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ORGAN ANTIBODIES IN SJÖGREN'S SYNDROME

By

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Clinically Sjögren's syndrome is characterized by xerostomia, xerophthalmia and frequently by swelling of the salivary and lacrymal glands. The syndrome is caused by a chronic sialoadenitis and dacryoadenitis. It is known that about two thirds of the cases are coexistent with rheumatoid arthritis, a fourth of the cases occurs as isolated phenomena, and occasionally it is found in patients with rhinitis, pharyngitis, laryngitis, achlorhydria, pernicious anemia, ulcerative colitis, cirrhosis of the liver, scleroderma, Raynaud's phenomenon, leukopenia, thrombocytopenia, disseminated lupus erythematosus (D.L.E.) and Hashimoto's thyroiditis (2, 4, 7, 12, 17). In most of these disease auto-immune mechanisms may be of pathogenetic importance. Auto-immunity may also play a role in the development of Sjögren's syndrome. Precipitating and complement fixing organ antibodies have been found in sera from patients with Sjögren's syndrome (15, 1). These antibodies are not organ specific since positive results can be obtained by means of extracts of salivary tissue as well as several other organ extracts. Antinuclear factor is a frequent finding in sera from patients with Sjögren's syndrome (7, 5, 3). Recently a phenomenon has been described which might represent an organ specific antibody against a salivary antigen (*Bertram & Halberg 1964*). Using an immunofluorescent techni-

que an antibody can be demonstrated which reacts specifically with a cytoplasmic antigen in the epithelial cells lining the excretory ducts. No antigenecity has been found in the acini by this technique.

In the following paper an account is given of the occurrence of organ specific and non-organ specific antibodies in sera from patients with Sjögren's syndrome. The sera were tested for the occurrence of salivary gland-, thyroid-, adrenal-, gastric-, parietal cell, nuclear, non-organ specific complement fixing antibody, and non-organ specific precipitin. The results are compared with the findings in sera from patients with D.L.E., Hashimoto's disease, pernicious anemia, and Addison's disease and with a group of control persons. The results relating to the Addison sera are quoted from a paper by *Halberg, Bertram, Søborg & Nerup* (11).

MATERIAL

Sera from 32 patients with Sjögren's syndrome were part of the material. Sjögren's syndrome was diagnosed by means of sialometry, sialography, Shirmers test, staining with Rose Bengale and the occurrence of changes of the oral mucous membrane characteristic of the disease (4). In eight cases the diagnosis was confirmed by biopsy of salivary glands which showed the type of chronic sialoadenitis called benign lympho-epithelial disease.

Thirty of the patients were women, 2 men. Fig. 1 shows the age distribution; the average age was 59 years. The material further included sera from 29 patients with D.L.E., 37 patients with Hashimoto's thyroiditis, 29 patients with pernicious anemia and 48 patients with non tuberculous Addison's disease (Quoted from no. 11). The control sera were drawn from 24 blood donors aged 20-40 years (12 women, 12 men), 98 healthy persons aged 50 years (44 women, 54 men), 29 women hospital patients without signs of disease of a possible auto-immune origin and 37 hospital patients (25 women, 12 men), more than 65 years of age, without signs of auto-immune diseases.

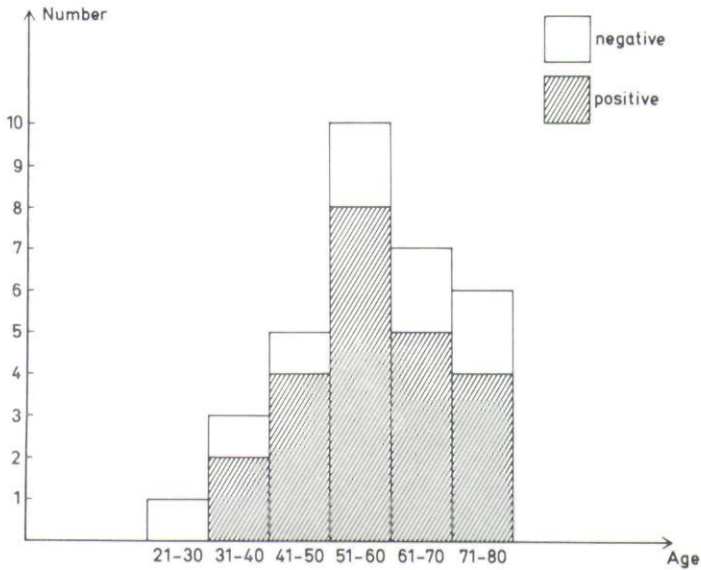


Fig. 1.

