EXPERIMENTALLY INDUCED CHANGES IN NASAL MUCOUS SECRETORY SYSTEMS AND THEIR EFFECT ON VIRUS INFECTION IN CHICKENS

I. Effect on Mucosal Morphology and Function*

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The recognized role of the nose as an entry into the respiratory tract obscures the fact that many of the organ systems associated with the nose evolved to ensure function of the olfactory membrane. The functional association between nose and lungs in the air-breathing vertebrates has obviously been successful in providing preconditioned air for both systems, but the nasal organ systems function separately from the respiratory system to a notable degree: the nerve supply (1) and the vascular (2), lymphatic (3), and mucociliary (4) systems have considerable local autonomy, and infections to which the lower tract tissues are susceptible may be confined entirely to the nose (5, 6). We have found also that within the nose the mucus-producing systems associated with lacrimal, respiratory, and olfactory functions secrete particular types of mucus focally—types which while miscible do not homogenize as they form the surface blanket of nasal mucus.

This area specificity of mucus secretions raised the question whether particular areas of mucosa might respond differently to physiological changes in a host animal, and a corollary question whether such changes would affect susceptibility to respiratory virus infections. We have induced physiological changes in chickens by eight basically different means in order to observe the effects on nasal secretory systems and, when feasible, the effects on mucociliary flow rates. Some, but not all, of these changes have been correlated with experimental viral pathogenesis and will be discussed in the succeeding paper (7).

Materials and Methods

Unless otherwise specified, white Leghorn commercial stock chickens were used. Most were from 1 day to 2 wk old; a few were 8 wk. For timing mucociliary flow rates, we had previously found that rates could be timed directly on the flat septal mucosae of heads sagittally sectioned

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and timed within 30 sec of death (4). Since the virus studies are chiefly on baby or young chicks whose septa are too small for this method, it was found more practical to time clearance of 1 drop of India ink solution from the nose in vivo. 1 drop of a 50% solution of India ink in normal saline was placed on an external naris until it was inhaled by the chick in normal breathing. Counts began from the moment of inhalation, and "clearance time" was the time required for the total load to clear out of the terminal attachment of the maxillary concha. The method had been developed in 24 clinically healthy chicks from the same commercial source, ranging in age from 2 to 10 days. In these, the individual variation in total clearance time ranged from 3.5 to 15 min. 15 min was therefore used (unless otherwise stated) as an arbitrary maximal clearance time. Thus, in most test chicks, heads were sagittally sectioned 15 min after ink was instilled, and the amount of residual ink in the inner scroll of the maxillary concha was recorded as either entirely full, moderately full, showing a terminal trail still clearing out, or entirely clear.

Whole tissue mounts were prepared for evaluation of the over-all effects of physiological changes on the mucous acini of the septum and maxillary conchae. For these, heads were fixed in formol-alcohol (1:2), stained with periodic acid–Schiff, and cleared in oil of anise according to the method of Moe (8) for viewing in the dissecting microscope by transmitted light.

For histology and histochemistry, heads of freshly killed birds were fixed in 10% formalin, 10% formalin incorporating 2% calcium acetate, or Bouin's fluid, decalcified in 2% nitric acid, and neutralized in 5% sodium sulfate. 6 μ sections were stained in hematoxylin and eosin and/or the combined Alcian blue–periodic acid–Schiff stain with the Alcian blue at 1% and at pH 1.0 (9).

Functional Morphology.—

Nasal structure and function: From the point of view of a virus, the nasal chamber is a dynamic environment in which air, particles, and mucus move at varying rates of speed and often in countercurrent directions. Inhaled air is probably vaporized as it passes through the vestibule, since serous nasal gland secretions accumulate there and are evidently atomized at inspiration (10). Airborne particles usually enter the nose by way of the nostrils; less often by way of the lacrimal ducts or mouth. Most inhaled particles are trapped in mucus and dumped into the throat within about 10 min (4), so that a virus particle must penetrate the mucus blanket within narrow time limits if it is to infect a cell.

In Fig. 1 we have attempted to diagram the mucociliary currents of the lateral wall of the chicken nose, as well as a concept of the initial thrust of air currents which enter narially. Fig. 1 A shows that traction removes olfactory secretions from the nonciliated receptor membrane (4), and that all mucociliary flow is initially directed away from that membrane. The mucus sheet then moves over the outer convexity of the maxillary turbinal scroll, then inside its concavity and out of the terminal attachment (inset), where it converges with sheets coming from the septum, lacrimal ducts, and sinuses, and moves into the pharynx to be swallowed. We have observed (4) that mucociliary rates are significantly slower on the anterior part of the septum and significantly swifter in areas surrounding the olfactory membrane and along the roof and floor of the nose.

Fig. 1 B indicates that the inhaled airstream is initially countercurrent to the direction of mucociliary flow on the maxillary concha, which would increase the force of impact of inhaled particles onto the mucus blanket. The pitch of the floor of the vestibule, plus constriction of the airstream between conchae and septum (cross-sections 1 and 2), would send part of the stream fanwise toward the arch of the roof and the olfactory chamber, while the lower portion of the stream would be released into the main airway leading to the pharynx; Proetz (11) calls this constriction and release the "nozzle effect." The rate of breathing would affect impact sites, in that rapid breathing or sniffing causes eddying. Much of the inhaled particulate matter seems to be intercepted by the large mucociliated concha; we found that in four chickens which in-

haled osmium vapor for 2 min, the most intensive staining was produced on the upper anterior convexity of that concha.

