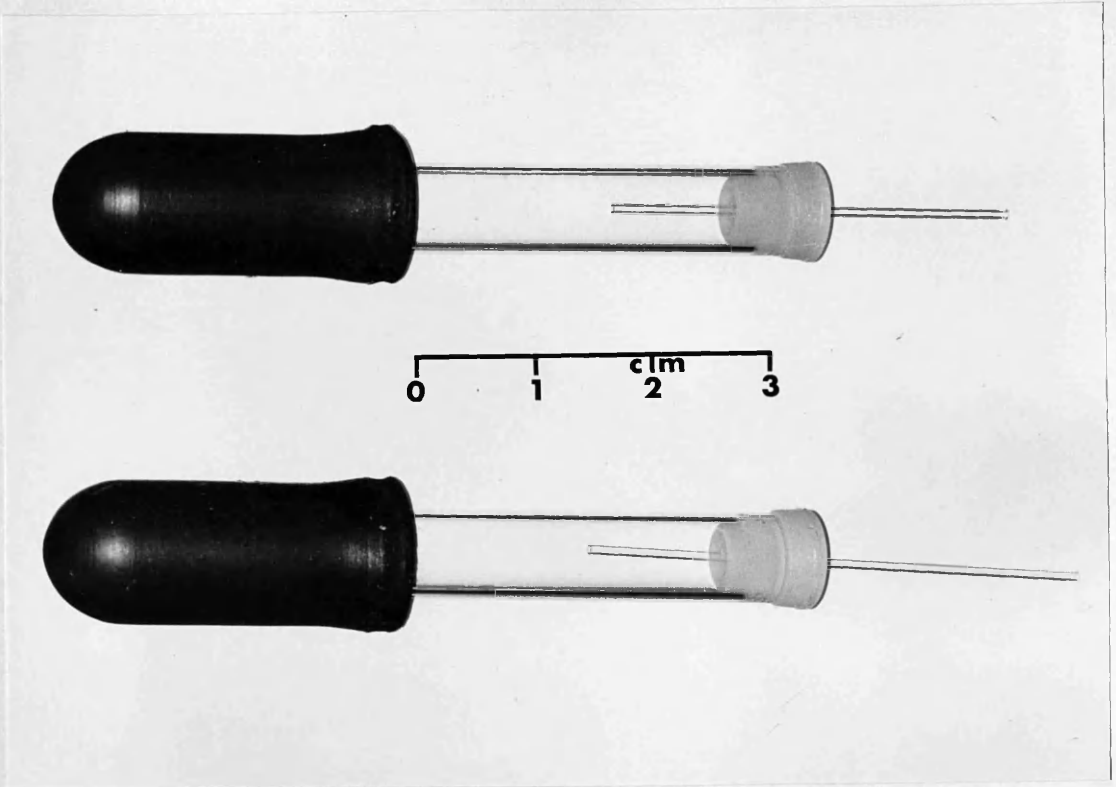
FIGURE IV, 22.

Note abnormal band pattern beyond the x-band region.



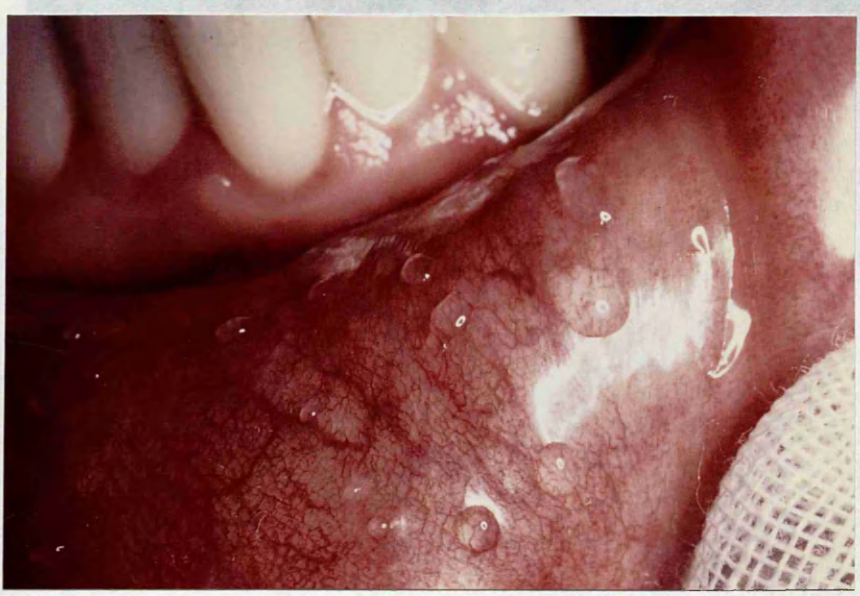
Band patterns from four patients with osteoarthritis.

