

THE PATHOGENESIS OF INFECTION WITH A VIRULENT (CG 179)
AND AN AVIRULENT (B) STRAIN OF NEWCASTLE
DISEASE VIRUS IN THE CHICKEN

I. COMPARATIVE RATES OF VIRAL MULTIPLICATION*

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PLATES 13 AND 14

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Interest in the Newcastle disease virus (NDV) stems from diverse sources. Apart from its importance as the agent of a widespread disease of domestic gallinaceous birds (1), it has recently been proved to cause a conjunctivitis (2-6) and possibly a generalized systemic infection in man (7). Moreover, since Burnet demonstrated the affinities of NDV to the influenza-mumps group of viruses, it has been the subject of numerous basic virus studies. Despite the volume of work on this commonly employed laboratory model, there has been no extensive study of the pathogenesis of the NDV infection in its natural avian host.

Previous studies have reported the successful isolation of NDV from the blood, visceral organs, central nervous system, saliva, and feces after natural or experimental infection of the chicken (1, 9, 10). Wide variations were noted among different virus strains. No attempt was made to follow quantitatively the temporal relations of virus distribution or of antibody production.

In the present paper, the comparative growth rates of a virulent (CG 179) and an avirulent (B) strain of NDV in the chicken were analyzed. The strains were shown to be different in their rates of multiplication in the central nervous system and this was correlated with the manifest course of the acute infection. In their extraneural growth, however, the multiplication rates of the two strains were found to be essentially alike. Certain neurohistological observations were found to be correlated with the virus growth patterns. The immune response of the host will be discussed in an accompanying paper (11).

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Materials and Methods

Virus Strains.—NDV was used for the entire study. The CG 179 strain was isolated from a natural outbreak in California by Dr. J. R. Beach. After 179 serial passages of this strain on the chorio-allantoic membrane of chick embryos, a highly virulent agent for adult chickens was obtained (9). This virulent strain is unusual in that it does not regularly agglutinate chicken RBC. The B strain (avirulent for adult chickens), recovered from an epidemic in New Jersey, was obtained from Dr. F. R. Beaudette as second egg passage material. Both strains are lethal for chick embryos (12).

Stock Virus.—In our laboratory both strains have been maintained by approximately 15 allantoic passages. After inoculation, embryos were incubated 40 to 48 hours at 35°C., chilled, and harvested. Stock virus was prepared by pooling allantoic fluid and storing in Lusteroid tubes in a CO₂ cabinet at -70° C. Virus was found to maintain its titer under these conditions for 5 months. All virus dilutions were made in 0.85 per cent NaCl buffered at pH 7.2 with 0.01 M phosphate.

Virus Titrations.—Virus was titrated on the chorio-allantoic membrane of 10 to 11 day chick embryos prepared by the "dropped membrane" technique. Four or five embryos were used per serial tenfold dilution. Deaths due to NDV occurred between the 2nd and 6th day. The cause of death was checked in a routine manner by bacteriological cultures, by hemagglutination when possible (B strain only), and by noting the presence of discrete tiny membranous foci characteristic of NDV (13). The LD₅₀ was calculated according to the method of Reed and Muench (14).

Virus Multiplication in Tissue.—Chickens inoculated intracerebrally or intramuscularly with varying quantities of virus were sacrificed at selected intervals. Under sodium pentobarbital anesthesia the animals were perfused with 300 ml. of normal buffered saline into the aorta and then directly into both carotid arteries. Individual tissues were removed aseptically with precautions to prevent cross-contamination and were washed with at least 10 volumes of saline from a syringe. Each tissue was ground in a chilled grinder in a final dilution of 1/3.2 (considered as 10^{-0.5} dilution). Muscle was ground in a mortar using sterile alundum.

In one experiment 0.5 ml. of virus was inoculated into the right gastrocnemius muscle and at intervals, thereafter, a segment of muscle weighing 5 gm. was excised at the site of inoculation. The contralateral muscle served as a control.

In early experiments three portions of the central nervous system (whole brain, cervical enlargement, and lumbosacral enlargement) were titrated. Eight such preparations disclosed no significant constant difference in rate of virus growth or final titers attained in brain and cord. Therefore, in all subsequent work, the brain titer alone was analyzed. Preliminary studies of washed rectal wall as compared with rectal contents indicated the same virus titer in each. Therefore, in all data shown the term, rectum, represents a ground mixture of wall and contents removed *in toto*.

Five hundred units of penicillin and 500 µg. of streptomycin were incorporated per ml. of final suspension of lung and rectal tissue. Gentian violet, in a final dilution of 1:50,000, was also added to the diluent in titrating rectal tissue. These concentrations of antibiotics, either singly or in combination, had no effect upon NDV infectivity, as determined in duplicate tests. Tissues stored at -20° C. in an electric freezing unit, maintained constant infectivity titers for at least 3 months.

The uncentrifuged unpooled suspension was titrated by inoculation of 0.1 ml. of tenfold serial dilutions on the chick chorio-allantoic membrane as described for virus titrations.

Reproducibility of End-Points in Tissue Titrations.—Duplicate and replicate titrations were performed on different days with 14 individual tissues (2 lung and 12 central nervous system specimens). Table I summarizes the results of 32 separate experiments. The standard deviation is calculated to be 0.434 log (15-17). The figures reflect error in mensuration of a

TABLE I
NDV Tissue Titrations. Summary of 32 Duplicate and Replicate Titrations and Calculation of Standard Deviation

Tissue No.	Titer* (X)	Mean titer (\bar{X})	(X - \bar{X})	(X - \bar{X}) ²
1	3.5	3.15	0.35	0.12
	2.8		0.35	0.12
2	2.8	3.15	0.35	0.12
	3.5		0.35	0.12
3	4.2	3.85	0.35	0.12
	3.5		0.35	0.12
4	0.6	0.30	0.30	0.09
	0.0		0.30	0.09
5	2.7	2.25	0.45	0.20
	1.8		0.45	0.20
6	2.7	2.45	0.25	0.06
	2.2		0.25	0.06
7	2.5	2.15	0.35	0.12
	1.8		0.35	0.12
8	1.5	1.35	0.15	0.02
	1.2		0.15	0.02
9	2.0	2.10	0.10	0.01
	2.2		0.10	0.01
10	3.7	3.35	0.35	0.12
	3.0		0.35	0.12
11	3.0	3.00	0.00	0.00
	3.0		0.00	0.00
12	3.8	4.10	0.30	0.09
	4.3		0.20	0.04
	4.2		0.10	0.01
13	5.2	5.57	0.37	0.14
	6.2		0.63	0.40
	5.3		0.27	0.07
14	3.8	3.10	0.70	0.49
	3.0		0.10	0.01
	2.8		0.30	0.09
	2.8		0.30	0.09
$\Sigma (X - \bar{X})^2$				3.39
S. D. (σ) = $\sqrt{\frac{\Sigma (X - \bar{X})^2}{N \ddagger}}$				0.434

* All titers are expressed as log LD₅₀/0.1 gm. of tissue.

