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SOME IMMUNOLOGICAL ASPECTS OF NEWCASTLE DISEASE VIRUS

by

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INTRODUCTION

Since its isolation in 1926 Newcastle disease virus has been studied by many investigators throughout the world. Because of its economic importance to the poultry industry many of these studies have not been limited only to the academic but rather to the practical level.

Veterinarians engaged in poultry practice in areas where Newcastle disease is enzootic are sometimes faced with the problem of protecting a flock of chickens close to another in which an active outbreak of the disease has been diagnosed. It is evident that such factors as the vaccination program, proximity of the infected to the noninfected flock, time elapsed since the outbreak started, number of birds affected, and the possibility of carriers must be considered in evaluating the true status of the flock. Some flocks can be helped while others cannot, depending on the above cited circumstances. However the fact remains that no effective therapeutic agent has been found against Newcastle disease, or any viral disease. Furthermore, the use of hyperimmune serum and gamma globulin, which sometimes are of value in viral infections, have not been used in poultry, at least commercially. Under these circumstances the only alternative given to the veterinarian for protecting the threatened flock is the use of inactivated or attenuated live Newcastle

disease vaccines. At this point, it is also difficult to recommend the type of vaccine to be used since no concrete experiences dealing with the outlined problem are found in the literature. Experiment 1 was designed to compare the rapidity and degree of protection induced in chickens by an inactivated and an attenuated live virus vaccine against a challenge Newcastle disease virus.

Repeated laboratory observations have been made that challenge virus can frequently be isolated from the blood, tissues, and feces from chickens which have been vaccinated with inactivated or live Newcastle disease virus vaccines. These isolations can be made even though the birds survive the challenge with no apparent symptoms or deaths. The academic question arises as to the fate of the challenge virus. Does the virus persist in some of the tissues and for what periods? What is the titer of the virus in the tissues as compared to that of unvaccinated controls? An attempt to follow the fate of the challenge virus in the chickens previously vaccinated with an inactivated vaccine is described in Experiment 2.

Another of the questions encountered by practicing veterinarians in areas of heavy poultry population, where dams are vaccinated frequently against Newcastle disease, is whether or not to vaccinate passively immune chickens the first days of life. The problem has two very interesting

aspects. One refers to the degree and extent, in terms of protection, given to the chickens by these passive antibodies, against a field strain of the virus. The other one is the effect of these passive antibodies upon the development of a proper immunity when vaccinated with a live virus vaccine. The purpose of Experiment 3 was to learn more concerning this aspect of Newcastle disease immunization.

MATERIALS AND METHODS

General

Chickens

The chickens used in these experiments were hatched from eggs laid by inbred White Leghorn hens obtained from the normal flock maintained at the Veterinary Medical Research Institute (VMRI), Iowa State University, Ames, Iowa; there had been no history of infection or vaccination with Newcastle disease virus in this flock. Also for the past 5 years no reactors to *Salmonella pullorum*, *Salmonella typhimurium* or *Mycoplasma gallisepticum* (S6 serotype) have been found in the flock. The chickens were started in electrically heated Oakes No. 801 brooders[1] and when they were 4 weeks of age they were transferred to conventional growing batteries. Except for the controls, all chickens were wing banded as a means of identification. In all lots, in each trial, division as to sex was approximately equal. A non medicated mash was fed throughout the experiments. In addition, some of the chickens used for Experiment 3 were of Ames-in-cross breeding obtained from a hatching flock which had been vaccinated twice by way of the drinking water with

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[1]Oakes Manufacturing Company, Inc., Tipton, Indiana.

the Bl strain of Newcastle disease virus.

Housing

The chickens for each experiment were maintained in separate isolation rooms until they were brought to special quarters in the laboratory for challenge.

Embryonated chicken eggs

All eggs used throughout these experiments were produced by the VMRI supply flock. Uniform size eggs were selected and incubated for 9-11 days at 37°C at a relative humidity of 60%, prior to inoculation.

Chicken embryo inoculations and detection of Newcastle disease virus

The general procedure used to inoculate embryonating eggs was that described by Cunningham (23). The inoculum was either 0.1 ml or 0.2 ml injected into the allantoic sac of 9-11-day-old chicken embryos. The inoculated eggs were candled daily until death of the embryos, or through the nineteenth day of incubation. All embryos dying before 24 hours were discarded as non specific deaths.

The presence of Newcastle disease virus in the inoculated embryos was determined by the ability of the allanto-amnionic fluid of dead embryos to agglutinate a 10

per cent suspension of washed chicken erythrocytes. This was done by mixing one drop of each on a metal plate and observing for hemagglutination.

When bacterial contamination of the inoculum was suspected a penicillin-streptomycin mixture was added as described for each experiment.

Challenge virus

The Newcastle disease virus used in this work was the GB strain (16) obtained by the VMRI originally from the University of Kentucky in 1951. It was selected for challenging because of its virulence and neurotropic characteristics making it easier to visually identify affected birds.

The virulence of the virus was maintained by inoculating intranasally two, 10 week old susceptible chickens with 0.1 ml of infective allantoic fluid. After 72 hours of exposure the chickens were bled by heart puncture, using 20 USP units of heparin[1]/ml of blood to prevent clotting. This blood was inoculated into the allantoic sac of sixteen 11 day old embryonated eggs. After 48 hours of incubation all dead and surviving embryos were chilled for 4 hours at $4^{o}C$ and the allantoic fluid harvested and pooled.

[1]Panheprin, a product of Abbott Laboratories, North Chicago, Illinois.

The pooled allantoic fluid was considered to be free of bacterial contamination when no growth was obtained on blood agar plates incubated 48 hours at 37°C. Approximately 5 ml of this pool were delivered into several screw cap tubes and stored at -35°C. At every challenge time the contents of one tube were utilized and any remainder discarded. The chicken infective dose ($c.i.d._{50}$) of this pool was established by inoculating intranasally susceptible chickens 13 weeks old with virus dilutions 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, using 5 chickens for each dilution (76). Each of the 4 groups of birds was held in a separate Horsfall-Bauer unit (53) for 96 hours. At this time blood from each chicken was collected and injected into embryonated eggs to determine which birds had become infected.

Vaccines

The inactivated vaccine was made by inoculating thirty 11 day old chicken embryos in the allantoic sac, each with 0.1 ml of a 10^{-2} dilution of the Manhattan strain of Newcastle disease virus. Those embryos dying 24 hours after inoculation were discarded. Forty hours after inoculation all dead and surviving embryos were held at 4°C for 4 hours and then the embryos, fluids, yolk and membranes ground in an electric Waring blender for about 5 minutes. The resulting suspension was clarified through a pad of sterile cotton and

gauze in a Buchner funnel using a vacuum system. Then 0.1 per cent beta-propiolactone[1] (BPL) was added and the mixture was held at 37°C for 2 hours with occasional shaking. This vaccine was kept at 4°C until used. In order to prove total inactivation of the virus, forty 10 day old chicken embryos were inoculated in the allantoic sac, each with 0.2 ml of this vaccine. None of them died after 9 days of incubation. Before inactivation the virus had a titer of $10^{8.5}$ embryo lethal doses (e.l.d.$_{50}$)/0.1 ml.

The Bl strain of Newcastle disease virus, obtained from a commercial source, was propagated in 10-day old embryonating eggs by inoculating 0.1 ml/chicken embryo. After 72 hours incubation the chicken embryos were killed by placing them at 4°C for 4 hours. The allantoic fluid was harvested and pooled. Approximately 5 ml of the pool were delivered into each of several screw cap tubes and stored at -35°C until used. The titer of this pool was found to be 10^9 e.l.d.$_{50}$/0.1 ml.

Erythrocyte suspension

Chicken erythrocytes were aseptically collected by heart puncture from a semi-mature rooster using a 10 ml syringe containing 2 ml of a 2 per cent solution of sterile

[1]B-propiolactone, a product of Eastman Organic Chemicals, Rochester, New York.

sodium citrate. The mixture was delivered into 2 graduated centrifuge tubes, which were then filled with physiological saline and carefully shaken. The tubes were centrifuged at about 1500 RPM for 10 minutes, the supernatant fluid discarded and the tubes refilled again with saline. The washing was repeated for a total of 3 times. After the third centrifugation the volume of packed cells was read and enough Alsever's solution (2) added to make a 10 per cent suspension. The cell suspension was stored at 4°C until used.

Serological tests

The hemagglutination inhibition, beta procedure, and serum neutralization tests were conducted according to the methods described by Salk (78) and Cunningham (23), respectively. The Manhattan strain was the antigen used in both tests.

In the hemagglutination inhibition test the 2-fold serum dilutions were prepared directly in 0.5 ml virus suspension of 10 hemagglutinating units. Washed chicken erythrocytes were used at a concentration of 0.25 per cent in 0.5 ml amounts to detect specific inhibition.

End points

End points were calculated by the method of Reed and Muench (76) from mortalities proved to be due to

Newcastle disease virus by the hemagglutination plate test.

Inocula diluent

The diluent for all inocula was sterile Difco[1] tryptose phosphate broth.

[1]Difco Laboratories, Detroit, Michigan.

EXPERIMENT 1: THE RAPIDITY OF PROTECTION INDUCED BY LIVE AND INACTIVATED NEWCASTLE DISEASE VACCINES

Review of Literature

What is now known as Newcastle disease of fowls was first recognized in the Dutch East Indies in 1926 (58). The same year Doyle (35) recognized the disease in Newcastle-on-Tyne, England, naming it after that place; in his report the filterability of the causative agent was also recognized. The outbreak in England was promptly stamped out by quarantine and slaughter methods (35); however this was not the case in the Dutch East Indies, and from this point of origin the disease became disseminated (41) throughout the world (13). The disease is principally important in chickens and turkeys, but it has been diagnosed in many species of birds (18).