Age of 32 patients with Sjögren's syndrome.

(Positive: Patients serum contain specific antibody against the cytoplasm of the salivary excretory ducts).

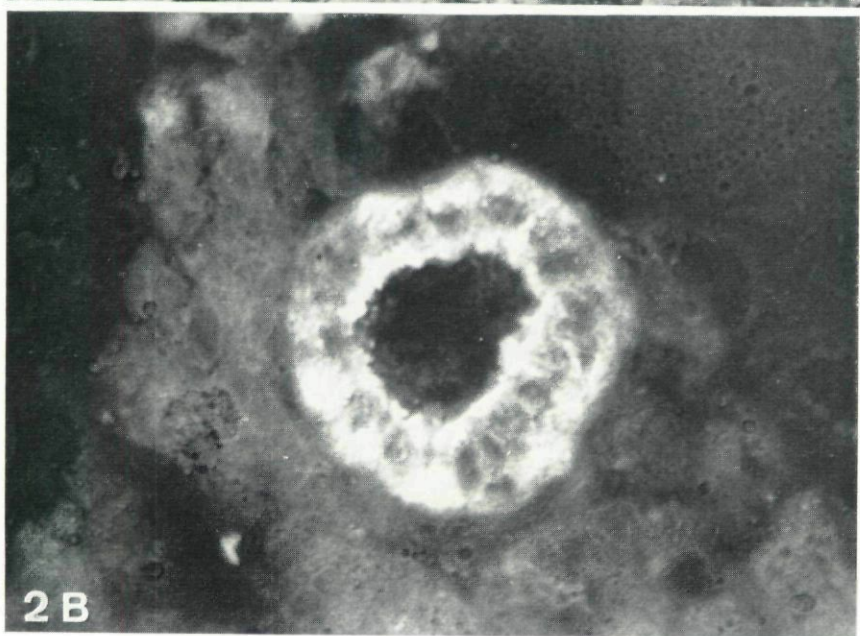
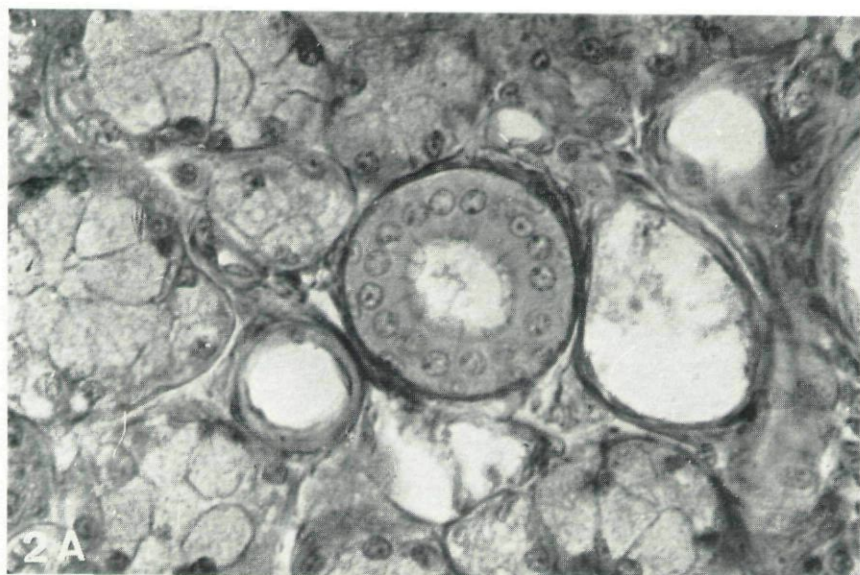
METHODS

Antibodies against salivary excretory ducts, thyroid follicular epithelium, gastric parietal cells, and adrenal cortex were demonstrated by means of Coons & Kaplan's immunofluorescent technique (8) as described by Bertram & Halberg (5), Holborow, Brown, Roitt & Doniach (13), Irvine (14) and Blizzard, Chandler, Kyle & Hung (6), respectively. Non-organ specific complement fixing antibody and non-organ specific precipitin was demonstrated as described by Gajdusek (10) and Jones (15) and Anderson, Gray, Beck & Kinnear (1). Antinuclear factor was demonstrated by the various modifications of the immunofluorescence technique stated above. The thyroid sections were the most useful for this purpose.

Figs. 2A-2B.

Fig. 2A. Histological section from normal submandibular gland.

Fig. 2B. Fluorescence of cytoplasm of the cells of a salivary excretory duct. $\times 400$.