‡ N = number of "degrees of freedom" or 18 in this instance.

given tissue sample but not the biological and sampling variation among the experimentally infected birds.

Experimental Animals.—Stock chick embryos were obtained from two local hatcheries. Virus titrations were identical in the two groups. Variable amounts of NDV neutralizing antibody were found in the yolk sac of stock eggs during the course of this study. Virus titrations on stock embryos were compared with titrations on embryos derived from two flocks with different immunological backgrounds: one group, known to be free of Newcastle disease (Rockefeller Institute, Princeton, New Jersey); the other, recently convalescent from an

TABLE II
Effect of Congenital Passive Immunity upon NDV Titrations in the Embryo

Experiment No.	Route of inoculation	Stock embryos*	Embryos from known NDV-free flock	Embryos from known immune flock
1	Allantoic sac	8.5	8.5+	
	Membrane	7.7		
2	Allantoic sac	8.3	9.4	
	Membrane	8.6		
3	Yolk sac	8.7	9.2	
	Membrane	7.6		
4	Membrane	9.4	9.7	
5	Membrane	7.8		7.2
6	Allantoic sac	6.8		7.0
7	Yolk sac	8.3		5.0
	Membrane			8.2
8	Yolk sac	8.7		5.8

* All figures given as log LD₅₀.

NDV epidemic in Maryland (Table II). The titrations on the chorio-allantoic membrane or *via* the allantoic sac were found to be unaffected by yolk antibody. However, when employing eggs from immune flocks the use of the yolk sac route of inoculation or of older embryos (15 days) did result in a lower virus titer (18).

Rhode Island Red chickens, 10 weeks old and weighing approximately 1,000 gm., were supplied by the Department of Poultry Husbandry, University of Maryland, and were isolated in a separate building. These stock chickens were free of NDV antibody and uniformly susceptible to infection. Strict isolation of experimental birds was maintained in three specially designed rooms.

Growth Rates of the Virulent and Avirulent Strains Inoculated by the Intramuscular Route

Intramuscular inoculation of the virulent strain (CG 179) in chickens produced a uniformly fatal disease. After an incubation period 3 to 4 days the birds

suddenly developed an acute illness, ushered in by ruffled feathers, anorexia, and weakness with occasional diarrhea and respiratory difficulty. Within a few hours a characteristic rhythmical gross involuntary jerking occurred in the majority of cases. The movements involved the head and neck, one or more limbs, or the entire musculature. Progressive weakness and paralysis ensued, resulting in death within 12 to 24 hours after the onset of symptoms. The following description of one bird was taken directly from laboratory notes:—

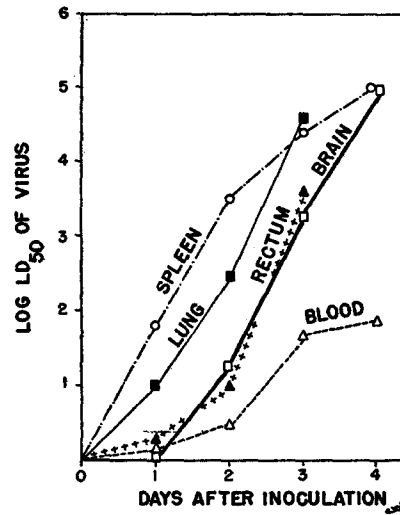
“Day 4, 4.30 p.m.: bird appears normal; 7.30 p.m.: was rather slow, jerking movements of body, can support itself on feet if provoked; 8.00 p.m.: brought to laboratory for study. Jerking is at rate of 30 to 36 per minute, and although irregular, count is repeatable. Jerking is largely on right side. This remains consistent. Jerking of the legs is caused by spasm of proximal leg muscles with resultant fast flexion and slow relaxation. During contraction muscle bundles fibrillate. Right proximal wing shows slight contraction, peripheral wing very slight, left wing none. Tail moves laterally to right on each contraction. 10.00 p.m.: the rate has gradually increased to 60 to 80 per minute. Contractions now involve neck, flexing neck to ‘S’ shape. Pupils are small and do not move. Trunk, abdominal, and pectoral muscles, especially latter, hardly contract at all. Movement is not related to respiration. Righting reflexes seem unimpaired. Bird can and does make organized movements with any and all parts of body in response to noxious stimuli and this, for 5 to 10 seconds, may stop jerking motions. After 10.00 p.m., however, jerking motions could not be stopped. Sacrificed at 10.30 p.m., almost moribund.”

The avirulent strain (B), after intramuscular inoculation, caused an asymptomatic infection in adult chickens (over 8 weeks old). Occasionally there was slight anorexia on the 3rd and 4th days after inoculation but all birds recovered uneventfully. Younger birds frequently developed a mild paralysis followed by recovery.

The rates of virus multiplication of the two contrasting strains were studied in the central nervous system, blood, spleen, lung, rectum, and muscle.

The data on individual titrations of the virulent (CG 179) strain are shown in Table III and the means are plotted in Text-fig. 1. This strain multiplied most rapidly in the spleen and lung, reaching titers of 1.0 to 2.0 logs within 24 hours. Titers in the rectum rose somewhat more slowly. The viremia was never marked. After a lag period of 24 hours, virus appeared in the brain on the 2nd day. The onset of paralysis occurred on day 3 when the brains contained a mean titer of 3.3 logs. The muscle titer at the site of inoculation remained constant after the 6th hour and did not rise above 4.7 logs.

