ELUTION PROPERTIES OF NEWCASTLE DISEASE VIRUS FROM DEAE-CELLULOSE

by

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INTRODUCTION

Numerous methods have been used for the separation of viruses from the normal constituent of the hosts. These separations lead to partial purification of the virus or separation of the virus sub-units into many fractions.

Physiochemical methods that have been employed are electrophoresis and ultra-centrifugation which separate groups of similar charge or weight, while chromatographic methods with ion-exchange adsorbents have been used with more complex high molecular weight compounds. One such chromatographic technique is the DEAE-cellulose (N,N-diethylaminoethyl-) column which has been used extensively for purification and the fractionation of different types of viruses.

The studies described in this thesis are concerned chiefly with the fractionation of Newcastle Disease Virus (NDV) on DEAE-cellulose columns. By using NDV cultivated on different hosts it was possible to obtain information on the specificity of this method of separating viral components.

The virus was cultivated and harvested from (1) the chorioallantoic fluid labelled with H^3-uridine and H^3-valine from embryonated chicken eggs, (2) tissue culture cells of chicken embryo, and (3) tissue culture cells of quail embryo. Fractions of each sample were collected and assayed for hemagglutinin (HA), plaque-forming units (PFU), protein concentration, and radioactivity (cpm). In addition, cells from the quail embryo were cultivated and studied for susceptibility to infection with NDV. Growth curves of the virus in this tissue were determined. Finally, it was shown that kidney tissue from baby hamsters (cell line BHK-21) is susceptible to infection with NDV.

LITERATURE REVIEW

THE NEWCASTLE DISEASE

Newcastle disease is an acute, highly infectious and frequently fatal disease of domestic fowl. It may be acquired by man through contact with the virus.

The disease was first described by Kraneveld in Indonesia in 1926, and the causitive agent of the disease was isolated by Doyal in England in 1927. It was first observed in the United States (California) in 1945 by Beaudette and Black, 1945.

THE NEWCASTLE DISEASE VIRUS

The Newcastle Disease Virus (NDV) has been studied by many workers. In his first report Bang (1946) described the virus as being pleomorphic. However, he soon showed (1948) that this pleomorphism was an artifact due to differences in salt concentration. NDV prepared in distilled water is spherical. The size of the NDV was estimated to range in size from 1200-3000A (Waterson and Cruickshank, 1964). It was shown by electron microscopy and by biochemical analysis (Horne, et al., 1960), that the outer part of the virus was the lipoprotein envelope with spikes surrounding it, and a central nucleoprotein core. This core was named NP-antigen or G-antigen and later named as soluble antigen (Rott, et al., 1963). They found that the soluble antigen derived from cells infected with NDV was the internal component of the virus, which consisted of viral ribonucleoprotein.

The genetic material of NDV is ribonucleic acid (RNA), which represents 10% of the virus particle (Waterson, 1964). It has been reported that the nucleic acid of the virus is single stranded (Kingsbury, 1963). By means

of autoradiography Wheelock (1963) demonstrated that the viral RNA is synthesized in the cytoplasm of infected cells.

NDV can be inactivated by many chemical compounds such as subtilin. As subtilin, this polypeptide can be used to inactivate NDV from egg allantoic fluid (Lorenz and Jann, 1964). The report suggests that the action of this material was due to a non-specific aggregation of virus in a subtilin-allantoic fluid protein complex, rather than a specific action against the virus itself. Rott, et al., (1961) and Waterson, et al., (1964) showed that the virus particles were easily disintegrated by the use of liquid solvents like ether, which broke the virus into many fragments.

The protein coat of the virus has been of interest to many investigators, due to its affinity for mucoprotein, its hemagglutinating properties, the presence of neurimindase enzyme and lipid content. Because of these characteristics, the virus was grouped with the myxoviruses.

The envelope protein refers to the protein associated with a lipid envelope of myxovirus which contributes a major share of the immunological properties to the virus surface. It is not clear whether these antigens have an exposed surface in addition to a matrix with viral lipids, which are responsible for the structure of the envelope (Eckert, 1966). The phospholipid of NDV was susceptible to the "lecithinase" and was inactivated by the enzyme (Franklin, et al., 1957).

The protein coat of NDV has the property of agglutinating the various types of animal red blood cells due to the presence of the hemagglutinin antigen on the spikes of the protein coat. Nuriminadase enzyme was located between these spikes (Rott, et al., 1962).

Granoff, et al., (1959) reported two noninfectious hemagglutinins. A small noninfectious component called the viromicrosomes, which were

described by Rott, (1964) as the viral protein coat devoid of nucleoprotein. The viromicrosomes were also described (Schafer, 1963) as cellular sites of hemagglutinin production.

EFFECTS OF NDV ON ANIMAL CELLS

Topacio (1964) was the first to propagate NDV in fragments of chick embryo tissue suspended in Tyrode's solution. Bankowski (1964) reported that NDV can be propagated in a variety of tissue cultures from various organs and neoplastic tissue of animal species such as chicken, bovine, porcine, rabbit, rat, mice, guinea pig, monkey and man.

Granoff, et al., (1950) noticed that embryonated eggs infected with NDV contained noninfectious hemagglutinin in the allantoic membrane. In 1955 he suggested that the noninfectious particles of hemagglutinin was a developmental stage of virus production.

Squib (1964) noticed that NDV infection was associated with a decrease in vitamin "K" and an accompanying increase in prothrombin times. Vitamin "A" can alter the phenotypic expression of several myxoviruses by producing filamentous forms. It has been suggested that the mode of action of vitamin "A" on the virus is twofold. It acts first as a surface-active agent, and second as a regulator of mucopolysaccharide biosynthesis (Blough, 1966).

It has been demonstrated (Granoff, et al., 1950), that there are three types of hemagglutinins in tissue culture cells infected with NDV. They are, the complete virus, small non-infectious components and viromicrosomes.

