ACUTE DISEASE OF THE SUBMAXILLARY AND HARDERIAN GLANDS (SIALO-DACRYOADENITIS) OF RATS WITH CYTOMEGALY AND NO INCLUSION BODIES

With Comments on Normal Gross and Microscopic Structure of the Exocrine Glands in the Head and Neck of Rats

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Many papers dealing with disorders of the salivary glands in laboratory rodents have been read; no description has been found of clinical and pathologic features comparable to those recorded in this article. Further, in the course of lecturing (J.R.M.I.) many times in the United States and Great Britain on disease of laboratory animals, slides illustrating the macroscopic and microscopic lesions have been shown to hundreds of workers interested in laboratory animal diseases without any indication that others have recognized the condition.* There are no data on this "new disease" in texts on diseases of laboratory animals.[1] It is thus remarkable that the disorder has been seen on two separate occasions (1957 and 1960), affecting many rats in two institutions hundreds of miles apart, and involving two different commercial strains of

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* Since writing this paper, we have learned that two other workers have found the disease in their rat colonies. The first, V. J. Rosen, M.D. (United States Naval Radiological Defense Laboratory, San Francisco) presented us with a section, in which all the changes described by us were found. Later, Dr. Hans Meier (Jackson Memorial Laboratory, Bar Harbor, Maine) intimated in a letter that he had seen the condition. Meier published a letter in *Nature, London* (1960, **188**, 506), on "Spontaneous cytomegalic inclusion-body disease involving lacrimal glands of Caesarian-derived (so-called pathogen-free) rats." He pointed out that all male and female rats from this colony had evidence of inclusion-body disease at 6 months of age. Sections of our material were sent to him. He confirmed our opinion that the sialo-dacryoadenitis bore no resemblance to the cytomegalic inclusion-body disease in rats, nor did there seem to be any etiologic relationship. In some transmission experiments by him with the inclusion body virus, no sialo-dacryoadenitis was produced. He considered that the latter might be induced by an extraneous factor, possibly a toxic chemical. This latter thought was of interest, because in one of our outbreaks the commercial breeder suspected contamination of the food pellets or bedding by ethylene dioxide used in sterilization. However, my (J.R.M.I.) experience of previous years with the chronic exposure of rats to ethylene dioxide in a gassing chamber indicated that "red tears" were common, but no submaxillary gland involvement was ever noted.

rats. The condition has a low mortality and might not be detected in affected animals when alive. Thus the problem is important to workers who pursue transmission experiments in rats, especially with the virus of the cytomegalic inclusion-body disease, for it is impossible to believe the two outbreaks have been the only ones which have occurred. The disease is specific in that the lesions are distinct and easily recognizable, but the cause is unknown. It has some marks of infection. However, any relationship to salivary gland cytomegalic inclusion-body disease encountered in different laboratory species is speculative. The combination of involvement of the submaxillary (but not sublingual or parotid) and the harderian (and not exorbital or intra-orbital lacrimal) glands is an added bizarre feature.

Most of the papers on salivary gland infection of laboratory animals have dealt exclusively with infection by the salivary gland virus group.[2-9] The topic of salivary gland virus disease in human subjects was extensively reviewed by Nelson and Wyatt[10] and is a useful source of directional literature, but there is almost no reference to animals other than investigations concerned with transmission. Much of this work is concerned with cytologic changes in the acinar and duct epithelium, and with the acidophilic intranuclear inclusion bodies rather than pathologic alterations. Host specificity, at least in the case of the salivary gland virus of the guinea pig, seems to be established. In the guinea pig, our experience and that of others indicates that this disorder, under natural conditions, provokes few or no clinical signs, and morphologic changes are generally limited to mild inflammation and intracellular inclusions in the chronically involved salivary glands.[9]

Lyon, Christian and Miller[11] found cytomegalic inclusion bodies in both the intra- and extra-orbital lacrimal glands of adult male rats from 8 separate colonies in the United States; thus, this viral infection is widespread. The infection was mild, and although there was mention of increase in stromal cells and lymphocytic infiltration, the reaction could not have been very intense, for the authors did not stress this or any pathologic aspect of the condition. The salivary glands were not mentioned, so presumably they were not involved; this feature offers the first item that may distinguish the disease described by Lyon and coworkers[11] from the disease described here.

Structure of the Superficial Exocrine Glands of the Head and Neck of the Rat

It is appropriate to refer briefly to the normal anatomy and histologic structure of glands in the head and neck of rats because the facts are not well known except to those whose work has been concerned with

these structures (Figs. 1 to 7). Within the past year, two reports have been brought to our attention in which the exorbital lacrimal gland has been confused with the parotid gland in rodents.