FIGURE IV, 23.



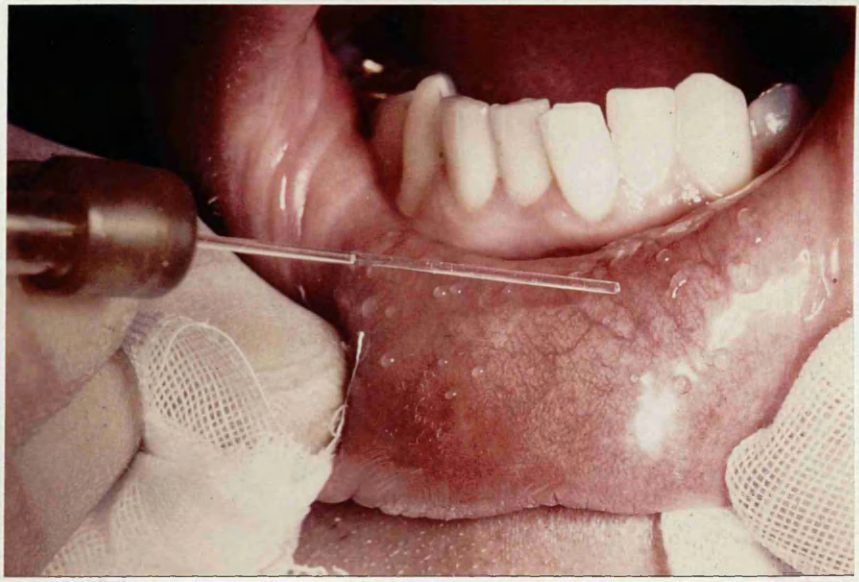
Collection device for minor gland saliva with 5 and 10 lambda capillary tubes ('Microcaps' Drummond Scientific Co., U.S.A.)

FIGURE IV, 24.



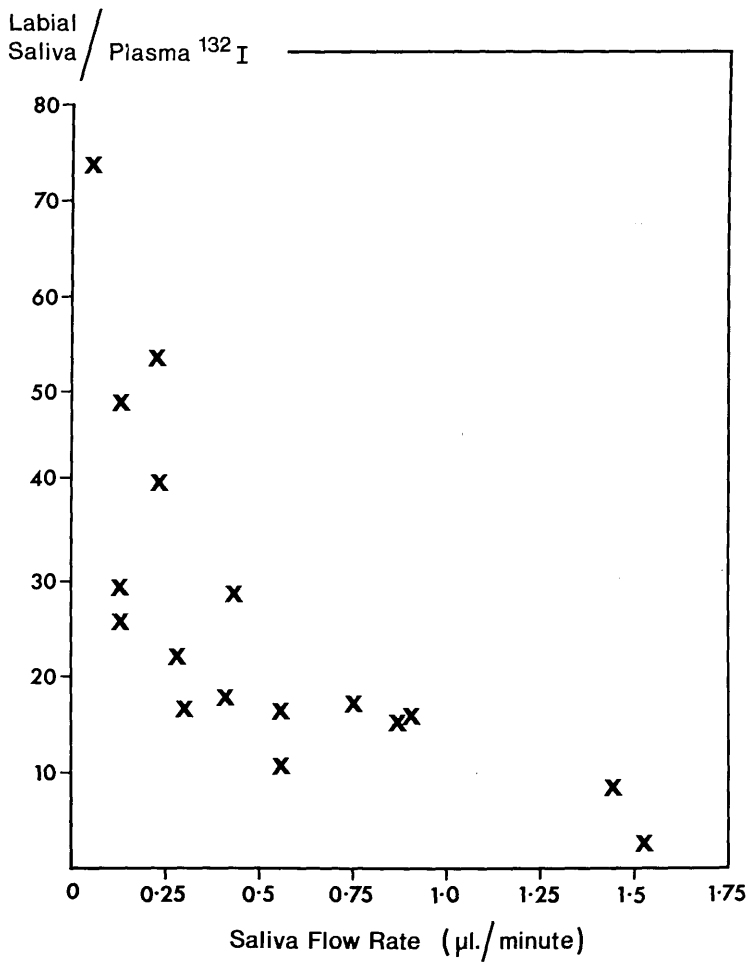
Beads of minor gland saliva on lower lip, in response to a drop of lemon juice applied to dorsum of tongue.

