FSTC-HT-23-658-68

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COUNTRY: USSR

TECHNICAL TRANSLATION

17

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FSTC-HT-23-658-68

EXPERIMENTAL VARIATION OF NEWCASTLE DISEASE VIRUS

by

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SOURCE: VOPROSY VIRUSOLOGII
No. 4, pp. 443-451, 1964
USSR

Translated for FSTC by Techtran Corporation

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In the domestic and foreign literature are a series of reports on modification of viruses during their adaptation to the central nervous system of susceptible or unsusceptible animals.

In 1947 Newcastle disease virus was adapted to a sirenian hampster brain [17], and as a result of this, its virulence for birds decreased and the "brain" virus was recommended as a live vaccine. However, during culturing of this virus in chick embryos, it comparatively rapidly re-established its virulence for birds.

Attempts of several investigators to attenuate this virus by means of adaptation to the organism of white mice and guinea pigs [9, 12, 21], did not give the expected results. In 1949 to 1956, V. N. Fyurin studied modification of Newcastle disease virus (virulent strain T) in the process of its long adaptation to guinea pigs and other mammals. As a result, a neurotropic variant was obtained, which regularly causes the destruction of guinea pigs upon intra-cerebral infection. However, this strain in the form of a guinea pig brain suspension, was avirulent and completely non-immunogenic for birds. Thus, the "brain" virus proved to be unsuitable as immunizing material. During a series of passages in chick embryos, this virus showed marked immunogenic properties, and some virulence for chicks, which usually did not exceed the residual virulence of the widely known vaccine strain H. However, the genetic similarity of the adapted Newcastle disease virus (strain GNKI) until recent times remained unclear, which to a certain extent prevented its wide use as a live vaccine.

The aim of our search was to fill this gap in the study of the properties of the adapted GNKI strain, and, in particular, to study its genetic similarity by the method of experimental selection.

Materials and Methods

In the experiments, the GNKI strain of Newcastle's disease virus was used by way of guinea pig brains of the 302nd intra cerebral passage. After three subsequent passages in guinea pig brains, the virus was transferred to 10 to 11 day-old chick embryos, and serially passaged 20 times. The hemagglutination reaction was carried out by the generally accepted method in a volume of 1 ml with a 1% suspension of chick erythrocytes. The hemadsorbtion reaction was carried out by the method of Vogel and Shelokov [22] in infected tissue cultures of chick embryo fibroblasts. Negative colonies were obtained by the method of Hsiung and Melnick [11] in growing culture of chick embryo fibroblasts. Adsorbtion of the neurotropic variants was carried out by the method of Piraino and Hanson [15]. The method of threshold infecting doses of virus was used for separation of virus clones. The passaging of virus in embryos was carried out in two variations: embryos of serial passage were infected in the allantoic cavity by concentrated virus-containing allantoic culture ($10^{-1} \times 0.1$ ml)--variant A; in the other case, for successive infection of embryos, virus was used in dilutions above the LD_{50} (variant B). The enzymatic activity of the virus was established on the basis of its ability to be adsorbed and to elute from formalinized chick embryos, and also in the hemolysis reaction of the latter according to the method of Khou Yun'-de [5].

Results

The summarized data of the experiment in the study of the genetic homogeneity of the GNKI strain are presented in Table 1, from which it is clear that the "brain" virus in the first passage in chick embryos cause destruction of them after 144 hours, in further passages this period (for variant A) was shortened to 48 to 56 hours. The virulence of the virus increased from 2.75 to 8.26 and 8.74 log ELD_{50} (for variants A and B). Variant B, which was obtained using the method of maximum dilution, possessed the same infectious titre for the embryos as variant A; however, in the progress of the serial passages, the period of destruction of the latter was not shortened, but stabilized in the range 96 to 144 hours. In comparative experiments in chicks, a clear difference in pathogenicity of the indicated variants was observed (Table 2).