Zhdanov, et al., (1966) showed that both protein components, soluble antigen, and the protein coat are synthesized in the cytoplasm of the infected cells. Reda, et al., (1964) showed by the immunofluorescence method

that in some experiments the soluble antigen was found also in the nucleoli of the infected cell. Waterson (1961) suggested that the infection of animal cells by viruses followed these events: adsorption, penetration, uncoating, RNA synthesis, viral protein synthesis, viral RNA synthesis, aggregation of viral components and release of progeny. Barry (1965) showed that the composition of the medium affected the rate of release and the burst size. Rubine, et al., (1957) showed that there was a lag period of three to four hours, as measured by the first appearance of progeny virus. Protein synthesis in infected cells increases during the period of 5-12 hours after infection. Gelenczei and Bordt (1960) showed that the growth and the behavior of several strains of NDV in different cell culture systems could be modified with a change of the host. Stenback and Durand, (1963) reported that the density of NDV was influenced by the amount or composition of host material incorporated directly into the virus structure, or variation in packaging of virus subunits. By comparing NDV grown in tissue cultures from chick embryos with that grown in chick chorioallantoic membrane, Drake and Lay (1962) were able to demonstrate that the host controls variation of the virus. The viruses differed in their sensitivity and ultraviolet irradiation. These authors suggested that the host-induced characteristics of the virus may be due to inclusion of materials in the virus particle, which are host specific. Durand and Eisenstark (1962) also showed that the host cell contributed physical and chemical properties to the virus.

In certain unsuitable cell lines, such as HeLa cells infected with NDV, an excess of viral protein and RNA are produced (Wheelock, 1962). In 1957, Cierciura, et al., infected HeLa cell cultures with NDV, isolated resistant cells, and found that the progeny of these cells are chronically infected

with NDV.

Wilcox (1959) infected strain "L" cells with NDV and observed cell destruction in the absence of infective virus production. The cytopathic effect was non-transmissible and was described as viral toxicity.

Wheelock (1962) prolonged the eclipse period of NDV in HeLa cells by two hours by treating the cells with puromycin for four hours. Polykaryocytosis, induced by NDV in monolayers of animal cells was reported by Kohn, (1965). He treated a monolayer of various continuous lines of animal cells with NDV. A high multiplicity of the virus resulted in the fusion of these cells into polykaryocytosis containing 3-150 nuclei per cell. Reaction began within an hour after the addition of NDV and reached its maximum within two to four hours. He suggested that the integrity of phospholipids of the viral membrane is the main requirement in the fusion of cells mediated by NDV.

The plaque morphology produced in tissue culture was studied by Durand and Eisenstark, (1962). They reported the single and multiple passages of NDV in cell cultures of different origin. The pathogenicity proved to be stable, but plaque morphology was different. They suggested that plaque size depends upon the number of genetic units present in a virus particle.

CHROMATOGRAPHY OF THE VIRUS ON DEAE-CELLULOSE

In 1956, Peterson and Sober used chromatographic analysis on a cellulose anion-exchange column for serum protein fractionation. Hoyer, et al., (1958) reported that the chromatographic behavior of the animal virus and rickettsiae appears to depend more upon the chemical nature of the surfaces of these agents than upon their size. The chromatographic procedure may prove useful in the preparation of purified labelled animal viruses for further studies.

It may also prove useful for removal of unwanted host material in the preparation of vaccine.

Klemper and Pereira (1959) and Haruna, et al., (1961) fractionated adenovirus by chromatography on a DEAE column and elution with increasing sodium chloride concentrations. They found that three types were eluted with different salt concentrations. Different kinds of cellulose were used, such as DEAE-, ecteola-, and P-cellulose. They found that DEAE-cellulose was the most suitable for the separation of the viruses. McCrea and Loughlin (1959) also separated vaccinia hemagglutinins from infectious virus particles by chromatography on a DEAE column. In 1962, Wilson separated NDV from chicken allantoic fluid by chromatography on DEAE-cellulose and showed that the L-Kansas strain had one peak of hemagglutinin and one of infective particle, but N-Kansas strain had two peaks of hemagglutinin and one peak of infective virus.

MATERIALS AND METHODS

VIRUS

The Roakin strain of NDV was used in this work. The virus was infected into the following susceptible hosts. Ten or eleven day old embryonated chicken eggs, quail tissue culture, BHK-21 cells and chick embryo tissue culture.

TISSUE CULTURE

Quail embryo tissue culture cells were made from 6-7 day old embryonated eggs. The blunt ends were sterilized with 95% alcohol and burned three times. The shells were cut with sterile scissors and the small embryos were hooked around the neck and placed into a 100 mm Falcon plastic Petri dish. The heads and limbs were removed and transferred to another sterile Petri dish where they were minced with sterile scissors and washed three times with phosphate buffered (0.01M,PH.7.2) saline PBS. The cells were trypsinized with 0.25% trypsin (Difco 1:300) for ten minutes and then collected by centrifugation for ten minutes at 586 x g. This process was repeated 3-4 times until a sufficient number of cells had been collected. The cell suspensions were counted by a hemocytometer and diluted to 1.0-1.4 x 10^6 cells/ml, with Eagle's medium containing 10% calf serum, and then seeded on a 100 x 20 mm glass or Falcon plastic Petri dish. They were incubated for 48-72 hours at 37 C in a humidified 5% CO_2 atmosphere. Complete monolayers of cells were infected with NDV, either for virus stock or experimental work. The chick embryo tissue cultures were made the same as above except that the embryo were 10-12 days old. The BHK-21 cells were trypsinized with 0.1% trypsin for five minutes. Sufficient Eagle's medium containing 10% calf serum was

added to the cells to make a final concentration of 0.7-1 x 10^6 cells per ml of medium. Five ml were added to each 60 x 15 mm Falcon plastic Petri dish for the experimental work.

INFECTION OF THE CULTURE

The confluent cell cultures were infected with NDV at a cell ratio of 1-PFU/cell. After a 30 minute adsorption time, the cultures were covered with Eagle's medium containing 10% calf serum, and then incubated at 37 C in a CO_2 humidified incubator for 48-60 hours or until complete cellular degeneration was observed. The cells and medium were harvested, frozen and thawed three times, and then centrifuged for thirty minutes at 3000 x g. The clear supernatant was collected and stored at -20 C until used. Quail cell tissue cultures, chick cells and BHK-21 cells were infected and the virus collected in the same manner.

