PATHOLOGY OF INFECTIOUS BRONCHITIS AS INFLUENCED BY VITAMIN A

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ABSTRACT

PATHOLOGY OF INFECTIOUS BRONCHITIS
AS INFLUENCED BY VITAMIN A

by Ricardo Moreno Chan

An experimental work was conducted in an effort to determine whether or not a vitamin A deficiency or a high vitamin A level in the diet, may influence the pathology of infectious bronchitis in chicks.

Three groups of 40 Dekalb cockerel chicks were used in this experiment. Each group was fed a different diet and a control group was maintained for each experimental group. One group was fed a basal diet low in vitamin A; a second group was fed the same basal diet plus 1,200 I.U.'s of vitamin A/lb of feed, as required by the National Research Council of Washington D.C. for poultry nutrition; and a third group was fed the basal diet plus 6,000 I.U.'s of vitamin A/lb of feed (or 5 X NRC).

The control groups of 40 chicks were not exposed to infectious bronchitis virus (IBV) and were kept in isolation from the inoculated groups. The same environmental conditions were furnished for both IBV-exposed groups and controls. The feed and water were supplied ad libitum. One day old chicks were reared in electric, wire floored, battery brooders of commercial design, starting at a temperature of 94° F with a decrease of five degrees in temperature per week. At three weeks of age, the chicks were

exposed to IBV Massachusetts 41 by the intratracheal route using 0.15 ml of inoculum per chick.

The experiment was divided into two periods: the pre-inoculation period which comprised the first three weeks of age; and the post-inoculation period, covering the time from the date of inoculation until the end of the experiment. The symptoms and mortality rate of the exposed groups as well as the control groups were recorded during both the pre-inoculation and the post-inoculation periods. Two chicks from each exposed group and two chicks from each control group were sacrificed for tissue samples for the histopathological studies at selected periods of time after inoculation. Samples from the trachea, lungs, air-sac membranes, esophagus and tongue were taken and fixed in Bouin's fluid. All the tissues were embedded in paraffin and stained with Harris' hematoxylin and eosin.

From the results obtained in this experiment, it appears that vitamin A deficiency in three-week old cockerels results in increased severity of infectious bronchitis experimentally produced. In addition to this, a high vitamin A level (5 X NRC) in the diet showed a beneficial effect in enhancing the recovery of the mucous epithelium lining the trachea of the chicks suffering from experimentally produced infectious bronchitis.

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by

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INTRODUCTION

A great deal of literature has been published on the role of vitamins in resistance to infections (6,13,17,45).

Many data have been concerned with the effects of vitamin-A deficiencies in resistance to some diseases caused by bacteria (22,25,32), protozoa (8,46), helminths (45), and viruses (50,52). In some of these experimental trials, vitamin-A deficiencies have resulted in synergism[1], whereas other experimental deficiencies of vitamin A have resulted in antagonism[2], or in no effect. On the other hand, some other papers (6,34) suggest that administration of vitamin A in an excess of that contained in an adequate diet may have a beneficial effect on the course of infective processes in decreasing their severity.

Infectious bronchitis is an acute respiratory disease caused by a virus (Tarpeia pulli). Its pathology as well as the pathology of vitamin-A deficiency have been carefully studied (26,47). The experimental work which is herein discussed was conducted in an effort to determine whether or not a vitamin-A deficiency or a high vitamin A level in the diet may influence the pathology of infectious bronchitis in the chickens.

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1 that situation in which a nutritional deficiency results in an increased frequency or severity of an infection (Scrimshaw, 1959) (45).

2 that situation in which a nutritional deficiency results in decreased frequency or severity of an infection (45).

REVIEW OF LITERATURE

Infectious bronchitis is an acute, highly contagious respiratory disease of chickens of all ages, first reported by Schalk and Hown in 1931 (40). They observed the disease in North Dakota in the spring of 1930. It soon became widespread through several states as indicated by other reports (5,10).

The disease occurs in the United States and it has been reported in England and Japan (1,38). It might also be present in many other countries, but no reports have been recorded.

The etiological agent has been demonstrated to be a filterable virus (Tarpeia pulli) which passes through all grades of Berkefeld filters, and grows well on the developing chick embryo (2,5).

The incubation period of infectious bronchitis is 18 to 36 hours depending on the dosage and route of inoculation (27). Chickens exposed to undiluted infective allantoic fluid, regularly have tracheal rales within 18 to 24 hours.

The symptoms of infectious bronchitis in young chicks have been described as depression, nasal discharges, gasping, tracheal rales and coughing (2,10). Wet eyes are commonly seen and the chicks tend to crowd beneath the hover to keep warm. In adult birds the symptoms have been reported to be gasping, sneezing and tracheal rales (26). The mortality rate in adult birds is negligible, while in young chicks it might be 25 percent (27) or higher. The course of

the disease usually ranges from one to two weeks.

Some aspects of the pathology of infectious bronchitis were described as early as 1931 by Schalk, et al., (40).