The data on the avirulent (B) strain are shown in Table IV and the mean titers plotted in Text-fig. 2. Three general types of curves emerged: (a) the titers of virus in the viscera, represented by the spleen, lung, and rectum, rose most rapidly, attained the highest level of any of the tissues, and fell to 0 by the 8th day of infection; (b) the viremia rose fairly promptly but was truncated and disappeared by the 5th day; (c) the brain titer increased most slowly, reaching 3.0 logs on the 5th day. However, virus in the brain persisted through the 8th day.



TEXT-FIG. 1. Amounts of virus present in tissues of individual chickens sacrificed after intramuscular inoculation with virulent (CG 179) strain of NDV.

TABLE III

Chicken Tissue Titrations after Intramuscular Inoculation with 100,000 Embryo LD₅₀ of Virulent NDV, Strain CG 179

Experiment No.	Day of sacrifice	Condition*	Virus titers†						
			Brain	Spleen	Blood	Lung	Rectum	Muscle site inoculated	Muscle contralateral
1	1	N	0	1.3	0	1.0	0		
	2	N	0.7	4.0	1.0	2.7	1.0		
	3	P	3.5	3.5	2.5	5.0	4.0		
2	6 hr.	N	0	0	0	0	0	3.8	0
	1	N	0	2.3	0	0.9	0.6	4.7	0
	2	N	0.6	3.0	0	2.3	—	4.7	0.9
	3	P	2.7	5.0	1.7	4.1	3.2	4.3	2.8
3	1	N	0	1.7	0.2				
		N	0.2	3.0	0.7				
	2	N	1.8	—	—				
		N	3.2	4.1	0.2				
		N	3.2	3.9	1.1				
	3	N	3.7	4.8	1.0				
		P	4.2	4.5	2.0				
		D	2.6	—	—				
4	P	5.0	5.0	1.9					

* Condition of bird at time of sacrifice: N = normal; P = paralyzed; D = dead.

† Titers expressed as log LD₅₀/0.1 gm. of tissue.

A direct comparison between the CG 179 and B strains is given in Text-fig. 3 which shows the mean curves during the first 4 days of infection. It may be seen that the growth rates of both strains were not significantly different in any of the visceral organs or blood. However, in the brain the CG 179

TABLE IV
Chicken Tissue Titrations after Intramuscular Inoculation with 100,000 Embryo LD₅₀ of Avirulent NDV, Strain B

Experiment No.	Day of sacrifice	Virus titers*					Serum antibody titers‡		
		Brain	Spleen	Blood	Lung	Rectum	Sensitized cell agglutinin	Hemagglutination inhibition	Neutralization index
1	1	0	0.5	0	0	0	0	0	0
	2	1.0	4.5	2.0	2.0	2.3	0	0	
	3	1.3	4.5	2.2	4.7	3.5	0	0	
	4	3.0	4.8	1.8	5.0	4.0	0	0	
	5	3.1	4.3	0.2	4.0	3.7	10	0	0
	6	2.0	2.2	0	2.3	1.7	1280	160	1.8
	7	2.5	0	0	0	0	640	320	1.6
2	1	0	0	0				0	
		0	1.3	0			0	0	
	2	0	3.6	1.0			0	0	
		0.3	3.7	0.5					
		0.7	3.3	1.2					
	3	0	—	2.3					
		0	4.0	0					
		0.8	4.5	2.0			0	0	
	4	1.3	5.1	2.0					
		1.0	3.7	0.4			0	0	
		1.7	5.0	1.0					
	5	3.0	3.7	0				10	
	6	1.5	3.8	0			160	0	0.8
7	0.3	0.2	0			1280	80	2.4	
8	2.0	—	—			1280	320	3.1	
9	0	—	—			2560	320		

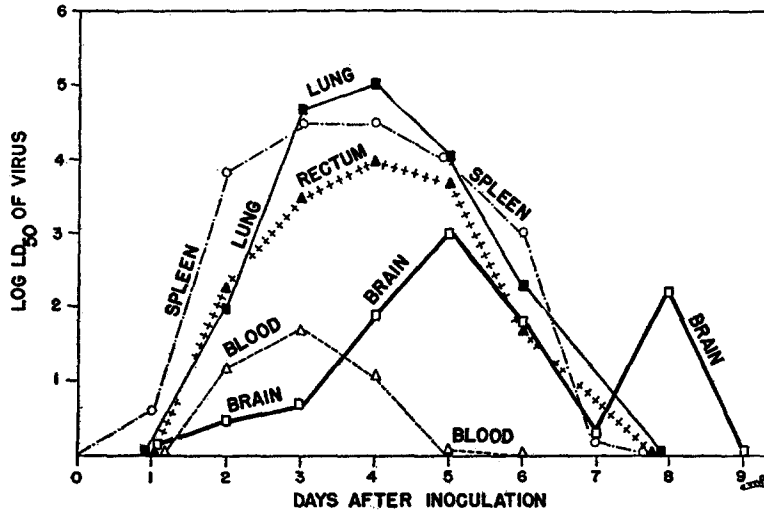
* Titers expressed as log LD₅₀/0.1 gm. tissue.

‡ Serum antibody data discussed in accompanying paper (11).

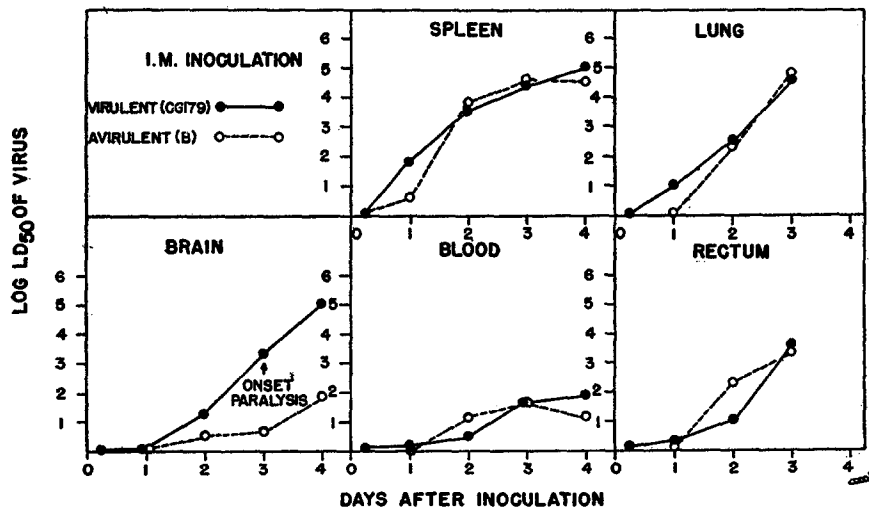
multiplied more rapidly than the B strain. Thus, the brain was the one site in which the virulent strain grew preferentially when compared to the avirulent strain.

