LARYNGOTRACHEITIS VIRUS IN CHICKENS

A Model for Study of Acute Nonfatal Desquamating Rhinitis*

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Acute upper respiratory infection may produce mild or severe epithelial destruction. Following acute rhinitis in humans, it is not always possible to judge the nature or severity of immediate or long-term aftereffects of acute desquamation. This report summarizes a series of experimental studies of the gross and histological pathology of an acute desquamating virus infection of the upper respiratory tract of domestic chickens. The pathology of the nasal tissues of chickens inoculated intranasally with the virus of laryngotracheitis has been followed sequentially from the earliest demonstrable lesions through the 4th month of convalescense. The study is, in effect, a morphological appraisal of the effects of the virus on the tissue systems which are in or which have developed embryonically from, the nasal fossa. Since some concept of the normal functional anatomy of these systems in the chicken is prerequisite to interpretation of the pathological sequences, a brief review of certain features of the structure and function of the chicken nasal fossa will precede the pathological record.

Materials and Methods

White Leghorn chickens were commercially obtained as embryonated eggs or 1-day-old chicks. Neither Newcastle disease nor laryngotracheitis has been reported in any of the farm flocks which supply chickens to this commercial agency.

Laryngotracheitis virus was obtained originally from Dr. E. L. Jungherr of the University of Connecticut. Virus was maintained by continuous passage on the chorio-allantoic membranes of 10 day chick embryos or in the allantoic fluid of 10 day embryos. Membranes were harvested on the 4th day, allantoic fluid on the 3rd day. These were either repassaged or frozen for subsequent continued passage. When used for inoculation, infected membranes were ground under sterile conditions in 2 ml of cold normal saline in a cold TenBroeck grinder; 100 mg each of penicillin and streptomycin were usually added. The virus suspension was kept in ice water and the relatively clear supernatant drawn off in a syringe and used as the inoculum. Allantoic fluid was inoculated per se; undiluted. Using a sterile 1 ml syringe and a size 22 to 23 needle, one drop of the inoculum was placed on each nostril of an unanesthetized

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chick until it was seen to be inhaled. In any given experiment all chicks were inoculated by one investigator.

Methods of studying the capacity of the mucociliary blanket to carry India ink, and techniques of whole mount preparation, have been previously described (3) and will be referred to or described in context. Whole mount material was fixed immediately in formol-alcohol (1 part formol 40% to 2 parts alcohol 95%); histological material was also fixed immediately in large volumes of Bouin's fluid, decalcified in 2% nitric acid, neutralized in 5% sodium sulfate, processed routinely, and stained either with Harris' hematoxylin and eosin Y, hematoxylin and periodic acid–Schiff stain (PAS) or combined Alcian Blue (AB) and PAS.

Anatomy

The chicken was chosen as the experimental host because the nasal fossae of the common laboratory mammals are highly specialized for olfaction (1, 2) while those of man and chicken—both of whom depend less on olfaction—are quite similar in their general structure and in their tissue systems. In common with other land vertebrates, the first lines of defence against infection of the nasal tissues in chickens are the mucociliary blanket and the lymphatic system.

The mucociliary blanket.—The dynamics of mucociliary clearance in the chicken fossa are shown in Fig. 1. We have observed that the nonciliated olfactory surfaces are cleansed by the traction exerted by the cilia of the surrounding mucociliary sheet, and traction is maintained because the olfactory gland secretions blend without interruption with those of the mucous glands.

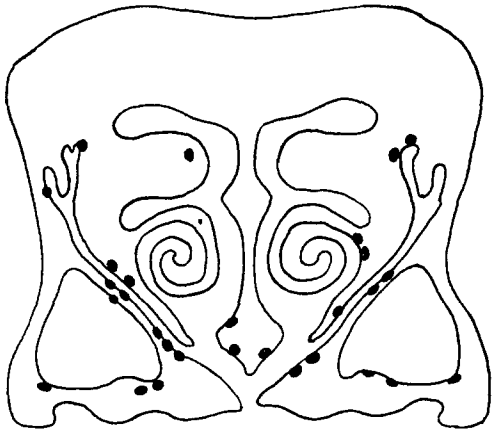
The mucous sheet moves over the septum in an arc; on the lateral wall it sweeps over, then inside, the maxillary concha (3). These streams merge with those from the lacrimal ducts and macillary sinuses and move into the pharynx to be swallowed. The rate of motion is not the same in all parts of the fossa simultaneously; it is swiftest in the areas least exposed to the main airstreams (3). In normal test chickens ranging in age from 2 days to 6 months, the average overall rate of motion has been 10 mm/min. About 18 normal chicks from 3 days to 1-month-old were tested for ink clearance in the present experiment. As in humans, mucus in the respiratory portion of the mucosa is produced by two types of gland cells: epithelial goblet cells which are not innervated, and acinar gland cells which are autonomically controlled (4).

While histological sections show that cilia and acini alternate with some regularity, it is only in whole mounts of mucous membranes that the entire surface pattern of acinar and gland distribution can be seen. Fig. 2 is a photograph of the septal mucous acini of a young chicken. The whole mount was prepared by peeling the mucous membrane from one wall of a freshly dissected septum, staining the remaining side with periodic acid–Schiff stain, clearing in anise oil, and viewing the result by transmitted light, according to the method of Moe (5). Similar preparations have been made of each area of the mucous membranes which line the fossa.

The motion of the mucociliary blanket shown in Fig. 1 was established by following the path of individual dots of India ink and of entire washes of India ink (3) as they were cleared from the surface of the mucous membranes repeatedly in freshly killed, sagittally sectioned chickens. The lineation of the acini shown in Fig. 2 coincides completely with the direction of motion of the mucociliary blanket as shown in Fig. 1. This functional synthesis is evidently established ontogenically; we did histological sections on duplicate sets of chick embryos from the 9th to the 18th day and found that differentiation of the respiratory area and localized anlagen of secreting mucous acini were demonstrable on the 11th day (Fig. 15 *b*), but only in areas in which the prospective epithelium would be out of the main air stream and would be deepest; by the 16th day patches of cilia appeared in these same areas, and by the 18th day cell differentiation was complete.

Lymphoid Nodules.—Since lymphocytes increased tremendously in experimental laryngotracheitis throughout pathogenesis and convalescence, we will specify the criteria by which "small" and "medium" lymphocytes are designated. Small lymphocytes were found in great numbers as infiltrations at the sites of infection. They ranged in size between about 5 and 7 μ. Medium lymphocytes appeared later in the infection as aggregations or encapsulated nodules in the submucosa; their sizes ranged from about 10 to 12 μ, and they stained somewhat more intensively than did the small lymphocytes. Mature plasma cells were readily distinguishable from either of these, by their large size (>13 μ) and greater amount of heavily stained cytoplasm.

The *Galliformes* are among the orders of birds which do not have cervical lymph nodes (6). We have, however, accumulated histological data over several years (unpublished) on lymphoid tissues in over three dozen species of wild birds and in Japanese quails which have been raised in both conventional and germfree[1] environments. The combined evidence of these



TEXT-FIG. 1. Composite diagram of sites of small lymphocyte nodules found in 22 individual germ free Japanese quails at ages from 3 to 171 wk.

and of healthy and infected chickens, leave little doubt that aggregations and/or nodules of small lymphocytes are normal structures in the submucosae of the nose and nasopharynx of birds. Text-fig. 1 is a composite diagram of the nodules found in 22 germfree Japanese quails.

The lateral nasal gland is a normal structure in most nonprimate vertebrates (7) and is quite prominent in chickens, in which it discharges serous fluid at the mucocutaneous junction of the nasal vestibule. In humans it is a transitory embryonic structure (7) but human nasal acini comprise both serous and mucous glands, and the primary acini also discharge into the mucocutaneous junction of the vestibule (8, 9). There is much evidence to support the hypothesis that serous secretions are atomized at inspiration so that they act as a continuing source of water vapor for the olfactory and mucociliary systems (9). Thus, while there has been evolutionary adaptation in the distribution of the nasal serous glands, the chicken lateral nasal gland is the functional analog of the serous gland units in the human nasal acini.

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[1] These quails were obtained through the kindness of Dr. James A. Reyniers and Miss Muriel Sacksteder of the Germfree Research Center, Tampa, Florida. Thirty-two specimens of each environmental source, ranging from 3 to 171 days in age, were examined histologically.

Establishment of Experimental Infection, and Reproducibility of Results

Since acute destructive infection of the upper respiratory tract of chickens has not been reported, nor, except for influenza in ferrets (10–12) have any fully destructive nonfatal experimental infections in the upper tract of any laboratory animals been reported, it is relevant to describe the methods of establishing the infection and testing infectiousness of the standard inocula.

TABLE I

Résumé of Experiments

Check marks designate that from two to six specimens were studied by a given method.

Each histological study represents about 18 sample slides from the anterior, middle, and olfactory area of at least two chicks.

Exp. No.	Age of chicks	Duration of exp.	No. of chicks	Ciliary motion	Whole mounts	Histology (days after infection)				Death rate
						1–3	4–7	8–10	more	
	days	*days*								%
1	3	7	8		√	√	√			0
2	18	24	10	√	√		√	√	√	0
3	5	33	12	√	√		√	√	√	0
4	8	32	20	√	√		√	√	√	0
5	2	27	20	√	√			√	√	0
7*	2	10	13		√		√			54
8	2	9	24		√	√	√			0
9	2	43	13	√	√		√	√	√	23
10	3	54	26	√	√		√	√	√	11.5
11	3	6	27	√	√	√	√			0
12	2	5	27	√	√	√				0
13	2	138	22	√	√	√			√	27
14	2	7	65		√	√	√			53.8
15	1½	21 hr	16			√				0
16	21	1½	5	√	√	√				0
17	2	6	43				√			18.6
18	2	22	34			√	√	√	√	0
19	2	4	24			√				0

* All histological material from Experiment 6 was accidentally discarded.

Source of Virus.—Amounts of virus present in the chorio-allantoic membrane or in the allantoic fluid of infected embryos were determined by titrations on the chorio-allantoic membrane of 10- to 11-day-old embryos. Although discrete pocks could be counted in the embryos inoculated with the least amount of virus, these were often not sharp and for this reason 50% end points were determined. Peak amounts were harvested on day 3 or (more usually) day 4 for use as inocula. However, in two of eighteen experiments initiated with such inocula (Nos. 4 and 8), no infection occurred (Table I). For this reason one of the last experiments was designed to test the infectiousness of different types of virus source material in intranasally inoculated chicks.

Test of Infectiousness of Inocula (Experiment 17). —Two types of preparations were used: undiluted 6th passage membrane virus, and undiluted 3rd passage allantoic fluid virus. Five

categories of undiluted inocula were prepared and the amount of virus in each was determined by titrations on chorio-allantoic membranes (Table II). End points were determined by the presence of characteristic lesions on the dropped membranes on the 4th or 5th day. Each of the undiluted preparations was then diluted 10^{-1}, so that there were ten sources of inocula in all (Table III). Five 2-day-old chicks were each inoculated with one drop per nostril of virus from each of the ten sources. Each of the ten groups was maintained in a separate cage. All chicks were killed on the 6th day, when sloughing was known to be most severe in established acute infections.

About 25 histological sections from the midfossa of each chick were examined. Each chick had been given a code number, so that slides could be evaluated without knowledge of the inoculum received by a given chick. The severity of the virus infection (which was identified by the presence of syncytia or intranuclear inclusions) was graded ++++ if all mucosae in the main fossa showed inclusions and had sloughed, +++ if a few remnants of intact mucosa remained, and so on to 0 if there was no histological evidence of infection. The results are

TABLE II

Titrations of Laryngotracheitis Stock Virus

Stock source	1 day	2 days	3 days	4 days
Membrane virus	$10^{-2.2}$	$10^{-3.3}$	$10^{-5.5}$	$10^{-6.2}$
Membrane virus	$10^{-4.0}$	$10^{-4.3}$		
Allantoic fluid virus	$10^{-3.0}$	$10^{-3.6}$	$10^{-3.0}$	$10^{-3.7}$
				$10^{-4.0}$

End points were determined by the presence of characteristic lesions on the dropped membrane at 4 to 5 days.

Since the highest titers were obtained at 4 days, inocula were prepared from membranes and allantoic fluid harvested on the 4th day.

shown in Table III. Assuming that the basic difference in the original virus material in each group was only in the amount of virus present in the material, then from all the data one may calculate a 50% end point of 3.1 logs. With an ordinary Poisson distribution and one hit curve, an amount of virus 1.5 logs dilution less than this would be expected to infect very few chicks. This may be what happened in the experiments in which no chicks were infected. The amount of virus recovered from the nasal tissues at daily intervals after inoculation in two experiments is shown in Table IV.

Of the sixteen experiments in which acute infections were established there were no deaths in ten, yet death rates of 11.5 to 54% in six (Table I). The deaths, like the two failures to infect, suggest a possible variation in the amount of virus in particular inocula, and inocula of 100 times the LD_{50} might be expected to cause excessive mortality. But the deaths also raise the possibility of secondary bacterial infection, because in each case peak mortality was at a stage when cilia were essentially lacking. An experiment was therefore designed to obtain bacterial counts and to identify bacteria in the nasal tissues of virus-infected chicks at successive periods after inoculation.

Bacterial Counts in Nasal Fossae of Virus-infected Chicks (Experiment 18).—Thirty-four 2-day-old chicks were each inoculated intranasally with one drop per nostril of a saline suspension of membrane virus. Beginning the 1st day after inoculation and continuing on days 3, 4, 6, 8, 13, and 22 after inoculation, the nasal conchae were removed from two chicks, ground, and inoculated onto bacteriological plates containing blood agar, desoxycholate, phenol–ethyl

TABLE III

Test of Infectiousness of Different Inocula

Though fifty chicks were inoculated eight died before the 6th day from accidents due to a structural defect of the cages

Category	6th passage membrane virus undiluted (thawed) Titer: $10^{4.5+}$	Same preparation diluted 10^{-1}	7th passage membrane virus undiluted Titer: $10^{4.5+}$	Same preparation diluted 10^{-1}	3rd passage allantoic fluid virus undiluted (thawed) Titer: $10^{1.7}$	Same preparation diluted 10^{-1}	4th passage allantoic fluid virus undiluted Titer: $10^{3.8}$	Same preparation diluted 10^{-1}	4th passage allantoic fluid virus grown on fresh membrane undiluted Titer: $10^{4.6}$	Same preparation diluted 10^{-1}
Severity of infection	No. 1 dead day 6	No. 1 ++++	No. 1 ++++	No. 1 ++++	No. 1 0	No. 1 0	No. 1 +++	No. 1 0	No. 1 ++++	No. 1 ++++
	2 +++−	2 +++−	2 ++++	2 ++++	2 0	2 0	2 +++	2 0	2 ++++	2 +++
	3 dead day 6	3 ++++	3 ++++	3 dead day 6	3 0	3 0	3 dead day 6	3 0	3 ++++	3 +++
	4 dead day 6	4 ++++	4 dead day 5	4 ++++	4 +++	4 dead day 4	4 +++	4 0		
	5 dead day 6	5 ++++			5 0		5 +++			

alcohol, and chocolate agar incubated in CO_2. In addition, tenfold dilutions of the ground material were made in Brewer's thioglycollate media to determine roughly the numbers of bacteria.

On day 3 at the peak of the virus infection, and again on day 13, bacteria grew in a 10^{-5} dilution in thioglycollate, but no more than 100 to 1000 bacteria were found on any of the other days. A variety of bacteria, including several which are present in the enteric system of domestic fowl, were identified: *Escherichia coli*, *Streptococcus faecalis*, *Klebsiella* species, and *Staphylococcus aureus*. All appeared in "light" or "moderate" numbers on the plates. These results do not conflict with the findings of Beach (13) in bacteriological studies of chickens infected in nature and experimentally with laryngotracheitis. Tests of nasal conchae of two chicks from this series on the 3rd day of infection showed virus present ($>10^{-1}$) in both.

TABLE IV

Virus Recovery from Chick Nasal Tissues at Intervals after Inoculation

Experiment No.	12 hr	1 day	2 days	3 days	4 days
14	0	$10^{-2.8}$	$10^{-4.0}$	$10^{-5.2}$	Bacterial contamination
	0	$10^{-3.2}$	$10^{-5.0}$	$10^{-4.0}$	
16	—	$10^{-2.5}$	$10^{-3.5}$	Bacterial contamination	
		$10^{-3.0}$	$10^{-3.5}$		
		$10^{-3.8}$	$10^{-2.8}$		

Amount of virus in nasal tissues could not be determined after 3 to 4 days because of the nature of bacterial contaminants which killed the chick embryos despite the use of antibiotics.

