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Experimental Allergic Sialoadenitis

I. Acute Sialoadenitis Induced by a Local Immune Reaction

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Summary. Acute sialoadenitis was induced by instillation of normal human plasma into the parotid gland of specifically sensitized rats. Histologically, the sialoadenitis was characterized by an acute inflammatory infiltration and parenchymatous destruction. All rats had precipitating antibodies, but less than half exhibited delayed skin reactivity. It is suggested that antigen-antibody complexes formed in situ elicit an Arthus-type of reaction.

Zusammenfassung. Verursachung einer akuten Sialoadenitis durch Instillation von normalem menschlichen Plasma in die Ohrspeicheldrüse spezifisch überempfindlich gemachter Ratten. Die Sialoadenitis war histologisch gekennzeichnet sowohl durch akut-entzündliche Infiltrate als auch durch Destruktion des Parenchyms. Alle sensibilisierten Ratten verfügten über präcipitierende Antikörper, aber nur eine Minderzahl zeigte eine Überempfindlichkeit vom verzögerten Typus. Es wird vermutet, daß an Ort und Stelle gebildete Antigen/Antikörperkomplexe eine Überempfindlichkeitsreaktion vom Arthus-Typus auslösen.

Perusal of the literature discloses but scant information on the pathogenetic role of immune mechanisms in the production of experimental sialoadenitis. Immunization with preparations of species-homologous salivary glands elicits formation of antibodies (Beutner, Djanian, Geckler, and Witebsky, 1961) and an inflammatory reaction in the glands (Haferkamp, 1962a; Haferkamp, 1962b; Chan, 1964). Sialoadenitis develops consequent to injection of heterologous antiserum against salivary gland if followed by the homologous antigenic preparation (Haferkamp, 1962). The present investigation describes the induction of sialoadenitis in immunized animals by direct challenge of the salivary gland with the sensitizing antigen.

Materials and Methods

Animals. Albino rats of both sexes of the Hebrew University (Sabra) strain, weighing 150–200 g, were used. The animals were housed two to a cage and given regular chow and water ad libitum.

Experimental Groups. Thirty one rats were injected subcutaneously, in the neck, with 0.5 ml of normal human blood plasma (NHP) emulsified in 0.5 ml of Freund's complete adjuvant (Difco). As of the second week, five intraperitoneal injections of 1 ml of NHP were given every third day. Twelve control rats, treated according to the same schedule, were injected with saline-in-adjuvant emulsion followed by five injections of saline. Two additional control groups consisted of ten immunized animals and ten rats injected with saline (Table 1).

Testing of the Immune Response. Two days after the last injection the rats were bled from the tail vein. The sera were assayed for precipitating anti-NHP antibodies by Ouchterlony's double immunodiffusion technique in agarose gel. A serum sample was considered positive

Table 1. Experimental groups

Group No.	Number of rats per group	Treatment	Challenge of parotid gland
I	31	NHP	NHP
II	12	Saline	NHP
III	10	NHP	Saline
IV	10	Saline	Saline

when one or more precipitin bands developed. Cutaneous hypersensitivity was tested by intradermal injections into the shaved abdominal skin of 0.1 ml of undiluted NHP, 1/10 and 1/100 diluted NHP as well as physiological saline. The test sites were examined after 1, 2, 24 and 48 hours. A skin test was considered positive when an indurated lump, 6 mm or more in diameter, developed.

Instillation of Fluid into the Parotid Gland. The technique of cannulation of the duct of the parotid gland was described in a previous publication (Ulmansky, Sela, Dishon, Rosenmann and Boss, 1972). Briefly, anaesthetized rats were placed on their back, the mouth was opened and the orifice of the right parotid duct visualized through a stereoscopic microscope. A Bardic® 1919[1] polyethylene catheter with its guiding stainless steel stylet was introduced into the duct to a depth of about 2 cm. The stylet was removed and 0.5 ml of NHP or saline were slowly injected without exertion of undue pressure.