Gross anatomic and histologic differences in the structure of the salivary glands of rats from that in other animals were described in the last century.[12-13] Greene[14] has supplemented these descriptions with modern terminology and excellent diagrams. The paired submaxillary glands of rats are the largest salivary glands. Each gland is a lobulated, encapsulated ellipsoid that is flattened in a dorso-ventral direction. The caudad pole is broader than the cephalad, and the two glands are loosely bound together with connective tissue at the midline. A major sublingual gland is lateral and closely adherent to the cephalad pole of each submaxillary gland. Generally, 4 lymph nodes lie superficial to the two pairs of ventral glands. Lateral to each submaxillary gland is the finely lobulated, uncircumscribed flat tissue of the parotid gland. This may extend laterally from the submaxillary glands to the ear and is partially enveloped by fatty areolar tissue. The latter can be separated from the parotid and is distinguished by its pale white color, in contrast to the gray-pink color of parotid tissue. Well circumscribed lymph nodes may be found also in this region. Lateral to the parotid tissue and just ventral to the ear canal is the encapsulated exorbital lacrimal gland. This is second in size only to the submaxillary gland and can be distinguished from adjacent lymph nodes by this fact; it is also a much darker shade of brown. In location and cell structure, the exorbital lacrimal gland of rodents resembles somewhat the parotid gland of man; hence the confusion sometimes noted in the literature. The remaining pair of intra-orbital lacrimal glands lies at the rim of the bony orbit. They are triangular in shape and similar in color to the exorbital lacrimal glands. Each lies superficial to the bony orbit but immediately adjacent to its more caudad margin.

We could find little information on the normal histologic features of the harderian glands, and nothing on their pathologic anatomy. There is very brief mention in von Möllendorff's text[15] that these structures are more developed in rodents and ruminants (see also Wolff[16]). We could not find any word to denote inflammation of the gland. However, as it is really an accessory lacrimal structure, there is no reason to object to the use of the term "dacryoadenitis" to refer to its inflammation. The harderian glands are yellow-orange in color and occupy much of the orbit deep to the eyeball. The histologic pattern in each gland is briefly described in the legends to Figures 2 to 7.

References to age changes in the parotid glands of rats will be found in Andrew.[17] Appleton[18] referred to the high variability in the size of

the submaxillary glands, not only in untreated control rats of the same sex and litter, but also between the right and left glands. This was also apparent to us after examination of some 40 normal rats.

The Disease

Two separate outbreaks were seen, no whit different from each other from any angle. In the first one, a large number of animals were affected, and over 10 animals were examined at necropsy. In the second outbreak the condition occurred in another strain of rats purchased from another commercial source. Unfortunately, the groups of rats in the second outbreak had been received both in November, 1959, and in January, 1960, and by the time the disease was noticed, the groups had been mixed and some had been treated with whole body x-irradiation. We do not know, thus, whether the condition was present in both groups, whether they acquired it in our own laboratory, or even whether the irradiation had ignited some latent infection brought in or indigenous to our own experimental animals. No rats were bred in our laboratory. Transmission experiments and bacteriologic studies could not be done. There were certainly no deaths in the outbreak in our laboratory, and if any deaths occurred in the first outbreak, they must have been few or had occurred before the condition was detected. No comparable process has been identified in guinea pigs or mice housed in the same animal quarters.

In the well developed stage, the disease was clinically unmistakable. The rats (all immature, noninbred, albino males and females) remained active and seemed to eat well. The neck was grossly swollen, with the head sunk into the neck, so that the rats had the short, hunched head-and-neck appearance of guinea pigs. Many showed "red tears" and red staining around the eyelids due to porphyrin excretion presumably from the harderian glands. The neck was thickened by obviously enlarged submaxillary glands and pronounced edema; both of these combined to produce a "mumps-like" appearance.

Macroscopically, gross gelatinous edema involved the intermandibular space from the front to the base of the neck (in the midst of which lay the swollen, tense submaxillary glands; Fig. 8). Pressure on the great veins entering the thorax caused them to be enormously dilated. All other viscera were normal.

The salivary glands and the harderian glands from a number of affected rats in both outbreaks were examined histologically. Paraffin sections after formol-saline fixation were stained with hematoxylin and eosin, eosin and methylene blue, Lendrum's method, and by the periodic acid-Schiff (PAS) and Giemsa stains.

Histologically, involved submaxillary and harderian glands showed

an acute inflammatory process affecting all parts (Fig. 9). In a few sections the mucin-secreting lobules of the adjacent major sublingual gland were unaffected. The lobules were widely separated from each other by edema and fibrinous exudate; there was infiltration by neutrophils, lymphocytes, histiocytes, and stellate and spindle-shaped connective tissue cells (Figs. 10 and 11). Mast cells were present in small numbers. There was no hemorrhage. The same edematous cellular exudate involved the capsule and the periglandular fat and connective tissue. The parenchyma of the gland was almost unrecognizable. Massive cellular infiltration obscured degenerated alveolar epithelium, but there was no massive necrosis (Fig. 11). Cytologic alterations in the acinar cells were remarkable. The cells and their nuclei were sufficiently enlarged to be considered "cytomegalic." In the many sections studied, however, no inclusion bodies of the types characteristic of salivary gland virus infection could be found in either ductular or acinar epithelium, although some nucleoli were very prominent. The latter may not be unusual in rats of advanced age (see Figs. 7 and 8 in Andrew [17]). There were also remarkable deposits of dense basophilic material in the cytoplasm of the hypertrophied acinar cells. This commonly occurred adjacent to the nuclear membrane or in the basal portion of the cell (Fig. 11). Some degree of this change is normally present in the exorbital lacrimal gland, but it is not observed in the cytoplasm of the submaxillary gland under normal conditions.

