Section of Odontology

President G L Roberts MB FDS RCS

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Paper

Focal Adenitis in Salivary and Lacrimal Glands[1]

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The mechanism of production of Sjögren's syndrome (of which an oral manifestation is commonly dryness of the mouth) has been tentatively linked with that of rheumatoid arthritis by Morgan (1954) and with that of a 'broader spectrum of auto-immune disease' by Bunim (1961). The present work was designed to investigate the hypothesis that foci of lymphatic infiltration seen in salivary and lacrimal glands might be more common than was realized, that they might represent a focal form of Sjögren's syndrome and that they might have the same associations with systemic disease entities as Sjögren's syndrome. The problem was approached in two ways: (1) by examination of post-mortem material and (2) by experimental attempts to reproduce the lesions in two species of laboratory animal.

In regard to terminology: the view of Bunim (1961) that the term Sjögren's syndrome should be used in a comprehensive sense and should include 'Mikulicz's disease' is accepted. Focal adenitis of salivary and lacrimal glands will be defined as chronic inflammation of these glands apparently unconnected, at least until late in the process, with suppuration or duct obstruction and manifested by the presence of microscopical foci of lymphocytes, histiocytes and a few plasma cells, usually in association with small veins. Gland lobules, whether containing otherwise acceptable foci or not, in which polymorphonuclear leucocytes were found in any site outside blood vessels or in which there was any duct dilatation were specifically excluded from consideration.

Materials and Methods

Macroscopical and microscopical examination of a submandibular, a parotid and a lacrimal gland from 239 subjects undergoing autopsy in the Bernhard Baron Institute of Pathology, London Hospital, and of the submandibular and parotid salivary glands from 21 coroner's autopsy subjects who had died 'sudden' deaths was carried out. The glands were dissected free and one half of each fixed in 10% neutral formal saline, embedded in paraffin wax, and sectioned by standard techniques.

Lymphocytes present in the glands as part of 'anatomical' (see *Discussion*, p 916) or neoplastic aggregates or of infiltrations associated with suppuration were excluded; the incidence of foci of lymphocytes and histiocytes in large representative sections of the glands was expressed quantitatively. This allowed prevalence by sex and age, distribution between glands and the associations with other pathological conditions to be assessed. Only glands containing at least 2 foci, each of at least 50 cells, in a representative section were considered to show evidence of focal adenitis. The application of the criteria listed in Table 1 led to the exclusion from the series of thirteen subjects from the 239 examined.

The degree of involvement of a section was assessed quantitatively as follows: Histological sections were examined under × 50 diameters magnification and the number of foci of lymphocytes and histiocytes comprising more than 50 cells counted. The area of the section was then measured with calipers, and the number of foci

Table 1
Criteria for inclusion in the series

A	*Gland lobules* were accepted if they:
	(*a*) were free from duct dilatation
	(*b*) were free from extravascular polymorphonuclear leucocytes
B	*Glands* were accepted if –
	A representative section of the gland (approximately $(2 \times 2\ cm^2)$ for a salivary and $(1 \times 1\ cm^2)$ for a lacrimal gland) remained after exclusions
C	*Individuals* were admitted to the series if they:
	(*a*) were free from neoplasm involving lymphocyte-like cells
	(*b*) had not received a cytotoxic drug within recent months
	(*c*) had at least two of the three types of gland: submandibular, parotid and lacrimal, which were acceptable

[1]The work reported in this paper forms part of that approved by the University of London for the award of the MD degree

Table 2
Focal adenitis: details of grading of a gland

0–1 focus per section	: Grade 0	None
2–8 foci per section	: Grade 1	'Slight'
9–40 foci per section	: Grade 2	'Moderate'
Over 40 foci per section	: Grade 3	'Severe'
More than half the gland parenchyma replaced in a section	: Grade 4	'Very severe'

expressed arithmetically as a count per standard size section (4 sq. cm for salivary, and 1 sq. cm for lacrimal glands, in adults, and proportionately for children). Each gland was graded as detailed in Table 2.

The grading of the worst gland of an individual was termed the 'summary grade' of that individual, or alternatively he was described as being in e.g. 'summary grade 2'.

The ABO groups of all patients in the hospital autopsy series with moderate or severe focal adenitis (grades 2–4), whose blood had been grouped, were extracted from their records and the prevalence of focal adenitis in them compared with that in sex-paired subjects from the same autopsy series whose glands were free from focal adenitis.