RESULTS

Twenty-three of the 32 patients with Sjögren's syndrome had sedimentation rates above 15 mm/h (Westergren). Serum electrophoresis was performed in all of the 32 cases. 22 had hypergammaglobulinemia (1.35–4.64 grams per 100 ml). Eighteen out of 20 sera tested contained rheumatoid factor demonstrated by the RA test and/or Rose-Waaler's test. L.E. cells were found in 3 out of 30 cases.

TABLE 1
Occurrence of Antibody against Salivary Excretory Ducts.

<i>Origin of sera</i>	<i>Number of sera</i>	<i>Antibody</i>	<i>% Positive</i>
Sjögren's syndrome	32	23	72
D.L.E.	29	7	24
Control	188	20	11

Tables 1–6 shows the findings of organ antibodies. Antibody against the cytoplasm of the cells of the excretory ducts was found in approximately three fourths of the sera from the Sjögren patients, (Fig. 2). This antibody was also found in a quarter of the sera from patients with D.L.E. without signs of Sjögren's syndrome, and it was found in one tenth of the control sera. Table 2 shows that the old age group is mainly responsible for this high figure. As previously stressed by *Markson & Moore* (16) the incidence of organ antibodies increases in the old age groups (9). The age factor seems particularly important for the occurrence of the salivary gland antibody. The antigen was found in the ducts of parotid, submaxillary and sublingual glands. It was not found in the excretory ducts of the pancreas or in gall ducts. The antibody does not seem to be specific of species since the antigen could be demonstrated in a parotid gland from a guenon.

Fig. 3–4.

Fig. 3. Fluorescence of parietal cells of gastric glands. $\times 400$.

Fig. 4. Fluorescence of cytoplasm of thyroid follicular epithelium. $\times 400$.

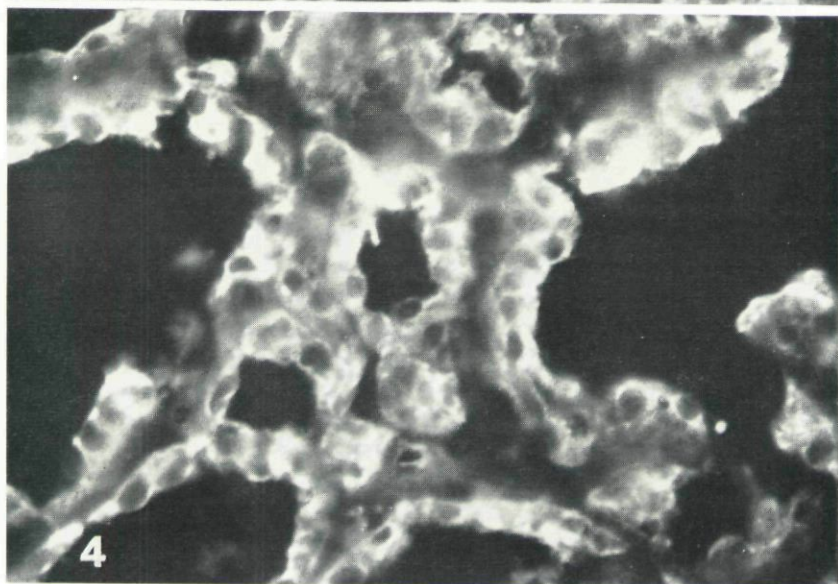
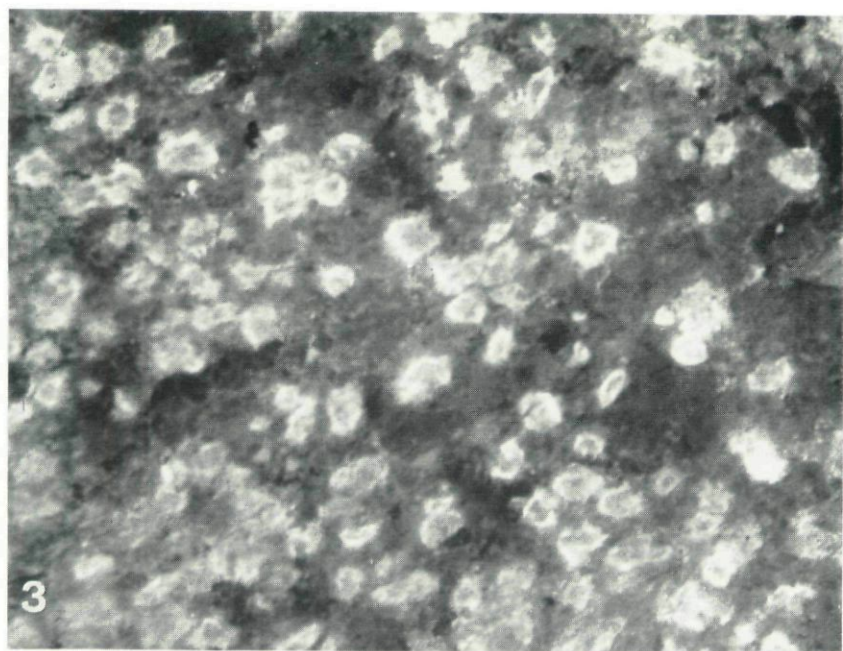