Text-fig. 4 presents the actual titers of virus in individual brains contributing to the mean curves. By the 3rd day there was a significant difference between the CG 179 and B strain titers, 3.3 as compared to 0.7 log, with no overlapping of individual points. Brain titers continued to rise on days 4 and 5 in

the B strain. Although symptoms occurred when the mean titer of the CG 179 strain in the brain was 3.3 logs, there was no specific individual titer which



TEXT-FIG. 2. Amounts of virus present in tissues of individual chickens sacrificed after intramuscular inoculation with avirulent (B) strain of NDV.



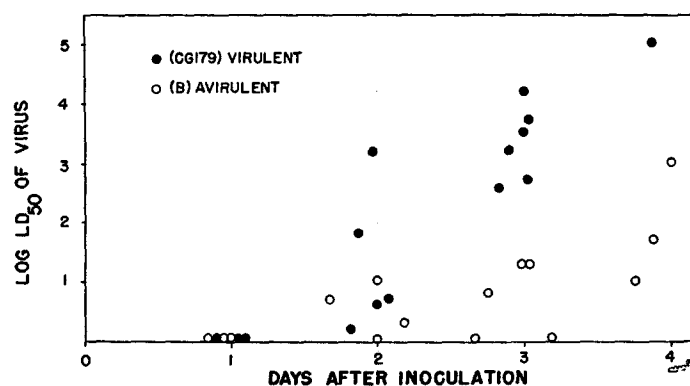
TEXT-FIG. 3. Comparison of the growth rates of the CG 179 and B strains of NDV after intramuscular inoculation.

determined the appearance of such symptoms. Rather, it was found that CG 179 caused death with brain titers as low as 2.6 logs on day 3 and that the B

strain, although reaching titers of 3.0 and 3.1 logs on days 4 and 5, did not even cause paralysis.

Growth Rates of the Virulent and Avirulent Strains Inoculated by the Intracerebral Route

In order to determine the growth propensities of both strains in the central nervous system, direct intracerebral inoculation was performed. With CG 179 this procedure shortened the incubation period to 48 to 72 hours. It was interesting to note that the gross jerking, which is so characteristic of NDV infection after intramuscular inoculation, was rare following an intra-



TEXT-FIG. 4. Titer of virus in brains of individual chickens after intramuscular inoculation with virulent (CG 179) and avirulent (B) strains of NDV.

cerebral inoculation. The birds developed generalized paresis and died within 12 hours after the onset of perceptible illness.

Intracerebral inoculation of the benign B strain resulted in a 50 per cent mortality. The incubation period was 4 to 5 days. Approximately one-half of the survivors had focal or generalized residual spastic paralysis. Here again the rhythmical jerking was not a prominent part of the manifestation.

Birds were inoculated into the right parietal region just anterior to the cerebellar prominence with 0.1 ml. of stock virus dilution and sacrificed at selected intervals. If it is assumed that the average chicken brain weighed 2.5 gm., the original inoculum was diluted 1/25 or 1.4 logs.

The data from four such experiments are shown in Tables V and VI. The patterns of growth in the brain after large inocula, $10^{3.9}LD_{50}$ of CG 179 and $10^{3.8}LD_{50}$ of B strain, are shown in Text-fig. 5. Allowing for an initial dilution of 1.4 logs, there was a further drop of 1.1 to 1.6 logs in the 1 hour samples. Both strains then rose rapidly, reaching concentrations of 4.0 to 5.0 logs within 24 hours. The CG 179 tended to have higher titers throughout the first 3 day period. This strain began to induce symptoms on the 2nd day and killed all

birds by the 3rd day. Neurological symptoms occurred at a mean titer of 5.0 logs.

Of the birds that received the B strain, those with brain titers above 5.0

TABLE V
Chicken Tissue Titrations after Intracerebral Inoculation with Virulent NDV, Strain CG 179

Experiment No.	Inoculum	Time of sacrifice	Condition*	Virus titer†			
				Brain	Spleen	Blood	
1	3.9	1 hr.	N	1.3	0	0	
			N	3.2	0	0	
			N	5.1	0.2	0	
		1 day	N	4.0	1.0	0	
			N	5.2	2.3	0	
			N	4.4	—	—	
		2 days	N	4.6	4.0	1.7	
			P	5.2	3.0	1.3	
			P	5.6	3.2	1.7	
			3 "	D	4.4	—	—
				P	4.7	2.2	0.9
				P	5.7	3.4	1.7
			4 "	D	5.8	—	—
				D	6.0	—	—
				D	6.0	—	—
2	1.8	1 day	N	4.7	0.9	0	
			N	5.0	0.0	—	
			N	5.5	0	0.3	
		2 days	N	4.0	2.4	0.5	
			N	5.0	2.9	0.9	
			N	—	3.1	2.3	
		3 "	N	3.9	3.5	0.7	
			P	5.0	4.3	2.8	
			D	5.0	—	—	
		4 "	D	5.3	—	—	
			D	6.2	—	—	
			D	3.5	4.2	2.2	
		5 "	D	5.4	—	—	
			D	5.4	—	—	
			P	4.0	5.2	1.7	

* Condition of bird at time of sacrifice.

† Titers expressed as log LD₅₀/0.1 gm. tissue.

logs by the 5th day became paralyzed or died, while those with brain titers of 1.3 and 1.6 logs on day 5 and day 6, respectively, were to outward appearances well.