The gross pathology has been observed in chicks to be catarrhal tracheitis and bronchitis, catarrhal inflammation of the nasal passages and sinuses, pulmonary congestion, small areas of pneumonia around the large bronchi, and fibrinous inflammation of the air sacs (2,5,10,27,40). In chicks that die, yellowish caseous plugs may be found in the lower trachea and bronchi.

Histopathological changes were briefly described by Bushnell and Brandly in 1933 (10). Two types of inflammatory reaction were observed: first, that of an acute inflammatory nature associated with a bacterial infection; and second, a chronic inflammatory reaction not associated with bacterial infection.

In the bacterial type in chicks there were areas of desquamation in the tracheal mucosa and edema with cellular infiltration in the submucosa. In the lungs there were areas of consolidation with necrotic centers. In the non-bacterial type of the disease in chicks, there was an exudate in the tracheal lumen and slight degeneration of the tracheal mucosa. Areas of consolidation were observed in the lungs. The spleen, liver and kidneys showed numerous microscopic hemorrhages.

Hofstad in 1945 (26) found a thickening of the tracheal mucosa and submucosa due to edema and diffuse cellular

infiltration as the principal microscopic lesion in the respiratory tract. There was no interruption of the continuity of the tracheal epithelium and the lumen contained an exudate in which cellular elements were usually sparse or absent.

Emmett and Peacock in 1923 (19) did an early study on the fat-soluble vitamin requirements of chicks. They concluded that a very large percent of the chicks fed a vitamin-A deficient diet (vitamin A and D were not differentiated at that time) develop symptoms of ophthalmia. Upon necropsy of their animals the presence of urates was revealed in the tubules of the kidneys, and at times on the surfaces of the heart, liver and spleen. Beach in 1924 (4) reported similar symptoms in a disease which he named poultry nutritional roup. Emmett and Peacock (19) concluded that both conditions are the same and that poultry nutritional roup is due to the absence of the fat-soluble vitamins in the ration.

The signs of vitamin A deficiency in chicks are cessation of growth, weakness, incoordination, ataxia, drowsiness, emaciation and ruffled feathers (18,44). In acute vitamin A deficiency, lacrimation may occur and a cheese-like material is seen under the eye lids. Xerophthalmia is a definite symptom of vitamin A deficiency, but in acute deficiency the chicks often die of other causes before the eyes become affected (44).

As a result of the studies of Wolbach, et al., (56) a

deficiency of vitamin A has been shown to affect the integrity of many epithelial tissues, as well as bones and teeth.

Vitamin A is essential in poultry rations not only for growth but also for optimum vision and for maintaining the integrity of the mucous membranes. The histologic changes are found in all epithelia whose cells have a secretory function in addition to the role of a covering layer (54). The epithelial cells comprising the digestive system, the respiratory system and the genito-urinary system, as well as the eye structures, have been reported to be affected.

Seifried in 1930 (48) made extensive histopathological studies on chicks suffering from avitaminosis A. The lesions first appear in the upper alimentary tract and are largely confined to the mucous glands and their ducts. Histopathologically, the original epithelium becomes replaced by a stratified squamous keratinizing epithelium which blocks the ducts of the mucous glands causing them to become distended with secretions and necrotic materials. The principal tissue changes in the respiratory tract of chickens caused by a vitamin A deficiency in the food are an atrophy of the cytoplasm and a loss of the cilia in the columnar ciliated epithelium (47). A pseudomembrane formed by the atrophying and degenerating ciliated cells may hang as tufts on the basement membrane. During this process, new cylindrical cells may be formed and appear as islands beneath the epithelium. Finally the columnar ciliated

epithelial lining of the trachea, bronchi and submucous glands becomes transformed into stratified squamous keratinizing epithelium (47). The protective mechanism inherent in the mucous membranes of the entire respiratory tract is seriously damaged by the degeneration of the ciliated cells at the surface, and the lack of secretion with bactericidal properties. Inflammatory processes are common, including purulent ones, especially in the upper respiratory tract, communicating sinuses, eyes and trachea. Seifried (47) established that the specific histological lesions make it possible to differentiate between avitaminosis A and some infectious diseases of the respiratory tract.

MATERIALS AND METHODS

Three groups of 40 Dekalb cockerel chicks of the same breeding were used in this experiment. Each group was fed a different diet and a control was maintained for each experimental group. The following diets were used:

I Basal diet (low vitamin A content)

II Basal diet plus 1,200 I.U.'s of vitamin A/lb of feed (adequate diet)*

III Basal diet plus 6,000 I.U.'s of vitamin A/lb of feed (high vitamin A diet)

Table I shows the composition of the basal diet (I) which is deficient in vitamin A.

The control groups of 40 chicks each were not exposed

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*diet which contains all the nutritional factors as established by the National Research Council in Washington D.C. for poultry nutrition.

Table 1. Composition of the Basal Diet, Deficient in Vitamin A.