Newcastle disease apparently reached California about 1940 (11). There, because the disease occurred as a respiratory-nervous disease in chickens, and not as an acute fulminating disease as was the case for the European and Asiatic type, it was not recognized as Newcastle disease. In the United States the disease occurred as a subacute mild inapparent disease. The respiratory and nervous symptoms were the most prominent manifestations (11). By 1947 the

infection had been reported in 43 states (22) making it necessary to initiate vaccination methods for its control.

There are 2 types of vaccines used in the control of Newcastle disease, namely, inactivated and live vaccines. The inactivated vaccines are prepared from tissues and fluids of chicken embryos; the mixture is treated with an inactivating agent and usually adjuvants are used to enhance its action. The live vaccines are obtained by repeatedly passing a field strain in homologous or heterologous animals, chicken embryos, or tissue cultures, until its original pathogenic characteristics are attenuated to the point of producing only a mild or unnoticed reaction in the vaccinated fowls.

The rate of protection induced by live Newcastle disease vaccines has been studied by some investigators. Hitchner and Johnson (47) designed an experiment to determine the rapidity of protection induced by the B1 vaccine instilled intranasally, against a virulent strain of Newcastle disease virus. They reported that birds vaccinated at 39 days of age were resistant to intranasal challenge with the California 11914 strain at 1, 3, 4, 5, 6, 7 and 8 days after vaccination. At 2 days half of the birds were resistant. When chicks were vaccinated at 1 day of age no protection was afforded until the fourth and fifth days after vaccination and it was not complete until the sixth day. In chicks vaccinated at 16 days of age, no

protection was detected until the fourth day after vaccination. The authors interpreted these findings as indicating an earlier antibody response in the older birds.

White and Appleton (84) studied the rapidity of immunity induced in chicks following vaccination with the Bl strain. In 1 day old chicks immunity against conjunctival sac exposure with the California 11914 strain was demonstrable 48 hours after vaccination. On the other hand resistance to intramuscular challenge was not found until the eighth day post vaccination.

The first specific report dealing with interference of Newcastle disease virus was that of Bang (5) who vaccinated 2 groups of 5 chickens each, intranasally with the Bl strain. One and 2 days after vaccination they were challenged intramuscularly with the neurotropic Cg 179 strain. One chicken died out of the 5 challenged at 24 hours, however none died in the group challenged at 48 hours. In fourteen, 2 month old chickens 100 per cent protection was obtained when the avirulent and virulent strains were injected intramuscularly and simultaneously into opposite legs. This blocking or interfering effect by the avirulent strain was not effective after symptoms of the disease had set in. Buzna and Hodosy (21) attributed to an interference phenomenon the protection given to young chickens when they were vaccinated subcutaneously with live Herdfordshire strain

and then exposed to challenge. In the group challenged at 24 hours only 1 chicken died while the rest either showed no symptoms or moderate symptoms from which they recovered. No symptoms occurred in the ones challenged 48, 72, and 96 hours after vaccination. Karczewski et al. (54) described several field outbreaks in which the ailing birds were killed and the remaining flock vaccinated with the Mukteswar strain of Newcastle disease virus. Only a small number of the vaccinates died. The same authors reported that under experimental conditions protection against challenge was established in a healthy flock 48 hours after vaccination with a live virus. Some birds were protected even 24 hours after vaccination.

Russeff (77) injected intravenously a vaccine using the Mukteswar strain of virus followed by virulent virus intramuscularly at varying intervals from 0 to 21 hours. In a second experiment the 2 viruses were injected simultaneously. In both instances evidence of interference was found if the dosage of the 2 types was the same, and a delayed interference if the vaccinal virus was given in a smaller dose. Hence a quantitative relationship could be established between the interfering and superinfecting viruses. Gupta and Rao (40) vaccinated intramuscularly a group of cockerels with the Mukteswar virus vaccine. At daily intervals for 10 days, a number of vaccinated and

unvaccinated chickens were challenged intranasally with the virulent strain of Mukteswar virus. All of the vaccinated chickens that were challenged 24 hours later were dead. Also 50 per cent of the ones challenged 48 hours after vaccination died. The groups challenged 72 hours and later were protected. Hemagglutination inhibiting antibodies with a titer of 80 units appeared in the serum of the chickens 7 days after challenge. This supports their statement that protection was due to an interference phenomenon. Attempts to demonstrate interference in a field outbreak by vaccinating the flocks early during the course of the disease were unsuccessful.

In contrast to the above cited reports, Pomeroy (74), using inactivated and live virus vaccines in 1 day old chicks, could not induce any protection against lethal effects when the same birds were challenged intranasally, with strain 447, 6 hours and 4 days after vaccination. However, when challenge was done 7 days after vaccination 14.3 per cent and 68.6 per cent of the chicks in the inactivated and live virus vaccinated groups, respectively, were resistant to challenge. The author concludes that this protection was probably due to circulating antibodies.

The experiment to be reported here was done to compare the degree of protection induced in chickens vaccinated with an inactivated and a live Newcastle disease

virus vaccine and exposed to a challenge virus 48, 72, 96, 120, 144, and 168 hours after vaccination. The degree of protection induced by the vaccines was determined by the presence or absence of the challenge virus in the blood of the vaccinated chickens, and by the presence or absence of nervous symptoms in the chickens up to 12 days after challenge. In addition to these criteria the degree of airsacculitis in both groups of birds was compared.

Procedure

In one experimental group each of 36 White Leghorn chickens, 9 1/2 weeks old, was injected in the muscles of the thigh with 1 ml of the beta-propiolactone (BPL) Newcastle disease virus vaccine.

Another similar group was vaccinated with 2 drops (0.05 ml/drop) of infective allantoic fluid of Newcastle disease virus, Bl strain, in each conjunctival sac.

A third group of 36 chickens of the same source and age as in the 2 previous groups served as unvaccinated controls. Forty eight hours after vaccination 6 chickens from each group were challenged intranasally with 2 drops (0.05 ml/drop) of a 1:1000 dilution of infective allantoic fluid of the GB strain having a titer of 10^7 c.i.d.$_{50}$/0.1 ml. This challenge procedure was repeated every 24 hours in

similar groups of chickens through 168 hours after vaccination.

Ninety six hours after challenge the chickens were bled by heart puncture, the clotting avoided with heparin, and 0.1 ml of the blood inoculated into the allantoic sac of five, 9-11-day old embryonating eggs, to detect Newcastle disease virus in the blood of the challenged chickens and thus to determine the degree of protection conferred by the two types of vaccines. Observations were also made for deaths and for the presence of nervous symptoms up to the tenth day after challenge. At this time the surviving chickens were killed and post mortem examinations were done to record the degree of airsacculitis.

It should be pointed out that no challenge exposure was done prior to 48 hours since preliminary experiments, carried out under similar conditions, revealed that no protection to challenge could be expected when vaccination was done 12 or 24 hours previously.

Results

According to the results presented in Table 1 it can be seen that the challenge (GB strain) virus could not be isolated from the blood of any of the chickens that had been vaccinated with the B1 vaccine. In contrast, the virus was recovered from all the unvaccinated control chickens. Of the

Table 1. Results of intranasal challenge[a] of chickens vaccinated at 9 1/2 weeks of age with a live and an inactivated Newcastle disease virus

Time of challenge[b]	Newcastle disease virus isolated from blood			Chickens exhibiting nervous symptoms or lameness			Air sac lesions		
	B1 vaccinated	BPL vaccinated	Unvaccinated controls	B1 vaccinated	BPL vaccinated	Unvaccinated controls	B1 vaccinated	BPL vaccinated	Unvaccinated controls
48	0/6[c]	6/6	6/6	1/6	6/6	6/6	No air sac observation was made		
72	0/6	4/6	6/6	0/6	2/6	6/6	2/6	3/6	
96	0/6	0/6	6/6	0/6	0/6	6/6	0/6	5/6	
120	0/6	3/6	6/6	0/6	1/6	6/6	0/6	3/6	
144	0/6	5/6	6/6	0/6	0/6	6/6	1/6	4/6	
168	0/6	5/6	6/6	0/6	3/6	6/6	0/6	6/6	

[a]0.1 ml per bird of a 1:1000 dilution of the GB strain having 10^7 c.i.d.$_{50}$/0.1 ml.

[b]Hours after vaccination.

[c]Number affected/number challenged.

birds vaccinated with the beta-propiolactone (BPL) inactivated Newcastle disease virus vaccine, 6, 4, 0, 3, 5, and 5 of the 6 birds in each group revealed virus in their blood when challenged at 48, 72, 96, 120, 144, and 168 hours after vaccination, respectively.

When the 2 groups were compared on the basis of protection against nervous symptoms following challenge, all but 1 of the chickens vaccinated with the Bl vaccine were protected. The only exception occurred at 48 hours after challenge. All control chickens exhibited nervous symptoms or some degree of lameness. For the birds vaccinated with the inactivated virus 6, 2, 0, 1, 0, and 3 of the 6 birds in each group were not protected against nervous manifestations at 48, 72, 96, 120, 144, and 168 hours after vaccination, respectively.

When the 2 groups were compared on the basis of protection against airsacculitis, 2, 0, 0, 1, and 0 of the 6 chickens in each group vaccinated with Bl vaccine exhibited gross air sac lesions at 72, 96, 120, 144, and 168 hours after vaccination, respectively. In the group of chickens vaccinated with the BPL inactivated Newcastle disease virus vaccine 3, 5, 3, 4, and 6 of the 6 in each group revealed gross air sac lesions 72, 96, 120, 144, and 168 hours after vaccination, respectively. No comparison can be made with the controls since these were killed as soon

as they exhibited nervous symptoms following challenge. None survived to the tenth day when the air sac observations were made. No air sac observations were made 48 hours after challenge.

Discussion

From the results of this work it can be stated that the B1 vaccine protected the birds completely against viremia following challenge. Though 1 bird showed nervous symptoms when challenged 48 hours after vaccination the protection against nervous manifestations by such a virus was also excellent. Very good protection was also induced against the presence of airsacculitis considering that only 10 per cent of the chickens showed air sac lesions. This experimental evidence on the early protection of chickens to a challenge virus seems to indicate that the protection was due to an interference phenomenon.