In order to measure with greater accuracy the difference between the growth rates of the two strains, the above experiment was repeated with a minimal

TABLE VI
Chicken Tissue Titrations after Intracerebral Inoculation with Avirulent NDV, Strain B

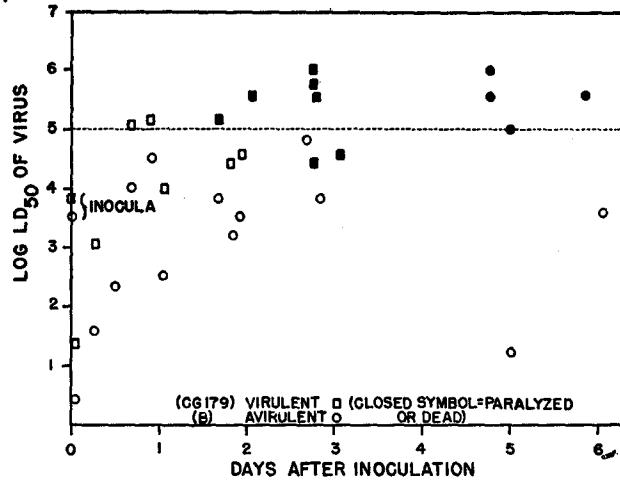
Experiment No.	Inoculum	Time of sacrifice	Condition*	Virus titers†			Serum antibody titers‡			
				Brain	Spleen	Blood	Sensitized cell agglutinin	Hemagglutination inhibition	Neutralization index	
1	3.5	1 hr.	N	0.4	—	—				
		6 hrs.	N	1.6	0	0				
		11 "	N	2.3	0	0				
		1 day	N	2.5	0	0				
			N	4.0	0	0				
			N	4.5	0	0				
			2 days	N	3.2	0	0			
				N	3.5	1.8	0			
				N	3.8	1.0	0			
		3 "	N	3.8	2.2	0				
			N	4.8	1.6	0	10	0		
		5 "	N	1.3	3.0	0				
			P	5.0	2.8	0.2	10	0		
			P	5.6	0	0				
			D	6.0	—	—				
		6 "	N	3.6	4.0	0	20	0		
			P	5.7	3.0	0	20	20	1.2	
			P	0	0.2	0	320	40	2.6	
		8 "	N	3.8	0	0	320	40	2.3	
		9 "	P	4.8	0	0	20	160	2.8	
		22 "	N	0	—	—	0	640	5.1	
		2	1.8	1 day	N	0.7	0	0		
N	1.2				—	—				
N	3.1				—	0				
2 days	N			3.7	—	—				
	N			3.8	0	0				
	N			5.7	—	—				
	N			5.7	—	—				
3 "	N			3.3	—	—				
	N			3.9	3.8	0				
	N			5.1	—	—				
4 "	N			2.4	—	—				
	N			5.0	3.2	0				
	P			5.9	—	—				
5 "	N			4.9	4.7	0				
	P			5.2	—	—				
	P			5.9	—	—				

* Condition of bird at time of sacrifice.

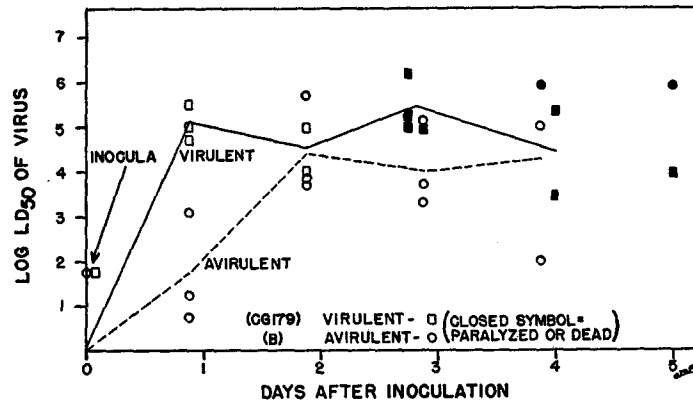
† Titers expressed as log LD₅₀/0.1 gm. tissue.

‡ Serum antibody data discussed in accompanying paper (11).

inoculum ($10^{1.8}LD_{50}$ of virus) intracerebrally. The resulting brain titers are shown in Text-fig. 6. Within 24 hours the CG 179 had a mean titer of 5.1 logs, the B strain a mean of only 1.7 logs or a mean difference of 3.4 logs. On the



TEXT-FIG. 5. NDV multiplication in brain tissue after intracerebral inoculation. Inocula: 9500 LD_{50} (CG 179 strain); and 7000 LD_{50} (B strain).

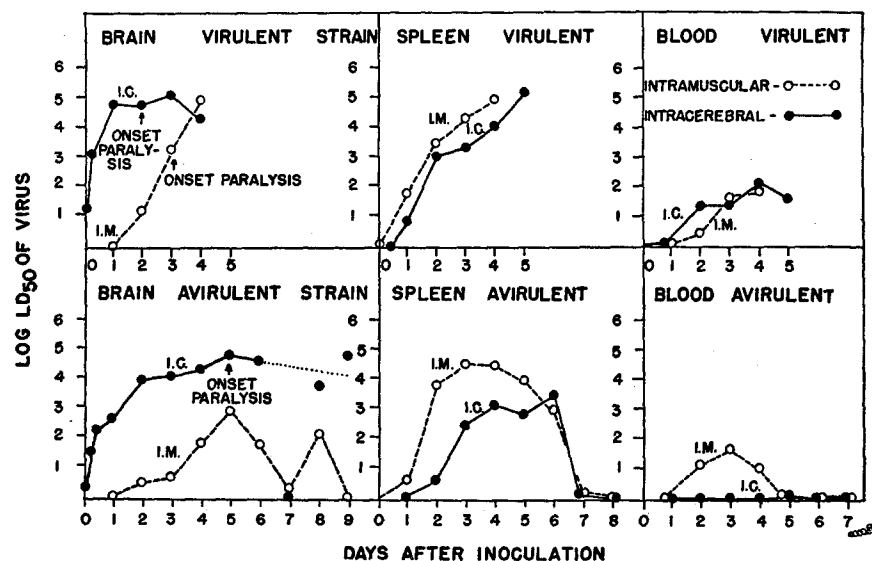


TEXT-FIG. 6. NDV multiplication in brain tissue after intracerebral inoculation of CG 179 and B strains. Inoculum: 90 LD_{50} .

2nd day the titer of the B strain approached that of the CG 179. Here again a titer of approximately 5.0 logs was the critical level in relation to symptoms although there was a tendency for symptoms to occur at lower titers of the virulent strain.