Looking at the pathways of mucociliary flow (Fig. 1 A), it is obvious that particles which land on or posterior to this scrolled concha would have the longest journey to the pharynx and the maximum time to penetrate the blanket. One would then expect the terminal part of the inner scroll to be most vulnerable to initial infection (2). Particles which hit the olfactory mem-

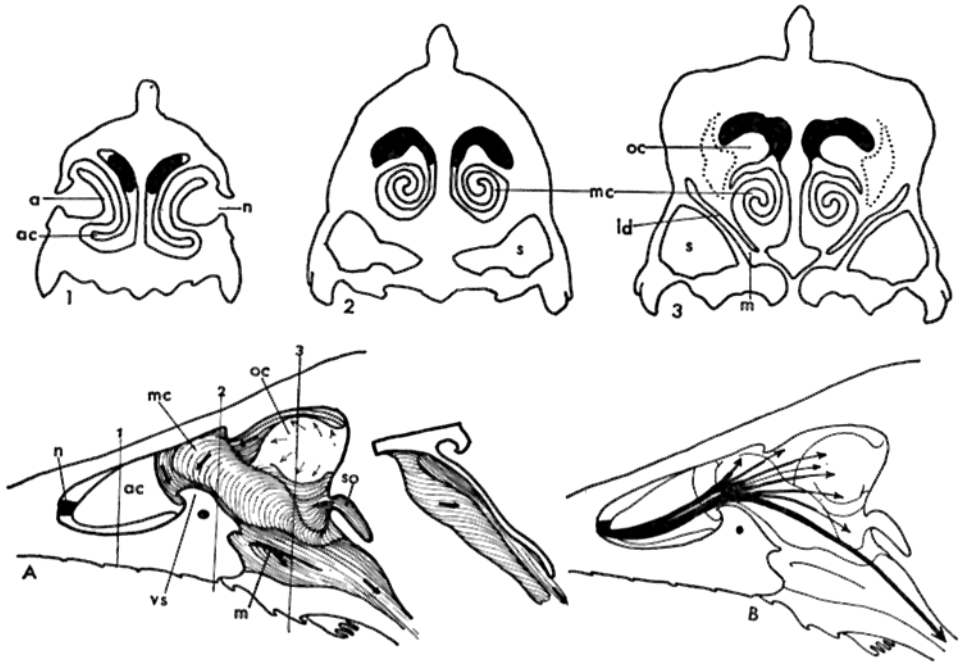


FIG. 1. Dynamic anatomy of chicken nasal fossa.

a, atrial concha	*n*, nostril
ac, anterior concha	*oc*, olfactory concha
ld, lacrimal duct	*s*, sinus
m, mouth of lacrimal duct	*so*, sinus ostium
mc, maxillary concha	*vs*, vestibular *schwelle*, or raised posterior limit

brane would have an even longer journey and would be stuck in acid mucus, as will be seen below.

In a normally functioning nose, then, the site of impact of a virus could determine the nature of the mucus in which it is stuck and the amount of time it might have to penetrate the mucous blanket and infect a cell. How much "rolling" of nasal mucus may take place, as described by Proetz for the trachea (11), is not known.

Nasal mucus: Histochemical staining with the combined Alcian blue and periodic acid–Schiff (AB-PAS) stain, with the AB at pH 1, selectively stains acid sulfomucins a clear blue-green and neutral mucopolysaccharides a bright fuchsia. Mucus comprising mixtures of the two types stains various shades of purple, in which either blue or red may predominate. In describing particular systems, the terms "AB mucus," "PAS mucus," etc., rather than the longer

"AB-positive . . .," will be used. Figs. 2 and 3 are histological sections at two levels in the nose which show most of the sources of the intransal mucous sheet.

Briefly, the olfactory (Bowman's) gland acini secrete a clear AB mucus which forms a distinct surface sheet. The complex of deep branched acini surrounding the olfactory membrane—including those of the sinus ostia—produces two morphologically distinct types of PAS mucus, one granular and one homogeneous and translucent. All glands of the lacrimal complex discharge ultimately into the nasolacrimal ducts, and thence into the nasal fossa on the lateral wall near the floor. While Harderian gland mucus is clear AB and that in the acini of the ducts proper is predominantly AB, the posterior lacrimal gland (which does not show in either section) and many of the goblet cells throughout the epithelia are PAS. The closely aligned, richly staining, flask-shaped acini along the main airway and nasal floor are quite distinctly zoned: in the base or bowl portion the mucus is mixed, with blue predominating, while the neck portion is almost entirely PAS, as are most goblet cells in the mucociliated epithelium. There is markedly less AB in the bowls in adult chickens than in young chicks.

In general, then, the olfactory and lacrimal systems produce principally acid mucus, while the paraolfactory areas, sinuses, and goblet cells produce principally neutral. The "respiratory area" acini are a mixed purple, and their neck cells are entirely PAS. The mucous blanket within the nose is thus a varying, unhomogenized, continually renewed mixture of mucous types.

EXPERIMENTAL RESULTS

Normal nasal dynamics were changed by drugs, systemic dehydration, temperature, diet, germfree environment, and cold.

Cocaine (Marked Temporary Arrest of Cilia).—Using two or three chicks for each time group, 8–10 day old chicks were inoculated with 4 drops/nostril of 20% cocaine solution in distilled water, and 15 min later with 1 drop of ink solution, and were killed at 15, 30, 60, and 90 min following the ink instillation. At 15 and 30 min ink was almost fully retained on and in the maxillary concha, and when surface mucus was removed essentially no ciliary beating was seen. At 60 min ink was retained only in the terminal part of the scroll, and roughly 75% of cilia were beating. By 90 min there was no retention of ink, and cilia appeared to function throughout the nose. Histologically there was no evident effect at 15 min, but at 60 min a number of acini on the lateral wall adjacent to the terminal scroll showed mucus anchored within cells (Fig. 5), presumably because of residual ciliary paralysis.

Repeated Cocaine Instillation (Acute Prolonged Acinar Depletion and Ciliary Paralysis).—Twenty-four 2 day old chicks were inoculated intranasally with 3 drops of 20% cocaine three successive times, the second 6 hr after the first, and the third 12 hr later. 24 hr following the last inoculation, both of two chicks failed to transport ink, and in each of four whole mounts the mucous acini of the septa were acutely depleted of mucus, especially in the posterior two-thirds of the fossa (Fig. 17). Even more extraordinary was the effect as seen on histochemically stained sections, in which the mucus in the acini of the maxillary conchae was so depleted that individual gland cells had lost definition, mucus was completely lacking from the acini of the inner lining of the conchae, and there was no continuous mucous blanket—large irregular heaps or piles

of mucus occurred in patches on the surface or free in the lumen. At 48 hr mucus was retained in all acinar cells and acinar lumens and there were great accumulations of surface mucus. Lacrimal duct acini were less abnormal than those in the main fossa. The division of acinar cells into differentially stained bowl and neck portions was lost. In this group the cocaine evidently had a direct effect on the process of mucous secretion, as well as paralyzing the cilia.