Ingredients	Percent	Total amount
Ground wheat	55	330 lbs
Soybean meal (44%)	25	150 lbs
Wheat midds	10	60 lbs
Whey (dried) 50% delactose	3	18 lbs
Meat scraps (50%)	3	18 lbs
Calcium carbonate	1.8	10.8 lbs
Dicalcium phosphate	1.6	9.6 lbs
Salt (iodized)	0.2	1.2 lbs
Vitamin B_{12} supplement (6 mg/lb)		270.0 gms
Vitamin D_3		60.0 gms
Manganese sulphate		48.0 gms
Choline chloride	0.2	540.0 gms
Riboflavin		8.0 gms
Calcium pantothenate		6.0 gms
Niacin		8.4 gms
Myvomix (vitamin E, 20 gms E/lb)		60.0 gms
DL methionine	0.05	136.2 gms

to infectious bronchitis virus (IBV) and were kept in isolation from the inoculated groups. The same environmental conditions were furnished for both IBV-exposed groups and controls. The feed and water were supplied ad libitum. One-day-old chicks were reared in electrical, wire floored, battery brooders of commercial design starting at a temperature of 94° F with a decrease in temperature of five degrees per week. At three weeks of age the chicks were exposed to infectious bronchitis virus by the intratracheal route using 0.15 ml of allantoic fluid taken from infected embryos as an infective dose per chick. The strain of virus was labeled as Massachusetts 41, and was supplied by Dr. C. H. Cunningham of the Department of Microbiology and Public Health, College of Veterinary Medicine at Michigan State University.

The experiment was divided into two periods:

I. The pre-inoculation period which comprised the first three weeks of age.

II. The post-inoculation period, covering the time from the date of inoculation until the end of the experiment.

During these periods, the symptoms and mortality rates of the exposed groups as well as the control groups were carefully recorded. Two chicks from each exposed group were sacrificed for tissue samples for the histopathological studies at the following selected periods:

24 hours after inoculation

48 hours after inoculation

96 hours after inoculation

144 hours after inoculation

192 hours after inoculation

240 hours after inoculation

288 hours after inoculation

The groups of chicks on the vitamin deficient diet was exhausted at the period of 248 hours because of the high mortality rate in this group. The last surviving chicks were samples at this period.

Tissue samples from the control groups were taken at the following periods

24 hours before inoculation

48 hours after inoculation

120 hours after inoculation

360 hours after inoculation

The tissue samples from the lower part of the trachea, lungs, air sac membranes, esophagus and tongue, were placed in Bouin's fluid. They remained in this fixative for about 20 hours, at which time they were removed and washed twice in 50 percent alcohol and temporarily stored in 70 percent alcohol. All tissues were embedded in paraffin. Sections were cut at six microns and stained with Harris' hematoxylin and eosin.

The gross changes found in the exposed and unexposed groups were recorded at the time of necropsy. Gross sections through the nasal cavities and communicating sinuses were performed. Examinations of some internal organs as

well as the air sac membranes were also made.

RESULTS

Pre-inoculation period. Most observations were concerned with the pathology of vitamin A deficiency in the group of chicks on the diet low in vitamin A.

Symptoms of incoordination, ruffled feathers, and weakness were first observed in some of the chicks after 12 days on the diet deficient in vitamin A. The number of sick chicks increased progressively as well as the severity of the symptoms. Furthermore, other symptoms, viz., drowsiness, conjunctivitis, ataxia, retarded growth and emaciation were apparent. At the age of 21 days, 65 percent of the chicks were showing some of the above described symptoms. The chicks fed the adequate diet as well as the chicks on the high vitamin A level did not show any evidence of nutritional disturbance throughout this period.

The mortality rate during this time in the group on the adequate diet as well as in the group on the high vitamin A level was 1.19 percent, whereas in the group on the vitamin A deficient diet the mortality rate was 2.3 percent. The microscopic lesions found in tissue samples which were taken at the end of this period were: loss of cilia in some areas of the tracheal epithelium and replacement of the original epithelium of the mucous glands of the tongue and of the esophagus by stratified squamous keratinizing epithelium (Figures 1 and 2).

Figure 1. Mucous gland in the esophagus of a chick on the vitamin A deficient diet. The glandular duct is obstructed resulting in enlargement of the gland. A stratified squamous keratinizing epithelium is replacing the original glandular epithelium. Hematoxylin and eosin. X 105.

Figure 2. Mucous glands in the tongue of a chick on the vitamin A deficient diet. The original glandular epithelium is undergoing stratified squamous metaplasia. Scanty mucinous material can be seen. Hematoxylin and eosin. X 105.