The Effect of the Route of Inoculation

The effect of the route of inoculation upon the distribution of virus is shown for both strains in Text-fig. 7. In the CG 179 infection, the route of inoculation did not materially affect the quantity of virus found in the blood or spleen. After intracerebral infection with the B strain, however, there was significantly less virus in the blood and spleen than after intramuscular inoculation. This was particularly evident in the blood titers in which there was detectable viremia in only one chicken.



TEXT-FIG. 7. The effect of the route of inoculation upon the growth of NDV in the chicken.

Thus, there were relatively lower extraneural titers after intracerebral inoculation of the avirulent strain than there were after the virulent strain. This raises the possibility that the avirulent strain is less capable than the virulent strain of penetrating from the central nervous system to non-nervous tissue.

Neuropathological Observations¹

The studies were based upon the serially sectioned brains of 14 chickens after intramuscular or intracerebral inoculation with the CG 179 or B strain and three normal control chickens. The birds were perfused *in vivo* under

¹The sections were obtained through the kindness of Dr. David Clark, Neuropathology Laboratory, The Johns Hopkins Hospital. The authors wish to express their appreciation to Dr. David Bodian, Poliomyelitis Research Center, Department of Epidemiology, The Johns Hopkins University, for his advice in the interpretation of the neuropathological data.

sodium pentobarbitol anesthesia with 10 per cent formol containing 1 per cent acetic acid. The brain and cervical and lumbosacral enlargements were removed, allowed to fix for an additional 48 hours and embedded in celloidin. The sections were cut 20 to 25 μ in thickness and stained with either aniline-thionine for nerve cells or the iron-hematoxylin myelin stain.

Certain of the observations that emerged from this small series could be correlated with the data presented above on viral growth. Following intramuscular inoculation there was a general dissemination of lesions throughout the brain. The lesions were of three types: first, a primary neuronal injury characterized by peripheral chromatolysis and clumping of nuclear material; second, an inflammatory infiltration which was either diffuse or clustered around a neurone; and third, perivascular "cuffing." The three types of lesions seemed to appear almost simultaneously. (Figs. 1 to 12.)

The lymphocyte was the cell type predominantly involved during the acute phase of the infection. An occasional macrophage was observed. A few of the older lesions (in birds sacrificed 2 weeks after inoculation) consisted of neuroglial foci. The endothelial proliferation reported as a prominent feature in this disease was not found in this material. Further, heavy perivascular lymphocytic infiltrations reported as infrequent (19) were observed to be numerous. No specific destruction of myelin was noted.

Birds which died on the 3rd or 4th day after a CG 179 infection with typical neurological symptoms and with a high virus titer in the brain, had very few lesions. In those birds which survived for a longer period, either spontaneously or due to partial protection from passive antibody administration, the lesions became more marked. After a typical B strain infection without paralysis, there was only an occasional "cuff" or inflammatory focus. However, in a young bird, in which the B strain caused a non-fatal paralysis, there were conspicuous lesions which were not distinguishable from a CG 179 infection.

The lesions were found in all major subdivisions of the central nervous system: forebrain; thalamus; hypothalamus; optic tectum; cerebellum; the nuclei and reticular formation of the midbrain and medulla; and spinal cord. There were, however, a few notable areas in which lesions were prominent and constant. The optic tectum was extensively involved, both in the superficial lamina and in the deeper inferior colliculus and isthmus nuclei. The cerebellar nuclei and subjacent vestibular nuclei were usually sites of severe chromatolysis and inflammatory infiltration. The cerebellar cortex showed a distinctive lesion consisting of an inflammatory accumulation of cells stemming radially outward from the Purkinje layer into the molecular layer. The Purkinje cells at the base of these lesions frequently showed various stages of peripheral chromatolysis, clumping of nuclear material or of neuronophagia.

The series was considered too small to establish definitely the nature of the neuropathological lesion related to the syndrome of gross tremors, although the above localization was suggestive.

After intracerebral inoculation the pattern of lesions was distinctive. The inoculum was found to be deposited in the hyperstriatum and neostriatum

regions of the forebrain (homologous to the basal ganglia of mammals). By the 3rd day there was a heavy diffuse and perivascular inflammatory infiltration in the hemisphere inoculated. In contrast, there were very few lesions in the contralateral hemisphere. Moreover, the pathological changes became bilateral caudal to the region of the supraoptic area and hypothalamus. In one intracerebral preparation the inoculum penetrated into the lateral ventricle. In this bird, only, an intense meningitis was present.

DISCUSSION

The data presented indicate that the nervous system was the primary locus of damage after parenteral administration of our strains of NDV. The specific neuronal and inflammatory changes in the brain and cord, considered in conjunction with the syndrome of gross rhythmical tremors, paralysis, and death, established the fact that the central nervous system was the "critical" organ in deciding the course of an NDV infection.²

The present study has led us to consider whether the property of virulence or pathogenicity is the resultant of a complex of factors, rather than of one factor which determines the outcome of an acute infection after peripheral inoculation with a given NDV variant. In our NDV-chicken system the factors would fall into five continuous phases: (a) the rate of virus multiplication peripherally; (b) the capacity of the virus to penetrate into the central nervous system; (c) the rate of multiplication of the virus in the central nervous system; (d) the extent of the brain destruction per infectious unit of virus in the central nervous system; and (e) the relative antigenic properties of the virus strains and the immune response of the host. Each of the first four factors has been found to play a decisive role in the pathogenesis of at least one virus infection. They will be discussed below and the fifth factor will be considered in an accompanying paper (11).

First, Fenner, working with ectromelia in mice (20), demonstrated that the virulent strain excelled in its ability to multiply peripherally in the skin and lymph nodes, resulting in a greater viremia and a consequent fatal necrosis of the liver. In contrast with NDV, it is unlikely that a differential peripheral multiplication is the decisive factor, since it was shown that the extraneural growth of the CG 179 and B strains was identical and that the amount of circulating virus was similar (Text-fig. 3).

Second, data indicating the existence of a functional block to the passage of vesicular stomatitis virus into the central nervous system has been presented by Sabin (21). There is some evidence that a similar phenomenon

²Intratracheal administration of NDV produced a severe tracheitis and sacculitis, a high titer of virus in the lungs and symptoms of respiratory obstruction. This syndrome, which resembles more closely the disease as it is often seen in nature, may follow different lines than those suggested here.

occurs with NDV. It was found that, following intramuscular inoculation of either strain, brain titers lagged markedly behind visceral titers, even though intracerebral inoculation of a small amount of virus showed the brain to be the potential site of extremely rapid multiplication (Text-fig. 7). Further, there was an indication, after intracerebral inoculation, that the B strain could not spread from the brain to the blood and viscera as well as did the CG 179 strain, as evidenced by the relatively low extraneural titers of the B strain.

