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

II. EFFECTS ON ADSORPTION OF NEWCASTLE DISEASE VIRUS*

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(Received for publication 6 March 1969)

The variety and number of viruses which cause infection of the upper respiratory mucosa of man (1) defy any attempt to generalize the important factors in their pathogenesis. In addition, it is impossible to determine rates of virus adsorption in man, or how effective the mucous blanket is in protection, or the types of cells infected in the early stages. For this reason we have turned to experimental models (2, 3) to explore the kinds of barriers which a virus may meet and the types of response of the host.

In the studies reported here, we have found several pharmacological agents which affect the adsorption of Newcastle disease virus to the intact nasal mucosa of chicks, and have adduced evidence that virus which is adsorbed during the first hour to the cells of the mucosa may be reexcreted when mucus secretion is stimulated. The virus presumably comes from mucous cells as part of the continuous process of mucus secretion. Studies on mucus secretion itself, in which rates of nasal clearance were determined in intact animals and mucosae were studied histochemically after exposure to drugs and cold, reported separately (4), support this interpretation.

Materials and Methods

The chicks used in this study varied in age from 3 to 21 days; the exact age is indicated in each experiment. The methods of infecting the upper respiratory tract, described in a previous study of the virulent or velogenic strain of Newcastle disease virus (5), was adapted to the present study.

3 wk old chickens were infected with intranasal inocula of the mesogenic (Beaudette) strain of virus, and at intervals beginning 12 hr later the middle turbinates were resected, fixed in neutral formalin, stained with hematoxylin and eosin, and examined for the earliest lesions. The virulent strain (cg 179) was used for the adsorption studies because of its greater capacity to produce plaques.

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* This investigation was supported by the Council for Tobacco Research, United States of America.

Since 1963 we have purchased chicks from a commercial flock in which Leghorn roosters are crossed with Kelab hens, a flock not vaccinated against Newcastle disease virus. Chicks were checked for antibodies to the virus in each experiment, and were found negative.

Plaque Method.—10 day old chick embryos were used to prepare trypsinized monolayer cultures containing 20–25 million cells/Petri dish (100 × 20 mm) or 10–12 million cells/60 × 15 mm plate. Medium for maintaining the cells consisted of 4% calf serum (not inactivated) and 96% Earle's balanced saline solution (BSS) containing 5% lactalbumin hydrolysate and 1% yeast extract. After growth for 48 hr at 37°C in the presence of 10% CO_2, the cells were washed once with phosphate-buffered saline, inoculated with 0.5 ml of inoculum, adsorbed for 1 hr at 37°C, then overlaid with a 1.9% neutral red agar (6 ml), 2-fold Earle's BSS (6 ml), and 50% embryo extract (1 ml). Plates were read after incubation with 10% CO_2 for 4 days at 37°C. The number of plaques present at this time was determined on two plates, each of which received 0.5 ml of inoculum. Final figures in all tables represent the calculated number of infected cells, infectious virus particles, etc., in the total sample. If the counts were high in the more concentrated plates, and exceeded 10 in the more dilute, the more dilute inoculum was taken as the final reading, which was corrected for the dilution. If the counts were low, the highest count was used in calculation. The amount of medium and the inoculum were reduced to half the volume when the 60 × 15 mm Petri dishes were used. Final figures of all experiments were calculated on the basis of 1 ml inoculum.

Infection of Chicks.—Unanesthetized chicks were inoculated by the intranasal route with 1 or 2 drops of undiluted allantoic fluid stock Newcastle disease virus which regularly titered to about $10^{8.5}$ LD_{50} in embryos or on trypsinized chick embryo cells. At intervals the chicks were decapitated, and some of the blood from the body was saved for antibody tests. The middle turbinates were dissected by removing the lower jaw, beak, and posterior skull, and cutting the nasal capsule sagittally just to one side of the septum to expose one turbinate. Under a dissecting microscope the turbinate was removed; then the septum was cut away and the other turbinate was cut out. Turbinates were placed in 2 ml of Earle's BSS containing 100 units each of penicillin and streptomycin. After a few minutes' shaking, the sample of six turbinates was transferred by adherence onto the end of a clean pipette to a 1:10 dilution of Newcastle disease virus chicken antiserum, kept there for a minimum of 5 min, and then washed. Serial washings, using three 10 ml tubes of Earle's BSS, were used for each sample. Each change of 10 ml saline was started in a clean tube and with clean pipettes, and was broken up into series of three to five steps. During these steps 2 ml of saline was removed from the clean tube containing 10 ml, placed with the tissue sample, mixed, and removed again. This was repeated until the 10 ml was used up. The final washed sample was placed in a clean tube and washing was repeated. Thus dilution of any contaminating residual virus far exceeded the calculated 1:300 which would automatically occur with each 10 ml change. At the end of the three sets of washings, the tissue was placed in a solution of 1% trypsin and the mucosa was scraped off with a sterile knife, again under the dissecting microscope. After incubation in trypsin for 1 hr at 37°C, the mucosal material was washed twice by centrifugation in cold Earle's BSS. The suspension of the centrifugate was then titered; the number of infected cells was determined by plating them on chick embryo monolayer cell cultures.

Adherent virus was considered as that virus found in the trypsin solution after tissue had been incubated in it. It was determined by first neutralizing the trypsin dilution with 0.25% soybean inhibitor and then plating the solution on the monolayer.

Cell virus was determined in the following way. The trypsinized cells which comprised the centrifugate after washing were suspended in distilled water to rupture the cells, the suspension was ground in a glass grinder and gently centrifuged, and the supernatant saline concentration was readjusted by dilution in double-strength BSS. The suspension was then plated on a chick embryo monolayer. Since there was individual variation from chick to chick, all of the experiments, unless specifically stated otherwise, were done with pools of three chicks. Sterile

conditions were maintained throughout the washing procedures, and bacterial contamination from the respiratory tract did not interfere with the titrations.

Histological and histochemical procedures followed the methods described in the preceding paper (4).

RESULTS

Location of Early Lesions.—We have shown previously that the middle turbinates had the most consistent pattern of virus adsorption (5), and that the virus of laryngotracheitis also produces its first effects on the middle turbinates (3).

Two chickens killed 24 hr after inoculation showed characteristic histological changes. One had such extensive spread of infection that many of the acini of the turbinates were collapsed and the acinar cells were in early stages of destruction. Moderate inflammation was present in the submucosa of both middle turbinates, but the ciliated surface seemed intact throughout.

The other chicken had changes apparently limited to the inner scroll of one middle turbinate. These are illustrated in Figs. 10 and 11; controls are shown in Figs. 12 and 13. Destroyed mucus cells were found particularly at the periphery of the collapsed acini. Stains for mucosubstances (4) showed that only remnants of mucus persisted in this area. The ciliated surface mucosa seemed intact, even directly over the areas of altered acini. It would therefore seem that the mucus cells are the ones predominantly affected by the virus early in the course of infection.