Pathological Changes

Throughout these experiments, pathological studies were done on chicks selected at random from infected batches, not on chicks selected on the basis of severity of symptoms. In all but two of the experiments the virus consistently produced complete or subtotal epithelial sloughing, so that within a given experiment if all chicks which were autopsied during the acute phase showed severe sloughing, it is assumed that those killed at later intervals were convalescent from equally severe effects. Of the 16 successful experiments, desquamation was severe in 11 and subtotal in 5. There was a very occasional unilateral infection.

The upper respiratory tract is understood as the area between the mucocutaneous junction in the nasal vestibule and the posterior limit of the nasopharynx; the nasal fossa proper includes the maxillary and olfactory conchae, the sinus ostia, the nasal septum, and the floor, roof, and lateral walls. Paranasal systems include the lacrimal ducts, maxillary sinuses, and lateral nasal glands and their ducts.

The morphological changes during pathogenesis will be described in chrono-

logical sequence since the course of the infection was quite consistent. Pathogenesis has been roughly divisible into phases of primary lesions, early spread, acute sloughing, and regeneration. The conclusions are based on histological slides, whole mounts, and ciliary motion studies in 16 individual experiments (Table I).

Primary Lesions (21 to 24 Hr).—

Gross effects: (ciliary motion and whole mounts). There were no significant gross changes at 21 to 24 hr.

Histology: Effects of the virus were not demonstrable histologically at 12, 15, or 18 hr in six chicks per time phase. At 21 hr scattered discrete foci of intranuclear inclusions were found in the shallow epithelium of the inner scroll of the maxillary concha and in the free edge of the scroll (Fig. 3) in three of six chicks. Most foci were covered by ciliated cells and were *in situ* in intact mucosa in which there was no evidence of separation or loosening of the syncytial mass. In this shallow epithelium it was not possible to determine whether the infected cells had orginally been mucous or ciliated, but in 24 to 30 hr specimens in which deeper epithelia were affected there were several syncytia in mucous acini which were surrounded by unaffected ciliated cells (Fig. 4).

There was some overlap in the degree of progress of syncytial formation in the 21 and 24 hr specimens. There were multiple foci in 5 of the 6 24-hr specimens.

Early Spread (24 to 72 hr).—

Gross effects: At 24 hr the mucous was clear and elastic, motion of the mucous blanket was smooth (as in Fig. 5), and whole mounts showed normal mucous glands and no sloughing. By 48 hr, however, there was a quantity of thin inelastic exudate. By 72 hr, the clearance of India ink was significantly retarded (Fig. 6), though eventually successful (Table V). Whole mounts showed very little mucous in the goblet cells or the acini (Fig. 6) though refractile "ghosts" of acini were clearly visible. The histological basis for this effect will be described below.

Histology: At *24 hr* foci were still mostly confined to the scroll of the maxillary concha (Text-fig. 2). Some syncytia had sloughed free in the lumen as typical "giant cells" (Figs. 7 *a* and 7 *b*). These syncytia showed a distinct change in the staining properties of the mucous. In sections stained with Alcian blue–PAS, the paranuclear mucous of individual syncytia stained only with the Alcian blue component, though elsewhere in the fossa the mucus was the typical magenta of the combined stain. This selective change in staining obtained throughout the formation, sloughing, and terminal breakdown of giant cell syncytia.

By *48 hr*, syncytial formation and sloughing increased markedly, and numbers of eosinophils[2] appeared at affected sites. By *72 hr* all nasal and paranasal mucosae were usually involved though there was wide variation in the progress of the infection in individuals. Specifically at the sites of sloughing there were infiltrations of small lymphocytes and numbers of eosinophils, and a few plasma cells were found circulating usually near the lumen of the fossa. Giant cells, exudate, and cell debris—often predominantly eosinophils—were abundant in the lumen.

At this same stage another direct effect of the virus was depletion of acinar mucus, as though secretion was exhausted or inhibited. In such areas, there were inclusions in some groups of cells, but in immediately adjacent areas there was equal depletion of mucus and no inclusions.

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[2] These eosinophils were spherical cells with several large basophilic nuclei, containing brilliantly eosinophilic, apparently spherical or ring-shaped, granules. They were almost never found in healthy chicks. We did not attempt differential heterophil-eosinophil cytology, so homology with mammalian eosinophils cannot be assumed (14).

The effect showed most clearly in a unilateral infection (Figs. 8 *a* and 8 *b*) and would account for the refractile "ghosts" of acini described above.

Acute Sloughing (3 to 8 Days).—

Gross effects: Of the 5th and 6th days 60 to 80% of tested specimens failed to clear India ink from the fossa (Table V). In whole mounts, conchae and septa were nearly devoid of acini

TABLE V

Ciliary Rates on the Septum of Individual Chicks

Average normal rate is 10 mm/min.

Days after inoculation				
1–4	5–7	8–13	14–25	32
+	+	+	++	+++
	−			
++	+	+	+	+++
+	+	++	+	
±	±	−		
	−	−		
++	−	+	++	
±	+	+	±	
	−	−		
++	−	±	++	
	−			
+	−			
++	−			
	−			
++	−			
	−			
	+++			
	−			
	+			
	++			
	+			
	±			
	−			

+++, 10 mm/min; ++, 17 mm/min; +, 5 mm/min; ±, moved but stopped; and −, no motion in 20 min.

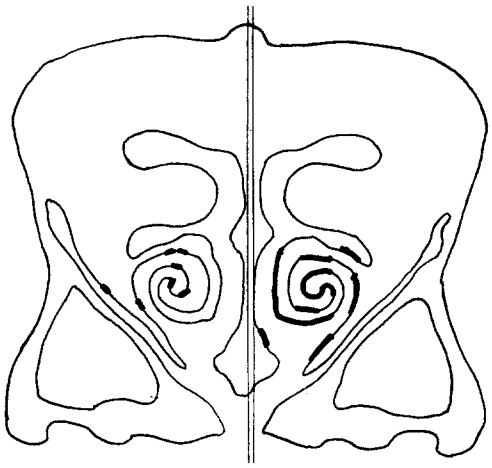
(Fig. 10). During this acute phase of sloughing, the conchal cartilages lost rigidity to such an an extent that during dissection they cut like soft butter. The histology of this effect is described below.

Histology: By 3 days the infection had spread to involve all epithelia in the main fossa and usually the sinuses as well. Desquamation was severest between the 4th and 7th days (Figs. 9 and 10).

Changes were so rapid during peak sloughing that at 7 to 8 days there was simultaneous sloughing and repair (Figs. 11 and 13 *a* and 13 *b*). The venules lying close along the periosteum

were greatly dilated and the outgrowth of new vascular channels was concomitant with evidence of beginning surface repair.

In the submucosa the numbers of circulating plasma cells increased and beginning about the 4th day plasma cells began to collect around and among the cells of the duct walls of the lateral nasal glands. Up to the 4th day there was no evidence of the aggregation of medium lymphocytes, but by the 5th day the formation of secondary nodules was under way and by the 6th day there were well formed encapsulated secondary nodules. In one specimen on the 6th day, syncytia were found in the squamous epithelium of one main duct of the lateral nasal gland, the only case in which inclusions were found in squamous cells.

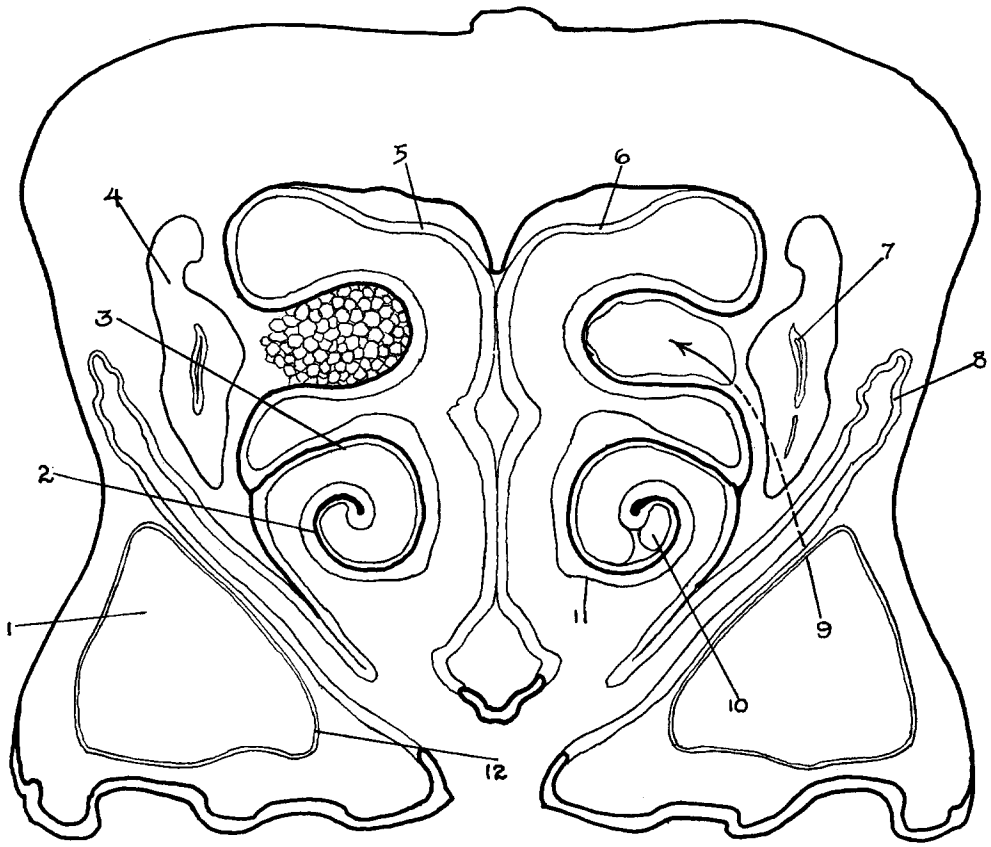


TEXT-FIG. 2. Left: sites of syncytia found at 21 to 24 hr, combined specimens from three experiments. Focus in lacrimal duct probably resulted from having some of the inoculum run into the eye; during inoculation chicks are held with beaks pointing up, and if the drop is not inhaled promptly it occasionally breaks and runs into the eye.

Right: composite of five experiments, showing sites of syncytia and of sloughing at 48 hr, indicating lateral spread of initial discrete foci.

Changes in the properties of the conchal cartilages were most clearly defined between the 3rd and 7th days. Sectioned cartilages were often distorted in contour (Fig. 12), but even more striking were changes in the staining properties of the matrix and nuclei. In all hematoxylin and eosin preparations, basophilia was lost from the nuclei and matrix (Figs. 14 *a* and 14 *b*) and in all AB-PAS–stained preparations, Alcian blue was lost from both nuclei and from matrix and the intercellular partitions were reduced (Figs. 14 *c* and 14 *d*). In cases of focal infection, the changes in the staining properties of the cartilage were also focal, sandwiched between normally stained areas. By the 8th day both basophilia and Alcian blue staining began to reappear, and cartilage changes were not seen in any specimens at later stages.

At the surface of the epithelial lining of the fossa, especially on the maxillary conchae, repair processes took place simultaneously with sloughing, beginning with rapid horizontal spread of a sheet of very thin flat cells on the surface, overlying the damaged submucosa (Fig. 13 *a*). At times, exudate and fibrin formed bridges across which some of these rapidly growing surface cells had spread, come in contact, and coalesced (Fig. 13 *b*). In the same speci-



TEXT-FIG. 3. Summary diagram of epithelial tissues affected following intranasal inoculation of laryngotracheitis virus; infection limited to nasal and paranasal tissues.

1. Maxillary sinus: may become chronically infected.
2. Maxillary conchal cartilage: becomes flaccid, is changed histochemically.
3. Epithelium of inner lining of scroll: site of most initial lesions.
4. Lateral nasal gland: occasionally atrophies.
5. Olfactory epithelium: sloughs later than respiratory epithelium.
6. Olfactory organ: undergoes complete metaplasia to mucociliary epithelium.
7. Nasal gland ducts: inclusions and sloughing of squamous epithelium (very rare).
8. Lacrimal duct: most resistant area of mucociliated epithelium.
9. Sinus epithelium may invade olfactory conchal hollow, replacing normal areolar tissue (indicated on opposite side).
10. Temporary mucosal adhesions: form during early repair.
11. Functionally effective regenerated surface epithelium: usually covers abnormal submucosa by about one month after inoculation.
12. Sinus epithelium: may convert to granulation tissue after acute phase.

mens a recognizable cuboidal epithelial layer had formed, in which the anlagen of acini were discernible (Fig. 13 *b*). At about 8 days, then, there was a gradual shift toward functional regeneration of the epithelium, with some individual variation in the rate and degree of repair.

TABLE VI

Sequence of Pathological Changes

Time after inoculation	Pathology
21–24 hr	Focal syncytia on maxillary conchae (initial nuclear changes in mucous gland cells?)
24–72 hr	Ciliary clearance rate retarded; "giant cell" formation and sloughing; sloughing of all epithelial areas; depletion of ancinar mucus; histochemical changes in infected mucous cells; small lymphocytes in submucosa; few plasma cells; exudate in fossa.
3–8 days	Ciliary clearance poor or lacking; continued sloughing of syncytia; intensive sloughing of olfactory epithelium; sloughing of capillaries (and probably small vessels); beginning formation of new epithelium; epithelial adhesions; many eosinophils and small lymphocytes in submucosae; formation of secondary lymphoid nodules; numbers of plasma cells circulating in tissues; loss of cartilage rigidity; loss of basophilic and Alcian blue staining of cartilage
8–21 days	Ciliary clearance irregular; acinar whole-mount pattern variable; no syncytial formation after 8th day; rapid epithelial growth differentiation; mucosal adhesions, cleared by day 16; greatly dilated blood vessels; hypertrophy of lateral nasal gland ducts abnormal extension of sinus epithelium into airspaces which line the olf. conchae
3 wk–4½ months	Ciliary clearance essentially normal; respiratory ciliary surface restored; acinar regeneration uneven, frequently abnormal; acinar lineation often distorted; sinuses inflamed, infrequently granulated; eosinophils in sinus exudate; complete mucous metaplasia of olfactory epithelium; beginning atrophy of olfactory nerves; some large "active" secondary lymphoid nodules

An occasional chick had pus in the larynx. Eight of these were examined histologically. While there was some loss of cilia, and moderate lymphocytic infiltration of the submucosae of the larynx and upper tracheal rings, inclusions were not found below the nasopharynx.

Regeneration (8 to 21 Days).—

Gross effects: In the first 2 wk of epithelial regeneration, of thirteen chickens tested for the capacity to clear India ink from the fossa, two cleared smoothly and in good time, seven unevenly and at less regular rates, and four failed to clear within the 20 min test time (Table V). A series of septal whole mounts prepared at intervals of about 2 days showed distorted alignment of acini which in turn were of irregular shapes and sizes. The olfactory areas showed

partial mucous metaplasia and occasional cystic degeneration of the olfactory glands. Mucous acinar regeneration on the septum of several specimens seemed similar to ontogenic development of acini (Figs. 15 *a* and 15 *b*).

Histology: After the 8th day, syncytia were not found in the lumen of the fossa.[3] Submucosae were swollen with loosely packed lymphocytes, plasma cells, eosinophils, dilated venules, and nonproductive acini. Regenerating acinar anlagen often contained groups of eosinophils in the acinar lumen, a situation seen in abnormal epithelia throughout regeneration, even at 4½ months.

Between about 8 and 15 days, mucosal adhesions were fairly common but were not manifest after the 16th day. Mucous acini were of irregular shapes and sizes (Figs. 16 *a* and 16 *b*) and were densely stained. There were varying degrees of destruction, and little regeneration, in the maxillary sinus and olfactory organ epithelia. Sinus epithelia were not only disorganized, but often invaded the lining of the olfactory concha to replace the normal areolar-tissue lining (Fig. 17). Fig. 18 indicates the degree of olfactory organ desquamation. Olfactory nerve branches could not be found in any part of the olfactory area during this phase, except the main trunks at the stem of the concha (Fig. 18).

Lateral nasal gland duct cells were often hypertrophied. Numbers of plasma cells were found between the cells of the duct walls, aligned along the basement membranes (Fig. 19), congregated in the areolar tissues and along the major venules, and circulating throughout the submucosa. In preparations stained with hematoxylin-PAS, their paranuclear area was brightly PAS-positive.

Convalescence (3 Wk to 4½ Months).—

Gross effects: The clearance of India ink from the main fossa was quite efficient after about the 3rd wk (Table V). Whole mounts at 2 months and at 4½ months showed no entirely normal septa or conchae; acini were of irregular shapes and sizes and the pattern of lineation often abnormal (Fig. 20). Disorganized lineation was more common on the maxillary conchae than on the septum, and more pronounced on the inner than the outer surface of the conchal scroll (Figs. 21 *a* and 21 *b*). Olfactory mucosae showed mucous metaplasia in which the distribution of acini and epithelial gland cells was random (Fig. 22 *b*).