CONCENTRATIONS OF THE VIRUS FROM INFECTED TISSUE CULTURES

The tissue culture cells and the growth medium was frozen and thawed 4-5 times to release cellular virus and then centrifuged for thirty minutes at 1465 x g. The clear supernatant was removed and saved. The cell debris was resuspended in 10 ml of clear supernatant and treated with 50 units of receptor destroying enzyme (RDE, Microbiologicl Associates, Bethesda, Md.) overnight at room temperature with magnetic stirring, and then centrifuged at 3000 x g for one hour. The supernatant was collected and added to the original supernatant fluid. This clarified supernatant fluid, which contained the virus particles, was precipitated with saturated ammonium sulfate solution. The final concentration of $(NH_4)_xSO_4$ was 33%. This was accomplished by mixing two parts of virus solution to one part of saturated

$(NH_4)_2SO_4$. The pH was adjusted to 7.5-8.0 with 1N. NaOH solution (Consigli, et al., 1965). The mixture was centrifuged at 10,400 x g at 4 C. The sediment was re-suspended with 0.01M tris-acetate buffer (pH 7.2) and dialyzed against tris-acetate buffer overnight at 4 C to remove the ammonium sulfate. The tris-acetate solution was changed every six hours during this period.

RADIOACTIVE VIRUS

Radioactive NDV was obtained from infected chorioallantoic fluid of embryonated chicken eggs, 9-10 days old. This was accomplished by inoculating the intra-allantoic fluid with 0.1 ml of H^3-valine (50µc/egg) 24 hours before they were infected with 10^4 PFU of NDV. This labeled the protein coat of the virus with the H^3-valine. The same labeling procedure was performed using H^3-uridine, to label the nucleic acid of the virus. The eggs were then incubated at 37 C. After 24 hours the eggs were candled and the eggs with dead embryos were discarded. The live ones were kept incubated for 2-3 days until the embryos died. The chorioallantoic fluid was collected aseptically and centrifuged for one half hour at 3000 x g. The supernatant was taken and assayed for hemagglutination activity (HA) and cpm, and then frozen until fractionated on a DEAE-cellulose column.

CHROMATOGRAPHIC PROCEDURE

The diethylaminoethyl-cellulose powder was prepared by thoroughly washing three times with 1N NaOH, and allowing six hours for settling each time. It was further washed three times with 1N HCl to neutralize the NaOH, and then three times with distilled water (deionized). It was then stored in 95% ethanol until used. This thoroughly washed DEAE-cellulose

was packed in a column 1.1 cm in diameter and 10 cm high. The packing was under 2 psi of air pressure and equilibrated with 0.01M tris-acetate buffer (pH 7.2) prior to the addition of the concentrated virus preparation. Following this, a program of step-wise elution employing increasing molarities of NaCl at constant pH was carried out. The elution was carried out under 2 psi of air pressure. For each molarity, 15 ml eluates were collected. The eluates so obtained were tested for PFU, protein concentration, radioactivity (cpm), and HA.

PLAQUE ASSAY

Virus titres were determined by a modified method of plaque assay for animal viruses (Delbucco, 1952). Monolayers of chick embryo tissue culture were grown in 60 x 15 mm Falcon plastic dishes. The media was discarded and the cells were infected with 0.5 ml of different log dilutions of virus after a 30 minute adsorption period at 37 C in a humidified 5% CO_2 atmosphere. The infected monolayer of cells was covered with 8 ml of an equal mixture of 2X-adeno medium (Consigli, et al., 1966) plus 2% purified agar (Difco). After 60-72 hours of incubation, 2.5 ml of adeno medium and purified agar mixture containing 0.01% neutral red was added. The plaques were readily visible 12-24 hours after the addition of dye.

HEMAGGLUTINATION

For a rapid estimation of the number of virus particles, the HA titrations were carried out in plastic trays. Two-fold dilutions of NDV were made in 0.5 ml PBS. Then 0.5 ml of a 1% suspension of chicken red blood cells was added. The final concentration of the RBC was 0.5%. The results were read 30-45 minutes after incubation at room temperature.

MEASUREMENT OF RADIOACTIVITY

The radioactivity was measured in scintillation vials which were filled with 10 ml of scintillation fluid of the following composition: 360 ml dioxane, 360 ml toluene, 216 ml absolute ethanol, 5g PPO, and 80g of naphthalene. They were cooled to 4 C for 3-4 hours. The radioactive samples were added and counted in a Packard Tricarb liquid scintillation counter.

MEASUREMENT OF PROTEIN

The protein concentration was determined by the method of Lowry, et al., (1951). One half ml aliquots of the samples were hydrolyzed with 0.5 ml of 1N NaOH (30 min. at 100 C). To this was added 5 ml of a solution consisting of the following mixture: 10 ml of solution A (2% Na_2CO_3 in 0.1N NaOH), plus 1 ml of 1% copper sulfate and 1 ml of 2% Na,K-tartrate.

The mixtures were incubated for 10 minutes at room temperature. Then 0.5 ml of diluted Folin reagent (50% diluted with distilled water) was added with rapid mixing. The mixture was incubated at room temperature for 30 minutes. Optical density readings were made using a Klett spectrophotometer with number 66 filter. Quantities were determined by using a crystalline bovine albumin standard.

ONE-STEP GROWTH CURVE OF NDV IN QUAIL CELLS

The growth characteristics of NDV in chick fibroblasts in tissue cultures have been described by Granoff, (1955), and Levine and Sagik, (1956).

This experiment was performed to determine various features of the multiplication of NDV in quail tissue culture, including the time required for release of newly formed virus particles from an infected cell. The experimental technique was similar to that used for chicken tissue cultures

(Rubbin, et al., 1957). Three day old monolayers of quail embryo cells were infected with NDV (2/PFU/cell). After 30 minutes adsorption, the monolayers were washed four times with PBS to remove the unadsorbed virus. Then 2 ml of Eagle's medium was added to each Petri dish and incubated at 37 C. Infected cultures were sequentially harvested to determine the latent period. The harvested cultures were stored at -20 C until assayed by the plaque method.

RESULTS

NDV FROM INFECTED ALLANTOIC FLUID OF CHICK EMBRYO

NDV preparations used in this experiment were respectively labeled with H^3-uridine and H^3-valine and obtained from allantoic fluid of embryonated eggs. Samples containing 8 ml each (2.6×10^9 total PFU, and 2560 total HA-units) were loaded onto a DEAE-cellulose column. The radioactivity of the respective samples was 613,920 cpm for H^3-valine and 19,248 cpm for H^3-uridine. The column loaded with the virus was washed three times with a tris-acetate buffer. Then the step-wise elution with sodium chloride solutions of different molarity, in tris-acetate buffer (pH 7.2) was carried out. Fifteen fractions were collected. The effluent fractions were assayed for HA, PFU, cpm, and protein.