The inactivated vaccine also induced some degree of protection, but not as good as that given by the live vaccine. When this protection was estimated by the absence of nervous symptoms 67 per cent of the chickens withstood challenge; 36 per cent did not reveal virus in their blood, and 30 per cent were found free of airsacculitis.

The observation made by Hofstad (49) was confirmed

that even though most of the chickens vaccinated with an inactivated vaccine do not die or show symptoms following challenge, they are shedders of the virus. In this experiment about half of the chickens in the group immunized with inactivated virus, which did not show nervous symptoms, yielded virus from the blood. The same observation applied when the criterion was the degree of protection against airsacculitis.

According to the results, the highly neurotropic characteristics of the GB strain were confirmed since all inoculated controls exhibited some degree of nervous symptoms or lameness by the fourth day after challenge.

The results regarding protection induced by the live vaccine are generally in agreement with those results obtained by Bang (5), Buzna and Hodosy (21), Karczewski _et al_. (54), and White and Appleton (84). In their experiments deaths and symptoms induced by a challenge virus were prevented as early as 48 hours following vaccination. This protection was ascribed to an interference phenomenon and was effective even though the challenge virus was given intramuscularly rather than intranasally.

The protection observed in the vaccinated chickens in the present experiment may also have been due to an interference phenomenon. It is difficult to explain the results obtained on the basis of circulating antibodies,

since it has been reported (18) that specific antibodies in chickens vaccinated with the live virus do not appear before 5 to 7 days after vaccination. In the present experiment protection with a live vaccine was obtained as early as 48 hours after vaccination.

Furthermore, White and Appleton (84) reported protection against a challenge virus within 48 hours following vaccination in 1 day old chicks. Since day old chicks are poor subjects to immunize, and produce little if any antibodies, the results of their experiments were ascribed to an interference phenomenon. Thus, this is additional evidence that the results in the present experiment could have been due to the interference phenomenon.

A search of the more pertinent reports on inactivated Newcastle disease vaccines (11, 12, 19, 24, 36, 42, 59, 60, 74, 82, 83) revealed that very few studies have been conducted to ascertain the status of immunity to challenge in chickens up to 1 week or less after vaccination. This is understandable since the workers in this field were more concerned with the length and degree of the immunity rather than with the relative rapidity at which it was induced. It appears from these reports that specific immunity against Newcastle disease develops, in healthy 10 day old or older chicks, 1 week after vaccination and reaches its peak 2 or 3 weeks later. In the present experiment partial protection

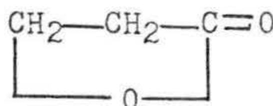
was observed 72 hours following vaccination.

Bang (5), Groupé (39), Henle and Henle (45) and Ziegler *et al.* (89) used chicken embryos as experimental subjects. In such experiments delay against death or complete suppression of the killing effect of a virus were demonstrated within 0 to 120 hours after the injection of the embryos with inactivated vaccines, and subsequent challenge with a virulent virus. Since no detectable antibody formation occurs in the chicken embryo the statement of the authors was that the protection in the chicken embryo was due to an interference phenomenon. If such a phenomenon can occur in the chicken embryo it very likely can occur in the living chick, as well, which apparently was the case in the present experiment.

EXPERIMENT 2: THE FATE OF A CHALLENGE VIRUS IN CHICKENS VACCINATED WITH INACTIVATED NEWCASTLE DISEASE VACCINE

Review of Literature

The search for a good virus inactivating agent has been focused to one that causes a complete loss of infectivity without destroying the immunogenicity of the virus; at the same time the vaccine or end product when given to the host should not be toxic or produce unwanted reactions. Beta-propiolactone (BPL) appears to have met these requirements and for this reason was used as the inactivating agent in the vaccine used in some of the experiments described here. BPL is an internal ester of beta-hydroxy propionic acid. Its chemical formula (38) is



Compounds like BPL possessing a four-membered ring are characterized by extreme activity due to the tendency of the ring to open, so almost any reagent having an active hydrogen or its equivalent can be the precursor of a large number of other beta-substituted propionic acids and their derivatives (62). BPL is known to react with groups associated with proteins including amino, carboxyl, and sulphydryl

(43). The virucidal and bactericidal properties of BPL were demonstrated first by Hartman _et al_. (44). Its toxicity in mice is 1/100 that of the nitrogen mustard (56, 67). Mangun _et al_. (67) found, after screening 140 compounds, that BPL was as good a virucidal agent as the best in their study but less toxic; Hartman _et al_. (43) after working with some 400 antiviral compounds had similar results. The MM strain of murine encephalomyelitis, eastern equine encephalomyelitis, and rabies virus vaccines treated with BPL showed a higher degree of antigenicity when compared with phenol and formalin inactivated virus vaccines (64). Viral inactivation occurred in a pH range of 5.0 to 9.0 (63). Polley and Guerin (73) found that the infectivity of influenza, mumps and poliomyelitis viruses was destroyed faster as the pH was raised from 6.0 to 8.0 and that hemagglutination titers of mumps and influenza virus suspensions were more stable to BPL treatment at pH 7.0. When influenza virus was treated with BPL and used to immunize mice and guinea pigs it resulted in protection of mice against challenge virus and production of specific antibodies in mice and guinea pigs (72). So far, a large number of viruses have been inactivated with BPL (27, 62, 81).

Mack and Chotisen (65) reported that 0.025 per cent by volume of BPL destroyed Newcastle disease virus infectivity; the vaccine protected 10-month old White Leghorn chickens

against intramuscular challenge with a field strain of Newcastle disease virus 16 days after vaccination. Serum neutralizing and hemagglutination inhibiting antibodies in the blood serum collected before and after vaccination showed a marked rise (66). Sullivan et al. (82) reported that one injection of BPL inactivated Newcastle disease virus gave variable degrees of protection to birds challenged intramuscularly with the GB strain of Newcastle disease virus 2, 4, 6, 8, and 10 weeks after vaccination. When 2 doses of vaccine were given a substantially higher degree of protection was afforded. Production of hemagglutination inhibiting and serum neutralizing antibodies was also stimulated by the vaccine. Winmill and Weddell (86) inactivated a native strain of Newcastle disease virus with BPL in a 1:4000 concentration. The vaccinated chickens were resistant to intramuscular challenge of a virulent strain up to 3 months after vaccination. Some waning of immunity was apparent at 4 and 5 months. Seven days after vaccination hemagglutination inhibiting titers were variable and had disappeared in all chickens 1 month after vaccination. Twelve months after vaccination 20 birds not having hemagglutination inhibiting antibodies were challenged by direct contact with infected birds. Only 1 bird died with symptoms of the disease whereas all the unvaccinated controls perished.

Quantitative estimations of Newcastle disease virus

in different tissues, blood, and feces of infected chickens have been reported by Asdell and Hanson (3), Hofstad (51), Karzon and Bang (55), and Sinha et al. (80).

Concerning the recovery of challenge Newcastle disease virus from the tracheal epithelium and feces of birds previously vaccinated with a live vaccine, Asplin (4) found that fowl vaccinated intranasally with the strain F of Newcastle disease virus failed to show symptoms when challenged intramuscularly with the Hertfordshire strain. Those birds whose hemagglutination inhibiting titers had fallen to 10 or less were found to excrete virus in their feces not earlier than the third and no later than the ninth day after challenge. The periods between vaccination and challenge ranged from 65 to 392 days. White et al. (85) showed that in birds challenged by aerosol exposure with the GB strain of Newcastle disease virus following spray vaccination, virus could be recovered from tracheal swabs for 3 to 4 days post exposure. However, the challenge virus could not be isolated from the blood of the birds at the same time intervals. Doll et al. (33) showed that susceptible chickens vaccinated intranasally at 3 weeks of age with the B1 strain of Newcastle disease virus were reinfected with the GB strain at 51 days following vaccination. The authors suggest that the epithelial surfaces of the respiratory tract were in a susceptible condition which permitted establishment and

growth of Newcastle disease virus. However, further invasion by Newcastle disease virus appeared to have been prevented by the circulating antibody and systemic infection did not occur. Winterfield _et al_. (87) recovered the challenge virus from tracheal swabs of chickens 96 hours after they were challenged intratracheally with the GB strain. The birds, 4 1/2 weeks of age, had been vaccinated in the drinking water, 3 weeks before, with Bl, F, and LaSota strains. Zuydam (90) found virus to be excreted in the feces up to 11 days after per os challenge of chickens vaccinated 3 months previously with live virus (Komarov's Haifa strain). Bankowski _et al_. (10) were able to recover the challenge virus, strain C, from the trachea of challenged birds 96 hours after aerosol or intramuscular administration. The birds in the experiment had been vaccinated 3 weeks before by way of the drinking water. Bankowski _et al_. (8) recovered virus irregularly from the trachea 96 hours after aerosol or intramuscular challenge of chickens vaccinated with live tissue culture attenuated vaccine 4 to 5 weeks previously.

Isolations of the challenge virus in birds which had received inactivated Newcastle disease virus vaccines have been reported by several authors whose work will be described briefly. Dardiri and Yates (25) in their study of the duration of immunity in layers after the use of live or inacti-

vated Newcastle disease virus vaccines recovered the intramuscularly injected challenge virus, GB Texas, from tracheal swabs 4 or 5 days after challenge. Every month, starting 4 months after the last dose of vaccine was given, a number of hens was challenged in each of the different vaccine tests. The virus was recovered from the groups of hens vaccinated 7 to 14 months before. Besides, 4 out of 62 eggs collected during the 10 day period after challenge yielded Newcastle disease virus from the yolks.