One curious feature concerned both the intralobular and interlobular ducts (Figs. 12 and 13). In many areas, their usually well defined columnar epithelium was converted into a mosaic of stratified epithelium that appeared sufficiently extensive to occlude the lumens. This hyperplastic epithelium was not keratinized, and many mitotic figures were present. Some of the parenchymal changes may have resulted from the partial obstruction of these altered intralobular and interlobular ducts. One could postulate, in fact, that the bizarre cytologic alterations might be due to stasis induced by obstruction.

The only other lesion of note was an intense sinus edema and leukocytic infiltration of the regional lymph nodes. This presumably was a phenomenon reflecting drainage from the affected glands.

Why the harderian glands were examined particularly in the first group of affected rats cannot be remembered, except that the production of "red tears" in rats was noted years ago following the administration of different toxic chemical agents. In the rats in both outbreaks an acute inflammatory process also affected the harderian glands (Fig. 12) in the few examined; how frequently this was associated with the acute sialoadenitis is unknown.

COMMENTS

This report is published primarily to draw attention to what we believe to be a new disease of rats. The disorder may be of interest to those in charge of rat colonies or interested in salivary gland virus (cytomegalic inclusion) disease. The cells and nuclei of some degenerated ducts and alveoli were certainly cytomegalic, but beyond this feature any resemblance to the salivary gland virus infection of laboratory rodents ceased. With the possibility in mind that the salivary gland virus still might be at work, and that infection had been introduced from a source of supply of rats, submaxillary glands from a large number of discarded adult breeders (provided by one commercial breeder) were examined; no additional information was forthcoming. However, it is difficult to believe the lesions are anything else but those due to some form of infection.

The metaplastic changes in the epithelium of the ducts and ductules have some similarity to those produced by vitamin A deficiency. However, vitamin A deficiency affects not only salivary glands but many organs.[19] Sections of submaxillary glands of vitamin A-deficient rats from work done many years ago[20] were re-examined with this in mind. Comparison of a gland from a rat, vitamin A-deficient for 3 months, is of interest. There is some resemblance in the parenchymal changes, but the duct alterations are different. In the present disease, metaplasia is complete without keratinization, and ducts are blocked by cell proliferation. In the vitamin A-deficient rat the epithelium shows keratinized metaplastic changes and gross dilation of lumens, perhaps from more distal duct obstruction. To our knowledge, there has been no report of any natural outbreaks of severe chronic vitamin A deficiency in rats. Further, if by chance batches of our pellet diet had been deficient in vitamin A, we would be at a loss to explain why all rats in the same room did not suffer to some degree. Moreover, in each outbreak, there was a sudden upsurge of cases and an equally precipitate disappearance. Furthermore, other manifestations of vitamin A deficiency in different tissues were absent. The same observations hold in the much larger first outbreak which occurred in a colony interbred for commercial rat production and in which thousands of rats of all ages were kept in one room.

To some extent, the appearance of the salivary glands in the present condition was like those at an early stage of ductal obstruction or those in which extracellular destructive phenomena prevented release of the gland secretion. It was totally unlike the progressive atrophy and sclerosis that followed chronic obstruction of major (salivary gland or pancreatic) ducts by stones or after experimental duct ligation.

From the comparative viewpoint the lesions are not without interest. In the colossal tomes written by Duke-Elder,[21] there is a lengthy chapter on conditions of the human lacrimal glands. Here it is stated that acute infection (dacryoadenitis) is very rare in man, and naturally pathologic reports are even more rare. The gland is, however, concomitantly involved in many specific infections, e.g., tuberculosis, syphilis, etc. It is of some moment that Duke-Elder illustrates an altered duct (his Fig. 4707) which shows considerable resemblance to the lesions in rat submaxillary glands.

It may be irrelevant, but Appleton[18] in his studies dealt with the simple hypertrophy of the submaxillary gland following experimental parathyroidectomy. The enlarged gland, other than its increase in actual size, showed no pathologic changes. The enlargement was due to an increase in the volume of individual parenchymal cells. This was estimated by nuclear counts and cell diameters, and by weighing the glands; the weight of the latter was about 0.212 gm. in the normal, and 0.3244 gm. in parathyroidectomized rats.