The results of the chromatographic analysis for the NDV infected allantoic fluid are shown in Figure I for H^3-valine labeled viral preparation and in Figure II for H^3-uridine labeled virus. Figures I and II show that the protein is mainly eluted in the 0.15 and 0.18M fractions. These figures also show that the cpm plot for H^3-valine is nearly coincident with the protein curve, but H^3-uridine showed an independent peak of elution at 0.15M concentration.

Hemagglutination activity was eluted early at 0.15M and 0.25M. These fractions were high in HA-units and low in PFU. The infectious virus particles were eluted at 0.33 M, where the peak of the total PFU reached the ratio of 1.1×10^6 PFU/HA-units.

NDV FROM INFECTED QUAIL TISSUE CULTURES

Different results were obtained when the virus from infected quail cells

EXPLANATION OF FIGURE I

Fractionation of H^3-valine labeled NDV preparations from infected egg allantoic fluid on DEAE-cellulose by gradient (stepwise) elution. For each NaCl molarity, 15 mo were collected, assayed for HA, PFU, and protein concentration. Each point on the graph represents the total content of the respective fraction.

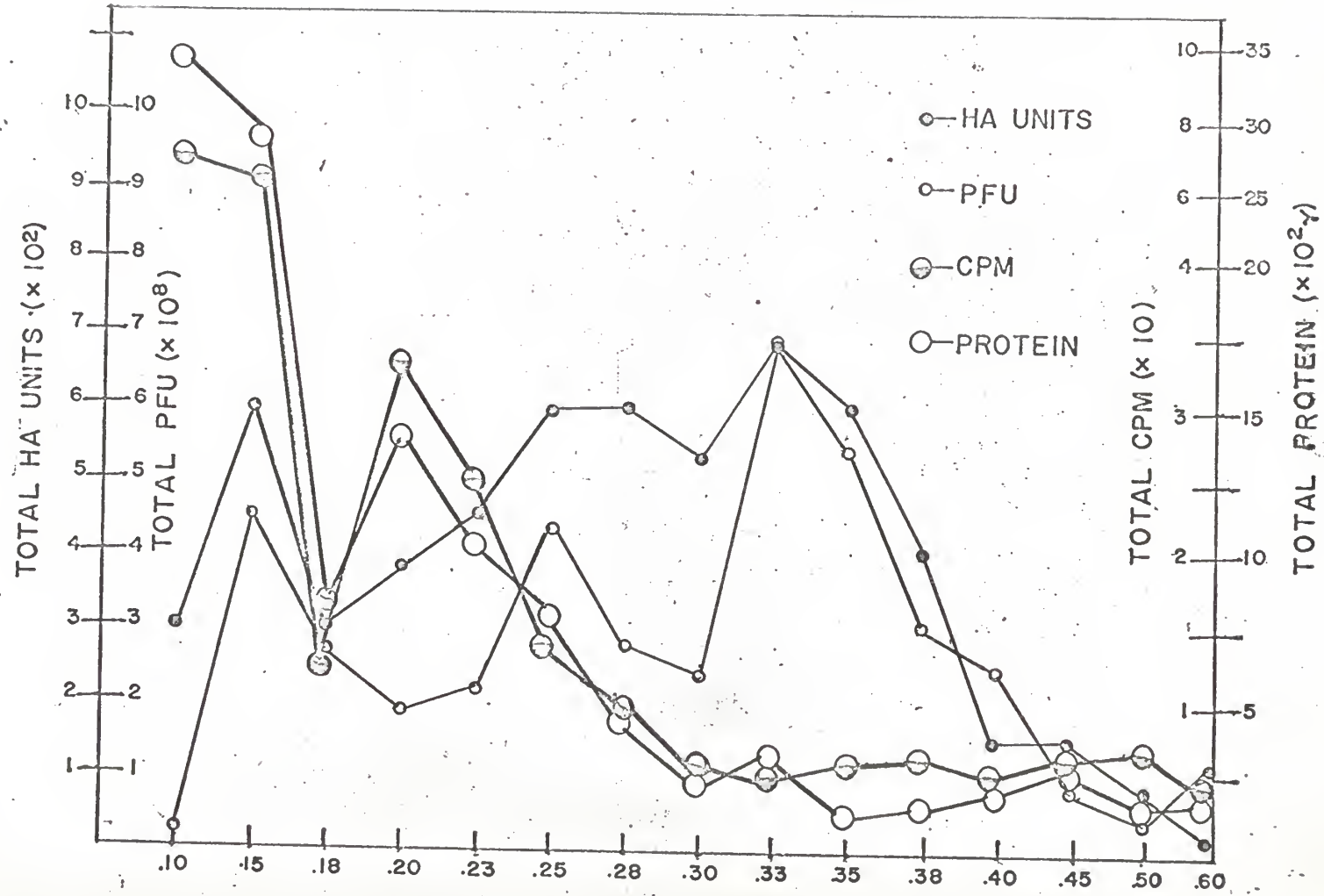
HA UNITS
PFU
CPM
PROTEIN
TOTAL HA UNITS (× 10²)
TOTAL PFU (× 10⁸)
TOTAL CPM (× 10)
TOTAL PROTEIN (× 10²γ)
NaCl MOLARITY
.10
.15
.18
.20
.23
.25
.28
.30
.33
.35
.38
.40
.45
.50
.60

Fig. I

EXPLANATION OF FIGURE II

Fractionation of H^3-uridine labeled NDV preparations from infected egg allantoic fluid on DEAE-cellulose by gradient (stepwise) elution. For each NaCl molarity, 15 ml were collected, assayed for HA, PFU, and protein concentration. Each point on the graph represents the total content of the respective fraction.