Dinter and Bakos (28) vaccinated 15 hens with formalin inactivated vaccine and subsequently challenged them with a virulent strain of Newcastle disease virus. This virus was isolated from the feces 3 to 13 days after challenge. Doll *et al*. (34) found that chickens 6 to 8 weeks old were susceptible to infection of the respiratory tract by the GB Texas strain 3 to 4 weeks after a single dose of inactivated Newcastle disease virus and at 2 weeks after 4 consecutive doses at weekly intervals. The challenge was effected by intranasal inoculation, direct contact exposure, or air borne contact exposure. The authors point out that a high number of the challenge birds were refractory to fatal infection.

Sullivan *et al*. (82) while studying the antigenic properties of 3 strains of Newcastle disease virus inactivated with 0.1 per cent BPL reported that virus was recovered

72 hours after challenge from the blood of 4 out of 12 chickens that had been vaccinated 6 weeks previously; from 1 bird out of 12 after 10 weeks of vaccination and no virus was recovered in birds vaccinated 4 and 8 weeks before. No virus could be isolated in birds twice vaccinated at the challenge intervals cited above. Winmill and Weddell (86) after vaccinating a group of chickens with a BPL inactivated native strain of Newcastle disease virus, isolated the challenge virus from the blood of the chickens on the second day after intramuscular administration of challenge, but not from the spleen, brain or intestinal contents on the fourteenth day. Zuydam (90) has indicated that in birds vaccinated 3 months before with formol and crystal violet inactivated virus vaccines he recovered the challenge virus, after per os infection, in the feces up to 35 days after infection. None of the birds showed clinical evidence of Newcastle disease.

Hofstad (50) has described a method to evaluate the status of immunity in birds vaccinated with inactivated Newcastle disease virus vaccines. The method determines the ability of the challenge virus, given intranasally, to multiply in the tissues of the vaccinated chickens. Failure to isolate the virus, 72 to 96 hours after challenge from the blood or tissues of the experimental birds was regarded as evidence of immunity. A comparison of the concentration of the virus in tissues or blood from vaccinated birds with

that of unvaccinated controls gives information of the degree of immunity in the vaccinated birds. This same method has been used in subsequent experiments (48, 49, 52). Based on the above described work it was considered of interest to design an experiment to study the fate and concentration of a Newcastle disease challenge virus in the tissues and blood of chickens vaccinated previously with a BPL inactivated vaccine.

Procedure

Group 1

Twenty 5 week old White Leghorn chicks were injected intramuscularly with 1 ml of the beta-propiolactone (BPL) inactivated Newcastle disease virus. Eight weeks after vaccination each was challenged intranasally with 0.2 ml of a 1:1000 dilution of infective allantoic fluid of the GB strain having a titer of 10^7c.i.d.$_{50}$/0.1 ml. An equal number of nonvaccinated control chickens were also challenged at the same time.

Twelve hours after challenge 2 vaccinated and 2 nonvaccinated chickens were bled by heart puncture. The clotting of blood was prevented by using heparin (10 USP units/ml of blood). Then the chickens were killed and the lower 2/3 of the trachea, the left lung, spleen, anterior lobe of the

left kidney, and cecal (proximal third to the cecal tonsils) and rectal contents from each bird were removed, each ground in a mortar with alundum and diluted approximately 1:5 with tryptose phosphate broth. Such material was stored in tubes at -35°C.

The same procedure was followed with the rest of the vaccinated chickens and corresponding controls at 24 hours intervals through 216 hours post challenge.

Detection of the virus After bleeding the chickens, the blood was delivered into tubes and chilled at 4°C for about 15 minutes. These tubes were then centrifuged in chilled cups at 1800 RPM for 5 minutes, the plasma decanted and replaced by tryptose phosphate broth, in an attempt to avoid the neutralizing effect of the immune serum in the blood. The blood treated in this way was stored at -35°C.

All tissue suspensions (cecal and rectal contents will be included under the term tissue) and blood belonging to 1 bird were thawed at one time and centrifuged at 1800 RPM for 5 minutes (except blood) to sediment the larger tissue particles. The supernatant fluid of each was employed to make tenfold dilutions in tryptose phosphate broth containing 1000 IU of penicillin and 2 mg of streptomycin/ml. Each of the tissue suspensions was mixed with a solution containing 5000 IU of penicillin (pen.) and 10 mg of streptomycin (strep)/0.1 ml (to prevent bacterial contamination)

according to the following scheme:

1.4 ml of blood + 0.1 ml of pen.-strep. mixture (mix.)

1.25 ml of tracheal suspension + 0.25 ml of pen.-strep. mix.

1.25 ml of lung suspension + 0.25 ml of pen.-strep. mix.

1.4 ml of spleen suspension + 0.1 ml of pen.-strep. mix.

1.4 ml of kidney suspension + 0.1 ml of pen.-strep. mix.

1.1 ml of cecal and rectal contents suspension
+ 0.4 ml of pen.-strep. mix.

Using a 1 ml pipette 0.25 ml of each of these tissue suspensions was transferred into 2.25 ml tryptose phosphate broth blanks to make ten-fold dilutions. Two patterns of dilutions for vaccinated and for nonvaccinated chickens were used. For the vaccinated chickens each tissue and blood was diluted through 10^{-2}, and in addition the trachea and lung were diluted through 10^{-3}.

For the nonvaccinated control chickens each tissue and blood, obtained at intervals from 12 to 120 hours after challenge, was diluted through 10^{-3}; in addition the trachea, lung, spleen, and kidney were diluted through 10^{-5}. For the tissues and blood of the nonvaccinated chickens, collected from 144 to 216 hours after challenge, dilutions through 10^{-2} were made; in addition the trachea, lung, spleen, and kidney were diluted through 10^{-3}. This procedure was done because higher titers of the virus were expected to occur in the nonvaccinated birds than in the vaccinated ones, and

within the nonvaccinated group the titer of the virus was expected to decline as the time since challenge was extended. Also concentrations of the virus in the different organs selected for isolation were expected to vary. For each of the above cited dilutions 0.1 ml was inoculated into the allantoic sac of each of five, 9 - 11-day-old chicken embryos. The allanto-ammionic fluids of embryos dying between 24 and 192 hours after inoculation were checked for erythrocyte agglutination by a rapid plate test.

Group 2

This group of chickens was also vaccinated intramuscularly with 1 ml of the BPL inactivated vaccine at 5 weeks of age and revaccinated by the same route and dose when 13 weeks old. Six weeks following the second injection, the vaccinated chickens, along with an equal number of nonvaccinated controls, were challenged as in Group 1, differing only in that this group was infected with a 1:10 dilution of the GB strain instead of a 1:1000 dilution given to chickens in Group 1. This was done since an increased immunity was anticipated by the greater age and additional vaccination.

Serology

In order to learn more about the immune status of the vaccinated birds, individual hemagglutination inhibition and

serum neutralization tests were performed with the blood serum from some of the chickens in Groups 1 and 2. For each of these groups the blood serum samples were obtained on the day of their corresponding challenge. Five and 10 chickens were bled for Groups 1 and 2, respectively.

Results

The results of this experiment are presented in Tables 2 through 5.

In birds vaccinated once with 1 ml of the beta-propiolactone (BPL) inactivated Newcastle disease vaccine at 5 weeks of age and challenged 8 weeks later virus could not be isolated, at the time intervals used, from the blood or tissues of any of the chickens used in the experiment (Table 2).

In the corresponding unvaccinated control chickens (Table 2) no virus was isolated from the blood or tissues of the chickens 12 hours after challenge, but it was found to be present in the lung and spleen at 24 and 48 hours after challenge; in all tissues and blood at 72 and 96 hours; in all tissues excepting blood at 120 and 144 hours; in kidney, and cecal and rectal contents at 168 hours. At 192 hours only the kidney contained virus. No virus was isolated at 216 hours after challenge.

Table 2. Concentration of Newcastle disease virus in tissues of vaccinated and control chickens[a] following intranasal challenge[b]

Hours after challenge	e.l.d.$_{50}$[c] per 0.1 ml of tissue suspension											
	Blood		Trachea		Lung		Spleen		Kidney		Cecal and rectal contents	
	V.[d]	C.[e]	V.	C.	V.	C.	V.	C.	V.	C.	V.	C.
12	0.0[f]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.3	0.0	0.0	0.0	0.0
72	0.0	0.3	0.0	3.8	0.0	3.7	0.0	2.4	0.0	3.6	0.0	0.1
96	0.0	0.1	0.0	3.4	0.0	4.4	0.0	3.9	0.0	3.1	0.0	1.2
120	0.0	0.0	0.0	5.3	0.0	4.5	0.0	0.2	0.0	4.0	0.0	1.4

[a]Chickens were 13 weeks old at time of challenge.

[b]0.2 ml per bird of a 1:1000 dilution of the GB strain having 10^7 c.i.d.$_{50}$/0.1 ml.

[c]Embryo lethal dose at logarithm base 10.

[d]Vaccinated intramuscularly with 1 ml of BPL inactivated Newcastle disease vaccine 8 weeks previously.

[e]Nonvaccinated controls.

[f]Average e.l.d.$_{50}$ of 2 inoculated chickens.

Table 2. (Continued)

Hours after chal-lenge	e.l.d.$_{50}$[c] per 0.1 ml of tissue suspension											
	Blood		Trachea		Lung		Spleen		Kidney		Cecal and rectal contents	
	V.[d]	C.[e]	V.	C.	V.	C.	V.	C.	V.	C.	V.	C.
144	0.0	0.0	0.0	1.6	0.0	1.9	0.0	2.4	0.0	2.3	0.0	1.2
168	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.1
192	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
216	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

In the case of chickens vaccinated twice (Table 3) with the BPL inactivated vaccine and exposed to challenge 8 weeks after the last dose of vaccine the challenge virus was isolated from all tissues and blood of 1 chicken 96 hours after challenge, and from the kidney of another bird at 120 hours. When the unvaccinated controls (Table 3) for the twice vaccinated group were challenged, no virus was found in the tissues or blood in the first 12 hours. But the challenge virus was found to be present in the blood at 24 hours after challenge; in all tissues and blood excepting cecal and rectal contents at 48 hours; in all tissues and blood at 72, 96, 120, and 144 hours; in blood, kidney, and cecal and rectal contents at 168 and 192 hours; in kidney the virus was found to be present 216 hours after challenge.