Histology: Between the 3rd and 8th wk of convalescence it was extremely rare to find any consecutive areas of normal mucosa or submucosa in the main fossa or sinuses. Most common were mucous metaplasia of the olfactory organ (Fig. 23 *b*); abnormally shaped acini; infiltration of submucosae with small lymphocytes, eosinophils, and plasma cells; uneven clumps of mucus in the lumen; sinuses marked by desquamation, heavily infiltrated submucosae, and exudate packed with eosinophils and plasma cells. Less common were cystic degeneration of olfactory glands (Fig. 23 *c*) atrophy of the lateral nasal gland (Fig. 24), and partial to nearly complete effacement of the sinus air spaces by hyperplastic granulation tissues. Figs. 25 *a* and 25 *b* are typical of this stage. Fig. 26 diagrams the sequence of infection and repair in maxillary conchal tissues. Figs. 22 *a* and 23 *a* show the normal conchal histology.

By 4½ months (no specimens were examined between 2 and 4½ months), much of the histopathology had been repaired. Even though many mucous acini were of odd shapes and sizes, the mucociliary epithelial surface of the main fossa was intact in every specimen. There was much less residual abnormality in the respiratory part of the fossa than in the olfactory area and sinuses. However, all four histological specimens showed complete mucous metaplasia of olfactory epithelia and many large encapsulated secondary lymphoid nodules (most of which had several mitotic figures in each large nodule per 6 μ section).

Six of the eight maxillary sinuses showed active inflammation. The walls were very heavily

[3] In related experiments which have not been published, a group of chicks which had been moderately chilled (68°C) before and during infection had syncytia in the lumen at 10 days.

infiltrated with small lymphocytes, and much of the debris mixed in the sinus exudate consisted of intact and broken eosinophils. The postcapillary venules of the affected sinuses contained many small lymphocytes and moderate numbers of eosinophils. The contours of the sinus linings were uneven, and from three sinuses pathological sinus epithelium had invaded the olfactory conchal hollow.

The olfactory bulb and proximal part of the olfactory nerves of one 4½ month convalescent chicken which had complete mucous metaplasia of the olfactory organs was preserved and sectioned. While fixation and staining (Bouin's–hematoxylin and eosin) were not adequate for detailed study of pathology, sections through the midportion of the olfactory nerves showed definite breakdown of nerve cell nuclei, apparent tissue edema, and signs of changes in the nerve fibers.

Text-fig. 3 is a summary diagram of the areas affected by pathological changes.

DISCUSSION

Laryngotracheitis virus infection of chickens is a useful model in which to study acute desquamating rhinitis and its after effects. There have been several studies of experimental influenzal rhinitis in ferrets (10–12), but few other models have been exploited. The effects of recently isolated parainfluenza 3 virus on hamster mucosa (15), and of respiratory syncytial virus on ferret mucosa (16) have been reported, but sequential pathogenesis was not described. The mild strain of Newcastle disease virus, when intranasally inoculated in baby chicks, is a satisfactory model in which to study the epidemiology of this infection, but it produces only mild rhinitis and no severe desquamation (17). Since severe rhinitis in humans is produced by many different viruses (18) it seems clear that experimental viral rhinitis should be explored in a variety of models.

In relation to human rhinitis, certain features of the host animals, the natural disease, and the viral lesion in the present study should be pointed out. There are two important anatomical analogies in the chicken model. First, in both chickens and humans the nasal respiratory epithelium is a mucociliated surface sheet overlying a submucosa rich in secretory acini which depend upon a coordinated vascular and neural complex. The laboratory mammals except primates have no deep mucous acini along the main airway. Secondly, they do not have true sinuses, but recesses in the lateral fossa walls which accomodate accessory olfactory conchae and are in wide communication with the main fossa (2). Sinusitis therefore, by definition, could not be a complicating factor in rhinitis or in postinfectious chronicity in these animals. Humans and chickens have true sinuses; and sinusitis is a frequent aftermath of rhinitis in both species.

Infectious laryngotracheitis in nature is transmitted via the respiratory route, and is reported to produce inflammation and excessive mucus in the lower tract (19); if there is nasal pathology it has evidently not been documented. If nasal tissues are not infected in nature, it may be because virus is inhaled in small droplets that pass directly into the lower tract to penetrate a thin mucous sheet and infect a shallow mucociliated epithelium. The experimental infection was in all cases limited within the nasal fossa where the virus had to penetrate a rela-

tively thick blanket of mucus to infect a susceptible cell within 20 min, the maximum time required for a particle to be carried from the peripheral limits of the fossa into the nasopharynx by the longest route: around the full coil of the maxillary concha. It may thus be no accident that the earliest lexions were on the terminal inner channel of this concha, where virus had had the maximum time to penetrate the mucus sheet and where the mucociliary epithelium is shallowest.

Little is known about penetration of viruses through the mucous blanket. One of our late experiments indicated that about 10,000 ID_{50} for the chick embryo were required to produce consistent infections by intranasal inoculation—the same magnitude required for intranasal infection with Newcastle disease virus (20). Therefore in retrospect the type of source virus preparations in our experiments may not have been too important. Whether the variable mortality was primarily due to the amount of virus in the inoculum or whether bacterial infection was sometimes a significant factor remains to be determined.

The viral lesion is the characteristic herpes virus type of eosinophilic "inclusion" in cell nuclei. Goodpasture (21) used this lesion to mark progressive herpes virus infection from an area of experimentally produced keratitis along the fifth nerve to the brain. We found no single, or isolated, cells which contained an inclusion, but found inclusions in small groups of cells within 21 hr after inoculation. Such groups of infected cells have been found in experimental infection of the lower tract at 12 hr (22). Virus evidently spreads through the mucociliary epithelium from cell to adjacent cell, so that antibody (which has no effect on the spread of herpes virus in tissue cultures (23)) might not be efficient in controlling the infection in the nose. The formation of syncytia may well be a property of certain viruses which promotes transmission from cell to cell, and ensures survival.

The present study has raised five general questions. The first is whether this virus has a specific effect on the mucus-secretory process. While it was not established that mucous cells were the first to be infected, there is clear evidence that mucus and mucous secretion are directly affected. The thick nonelastic mucus early in the infection may reflect virus-induced alteration in the property of the surface mucus. Exhaustion or inhibition of secretion in areas of intact epithelium suggest an enhancing effect of some product released by infected cells. The most striking direct effect, the histochemical change in the mucus in infected syncytia, may indicate rapid changeover to synthesis of nonsulfated acid mucopolysaccharides, or inhibition of secretion of neutral or sulfated mucopolysaccharides (24).

The second question is the role of the virus in altering the property of the maxillary conchal cartilage. The gross softening, consistent loss of basophilia and of Alcian blue staining, reduction of intercellular partitions, and the duration of the effect are reminiscent of the effects of papain and of excess vitamin A

in living rabbits (25) and in tissue cultures (26). The effects produced by the virus infection are evidently as severe as the combined effects of papain and excess vitamin A (25). Loss of Alcian blue from cartilage evidently represents loss of chondroitin sulfate (27). Thomas and his associates (25) suggested that the changes produced by hypervitaminosis A might represent exaggeration of a normal process of activation of lysozomes. In our model, exacerbation of normal enzyme activity may have been induced by the viral infection, a point of particular interest in relation to atrophy of nasal cartilages following some diseases in humans. The remarkable atrophic rhinitis of baby pigs is also characterized by early and permanent atrophy of nasal cartilages.

The third question is raised by the process of repair. As in other models of acute viral desquamation (10), this is initiated by spread of a thin new sheet of epithelial cells, originating presumably from the basement membrane, over the denuded and inflamed mucosa. Since the alignment and coordination of the acinar and surface elements are ontogenically established, and the acinar alignment is probably induced by the underlying vascular pattern, the degree of damage to the vascular bed during sloughing may influence the degree of regeneration. The mesenchyme-epithelial interaction, so fully established in tissue culture studies (28), needs study during the repair process both at cellular and organ-system levels.

The fourth question is raised by the complete, possibly permanent, mucociliary metaplasia of the olfactory organ. In acute rhinitis produced by experimental influenza in ferrets the olfactory epithelium was either minimally affected (10) or was described as desquamated in focal areas and perhaps restored by the 19th day (11). In our study there was no sign, after 4½ months, of olfactory epithelial regeneration, and atrophy of olfactory nerves was evident. The olfactory end organ develops ontogenically independently of the central nervous system, and induces development of the olfactory portion of the brain (29), and experimental destruction of olfactory epithelium is followed by degeneration of the fiber plexus on the surface of the bulb (30). A much longer followup will be needed to determine whether the neural elements regenerate.

The final question is whether the residual sinusitis in our late convalescent cases were reservoirs of virus. Virus isolation was not attempted on these birds. It has been reported that in nature this virus is sometimes shed up to 15 months after the acute infection (31). We have found no nuclear lesions in the cells of the actively inflamed sinus epithelium or adjacent submucosa, but there are moderate numbers of plasma cells throughout these tissues. Fluorescent antibody might resolve the reservoir question.

SUMMARY

Infectious laryngotracheitis can be produced in chickens as an experimental model of severe nonfatal rhinitis and sinusitis. Inoculated intranasally into un-

anesthetized baby chicks it remains limited to the nasal fossa, produces acute desquamation of all nasal epithelia, results in functional recovery of the respiratory epithelium, but leaves important residual abnormalities. From the earliest recognizable lesions through 4½ months' convalescence, the principal changes are as follows:

1. Initial lesions, or small syncytia of intranuclear "inclusions", first identifiable in the mucociliated cells of the shallowest portion of the epithelium at about 21 hr postinoculum (the inner surface of the maxillary conchal scroll).

2. Acute sloughing, (about 3 to 7 days), marked by: (*a*) spread of lesions from cell to cell via multinucleated "giant cells" which progressively slough and desquamate respiratory, olfactory, and sinus epithelia, epithelial neural elements and blood vessels; (*b*) appearance of numbers of eosinophilic leukocytes along the basement membrane at the sites of lesions just previous to sloughing; intensive infiltration of the submucosa with small lymphocytes after sloughing begins; (*c*) histochemical change in the intracellular mucus of the cells which comprise the syncytia: this mucus stains with Alcian blue alone when stained with AB-PAS; and (*d*) all cartilages of the maxillary conchae become flaccid, and the cell nuclei and matrix lose both basophilic and Alcian blue staining properties, effects which recede by about the 8th day.

3. Repair (about 8 to 21 days), marked by rapid initial spread of a sheet of epithelial cells over the infiltrated submucosa, appearance of numbers of plasma cells circulating in the tissues, formation of encapsulated secondary nodules, and mucosal adhesions.

4. Convalescence (about 1 to 4½ months when experiments terminated), marked by functional restoration of the mucociliary lining of the nasal fossa. However, at 4½ months eight specimens all show complete metaplasia of the olfactory organ (end nerves, supporting cells, and glands of Bowman) to mucociliated epithelium, all show abnormal formation and alignment of mucous acini, and about 50% have severe persistent sinusitis.

We are indebted to Miss Marie Foard for maintaining the virus and for performing many of the titrations and inoculations. Mr. Raoul Spicker has carried out all technical procedures for whole mounts and histology. Dr. N. N. Pal, of the Seth Sukhlall Karnani Memorial (SSKM) Hospital, Calcutta, took the microphotographs for Figs. 8 and 14; all other photography and microphotography was done by Mr. Chester Reather, Photographer-in-Chief of the Johns Hopkins Hospital. Mrs. Louise Knocke carried out the bacteriological procedures.

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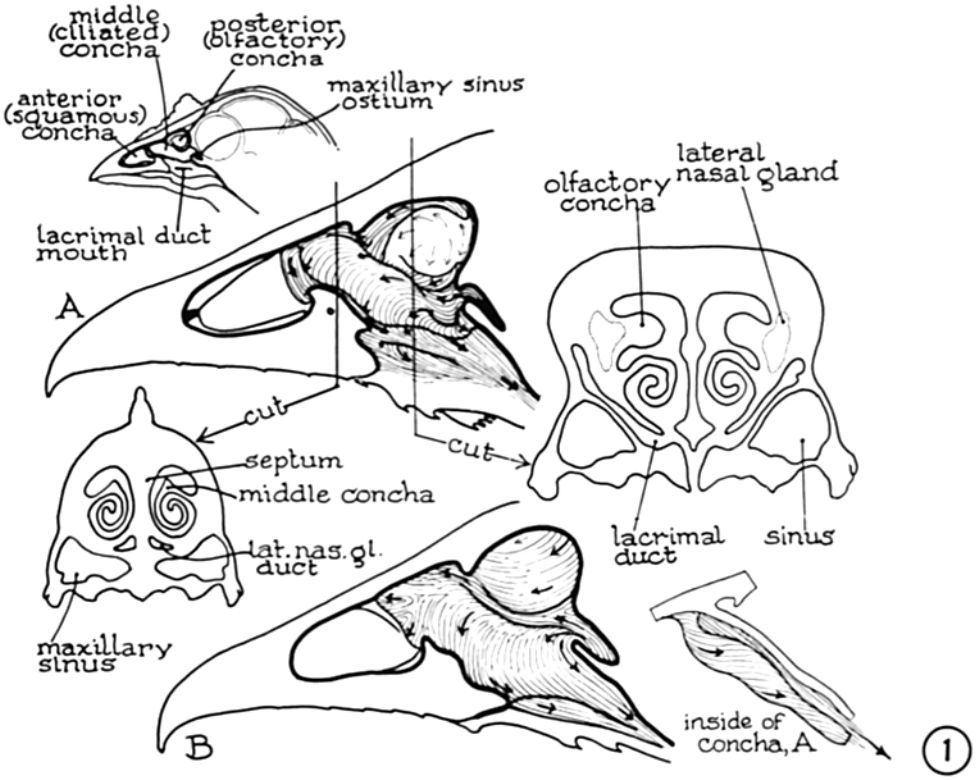
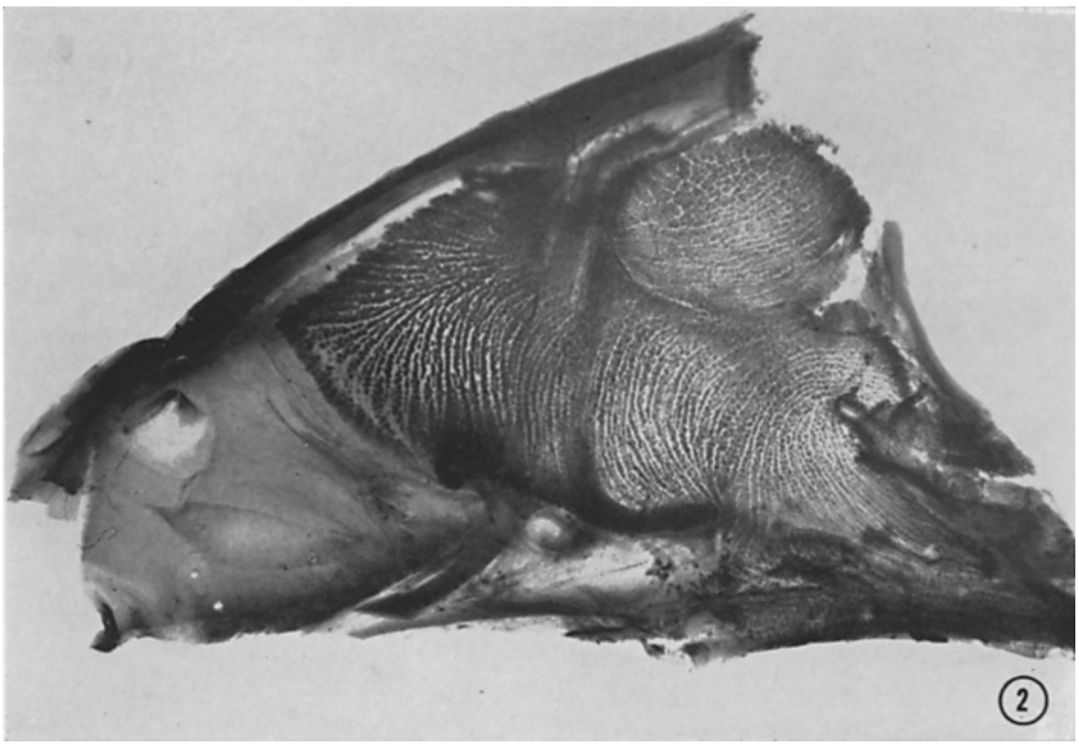
EXPLANATION OF PLATES

PLATE 25

FIG. 1. Anatomy of chicken nasal fossa. (*A*) Dynamics of mucociliary clearance of lateral wall. Lines and heavy arrows show pattern of lineation of acini and direction of flow of mucous sheet. Fine arrows on nonciliated olfactory concha show how olfactory gland secretion is pulled off of concha by traction of surrounding cilia. The vertical sections will orient most of the histological sections. (*B*) Lineation and clearance of septum. Lower right, detail of inside of maxillary concha of *A*, to show flow of mucus around free edge of concha into the funnel-like channel leading to the nasopharynx. Approximately × 2.