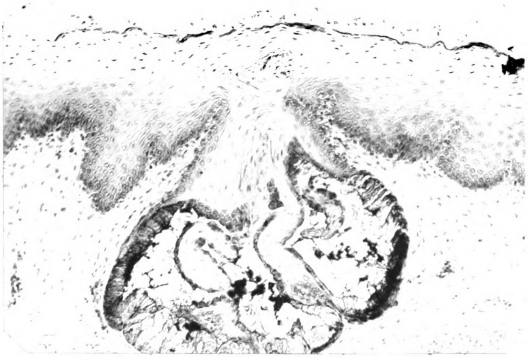


Figure 1

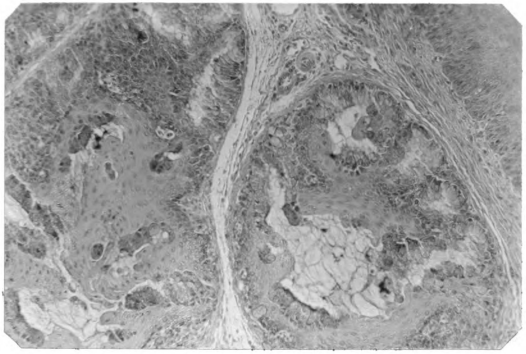


Figure 2

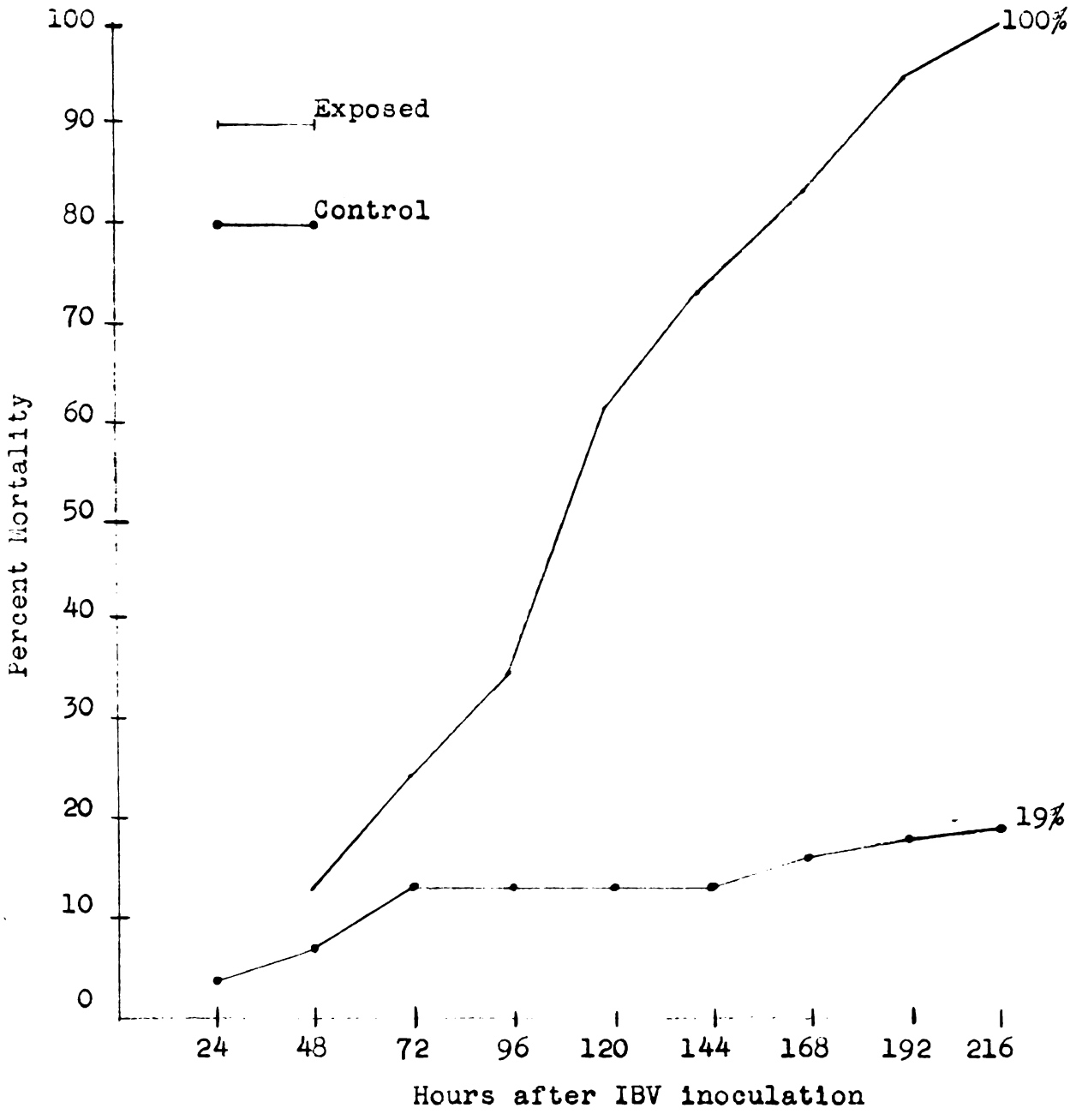
Post-inoculation period

Symptoms. Once the chicks were inoculated with IBV, the first evidence of respiratory infection was observed 17 hours after inoculation in the three experimental groups. Some of the chicks had tracheal rales, and were gasping.

Twenty-four hours after inoculation the number of chicks showing symptoms increased, and at this time most chicks had respiratory disturbance.