Histological Examination of the Parotid Glands. The animals were killed by ether 24 to 48 hours following instillation of NHP or saline. Both parotid glands were removed and fixed in buffered formalin. Sections of paraffin embedded samples were cut at 6 μ and stained with haematoxylin and eosin.

Experimental Design. The parotid glands of four groups of rats were examined (Table 1). Groups II, III and IV served as controls to determine the effects of instillation of NHP and fluids per se on the glandular parenchyma.

Results

The results of the immunodiffusion and skin tests and histological findings in the right salivary glands are summarized in Table 2. Sera of all immunized rats were positive, developing in the majority of cases two precipitin lines. In both sensitized and unsensitized animals the intradermal injection of NHP did not elicit a reaction within two hours. A positive skin test was observed in 19 of 41 sensitized rats, 24 or 48 hours following injection of undiluted NHP and/or 1/10 diluted NHP solution. No reaction was elicited by NHP diluted 1/100 or saline. In rats injected with saline and tested with NHP cutaneous swelling 2–4 mm in diameter occasionally developed. Positive skin reactions were characterized by an indurated and elevated lump, 6 mm or more in diameter. Histological sections of representative positive test sites revealed marked perivascular and periadnexal inflammatory infiltration consisting mainly of histiocytes with some admixed lymphocytes and granulocytes.

As seen in Table 2, 15 of the 31 rats immunized and challenged with NHP developed moderate to severe sialoadenitis. On the other hand, of the 32 control

1 C.R. Bard Inc., Murray Hill, N.J., U.S.A.

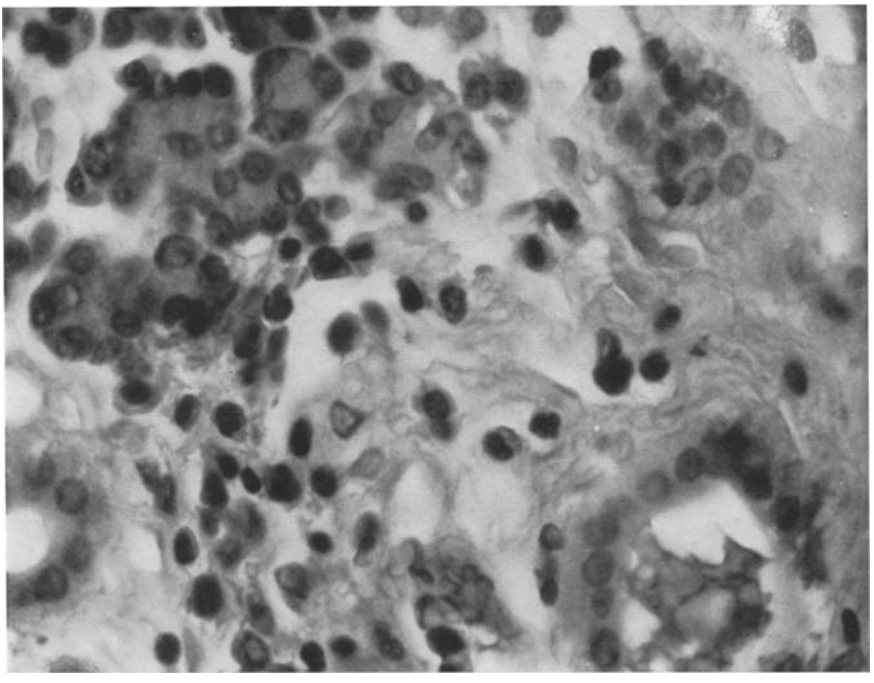

Fig. 1. Mild sialoadenitis in rat treated with saline and challenged with NHP. The parenchyma is intact. Few inflammatory cells infiltrate the interlobular connective tissue. Haematoxylin and eosin ×590