Table 1

Biological Properties of 2 Variants of Newcastle Disease Virus, Isolated from GNKI Strain

Passage	Variant A					Virus after Treatment by the Piraino and Hanson Method		Variant B				
	Period of Destruction of Embryos (in hrs)	Log ELD_{50}	Hemagglutination Titre	Cytopathic Effect	Hemadsorption	Log ELD_{50}	Period of Destruction of the Embryos (in hrs)	Period of Destruction of Embryos (in hrs)	Log ELD_{50}	Hemagglutination Titre	Cytopathic Effect	Hemadsorption
1st	144	2,75	0	0	+	—	—	144	2,75	0	0	+
2nd	96	—	0	0	+	—	—	120	2,6	0	0	+
3rd	72	—	0	0	+	—	—	120	4,5	0	0	+
4th	56	—	0	+	+	—	—	144	6,48	0	+	+
5th	72	6,0	128	+	+	—	—	120	6,5	0	+	+
10th	60	7,5	1 024	+	+	5,23	72	120	7,28	0	+	+
11th	48	—	512	+	+	6,50	60	96	7,00	128	+	+
12th	48	—	1 024	+	+	6,23	56	120	7,5	256	+	+
13th	56	—	1 024	+	+	6,66	56	120	7,76	128	+	+
14th	48	—	512	+	+	6,00	56	144	7,76	256	+	+
15th	56	8,23	512	+	+	—	—	120	7,60	256	+	+
20th	56	8,26	512	+	+	—	—	120	8,74	512	+	+

Note. Hemagglutination titres are presented in the form of inverted fractions.

As a result of the research carried out, it was established that the "brain" virus did not possess cytopathogenicity and did not form negative colonies in the tissue culture. During serial passage in chick embryos, the virus increased its pathogenicity for the embryos, tissue culture, and also acquired a hemagglutinating property. Virus of the fourth passage in chick embryos caused cytopathic changes and the formation of patches. Negative colonies were not distinguished by form and quantity. Virus isolated from certain colonies was analogous to the control. By applying the method of Piraino and Hanson, we succeeded in experimentally obtaining the aneurotropic variant.

Using the method of serial dilutions during passage of the "brain" virus in chick embryos, we selected the avirulent variant B of the GNKI vaccine strain. Probably, the long adaptation of the virulent, epizootic T strain to a guinea pig brain is accompanied by the appearance of heterogeneous elements (virions) in the virus population, which can be revealed by two methods of culturing of the prototypical virus in the developing chick embryos.

Table 2

Comparative Virulence for Chicks of Two Variants (A and B) of the GNKI Vaccine Strain of Newcastle Disease Virus

Variant	Passage	Titre (-log LD_{50})	Method of Infection	Dose (in $El.D_{50}$)	Growth of Chicks (in Days)		
					2	15	22
A	20th	8,26	Intramuscularly	$10^5 \times 02$	4/4	14/17	3/4
B	20th	8,74	»	$10^5 \times 02$	0/25	0/35	0/23

Note. Denominator--number of chicks taken in experiment; Numerator--number of stricken chicks.

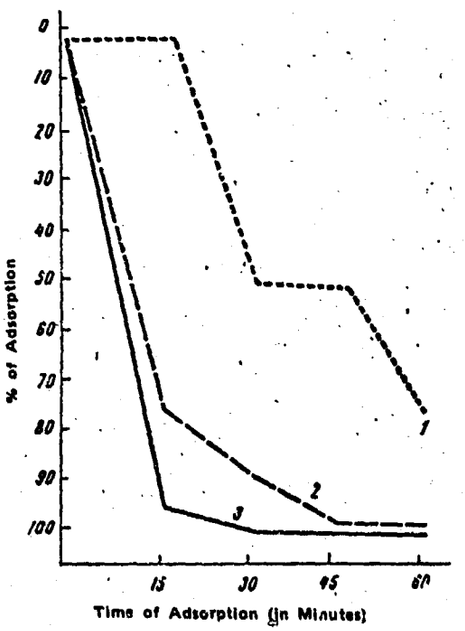

Figure 1. Adsorption of Variants of Newcastle Disease Virus by Formalinized Chick Erythrocytes. 1--Variant B; 2--Variant A; 3--T Strain.

Enzymatic Activity of the Virus as a Test of the Selection of Avirulent Clones

Clear distinctions in ability to be adsorbed by formalinized chick erythrocytes (Figure 1) were detected in three strains of Newcastle disease virus (A, B, and T strain), which differed in virulence. A difference was also detected in the eluting ability of the indicated variance of the virus. Elution occurs more rapidly and more completely in the virulent T strain. With a reduction in virulence, there is also a decrease in the speed of elution of the virus (Table 3).

Evidently, both adsorption and elution of virus from erythrocytes is in some measure connected with the enzymatic activity of the virus, since loss by the latter of virulent properties is accompanied by diminished enzymatic activity. Having observed this, we use the adsorption method for the experimental selection of variants differing in virulence from the original pathogenic T strain.