Forty-eight hours after inoculation, wet eyes, ruffled feathers, sneezing, and tendency to crowd beneath the hover, were also observed. The first dead chicks, three in number, were found at this time in the group on the diet low in vitamin A. The chicks on this diet continued showing the above described symptoms until the end of the experiment at 240 hours, at which time the cumulative mortality rate reached one hundred percent (Graph I). On the other hand the severity of the symptoms in the group on the adequate diet as well as in the group on the high vitamin A level, began to subside in intensity between 96 and 120 hours after inoculation. The chicks in these latter groups were almost completely recovered between 216 and 240 hours after exposure. No chicks in these groups died as a result of the infection.

Evidence of respiratory disturbance was not detected in the control groups. The symptoms observed in the group on the vitamin A deficient diet were recorded as retarded growth, ruffled feathers, emaciation, conjunctivitis,



Graph I. Mortality rate in vitamin A deficient groups.

Table II. Comparative Mortality Rate Among Groups (*).

	Vitamin-A deficient diet	Adequate diet	High vitamin-A diet
Exposed	100	0	0
Control	19	0	0

* percent of mortality

incoordination and ataxia. Graph I shows that the cumulative mortality rate in this group, at 216 hours after inoculation was 19 percent.

Gross pathology. The gross lesions in the three experimental groups were almost completely confined to the respiratory organs. They were catarrhal inflammation of the mucous membranes of the infraorbital sinuses, nasal passages, trachea and the large bronchi. Thickening and cloudiness of the abdominal air sac was observed as well as congestion of the lungs. Lesions such as cheese-like material under the eye lids, and pustule-like patches in the esophagus were recorded in about 20 percent of the exposed chicks on the vitamin A deficient diet. The lesions present in chicks on the adequate diet as well as the group on the high vitamin A diet, almost completely disappeared at 216 hours after exposure. The only remaining observable lesion at this time was cloudiness of the abdominal air sac membranes. At 240 hours after inoculation, no gross lesions were found in these groups.

In the control groups, no gross lesions were found in the group on the adequate diet nor in the group on the high vitamin A level, but, in the control group on the vitamin A deficient diet, gross lesions such as whitish material plugging the upper part of the tracheal lumen, and deposits of urates covering all the internal organs were found in some of the necropsied chicks.

Histopathology. Histologic changes in tissue samples from

the chicks sacrificed throughout the course of the infection were studied in the tracheal and bronchial mucosae, lungs, and air sac membranes. Twenty-four hours after inoculation there was extensive edema, capillary congestion and diffuse lymphocytic infiltration in the tracheal and bronchial mucosae and submucosae, causing the thickening of these structures to several times their normal size (Figure 3). Large numbers of acidophilic granulocytes were now present. The cilia of the columnar epithelial cells lining the trachea and bronchi disappeared in extensive areas. A mixed mucinous and fibrinous exudate containing inflammatory cells such as macrophages, lymphocytes and acidophilic granulocytes were present plugging the tracheal and bronchial lumens (Figures 4 and 5).

In the tissue surrounding the large bronchi, there were areas of congestion and consolidation. The abdominal air-sac membranes showed some thickening due to diffuse lymphocytic infiltration. The degree of severity of these lesions at this time was somewhat similar in all experimental groups.

Forty-eight hours after inoculation, the histopathologic appearance as observed at 24 hours remained essentially the same in all the groups. Some desquamation of the columnar epithelium of the trachea and bronchi was observed at this time in the chicks on the vitamin-A deficient diet (Figure 6). This microscopic picture remained about the same in all the groups and no additional inflammatory changes were observed in the group until 96 hours after inoculation, at

Figure 3. Tracheal section showing extensive edema in the submucosa, diffuse lymphocytic infiltration and capillary congestion. The cilia of the columnar epithelium have been lost and some inflammatory cells are observed in the lumen. (24 hours after inoculation) Hematoxylin and eosin. X 215.

Figure 3

Figure 4. Tracheal section showing a mucinous and fibrinous exudate containing inflammatory cells plugging the tracheal lumen. Hematoxylin and eosin. X 105.

Figure 5. Bronchial section showing extensive edema, loss of the cilia and a serous exudate containing inflammatory cells plugging the bronchial lumen. Hematoxylin and eosin. X 215.

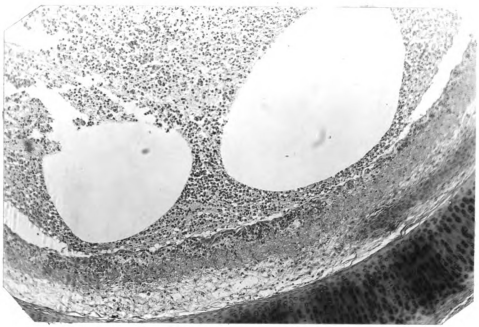


Figure 4

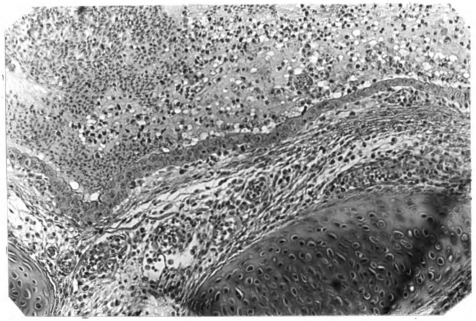


Figure 5

Figure 6. Section of a large cranium showing desquamation of the epithelial lining and thickening of the mucosa and submucosa due to edema and lymphocytic infiltration. Hematoxylin and eosin. X 215.