Table 2. Results of immunological tests and histological findings

Pre-treatment	Instillation into parotid gland	Number of rats with precipitating antibodies	Skin test		Histological findings		
			Positive	Negative	Normal	Mild inflammation	Moderate to severe inflammation
NHP	NHP	31	14	—	5	1	8
			—	17	8	2	7
Saline	NHP	0	0	—	—	—	—
			—	12	9	2	1
NHP	Saline	10	5	—	4	1	0
			—	5	4	1	0
Saline	Saline	0	0	—	—	—	—
			—	10	8	2	0

animals in only one, which was injected with saline and challenged with NHP, was a moderate sialoadenitis observed.

The histological alterations were evaluated semiquantitatively on an arbitrary scale of 0 to 3+. Salivary glands in which there were neither inflammatory infiltration nor paren-

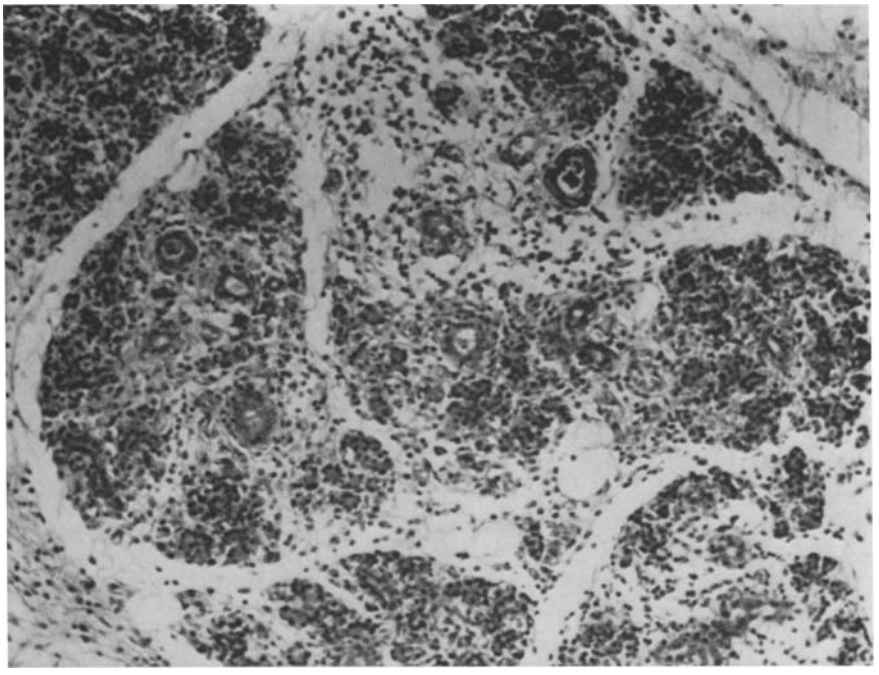

Fig. 2. Severe sialoadenitis in sensitized rat challenged with NHP. Marked acinar loss and severe inflammatory infiltration. Haematoxylin and eosin ×118

chymatous changes were scored 0. Mild sialoadenitis (1+) was characterized by few granulocytes infiltrating the septal connective tissue, the glandular parenchyma being usually intact (Fig. 1). In some rats, however, nuclear debris was seen in a few acinar cells. It should be emphasized that in several control rats pertaining to Group II to IV nuclear debris in acinar cells was seen in the absence of an inflammatory response. Moderate (2+) and severe (3+) sialoadenitis were essentially similar, the difference being one of degree. The inflammatory cells infiltrated the septal as well as intralobular connective tissue and invaded the acinar and ductal epithelium. The inflammatory infiltration consisted mainly of neutrophilic granulocytes; a moderate number of histiocytes and lymphocytes together with a few plasma cells were also present. Loss of acini was conspicuous in severe sialoadenitis (Fig. 2), some distorted acini persisting in the vicinity of the ducts (Fig. 3). Focal coagulation necrosis of parenchymal cells occurred in some lobules (Fig. 4). The left salivary glands, the ducts of which were not intubated, were histologically normal in all instances.