Table 3
Dynamics of Elution of Variants of Newcastle Disease Virus

Virus	Time of Elution (in Minutes)							
	30		60		120		180	
	GAU	%	GAU	%	GAU	%	GAU	%
GNKI-B	0	0	16	12	32	26	64	50
GNKI-A	64	25	256	100	256	100	—	—
T Strain	128	50	256	100	256	100	—	—

Note: GAU = the number of hemagglutinating units in 1 ml.

With this aim, the virus was precipitated five times by formalinized erythrocytes in order to fully deplete possible virulent virions in the population. After the fifth successive adsorption, the virus found in the supernatant liquid, was titrated and selection of avirulent clones was carried out by the method of serial dilutions in chick embryos. During titration of the non-adsorbed virus particles in the chick embryos, a significant difference was established in the period of their death (Table 4). Embryos infected by the original virus, were destroyed after 48 hours, whereas the virus after adsorption caused their destruction in 96 to 120 hours. After 120 hours, all the

living embryos were killed by cold, and each embryo was separately studied for the presence of virus with the help of a drop of RGA on the glass. As a result, two surviving embryos (No. 4 and 10) were detected, which were infected by a virus at dilution 10^{-4}, the allantoic fluid of which contained hemagglutinin. The organs of these embryos did not have visible pathological changes. The allantoic virus culture (TA_1) of embryo No. 10 was passaged five times in chick embryos, and towards the fifth passage, the variant reestablished virulence (up to the original extent) and enzymatic activity (Table 5 and 6).

Table 4
Titration of Non-adsorbed Virus in 10-Day Old Chick Embryos

Dilution of Virus	Number of Embryos										Original Virus	
	1	2	3	4	5	6	7	8	9	10	1	2
	Period of Destruction of Embryos (in hrs)											
10^{-2}	96	96	96	96	108	108	108	120	120	+	48	48
10^{-3}	96	108	120	120	120	—	—	—	—	—	48	48
10^{-4}	—	—	—	+	—	—	—	—	—	+	48	48
10^{-4}	—	—	—	—	—	—	—	—	—	—	48	48

Note: - equals negative reaction of hemagglutination;
+ equals positive.

Table 5
Passage of TA_1 Virus in Chick Embryos

Passage	RGA Titre	Log ELD 50	Period of Destruction of Embryos (in Hours)	Pathomorphological Changes	Log LD_{50}	Ratio of ELD_{50} to LD_{50}
1st	256	7,5	72—120	0	—	—
2nd	128	8,0	96	+	5,4	2,6
3rd	512	—	48—72	+++	—	—
4th	256	—	48—72	+++	—	—
5th	256	8,5	48—72	++++	—	—

Note: +, +++, ++++ are different degrees of pathomorphological change.

Table 6
Changes in the Enzymatic Activity of the Virus (Variant TA_1) During Passage in Chick Embryos

Passage	Adsorption				Elution			
	Reaction Time (in Minutes)							
	15	30	45	60	30	60	120	150
1st	128	128	64	16	4	32	128	128
2nd	128	64	4	0	16	64	256	256
5th	8	0	0	0	64	128	256	256

Note. 256 GAU/ml were taken in the experiment; the numbers represent the number of GAU in the supernatant fluid.

We assumed that the reversion of variant TA_1, apparently, occurred because of incomplete precipitation of the virulent virions, and therefore the experiment was repeated with subsequent adsorption of the TA virus on the first passage by the same method. The results of the repeated experiment are presented in Table 7.

Table 7
Results of Titration of Non-adsorbed Virus in 10-Day Old Chick Embryos

Dilution of Virus	Number of Embryos									
	1	2	3	4	5	6	7	8	9	10
10^{-1}	72	72	96	72	96	96	96	96	96	96
10^{-2}	96	96	96	96	96	96	96	+	+	+
10^{-3}	+	+	—	—	—	—	—	—	—	—
10^{-4}	—	—	—	—	—	—	—	—	—	—

Note: Symbols are the same as in Table 4.

Upon dissection of the destroyed embryos, it was noted that in the majority of them hemorrhage in the body and oxypital hematoma were absent. Allantoic culture of the virus was taken from embryo No. 1 (infected with virus at a dilution of 10^{-3}), and was passaged three times in embryos by the serial dilutic method. As a result, a variant of the virus (TA_2) was obtained, differing from the original (T) in the period of destruction of the embryos (Figure 2). Not one of the embryos destroyed by the TA_2 virus variant, had blood flow and oxypital hematoma; moreover, this was characteristic for the original virulent T strain. Sharp differences were detected between the TA_2 variant and the original T strain in pathogenicity for 1- and 10-day old chicks upon intracerebral and intramuscular methods of infection (Table 8).