In Tables 4 and 5 the hemagglutination inhibition titers and neutralization indexes of some of the chickens in this experiment are presented. For the once vaccinated chickens hemagglutination inhibition titers ranged from 0 to 20 whereas in the controls a titer of 10 was found. The neutralization indexes ranged from 0.0 to 0.9. No neutralization index was found in the controls. For the twice vaccinated chickens the hemagglutination inhibition titers varied from 80 to 2560. The controls had a titer of 10. The corresponding neutralization indexes for the birds in this group ranged from 1.3 to 5.0. The neutralization titer

Table 3. Concentration of Newcastle disease virus in tissues of twice vaccinated and control chickens[a] following intranasal challenge[b]

Hours after challenge	e.l.d.$_{50}$[c] per 0.1 ml of tissue suspension											
	Blood		Trachea		Lung		Spleen		Kidney		Cecal and rectal contents	
	V.[d]	C.[e]	V.	C.	V.	C.	V.	C.	V.	C.	V.	C.
12	0.0[f]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	1.4	0.0	1.7	0.0	1.5	0.0	1.7	0.0	0.6	0.0	0.0
72	0.0	2.4	0.0	3.5	0.0	3.4	0.0	2.7	0.0	2.2	0.0	0.1
96	0.2	1.7	1.7	5.0	1.7	4.4	1.2	3.5	1.2	3.5	0.6	0.9
120	0.0	0.7	0.0	4.0	0.0	3.1	0.0	2.6	0.1	2.6	0.0	0.8

[a]Chickens were 19 weeks old at time of challenge.

[b]0.2 ml per bird of a 1:10 dilution of the GB strain having 10^7 c.i.d.$_{50}$/0.1 ml.

[c]Embryo lethal dose at logarithm base 10.

[d]Vaccinated intramuscularly. Second vaccine given 8 weeks after first vaccine. Challenge at 6 weeks after last vaccine.

[e]Nonvaccinated controls.

[f]Average e.l.d.$_{50}$ of 2 inoculated chickens.

Table 3. (Continued)

Hours after challenge	e.l.d.$_{50}$[c] per 0.1 ml of tissue suspension											
	Blood		Trachea		Lung		Spleen		Kidney		Cecal and rectal contents	
	V.[d]	C.[e]	V.	C.	V.	C.	V.	C.	V.	C.	V.	C.
144	0.0	0.1	0.0	2.6	0.0	2.6	0.0	2.3	0.0	3.0	0.0	1.2
168	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.3
192	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.1
216	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0

Table 4. Serological response in chickens vaccinated once with a Newcastle disease virus vaccine inactivated with beta-propiolactone

Vaccinated with 1 dose of vaccine[a]		
Chicken	HI[b] titer	NI[c]
1	10	0.3
2	10	0.0
3	0	0.9
4	20	0.9
5	10	0.2
Control pool[d]	10	0.0

[a]Eight weeks previously.

[b]Hemagglutination inhibition, expressed as the reciprocal of the serum dilution.

[c]Neutralization index.

[d]Mean titer of 2 chickens.

for the controls was negligible.

A question arose regarding the effect of a more severe route of inoculation (intramuscular) upon the vaccinated chickens. To seek an answer for the question, a very simple trial was designed. On the same day when the group of twice vaccinated chickens was challenged intranasally 8 additional chickens, along with a similar number of nonvaccinated controls, were injected into the muscles of

Table 5. Serological response in chickens vaccinated twice with a Newcastle disease virus vaccine inactivated with beta-propiolactone

Vaccinated with 2 doses of vaccine[a]		
Chicken	HI[b] titer	NI[c]
1	640	4.0
2	1280	3.0
3	160	3.3
4	640	2.1
5	320	2.2
6	80	4.1
7	320	5.0
8	640	1.3
9	1280	3.7
10	2560	1.3
Control pool[d]	10	0.1

[a]Second dose given 8 weeks after first dose.

[b]Hemagglutination inhibition, expressed as the reciprocal of the serum dilution.

[c]Neutralization index.

[d]Mean titer of 2 chickens.

the thigh with 0.2 ml of the GB virus. These chickens were housed in the same quarters, but in separate cages, as those inoculated intranasally. At intervals of 12, 24, 48, and 72 hours their tissues and blood were processed according to the method described under Procedure.

No challenge virus could be isolated in chicken embryos inoculated with the tissues and blood from these vaccinated chickens. In contrast the virus was isolated from all the nonvaccinated controls. These results provide further evidence of the immunogenic properties of the BPL inactivated vaccine, since in spite of the more drastic route of challenge employed, the vaccinated chickens were protected enough to prevent replication of the virus at the intervals described.

Discussion

The results in the groups of birds receiving only 1 dose of beta-propiolactone (BPL) inactivated Newcastle disease vaccine reveal that no detectable replication occurred in the birds. Perhaps a longer interval between vaccination and challenge would have permitted isolation of the virus from the partially immune birds as it was the original purpose of the experiment.

The above mentioned results exceed those of Sullivan

et al. (82). In their studies, when challenge was effected 8 weeks after vaccination the virus was isolated in 12 per cent of the chickens. Perhaps the difference is due to the fact that they vaccinated the chickens when 1 day old whereas in the present experiment the chickens were 5 weeks old, when a better immunological response can be expected.

In the report of Mack and Chotisen (65) their criterion in evaluating immunity following injection of BPL inactivated virus was survival of the chickens after challenge with a virulent strain. Complete protection was obtained when the 10 month old chickens were challenged 16 days after vaccination. The results of the present experiment concur with the results of Mack and Chotisen (65) and also agree with those of Winmill and Weddell (86) in which they reported complete protection of chickens to intramuscular challenge 1 and 2 months after vaccination with a BPL inactivated virus.

The data presented, representing in each case the mean titer of the virus found in two 13 week old nonvaccinated control chickens, indicate that the highest concentration of the virus was found in the lung, followed by kidney, trachea and spleen. Titers in the cecal and rectal contents rose somewhat more slowly. Viremia was never marked in the chickens. Similarly Sinha et al. (80) reported, while testing 6 different strains of Newcastle disease virus,

that the highest concentration of virus for all strains was always found in the lung. Karzon and Bang (55) also found the highest titer of the virus in the lung, when the chickens were challenged with the GB strain. In general, the results of this experiment, regarding the concentration of virus in tissues of unvaccinated chickens, are in agreement with those of Asdell and Hanson (3); however, somewhat lower concentrations of virus were found in the present experiment. Similarly the results concur with those of Hofstad (51) in the demonstration of virus at 24 hours through 168 hours; however, in the present experiment virus was isolated from the kidney at 192 hours from the controls of Group 2. Virus concentrations were found to be slightly less in this experiment compared to that found by Hofstad (51).

The results obtained after challenge of the chickens vaccinated twice with the BPL inactivated vaccine were rather surprising in that 1 bird yielded virus from the blood, trachea, lung, spleen, kidney, and intestinal and cecal contents 96 hours after challenge. In addition the virus was isolated from the kidney at 120 hours after challenge from another bird. The fact that the virus was in the process of replication in the affected chicken and was not residual challenge virus is supported by the statement of Beaudette (14) who indicated that "the Newcastle disease virus recovered from the respiratory tract of chickens the

day after inoculation is probably residual virus. An eclipse period of 24 hours then follows when no virus is likely to be recovered. However, after 48 hours and generally up to 144 hours, virus resulting from multiplication within the birds is recoverable". The failure of these 2 birds to develop an effective immunity might be explained by an absence of individual response of the chickens to the antigen. It should also be emphasized that the dose of challenge virus for the chickens in this group was 100-fold greater than for those in the group of chickens vaccinated once. Another explanation of the apparent failure of the vaccine to induce protection in 1 chicken when assayed 96 hours after challenge is that the referred chicken could have been missed when the chickens were vaccinated for the second occasion, which means that at the time of challenge it has been vaccinated once, 14 weeks previously, compared to the rest of its pen mates that had been vaccinated, for the second time, 6 weeks previously. Thus it is explainable that a waning of the immunity furnished by only 1 vaccine dose was evident at the time of challenge. Many authors, Brandly _et al_. (19), Dardiri _et al_. (26), Hofstad (49, 50, 52), Waller and Gardiner (83) have pointed out that a higher degree of protection should be expected by the use of 2 doses of inactivated vaccine at the appropriate intervals which apparently was the case for all but 2 chickens in the above

cited chickens in Group 2.

The data representing the titer of the challenge virus found in the tissues and blood of the control chickens at 19 weeks of age reveal that the highest concentration of the virus was found in the trachea and not in the lung as was the case for the controls at 13 weeks of age. A decreasing concentration of virus was found in the lung, spleen and kidney, blood, and cecal and intestinal contents respectively. The virus was found consistently in the blood of the birds in fairly high titers, even higher than those found in the intestinal and cecal contents; this is opposite to the findings in the controls 13 weeks of age. The differences seem to stem from the larger doses of challenge virus used in this second group.

Regarding the hemagglutination inhibition and neutralizing indexes, the data indicate that the serum antibody levels were negligible in the once vaccinated chickens. These results are in agreement with those of Sullivan et al. (82) in which vaccination of chickens with a BPL inactivated Newcastle disease vaccine was followed by a steady increase in hemagglutination inhibiting antibodies that reached their peak 2 weeks after vaccination. Beyond this time the hemagglutination inhibiting antibodies decreased in such a magnitude that 6 weeks after vaccination the titer at this time could be compared with the initial one. In contrast, the

virus neutralizing antibodies did not exhibit such a sharp increase and decline. At 8 weeks after vaccination they were able to detect a small amount of serum neutralizing antibodies which was not the case in Group 1. Regardless of the absence of hemagglutination inhibiting or virus neutralizing antibodies in the chickens vaccinated once with the BPL inactivated vaccine, they were refractory to challenge, as previously stated, according to the method of evaluating the immunity in this experiment. These results are in complete agreement with those of Sullivan _et al_. (82) and Winmill and Weddell (86).