Summary

Two separate outbreaks of an acute disease (sialo-dacryoadenitis) with low mortality affected the submaxillary and harderian glands of rats. There was a marked swelling of the neck due to enlargement of the salivary glands and inflammation and edema of adjacent areolar tissues. Characteristically, the animals developed "red porphyrin tears" from the harderian part of the complex. Within the submaxillary glands the process was one of acute inflammation with enlargement of parenchymal cells and hyperplasia of duct epithelium but without inclusion bodies. The cause of the disease was not determined, but the inflammatory reaction and epidemic pattern suggested an infectious agent. The condition is distinct from the so-called cytomegalic or salivary gland virus diseases of rodents.

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LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1. Anatomy of the major exocrine glands of the neck, male rat, strain Osborn-Mendel, age 12 months. Ventral view, showing the lobulated submaxillary gland (SM), the major sublingual gland (SL), the parotid gland (P), the exorbital lacrimal gland (E), and lymph nodes (N). × ½.

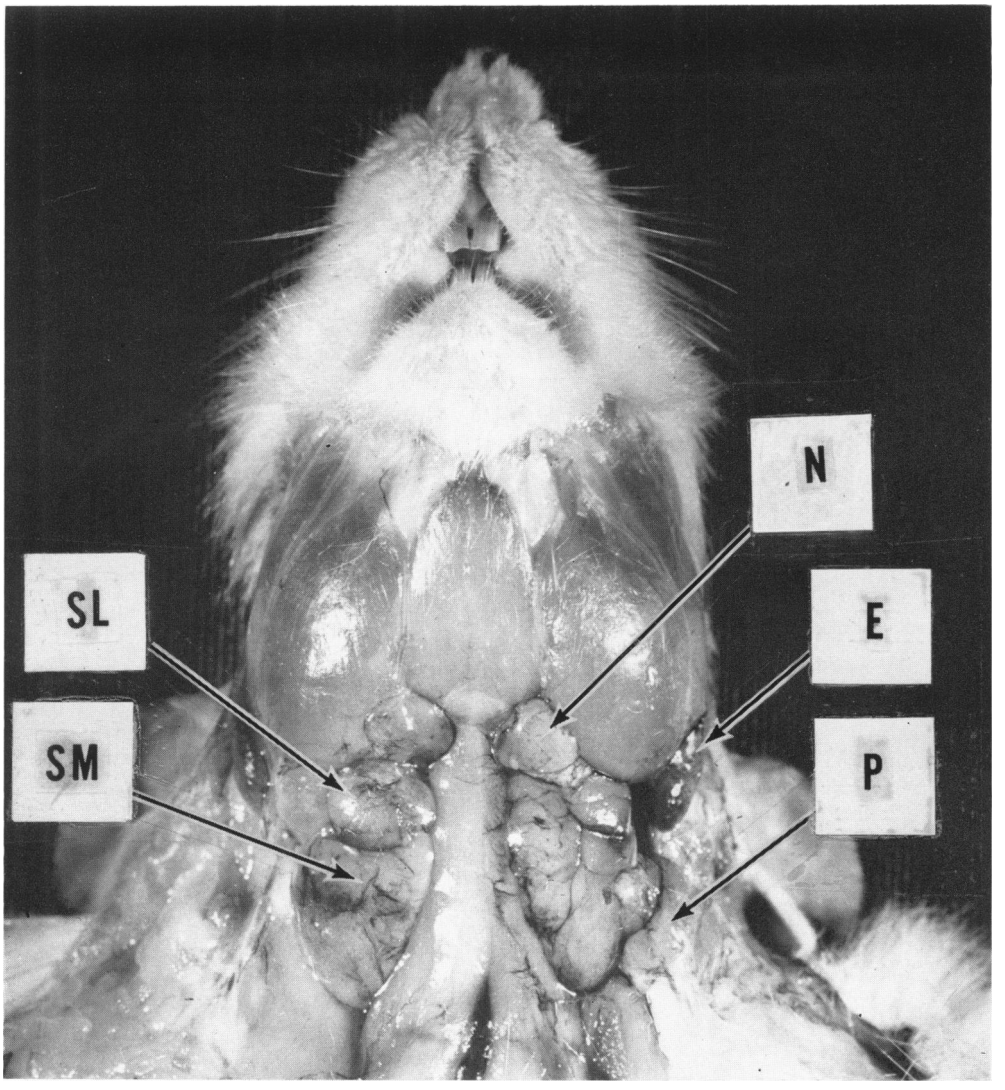

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FIG. 2. Submaxillary gland. The serous cells of the acini contain a granular basophilic cytoplasm and basally placed chromatin-filled nuclei. The ducts are lined by columnar cells with basal striae and intensely eosinophilic cytoplasmic globules. × 340.