The submandibular and parotid glands of 21 coroner's autopsy subjects, aged between 8 months and 83 years who had died 'sudden' deaths, were examined in order to exclude the possibility that focal adenitis found in the hospital autopsy subjects might be a terminal phenomenon resulting from a somewhat slow death. It seemed conceivable that, for instance, a latent virus infection might become active terminally. Of the 21, all had died from a cause of death which had not, it was considered, operated for more than forty-eight hours and were free from significant systemic disease.

In order to establish the significance of differences between prevalences in different groups, a χ^2-test using throughout Yates's correction for small samples, or the Mann-Whitney U-test, has been used. The Mann-Whitney U-test incorporates the concept of ranking and thus takes into consideration the degree to which a trend exists consistently throughout a set of data. Both these tests have the advantage that they are non-parametric and therefore no assumption as to the normality of a distribution needs to be made when they are used.

In addition, preliminary experiments to produce comparable lesions in two species of experimental animal by sensitization using freeze-dried saline extracts of homologous salivary and lacrimal glands homogenized with Freund's complete adjuvant were carried out.

Freund's complete adjuvant, comprising a mineral oil, an emulsifying agent and killed myco-

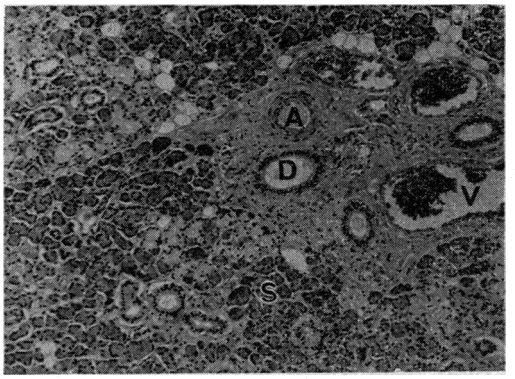

Fig 1 *Human submandibular salivary gland of woman, aged 42. Normal histology. The connective tissue surrounding vessels and ducts is free from all but a small number of isolated lymphocytes. A, artery. D, duct. S, serous acini. V, vein. H & E.* ×55

bacteria, is to be regarded as an experimental aid in causing an animal to make autoantibodies – that is, antibodies that would react equally well against the tissues of its own body as against foreign antigens.

In the course of these experiments the prevalence of spontaneous focal adenitis in the salivary and lacrimal glands of the albino rat and guinea-pig was determined in healthy untreated animals. It was also determined in those receiving injections intradermally either of gland extract plus Freund's complete adjuvant, or of Freund's complete adjuvant plus physiological saline. The gland extracts were made from salivary and lacrimal glands dissected from healthy untreated animals in each case of the same species as the future recipients. The glands were either used immediately or stored at −20°C until required. They were minced finely, extracted in chilled saline and the extract either used at once, or dialysed, freeze-dried and stored in a desiccator at −20°C until used. Injections were made intradermally on each injection day in multiple separate sites of the skin of the back near the tail and/or footpad. The effect of different injection schedules involving weekly or monthly injections over periods of time ranging from two weeks to eight months, and of using extracts from single gland types or various combinations of gland types was investigated.

At the end of each experiment the animals were killed by ether and the organs of the mouth, neck and thoracic and abdominal cavities examined. The salivary and lacrimal glands were dissected out, bisected and placed in chilled 10% neutral formal saline. Samples were removed from the skin granulomas and regional lymph nodes of the cervical and inguinal regions also. From a proportion of the animals, specimens were taken also from the thyroid, liver, pancreas, ovary, spleen

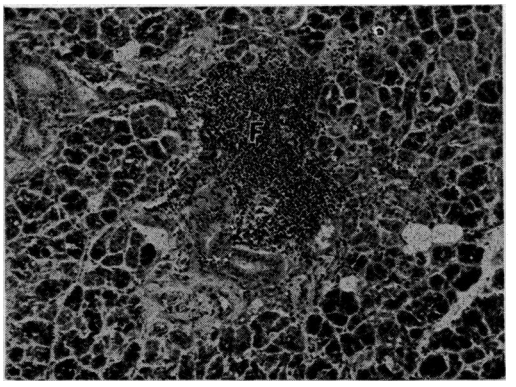

Fig 2 *Human submandibular salivary gland of woman aged 53. Myocardial insufficiency and hypertension. Replacement of acini by a focus of lymphocytes and histiocytes.* F, focus. *H & E.* ×80