Hexylcaine Hydrochloride (Epithelial Destruction, Acinar Depletion).—After preliminary steps to establish a dosage which would desquamate the epithelium but would be nonfatal, 4 drops/nostril of hexylcaine hydrochloride 5% (12) were nasally instilled in twenty-four 10 day old chicks, followed 5 min later by 1 drop of ink solution; effects were observed at intervals from 5 to 5½ days. As observed by the combined methods of flow rates, whole mounts, and histology, these may be summarized as follows: within 5–10 min there was immediate general epithelial sloughing, including the olfactory membrane; appearance of viscous amber floccular mucous exudate; and partial or severe exhaustion of acinar mucus (Fig. 8). By 3½ days acinar mucus was still exhausted (Fig. 18), and by 5½ days regeneration of surface epithelium not only was incomplete, but in one specimen the glands of Bowman were necrotic and filled with lymphocytes.

Four additional chicks were similarly instilled, immediately given a thorough nasal douche with warm saline, then inoculated with 1 drop of ink. Results were the same as those obtained without the nasal douche.

Ether (No Apparent Effect).—Six 12 day old chickens were anesthetized with ether by placing them in a closed, 2 gal container with a small wad of ether-saturated cotton. As soon as they lost consciousness, they were removed, 1 drop of ink solution was instilled nasally, and they were killed 2 min later. In all cases ink was moving swiftly, smoothly, and at normal rates. Three additional chicks were reanesthetized three additional times each, with the same lack of effect on mucociliary flow rates

72 hr Water Deprivation (Deceleration or Arrest of Mucociliary Flow and of Mucus Secretion).—Chickens aged 1 or 2 days and 8 wk were deprived of water but not of dry mash food for 72 hr; all ages survived without fatality, even though the baby chicks showed an average weight loss of 11.1 g. In 8 wk chickens, flow was entirely arrested on most maxillary conchae at about 72 hr. Histologically, acinar cells were completely gaping, individual cells had lost the limiting membranes, and stringy mucus, stuck in all of the acini, was anchored to the immobilized surface sheet (Fig. 15). Effects were less regular in baby chicks, and were evidently less severe, for in no case was there complete arrest of blanket motion. However, histochemically stained slides showed marked reduction in the height of all mucosae, and the engorged yet compressed acini were so intensively stained dark blue-violet that all distinction between the acid and neutral components was lost.

Six of the chicks which had been dehydrated for 72 hr were then given water ad libitum and killed 30 min later, and their heads were stained histochemically. Acini along the airway were a translucent, rich blue, with all lumens full of lighter blue secretion; the paraolfactory PAS acinar complex also had secretion in the lumens, but relatively less. In this case there seemed to be intensive hypersecretion of AB mucus and relatively normal secretion of the PAS component.

Six of the chickens which had been dehydrated for 72 hr were given 0.25 mg of 6:1000 pilocarpine and killed after 45 min. Acini throughout the fossa were extraordinarily depleted, so that only a few cells in the base of each acinus contained stained secretions. There seemed to be relatively fewer AB than PAS cells generally in these acini.

Vitamin A Deprivation (Reduction of Mucosal Depth, Loss of Staining Intensity, Moderate Deceleration of Flow Rate).—From the time of hatching until the age of 18 or 21 days, 15 chicks were fed on a diet lacking vitamin A. At 21 days, flow rates were tested on six of the 21 day group by direct examination of sagittally sectioned heads 15 min after narial instillation of a drop of India ink solution. In each of these a trail of ink-laden mucus continued to remain inside the inner scroll of the turbinate, where it could be clearly observed to move slowly but steadily, requiring 30–45 min total time, in contrast to complete clearance by 15–20 min in six controls.

Whole mounts showed acini in the anterior portion of the nasal septum to be less affected than those elsewhere in the fossa (Fig. 19), while those of the conchal inner lining were exceptionally depleted of mucus and also reduced in diameter (Fig. 20). Histology (Fig. 10) and histochemical staining together showed marked reduction in the height of all mucosae, an actual 50% reduction in the numbers of gland cells per individual acinus (from an average of 40 to an average of 18 on the outer surface of the maxillary concha), and about 50% reduction in staining intensity.

While there was no evidence of actual keratinization of the epithelium, the effects conform to a description of the first phases of keratinizing metaplasia (13).

Three chickens which had been raised for 18 days on a vitamin A–deprived diet were put on a normal diet for 5 days; at the end of the 5 day period, mucociliary flow rates were essentially normal, and whole mounts showed good progress toward normalcy even on the conchal inner lining (Fig. 20), effects which also conform to the reversibility of keratinizing metaplasia after 5 days of normal diet in rats (13).

Pilocarpine (Intensive Acinar Hypersecretion, Followed by Depletion and Gradual Recovery).—After preliminary tests to determine effective but nonlethal dosages, 0.25 mg of a 6:1000 solution of crystalline pilocarpine in normal saline was injected subcutaneously under the scalp of 10 day old chickens, and

0.2 mg of the same solution was injected the same way into 2–3 day old chicks. While flow rates can be successfully timed after administering this drug to 8 wk old chickens, we have not yet obtained satisfactory flow rate measurements with either individuals or averaged groups of younger chicks. By simple visual observation, when ink was placed along the maxillary conchal attachment, it was rapidly carried away into the pharynx in a great outpouring of mucus. This initial hypersecretion of mucus, which seemed of normal consistency when picked up on a pin point, lasted 5–10 min and gradually slackened pace by 30 min. By 45–60 min, the rate of flow was much decelerated and the mucus was very tacky when picked up with a pin point. In most, but not all, chicks the secretion was relatively normal at 90 min.