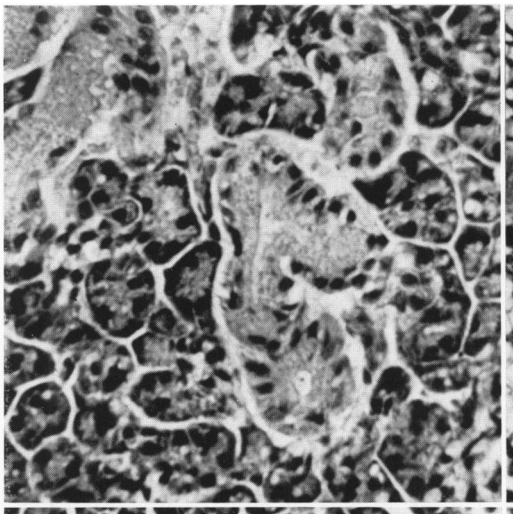
FIG. 3. Sublingual gland. The acini are composed of pyramidal-shaped, mucin-secreting (clear) cells with flattened basal nuclei. The intralobular ducts are lined by columnar cells with eosinophilic striated cytoplasm. × 340.

FIG. 4. Parotid gland. The acinar serous cells are smaller than those in the submaxillary gland and contain a more intensely basophilic cytoplasm that is basally striated and filled with chromophilic granules. The intralobular ducts are lined by striated, eosinophilic columnar cells. × 340.

FIG. 5. Harderian gland. The tubulo-alveolar gland is often confused histologically with the salivary and lacrimal glands. The acini are composed of tall clear cells with vacuolated fat-filled cytoplasm, and with much variation in nuclear size. The lumens of acini and ducts contain deposits and laminated concretions of golden brown pigment not shown in the picture. Ducts are lined by a pseudo-stratified epithelium. × 340.

FIG. 6. Exorbital lacrimal gland. A tubulo-alveolar gland like the major salivary glands, and rather similar to the parotid gland. The acinar cells are much larger, and the nuclei range in shape and size from small ovoid to multilobulated structures which occasionally show mitosis. Note some vacuolated nuclei, a common finding in the lacrimal acinar cells. Intralobular ducts are lined by flattened cuboidal epithelium. × 340.

FIG. 7. Intra-orbital lacrimal gland. The gland is identical in structure to that of the exorbital lacrimal gland. However, this section illustrates the degree of artifactual atypism which may occur in a gland left unfixed for 4 hours after death. Such changes have led to the mistaken identification of this gland as a tumor. × 340.



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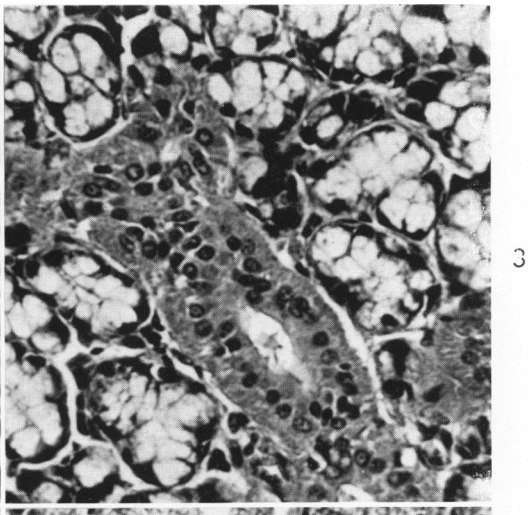

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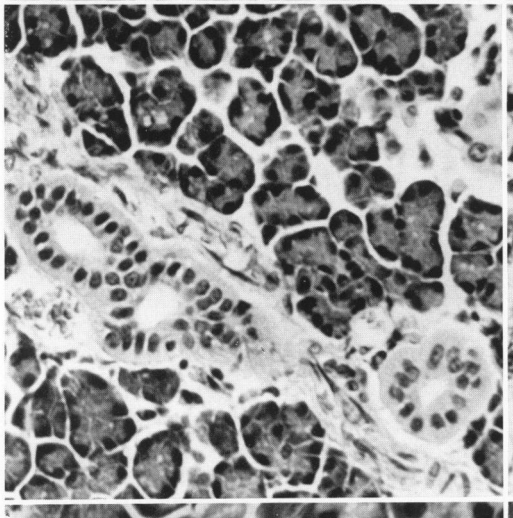

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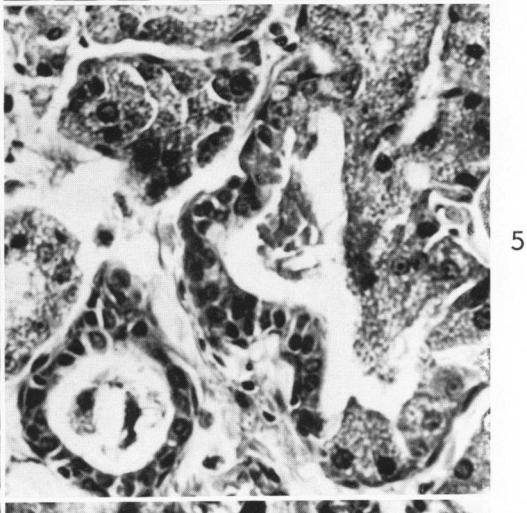