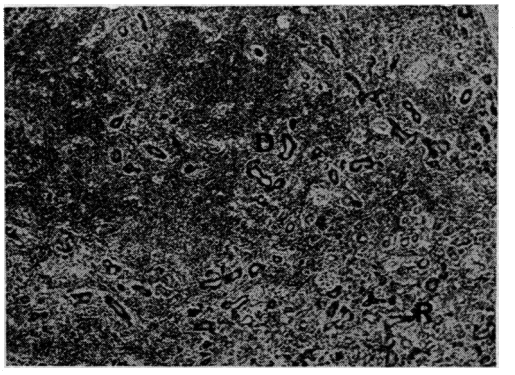

Fig 3 *Human submandibular salivary gland in man aged 66. Rheumatoid arthritis, pancytopenia. Marked parenchymatous replacement by lymphocytes and histiocytes. Acinar loss, duct hyperplasia and some fibrosis are seen. D, ducts. R, remnants of acini. H & E.* ×40

and adrenal gland. Tissues were embedded in paraffin and sections cut at 5μ using standard techniques. A skin sensitivity test, a precipitin test and a fluorescent antibody technique were used to investigate the immunological state of some of the guinea-pigs in the experiments. In these experiments the criteria for inclusion of a gland and of an animal in the rat and guinea-pig experimental series were similar to those used in humans, and the method of grading glands was also similar.

RESULTS

Naked eye and microscopical appearances of normal glands and of those affected with focal adenitis: None of the submandibular, parotid or lacrimal glands admitted to the series after the application of the criteria listed in Table 1 had a macroscopical appearance deviating from the normal, i.e. the cut surface showed a regular lobular pattern and had a fleshy consistency.

The microscopical appearances of the submandibular gland are illustrated in Figs 1, 2, 3. The normal submandibular gland (Fig 1) is a compound tubulo-alveolar gland containing both mucous and serous acinar cells. The connective tissue surrounding ducts and blood vessels was free from aggregates of lymphocytes. Although lymph nodes constantly lay on the surface of the submandibular gland none were found within the gland substance, but were, however, an almost constant finding inside the parotid gland.

The foci (considering only those containing more than fifty cells) consisted (Fig 2) of aggregates of lymphocytes and histiocytes with a few plasma cells peripherally. Larger foci became confluent and, in glands containing many areas of infiltration, much destruction of gland parenchyma was apparent (Fig 3). Lymphocytes of the foci appeared to have infiltrated between, and then to have entered, the acinar cells at the edge of a focus, which suffered the loss of their serozymogenic granules and, apparently, the continuity of their basal membrane. Hyperplasia of the epithelium lining ducts in relation to large foci was commonly seen.

Human Autopsy Data

The results of analysis of the human autopsy material are presented in Figs 4–8 and Tables 3 and 4. The types of statistical test used in the present work to establish the significance of differences or correlations between prevalences in different groups of subjects or glands, which were apparent from the diagram and tables, have been considered above (see '*Materials and Methods*', p 911).

Overall figures for the prevalence of focal adenitis, as measured in the way described above, in the most severely affected gland out of the submandibular, parotid and lacrimal glands of each

Table 3
Focal salivary and lacrimal adenitis

	Grades					*Total*
	0	*1*	*2*	*3*	*4*	
Percentages of 159 female subjects	30	43	24	3	0	100
Percentages of 67 male subjects	70	21	6	2	1	100

Table 4
Focal adenitis of any degree. Percentages of the age groups affected

	Age groups				
	0–44	*45–54*	*55–64*	*65–74*	*Over 75*
159 female subjects	50	74	73	68	86
67 male subjects	33	36	22	20	67

individual subject and in different age groups of the 159 female and 67 male subjects are shown in Tables 3 and 4.

The block diagrams shown in Figs 4 and 5 illustrate the prevalence in age groups of focal adenitis in each sex. When considered both together and singly, they indicate a higher prevalence in women than in men, and in women over 45 years than in other subjects. The significance of these differences was confirmed in statistical analysis by χ^2-tests, applying Yates's correction for small samples throughout. Analysis of the distribution in groups within the series when all degrees of focal adenitis including slight were considered, showed that the prevalence was highly significantly greater ($P<0{\cdot}001$) in women of all ages than in men and higher in women over 45 years than in all other subjects. The prevalence in women of 45 and above was higher than in younger women. This difference was significant at the 5% level (Mann-Whitney U-test).