Adsorption of Virus to the Turbinates.—The initial purpose in studying the interaction of mucosa and virus was to determine the number of infected cells, the period of intracellular multiplication, release, etc., in these specialized cells, and the effects of drugs on these phenomena. However, both drugs and environmental changes had a marked effect on the behavior of virus during the very earliest phase of adsorption, and it appears that important new phenomena occur in the acinar cells during the first 5 hr of interaction. We have therefore been concerned not only with initial adsorption but with the early distribution of virus within the cell.

A number of variables in the experiments are still not understood. One is the large variation from chick to chick in the amount of adsorption of virus to the intact mucosa. In one experiment in which each pair of turbinates from one chick was handled individually but in an identical fashion, and in which little difference should be obtained between 1, 3, and 5 hr after absorption, there was nevertheless as much as a 75-fold variation from chick to chick (Table I).

In order to decrease the effect of this, all of the routine experiments in this report have used a pool of turbinates from three chicks to determine each point. This generally produced consistent results within an experiment, but there was nevertheless variation from experiment to experiment in the total amount of adsorbed (Fig. 1). While seasonal variation may play some role, it is clear it is not the sole factor in variation. There was no apparent effect of age (Fig. 2).

That variation was not due to the method of handling the tissue or to the titration themselves is indicated by the consistency of results obtained for the numbers of infected cells and of adherent virus within a particular experiment. One interpretation is that the variation may be associated with concomitant infection with one of the many types of respiratory disease in the individual flocks which are commercially obtained. It presents both a fundamental problem in determination of susceptibility to virus infection and a bothersome limitation on the interpretation of individual experiments.

The variation in total amount adsorbed has been overcome by repeating essential experiments several times, and by always running a control series at the same time. In the first part of this study, out of 15 experiments, five had control values which dropped at 3 or 5 hr more than 10-fold from the initial 1 hr value. In the subsequent experiments, in which particular care was taken to standardize the temperature at which the chicks were held, there has been less variation in the controls. Of 12 experiments, two showed a rise at 5 hr, and one a drop at 3 hr, followed by a return to base line values. Thus, following temperature adjustment, none has shown a consistently maintained drop in the number of infected cells during the 5 hr period.

TABLE I

Numbers of Infected Cells and Adherent Virus Obtained from the Middle Turbinates of Individual 12 Day Old Chicks

Time	Infected cells	Adherent virus
1 hr	292	1280
1 hr 20 min	180	216
3 hr	40	368
3 hr 20 min	108	880
5 hr	<4	<8
5 hr 20 min	148	144

Effect of Cocaine.—Cocaine is known to paralyze ciliary action, and since virus must gain access to susceptible cells by passing through the mucus blanket to the cells, we tested the effect of immobilization of the blanket on adsorption of virus. A series of four experiments was done, in which 1–3 drops of 10% cocaine were nasally instilled before virus was introduced. These are presented individually in Fig. 3 a–d, and the entire group is summarized in Fig. 3 e and f. From these it may be seen that in three of the four experiments there was a sustained, significant increase in the numbers of infected cells in those chicks given intranasal instillation of 5% and 10% cocaine before virus was given. In the fourth experiment, although most of the values for cocaine-treated chicks were above the control values, the differences between control and cocaine-treated values were not great. It is not clear whether the failure in this case to obtain a great

difference between experimental and control results was due to failure of the cocaine to paralyze the cilia, or whether for some reason the high value for the controls (close to the highest obtained in the 5 yr of work) reflects a limit on the number of cells which can be infected without removing the mucus blanket.

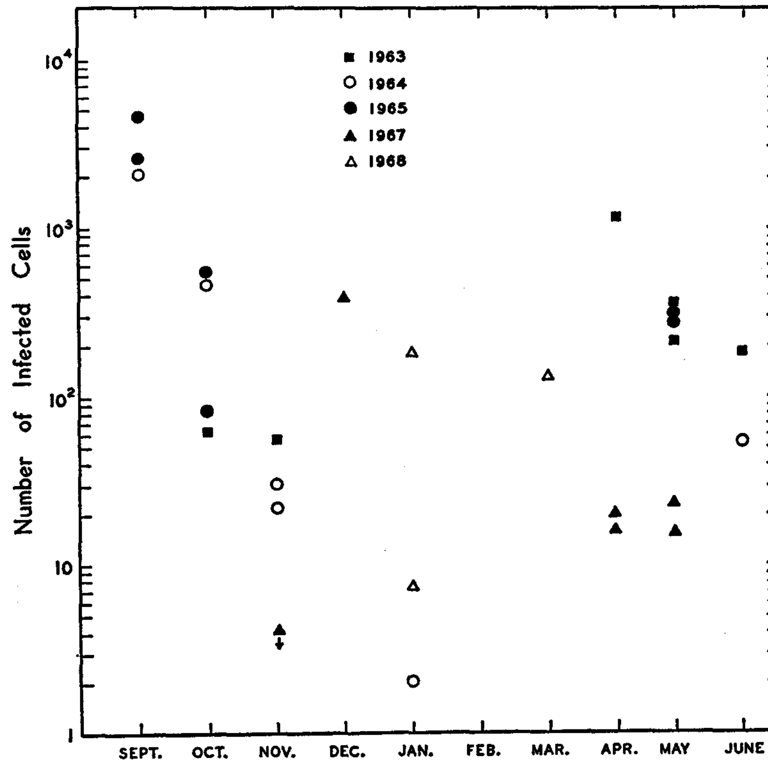

FIG. 1. Variation in the number of infected cells in turbinates of chicks inoculated with Newcastle disease virus 1 hr previously.

In general, however, cocaine was effective in increasing the number of infected cells whether it was given one, two, or three times before administration of virus, or at times varying from 10 to 30 min before the virus. Later times were not tested. In one experiment virus was given first, followed by cocaine. In this case the values for the cocaine-treated chicks at 1 and 3 hr after virus were below the control values, both for the number of infected cells and for adherent virus, but by 5 hr the value for cocaine-treated chicks was slightly (but not significantly) higher than the control values. In general, this experiment agreed with the assumption that if cocaine is given ½ hr after virus, adsorption will already have taken place.