The presence or absence of sialoadenitis did not correlate with the results of skin tests (Table 2). Mild sialoadenitis occurred in 3 of 31 rats of Group I and in 2 of 12 rats of Group II. These cases were excluded from our computations, since NHP instillation per se appears to be noxious to some extent to the tissue. Moderate and severe sialoadenitis occurred in 15 of 31 immunized and 1 of 12 control animals. The difference in the incidence of frank sialoadenitis between the two groups of rats is statistically significant ($X^2 = 4.3$, $0.01 < p < 0.025$). The salivary glands of sensitized (Group III) and nonsensitized (Group IV) rats challenged with saline showed only mild sialoadenitis in four instances.

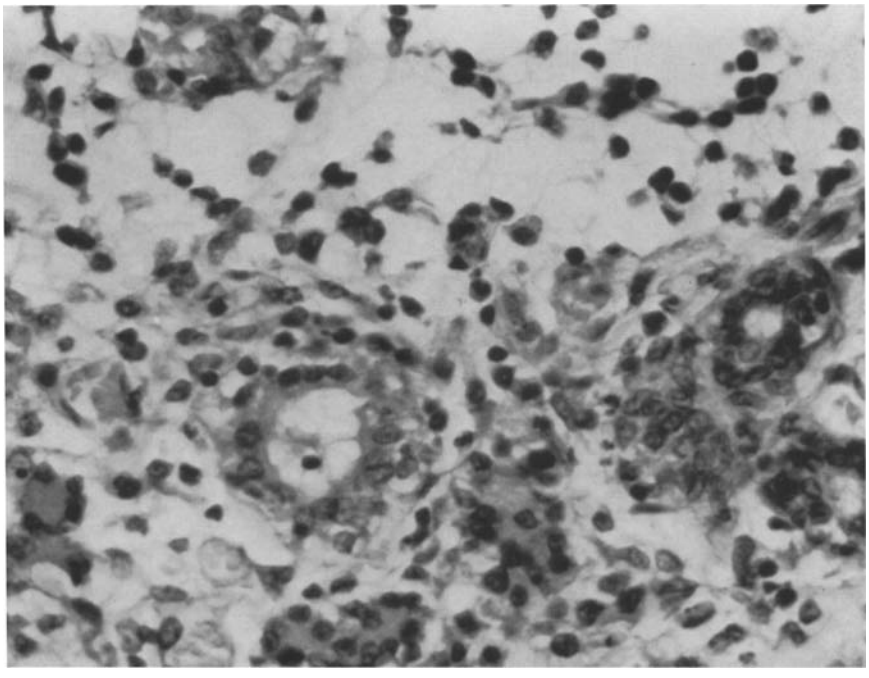

Fig. 3. Higher magnification of Fig. 2 showing an excretory duct surrounded by a mixed inflammatory cell infiltration. There are some distorted acini in the vicinity of the duct. Haematoxylin and eosin ×495

Discussion

Experimental sialoadenitis was induced in a significant number of sensitized rats by the direct challenge of the gland with the homologous preparation. The sensitizing normal human plasma contains a large variety of constituents, at least some of which appear to be noxious to the tissues. Statistical analysis of our results proves, however, that sialoadenitis results from instillation of the antigenic material into the parotid gland of sensitized animals. Histologically, the adenitis is characterized by acute inflammation and parenchymatous alterations leading to acinar loss. Instillation of antigens into the parotid gland of sensitized rats brings upon an immune reaction, which is injurious to the parenchyma.