For the chickens vaccinated twice with the BPL inactivated Newcastle disease vaccine a substantial increase in hemagglutination inhibiting titers was observed as compared with those of the chickens vaccinated only once. The same statement can be applied to the virus neutralizing titers although 2 of the chickens tested exhibited a low neutralization index. This increase in serum antibodies as a response to the injection of a second dose of vaccine is in agreement with the results of Mack and Chotisen (66), and Sullivan _et al_. (82).

The data presented indicate BPL to be a good inactivating agent for Newcastle disease virus since it rendered the virus inactive without destroying its immunogenic properties. The main advantage of an inactivated

vaccine is that there is no possibility of introducing Newcastle disease infection in the flock as may be possible with attenuated live virus vaccines.

Though the original purpose of this experiment, that of determining the fate of the challenge virus in partially immune birds, was not accomplished, apparently because of the good immunity produced by the BPL inactivated virus vaccine, the experience with the BPL inactivating agent and the results of the concentration of the virus in the control chickens rendered this experiment profitable.

EXPERIMENT 3: THE EFFECT OF PASSIVE ANTIBODIES IN CHICKS UPON CHALLENGE AND VACCINATION

Review of Literature

The transfer of hemagglutination inhibiting and virus neutralizing antibodies from dams to chicks has been demonstrated by Bornstein et al. (17), Brandly et al. (20), Doll et al. (32), Levine and Fabricant (61), and Schmittle and Millen (79). The effects of passive antibodies upon susceptibility of chickens to challenge with Newcastle disease virus has been studied by a number of investigators.

Brandly et al. (20) demonstrated the existence of serum antibodies in chicks hatched from hens immune to Newcastle disease. They challenged chicks with intramuscular injections of a lethal Newcastle disease virus and found a correlation between the serum antibody titer and survival of the chicks. The same authors reported that when chicks hatched from immune dams were challenged intramuscularly at 1, 8, 15, 22, 29, 36, and 43 days of age with the Hertfordshire strain, and the survivors rechallenged intravenously 21 to 35 days later with the same virus 53, 58, 45, 22, 19, 11 and 0 per cent, respectively, died from the second challenge that killed 100 per cent of the controls. This, according to the authors, demonstrates that the virus

injected intramuscularly is neutralized by the chicks with high levels of antibodies and that few become immune, while older chicks with little or no parental immunity do not neutralize the virus, thus resulting in active immunity as demonstrated by resistance to a second intravenous challenge.

Doll _et al_. (29) in studying the passive protection conferred to chicks through the yolk used breeder hens that were vaccinated intranasally with the B1 strain. Six months later, eggs were incubated and hatched; the 1-day-old chicks were challenged with the Texas strain GB. In groups of chicks challenged intramuscularly, intranasally or by contact the infection rates were 100, 100, and 93.7, respectively.

In a similar group, chicks hatched from eggs laid by dams vaccinated 4 months previously, the infection rates following challenge were 91.6, 76.1, and 73.9 per cent respectively. The authors concluded that the chickens, hatched from eggs obtained 4 to 6 months after vaccination of the breeders, have little or no passive immunity.

Alberts and Millen (1) determined the survival rate of passively immune chicks following challenge with the California strain 11914 by the wing web method of inoculation. Survival rates among the progeny of vaccinated layers were 91, 87, 77, 55, and 35 per cent respectively at 1, 7, 14, 21, 28 days of age. The survival rate among progeny of

layers which had recovered from the natural disease was 100, 96, 100, 82, and 53 per cent respectively for similar periods. The experiment supported the belief that when Newcastle disease virus is introduced directly into the tissues of passively immune chickens, the virus is neutralized by the circulating antibodies.

Waller and Gardiner (83) reported that chicks hatched from hens that had been revaccinated with inactivated virus at 13 weeks of age had sufficient parental immunity to protect 62.5 per cent of the chicks against a challenge with Newcastle disease virus at one day of age. Markham _et al._ (69) reported that chicks hatched from immune layers with high hemagglutination inhibition serum titers still had sufficient demonstrable antibodies at 5 weeks of age to protect 43 per cent of them against intramuscular challenge with the GB strain.

Olson _et al._ (71) reported on 5 outbreaks of Newcastle disease in chicks under 1 week of age which possessed parental antibodies. The chicks had been hatched from hens that had received the wing web type of live virus vaccine. Pooled sera collected from the hens 20 to 70 days following vaccination had hemagglutination inhibition titers greater than 1:40 in 64 per cent of the flock. Such results indicate that under field conditions the dams confer to their progeny varying degrees of protection.

Levine and Fabricant (61) could not demonstrate any protection against respiratory symptoms induced by a field strain when the virus was instilled in the nares of 1, 5, 7, 10, 14, and 17 day old chicks hatched from hens vaccinated with live virus 10 months before. Five out of 29 dams in this vaccinated flock had negative virus neutralizing and hemagglutination inhibition titers. Previous to challenge hemagglutination inhibition titers of the serum of the chicks were variable; serum neutralization titers were negative. Equivalent results were obtained in chicks hatched from hens naturally recovered from an outbreak 9 months before. In this case the chickens were challenged at 1, 4, 7, 11, 14, and 18 days of age. All hens in this group had positive hemagglutination inhibition and serum neutralization titers.

Doll et al. (30) used passively immune chicks from hens that survived a natural outbreak of Newcastle disease 8 to 10 months previously. At 1, 2, and 3 weeks of age, the chicks were exposed intranasally to the strain Ky-50. No lethal infections occurred but nervous symptoms were recorded in 2.5, 2.5, and 0 per cent respectively for the different challenge intervals. Of 22 susceptible chicks 7 weeks old used as controls 31.7 per cent perished. When the GB strain was used to challenge intramuscularly another group of the same hatch at 1, 2, 3, and 4 weeks of age, this inoculation produced nervous symptoms and/or death in 22.6,

86.8, 100, and 100 per cent of the chicks respectively. The experiment emphasizes mainly the variable introduced when 2 different routes of challenge are used.

Alberts and Millen (1) demonstrated that in passively immune chicks their resistance to challenge exposure declines inversely with age. By using the California strain 11914 of Newcastle disease virus for challenge, the per cent of resistant chicks at the different intervals was as follows: 91 per cent for 1 day old, 87.4 per cent for 7 day old, 77.2 per cent for 14 day old, 55.3 per cent for 21 day old, and 35.0 per cent for 28 day old chicks.

Komarov (57) challenged passively immune chicks with Newcastle disease virus by the intramuscular route. Day old chicks were 47 to 100 per cent resistant to challenge; 1 week old chicks 50 to 60 per cent; 2 and 3 week old chicks 30 to 60 per cent; 4 week old chicks 25 to 27 per cent. However at 6 weeks of age none of the 15 challenged chicks survived.

Markham _et al_. (69) have demonstrated, with the aid of the hemagglutination inhibition test, that a poorer anamnestic response resulted with revaccination by the parenteral route than with revaccination by the upper respiratory route.

Brandly _et al_. (20) reported that the passive antibodies in chicks hatched from immune dams were capable of

interfering with the normal production of antibodies in chicks that were injected with 1 ml of formalin inactivated virus vaccine at 21, 28, 35, and 42 days of age. The mortality in chicks following intramuscular challenge at 3 weeks and also at 6 weeks after vaccination was 100, 80, 24, and 38 per cent, respectively, as compared to 11 per cent in chicks, without passive antibodies, vaccinated at 21 and 49 days of age.

Markham et al. (70) compared the response to Newcastle disease virus vaccination by the intramuscular, wing web (subcutaneous-cutaneous) and intranasal routes in passively immune birds under experimental conditions. In their experiments they obtained the passively immune status by injecting subcutaneously 2 week old chicks with Newcastle disease immune serum using a dosage of 0.5 ml or 2.5 ml per bird. The chicks were vaccinated 48 hours after injecting immune serum. The immune response was uniformly negative or very poor as determined by the hemagglutination inhibition test. The authors concluded that the response to primary immunization with Newcastle disease vaccine was limited or suppressed in birds that possessed varying grades of residual passive immunity.

Winterfield and Seadale (88) studied the serological response of young chicks to B1 Newcastle disease virus administered through the drinking water. In their field trials

all the parent flocks of the chicks had been vaccinated against Newcastle disease prior to sexual maturity. Four-day old chicks, 75 per cent of which had positive serum neutralizing titers, were vaccinated with 10^8 embryo infective $dose_{50}$ ($e.i.d._{50}$) of the B1 strain by way of the drinking water. Four weeks later 60 and 4 per cent of them, respectively, exhibited positive serum neutralizing and hemagglutination inhibiting titers. When brooder mates of these chicks were vaccinated with 10^5 $e.i.d._{50}$ only 5.5 and 4 per cent exhibited positive serum neutralizing and hemagglutination inhibiting titers, respectively. In a third experiment 100 per cent of the chicks in the test had positive serum neutralizing titers at 4 days of age. Even though they were vaccinated with 10^8 $e.i.d._{50}$ of vaccine, the percentage of birds that possessed positive serum neutralizing and hemagglutination inhibiting titers 4 weeks after vaccination was 25 and 4 per cent respectively. The authors concluded that in view of the gradations and extremes of parentally conferred immunity that exist under field conditions the stimulation of a satisfactory immunity in many flocks vaccinated at an early age in the drinking water may be difficult. Also, the immunity stimulated was appreciably conditioned by the dosage of vaccine per bird. High dosages were apparently not always sufficient to give an antigenic stimulus in overcoming barriers of passive immunity.