FIG. 2. Whole mount of PAS-stained, cleared, septum of 3-wk-old chicken. Mucous acini are stained, and lines between acini show how ciliated cells are aligned in rows between acini. Compare with Fig. 1, *B*. Small amounts of mucus are deposited in anterior mucocutaneous junction. Approximately × 16.



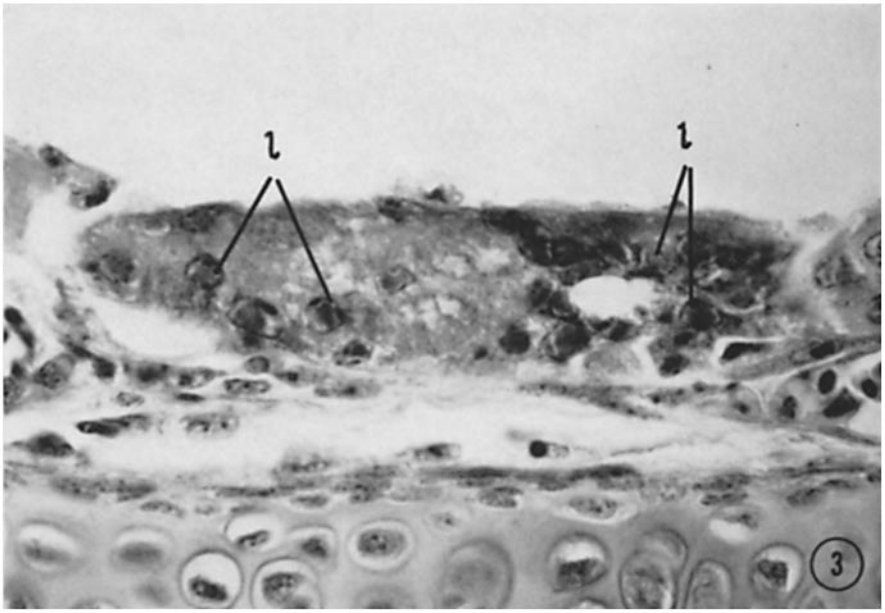


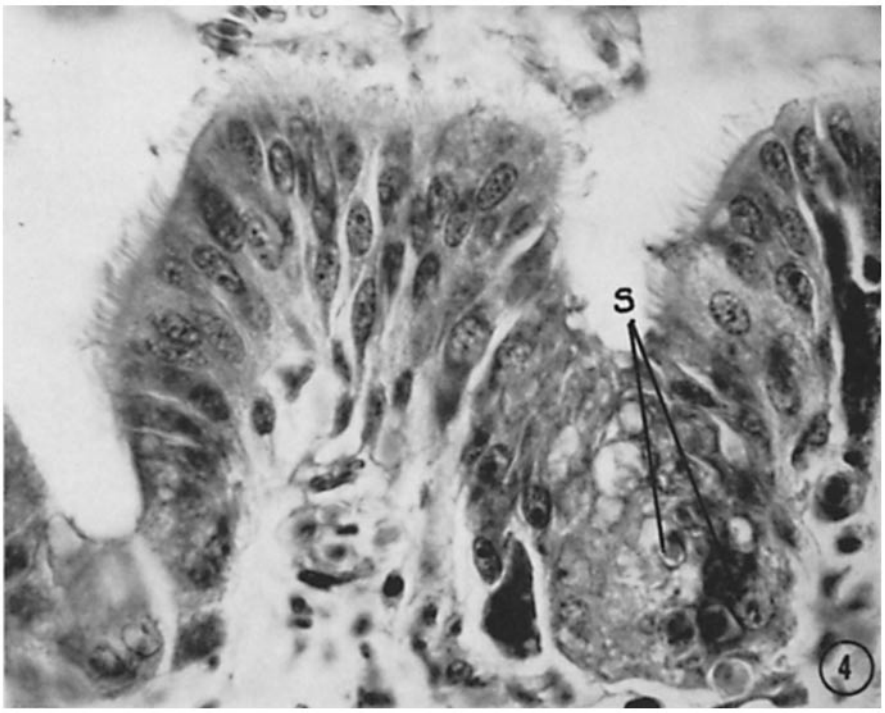
(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 26

FIG. 3. Histological section showing intranuclear lesions (*l*) in epithelial cells of inner surface of scroll of maxillary concha 21 hr after inoculation of virus. Hematoxylin and eosin. × 1000.

FIG. 4. Syncytium (*S*) of altered nuclei of mucus cells in acinus, lying between adjacent normal ciliated cells. 24 hr. Hematoxylin and eosin. × 1000.





(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 27

FIG. 5. Whole mount of septum of normal 5 day chick fixed in formol-alcohol 1 min. after India ink was applied along posterior limit of upper septal wall. A smooth forward arc is formed by concerted ciliary motion. × 15.

FIG. 6. Whole mount of 72 hr infected septum fixed 5 min after applying ink at same site. Note slow, incomplete clearance, and pallor of acini. × 20.

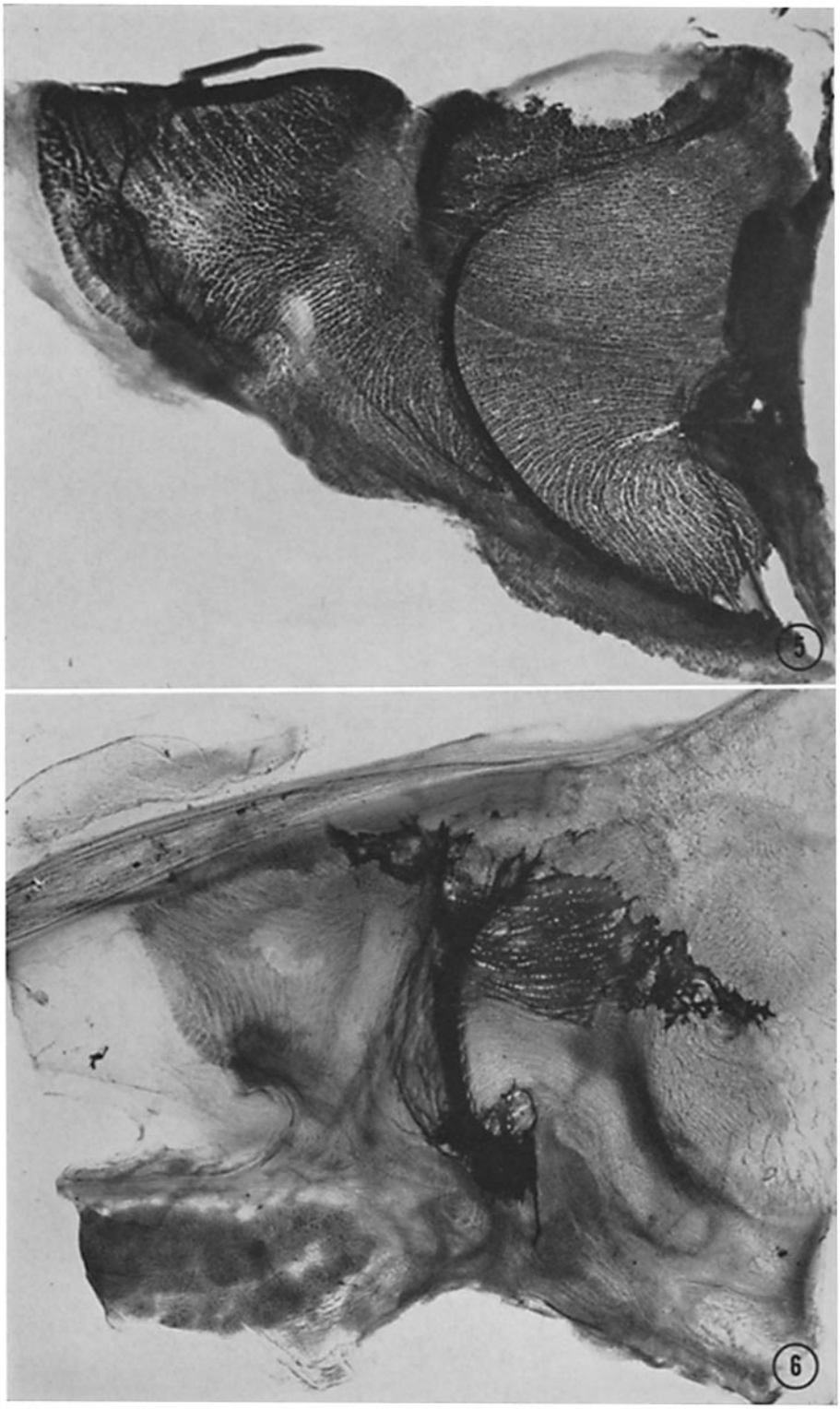


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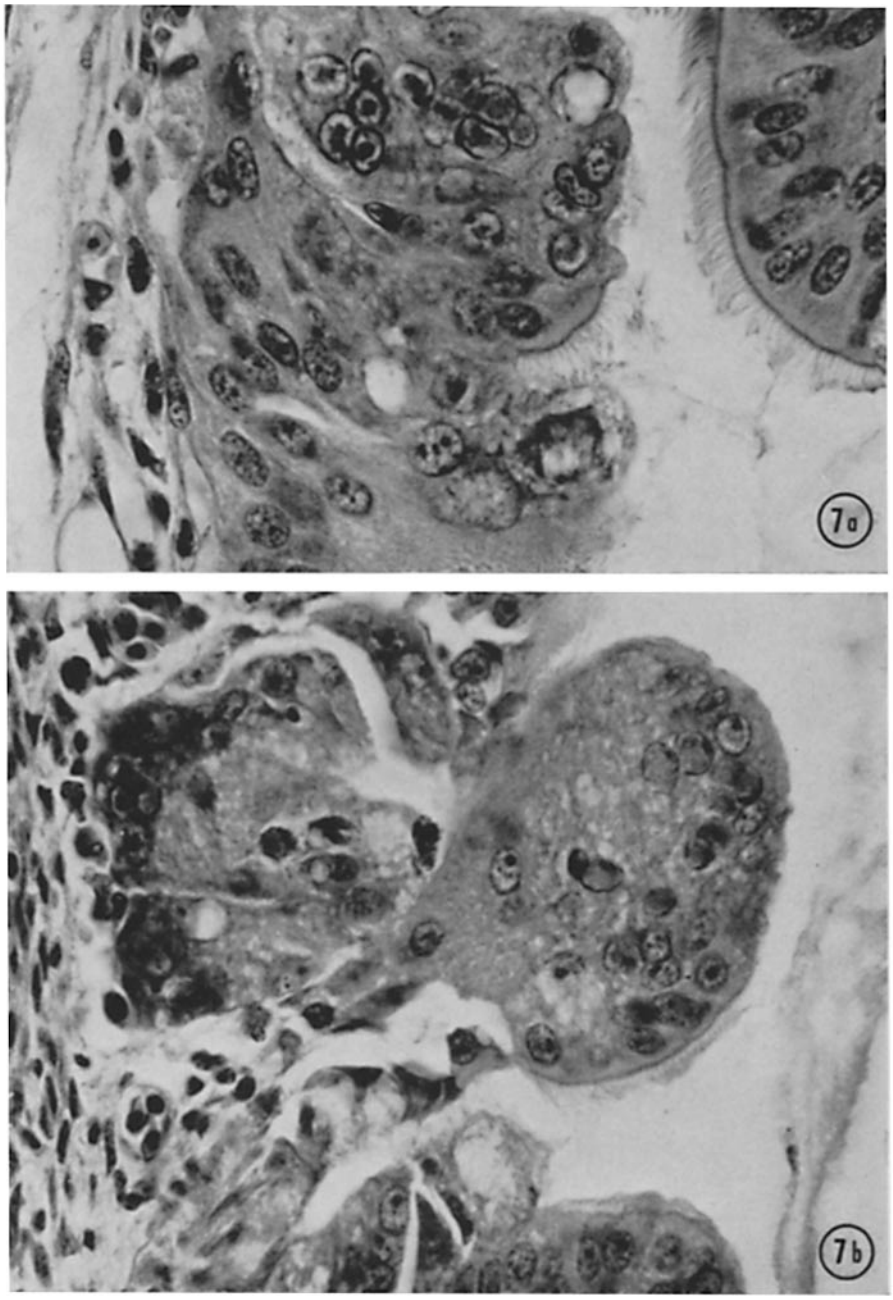
PLATE 28

FIGS. 7 *a* and 7 *b*. Syncytia of cells with nuclear lesions in process of becoming detached from epithelium to form "giant cells". Hematoxylin and eosin. × 1000.

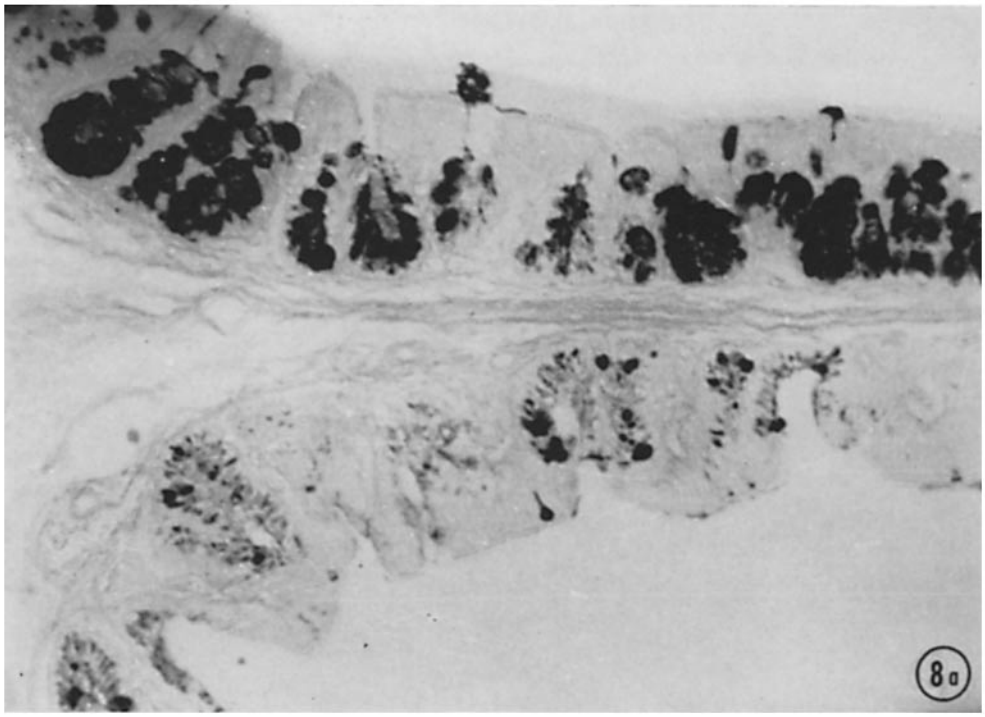


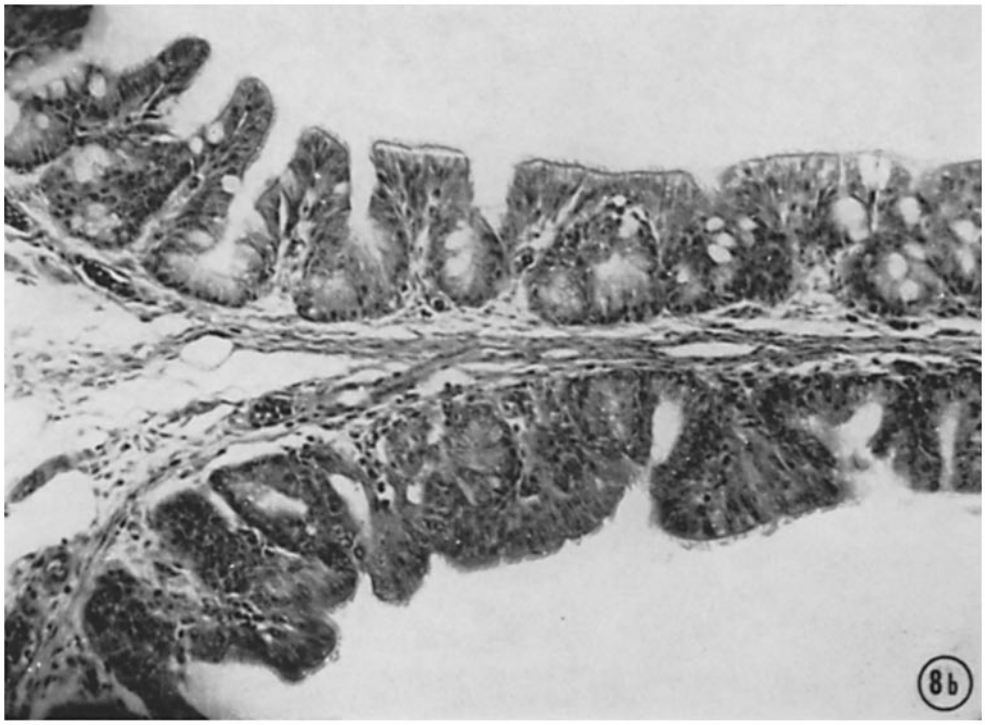
(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 29

FIG. 8 *a*. Base of septum, 72 hr unilateral infection stained with Alcian blue and periodic acid-Schiff (AB PAS), showing normally secreting acini on left (top), and markedly depleted acinar secretion on infection side on right. Approximately × 200.