When figures for moderate or severe grades were analysed, the same relationships emerged. Once again women were seen to be significantly more commonly affected ($P<0{\cdot}01$) than all other subjects. Women of 45 and over were significantly more commonly involved than younger women at the 5% level (Mann-Whitney U-test).

In the submandibular, parotid and lacrimal glands considered separately the prevalence of focal adenitis of all grades for male and female subjects is illustrated in Figs 6 and 7. The degree of involvement on the scale from 0 to 4 shown by each subject in at least one gland, i.e. the grading of his most severely affected gland, is indicated as the 'summary grade'. It will be noted that the trend of prevalence with advancing age in each gland closely resembles that in each of the other glands and in the worst gland of each individual ('summary') as evidenced by the similar shape of each curve in both diagrams.

Because the submandibular and lacrimal glands were all quite free from any 'anatomical' lymphoid tissue such as occurs in the parotid gland (see *Discussion*, p 917), detailed analysis of the results of these two glands will now be presented. A contingency table showing the distribution of focal adenitis of different grades between the submandibular and lacrimal glands was constructed, and the correlation coefficient (r) between the prevalence in the submandibular gland and the prevalence in the lacrimal gland for each grade of severity, was calculated. The value of $r=0{\cdot}45$, and a χ^2-test using Yates's correction showed $\chi^2=57{\cdot}20$, 9 d.f., $P<0{\cdot}001$. For such high numbers there can be little doubt of an association and it may therefore be said that either the submandibular or the lacrimal gland may be accepted as a very good indicator of the degree of involvement of the other two glands.

The results of considering only the submandibular gland from each subject will now be presented. Clearly a great economy of research time would result if from a subject one gland instead of three could be scrutinized and from it approximately the same assessment of the subject made as would have been obtained from the consideration of all three glands from that subject. The scrutiny of only the submandibular gland would have caused only 27 subjects with grade 1 (slight) focal adenitis to be missed, only 6 subjects (3%) with grade 2 (moderate) focal adenitis, and none from grades 3 or 4 (severe).

Although the number of subjects whose blood group was known in the present series is small it is considered desirable to present the results obtained, for the reason that a difference marked enough to be statistically significant in a small group is indicated. Moreover the presentation of the results of analysing a small series, when they support an apparently original hypothesis, might enable them to be considered by other workers in combination with figures from other sources. It must naturally be emphasized that isolated find-

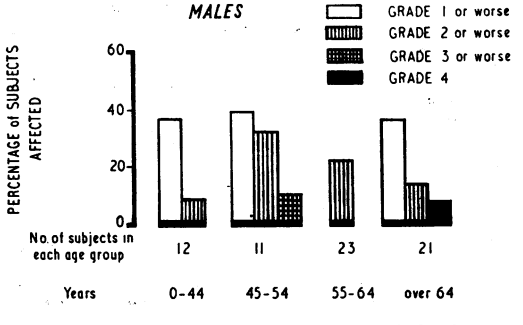

Fig 4 *Focal adenitis in salivary and lacrimal glands showing prevalence in age groups. All degrees of involvement. Male cases*

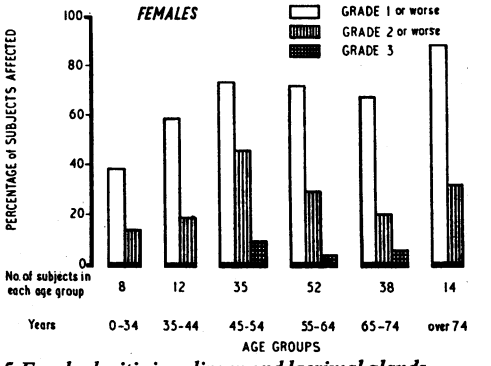

Fig 5 *Focal adenitis in salivary and lacrimal glands showing prevalence in age groups. All degrees of involvement. Female cases*