FIGURE IV, 25.



Method of collecting minor labial gland saliva, using capillary tubes.

FIGURE IV, 26.



Labial saliva/plasma ^{132}I-iodide ratio plotted against labial flow rate.

TABLE IV, 9

CONCENTRATION OF ^{132}I-IODIDE IN LABIAL SALIVARY GLANDS AND SALIVA AND IN PAROTID SALIVA

SUBJECT	LABIAL			PAROTID
	Flow rate µl/min	Saliva/ plasma (132_I)	Gland/ plasma (132_I)	Saliva/ plasma (132_I)
1	1.44	8.5	0.66	-
2	0.13	25.6	1.70	10.2
3	0.06	73.9	0.88	19.6
4	0.13	29.2	0.74	13.5
5	0.43	28.8	0.74	13.0
6	0.75	17.0	0.47	17.8
7	0.14	49.1	3.08	24.0
8	0.41	17.9	1.08	11.3
Mean	0.44	31.3	1.26	15.6
S.E.	0.17	7.4	0.31	1.9

TABLE IV, 10

EFFECT OF POTASSIUM PERCHLORATE ON LABIAL AND PAROTID SALIVA/IODIDE CONCENTRATION

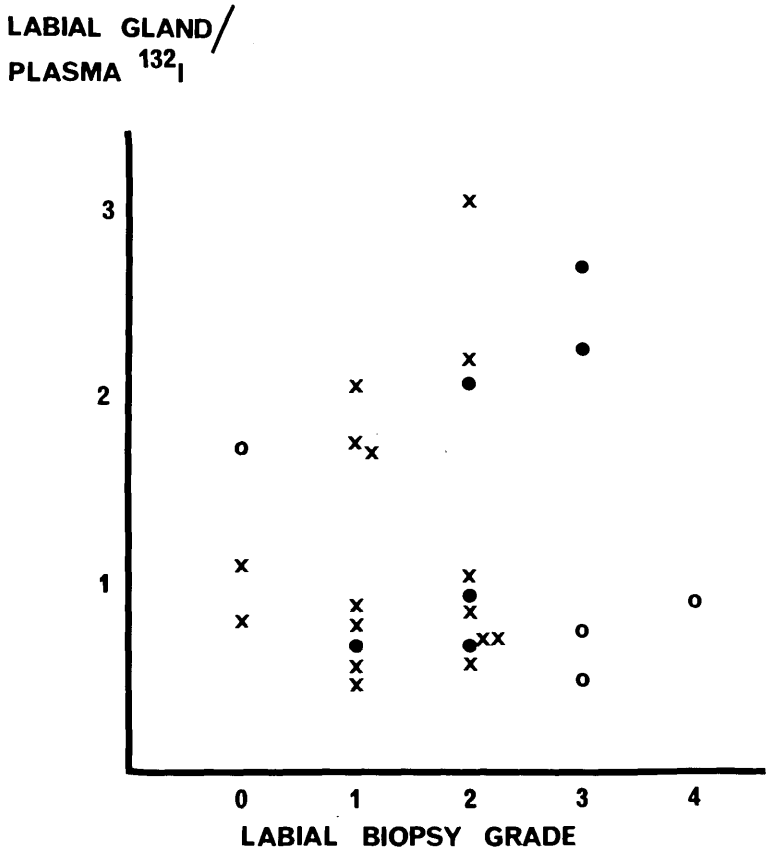
	SUBJECT	Before Perchlorate		After Perchlorate		per cent fall
		Flow rate μ l/min	S/P (132_I)	Flow rate μ l/min	S/P (132_I)	
LABIAL	1	0.23	53.6	0.11	40.2	25
	2	0.31	16.6	0.58	5.2	69
	3	1.20	5.6	0.59	2.9	48
	MEAN	0.58	25.2	0.43	16.1	47
		ml/min				
PAROTID	1	1.5	18.5		9.9	47
	2	1.5	9.2		2.8	69
	3	1.5	11.1		3.9	65
	MEAN	1.5	12.9		5.6	60

TABLE IV, 11

GLAND/PLASMA ^{132}I RATIOS AND LABIAL BIOPSY GRADES IN 12 PATIENTS WITH CONNECTIVE TISSUE DISEASE.