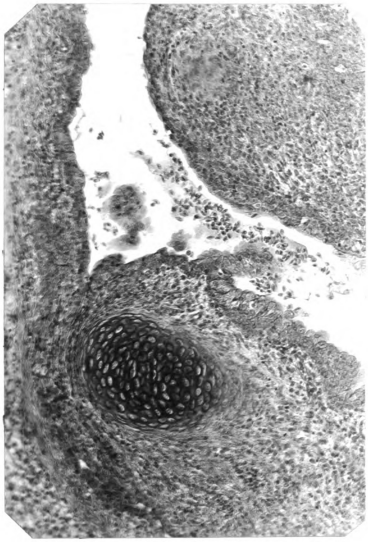


Figure 6

which time the intensity of the inflammatory reaction clearly began to subside. Some mitotic figures were observed in the cells of the columnar epithelium of the trachea (Figure 7). A mucinous and fibrinous exudate, cellular debris and inflammatory cells such as macrophages and lymphocytes were still present in the lumen of the trachea and bronchi.

One hundred forty-four hours after inoculation the reparative processes proceeded more rapidly in the chicks on the diet containing the high vitamin A level than in the chicks fed the adequate diet or the chicks fed the diet low in vitamin A. The cilia of the columnar epithelial cells had almost completely restored in the chicks on the high vitamin A diet, and the thickening of the mucosa and submucosa of the trachea and bronchi had been greatly reduced. On the other hand in the chicks on the adequate diet as well as in the group on the vitamin A deficient diet the cilia of the tracheal epithelium had not been restored at all and the lymphocytic infiltration of the mucosa and submucosa was still observable in these groups (Figure 8). In addition to this, degenerative alterations of the columnar epithelial cells such as vacuolization and ballooning degeneration were also observed. The abdominal air-sac membranes from the chicks of all the experimental groups were somewhat thickened due to edema and lymphocytic infiltration (Figure 9). Mucinous exudate in the lumens of the trachea and the large bronchi, as well as areas of consolidation in the lung, were still present in all the groups. The reparative processes

Figure 7. Tracheal epithelium showing some mitotic figures in the columnar epithelial cells 96 hours after IBV inoculation. Hematoxylin and eosin. X 615.

Figure 8. Lymphocytic infiltration in the tracheal mucosa from a chick on the vitamin A deficient diet 144 hours after IBV inoculation. Hematoxylin and eosin. X 215.

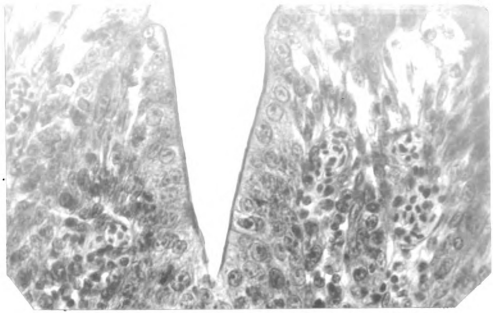


Figure 7

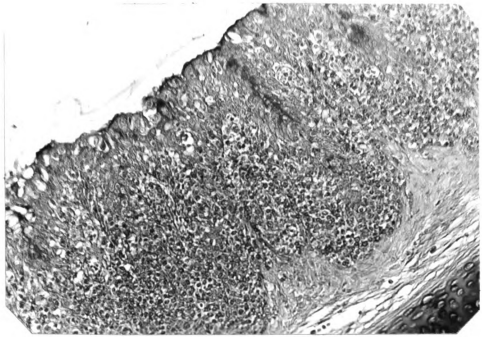


Figure 8

Figure 9. Thickening of the abdominal air-sac membrane due to edema and lymphocytic infiltration 144 hours after IBV inoculation. Hematoxylin and eosin. X 215.

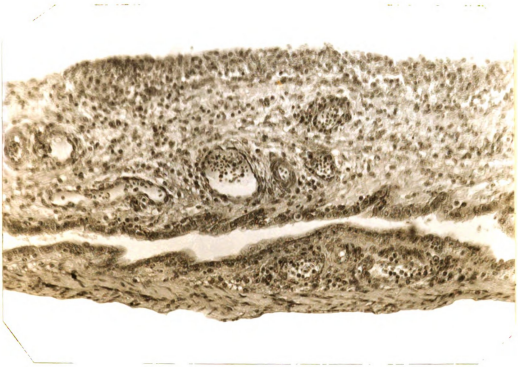


Figure 9

continued and, by the 192nd hour after exposure, the recovery of the group on the adequate diet was more noticeable. The cilia of the tracheal and bronchial epithelium had been restored to a great extent and only a slight lymphocytic infiltration of the mucosa was still present (Figure 10). While the tracheal and bronchial epithelium of the chicks on the diet containing a high vitamin A level had completely recovered its normal microscopic appearance (Figure 11), the cilia of the tracheal epithelium of the chicks on the vitamin A deficient diet had not been restored, and the thickening of the mucosa and submucosa was still noticeable. Lymphoid hyperplasia was present in the bronchial and tracheal lymphoid tissue of all the groups.