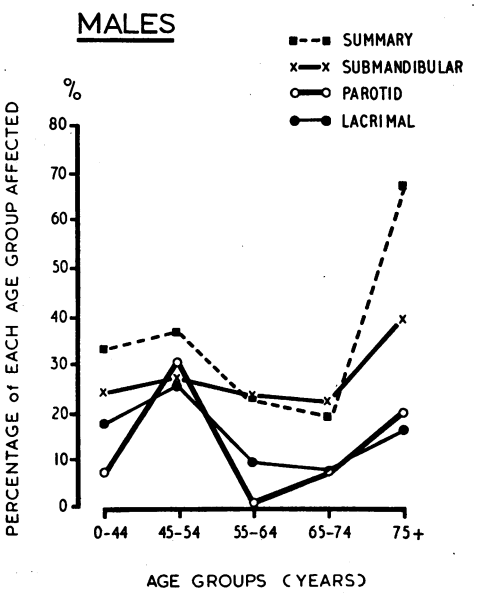

Fig 6 *Prevalence of focal adenitis in the glands considered separately*

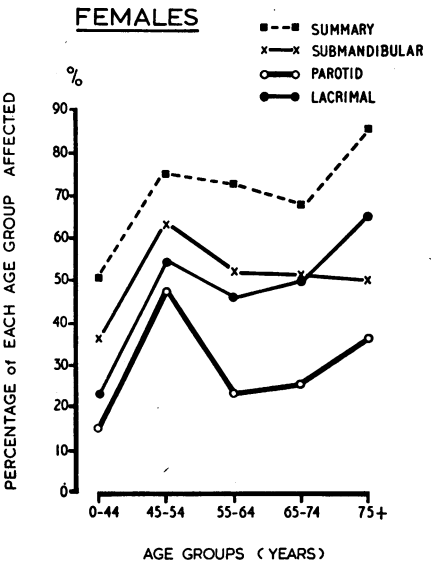

Fig 7 *Prevalence of focal adenitis in the glands considered separately*

ings (even based on large numbers) which are unsupported by other series or by associations in similar diseases must await confirmation, as was pointed out by Edwards (1962). The prevalence of focal adenitis in the 28 subjects in whom adenitis grade 2 to 4 had been found, and whose ABO blood group was known, was compared with that in a sex-paired group consisting of subjects in sequence within the hospital autopsy series and free from focal adenitis.

The results of the analysis of this (small) sample indicate a tendency for Group 0 and freedom from focal adenitis to be associated which is significant at the 5% level ($\chi^2 = 5{\cdot}3$, 1 d.f., $P < 0{\cdot}05$) using Yates's correction for small numbers. That is to say, the indication is that group 0 was less frequent in subjects with moderate or severe focal adenitis in at least one gland than in subjects free from focal adenitis. Thus, group 0 has been tentatively associated with a freedom from focal adenitis. Larger samples, which it is planned to obtain in the future, are required to investigate a possible association between the positive finding of moderate or severe focal adenitis and the individual groups A, B and AB.

Fig 8 shows the expected and observed prevalence of focal adenitis of salivary and lacrimal glands in subjects of the hospital autopsy series suffering from rheumatoid arthritis. The 'expected' figures were derived from that percentage of the total series who conformed to the description given for each column. The observed prevalence was higher than expected but the numbers were too small, in each group considered singly, for statistical analysis and no firm conclusions could be drawn. However, there appeared to be a consistent tendency for the incidence of slight and also of more marked degrees of focal adenitis to be increased in subjects with rheumatoid arthritis, and a similar association for subjects with amyloidosis.

The prevalence of focal adenitis in the coroner's autopsy group, which had died a 'sudden' death and that in the hospital autopsy group were compared in order to test the hypothesis that focal adenitis was a terminal event in a slow death. If this were so, then the prevalence in the coroner's group would be lower than in the hospital

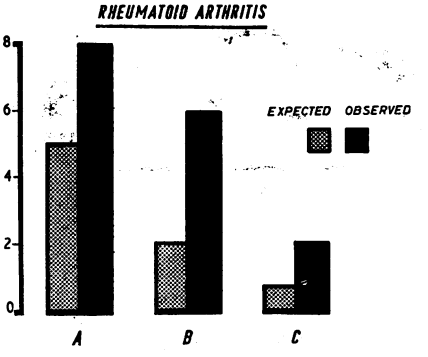

Fig 8 *Prevalence of focal adenitis in subjects of the hospital autopsy series with rheumatoid arthritis. A, total number with evidence of focal adenitis. B, number with more than 9 foci per section in at least one gland. C, Number with more than 40 foci per section in at least one gland*

autopsy group. It was in fact, even slightly higher in the coroner's than in the hospital group. Statistical analysis showed that there was no significant difference in the distribution of the two samples ($\chi^2=0{\cdot}87$, 1 d.f., $0{\cdot}4>P>0{\cdot}3$) with Yates's correction for small samples. The results therefore gave no support for a hypothesis that focal adenitis was a terminal phenomenon in persons dying slowly.