Table 3 shows that parietal cell antibody (Fig. 3) did not occur more often in sera from patients with Sjögren's syndrome than among the control sera. None of the 3 Sjögren patients whose sera contained parietal cell antibody had signs of pernicious anemia. All of them had normal levels of Serum B-12, and none of them had achlorhydria.

TABLE 2
*Age Correlated to the Appearance of Antibody against Salivary glands
(Positive) in Controls.*

<i>Age</i>	<i>Number of sera</i>	<i>Positive</i>	
		<i>Number</i>	<i>%</i>
17-40	35	1	3
50	99	7	7
> 65	44	12	27

TABLE 3
Occurrence of Antibody against Parietal Cells.

<i>Origin of sera</i>	<i>Number of sera</i>	<i>Antibody</i>	<i>% Positive</i>
Sjögren's syndrome	32	3	9
Pernicious anemia	29	22	76
Control	121	10	8

TABLE 4
Occurrence of Antibody against Adrenal Cortex

<i>Origin of sera</i>	<i>Number of sera</i>	<i>Antibody</i>	<i>% Positive</i>
Sjögren's syndrome	32	1	3
Addison's disease*	48	31	65
Control	29	0	0

* Quoted from Halberg, Bertram, Soborg & Nerup (11).

Table 4 shows that antibody against adrenal cortex was found in only one of the Sjögren sera. This patient had no signs of Addison's disease. Serum from this patient contained antinuclear factor, non-organ specific complement fixing antibody and three organ specific antibodies against adrenal cortex, parietal cells and salivary excretory ducts, respectively.

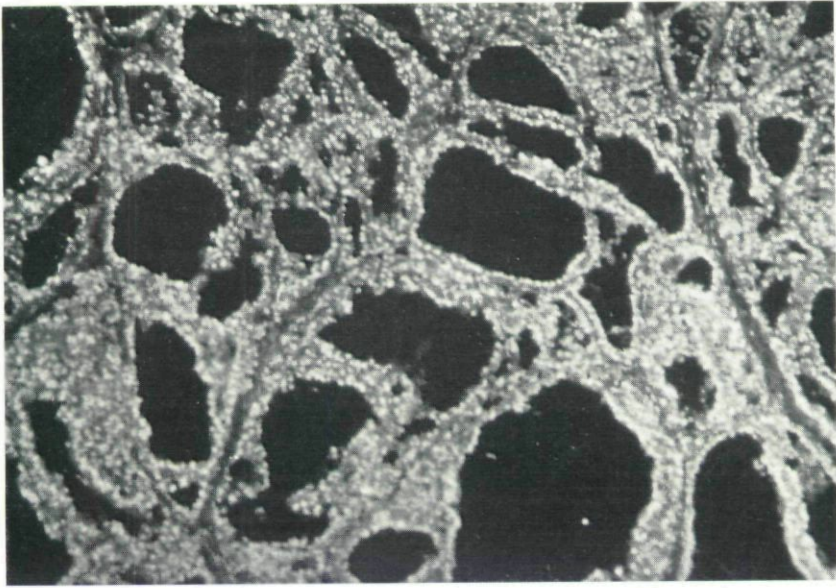


Fig. 5.

Nuclear fluorescence in a thyroid gland. $\times 50$.

TABLE 5

Occurrence of Antibody against the Follicular Cytoplasm of the Thyroid Gland.

<i>Origin of sera</i>	<i>Number of sera</i>	<i>Antibody</i>	<i>% Positive</i>
Sjögren's syndrome	32	4	12.5
Hashimoto's thyroiditis	37	35	94.6
Control	80	2	2.5

Table 5 shows that microsomal thyroid antibody (Fig. 4) was found in 4 of the 32 sera from patients with Sjögren's syndrome, whereas, it was found in only 2 of 80 control sera. Two of the four Sjögren patients with thyroid microsomal antibody in their sera had atoxic goitres of unknown origin.

Table 6 shows that approximately two thirds of the Sjögren sera contained antinuclear factor (Fig. 5). In about half of them non-organ specific complement fixing antibody could be demonstrated. One serum contained non-organ specific precipitin.