Third, Schlesinger, working with Western equine encephalomyelitis in mice, showed that the more rapidly growing variant was characterized by greater virulence and a shorter incubation period, whereas the slower growing strain was less virulent (17). A parallel situation was found to occur with NDV in which the rate of virus multiplication in the central nervous system was definitely faster in the CG 179 than in the B strain. The clearest demonstration of this was the experiment in which, after a small inoculum of virus intracerebrally (Text-fig. 6), the CG 179 rapidly attained much higher titers than the B strain. Similarly, after intramuscular inoculation, CG 179 reached definitely higher concentrations in the brain (Text-fig. 3).

Fourth, in studying the adaptation of influenza virus to mice, Hirst (22) demonstrated the potential dichotomy between the capacity of a virus to reproduce and its capacity to cause pathological damage. A similar dichotomy in NDV was suggested by the general trend for the birds infected with CG 179 to develop neurological symptoms at lower brain titers than birds infected with the B strain (Text-figs. 5 and 6). This would indicate that the CG 179 strain per infectious unit caused greater destruction of brain tissue.

Thus, it is possible that the last three factors discussed above may be involved in explaining the different properties of our two strains of NDV.

The route of inoculation introduced another variable and materially affected the level of virus in the brain at which symptoms occurred. After intramuscular inoculation, 3.3 or more logs of virus and, after intracerebral inoculation, titers of 5.0 or more logs, were related to obvious manifestations of damage. Pathological sections showed that after intracerebral inoculation there was an early massive local inflammation in the cerebrum. Presumably, the high intracerebral titers were attained in non-critical regions, while in contrast the lesions, although minimal, caused by blood-borne virus following peripheral inoculation, may have occurred at critical sites.

SUMMARY

The comparative growth rates of a virulent (CG 179) and an avirulent (B) strain of NDV in the chicken were analyzed. Following intramuscular inoculation, the CG 179 and B strains both increased at the same rate in the extra-

neural tissues, *i.e.* the blood, lung, rectum, and spleen, but the CG 179 strain showed an accelerated growth rate in the brain.

The CG 179 strain also multiplied more rapidly in the brain than the B strain following intracerebral administration of minimal inocula.

Recovery with the B strain was associated with a decline in virus titer, first in the circulating blood, then in the visceral organs, and lastly in the central nervous system.

Certain neuropathological observations were correlated with the pattern of virus growth.

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EXPLANATION OF PLATES

The photographs were made by Mr. Chester Reather. The tissues were fixed by *in vivo* perfusion with acetoformalin and the sections stained with aniline-thionine. All chickens were infected with Newcastle disease virus.

Figs. 4, 6, 8, and 12 are sections taken from birds infected intramuscularly with the virulent CG 179 strain. Few central nervous system lesions were found in birds dying shortly after the appearance of symptoms. The photographs selected here, showing extensive involvement of the central nervous system, were obtained from chickens surviving 2 or more days after the onset of paralysis.

Figs. 1, 3, 5, 7, and 9 are sections procured from birds inoculated intramuscularly with the relatively avirulent B strain. The 10 week old stock chickens, which typically did not develop paralysis, exhibited only minimal central nervous system lesions. The illustrated material is from a 6 week old bird which did become paralyzed. The lesions caused by the two NDV strains were qualitatively similar.

Figs. 2, 10, and 11 are sections taken from birds which became paralyzed after intracerebral inoculation with the B strain.

PLATE 13

FIG. 1. Primary degeneration of a nerve cell body without inflammatory reaction, in a section through the vestibular nucleus. The cell on the right has undergone a diffuse chromatolysis and disruption of nuclear contents. This may be compared with a normal cell on the left. $\times 375$.

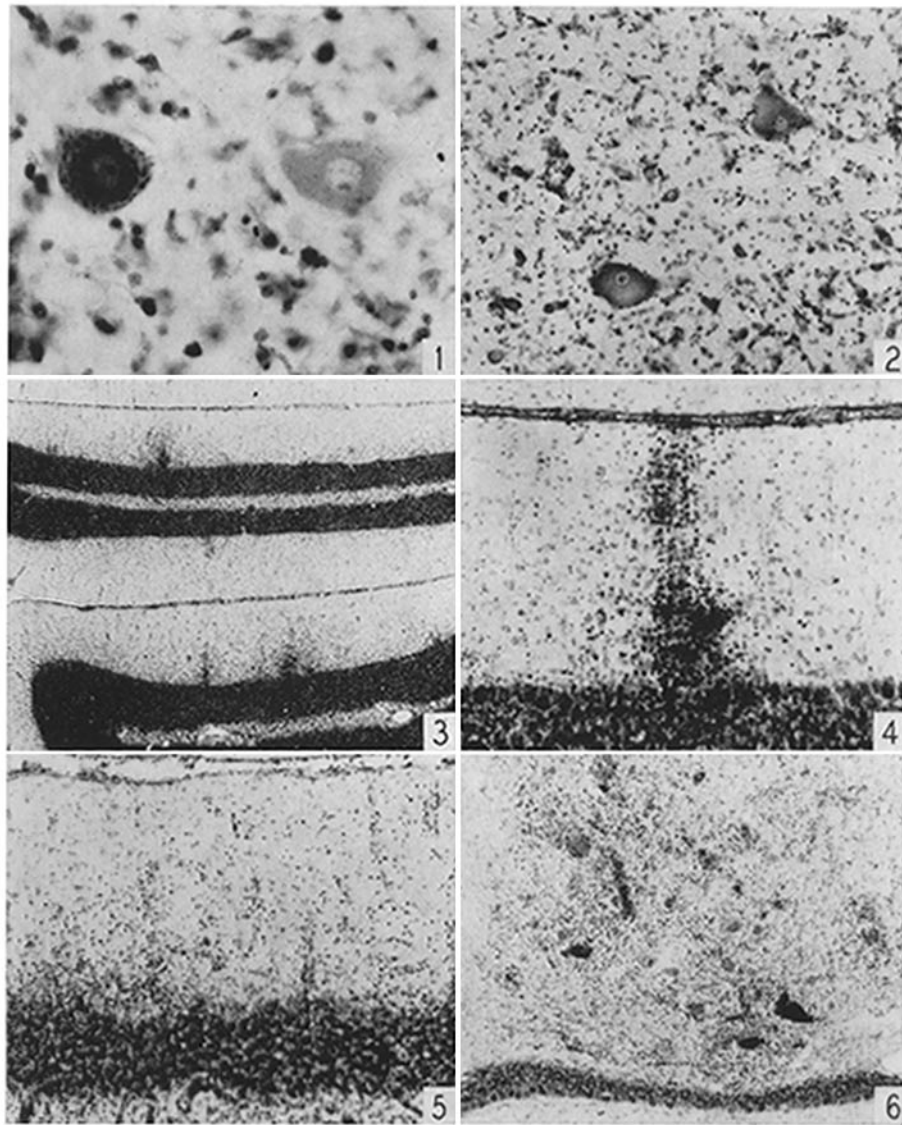
FIG. 2. Peripheral chromatolysis and clumping of nuclear chromatin in the region of the vestibular nucleus. $\times 125$.