Rat and Guinea-pig Pilot Experiments

Only animals which were healthy throughout life were included in the uninjected normal control series in these experiments. These animals had healthy cervical, thoracic and abdominal organs at autopsy. The histological appearances of the foci in the glands of the control rats and guinea-pigs (when foci were present) and in the experimental rats and guinea-pigs (Fig 9) were similar to those of small foci in human subjects. Potential differences between experimental and control animals lay in the numbers of foci present. Foci comprised aggregates of lymphocytes and histiocytes (by arbitrary definition only aggregates containing more than 50 cells were considered as foci).

The histological results from the rat and guinea-pig experiments are summarized in Table 5 and those of the guinea-pig experiments illustrated in Fig 10. Of interest are the presence of foci in some glands of a few of the untreated control animals, and of those animals receiving Freund's complete adjuvant alone, and in a numerically bigger fraction of those receiving Freund's complete adjuvant plus gland extract. Of these findings the first two are what would be expected. Numbers are too small for firm conclusions to be drawn although the contrast between the absence of foci in the submandibular glands of the untreated control guinea-pigs and their presence in the submandibular glands of 5 out of 7 of the animals injected with gland extract plus Freund's complete adjuvant (Fig 10) is of great interest. The investigation of the immunological state of some of the guinea-pigs in the experiments by a skin sensitivity test, a double diffusion gel precipitation test and a fluorescent antibody technique, using each animal's own serum and a rabbit antiguinea-pig serum conjugated with fluorescein isothiocyanate, did not provide firm evidence of sensitization.

Table 5

Summary of results of animal experiments
Percentage of animals with one or more foci (50+ cells) in a section of the gland named

Gland	*Uninjected normal control animals*	*Animals injected with saline plus Freund's complete adjuvant*	*Animals injected with gland extracts plus Freund's complete adjuvant*
Rat			
Submandibular	2 (50) ●	0 (5)	5 (22)
Parotid	0 (44)	0 (5)	5 (22)
Exorbital-orbital lacrimal	40 (15)	40 (5)	57 (21)
Harderian lacrimal	8 (12)	20 (5)	5 (22)
Guinea-pig			
Submandibular	0 (37)	33 (3)	71 (7)
Parotid	0 (37)	0 (3)	0 (7)
Orbital lacrimal	0 (36)	0 (3)	43 (7)
Harderian lacrimal	35 (37)	0 (3)	86 (7)

● The total number of animals in each group in whom the gland named was available is shown in brackets after the percentage value

DISCUSSION

Human autopsy material: The distinction between the findings and their interpretation is important. The findings were that microscopic foci of lymphocytes and histiocytes containing more than 50 cells occurred in salivary and lacrimal glands.

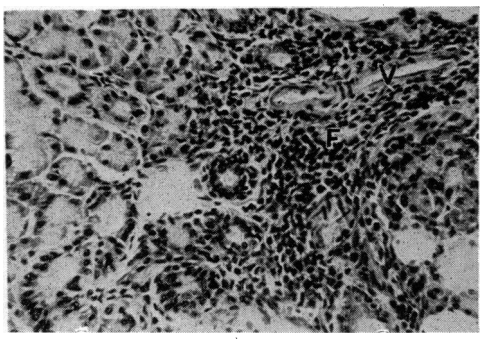

Fig 9 *Guinea-pig submandibular salivary gland. Female, 11 months. Injected with gland extract plus Freund's complete adjuvant. A focus composed of lymphocytes and histiocytes in relation to a venule. V, venule. F, focus. H & E. ×190*