CLINICAL DIAGNOSIS	Patient number	Age/ sex	Gland/ plasma ^{132}I	Biopsy Grade
RHEUMATOID ARTHRITIS	1	50/M	0.68	1
	2	70/F	2.69	3
	3	58/F	0.95	2
	4	49/F	2.08	2
	5	37/M	0.66	2
	6	47/F	2.25	3
		MEAN -	1.55	
		S.E. -	0.36	
SJÖGREN'S SYNDROME	7	59/F	0.49	3
	8	61/F	0.92	4
	9	51/F	1.76	0
	10	53/F	0.78	3
		MEAN -	0.99	
		S.E. -	0.27	
PROGRESSIVE SYSTEMIC SCLEROSIS	11	48/F	1.07	0
REITER'S DISEASE	12	40/M	0.66	2

FIGURE IV, 27.



Labial gland/plasma ^{132}I-iodide ratio plotted against histopathologic grade.

O = Sjögren's syndrome

● = Rheumatoid arthritis

X = Controls

TABLE V, 1

SALIVARY GLAND FUNCTION TESTS IN SYMPTOMATIC XEROSTOMIA

NO.	AGE/ SEX	PAROTID FLOW RATE ml/min	SIALOGRAM N= normal ND = not done	GRADE LABIAL BIOPSY	SALIVARY ANTIBODY - =negative	AETIOLOGICAL FACTOR
X1	73F	R 1.42 L 1.10	N	0	-	CHLOPROMAZINE HCl
X2	45F	R 2.25 L 1.88	N	1	-	SIALOLITHIASIS
X3	62F	R 1.32 L 0.92	N	ND	-	ANXIETY
X4	35F	R 1.15 L 1.10	N	0	-	PROMETHAZINE HYDROCHLORIDE
X5	73F	R 0.70 L 0.72	N	2	-	ANXIETY MYXOEDEMA
X6	55F	R 1.50 L 1.04	N	1	-	ANXIETY
X7	54F	R 0.90 L 1.05	N	1	-	IRON DEFICIENCY ANAEMIA
X8	54F	R 2.50 L 2.47	N	ND	-	SIALADENITIS
X9	72F	R 0.65 L 0.75	ND	0	-	AMITRIPTYLINE HCl
X10	59F	R 0.84 L 0.89	ND	0	-	AMITRIPTYLINE HCl

TABLE V,2 SALIVARY GLAND FUNCTION TESTS IN SYMPTOMATIC XEROSTOMIA

NO.	AGE/ SEX	PAROTID FLOW RATE ml/min	SIALOGRAM N = normal ND= not done	GRADE LABIAL BIOPSY	SALIVARY ANTIBODY - =negative	AETIOLOGICAL FACTOR
X11	74F	R 0.76 L 0.96	N	0	-	MECAMYLAMINE HCl
X12	60M	R 1.35 L 1.25	N	1	-	ANXIETY
X13	57F	R 0.81 L -	N	0	-	DIABETES MELLITUS
X14	77F	R 0.66 L 0.54	ND	1	-	PERNICIOUS ANAEMIA
X15	56F	R 1.27 L 1.30	N	1	+	CHLORPROMAZINE HCl
X16	67M	R 1.00 L 0.96	N	1	-	BELLADONNA
X17	64F	R 0.82 L 0.75	ND	ND	-	ANXIETY
X18	58M	R 1.05 L 0.19	calculus	2	-	PAROTID TUMOUR
X19	52F	R 0.72 L 0.70	ND	ND	-	AMITRIPTYLINE HCl
X20	75F	R 0.98 L 1.05	ND	ND	-	MEGAKARYOCYTIC MELOSIS
X21	35F	R 0.86 L 1.24	N	2	-	CHLORPROMAZINE HCl