Effect of Pilocarpine.—The secretion of mucus in the respiratory tract may be expected to influence virus absorption in at least two ways. First, an intact mucus blanket keeps virus from reaching susceptible cells, and traps virus in the moving sheet to be disposed of in the gastrointestinal tract. Second, since a

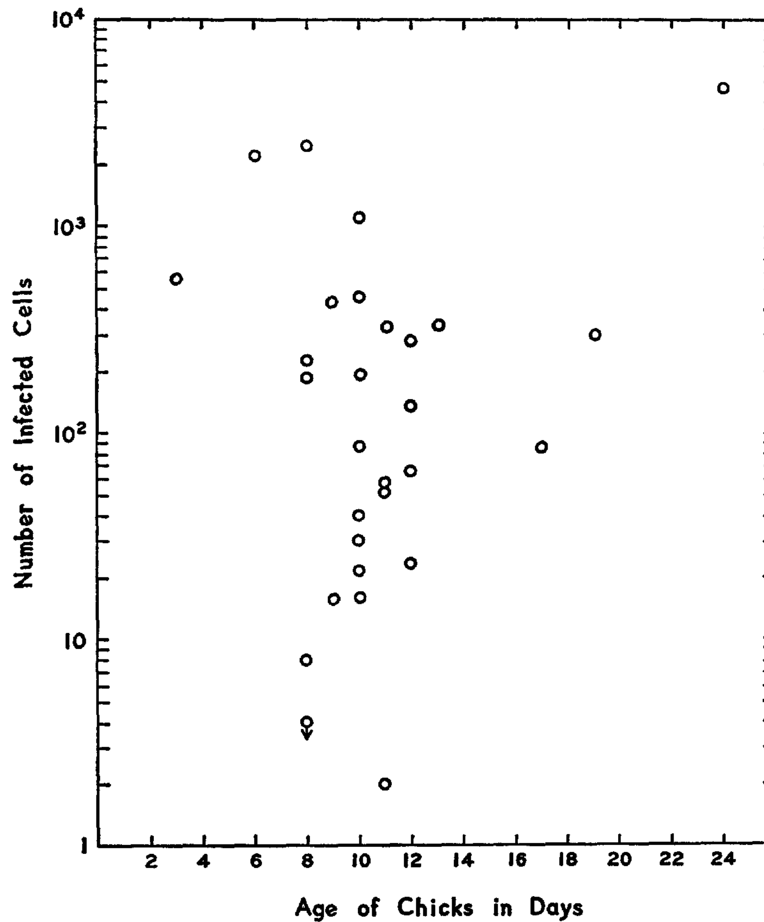

FIG. 2. Lack of relationship between age of chick and the number of infected cells found in turbinate 1 hr after inoculation. Chicks otherwise untreated.

myxovirus like Newcastle disease virus has a specific enzymatic interaction with mucopolysaccharide, one might expect some interaction at the gland cell level. Therefore, the number of infected cells might increase immediately after intensive hypersecretion, at a time when the gland cells are temporarily exhausted but the mouths of many acini are still gaping. An increased number of infected cells was indeed found when virus was given after pilocarpine injection (0.2 mg,

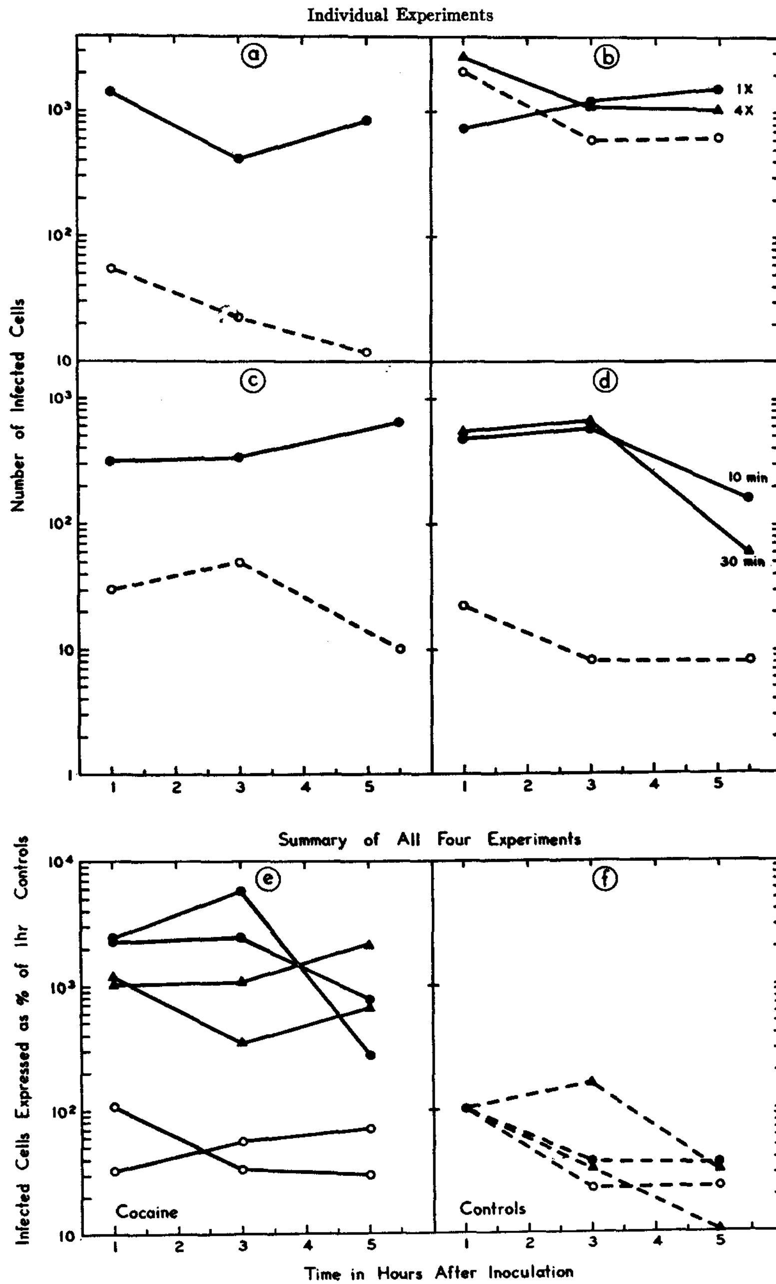

FIG. 3. Effect of cocaine before virus inoculation on the number of infected cells in the middle turbinate. In this figure and subsequent ones, the control values are represented by dashed lines.