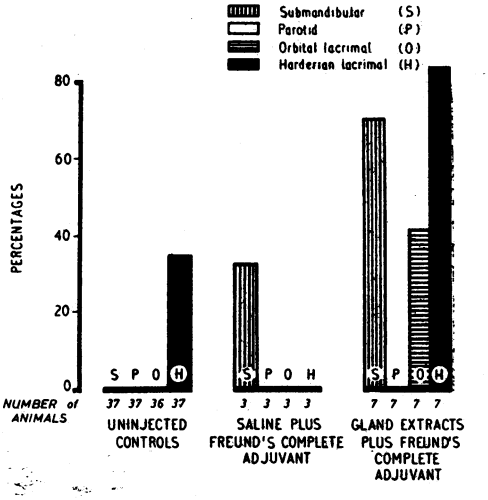

Fig 10 *Guinea-pig sensitization experiment. Percentage of animals with one or more foci (50 + cells) in a section of the gland named*

These glands were free from acute inflammation or evidence of duct obstruction, and, in the case of the submandibular and lacrimal glands, free from 'anatomical' lymphocytes. Larger foci became confluent and gland acini were involved and replaced. The large foci did not merge into lymphoid tissue or lymph nodes, which were quite absent, as has been stated, within submandibular and lacrimal glands. Foci occurred more often in the glands of women than men and in those of women over 45 than in younger women. The prevalence in a small series of coroner's autopsy subjects who had died 'sudden' deaths was similar. The degree to which the submandibular, parotid and lacrimal glands were involved corresponded well. These findings are, it is felt, by reason of the degree to which it has been possible to introduce quantitative and statistical methods into the analysis, free from the element of controversy and hypothesis which may attach to their interpretation. When their interpretation is considered, pointers are found which suggest an autoimmune basis for some if not all of the foci, and the evidence for this will now be considered.

The grounds for suggesting an autoimmune basis for focal adenitis of salivary and lacrimal glands are several but it must be stressed that in sum they amount to nothing more than an indication of the possibility. The histological appearances, merging as they do into Sjögren's syndrome and resembling focal thyroiditis, the uniformity of involvement between the salivary and lacrimal glands, the sex and age findings, in that women over 45 years are most commonly involved, as in Sjögren's syndrome and Hashimoto's thyroiditis, and the indication of an association with rheumatoid arthritis and amyloidosis can all be quoted. Moreover the evidence mentioned for regarding Sjögren's syndrome as part of the broad spectrum of immune diseases, that is, the finding of antinuclear factor and rheumatoid factor in the serum of Sjögren's patients, together with the production of experimental autoantibodies to rabbit submandibular by Beutner and co-workers in 1961 and the demonstration of antibodies to salivary and lacrimal glands in some patients with Sjögren's syndrome in 1958 by Jones, means that the possibility of an immune basis for focal adenitis is increased. The histological findings of parenchymal loss and focal infiltration with lymphocytes in relationship to small veins of this focal adenitis are seen also in many diseases known or suspected to have an autoimmune basis. Such diseases include chronic thyroiditis, some examples of Addison's disease, pernicious anæmia and certain types of cirrhosis, as was pointed out by Williams & Doniach in 1962.

The various other factors which could contribute lymphocytes or similar cells to the salivary glands may now be considered. The headings which might be called 'anatomical', 'neoplastic' or 'infective' have been mentioned. Briefly the findings of the lymphocytes are unlikely to be explicable on anatomical grounds since, as Denz (1947) showed, the lymphoid tissue of the body reaches maximum development at about puberty and thereafter declines, which finding contrasts sharply with the prevalence of focal adenitis in corresponding age and sex groups. Undiagnosed lymphocytic neoplasms are not common. Lymphocytes indicating infection are unlikely to account for the findings since all lobules showing duct dilatation or extravascular polymorphonuclear leucocytes were excluded. Viruses in general produce an acute febrile illness and after a period none of the histological evidence of a virus infection is to be found.

The characteristic histology of salivary gland virus infection was not seen in any subject. Thus, although it is not possible on histological grounds completely to exclude infection as a possible cause of some of the foci, there is no positive evidence to counterbalance that already adduced, which argues against infection as a significant factor.

The animal experiments: The object of these pilot experiments was to determine whether it was possible to immunize an experimental animal by intradermal injections of freeze-dried saline extracts of salivary and lacrimal glands. The production of autoantibodies to submandibular gland in rabbit had been reported by Beutner *et al.* (1961) but no report has been found of significant histological changes in these glands.