Histological sections of conchae of 8 wk chickens killed 5 min after pilocarpine injection showed all acinar glands simultaneously releasing mucus (Fig. 9). Histochemical staining of the younger chicks at 20 min showed distinct loss of the AB component, and at 30 min (the time of greatest apparent acinar exhaustion) many gland cells in each acinus lacked any secretion and most acinar mouths were gaping (Fig. 6). Lacrimal duct and Bowman's gland acini did not lose the AB component, but were reduced in size and stained less intensely.

Germfree Environment (Reduction of Mucosal Depth and of Acinar Size).—Six commercial stock chickens were hatched and raised under germfree conditions for 6 wk in the Lobund Laboratory, Notre Dame University, under the direction of Dr. Morris Pollard. In comparison with six commercial stock control chickens of the same age (Figs. 13 and 14), these showed histologically a remarkable reduction in over-all mucosal depth in all areas of the fossa, including the olfactory membrane and lacrimal ducts, and a most remarkable reduction in the depth of the normally deep-branched paraolfactory complex. There were two striking changes in the acinar neck area: a relative reduction in the total number of neck cells and a relative increase in the number of ciliated cells. While acinar depth along the nasal floor at about midfossa reached counts of over 100 gland cells per acinus in control chickens, acini in this area in the germfree group rarely contained more than 20 cells. Histochemically the mucus in the acini of the main airways was so dense and opaque that individual granules were scarcely distinguishable, giving the strong impression of inhibition of normal rates of excretion (a point that could be clarified only by radioautography).

Temperature of −20°F for 1 hr (Marked Hyposecretion in all Mucus Secretory Systems).—2 wk old chickens were exposed to a temperature of −20°F for ½ hr, and after being killed their heads were fixed for histochemical staining. Body temperatures were 26°C and 29°C at death; controls were 42°C. Mucosal depth was sharply reduced throughout the nasal fossa, goblet cells were almost entirely lacking, and mucous acini throughout looked shrunken, pale, and rather desiccated, especially along the main airway (Fig. 12). The deeply branched

paraolfactory complex of acini was much depleted. There was no relative depletion of either the AB or the PAS component. In this case the reduction in mucosal depth appears histologically to be due to the combined effects of vascular contraction and depression of the secretory process.

Six other 2 wk old chicks were exposed continuously for 2 hr to 41.5°F; two were then fixed, two were returned to room (83.5°F) temperature for 1 hr and then fixed, and the remaining two were fixed 2 hr after being returned alive to room temperature. Body temperatures in the three groups, respectively, were 36°C, 41.5°C, and 42°C.

The effects of vascular, mucosal, and acinar contraction were not nearly so marked as in the −20°F group. But even after 2 hr at normal temperatures, acinar secretions were of irregular intensity and surface secretions were thick.

DISCUSSION

Normal nasal mucociliary flow rates in any individual depend on ciliary rates plus secretion of mucus of a particular viscosity. Seven of the eight methods of altering host physiology which we tested affected either cilia or mucus or both. Cilia were directly affected when paralyzed by cocaine and, of course, when desquamated by hexylcaine. Mucus quantity and quality as judged morphologically by histochemical staining were affected in a different way by each of the seven methods.

There was no evidence that any of the area-specific (lacrimal, olfactory, etc.) systems was selectively affected quantitatively by any test method: if there was hyper- or hyposecretion or reduction in acinar size, it obtained generally. This was usually true also of mucus quality; the single exception was that germfree rearing induced intensive staining (reduced rate of excretion?) of the flask-shaped mixed acini, yet drastic depletion of the paraolfactory PAS complex.

With a few exceptions, there was no clear evidence that the normal balance between the AB and PAS components in the mixed acini was altered either. But there was distinct loss of the normal zoning of these components after exposure to intense cold, after repeated cocaine injections, and after both pilocarpine stimulation and rehydration of severely dehydrated chicks. In the latter case there was an apparent marked selective increase of AB secretion. Any less clear-cut differences were disregarded because of consistent moderate individual differences in staining in chickens in any one group, even though they were processed simultaneously.

The normal area-specific differences in mucus quality and acinar structure might be related to the fact that the surface mucosae in each area subserve either primary or accessory olfactory functions (14–16) and are innervated by branches of different cranial nerves (nn I, V, IX, X), as are the glands of Harder [n II (1)]. Thus specialized functions of odor detection may be subserved by particular mucin types.

At any rate, it is clear that the respective areas serve local functions to some extent, and it is known that innervation, secretory types, and lymphoid responses are also fairly localized. The spectrum of effects produced by altered physiology emphasizes how little is known about factors which control synthesis of mucous or serous secretions in the nose (17).

The individual variation of about 4-fold in control flow rates in young chicks could be due to normal individual variation as found in adult human nasal flow rates, which is over 10-fold (18), or to age, to chance incorporation of ink in a fast or slow ciliary pathway, to relative individual hydration, or to combinations of these. We are working to standardize procedure and environment further in order to minimize the variables.

SUMMARY

The domestic chicken was used as an experimental model in which to demonstrate morphological and functional relationships of nasal organ systems, principally of mucous systems. Mucous secretions of olfactory, respiratory, lacrimal, and accessory areas were found to have clear histochemical differences, yet were sufficiently miscible in normal circumstances to form an unbroken, synchronously moving sheet. Changes induced experimentally in host physiology did not all affect the mucous components of given areas in the same way or to the same degree. Mucosal changes were produced by the following methods:

Topically administered cocaine 20%, in a single application, temporarily paralyzed the cilia, and the consequently reduced traction apparently held mucus in the acini and effected a temporary lag in mucus excretion. Three successive applications caused acute acinar depletion and ciliary paralysis.

Hexylcaine chloride 5% immediately desquamated all intranasal epithelia, damaged the proximal portion of the acini, and induced acinar exhaustion and mucosal inflammation—effects not overcome within 5½ days.

Internal dehydration produced progressively viscous mucus, severe acinar gaping with mucus anchored in the acini, a heavy surface sheet, and deceleration or arrest of mucociliary flow.

Avitaminosis A induced reduction in the height (about 50%) of all mucosae and acini, especially the inner lining of the maxillary concha, caused an actual 50% reduction in the number of cells per acinus, and retarded the mucociliary flow rate about 50%.