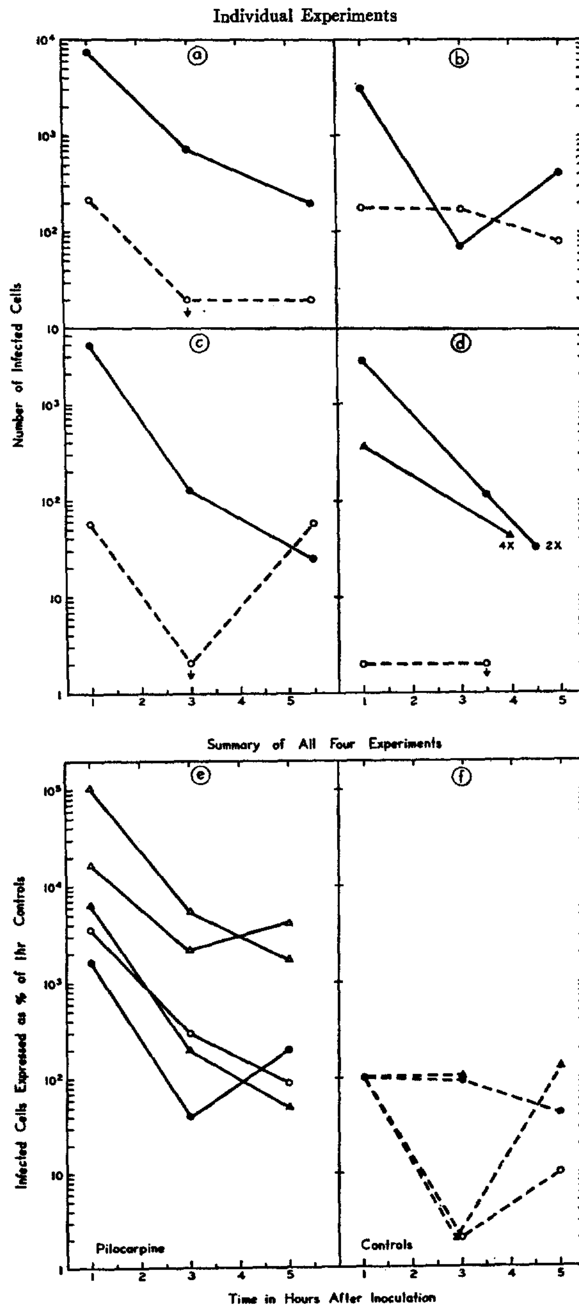

FIG. 4. The effect of pilocarpine before virus inoculation on the number of infected cells in the middle turbinate.

0.6%). But the subsequent events were a surprise. In all four experiments (five examples) there was a large drop in the number of infected cells between the 1 hr sample and the 3 or 5 hr samples (Fig. 4). This drop in three of the four experiments brought the number of infected cells at 5 hr after pilocarpine treatment back to the normal 1 hr control value. In the other experiment (Fig. 4 d), the 5 hr value failed to come near the very low control values. As in the cocaine experi-

TABLE II

Ratio of Infected Cells to Cell Virus at Different Times

Treatment	Expt. No.	1 hr		3 hr		5 hr	
		Actual values	Ratio	Actual values	Ratio	Actual values	Ratio
Controls	38	255/60	4.3	111/30	3.7+	192/450	0.4*
	76 (8 days old)	2,640/100	26.4	320/40	8.0	20,000/1,600	12.5
	76 (24 days old)	4,800/340	14.1	2,400/240	10.0	3,800/80	45.0
	63	58/20				76/20	
Pilocarpine ½ hr before virus	63	3,820/1,580	*2.3*‡	130/4	32.5	26/4	6.5
2× pilocarpine before virus	64	2,600/920	*2.9*	112/20	5.6	34/20	
Dehydrated 48 hr		800/330	*2.4*	1,160/780	*1.5*		
Excised		7,100/660	10.8	2,430/1,050	*2.4*	2,160/1,110	1.1

* This value, which is clearly not significantly different from 1, nevertheless may be expected, since new virus should begin to appear in the cell by 5 hr.

‡ Ratios which were unexpected, and which are refered to in the discussion, are italicized.

ments, results of individual experiments are given in the individual graphs (a–d) of Fig. 4 and are summarized in Table II on the basis of the percentage change from the original control value for 1 hr in Fig. 4 e and f. This loss in the number of apparently infected cells will be discussed later. Control values were less constant than in the experiments on the effect of cocaine, and this finding is also considered later.

If the increased number of infected cells at 1 hr were due to increased penetration through a less efficient blanket, resulting from temporary exhaustion of mucus and perhaps an opening up of the glands, quite different results might be expected if the pilocarpine were given after the virus had been inoculated. This was tested in four experiments. In three, pilocarpine was injected 15 min after

virus, and in the fourth, ½ hr after the virus. In contrast to the experiments in which pilocarpine preceded virus administration, there was no significant increase in the number of infected cells over the control at 1 hr. The results on the whole, however, did not show any consistent pattern, except that pilocarpine

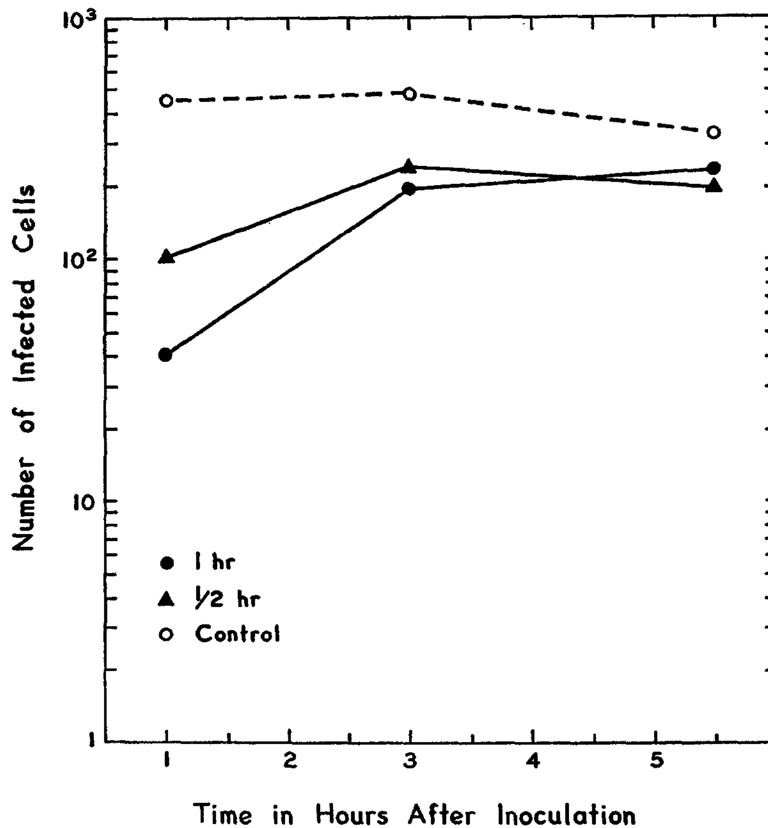

FIG. 5. The effect of hexylcaine before virus inoculation on the number of infected cells in the middle turbinates.

given ½ hr after virus seemed to have no effect. Again, control values varied a great deal.

Many topical drugs are used as local anesthetics in humans. The effects of some of these on the histology and function of the chick nasal fossa are presented in the accompanying paper (4). Xylocaine had no significant effect on the amount and course of absorption (one test). Hexylcaine (5%), however, produced striking changes physiologically and histologically, and therefore may be a more effective tool for studying the effect of morphological and physiological variables on virus-host interaction. Fig. 5 shows the effect of giving hexyl-