TABLE 6
Occurrence of Non-Organ Specific Antibodies
 (ANF = Antinuclear Factor, CTF = Complement Fixing Antibody).

<i>Origin of sera</i>	<i>Number of sera</i>	<i>ANF</i>	<i>CTF</i>	<i>Precipitin</i>
Sjögren's syndrome	32	22	14	1
D.L.E.	33	32	21	3
Control	95	15	0	0

DISCUSSION

Both organ specific antibody against the epithelium of the salivary excretory ducts and non-organ specific antibodies (antinuclear factor and non-organ specific complement fixing antibody) were found with considerable frequency in the 32 sera from patients with Sjögren's syndrome.

The salivary gland antibody occurred in a fourth of the sera from patients with D.L.E. and non-organ specific antibodies were often found in sera from Sjögren patients, *i.e.* a considerable serological overlap was found between Sjögren's syndrome and D.L.E. However, the overlap was also clinical. Three of the patients with Sjögren's syndrome had pronounced L.E. cell phenomena. Besides Sjögren's syndrome all of them had cirrhosis of the liver, two had rheumatoid arthritis and the third patient had Raynaud's phenomena. In these patients a diagnosis of D.L.E. could be made. 17 of the 32 patients had rheumatoid arthritis, six had cirrhosis of the liver, four patients had scleroderma, one had leukopenia, two had thrombocytopenia, seven had anemia, and two had Raynaud's phenomena. All these diseases are typical manifestations of D.L.E. and as emphasized by *Heaton* (12), Sjögren's syndrome may itself be a manifestation of D.L.E.

A third of the sera from the Sjögren patients did not contain the salivary gland antibody. Fig. 1 shows that contrary to the case in the control sera no correlation was found between the occurrence of the antibody and the age of the patients. Consequently the ages of the patients hardly explain the failure to demonstrate organ specific antibody in a third of the Sjögren sera. The stage of the disease might be of

importance to the occurrence of the antibody. As suggested by Jones (15) the antibody may disappear when the disease has progressed so far that the auto-antigen has been destroyed. This hypothesis is supported by the observation that the sedimentation rate goes down when the swelling of the salivary glands disappears and xerostomia develops. Table 7 shows, however, that in this material the occurrence of antibodies cannot be correlated to sedimentation rates, hypergammaglobulinemia or the presence of xerostomia. Consequently the varying stages of progress in the disease can not explain the missing antibodies.

Sera from 20 of the 188 control persons contained antibody against the epithelium of the salivary excretory ducts. 14 of the positive sera had been drawn from persons more than 65 years of age, *i.e.* the incidence of the antibody in control sera from persons under the age of 65 was 5 per cent (Table 2).

The occurrence of antibodies to gastric parietal cells and adrenal cortex did not exceed that of the control sera. The microsomal thyroid antibody was found more frequently in sera from Sjögren patients than in control sera, but the difference was not statistically significant. (χ^2 test Yates correction. $0.05 < p < 0.1$).

SUMMARY

Sera from 32 patients with Sjögren's syndrome contained in 72 per cent a specific antibody against an antigen in the cytoplasm of the cells lining the salivary excretory ducts which could be demonstrated by an immunofluorescence technique. Non-organ specific antibodies (antinuclear factor and non-organ specific complement fixing antibody) were found with a similar frequency. The finding of antibodies against thyroid follicular epithelium, gastric parietal cells and adrenal cortex in Sjögren sera did not significantly exceed the findings in control sera. Three of 32 Sjögren's patients had disseminated lupus erythematosus and 16 of the other patients had diseases which are characteristic manifestations of D.L.E. A consider-

TABLE 7
Antibody against Salivary Excretory ducts Correlated with Sedimentation Ratio (SR), Hypergammaglobulinemia and the Presence of Xerostomia (Determined by Sialometry).

<i>Antibody against salivary excretory ducts</i>	<i>Number of sera</i>	<i>SR</i>		<i>γ-Globulin</i>		<i>Xerostomia</i>	
		<i>Mean</i>	<i>Range</i>	<i>Mean</i>	<i>Range</i>	<i>Present</i>	<i>Absent</i>
Present	23	40	5-125	1.77	0.70-3.1	18	5
Absent	9	54	7-118	1.97	0.65-4.64	7	1

able serological overlap was found between Sjögren's syndrome and D.L.E. The occurrence of the salivary gland antibody in control sera was highly dependent on the ages of the control subjects, but neither this finding nor the varying stages of the 32 cases of Sjögren's syndrome explained the failure to demonstrate the salivary gland antibody in 28 per cent of the Sjögren sera.

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