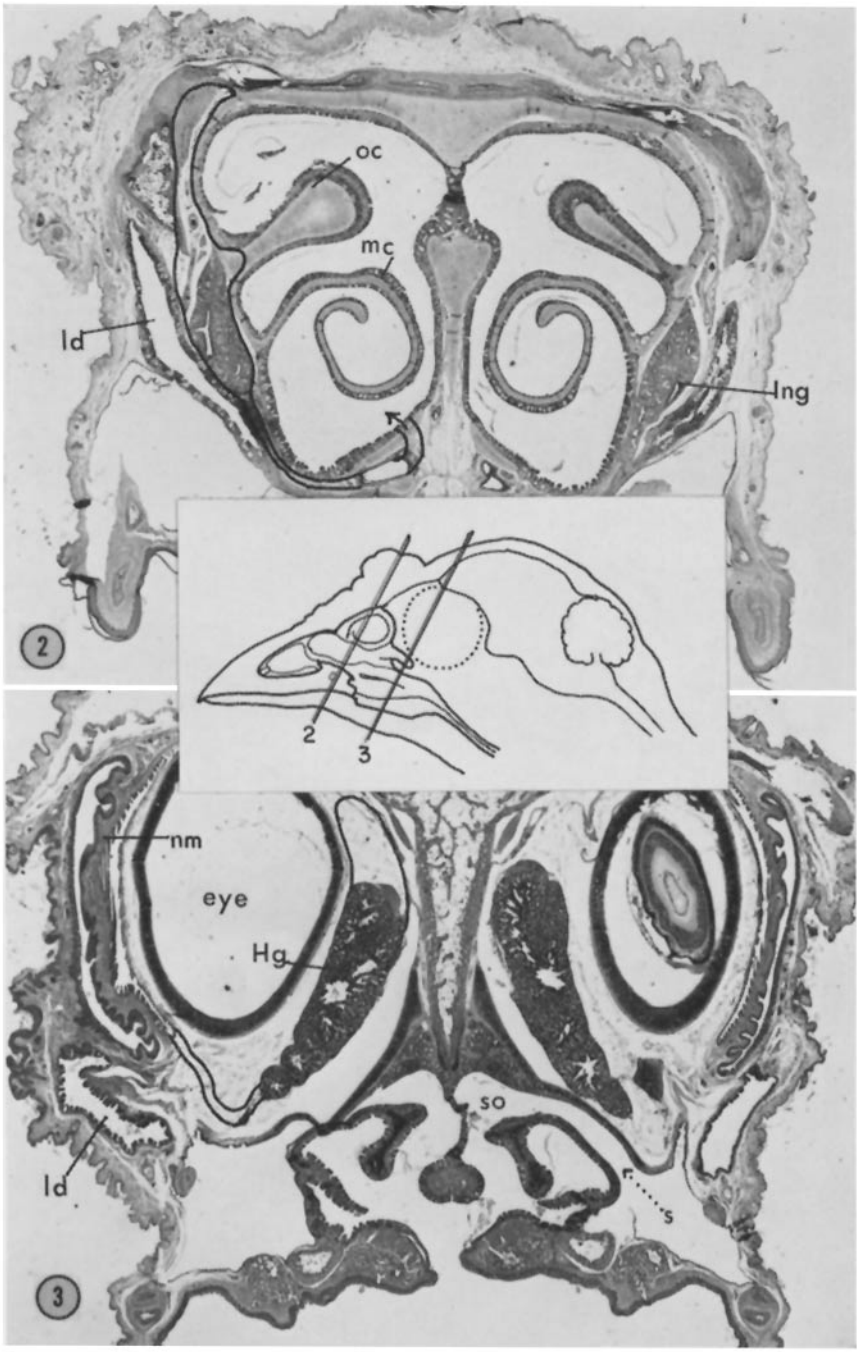
Pilocarpine induced initial hypersecretion, later exhaustion, and, still later, slow production of densely staining mucus in the acinar cells; also acinar gaping.

Breeding in a *germfree environment* produced a greatly reduced mucosal depth throughout the nasal fossa, an extraordinary reduction in the number of cells per acinus, relative reduction in the number of acinar neck cells, and concomitant increase in ciliated cells in that region.

Exposure to a *temperature of −20°C* for 1 hr caused blanching of all secretory cells, acinar gaping, and temporary reduction of mucosal depth.

BIBLIOGRAPHY

1. Stresemann, E., W. Kukenthal, and T. Krumbach. 1927–1934. Handbuch der Zoologie. W. de Gruyter and Company, Berlin. **7:**102.
2. Richards, S. A. 1966. Anatomy of the arteries of the head in the domestic fowl. *J. Zool. (London).* **152:**221.
3. Bang, B. G., and F. B. Bang. 1968. Localized lymphoid tissues and plasma cells in paraocular and paranasal organ systems in chickens. *Amer. J. Pathol.* **53:**735.
4. Bang, B. G. 1961. The surface pattern of the nasal mucosa and its relation to mucous flow—a study of chicken and herring gull nasal mucosae. *J. Morphol.* **109:**57.
5. Hotz, G., and F. B. Bang. 1957. Electron microscope studies of ferret respiratory cells infected with influenza. *Bull. Johns Hopkins Hosp.* **101:**175.
6. Bang, B. G., and F. B. Bang. 1967. Laryngothracheitis virus in chickens. A model for study of acute nonfatal desquamating rhinitis. *J. Exp. Med.* **125:**409.
7. Bang, F. B., and M. A. Foard. 1969. Experimentally induced changes in nasal mucous secretory systems and their effect on virus infection in chickens. II. Effects on adsorption of Newcastle disease virus. *J. Exp. Med.* **130:**121.
8. Moe, H. 1952. Mapping goblet cells in mucous membranes. *Stain. Technol.* **27:**141.
9. Leppi, T. J., and S. S. Spicer. 1966. The histochemistry of mucins in certain primate salivary glands. *Amer. J. Anat.* **118:**833.
10. Bojsen-Møller, F. 1965. Glandulae nasales anteriores in the human nose. *Ann. Otol. Rhinol. Laryngol.* **74:**363.
11. Proetz, L. W. 1941. Essays on the Applied Physiology of the Nose. Annals Publishing Company, St. Louis.
12. Beyer, K. H., A. R. Latven, W. A. Freyburger, and M. P. Parker. 1948. A comparative study of the activity and toxicity of hexylcaine (1-cyclohexylamine-2-propylbenzoate); a new local anesthetic agent. *J. Exp. Therap.* **93:**388.
13. Wolbach, S. B. 1954. *In* The Vitamins. W. H. Sebrell and R. S. Harris, editors. Academic Press, New York.
14. Moulton, D. G., and L. M. Beidler. 1967. Structure and function in the peripheral olfactory system. *Physiol. Rev.* **47:**1.
15. Tucker, D. 1963. Olfactory, vomeronasal and trigeminal receptor responses to odorants. *In* Olfaction and Taste. Y. Zotterman, editor. Pergamon Press, Oxford. 459.
16. Henkin, R. I. 1967. The definition of primary and accessory areas of olfaction as the basis for a classification of decreased olfactory acuity. *In* Olfaction and Taste II. T. Hayashi, editor. Pergamon Press, Oxford. 235.
17. Spicer, S. S. 1963. Histochemical differentiation of mammalian mucopolysaccharides. *Ann. N. Y. Acad. Sci.* **106:**379.
18. Bang, B. G., A. L. Mukherjee, and F. B. Bang. 1967. Human nasal mucus flow rates. *Johns Hopkins Med. J.* **121:**38.