TABLE V, 3

SALIVARY FUNCTION TESTS IN FIVE PATIENTS WITH MIKULICZ'S SYNDROME

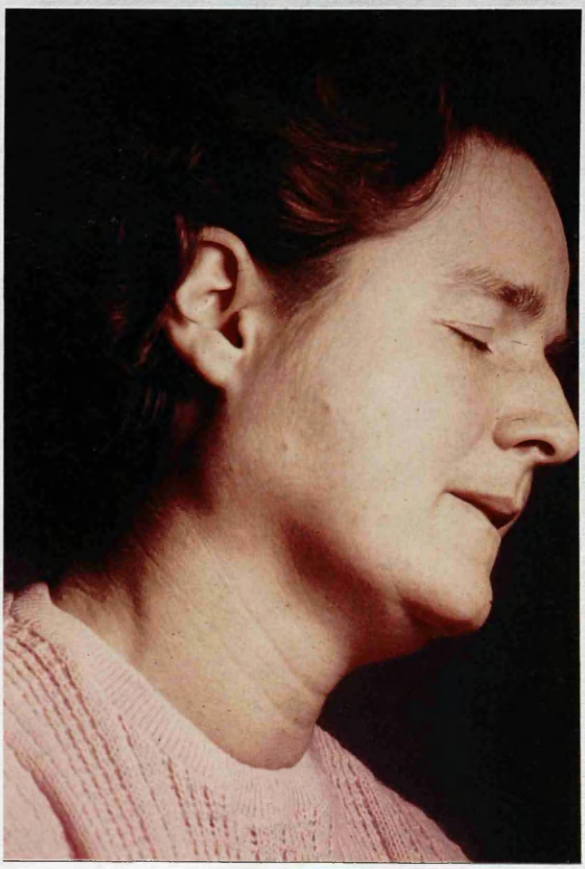
PATIENT NUMBER	AGE/ SEX	STIMULATED PAROTID FLOW RATE (ml/min)	SIALOGRAM	LABIAL BIOPSY GRADE	SCAN MEAN TOTAL %UPTAKE ^{99m}Tc (8 min.)
1	54F	R 0.0 L 0.0	CAVITARY	4	0.19
2	32F	R 0.70 L 0.80	CAVITARY	4	ND
3	53F	R 0.45 L 0.30	GLOBULAR	4	0.22
4	51M	R 0.70 L 0.80	ND	4	0.20
5	62F	R 2.00 L 0.78	ATROPHY	2	0.39

TABLE V, 4

SEROLOGICAL FINDINGS IN FIVE PATIENTS WITH MIKULICZ'S SYNDROME

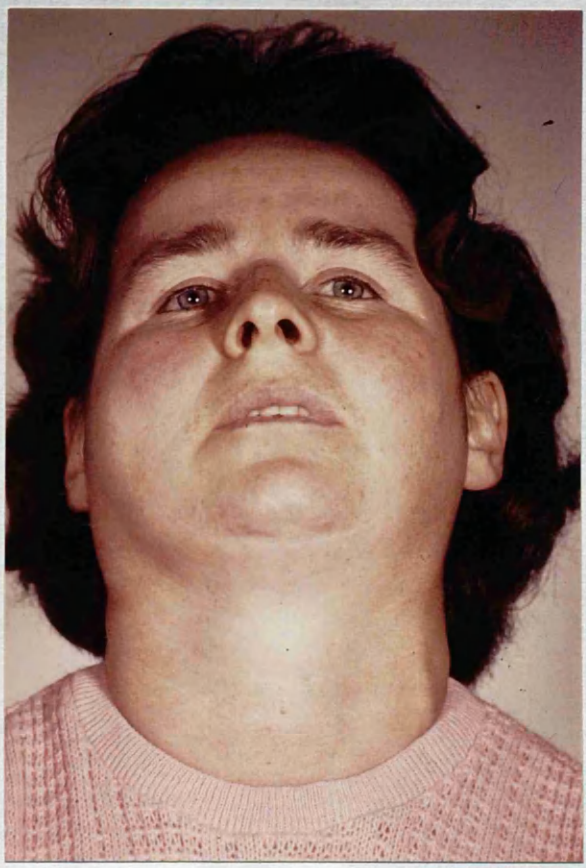
PATIENT NUMBER	SALIVARY DUCT ANTIBODY	ANTI-NUCLEAR FACTOR	RHEUMATOID FACTOR	ANTI-THYROGLOBULIN	NON-SPECIFIC TISSUE PRECIPITIN
1	-	-	1/256	-	-
2	-	-	1/512	-	-
3	+	+	1/128	-	+
4	-	-	-	-	-
5	-	-	-	-	-