FIG. 8 *b*. Same area shown on adjacent section stained with hematoxylin and eosin. Epithelium is beginning to show cellular changes on right, but is still intact *in situ*. Approximately × 200.

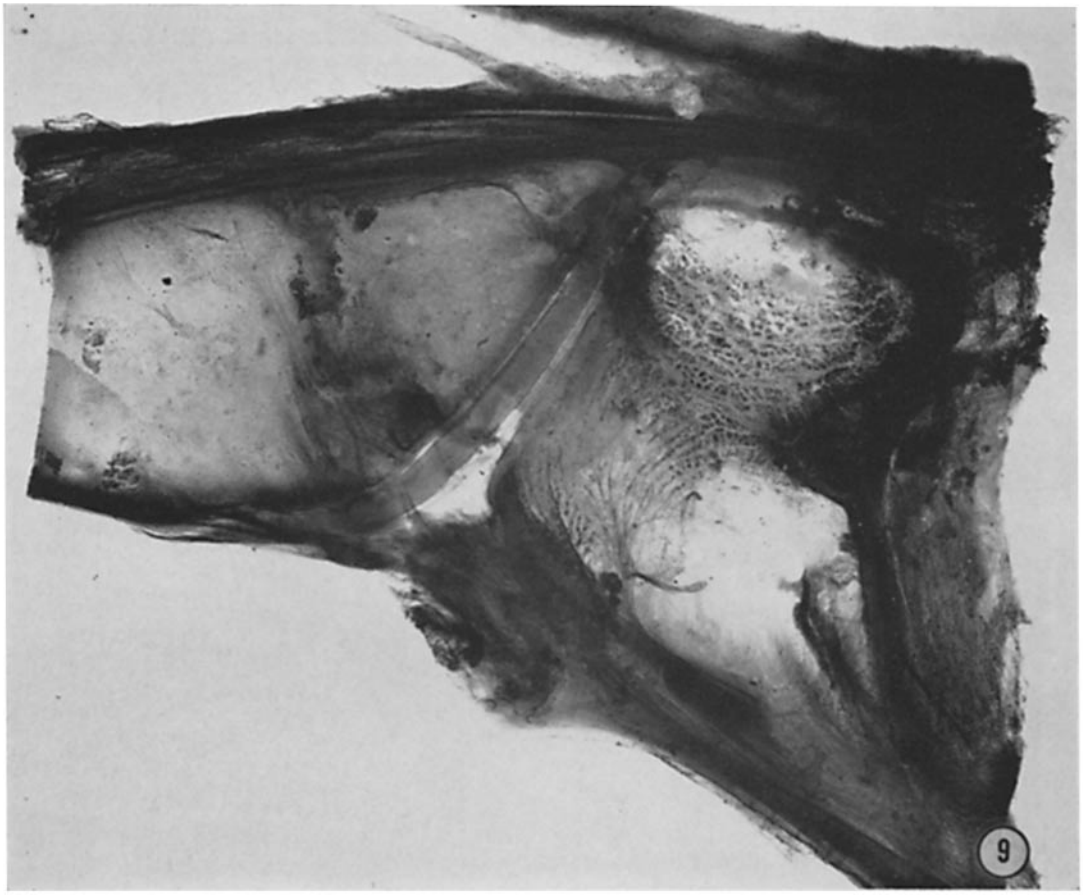




(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 30

FIG. 9. Whole mount of septum of chick 72 hrs postinoculum. Nearly all acini have sloughed, but deep parts of branched acini in juxta-olfactory area are partly intact. This can be seen in vertical section in Fig. 10. × 12.

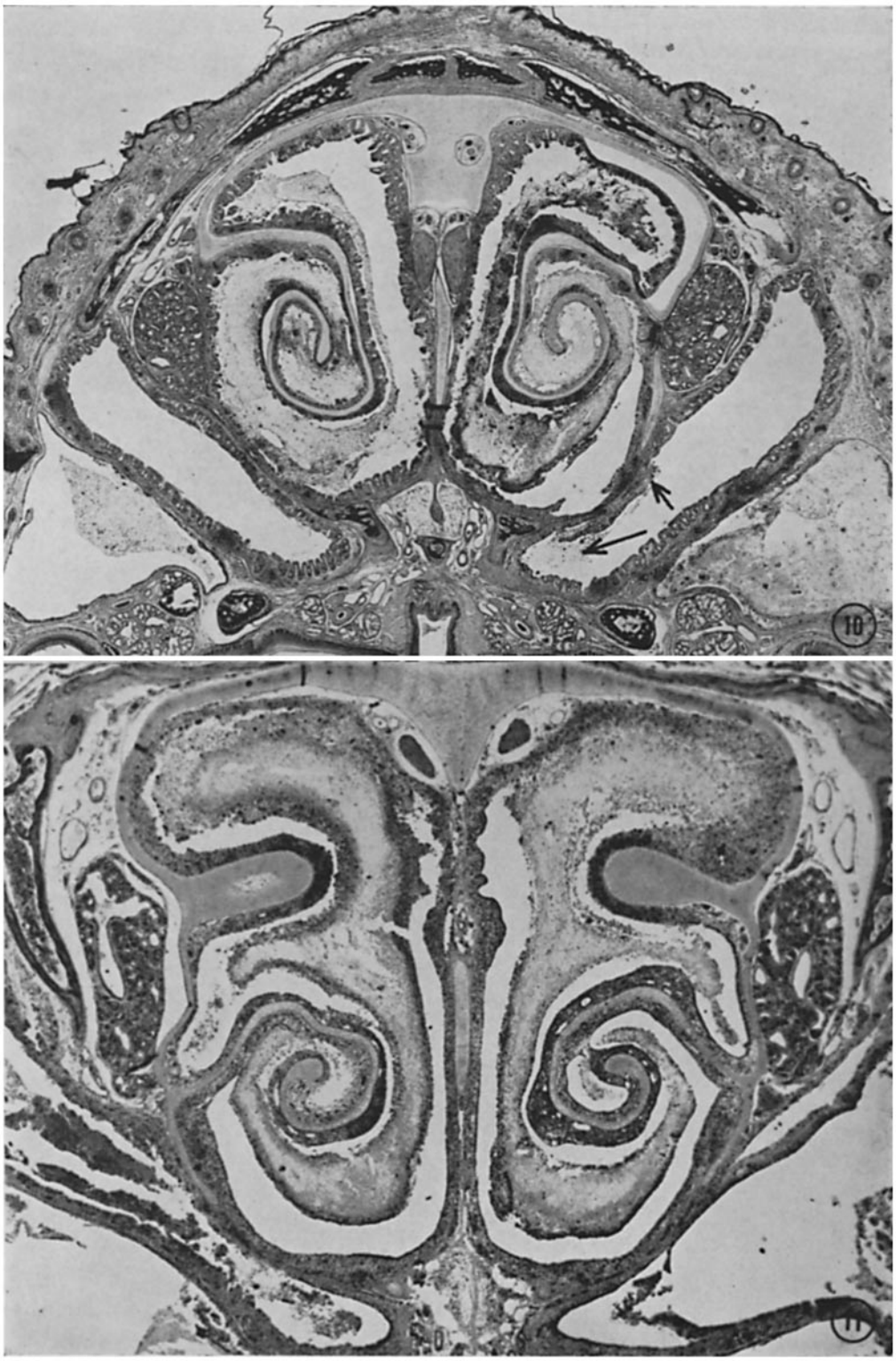


(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 31

FIG. 10. 72 hr postinoculum. Low power microphotograph through midfossa showing epithelial sloughing in respiratory area and sinuses, beginning sloughing in lacrimal duct (arrows). Hematoxylin and eosin. × 25.

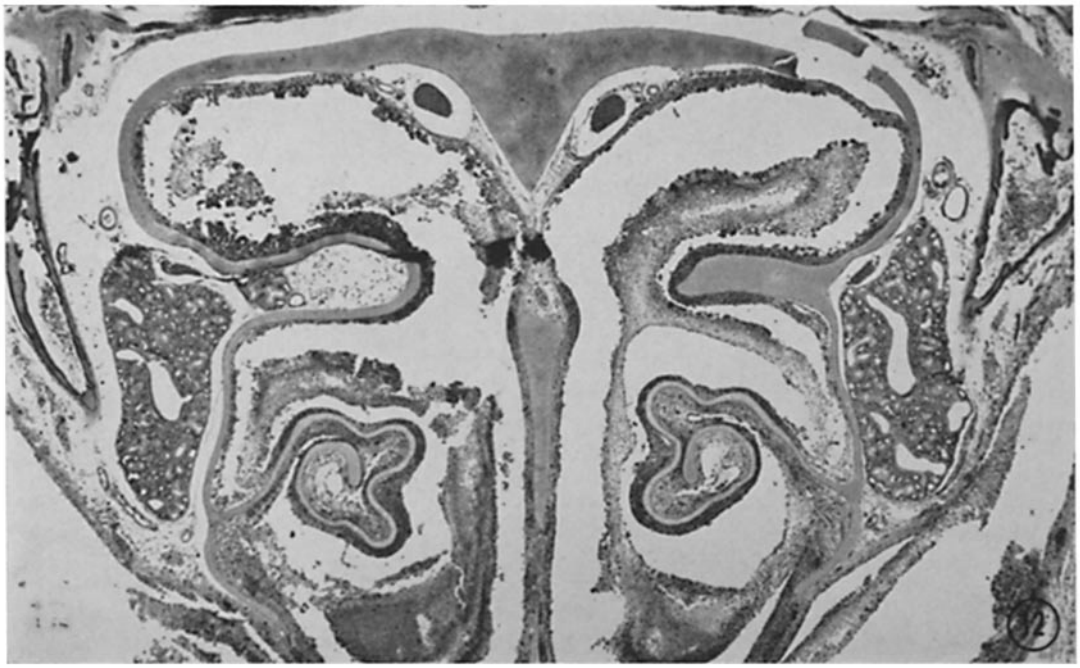
FIG. 11. Low power microphotograph of 8 day infection showing that severe sloughing of olfactory epithelium and beginning repair of respiratory epithelium occur simultaneously. Hematoxylin and eosin. × 25.



(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 32

FIG. 12. 7 day infection, showing distortion of cartilages of maxillary conchae during period of softening of cartilages. Hematoxylin and eosin. × 25.

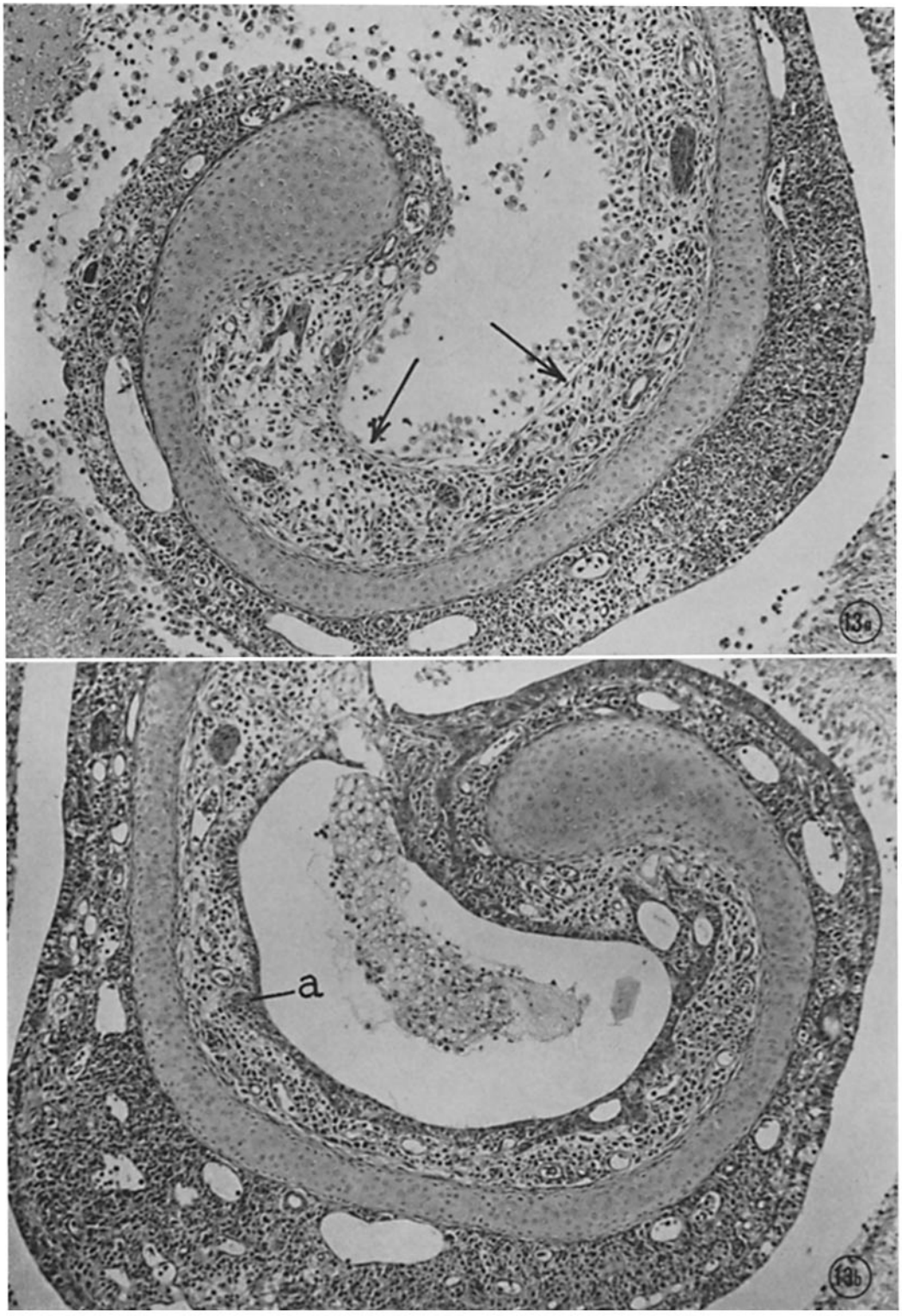


(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 33

FIG. 13 *a*. Higher power of right maxillary concha of Fig. 11. Transition state between sloughing and heavy infiltration shows on inner surface of scroll. Many post-capillary venules filled with cells. Thin line of new epithelial cell sheet is just discernible (arrows). × 162.5.

FIG. 13 *b*. Left concha of Fig. 11 showing more advanced repair. Epithelial-cell adhesion just beginning to break down. Squamous-cell anlagen of mucous acini (*a*), dilated venules, lacelike epithelium on outer surface of concha as cell differentiation begins. Hematoxylin and eosin. × 162.5.