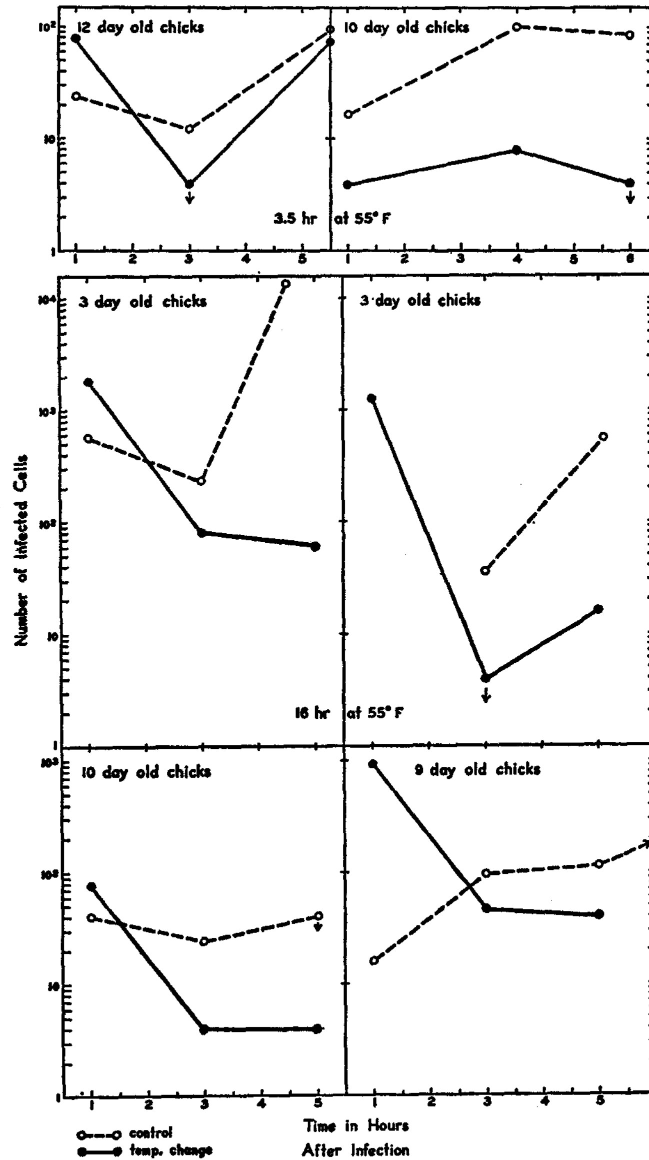

FIG. 6. The effect of previous cold exposure on the number of infected cells from the middle turbinate.

caine ½ and 1 hr before the administration of the virus. There was a decrease in the number of infected cells, perhaps caused by sloughing of cells as a result of the hexylcaine. The 20-fold increase in the number of infected cells between 1 and 5 hr in the chicks which were nasally instilled with 4–6 drops of hexylcaine

1 hr before virus inoculation can be explained by the failure of mucociliary flow following the extensive destruction of the mucosa. This would favor a continued increase in the amount of absorption.

Effect of Cold.—The failure of control chicks to show fairly consistent values during the first 5 hr of study led us to consider the temperature of the environment of the chick. The chicks were often kept at room temperature for an hour or so before and after inoculation. When chicks were kept in brooders throughout the experiments, much of the variation in controls disappeared. This led to a study of the effects on 3–12 day chicks of lowering the temperature well below

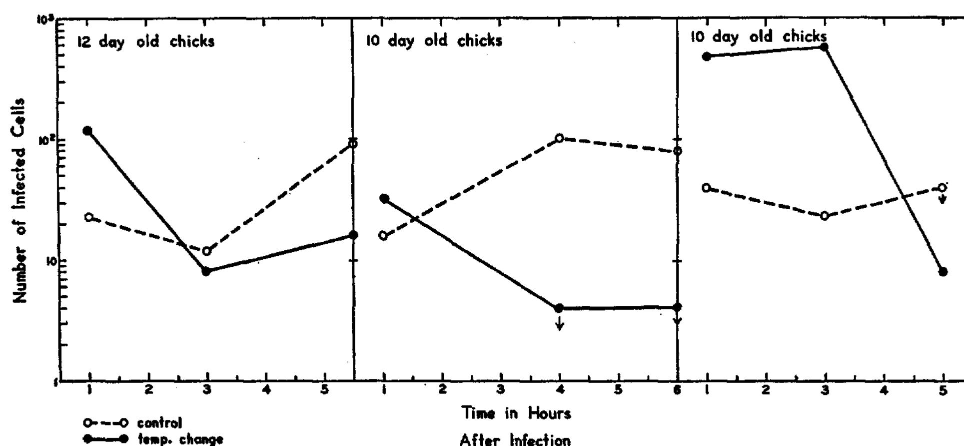

FIG. 7. The effect of cold exposure (55°F) after inoculation on the number of cells from the middle turbinate.

70°F. Results are presented in Fig. 6, which shows the results of six experiments. When 10–12 day old chicks were kept at 55°F for 3½ hr before inoculation, no consistent difference from the controls was apparent (Fig. 7). However, in four experiments, when younger chicks were kept for longer periods of time (16 hr) at lower temperatures (44–50°F), all initial values at 1 hr were above control values, and all 5 hr values were at least 10 times below the 1 hr value. This result was somewhat similar to that produced by previous injection with pilocarpine, and it is of some interest that the effects of prolonged mild cold and of pilocarpine after about ½ hr are histologically similar (4). The effect of lowering the temperature of the chicks immediately after inoculation with virus was studied in three experiments. In all three the initial value for the cold exposure group was above that of the controls, and in two the value of the cold-exposed group at 3 or 5 hr fell more than 10 times below the 1 hr value. In the third experiment it also fell, but because it had reached a minimum detectable value, the absolute fall could not be determined. Brooder temperatures were ±100°F.

In three of the experiments on the effect of prolonged mild cold, a group of

chicks was returned to the brooders after their original exposure to cold and immediately after virus inoculation (Fig. 8). In one group there was an immediate reversal of the cold effect, so that the 3 and 5 hr values were above the 1 hr value; in the other two, the 5 hr value returned to equal the 1 hr value. In other words, the consistent drop, which was apparently related to cold exposure before virus inoculation, was reversed by placing the chicks at brooder temperatures during the period of virus adsorption and development.

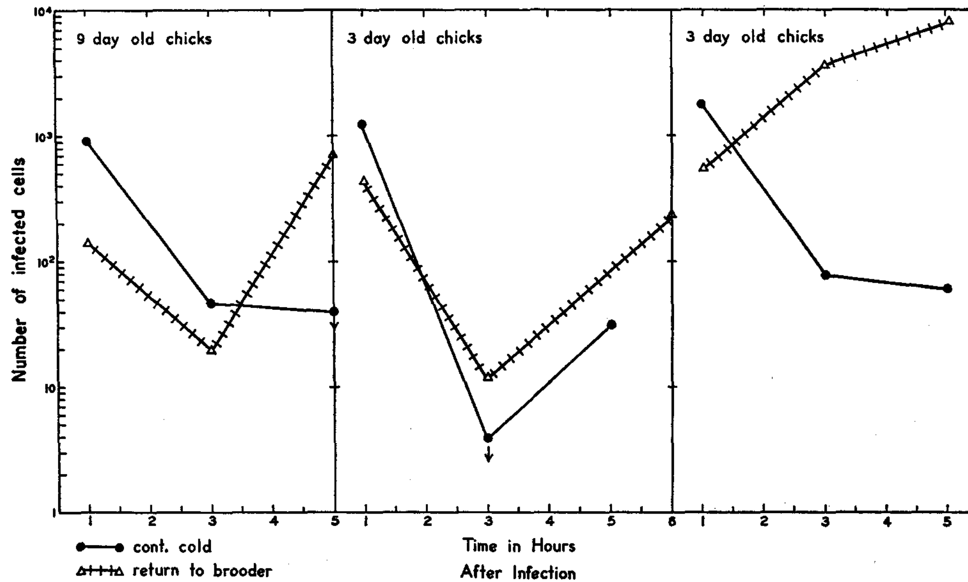

FIG. 8. The effect of return to brooder after 16 hr cold exposure on the number of infected cells from the middle turbinate.