FIGURE V, 1



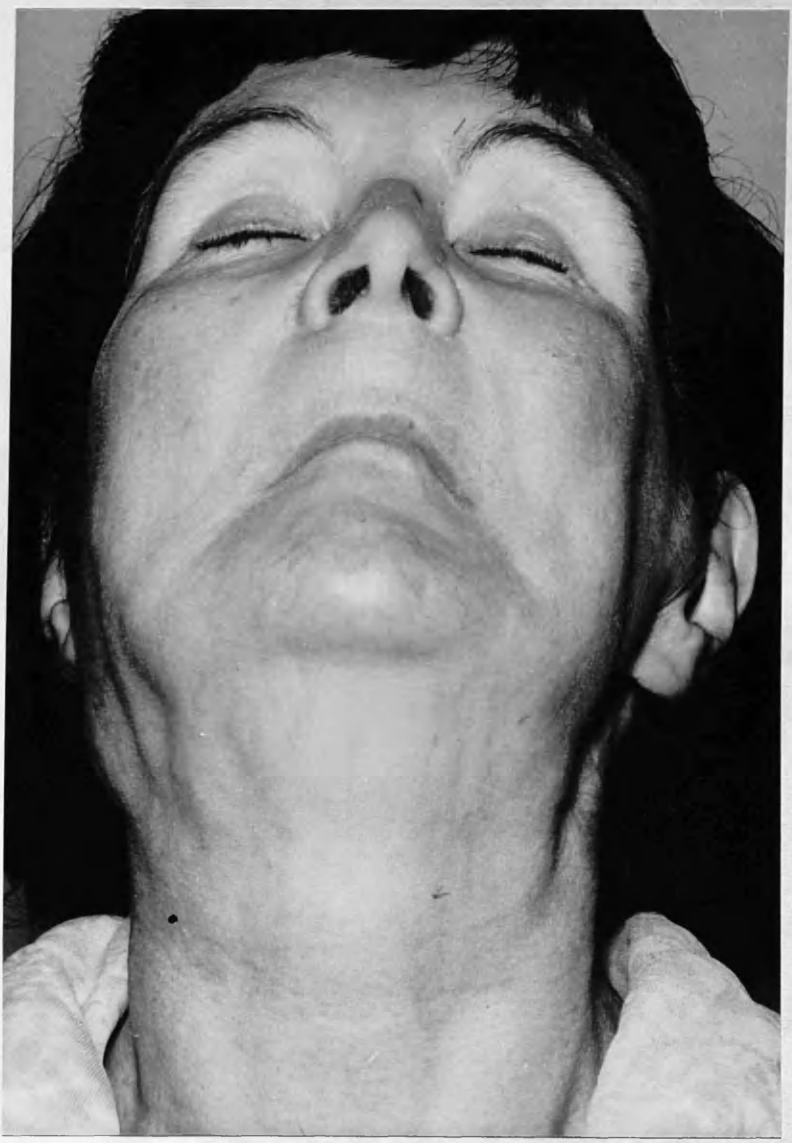
Female patient with 3 year history
of bilateral parotid gland enlargement.

FIGURE V, 2



Bilateral swelling of parotid glands
(Patient shown in Figure V, 1)

