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Acinar Cell Hydropic Degeneration Induced by Intraductal Instillation of Solutions into the Parotid Glands of the Rats*

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Summary. Saline or BSA were instilled 2, 5, 8 or 11 times at daily intervals into the parotid glands of rats, via Stensen's duct. Hydropic change of the acinar cells developed after 8 and 11 instillations. Histologically, the lesion was characterized by enlargement of the acinar cells, the cytoplasm of which contained vacuoles displacing the nuclei. In view of the negative results of various histochemical reactions the vacuolization was interpreted as hydropic in nature.

Introduction

A variety of non-inflammatory retrogressive changes of the salivary glands have been induced in experimental animals, with parenchymal atrophy as the most common histopathological manifestation. Prime among the non-inflammatory degenerative disorders is sialadenosis, which is morphologically expressed by qualitative alterations of the secretory granules (Seifert and Donath, 1975) and resulting hypofunction of the glands (Rauch and Gorlin, 1959). Animal models of sialadenosis have been produced by diverse insults (Simson, 1972; Leake et al., 1974; Ulmansky and Unger, 1967; Donath et al., 1973). In the present investigation, a peculiar degenerative change of the acini of the parotid gland of rats, hydropic vacuolization of the acinar cells, was induced by repeated intraductal instillations of solutions. Perusal of the literature failed to disclose previous observations of a similar nature.

Materials and Methods

Animals. Albino rats of either sex of the Hebrew University (Sabra) strain, weighing 150–200 gm, were used. The animals had free access to food and water at all times.

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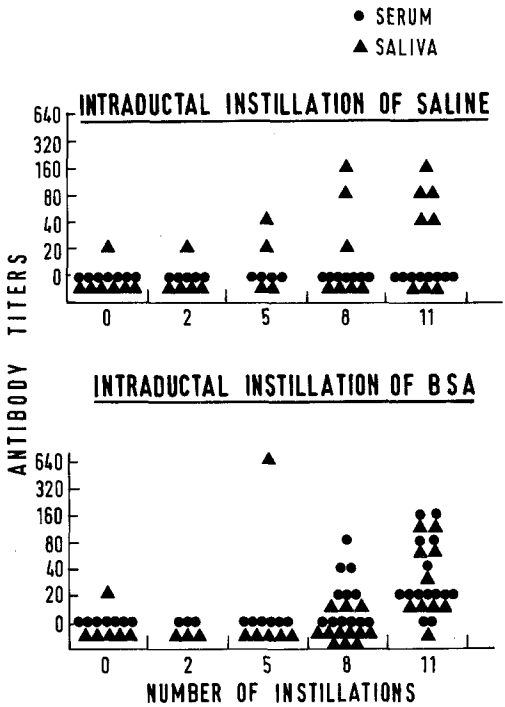

Fig. 1. Scatter diagram of titers of hemagglutinating antibodies in the serum and saliva following intraductal instillations of saline or BSA

Intraductal Instillation. Half a milliliter of physiological saline or a 2% solution of bovine serum albumin (BSA) in saline was instilled into the right parotid gland via Stensen's duct as described previously (Ulmansky et al., 1972). The instillations were repeated at 1 day intervals.

Experimental Design. Fourteen untreated rats served as controls. The parotid ducts of 24 and 35 animals were instilled 2, 5, 8 or 11 times with saline and BSA, respectively, as detailed in the accompanying scatter diagram (Fig. 1). The rats were killed 24 h after the last instillation.

Serological and Histological Examinations. The animals were anesthetized with pentobarbitone, saliva was collected by suction from the orifice of the right parotid duct (Ulmansky et al., 1971), and blood was drawn by cardiac puncture. Anti-BSA antibodies were titrated in the sera and saliva specimens by Avrameas' passive hemagglutination technique (Avrameas et al., 1969) as described in detail elsewhere (Dishon et al., 1975a). The right parotid glands were removed and fixed in buffered formalin. Paraffin embedded sections were cut at 6 μ and stained with hematoxylin and eosin, mucicarmine, colloidal iron and alcian blue. Representative samples of the parotid glands were fixed in alcohol and sections were stained with Best's carmine and periodic acid Schiff reaction. Others were quick frozen in liquid nitrogen, subsequently cryostat sections were stained with sudan IV, sudan black B and oil red O.