(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 34

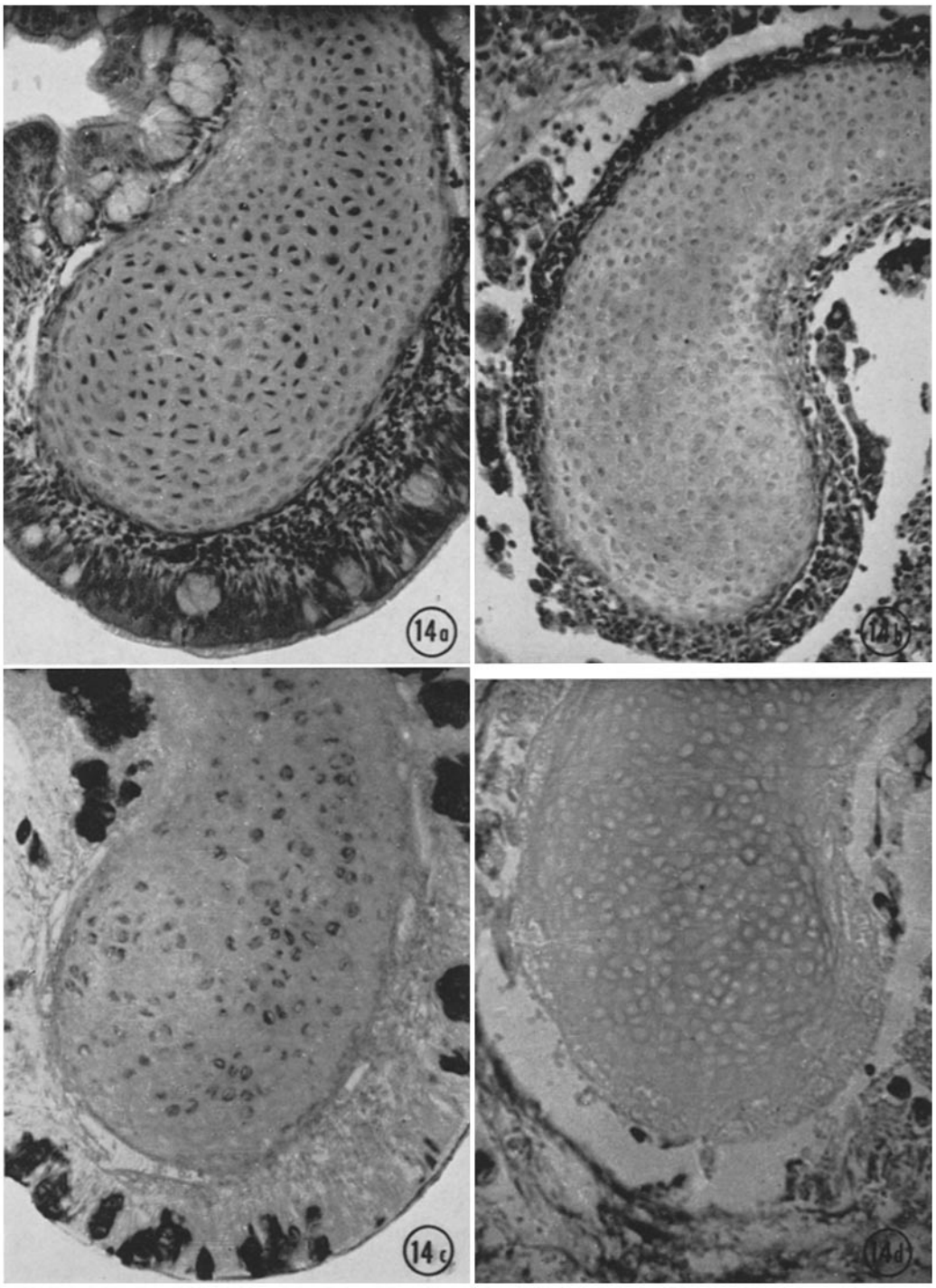
FIGS. 14 *a* to 14 *d*. Unilateral infection, 72 hr.

FIG. 14 *a*. Tip of maxillary concha of normal side, showing basophilic cartilage and intact mucosa. Hematoxylin and eosin. Approximately × 300.

FIG. 14 *b*. Tip of opposite, infected, concha showing loss of basophilia and severe desquamation. Hematoxylin and eosin. × 300.

FIG. 14 *c*. Same chick, normal concha stained with Alcian blue and periodic acid-Schiff stain, showing Alcian blue in cartilage nuclei. AB PAS. × 300.

FIG. 14 *d*. Opposite, infected, concha showing less of Alcian blue from cartilage nuclei. The loss of Alcian blue from the cartilage matrix does not show up in black and white photographs. AB PAS. × 300.

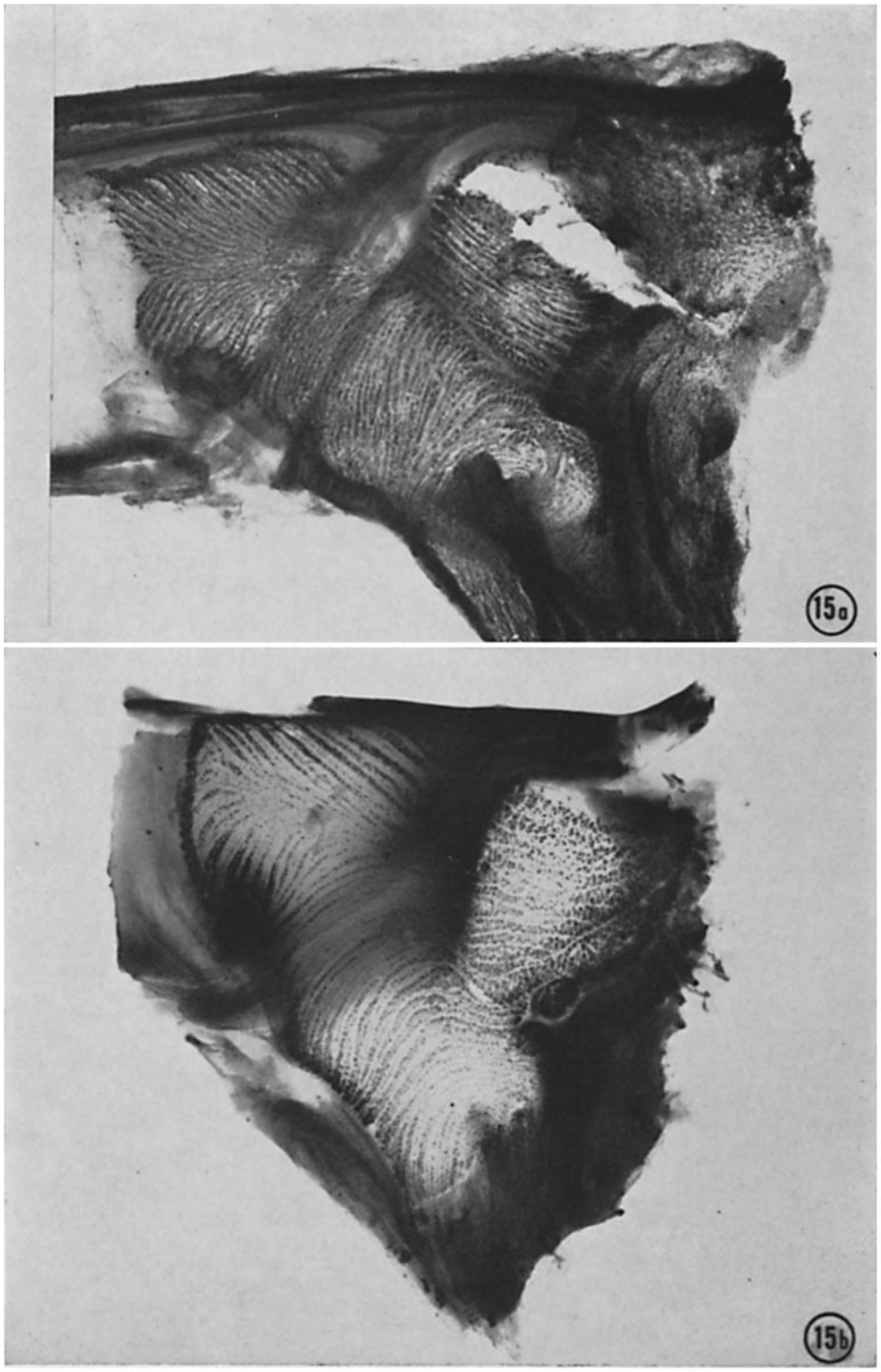


(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 35

FIG. 15 *a*. Whole mount of septum during early repair, day 11. Note that most advanced regeneration of secretory acini is in peripheral areas relatively sheltered from direct airflow, having the deepest epithelia and most rapid ciliary motion. Artificial tear in upper right. × 12.

FIG. 15 *b*. Whole mount of 11 day developing chick embryo, showing similarly advanced development in homologous areas. × 20.

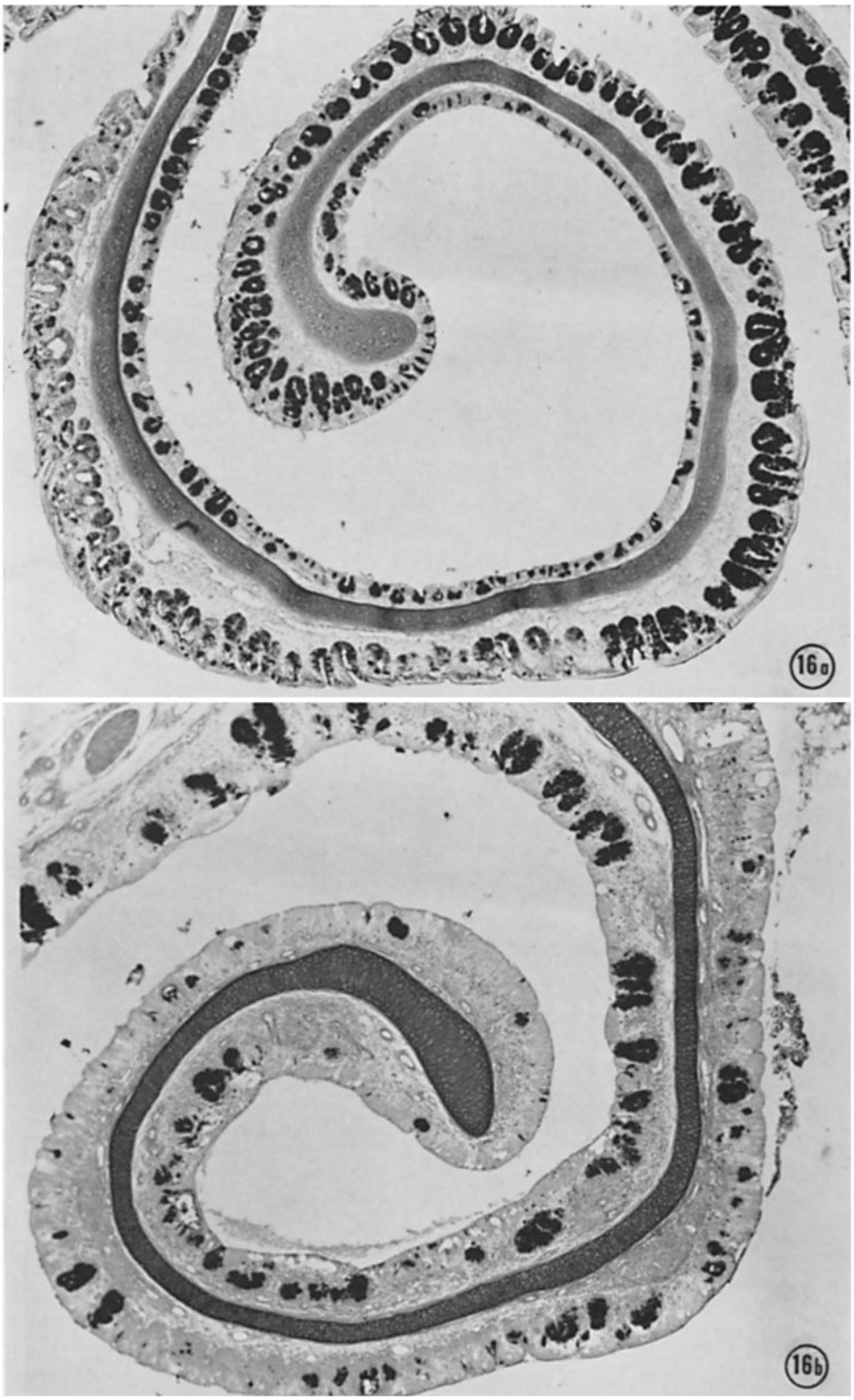


(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 36

FIG. 16 *a*. Maxillary concha of normal 3 day chicken. AB PAS. × 65.

FIG. 16 *b*. Maxillary concha of chicken 13 days after inoculation with laryngotracheitis virus: irregular shapes and sizes of acini, occasional mucous cysts, imperfectly regenerated epithelium. AB PAS. × 65.

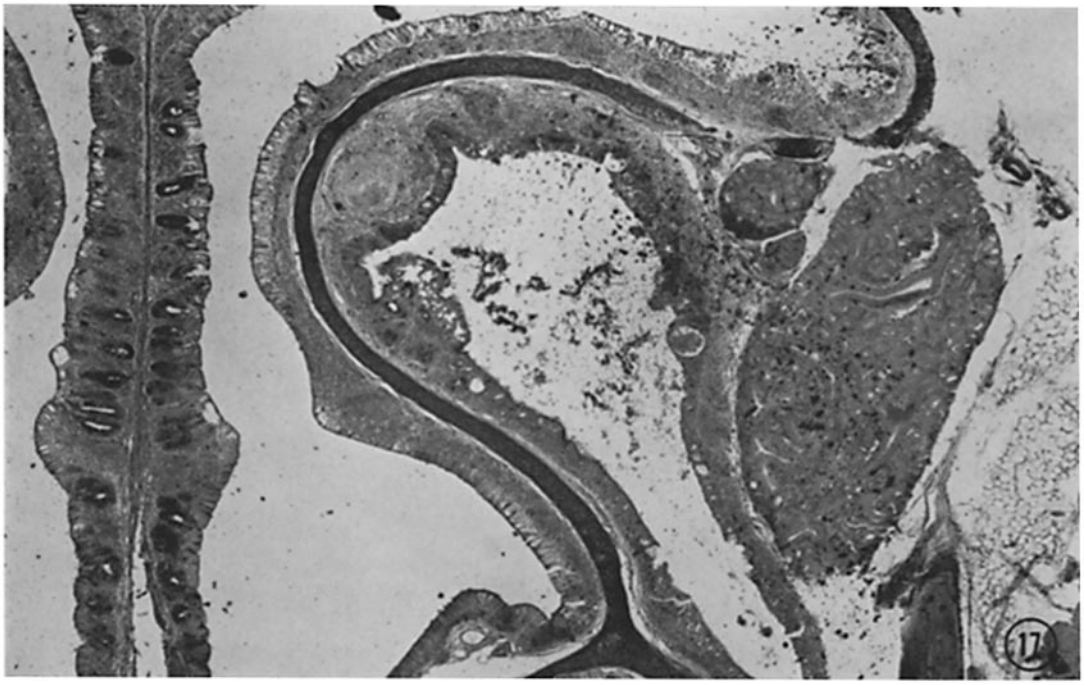


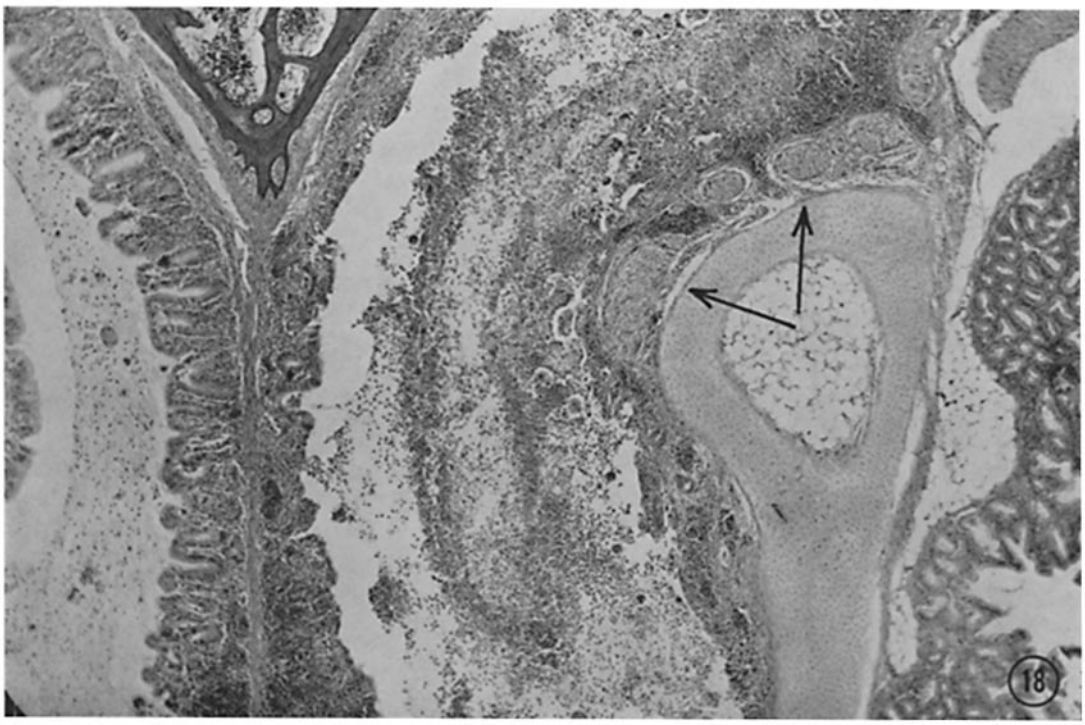
(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 37

FIG. 17. Olfactory concha of specimen shown in Fig. 16 *b*, showing invasion of normally areolar lining of concha by incursion of abnormal sinus epithelium into the hollow (orient on Text-fig. 3). Note replacement of olfactory epithelium by early stage mucociliary epithelium. Hematoxylin-PAS. × 50.