Precipitating antibodies appeared and delayed skin reactivity occurred in the sensitized rats. The sialoadenitis, therefore, may be related to humoral and/or cellular immunity, in the wake of in situ formation of antigen-antibody complexes and the tissue damaging activity of sensitized lymphoid cells, respectively. Our findings (Table 2) favour the role of humoral antibodies in the evolution of the disease. The effects of antigen-antibody complexes in causing tissue damage are well known (Baumal and Broder, 1968; Cochrane, Unanue and Dixon, 1965; Chan and Cebra, 1968; Ward, Cochrane, and Muller-Eberhard, 1966). Injury to the synovial membrane by an in situ antigen-antibody reaction had been previously described (Dishon, Ginsburg, and Boss, 1969).

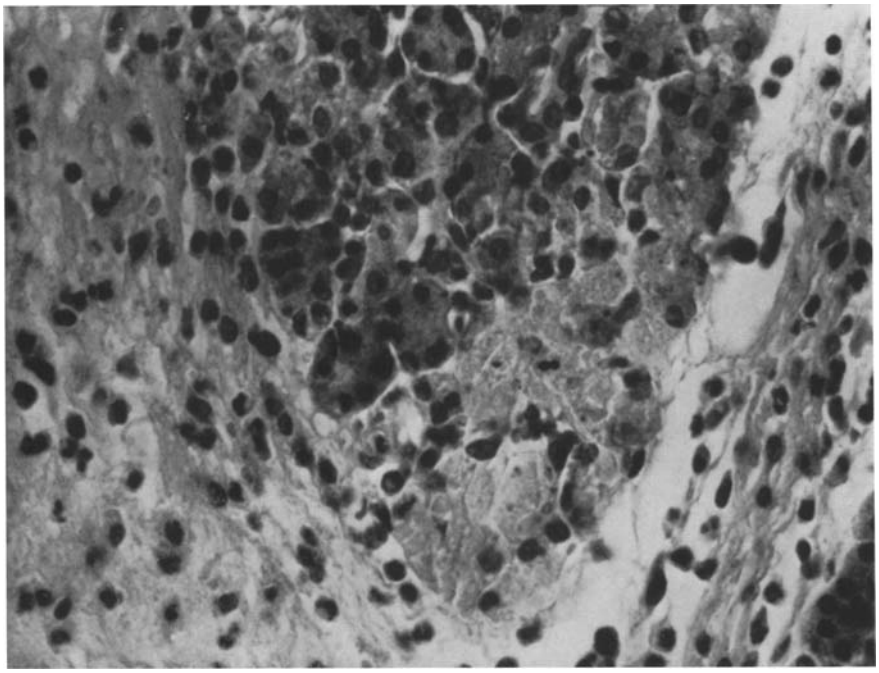

Fig. 4. Sialoadenitis in sensitized rat. Coagulation necrosis of several acini at the periphery of a lobule. Inflammatory cells surrounding and penetrating the necrotic tissue. Haematoxylin and eosin ×495

Information on allergic sialoadenitis in experimental animals is scarce. Spontaneous sialoadenitis, histologically reminiscent of Sjögren's syndrome, occurs in NZB mice (Kessler, 1968). Bevilacqua and Mosca (1968) and Mosca and Bevilacqua (1968) claim to have produced sialoadenitis by the parenteral administration of salivary gland antiserum. Haferkamp (1962b) could induce adenitis only by the successive injection of the antiserum and its homologous preparation. In another experimental setup, Haferkamp (1962a) has succeeded in producing autoimmune sialoadenitis by immunizing rats with an extract of homologous salivary glands. Similar results have been obtained by Chan (1964) in guinea pigs. A novel approach to experimental allergic sialoadenitis is described herein. Inflammation of the parotis results from instillation of antigens into the gland of sensitized rats. It is speculated that formation of immune complexes in situ triggers the chain of events eventuating in parenchymal damage. In view of the method employed, the inflammatory process is ascribed to an Arthus-type of reaction, since it is "produced when a soluble antigen is injected locally into animals which have precipitating antibodies" (Humphrey and White 1970).

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