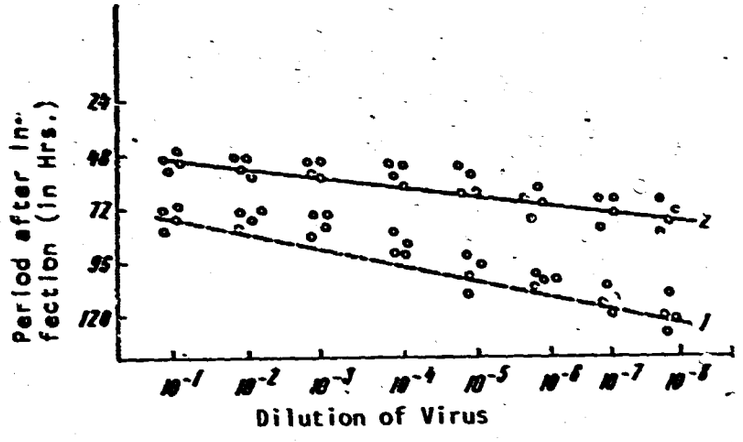

Figure 2. Period of Destruction of 10-Day-Old Embryos, Infected with Variants of the TA_2 (1) and T (2) strains.

The comparative antigenic activity of the TA_2 variant and the original T strain were studied with the help of RZGA with rabbit antiserum of the T strain. The serum inhibited hemagglutination of the TA_2 virus (1st, 3rd, 5th and 8th passages) and the T strain at dilutions 1:256--1:512.

Table 8
Comparative Characteristics of Virulence of TA_2 Variant and T Strain Upon Titration in Chicks and Chick Embryos

Virus	Log [illegible]	Log LD_{50} at Infection		
		Intramuscular		Intracerebral
		5-Day old Chicks	10-Day old Chicks	Day Old Chicks
TA_1	8.0	1.0	1.0	5.5
TA_2	9.0	0	0	6.0
TA_4	8.5	0	0	4.5
T original	8.0	7.0	7.5	7.5

Comparative Hemolytic Properties of the Virus Variants

By studying the hemolytic properties of Newcastle disease virus, Kilham [13] established the effect of the pH of the medium, temperature and form of erythrocytes on its activity. The author showed that the hemolytic properties of the virus may be inactivated by specific antisera and formalin. It was later established [7, 8, 12, 14, 19] that the majority of strains of Newcastle disease virus possess hemolytic properties, which increase after dialysis of the virus, its purification by the method of freezing and thawing, replacement of allantoic fluid with physiological solution, buffer, sodium citrate, etc. Hemolytic activity sharply decreased at a temperature of 4°. In our research, a comparative study of the hemolytic properties was carried out with virants A, B, and TA_2 of the Newcastle disease virus in comparison with the T strain (Table 9).

Table 9
Comparative Characteristics of Hemolytic Properties of Variants of Newcastle Disease Virus

Erythrocytes	Virus Strain		
	T	A	B
Horse	0,18	0,11	0,01
Guinea Pig	0,438	0,19	0,048
Chicken	0,07	0,03	0,011

Note. The numbers represent optical density, of a range of error of determinations of ±0.03.

From Table 9 it is clear that the virulent T strain possesses a higher hemolytic activity, the avirulent B variant possesses almost none, and the A variant occupies an intermediate position between the T strain and the B variant. Evidently, the changes in the virulent properties of the T virus in the process of adaptation to guinea pig brains, are accompanied by a reduction of hemolytic activity.

Interest has been shown in studying the inner connection of hemagglutinizing and hemolytic activity in variants of Newcastle disease virus by the method proposed by McCollum and Bradly and based on the use of chicken erythrocytes [14]. The interconnection between hemolytic and hemagglutinizing properties of the virus have not been established; evidently, this property is determined by various structures and biochemical components of the virus capsid. In the T strain clearly expressed hemolytic activity was observed up to a dilution of 1:40, and variants of this virus (TA, A, B) did not produce hemolysis, but possessed relatively high hemagglutinating properties.