FIGS. 2 and 3. Inset shows level of sections. Abbreviations are the same as in Fig. 1, except for: *Hg*, Harderian gland; *lng*, lateral nasal gland; *nm*, nictitating membrane. × 33.

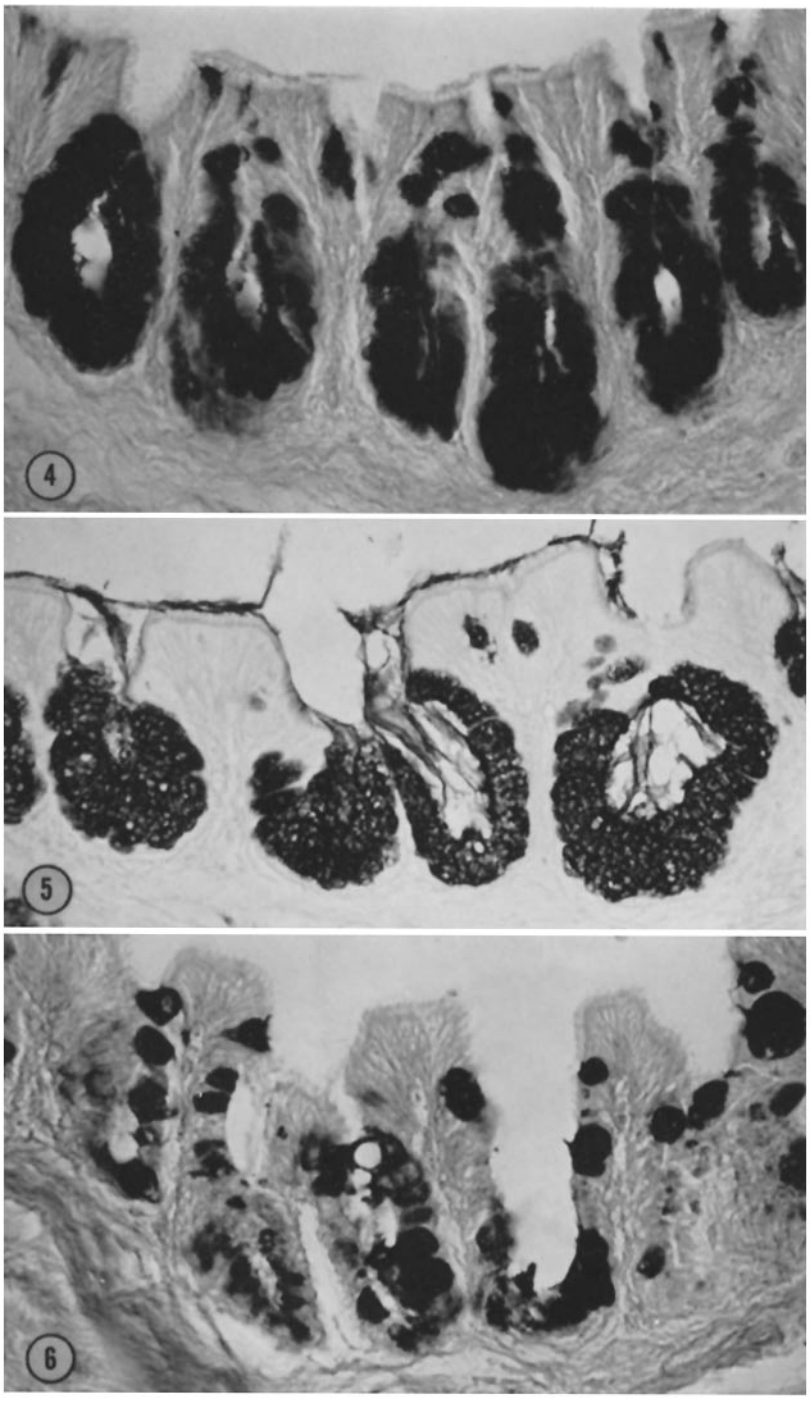


FIG. 4. Mucous acini of lateral wall, control. AB-PAS, × 370.

FIG. 5. Mucous acini, lateral wall, 60 min after nasal instillation of 0.25 mg of 20% cocaine. AB-PAS, × 370.

FIG. 6. Mucous acini, lateral wall, 15 min after subdermal injection of 0.25 mg of 0.6% pilocarpine. AB-PAS, × 370.