A slight degree of focal adenitis was found to be present in a small proportion of uninjected control rats and guinea-pigs, and injection of Freund's complete adjuvant with physiological saline produced lesions in a slightly larger proportion of the rats. This means that proof, that the number of lesions found in the experimental animals represents a significant experimental increase, can only be statistical. The establishment of the significance of a small difference involves the use of a large number of experimental animals and, in this situation, a desirable approach after preliminary experiments such as these is to take steps to increase the magnitude of the experimentally induced change, if that is possible.

The results of investigation of the immunological state of certain of the control and experimental guinea-pigs cannot satisfactorily be condensed into a few sentences. With the fluorescent antibody technique evidence of two antigen-antibody reactions was obtained but these were probably incidental to the main hypothesis. In brief, evidence of sensitization was not obtained from these tests but the possibility was not ex-

cluded by them. The possible presence of antigen near foci, for instance, was not excluded.

Summarizing the results of the animal experiments: it has not been possible to prove that sensitization occurred but the overall results suggest this in the guinea-pig.

CONCLUSION

Focal adenitis (foci of lymphocytes and histiocytes and the parenchymal changes associated with them) occurred commonly and equally in human post-mortem submandibular, parotid and lacrimal glands, especially in those of women of over 45 years. It may be a focal form of Sjögren's syndrome and the latter has several of the features of an autoimmune disease.

From the animal experiments it was not possible to prove that immunization occurred but the results suggest that this was achieved in the guinea-pig and indicate that, although more expensive to breed and maintain, this animal rather than the rat should be used for subsequent attempts. For these, isolation and purification of the 'antigen' is desirable.

Acknowledgments: I wish to thank Professor I Doniach, Director of the Bernhard Baron Institute of Pathology for advice and for granting generous departmental facilities – and also Professor A E W Miles, Head of my own department, for his support. I am indebted to Drs J A Heady and L Lipworth of the Social Medicine Research Unit of the Medical Research Council for help in the statistical testing of the data and to Mr G Bowden and the Photographic Department of the London Hospital for colour photography. I was able to study the glands from the coroner's autopsy subjects through the kindness of Drs H R M Johnson and J Horder of the Department of Forensic Medicine. I am very grateful to Dr R G White for help and tuition in fluorescent antibody techniques.

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Meeting May 27 1963
at the Royal College of Surgeons, London

At the Annual General Meeting of the Odontological Section, held at the Royal College of Surgeons, London, on Monday, May 27 1963, the Honorary Curator of the Odontological Museum, Professor A E W Miles, delivered his Annual Report and gave a talk and demonstration entitled 'Pigmented Enamel' of which an abbreviated version appears below.

Mr James W White arranged an exhibit as a tribute to the late Mr Frank Coleman, an Honorary Member of the Section since 1951.

Pigmented Enamel

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The enamel on the incisors of most rodents is yellowish-brown or orange in colour and the crowns of all the teeth of some species of shrews are tipped with enamel of reddish colour. The pigment in both cases is confined to an outer zone of the enamel of the order of 40 microns in thickness. What the purpose is of such a feature is a matter for speculation. One wonders whether it may have evolved as a means of species recognition, or as a means of intimidating enemies, but I am aware of no evidence on this point.

Many observations suggest that iron is an essential component of the pigment of the rat incisor; for instance, if rats are kept on an iron-free diet or are bled frequently enough to produce an anæmia the enamel being formed at the time and which, because the incisors are of continuous growth, in due course erupts into view, is unpigmented. Furthermore, pigment formation may be disturbed in many other ways not obviously connected with iron metabolism; for example, intoxication with selenium, cadmium or fluorine, and vitamin deficiencies have been described as associated with disturbed pigment formation (Pindborg 1953). Furthermore, when the incisors of rats are prevented from occluding for a few weeks by keeping the erupting crowns cut down near to the gum, a procedure which greatly accelerates the rate of formation and eruption of the teeth, the enamel which appears at the gum margin about three weeks later is often unpigmented (Bryer 1957). It seems possible that the process of pigment formation is, under these circumstances, unable to keep up with the increased rate of eruption of the tooth. Some preliminary observations on the histology of rat incisors in this condition do indeed suggest that tension is set up leading to detachment of pigment-forming ameloblasts.

As pointed out by Schmidt (1958), a simple way to show that the enamel pigment is rich in iron is to use the Perls reagent (potassium ferrocyanide acidified with hydrochloric acid) which is highly