Results

Anti-BSA antibodies were not detected in the sera of untreated rats, nor in animals given instillations of saline, or animals subjected to 2–5 injections of BSA into the parotid ducts. Antibodies were found in the serum of 6 of 12

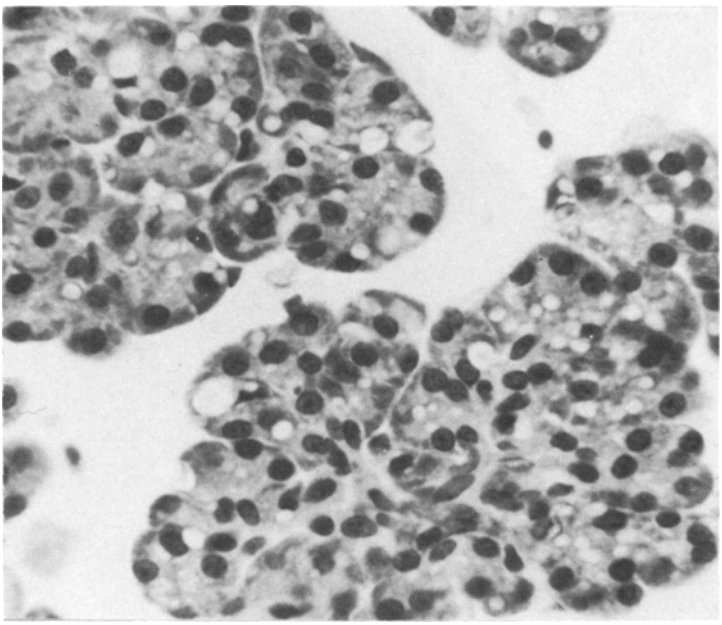

Fig. 2. Vacuolar change involving several acini of two adjacent lobules. The vacuoles vary considerably in size. Haematoxylin and eosin. ×640

and 12 of 14 animals after 8 and 11 instillations of BSA, respectively (Fig. 1). The titers ranged from 20 to 80 after eight and from 20 to 160 after eleven instillations. Salivary anti-BSA antibodies were found in 2 untreated rats. As seen from Figure 1, the incidence of salivary antibodies increased with the number of instillations of either saline or BSA. The antibody titers ranged from 20 to 640. The amount of saliva obtained from 1 untreated rat and 4 of 14 animals given instillations of BSA was insufficient for determination of antibody titers.

The parotid glands of the untreated rats were normal. Two or 5 introductions of saline into the parotid duct were without untoward effect on the glandular parenchyma. The interlobular septa contained a few polymorphonuclear cells in an occasional instance. The parotid glands of rats given 2 or 5 intraductal instillations of BSA were essentially indistinguishable from those treated with saline. A slight inflammatory infiltrate, predominantly comprising PMN's involved the septal as well as intralobular connective tissue of the parotid glands of 3 rats each after 8 and 11 instillations of BSA.

The examination of the salivary glands of rats in which Stensen's ducts were injected 8 or 11 times with saline or BSA revealed histological features consistent with hydropic degeneration in 26 rats. This change developed in 2 of 7 and 5 of 8 animals after 8 and 11 introductions of saline, respectively. It was also seen in 7 of 12 and 12 of 14 animals given 8 and 11 instillations of BSA, respectively. The presence or absence of serum or salivary anti-BSA

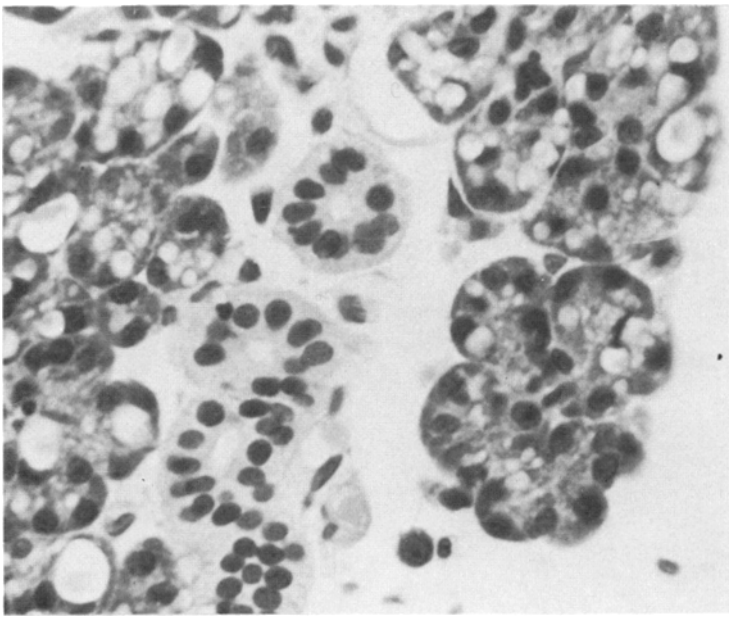

Fig. 3. Severe vacuolar change involving all acini of two adjacent lobules. Many vacuoles are large, displacing and distorting the cytoplasm and nuclei. The ductal epithelium is normal. Haematoxylin and eosin. ×640

antibodies did not correlate with the occurrence of the hydropic change. Lobular architecture of the affected glands was normal; in two instances the degenerative alteration was associated with an inflammatory infiltration. Some lobules were intact while others exhibited vacuolar change involving several or all acini in any one area of the lobules. The number of epithelial cells involved in a particular acinus varied widely. One or more round to oval vacuoles were present in the epithelial cells, and these varied considerably in size from one cell to another (Fig. 2). Some cells contained one or more small vacuoles, while others were greatly distended by several medium-sized vacuoles or a solitary large one. The large vacuoles occupied the apical portion of the cells; the nuclei were smaller than normal, distorted, occasionally crescent shaped, and displaced basalwards (Fig. 3). The myoepithelial cells were unaffected and the ducts and ductules were intact.