Discussion

In analyzing the data obtained, one may draw the conclusion that virulence and enzymatic activity are related in Newcastle disease virus. V. D. Solov'yev, T. G. Orlov, L. A. Porubel', and I. N. Vasil'yeva [4], in studying genetic markers of virus strains of the A2 group, obtained contradictory results. In their experiments, the non-pathogenic strains possessed high hemagglutinating and enzymatic activity; the pathogenic strains weakly agglutinated chicken and mammalian erythrocytes, and did not possess enzymatic activity. Similar data was also obtained by A. A. Kolcharina [2] with vaccine and pathogenic strains of group A2 virus. Evidently, the lack of agreement of the results of our study and that of the indicated authors may be explained by a difference in the biological properties of the viruses.

By studying the beginning stages of interaction of the virus with the cell, several authors [1, 6, 20] arrived at the conclusion that the adsorption of the virus by the cell depends slightly on its sensitivity to the virus and, evidently, occurs under the influence of a physico-chemical force, and inherent Brownian motion, which is general for all suspended particles. But in the hemagglutinating virus, the interaction begins between its enzyme and the surface receptors of the cell, as a result of which, the destruction of the neuraminic bonds occurs.

In our research, the adsorption process, besides the physical factors, evidently, still served as some specific bonds of the virus with the receptors of the cell, while the extremeties bonds depended on the virulence of the virus. The adsorption of the virus by the cell is so specific, that it does not serve as a framework of a simple physical phenomenon. The rate of elution of Newcastle virus from erythrocytes also depends on its virulence, which may be used in selective work. This hypothesis is confirmed by the results of experimental selection of the avirulent TA_2 variant from the original population of the epizootic T strain. The TA_2 variant differs in its enzymatic and virulent properties from the original. We also carried analogous experiments with the virus of the classical chicken plague (Rostok strain); as a result, the avirulent R_4 and R_5 variants were obtained [3].

In 1957, Padgett and Walker [16], using various periods of elution of virus of the group, obtained variants possessing different enzymatic activities.

The hemolytic activity of Newcastle disease virus was studied in experiments with erythrocytes of chicken, horse, and guinea pig. The guinea pig erythrocytes proved to be most sensitive. We did not succeed in establishing an interaction between hemagglutinating and hemolytic activity. Analogous results were presented in the work of V. M. Zhdanov and A. G. Bukrinskaya [1] and Khou Yun'-de [5], having studied the different variants of Senday virus. A direct dependence was established between virulence and hemolytic activity.

Results

1. The process of adaptation of Newcastle virus to a heterologous animal (guinea pig) was accompanied by the appearance of genetically heterogeneous virions, which were successfully isolated by the method of serial dilution.

2. The attenuation of the virus was accompanied by reduction of its enzymatic activity (adsorption, elution, hemolysis), while a direct dependence was established between virulence and enzymatic activity.

3. The possibility of obtaining weakly virulent variants of the virus from pathogenic strains according to their enzymatic activities was demonstrated. Published principles of experimental selection, probably, can be used in investigations on the direction of alteration of these viruses.

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UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Foreign Science and Technology Center
US Army Materiel Command
Department of the Army

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP

3. REPORT TITLE
Experimental Variation of Newcastle Disease Virus

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Translation

5. AUTHOR(S) (First name, middle initial, last name)
V. M. Kolosov, and V. N. Fyurin

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
MAR 10 1969	13	N/A

8a. CONTRACT OR GRANT NO.

b. PROJECT NO. 0703006

c. 9223628 2301

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
FSTC-HT-23-658-68

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
AOSI Control Number (None)

10. DISTRIBUTION STATEMENT
Distribution of this document is unlimited.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
US Army Foreign Science and Technology Center

13. ABSTRACT

Adaptation of Newcastle disease virus to guinea pigs was accompanied by the appearance of genetically dissimilar virions, which were successfully isolated by the method of serial dilution. Attenuation of the virus was accompanied by a reduction in its enzymatic activity (adsorption, elution, hemolysis), while a direct relationship was established between virulents and enzymatic activity. Enzymatically active variants were precipitated with formalinized erythrocytes, and an avirulent TA2 variant, isolated from the supernatent, was shown to be less active enzymatically. It is suggested that enzymatic activity could be used to select weakly virulent variants from pathogenic strains of the virus.

DD FORM 1 NOV 65 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

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14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Newcastle disease virus						
adsorption						
elution						
hemolysis						
virulence						
enzymatic activity						