It is known that older chicks rapidly acquire the capacity to regulate their temperature. A group of 12 day old chicks were exposed to severe cold by placing them for 1 hr (with one 10-min interruption) at −20°C. Their body temperatures dropped to near lethal levels (25–30°C), but the chicks revived when placed in brooders. As shown in Fig. 9, the severe cold had a greater effect, lowering the amount of infected cells recovered at 5 hr to about 1% of the value of the 1 hr specimen. All chicks were returned to brooder temperature during the period of virus adsorption and development.

DISCUSSION

These experiments were initiated with the thought that a study of the amount of virus adsorbed to the nasal respiratory mucosae would yield direct information concerning the susceptibility of the mucociliated epithelium. Such informa-

tion has indeed been forthcoming, but with the methods used it applies primarily to the initial stages of absorption and disposal of the virus.

The middle turbinates were selected originally as the test tissues because more virus was adsorbed to these specific structures than to others in the upper respiratory tract. This was a fortunate choice, for as knowledge of the normal

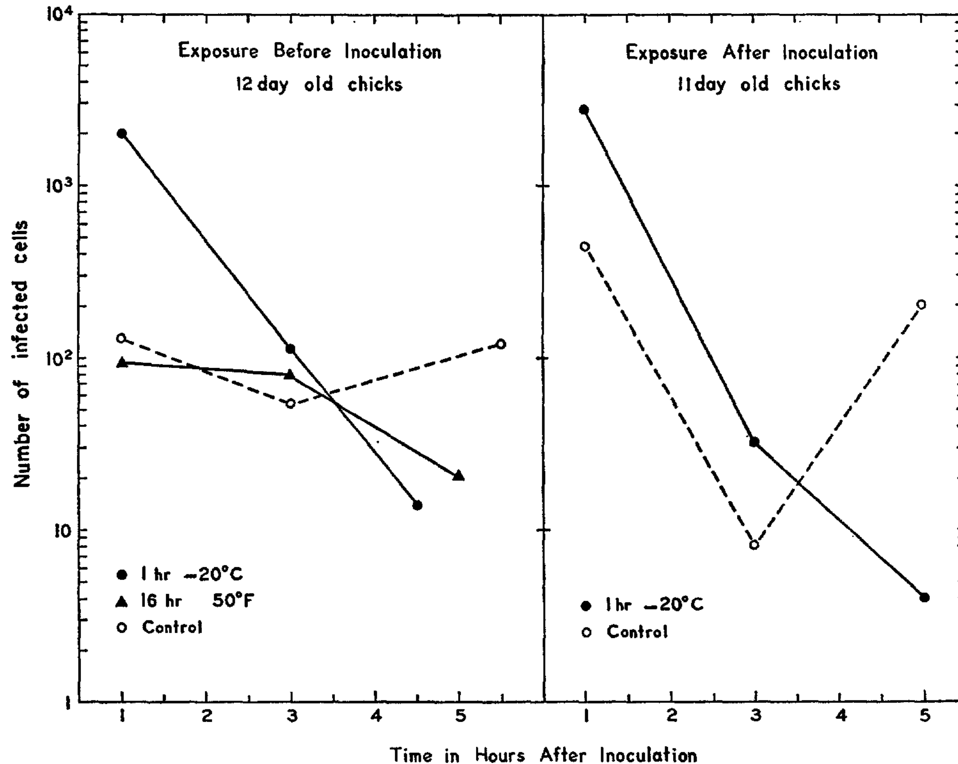

FIG. 9. The effect of exposure to severe cold (−20°C) on the number of infected cells from the middle turbinate.

histology has accumulated, it is clear not only that the middle turbinates are more free of extraneous inflammation than other areas of the upper respiratory tract (6), but that the higher amount of virus in these turbinates seems related to the fact that particles which localize there are stuck in the mucociliary flow channel, which takes the longest time to clear from the nose (3). Moreover, histological studies of laryngotracheitis (3) and of Newcastle disease virus (here reported) have shown that the middle turbinates represent the area in which active infection may first be detected.

The middle turbinates have only three types of cells initially exposed to in-

haled virus, and in the context of the present study it is important to know whether it is the goblet cells, the ciliated cells, or the acinar mucous secretory cells which are first infected. The earliest lesion detected histologically clearly involved acinar mucous cells at 24 hr, long after the initial 5 hr period for adsorption on which this study has concentrated. Since neither goblet cells nor ciliated cells were seen to be destroyed by then, we assume that acinar mucous cells have the chief role in early infection, though associated infection of ciliated cells cannot be excluded. Attempts to localize the infected cells with fluorescent antibody in the first 5 hr have so far failed.

It is unlikely that the cells in the middle turbinates all become infected simultaneously. Since complete clearance of the nasal passage in healthy chicks takes place within 15 min, new virus is probably not adsorbed to the respiratory mucosa more than ½ hr after the original inoculation.

While variations in data from control groups were frustrating, one category of variation has actually become part of the experimental data: the variation in the amount of virus recovered from the control pools of chicks at 1, 3, and 5 hr after inoculation. Theoretically, if virus is adsorbed during the first 15–30 min, then goes through a phase of incorporation into the cell, and does not multiply until 5 or 6 hr, the number of infected cells should not vary much, since no multiplication has taken place and presumably no infected cells are lost. The data, however, indicate that infected cells *are* lost. This ties in with the least expected finding in this study, which was the sudden and significant drop in the number of infected cells between 1 and 5 hr in chicks treated with pilocarpine before receiving virus, and in chicks exposed to cold before virus inoculation—a drop reminiscent of the effects of virus on organ cultures of recently excised trachea (5).

It is assumed that *adherent virus* represents virus which is in some way stuck to the cells but is freed by trypsin, which probably digests the surface mucus. For this reason it was not surprising to find in the first set of experiments (5) that the ratio of infected cells to adherent virus was low at 1 hr, tended to increase at 3 hr (as virus was taken into the cell), then fell again at 5–6 hr as virus started to multiply and was perhaps excreted from the cell. Pilocarpine stimulation of acinar cells before giving virus had little or no effect on these ratios.

There are several possible explanations for such a drop: there may be an actual decrease in the number of infected cells in the tissue, perhaps by sloughing of infected cells; infected cells may in some way become more sensitive to trypsinization; or there may be loss of infectivity of individual cells during the 5 hr period. If infected cells are more sensitive to trypsin, there would also have to be an increase to sensitivity to trypsin brought about by pilocarpine and cold. This seems unlikely. Although we originally proposed a sloughing of infected cells to explain the loss of infected cells from the explanted trachea, the new data on pilocarpine, exposure to cold, and mucus secretion support the idea that many

cells infected at 1 hr lose their infection by 5 hr without undergoing a cycle of infection which produces a new virus.