Paraffin embedded and frozen tissue samples were examined after applying special staining procedures (Luna, 1968) in an attempt to clarify the nature of the contents of the vacuoles. The presence of glycogen could not be demonstrated by Best's carmine or the PAS techniques. That the vacuoles did not contain mucin, mucosubstances or mucopolysaccharides was evident from the negative results of the mucicarmine, alcian blue and colloidal iron stains, respectively. The contents of the vacuoles were not stained by sudan IV, sudan black B or oil red O. It was therefore concluded that the vacuoles evolved by accumulation of fluid and that the change was hydropic in nature.

Discussion

The presence of anti-BSA antibodies in the saliva of untreated rats or animals given intraductal instillations of saline has been described previously (Dishon et al., 1975a). Formation of these natural antibodies has been ascribed to stimulation of the local immune apparatus by bovine milk constituents present in the food pellets tendered to our rats. The incidence and titers of the antibodies increase following introduction of saline of BSA into the parotid gland (Boss et al., 1975; Dishon et al., 1975b). It is of note that 8 and more particularly 11 instillations of BSA at daily intervals induced the formation of circulating anti-BSA antibodies. It is likely that immunocytes, stimulated by the BSA introduced into the parotid gland, emigrate to the lymph nodes and spleen, where they maintain synthesis of antibodies of the same specificity (Crabbe et al., 1969). The absence of an inflammatory response in most salivary glands negates, to our mind, the notion that the ensuing acinar hydropic change is related to an immunological event. The mild acute parotitis, which occurred in a few instances only, probably developed consequent on sensitization via the intraductal route and reflects an Arthus-like reaction (Sela et al., 1973).

The regressive lesion of the parotid glands evolving after 8 and 11 instillations of BSA or saline is characterized by a vacuolar change of the acinar epithelial cells. In any one gland, the alteration involved several lobules, in which either the majority or only occasional acini might be affected. The process appears to arise by the formation of a few small intra-cytoplasmic blebs, which grow in size and merge with each other, resulting in solitary large vacules which distend the acinar cells. The vacuolization causes displacement of the cytoplasmic constituents and nuclei towards the cell membrane. In view of the failure of histochemical procedures to demonstrate any specific vacuolar content we believe the observed change to be hydropic in nature. The alteration is a non-inflammatory degenerative condition of the salivary glands in the presence of a normal duct system. It is striking that we could not obtain sufficient saliva after the eleventh instillation of BSA in 4 of 14 collections, and would suggest that the observed hyposecretion is related to the underlying acinar degeneration. Obstruction of the excretory system did not ensue following manipulations of Stensen's duct, which was grossly patent for the duration of the experiment, and small ducts were histologically normal. Finally, ligation or injury of the parotid duct in rats causes parenchymal atrophy and mucocele formation (Leake et al., 1974; Donath et al., 1973), neither of which occurred in our rats. Cytoplasmic vacuolization after ligation of the duct is transient and rapidly progresses to destruction of the acini (Donath et al., 1973). It should be stressed that single cell necrosis or dissolution of acini were not evident in our animals. We submit, therefore, that the acinar hydropic degeneration described herein results from repeated introductions of solution under pressure into the parotid gland.

Perusal of the literature has not revealed a description of a similar disorder in experimental animals. One form of sialadenosis, in which the acinar cytoplasm presents a foamy appearance (Donath and Seifert, 1975), is somewhat reminiscent of our findings. However, in sialadenosis, the cytoplasm is foamy because

of a qualitative change of the secretory granules (Seifert and Donath, 1975). The parotid glands of rats, sacrificed a few hours after administration of isoproterenol, exhibit watery swelling of the acinar cells (Simson, 1972). It is believed that isoproterenol causes segmental disruption of the acinar cell membranes, and that transmembrane equilibrium is disturbed with the hyperpermeable membrane facilitating entry of water and other small molecules and ions into the cell. Vacuolization of acinar cells in rats with experimentally induced acute pancreatitis (Kakizaki et al., 1972) is believed to result from retrograde flow accompanied by elevation of the intraductal pressure (Howard et al., 1949). In view of the technique used here, instillation of solutions into the parotid gland may raise the intraductal pressure, damage and alter the permeability characteristics of the acinar cell membranes and bring about increased water entry and vacuolization. The hydropic change of the parotid gland described here has been produced by introduction of solutions into Stensen's duct of rats. Rising intraductal pressure causes escape of fluid through the intercellular spaces, mainly at the acino-ductal junctions (Bockman et al., 1971), and consequently increases the pressure in the interstitial tissue. Our observations point towards a pathogenetic role of disturbances of the interstitial pressure relationships in the causation of hydropic change of the acinar portion of the salivary gland parenchyma.

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