Beaudette and Bivins (15) studied the effect of passive immunity in chicks on subsequent immunization with live Newcastle disease virus. They injected 5 ml of hyperimmune Newcastle disease serum into each of 3 chicks, 5 weeks old 24 hours prior to intramuscular vaccination. Three different dosage levels of virus were used. Three additional birds were treated similarly but received the virus intranasally. After vaccination, attempts to isolate the vaccine virus from the trachea were negative after the intramuscular route. In the intranasally vaccinated birds virus was recovered from the trachea of 2 of the 3 chicks. The authors concluded that the vaccine virus is neutralized in passively immune birds when administered intramuscularly; no immune response is provoked, nor does the virus appear in the respiratory tract. Administered intranasally to passively immune birds, the virus is also neutralized even though it may appear briefly in the respiratory tract. There is no immune response except when large doses of virus are given.

Bankowski *et al.* (9) designed an experiment to determine the effectiveness of administering a tissue culture modified Newcastle disease virus vaccine by various routes to susceptible and passively immune chicks. At five days of age passively immune chicks revealed an hemagglutination inhibition geometric mean titer of 37 and an serum neutralization titer of 10^5. Susceptible chicks exhibited negative

hemagglutination inhibition and serum neutralization titers. At this time the chicks were vaccinated either intramuscularly, by way of the drinking water, or by instilling 2 drops of vaccine intranasally. By the fifth week after vaccination chicks from susceptible hens, vaccinated intramuscularly, developed substantial hemagglutination inhibition and serum neutralization titers. Lower hemagglutination inhibition and serum neutralization titers were detected in serum from birds vaccinated by way of the drinking water, or by intranasal inoculation. Comparable results were obtained as to the degree of resistance of the birds in each of these groups when challenged with the GB Texas 1948 strain either by the intramuscular or air borne routes.

In contrast, chicks hatched from immunized hens did not develop hemagglutination inhibiting and serum neutralizing antibodies following vaccination when tested at 5 weeks of age. However some birds vaccinated by each method were immune to challenge by intramuscular and air borne routes. This led the authors to conclude that susceptible chicks vaccinated at five days of age with a modified tissue culture vaccine were solidly immune whereas progeny from immune hens vaccinated at the same age acquired an irregular and less effective immunity.

Bankowski and Corstvet (7) determined the influence of residual resistance at an early age upon the quality and

duration of an immunity induced by revaccination. At 5 days of age passively immune and susceptible chicks were vaccinated intranasally with a commercial B1 vaccine. Individual serum samples from 5 day old passively immune chicks presented hemagglutination inhibition titers from 0 to 1:80. The serum neutralization titer of a pool of five serums neutralized 10^3 embryo lethal doses of Newcastle disease virus. No significant antibody titers were found in the chicks of the susceptible group. At 32 days of age the chicks were revaccinated intramuscularly with a live attenuated virus propagated in tissue culture. At this time no hemagglutination inhibition or serum neutralization titers were found in the passively immune group and when revaccinated at 32 or 39 days of age a good anamnestic response was obtained.

On the other hand chicks of the susceptible group still had hemagglutination inhibiting and serum neutralizing antibodies when tested at 32 days of age. This resistance resulted in poor anamnestic response when the chicks were vaccinated at this time with the tissue culture vaccine.

Komarov (57) reported on the interference of congenital passive immunity with Newcastle disease immunization using the "Mukteswar" strain of Newcastle disease virus. He found that intramuscular challenge at 44 days of age killed 100 per cent of chicks vaccinated when 1 day old, 40 per cent of those vaccinated at 1 week of age, but only

2 per cent of those vaccinated when 3 weeks old.

Bankowski _et_ _al_. (9) reported that progeny from immune dams possessing hemagglutination inhibiting and serum neutralizing antibodies at the time of live virus vaccination acquired an irregular and less effective immunity compared to chicks from immune dams which were held in isolation for a period of 3 weeks prior to vaccination. The latter responded well serologically and were solidly immune to intramuscular challenge with the GB strain when they reached 12 weeks of age.

Doll _et_ _al_. (31) studied the immunizing reaction of the B1 strain given intranasally and intramuscularly to 1 day old chicks hatched from dams that had survived an outbreak of the disease 50 days previously. The response in the chicks was determined by means of the hemagglutination inhibition test on pooled samples from day 3 to day 27. In general, a gradual fall in titer was observed in both vaccinated groups and in the control. chicks. On challenge 31 days after vaccination, 100 per cent of the chicks vaccinated intranasally and intramuscularly, respectively, resisted challenge of the Ky-50 strain of Newcastle disease virus instilled in the trachea. Seven of the 22 controls died.

In another experiment the effect of parental immunity upon vaccination was studied in which chicks hatched from

dams that suffered an attack of the disease 6 months previously were vaccinated intranasally with the Bl strain at 2 and 9 days of age. The hemagglutination inhibition titers 7 and 14 days after vaccination were higher in the chickens vaccinated at 9 days than in those vaccinated at 2 days, the latter being similar to the nonvaccinated controls. When intramuscular challenge was done with the GB strain at 31 days following vaccination the losses from death and paralysis were 60.8 per cent and 35.7 per cent, respectively, for the chickens vaccinated at 2 and 9 days of age, and 100 per cent for the controls.

The authors concluded that parentally immune chicks can be immunized by intranasal inoculation, however the chicks with low levels of passive antibodies are more effectively immunized than those with high levels of passive antibodies. The virulence of the vaccine strain is also a factor in the degree of immunity produced.

Markham _et al_. (68) demonstrated that chicks hatched from immune hens can be immunized by intranasal vaccination with the Blacksburg strain of Newcastle disease virus. Fifteen 1 day old chickens, with hemagglutination inhibition titers from 1:16 to 1:512 were vaccinated intranasally or in the conjunctival sac with the GB strain. No deaths or symptoms of the disease were observed in the birds challenged at 5 or 7 weeks of age, and only 8 per cent of combined

morbidity-mortality was observed in the group of 25 chickens challenged 10 weeks after vaccination.

Hitchner (46) stated that 1 day old chicks, possessing a passive hemagglutination inhibiting antibody titer of 1:160, could be immunized satisfactorily by the intranasal route with live virus vaccine. In his experiment chicks hatched from breeders immunized 126 days previously with a commercial vaccine by the wing web method were vaccinated at 1 day of age. Only 1.8 per cent showed evidence of infection when challenged later with the California strain 11914. Controls from the same group challenged simultaneously resulted in 70 per cent visibly infected birds.

Quesada _et al_. (75) were able to establish solid immunity in 1 day old chickens with passive antibodies by vaccinating the birds intranasally or in the conjunctival sac with the F strain of Newcastle disease virus.

Because of the different results obtained by the various authors in regard to the effect of passive antibodies on subsequent vaccination and challenge, a study was undertaken to obtain additional information on the susceptibility to Newcastle disease infection of chickens hatched from actively immunized hens, and to obtain information on the activity of Newcastle disease virus infection in groups of chicks with congenital passive antibodies.

Procedure

In this experiment a group of Ames-in-cross chicks was hatched from parent stock which had been vaccinated against Newcastle disease by way of the drinking water at 4 weeks of age, and against Newcastle disease and infectious bronchitis, by the same route, when 22 weeks of age. The breeding stock was 31 weeks old at the time the eggs were saved for the experiment.

The chicks were placed in battery brooders in an isolation room. These chicks will hereafter be called the passively immune chicks. At the same time another group of chicks were hatched from eggs obtained from breeders with no history of Newcastle disease virus infection or vaccination. These were placed in another isolation room and hereafter will be called controls.

At 2 days, and at weekly intervals for 4 weeks, 20 passively immune chicks and 20 control chicks were removed for intranasal challenge with 0.2 ml of a 1:1000 dilution of infective allantoic fluid of the GB strain having a titer of 10^7c.i.d.$_{50}$/0.1 ml. The chicks were observed daily for 12 days after challenge to detect deaths or nervous symptoms. At the end of this time all survivors were killed.

From the same group of passively immune chicks, 20 chicks were vaccinated at weekly intervals with 1 drop

(0.05 ml) of Bl vaccine in each nostril and conjunctival sac. Hereafter these will be called the vaccinated passively immune chicks. The control chicks of these will be called the nonvaccinated controls.

Four weeks after vaccination each group of birds, for the different intervals, along with 10 nonvaccinated controls were challenged intranasally with the same amount of GB virus as for the passively immune chicks. Ninety six hours after challenge each of the vaccinated chicks was bled by heart puncture, the clotting prevented with heparin, and 5 ml of this blood delivered into separate tubes. These tubes were centrifuged in chilled cups at 1800 RPM for 5 minutes, the plasma decanted and replaced by tryptose broth. This procedure was done in an attempt to avoid the neutralizing effect of immune serum in the blood. Then 0.2 ml of blood from each chicken was inoculated into the allantoic sac of four 9 - 11 day old embryonating eggs, to detect Newcastle disease virus in the blood of the challenged birds and thus to determine the degree of immunity induced by the vaccine in the chickens. Daily observations were also made for deaths and for the presence of nervous symptoms up to 12 days after challenge. At this time the surviving chickens were killed and post morten examinations were done to record the degree of airsacculitis.

Results

The results of the intranasal challenge for the passively immune and control chicks are presented in Table 6. When each of 20 passively immune chicks was challenged intranasally at 2, 7, 14, 21, and 28 days of age, 19, 18, 19, 19, and 20 of them, respectively died or exhibited nervous symptoms up to 12 days after challenge. All corresponding controls died or showed nervous symptoms.

The results of intranasal challenge of the vaccinated passively immune chickens are presented in Table 7. None of the chickens vaccinated with the Bl vaccine died or exhibited nervous symptoms as a result of challenge. Two of them in the group challenged at 4 weeks of age died during the bleeding operation. In contrast; all nonvaccinated controls died or exhibited nervous symptoms.

No virus could be isolated, by the assay method used, in embryonated eggs from any of the blood samples collected 96 hours following challenge from the vaccinated passively immune chicks. The blood of the nonvaccinated control chicks was not inoculated into embryonated eggs since by the fourth day after challenge most of the chicks were dead or exhibiting nervous symptoms. This was regarded as proof enough that infection had become established in these control birds.