It is thus now proposed that there are two possible paths for the virus after it is adsorbed to the cell surface: (*a*) it may, of course, be ingested, and then incorporated in some way into the metabolic activity of the cell, as has been shown in a variety of unspecialized tissue culture cell systems (7); (*b*) alternatively, the virus may be ingested by the cell, but adsorbed to particulates, probably mucus granules, remain there as infectious virus for undetermined periods of time, and then be reexcreted with the mucus granules. This virus would be measured in that portion of the total virus which we have called *cell virus*. It is clear that this in turn would be included in measurements of "infected cells." *Cell virus*, of course, cannot exceed the "infected cell" component, and in the seven experiments in which both "infected cells" and "cell virus" were determined, it did not.

We have four separate lines of evidence that unchanged virus is ingested and reexcreted.

1. Numbers of infected cells would be initially high if susceptible secretory cells were exhausted of their mucus, or were temporarily inhibited from secreting. This was the case when pilocarpine was administered before virus was inoculated, so that acinar cells were initially exhausted; with cold, which initially inhibited secretions; and with primary tracheal explants, which presumably were initially undernourished. In each case, numbers of infected cells which were high at first would be expected to drop, as they did, if normal mucus secretion was resumed so that the previously arrested granules with their attached virus were excreted.

2. If virus is reexcreted from the cells in mucus granules, it should be possible to find it in the mucus which is excreted 1–3 hr after infection of the cell. Virus was indeed found in strands of mucus obtained from pieces of middle turbinate of chicks infected 1 and 3 hr previously, after the turbinates had been repeatedly washed and thus presumably freed of the original inoculum. Furthermore, the natural process of mucociliary clearance would have removed the original inoculum. In addition, in past studies on infected trachea, as many as four saline washes yielded virus in the saline wash, yet in the nose the same method of washing yielded little or no virus from turbinates. This is probably related to the depth of the acini, which are very shallow in the trachea and very deep in the middle turbinate. Thus virus may be washed out of the shallow mucus cells.

3. If mucus hypersecretion was induced soon after virus administration, adherent virus should be decreased at the 1 hr reading because a large proportion would be excreted. Cell virus should be less affected. This was the case in five of eight experiments when pilocarpine was injected 15 min (in one group, 30 min) after virus inoculation, while it occurred only once in 23 control tests.

4. The ratio of *infected cells* to *cell virus* is important in view of the variation

in absolute values from experiment to experiment. This ratio was over 3.7 at 1 and 3 hr, when most virus was in the eclipse phase; but in the two experiments which involved recent sudden excretion of mucus, the 1 hr ratio was well below the control ratio, indicating that much virus was caught on newly formed granules before it had entered into the phase of normal replication. Furthermore, in dehydrated chicks (4) and in excised turbinates kept in plasma clots (5), ratios were below normal at 3 hr, presumably because of a retarded rate of secretion, which held virus attached to (adherent to) granules in many mucus cells (Table II).

To summarize this part of the discussion: it is proposed that a varying amount of the virus adsorbed to mucus cells is not incorporated into the general metabolic pathway of virus multiplication, but is adsorbed to the mucus granules and is then reexcreted from the cell. This hypothesis explains (*a*) the sudden drop in the number of infected cells during the first 5 hr of infection when chicks are treated with pilocarpine or exposed to cold; (*b*) the continued presence of virus in mucus secreted from the cells; (*c*) the change in the ratio of infected cells to adherent virus brought about by mucus stimulation; and (*d*) variations in the amount of infectious virus which were detected in the cell during the first 5 hr of infection.

SUMMARY

Chickens 3 wk old, inoculated intranasally with a mesogenic (moderately virulent) strain of Newcastle disease virus, developed necrotic lesions of the mucous acini, predominantly of the middle turbinates. The infection subsequently spread to involve much of the rest of the mucosa, including mucous and ciliated epithelial cells, and other acini.

The early phase of adsorption of a virulent strain of the virus to the middle turbinates of chicks 5–21 days of age was studied by giving a standard inoculum intranasally to unanesthetized animals.

Variation in amounts adsorbed by individual chickens was large, but was minimized by making measurements on pools of turbinates from three chicks at intervals of 1, 3, and 5 hr after exposure of the excised turbinates to antibody, by washing, and by trypsinization. The virus released from the cells into the trypsin was designated as *adherent virus*, and the infectious virus in the cells after destruction of the cells by water grinding, as *cell virus*.

Paralysis of ciliary action by cocaine increased the number of infected cells in the turbinates about 10-fold at all three time intervals.

Pilocarpine injection before virus inoculation caused a large increase in the amount of infected cells 1 hr after virus administration, but was followed by a sharp drop in infected cells by 3 or 5 hr. Pilocarpine given after the virus decreased the number of infected cells and changed the relationship of infected cells to adherent virus.

Exposure of chicks to sustained or severe cold caused a similar but less marked effect. The drop in infected cells was restored to control values if chicks were returned to brooder temperatures.

The marked drop of infected cells produced by pilocarpine and cold in living chicks, and in cultures of chicken trachea (previous study), is consonant with the idea that virus has been adsorbed on mucus granules in the mucous cells of the turbinates and then has been reexcreted, as unincorporated virus, into the moving mucous sheet. A series of accessory data support this interpretation.

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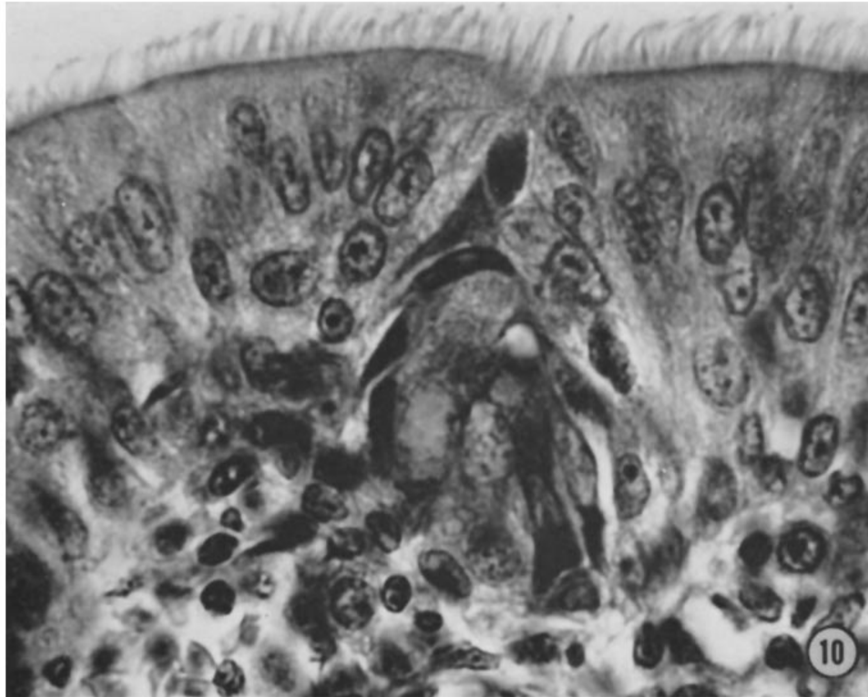