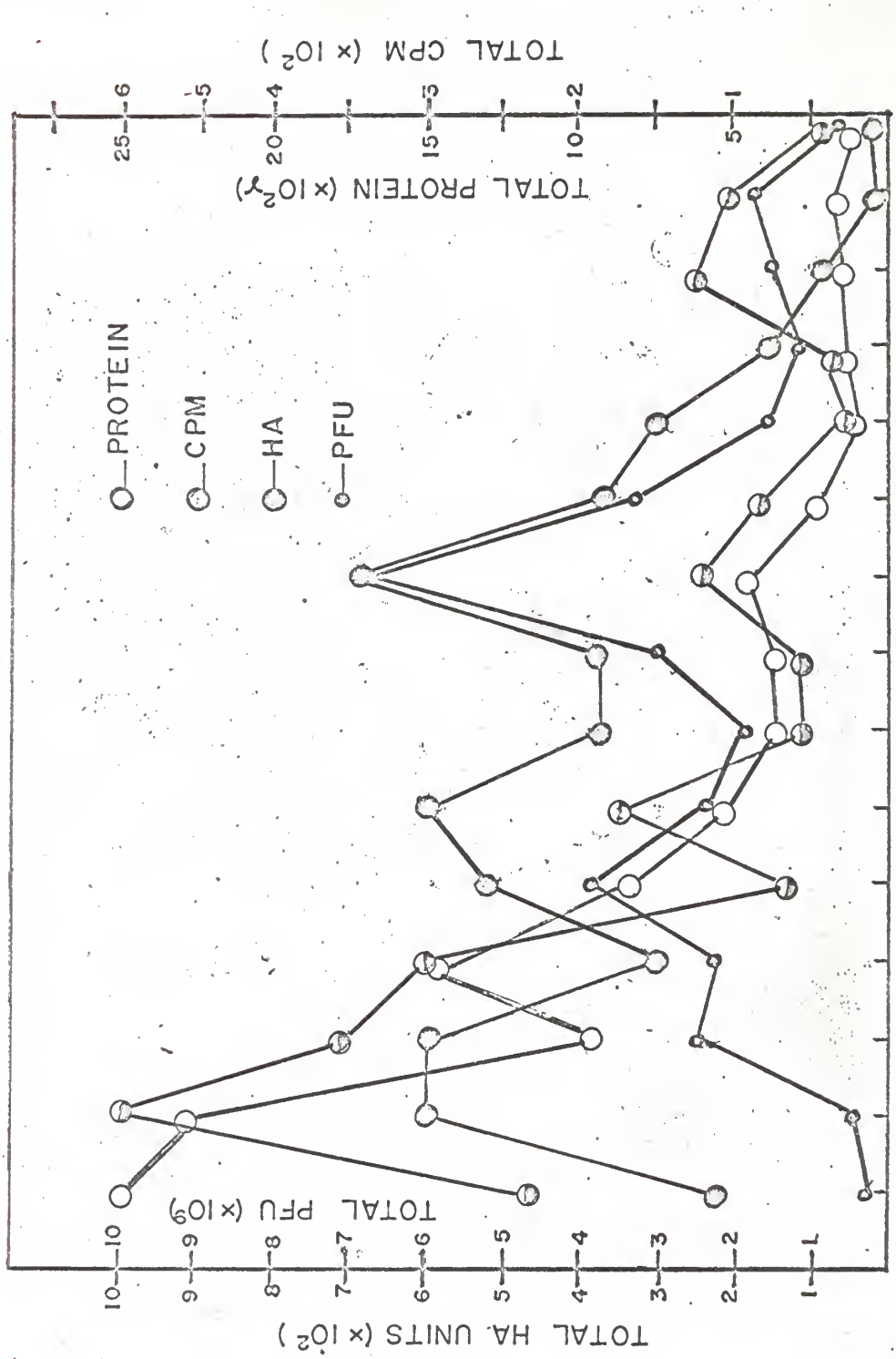
TOTAL HA UNITS (x 10²)
TOTAL PFU (x 10⁹)
TOTAL PROTEIN (x 10²γ)
TOTAL CPM (x 10²)
NaCl MOLARITY
PROTEIN
CPM
HA
PFU

Fig.11

was chromatographed (Fig. III). The concentrated tissue culture fluid from quail cells infected with NDV was dialyzed and added (1.8×10^9 total PFU and 2400 total HA-units for each sample) onto the DEAE-cellulose column. The loaded column was washed and fifteen fractions were eluted as mentioned before. The results show that most of the hemagglutinin activity was eluted at 0.18M and 0.25M, and the ratio between the total PFU and the total HA-units was 4×10^5. The infectious virus was eluted at 0.4M. The total PFU at this stage was 4.5×10^8 and the total HA-units was 255 or a ratio of 1.6×10^6 PFU/HA-units. The protein was eluted in the early steps, and consisted mainly of host protein mixed with noninfectious particles of hemagglutinin from the virus.

NDV FROM INFECTED CHICK EMBRYO TISSUE CULTURES

The concentrated NDV preparations from chick embryo tissue cultures were dialyzed and placed onto a DEAE-cellulose column in 15 ml amounts (5040 total HA-units and 4.2×10^9 total PFU), and then eluted with increasing concentrations of NaCl, as described previously.

Infectivity (PFU), HA, and protein assays were done on each eluate. The results are graphically summarized in Figure IV. The highest HA was eluted at 0.1M, and the PFU/HA-units ratio was 9×10^4. The infectious particles were eluted at 0.45M, and the ratio of PFU/HA-units was 0.5×10^6.

GROWTH CURVE OF NDV IN CELL CULTURES DERIVED FROM QUAIL EMBRYO

In recent years, several investigators have demonstrated the growth of NDV in different types of cell cultures. The quail cells described in this work adds another cell line to a growing list of tissue culture cells that are susceptible to the virus. This experiment was carried out to determine

EXPLANATION OF FIGURE III

Fractionation of concentrated NDV preparations from quail cell tissue cultures on DEAE-cellulose by gradient (stepwise) elution. For each NaCl molarity, 15ml were collected, assayed for HA, PFU, and protein concentration. Each point on the graph represents the total content of the respective fraction.