Table 6. Results of intranasal challenge[a] of passively immune[b] chicks

Chicks	Age in days	Dead or exhibiting nervous symptoms
Passively immune	2	19/20[c]
Controls	2	20/20
Passively immune	7	18/20
Controls	7	20/20
Passively immune	14	19/20
Controls	14	20/20
Passively immune	21	19/20
Controls	21	18/18
Passively immune	28	20/20
Controls	28	20/20

[a]0.2 ml per bird with a 1:1000 dilution of the GB strain having 10^7c.i.d.$_{50}$/0.1 ml.

[b]Hatched from Ames-in-cross dams previously vaccinated against Newcastle disease.

[c]Number affected/number challenged.

The results of examination for airsacculitis 12 days following challenge are presented in Table 7. Airsacculitis was found in only 3 of 20 chicks in the group vaccinated at 2 days and challenged 4 weeks later. The same results were found in the groups vaccinated at 1 and 2 weeks, when challenged 4 weeks later. In the group vaccinated at 3 weeks, and challenged 4 weeks later, only 1 of 20 chicks had air-

Table 7. Results of intranasal challenge[a] of vaccinated[b] passively immune[c] chicks

Chicks	Age in weeks	Dead or exhibiting nervous symptoms	Air-sacculitis
Vaccinated	4	2/20[d]	3/20
Controls	4	10/10	N.D.[e]
Vaccinated	5	0/20	3/20
Controls	5	10/10	N.D.
Vaccinated	6	0/20	3/20
Controls	6	10/10	N.D.
Vaccinated	7	0/20	1/20
Controls	7	10/10	N.D.
Vaccinated	8	0/20	0/20
Controls	8	10/10	N.D.

[a]0.2 ml per bird of a 1:1000 dilution of the GB strain having 10^7c.i.d.$_{50}$/0.1 ml.

[b]0.2 ml per bird of B1 vaccine, intranasally, 4 weeks previous to challenge.

[c]Hatched from Ames-in-cross dams previously vaccinated against Newcastle disease.

[d]Number affected/ number challenged.

[e]Not done since birds died or were killed as soon as nervous symptoms were detected.

sacculitis and for the group vaccinated at 4 weeks none had airsacculitis.

When this experiment was started it was understood that the eggs from which the chicks were hatched were laid

by hens vaccinated with live virus intramuscularly. In previous experiences this type of vaccination had endowed the dams with an appropriate level of antibodies which in turn was transmitted to their progeny. Since no protection was observed in the supposedly immune chicks exposed to a challenge virus an inquiry to the owner of the breeding flock revealed that he had furnished eggs from a flock vaccinated instead with the Bl vaccine, by way of the drinking water. This type of vaccine does not furnish as good an immunity as the vaccine previously mentioned. Thus, 8 of the breeding dams were secured to find out about their immune status. At this time the hens were approximately 13 weeks older than when the eggs were gathered for Experiment 3.

On the day of arrival to the laboratory the hens were bled from the wing vein, the blood allowed to clot, and the serum used to perform individual hemagglutination inhibition and serum neutralization tests. After bleeding, each of the 8 hens was challenged intranasally with 0.2 ml of a 1:100 dilution of infected allantoic fluid of the GB strain having 10^7 $c.i.d._{50}/0.1$ ml. Then at 96 hours after challenge the hens were bled by heart puncture, the blood processed as previously described, and for each hen 0.2 ml of blood inoculated into the allantoic sac of four, 10 day old embryonated eggs. Daily observations were made for the occurrence of deaths and nervous symptoms up to 12 days after

challenge. At the end of this period the hens were killed, and the degree of airsacculitis recorded.

The challenge virus was isolated from the blood of 2 of the hens 96 hours after challenge (Table 8), however no deaths or nervous symptoms were observed in the birds up to the twelfth day after challenge. This protection was apparently induced by the 2 previous doses of vaccine given to the breeder hens.

With such good results obtained in the challenge test it was hoped that the hens would exhibit fairly high serum neutralizing titers against Newcastle disease virus. However, this was not the case, as can be seen in Table 8. None of the 8 hens tested exhibited serum neutralization titers of 10^2 or greater. Also, the hemagglutination inhibition titers were practically negative. This is in agreement with the work of Bankowski and Corstvet (6) who stated that "immunity to Newcastle disease is composed of many measurable as well as immeasurable factors." Furthermore, they demonstrated that infection of the respiratory epithelium, immunity to systemic infection as denoted by clinical signs, and resistance to a decrease in egg production varied independently.

Table 8. Immune status of hens previously vaccinated[a] with live Newcastle disease virus in the drinking water

Hens	HI[b] titer	NI[c]	Virus isolation[d]
1	10	0.0	+
2	10	0.3	-
3	10	0.5	-
4	20	1.6	-
5	10	0.6	+
6	5	0.2	-
7	10	0.0	-
8	10	0.6	-

[a]For the first time 40 weeks previously; for the second time 22 weeks previously.

[b]Hemagglutination inhibition titer, expressed as the reciprocal of the serum dilution.

[c]Neutralization index.

[d]Ninety six hours following intranasal challenge with the GB strain.

Discussion

The results of challenge of the passively immune chicks revealed very little protection compared with the controls, although there was a tendency for the passively immune chicks to become affected a little later than the controls. All controls were dead by the fifth day after

challenge, however some of the passively immune chicks did not die until the tenth day after challenge, and few survived to the twelfth day exhibiting only slight nervous symptoms. An explanation for these results is that the chicks did not have enough antibodies at the time of challenge. Possibly if a lesser challenge dose would have been used, for example, one that killed only 80 or 90 per cent of the control chickens, the protective effect of the passive antibodies would have been detected more readily. The evidence presented seems to indicate that the dams did not have high antibody levels.

The excellent immunity to challenge possessed by the Bl vaccinated passively immune chicks supports the results reported in the literature that parentally immune chicks can be immunized by intranasal inoculation, however the chicks with low levels of passive antibodies are more effectively immunized than those with high levels of passive antibodies. The virulence of the vaccine strain is also a factor in the degree of immunity produced. The results of the present experiment were somewhat surprising since it was anticipated that the chicks would possess a substantial titer of passive antibodies thus interfering with the Bl vaccine in establishing a sound immunity.

When the information on the transfer of Newcastle disease protective antibodies from dams to chicks is analyzed as a whole it can be stated that when enough of these anti-

bodies are found in the blood serum of the dams they are transferred to the progeny through the egg yolk. In a flock of layers under field conditions, variations in the level of protective antibodies are found. If sufficient amount is transferred the chicks are protected for the first days of life. These protective antibodies decline inversely with the age of dams and progeny. In the chick antibodies can not be demonstrated after 4 to 5 weeks of age.

GENERAL SUMMARY

A study was made to compare the rapidity and degree of protection induced in groups of chickens vaccinated with live and with inactivated Newcastle disease virus. Six chickens from each group were challenged at 24 hour intervals thereafter, through the seventh day. Evaluation of the protection was based on the presence or absence of virus in the blood at 96 hours following challenge, deaths or nervous symptoms, and air sac lesions observed at necropsy 12 days after challenge. Chickens vaccinated with B1 live virus vaccine were resistant to intranasal GB challenge as early as 48 hours after vaccination. No virus was isolated from blood and only 1 of 6 birds challenged at 48 hours developed nervous symptoms. At necropsy 10 per cent had air sac lesions. Chickens vaccinated with beta-propiolactone (BPL) inactivated vaccine were susceptible to challenge 48 hours after vaccination; however, beginning at 72 hours through the 168 hour challenge, 43 per cent resisted challenge based on virus isolation from the blood, and 80 per cent resisted development of nervous symptoms compared to 100 per cent susceptibility in the controls. Air sac lesions were observed in 70 per cent of the birds vaccinated with inactivated vaccine.

The fate of the challenge virus in tissues of

chickens vaccinated 8 weeks previously with BPL inactivated vaccine was studied. Paired birds were bled and tissues collected at 12 hours after challenge; thereafter, collections were made at 24 hour intervals starting 24 hours after challenge through 216 hours. The challenge virus was not isolated from any tissues or blood from the vaccinated birds; however, virus was isolated from tissues of the control chickens beginning at 24 hours and continuing through 192 hours. The highest concentrations of virus were found in the lung and trachea at 96 and 120 hours after challenge. Approximately the same results were obtained following challenge of twice vaccinated chickens. Hemagglutination inhibition and neutralization tests conducted with the serum of the once vaccinated chickens revealed negligible antibody titers; however, in the twice vaccinated birds hemagglutination inhibition titers ranged from 1:80 to 1:2560. The neutralization indexes ranged from 1.3 to 5.0.

The influence of passive antibodies in resistance to challenge and development of immunity following vaccination in chicks was the subject of another study. Chicks were obtained from hens which had been vaccinated twice with B1 Newcastle disease vaccine. Another group of chicks from susceptible dams were used as controls. Challenge of the passively immune and non-passively immune chicks at 2 days and at weekly intervals through 4 weeks revealed only slight

protection afforded by the passive antibodies. The slight protection detected was in the delay of deaths and nervous symptoms compared to the controls which uniformly were dead by the fifth day.

When groups of the above passively immune chicks were vaccinated intranasally with the B1 vaccine at 2 days of age and at weekly intervals through the fourth week, no challenge virus could be isolated from the blood of any of them at 96 hours following challenge, 4 weeks after vaccination. The challenge virus did not kill or induce nervous symptoms in any of the vaccinated chickens, whereas all control birds died or exhibited nervous symptoms. Only a few of the vaccinated chickens revealed air sac lesions 12 days after challenge. Eight of the breeder hens, which provided the passively immune chicks, were challenged to ascertain their immune status. The challenge virus was isolated from the blood of 2 of the hens at 96 hours following challenge, however no deaths or nervous symptoms were observed. Hemagglutination inhibition and serum neutralization titers were essentially negative.

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