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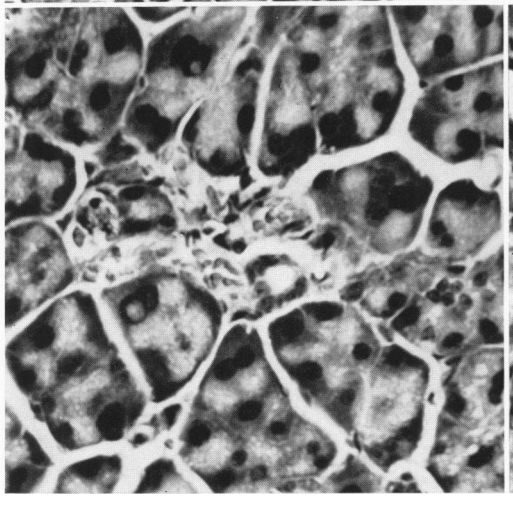

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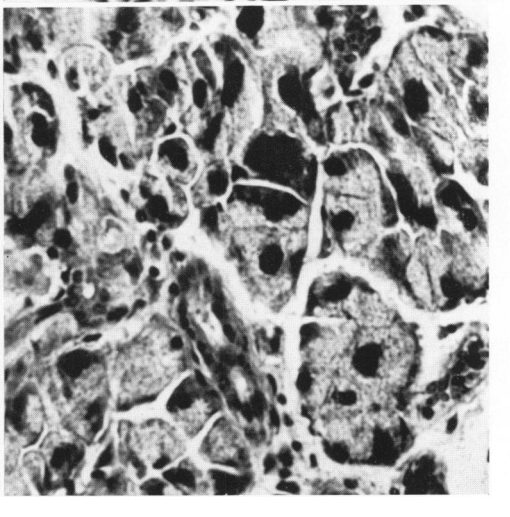

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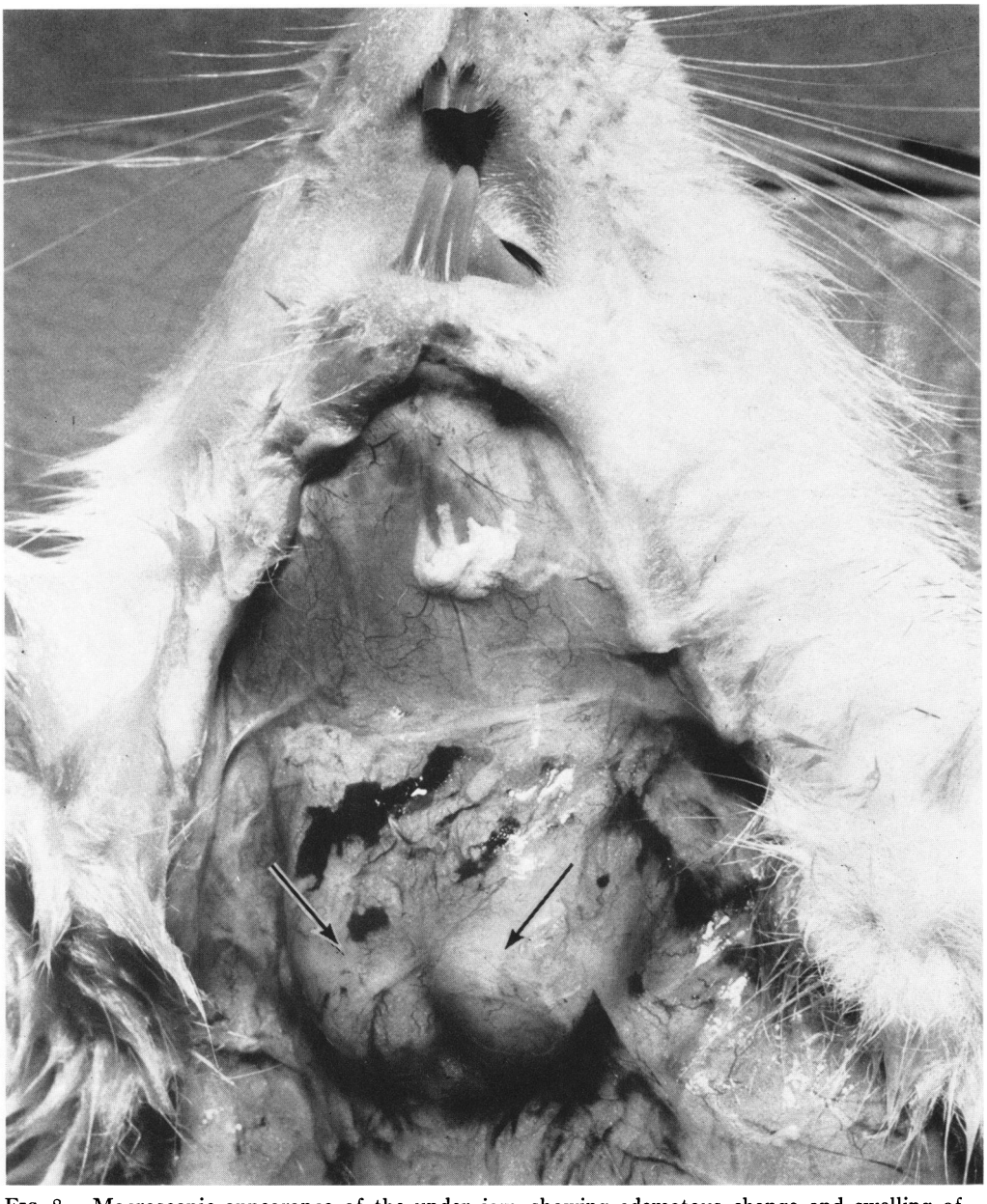
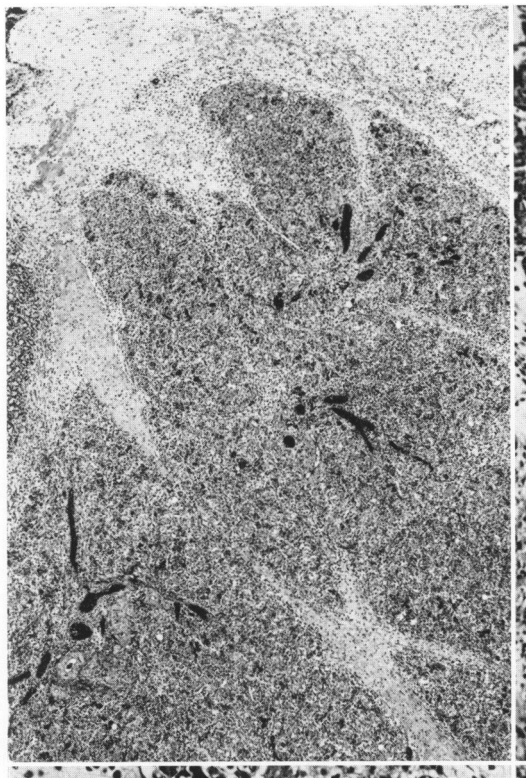