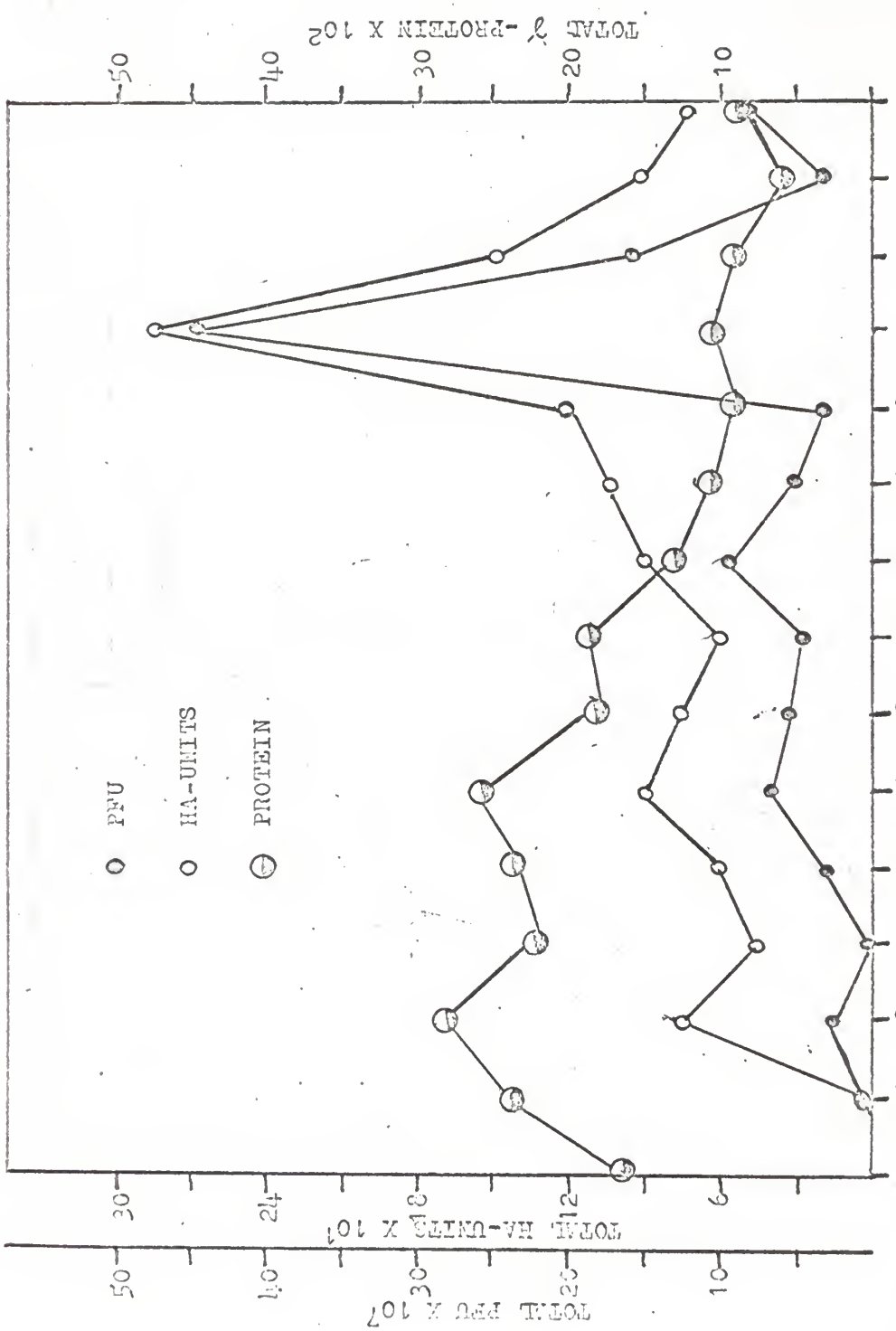
TOTAL PFU X 10⁷
TOTAL HA-UNITS X 10¹
TOTAL γ-PROTEIN X 10²
PFU
HA-UNITS
PROTEIN
NaCl Mol.
.10 .15 .18 .20 .23 .25 .28 .30 .33 .35 .38 .40 .45 .50 .60

Fig. III

EXPLANATION OF FIGURE IV

Fractionation of concentrated NDV preparations from chick cell tissue cultures on DEAE-cellulose by gradient (stepwise) elution. For each NaCl molarity, 15ml were collected, assayed for HA, PFU, and protein concentration. Each point on the graph represents the total content of the respective fraction.

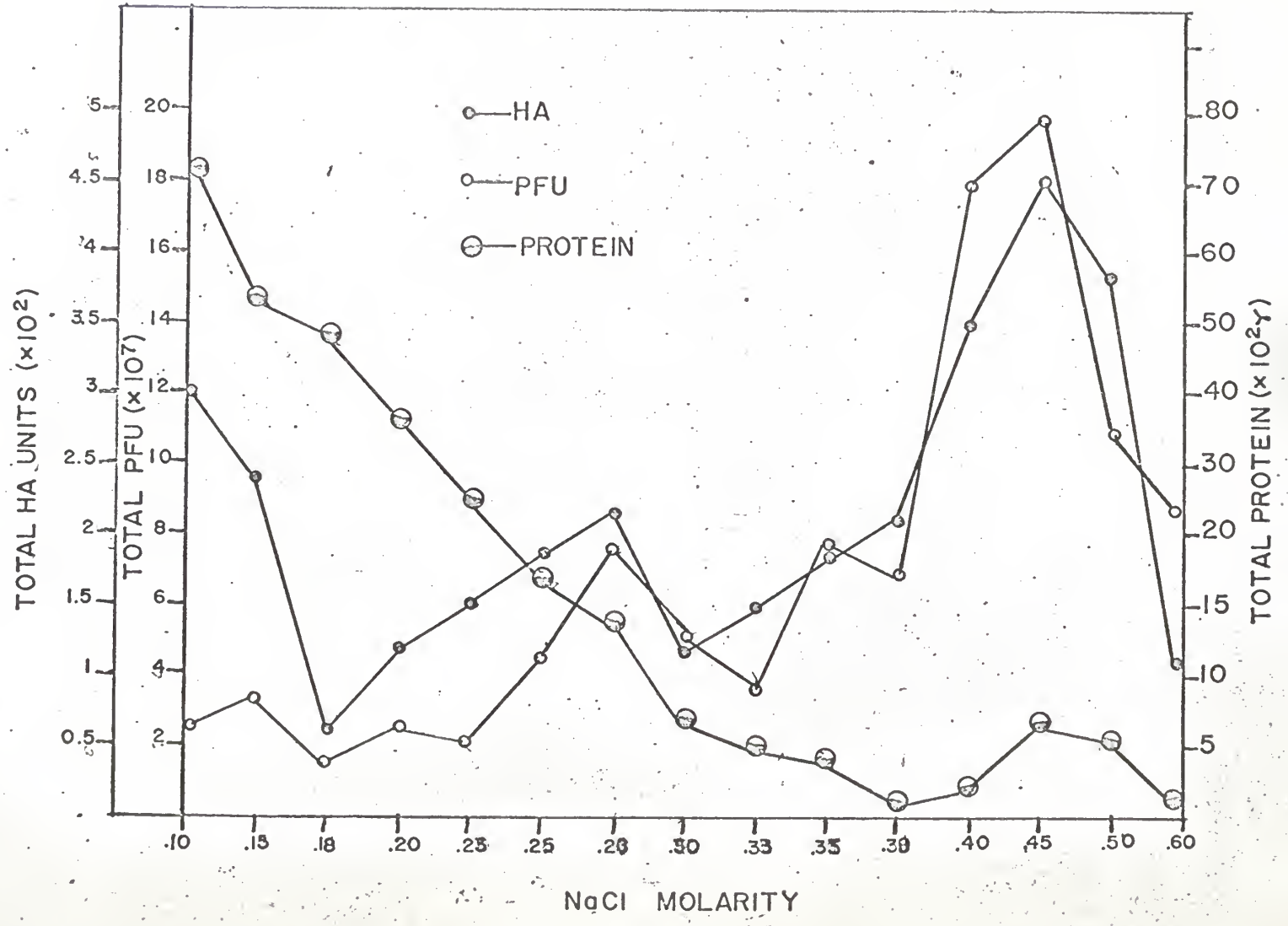
HA
PFU
PROTEIN
TOTAL HA UNITS (×10²)
TOTAL PFU (×10⁷)
TOTAL PROTEIN (×10²γ)
NaCl MOLARITY
.10
.15
.18
.20
.25
.30
.33
.35
.38
.40
.45
.50
.60