At 240 hours after intratracheal inoculation, the microscopic picture in each of the experimental groups was as follows:

I. Group on the vitamin A deficient diet.
   a. The cilia of the tracheal and bronchial epithelium had not been completely restored (Figure 12).
   b. Areas of consolidation in the lungs.
   c. The abdominal air-sac membranes slightly thickened due to lymphocytic infiltration.
   d. Vitamin A deficiency lesions in the mucous glands of the tongue and esophagus (Figures 1 and 2).

II. Group on the adequate diet.
   a. The tracheal and bronchial mucosae were almost completely recovered (Figure 13).

Figure 10. Tracheal section 192 hours after IBV inoculation. Slight lymphocytic infiltration and extensive restoration of the cilia. Group on the adequate diet. Hematoxylin and eosin. X 215.

Figure 11. Tracheal section of a chick on the high vitamin-A level diet 192 hours after IBV inoculation. The structure of the ciliated columnar epithelium has almost recovered its normal appearance. Hematoxylin and eosin. X 215.

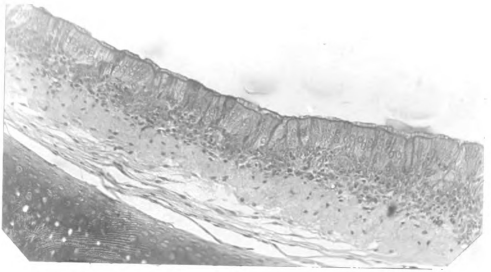


Figure 10

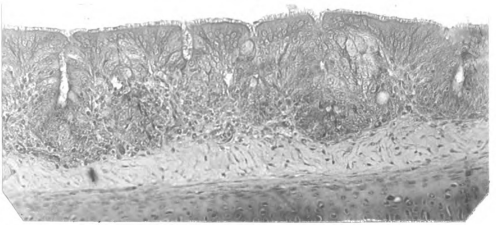


Figure 11

Figure 12. Tracheal section of a chick on the vitamin A deficient diet 240 hours after IBV inoculation. The cilia and columnar epithelial cells have not been restored. Hematoxylin and eosin. X 215.

Figure 13. Tracheal section of a chick on the adequate diet 240 hours after IBV inoculation. The ciliated columnar epithelium is almost recovered. Hematoxylin and eosin. X 215.

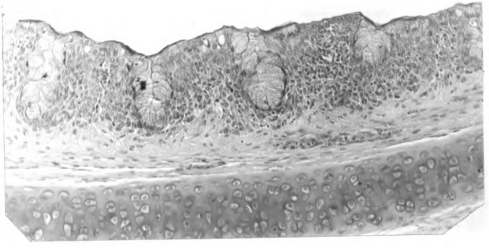


Figure 12

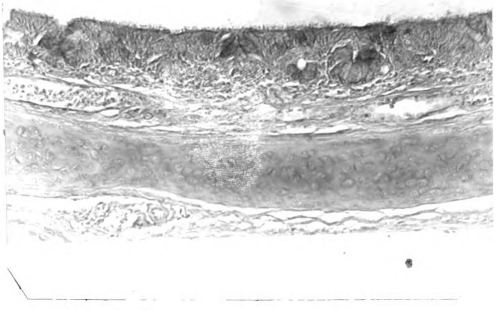


Figure 13

b. Areas of consolidation in the lungs surrounding the large bronchi.

III. Group on the diet containing high vitamin A level.

a. The tracheal and bronchial mucosae were found essentially well recovered in their histologic elements (Figure 14).

b. The lungs were showing areas of consolidation.

c. The abdominal air-sac membranes were somewhat thickened due to mild edema, and some lymphocytic infiltration.

In the control groups the histologic alterations of the tissues were carefully studied, and several samples were collected at the following periods:

24 hours before intratracheal inoculation
48 hours after intratracheal inoculation
120 hours after intratracheal inoculation
360 hours after intratracheal inoculation

The group on the adequate diet as well as the group on the diet containing a high vitamin A level, did not show any evidence of respiratory infection, and did not show any lesion similar to those described for vitamin A deficiency in the tongue and in the esophagus.

The group of chicks on the vitamin A deficiency diet, did not show any evidence of respiratory infection, but they did show the following histologic alterations: in the tracheal mucosa, some of the columnar epithelial cells had undergone atrophy of the cytoplasm and had a tendency to

Figure 14. Tracheal section of a chick on the high vitamin-A diet 240 hours after inoculation. The structure of the tracheal epithelium has completely recovered its normal histologic picture. Hematoxylin and eosin. X 215.

Figure 15. Tracheal section of a chick from the control group upon vitamin A deficient diet. Atrophy of the columnar ciliated cells and tendency of the epithelium to undergo stratified squamous metaplasia. The cilia are absent. Hematoxylin and eosin. X 615.