FIG. 8. Macroscopic appearance of the under jaw, showing edematous change and swelling of the submaxillary glands (arrows), lymph nodes, and interstitial tissues of a severely affected rat. Compare with Figure 1.

FIG. 9. Sialoadenitis, submaxillary gland. There is gross interlobular edema and inflammatory reaction; ducts (see Fig. 13) stand out as dark streaks. × 25.

FIGS. 10 and 11. Affected submaxillary glands (two separate outbreaks), showing diffuse inflammatory edema and cellular reaction. × 100.

FIG. 12. Affected harderian gland showing degenerating acini and interstitial inflammatory reaction. × 100.



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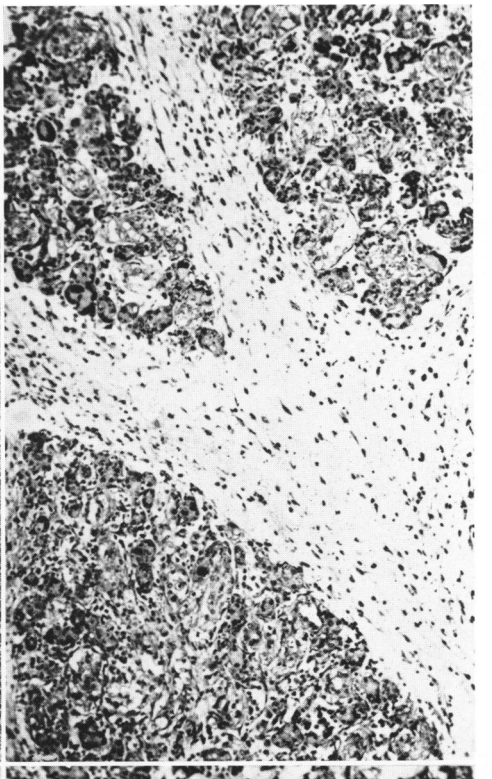

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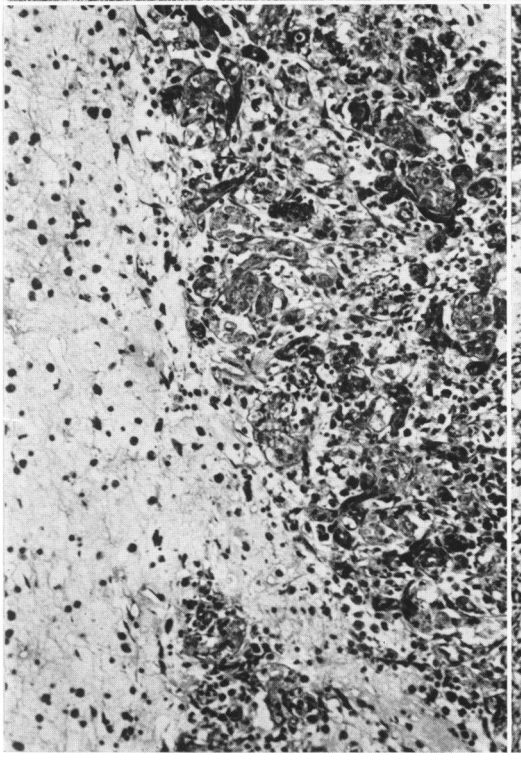