Fig. IV

various features of the propagation of NDV, including the time required for the release of newly formed virus particles after they were infected with NDV.

The results of this experiment are shown in Figure V. The first appearance of progeny virus occurs between 6-7 hours after infection, and increases in quantity during the last two hours. The curve shows that in the first 5.5 hours the virus is in the latent period. This result was different from the latent period of the NDV in chick tissue culture cells as reported by Levine and Sagik (1956). Rubin, et al., (1957) also showed different results when they used culture of chick embryo lung epithelium cells.

NDV GROWN IN BHK-21 CELLS

After serial passage of NDV on BHK-21 tissue culture cells, the virus production was assayed. The cytopathic effect of the virus on the BHK-21 cells appeared in the first passage after four days of exposure of the cells to the virus. This time was greatly reduced during the third and fourth passages. The number of virus particles increased and reached 1.5×10^6 PFU/ml at the end of the fourth passage. These results are shown in Figure VI. In the first and second passages there was little increase in virus number.

EXPLANATION OF FIGURE V

One-step growth curve of the Roakin strain of Newcastle disease virus in quail embryo tissue cultures.

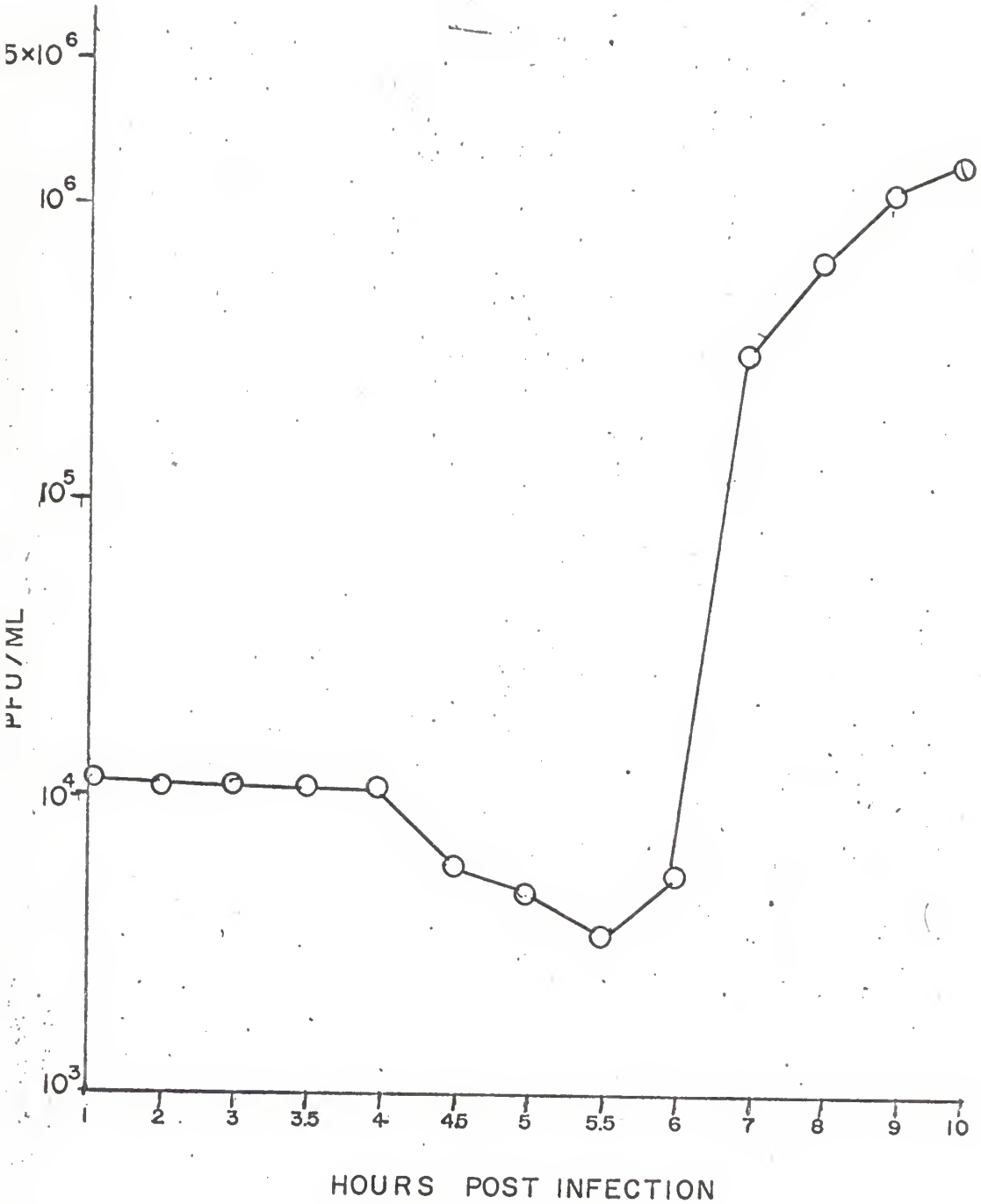
5×10^6
10^6
10^5
10^4
10^3
PFU/ML
1
2
3
3.5
4
4.5
5
5.5
6
7
8
9
10
HOURS POST INFECTION

Fig. V

EXPLANATION OF FIGURE VI

Titer of the Roakin strain of Newcastle disease virus of successive passages in BHK-21 cells.