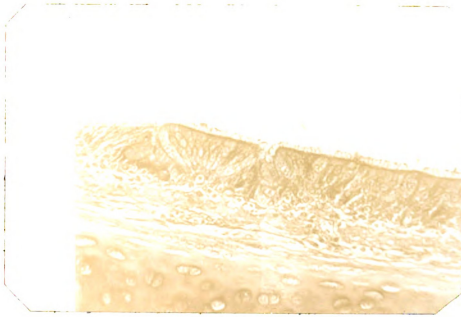


Figure 14

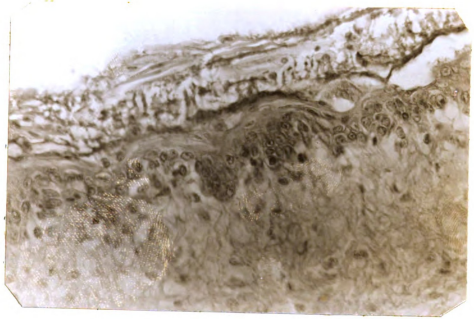


Figure 15

undergo stratified squamous metaplasia (Figure 15). In addition to this, the cilia had been lost. In the mucous glands of the tongue and esophagus, the glandular ducts were obstructed and the glands had become somewhat enlarged due to the accumulation of mucinous material. A stratified squamous keratinizing epithelium almost completely replaced the glands (Figures 1 and 2).

DISCUSSION

According to the data obtained in this investigation, the chicks on the low vitamin A diet were suffering from vitamin A deficiency at the time of inoculation (three weeks of age). The symptoms, gross lesions and histopathological findings recorded in this group, were consistent with the pathological picture described by Seifried in 1930 (48) in chicks suffering vitamin A deficiency. The group on the adequate diet (II) as well as the group on the high vitamin A diet (III) did not show any evidence of nutritional disturbance throughout the pre-inoculation period. During this period the cumulative mortality rate in group II as well as in group III was 1.19 percent; whereas in group I on the vitamin A deficient diet the mortality rate was 2.3 percent. The normal average of the mortality rate in rearing healthy chicks in the starting period ranges from three to four percent and it is considered as due to non-specific causes.

After inoculation with IBV (Mass. 41) the chicks fed the adequate diet, as well as the chicks fed the high vitamin A diet, apparently showed the same clinical signs during

the course of the disease. No significant differences were observed in the incubation period, in the severity of the symptoms or in the course of the infection. However, these groups showed some differences in the histopathological studies particularly in the time of recovery of the tracheal and bronchial columnar ciliated epithelium. The chicks on the high vitamin-A diet restored their tracheal mucous epithelium to its normal histologic appearance faster than the chicks on the adequate diet. Differences of 24 to 48 hours were observed. Such differences might be significant in the case of infectious bronchitis in which the course of the disease comprises a short period of time (10 to 14 days). Moreover, regarding secondary bacterial infections as a frequent complicating factor in poultry respiratory diseases, this difference in the time of recovery could be significant, inasmuch as a faster recovery would decrease in greater or lesser degree the dangers of complications with secondary invaders.

In regard to the data obtained from the group fed the low vitamin-A diet, the severity of the symptoms and the increased mortality rate in this group (Graph I) indicate a definite synergism under the conditions of this experiment.

The interactions between vitamin A deficiency and the infectious bronchitis virus infection are difficult to evaluate. Graph I shows the percent of mortality of the non-infected control, and the inoculated group on the low vitamin A diet. Vitamin A deficiency resulted in 19 percent

mortality in the non-infected control, whereas in the infected group the mortality was accelerated and increased in the presence of both infectious bronchitis virus infection and vitamin-A deficiency. These results seem to indicate that while vitamin A deficiency enhances the severity of infectious bronchitis virus infection, also the infection itself precipitates the deleterious effects of the deficiency.

CONCLUSIONS

I. Vitamin A deficiency in three-week-old Dekalb cockerels results in increased severity of infectious bronchitis experimentally produced.

II. It appears that some beneficial effects may be obtained in enhancing the recovery of the tracheal and bronchial mucous epithelium of chicks infected with infectious bronchitis virus, if high vitamin A levels (5 X NRC) are fed in the diet.

SUMMARY

1. Three groups of 80 one-day-old Dekalb chicks were placed on different diets. One group was fed a basal diet deficient in vitamin A. A second group was fed an adequate diet containing all the nutritive requirements established by the National Research Council. A third group was fed a high vitamin A diet (or 5 X NRC).

2. At three weeks of age, the chicks were inoculated by the intratracheal route with infectious bronchitis virus, strain Massachusetts 41. The infective dose per chick was

0.15 ml of infectious allantoic fluid, from infected embryos.

3. The symptoms, gross lesions and histopathological findings in the group on the vitamin A deficient diet were consistent with the pathological picture which has been described in the literature in chicks suffering from vitamin A deficiency.

4. The symptoms and course of the experimentally produced infection were more severe in the group on the vitamin A deficient diet.

5. The chicks on the high vitamin-A level diet restored their tracheal mucous epithelium to its normal histologic appearance faster than the chicks on the adequate diet.

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