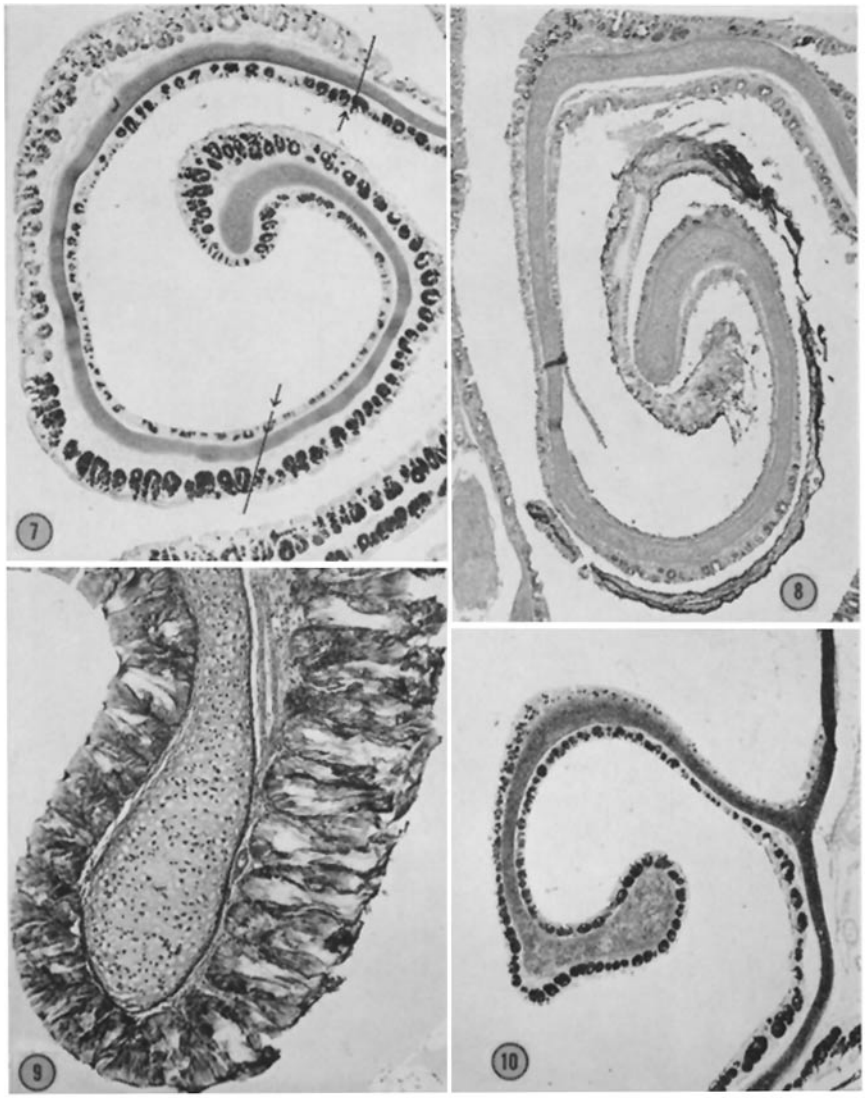


FIG. 7. Section through center of middle concha, control. Arrows indicate area of concavity of inner scroll shown in whole mounts in Fig. 20, after peeling away the acini from the outer convex surface. Note abrupt transition between pale, PAS-positive, branched acini on upper part of convexity, associated with the olfactory chamber, and richly staining mixed acini along the main respiratory airway. AB-PAS, × 33 (AB at pH 1).

FIG. 8. Same area 5 min after nasal instillation of 4 drops/nostril of 5% hexylcaine: acinar exhaustion, exudate, epithelial sloughing. PAS, × 33.

FIG. 9. Tip of middle concha 5 min after subdermal injection of 0.6% pilocarpine: intensive hypersecretion. PAS, × 100.

FIG. 10. Section through posterior part of middle concha of chicken deprived of vitamin A for 3 wk following hatching. Compare with height of mucosa and acini in Fig. 7. AB-PAS, × 33 (AB at pH 2.7).