FIG. 18. Severe desquamation of olfactory epithelium, 8 days postinoculum. Main nerve trunks intact (arrows) but all epithelial branches and end organs have sloughed. Hematoxylin and eosin. × 50.

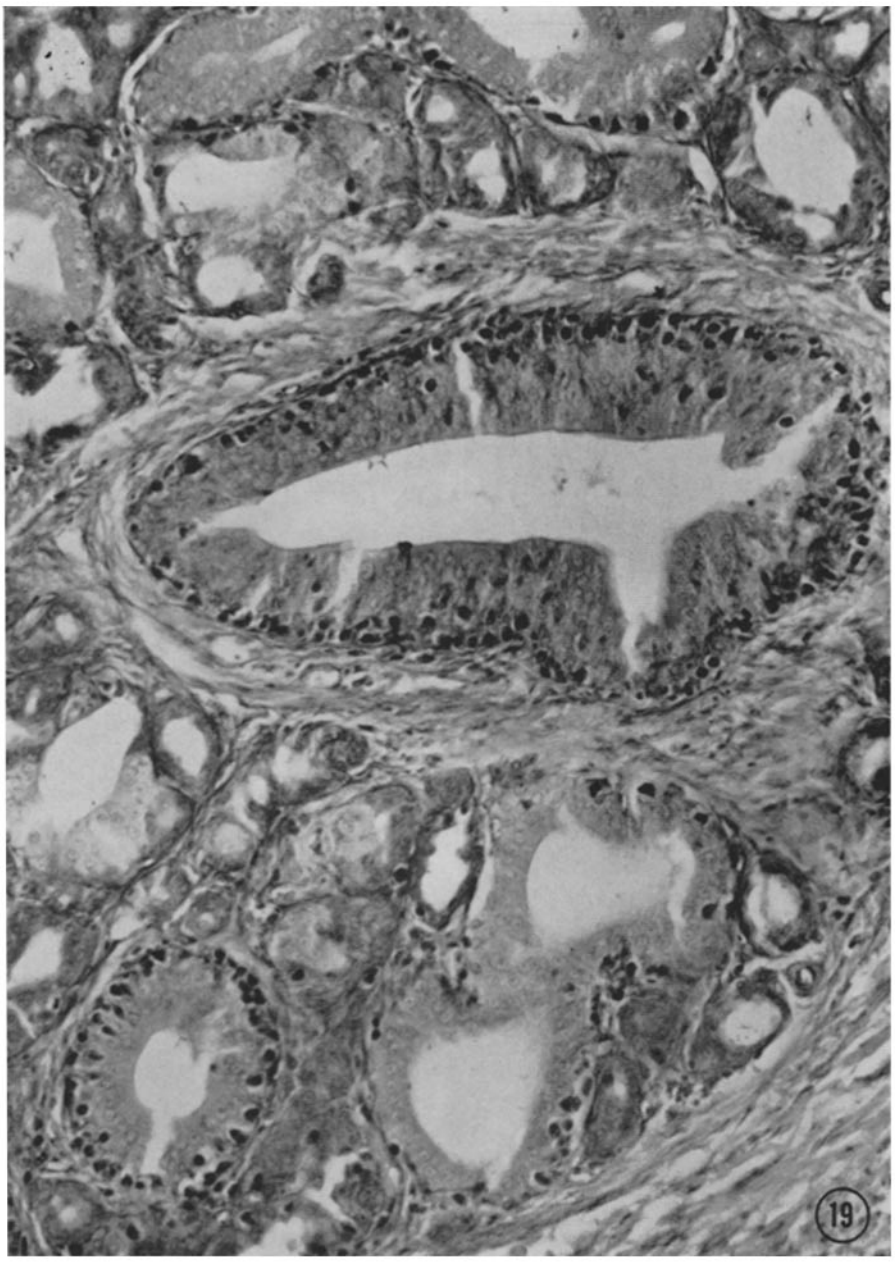




(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 38

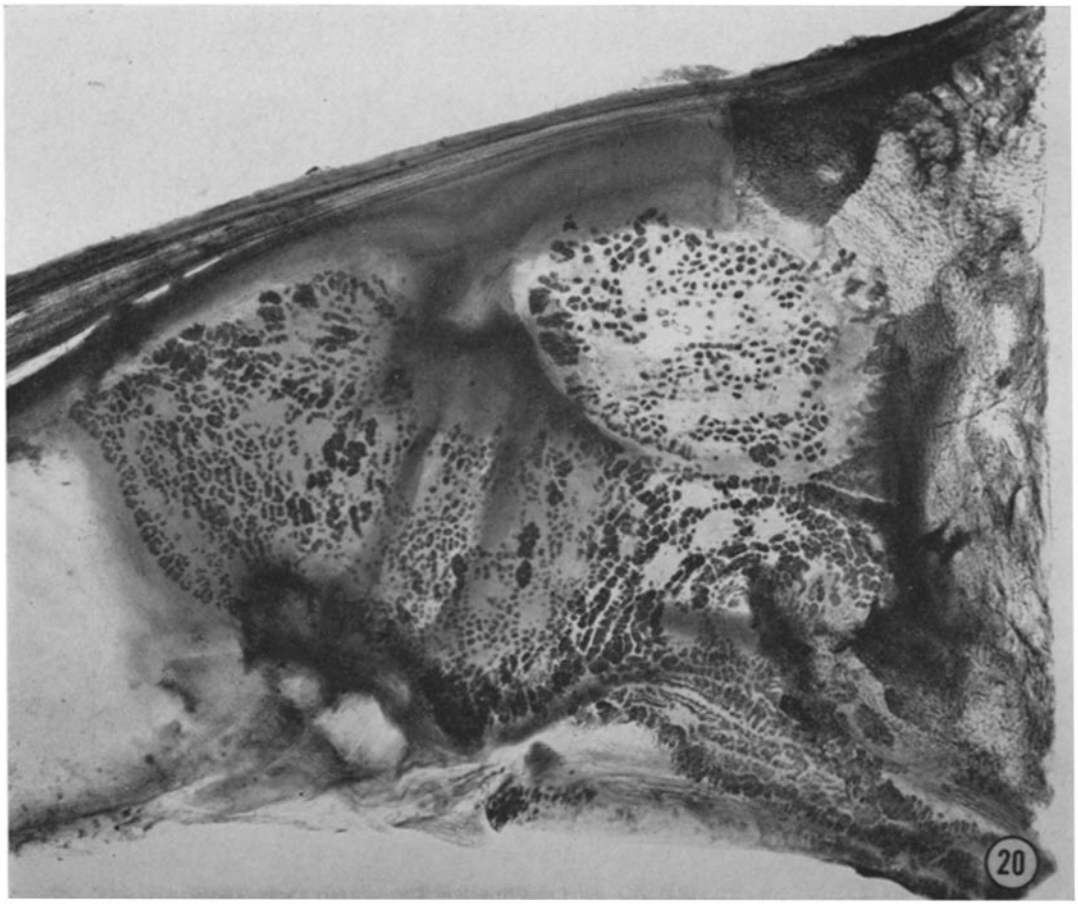
FIG. 19. Circulating plasma cells in duct walls of lateral nasal gland. Typical concentration of plasma cells at basement membrane of duct. While this orderly concentration of plasma cells around the ductules and main ducts of the lateral nasal gland was consistently found in chickens which had been acutely infected, plasma cell concentrations were also quite often found around the main ducts of control chickens, especially in birds which had heavy lymphoid infiltrations in the lacrimal ducts. Hematoxylin-PAS. × 250.



(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 39

FIG. 20. Abnormal pattern of acinar regeneration, whole mount of septum at six weeks convalescence. Rounded clear spaces are lymphoid nodules. × 12.



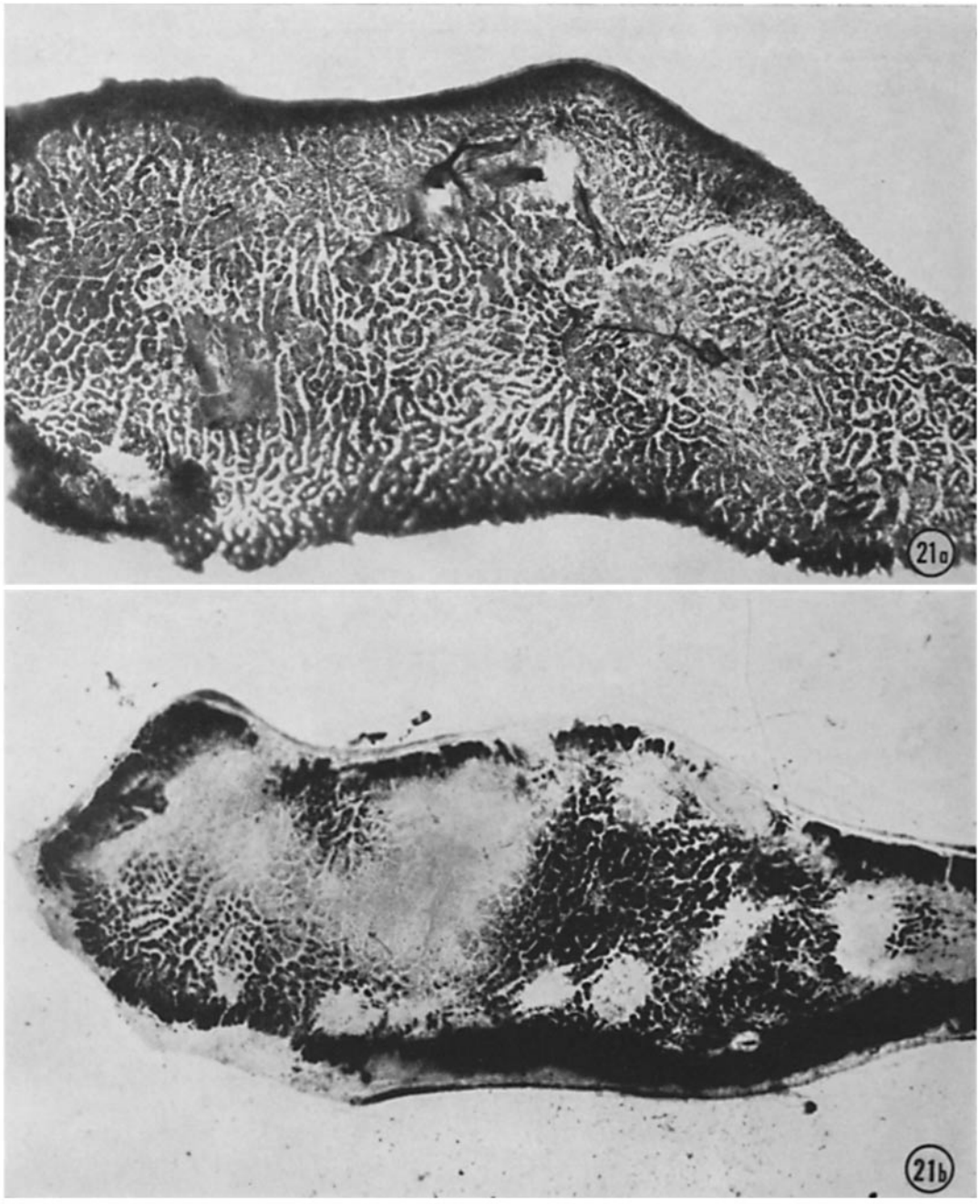
(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 40

FIGS. 21 *a* and 21 *b*. Distorted pattern of acinar regeneration, 4½ months.

FIG. 21 *a*. Whole mount of outer surface of maxillary concha: random lineation and frequent swirls or cowlicks. × 15.

FIG. 21 *b*. Inner surface of same conchal scroll; clear spaces are mixed lymphoid nodules and nonfunctioning acini. × 15.



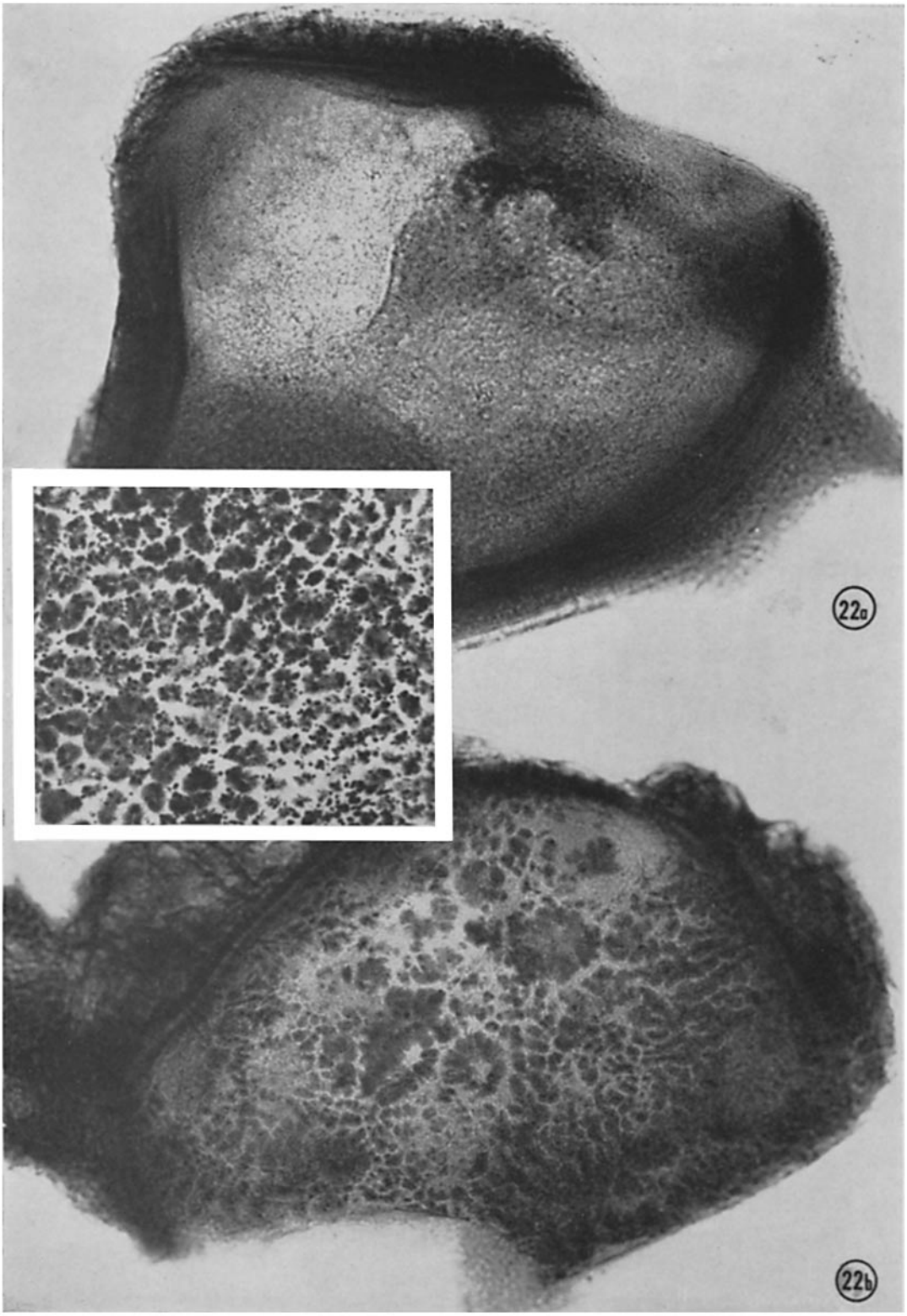
(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 41

FIG. 22 *a*. Whole mount of normal olfactory concha: clear, translucent, devoid of mucous glands. × 40.

FIG. 22 *b*. Whole mount of 27-day convalescent chicken: random, irregular sized abnormal mucous acini and glands. × 40.

Inset: Enlarged portion of metaplastic epithelium, showing goblet cells and acini. × 50.