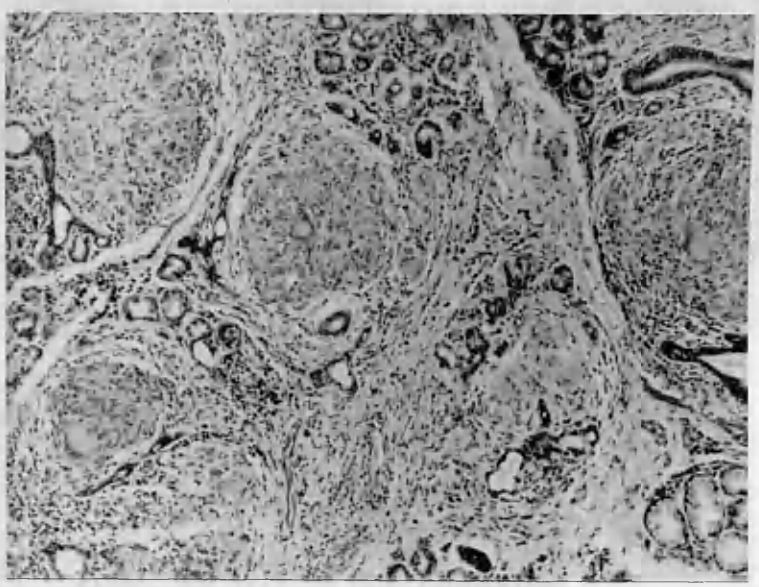
FIGURE V, 3



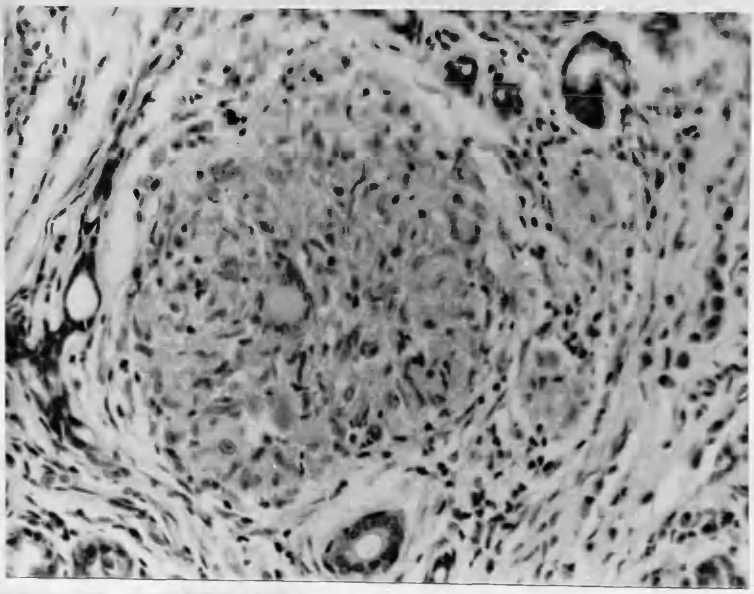
Bilateral parotid gland enlargement.

FIGURE V, 4

A



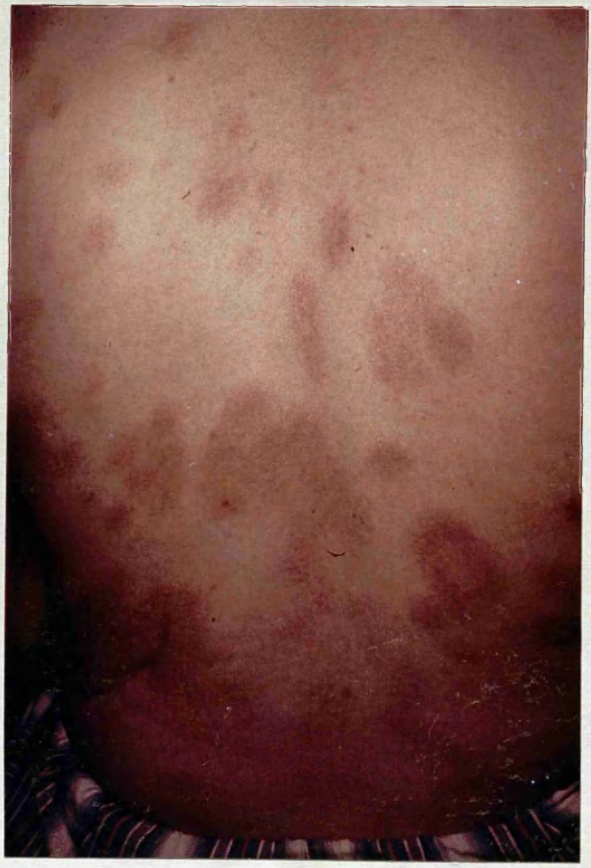
B



Marked replacement of minor labial salivary gland tissue by sarcoid granulomata, with centrally placed Langhan's type giant cells and peripheral chronic inflammatory cell infiltrate.

A x 30 B x 75

FIGURE V, 5



Back of patient showing the cutaneous lesions of Boeck's sarcoidosis. Scar on the left flank from old operation on kidney (calculus)