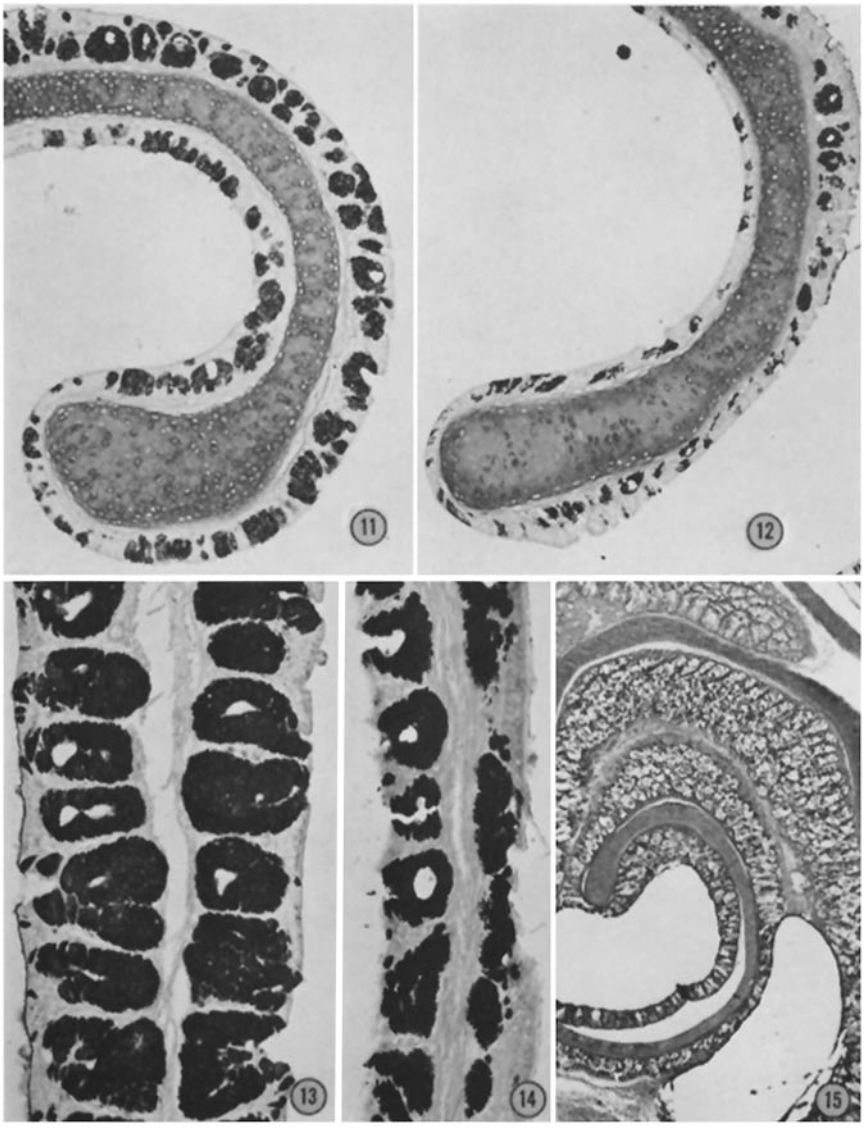


FIG. 11. Tip of middle concha, control. AB-PAS × 120.

FIG. 12. Tip of concha after −20°C cold for 1 hr. AB-PAS × 120.

FIG. 13. Area of main airway near mouth of lacrimal duct, control chicken, 6 wk old. AB-PAS, × 135.

FIG. 14. Same area in chicken raised for 6 wk in a germfree environment. AB-PAS, × 135.

FIG. 15. Portion of scroll of middle concha of 8 wk old chicken deprived of water for 72 hr: acini distended and exhausted, thickened layers of surface mucus adherent and stalled. The mass is released within 5 min after drinking water. PAS, × 34.

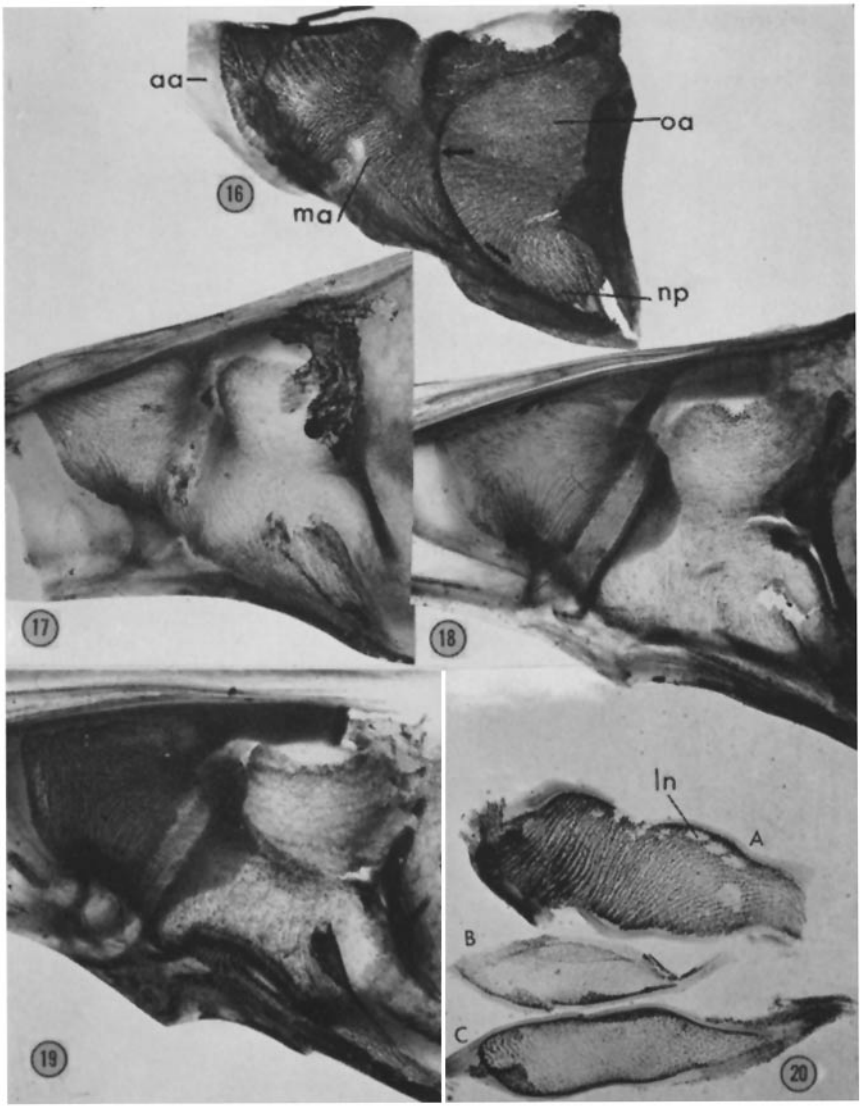


FIG. 16. PAS-stained, cleared whole mount of nasal septum of control chicken, fixed about 30 sec after India ink was placed along the posterior wall of the depression (*oa*) opposite the olfactory concha, showing normal arc of ink-laden mucociliary sheet. Arrows show direction of mucociliary flow. The dark striations are rows of stained acini; the light rows between them are rows of ciliated cells (4).

aa—area opposite anterior concha
ma—area opposite maxillary concha
np—nasopharynx
oa—opposite olfactory concha

FIG. 17. PAS-stained, whole mount of septum fixed 30 min after ink was placed in the same location in a chicken which had received three successive nasal instillations of 20% cocaine and killed 24 hr after the last instillation. Note that ink is stalled and acini are almost exhausted of mucus.

FIG. 18. PAS-stained, whole mount of septum 5½ days after nasal instillation of hexylcaine 5%: patchy partial acinar regeneration.

FIG. 19. PAS-stained, whole mount of septum of chicken deprived of vitamin A for 3 wk after hatching. Shadow in anterior portion is underlying cartilage, which stained quite heavily with PAS.

FIG. 20. PAS-stained, whole mounts of inner convex surface of maxillary concha of (A) normally fed, 3 wk old chicken; (B) 3 wk old chicken deprived of vitamin A since hatching; (C) 3½ wk old chicken deprived of vitamin A for 3 wk and on normal diet for 5 days. *ln*—lymphoid nodule

FIGS. 16–20. Photographed floating in oil of anise; all × 8.