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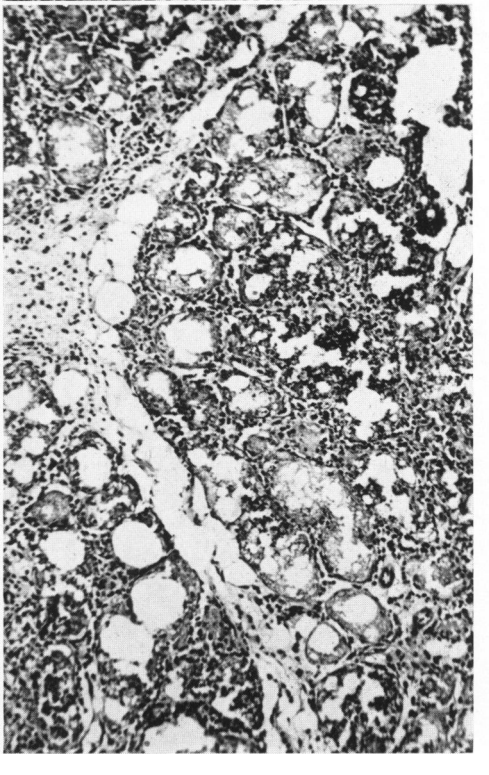

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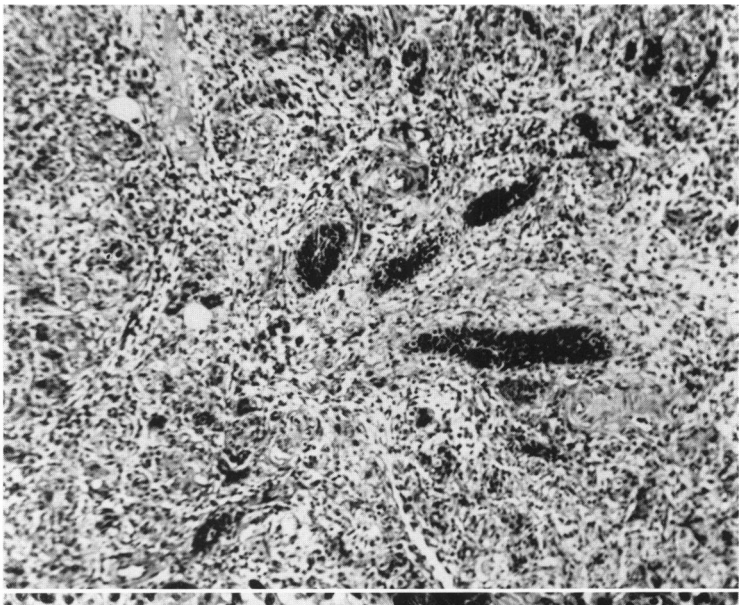

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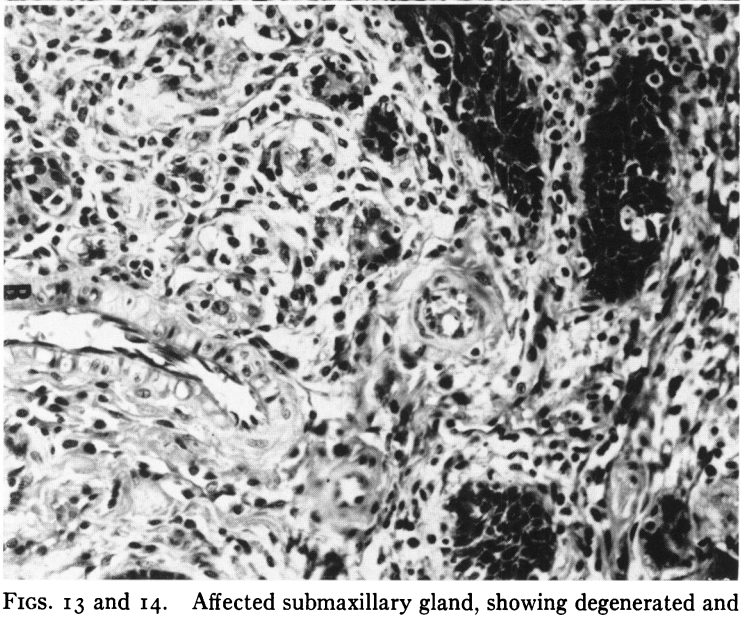

FIGS. 13 and 14. Affected submaxillary gland, showing degenerated and hypertrophied acinar cells and nonkeratinizing proliferation of duct epithelium with consequent blockage. Fig. 13, × 200; Fig. 14, × 400.