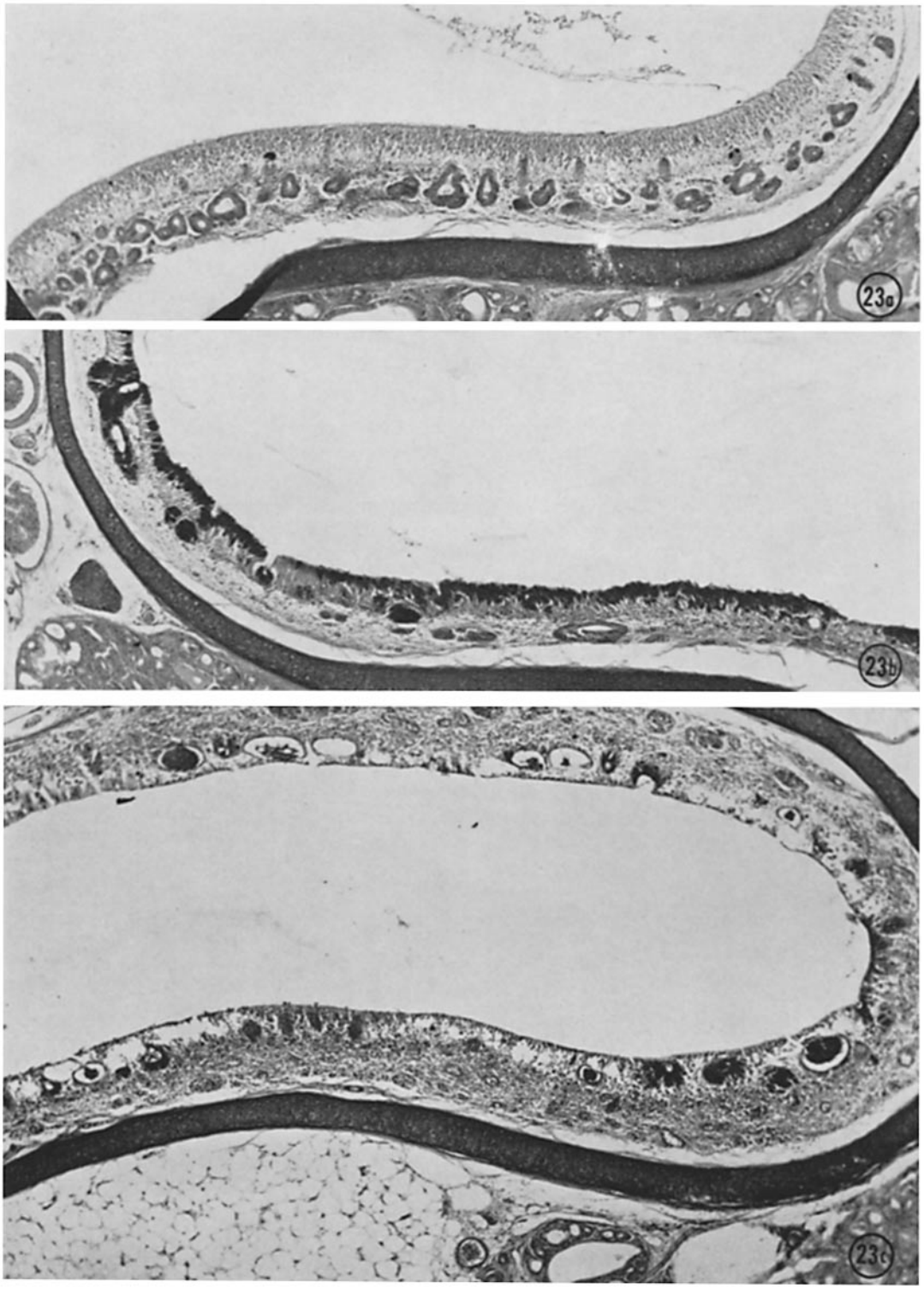
(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 42

FIG. 23 *a*. Histological section of upper surface of normal olfactory concha: olfactory epithelium and glands of Bowman. Hematoxylin-PAS. × 62.5.

FIG. 23 *b*. Upper surface of olfactory concha 27 days postinoculum: mucous metaplasia of surface epithelium and glands of Bowman. Hematoxylin-PAS. × 62.5.

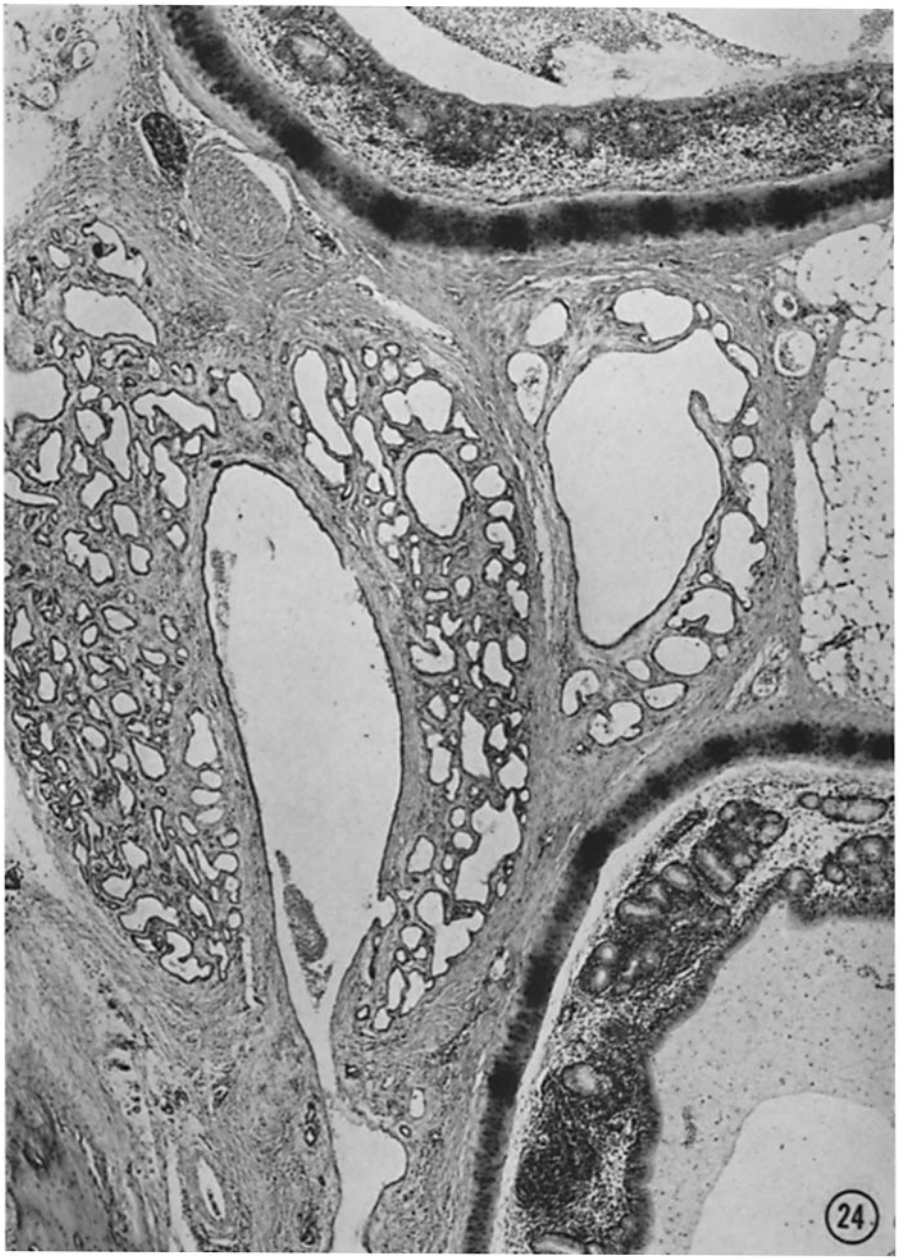
FIG. 23 *c*. Another specimen at 27 days: cystic degeneration of glands of Bowman, beginning mucociliary epithelium. Hematoxylin-PAS. × 62.5.



(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 43

FIG. 24. Atrophy of lateral nasal gland, 54 day convalescent chicken. Normal appearance of gland seen at lower magnification in Fig. 25 *a*. Hematoxylin and eosin. × 50.



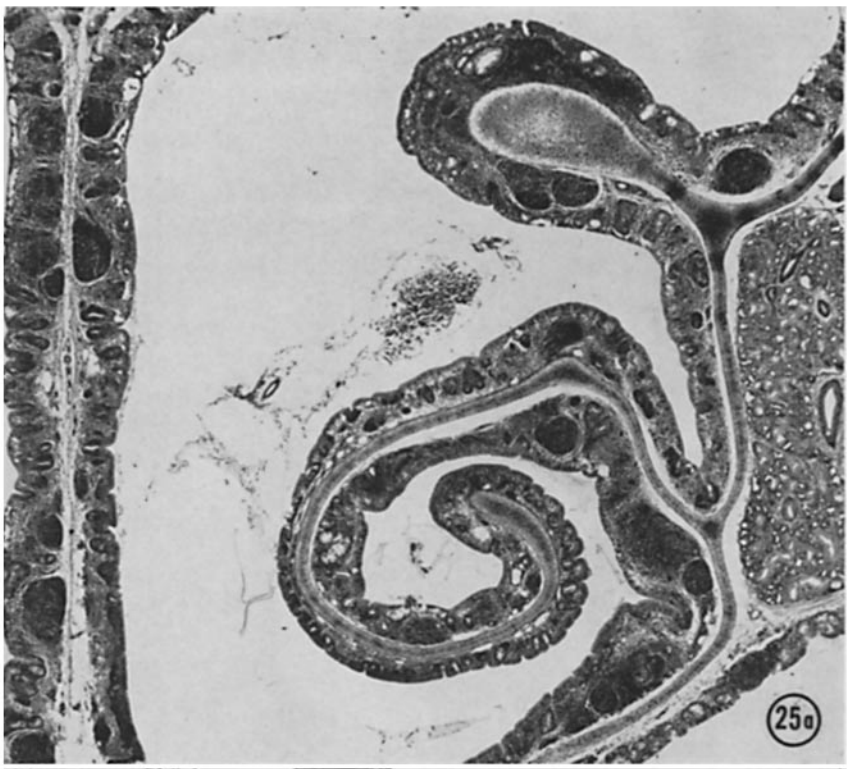
(Bang and Bang: Laryngotracheitis virus in chickens)

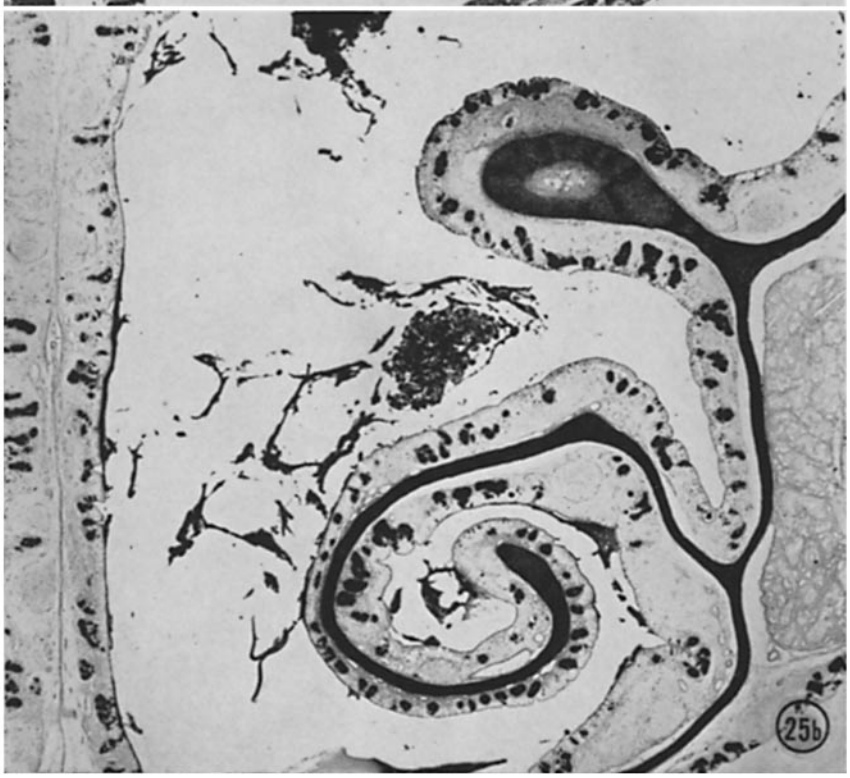
PLATE 44

FIGS. 25 *a* and 25 *b*. Histological sections through olfactory and maxillary conchae of 40-day convalescent chicken. Adjacent sections.

FIG. 25 *a*. Thickened submucosa, large secondary lymphoid nodules, incompletely regenerated epithelium. Hematoxylin and eosin. × 40.

FIG. 25 *b*. Irregular acinar regeneration, mucous metaplasia of olfactory concha, thick mucus in lumen. AB PAS. × 40.



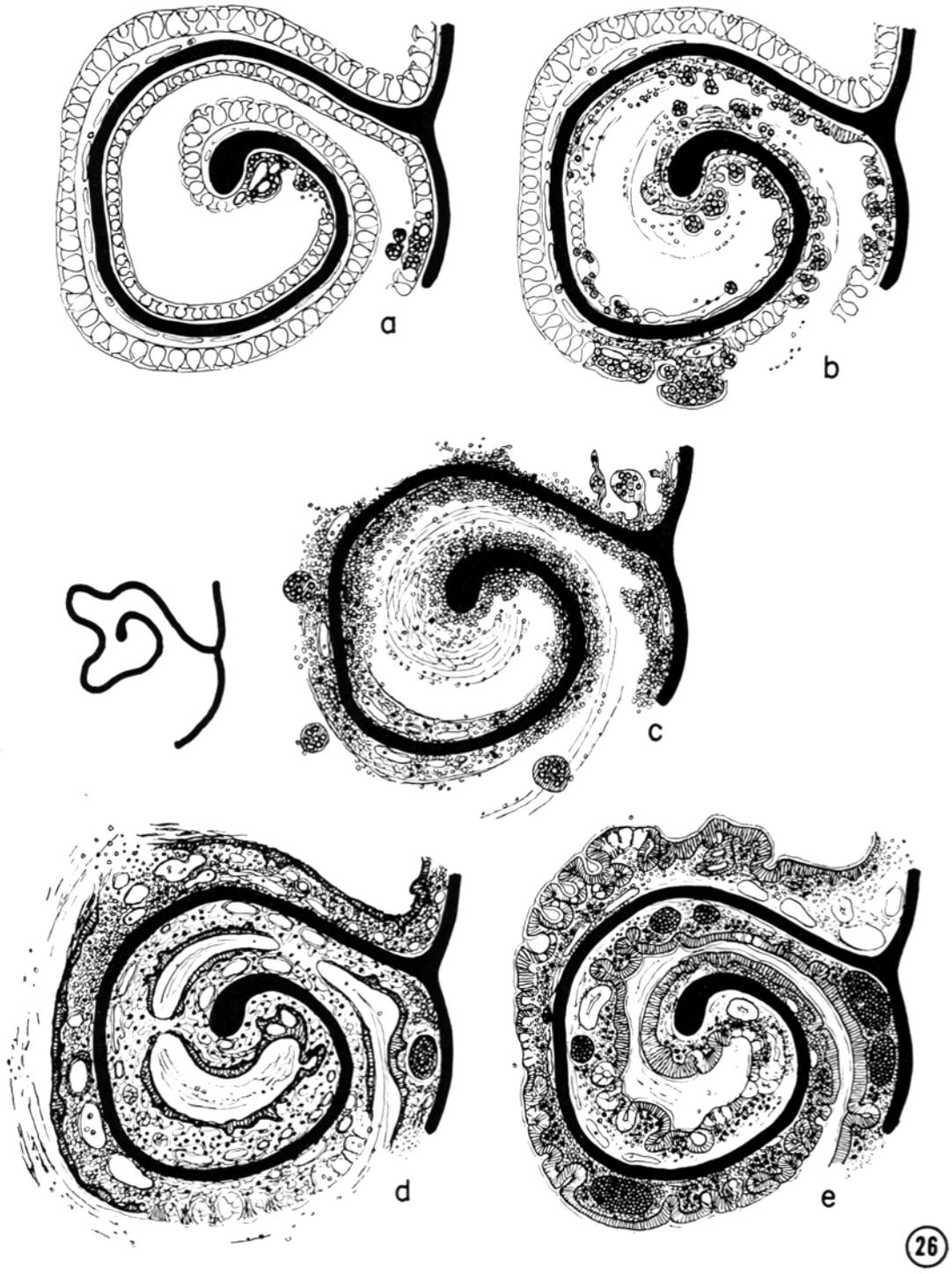


(Bang and Bang: Laryngotracheitis virus in chickens)

PLATE 45

FIG. 26. Diagrammatic sequence of changes in epithelium of maxillary concha. Black dots in 3, 4, and 5 represent plasma cells.

(*a*) Primary lesions (21 to 24 hr); (*b*) early spread (24 to 72 hr); (*c*) acute sloughing (3 to 8 days), inset: distortion of softened cartilage; (*d*) early regeneration (8 to 21 days); and (*e*) later regeneration (3 to 6 wk).



(Bang and Bang: Laryngotracheitis virus in chickens)