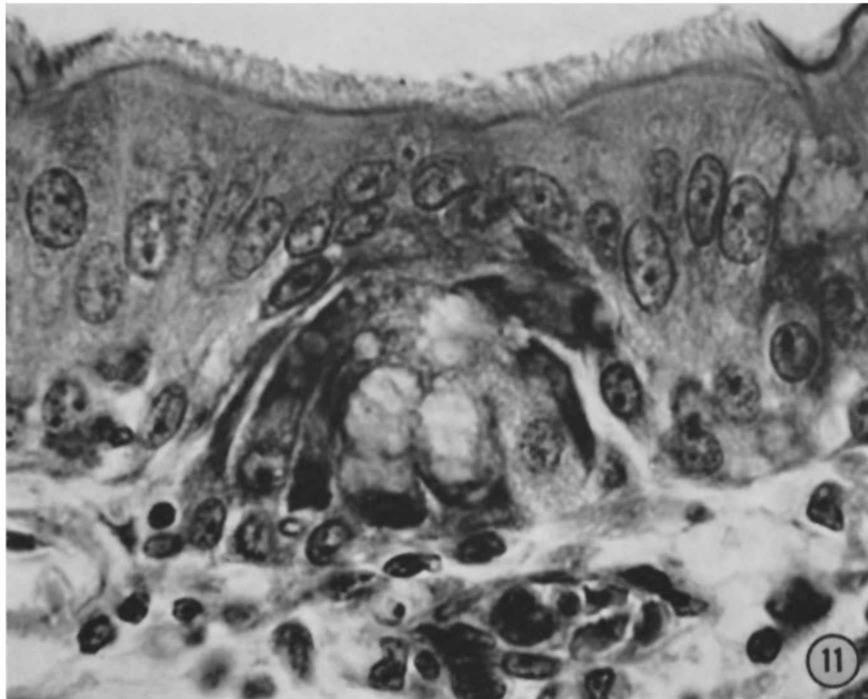
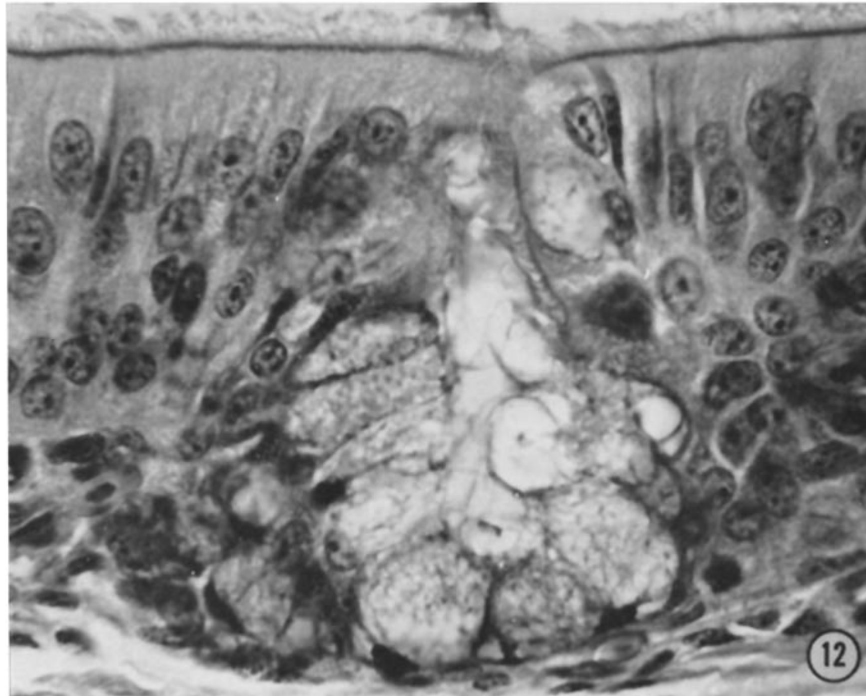


FIG. 10. Mucous acinus in middle turbinate of day old chick 24 hr after infection with Newcastle disease virus. Peripheral cells are dark and collapsed. A few inflammatory cells are seen to the lower left. No destruction of ciliated cells is apparent. × 1500.

FIG. 11. Similar early effect on another acinus. Mucus still present in two cells, some cells vacuolated. Ciliated cells intact. × 1500.



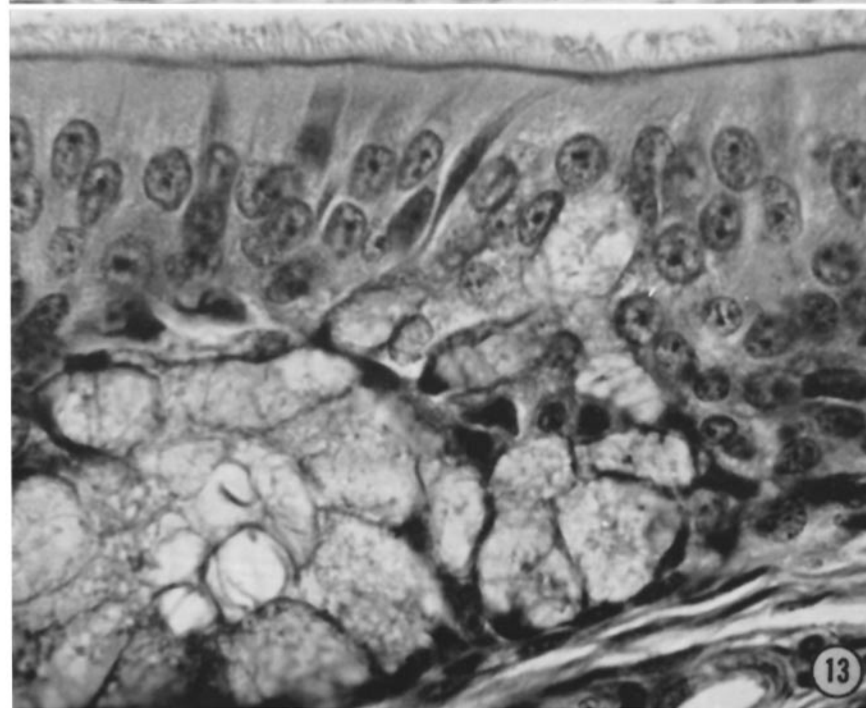


Fig. 12. Normal day old chick turbinate mucous cells, fully distended. Photograph taken at slightly lower magnification in order to include normally distended acinus not present in infected area. × 1250.

Fig. 13. Normal day old chick, showing larger acinus cut near periphery. × 1250.