FIG. 3. A section through the cerebellum illustrating typical lesions extending from the Purkinje layer radially into the molecular layer. $\times 20$.

FIG. 4. A single inflammatory lesion in the cerebellum extending linearly from the Purkinje layer to the meninges. In this preparation the Purkinje cells were largely preserved. $\times 100$.

FIG. 5. A cerebellar folium with complete destruction of the Purkinje cell layer accompanied by an inflammatory infiltration into the molecular layer. $\times 75$.

FIG. 6. Diffuse cellular infiltration in the cerebellar nuclei. $\times 50$.



(Karzon and Bang: Virulent and avirulent Newcastle disease virus. I)

PLATE 14

FIG. 7. The ventral horn region of the lumbosacral cord revealing widespread chromatolytic changes. $\times 75$.

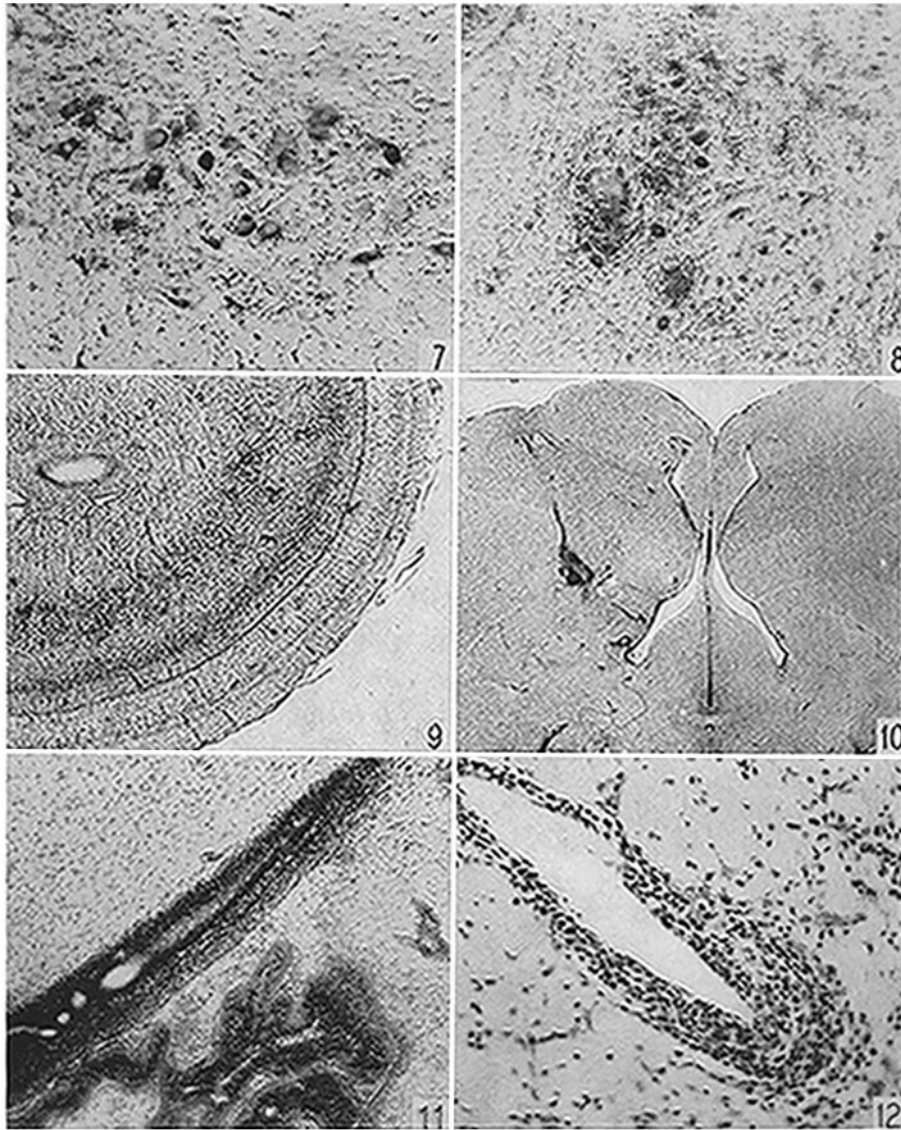
FIG. 8. Heavy inflammatory reaction and severe chromatolysis of several cells in the region of the vestibular nuclei. $\times 50$.

FIG. 9. A low-power view of the visual cortex demonstrating numerous inflammatory foci and perivascular infiltrations in the cortical and subcortical layers. $\times 20$.

FIG. 10. A localized inflammatory response in the right cerebral hemisphere at the site of the intracerebral inoculum. Note the paucity of lesions in the contralateral hemisphere. $\times 6$.

FIG. 11. Meningitis and choroiditis found only in the one bird in which the intracerebral inoculum had penetrated into the ventricular system. $\times 50$.

FIG. 12. A typical "cuff" in the forebrain showing heavy perivascular lymphocytic infiltration. There was no demonstrable endothelial proliferation. $\times 200$.



(Karzon and Bang: Virulent and avirulent Newcastle disease virus. I)