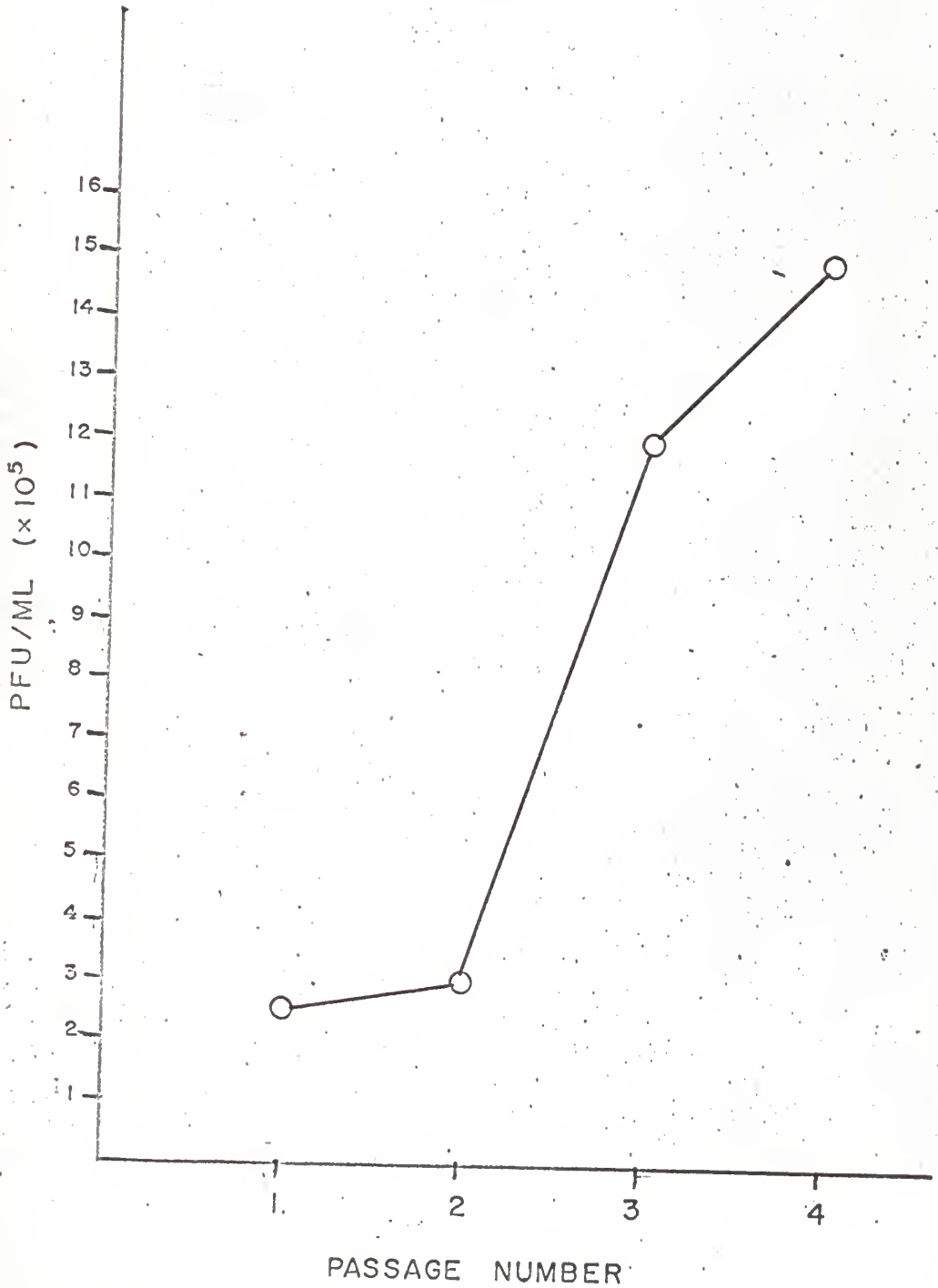

Fig. VI

DISCUSSION

Ion-exchange chromatography has been employed for the fractionation and purification of viruses by several investigators. Hoyer, et al., (1958) used this chromatographic method for animal viruses and rickettsiae and demonstrated that it was a good way to remove the contaminating host material. Klemper and Pereira (1959) fractionated adenovirus by DEAE-cellulose chromatography. In 1961, Haruna, et al., suggested that DEAE-cellulose chromatography was the best method of separating adenovirus. Wilson (1962) used DEAE-cellulose chromatography for fractionation of L-Kansas strain and N-Kansas strain of NDV from allantoic fluid. Taniguchi (1964) also separated components of plant viruses on different types of chromatographic columns. The majority of these investigators applied ion-exchange chromatography only as a means for the partial purification of viruses. This method has also been used for studies of the physicochemical properties of the viruses.

Sober and Peterson (1964) have given a clear picture of the probable mechanism of chromatography on modified celluloses, and the principles of elution. The adsorbed protein is tightly bound on a substance with a singly charged group. The elution of the protein by changing pH, reduces the number of charges on the protein or cellulose. The increasing salt or buffer-ion concentration of the eluant competes for the charges on the ion-exchange cellulose. The factors affecting the chromatographic behavior of a protein are, the net surface charge density, the arrangement of the charge in space, and molecular size.

The results presented in this thesis demonstrate that fractionation of NDV from different type tissue culture hosts was possible by continuous elution of the virus from a DEAE-cellulose column by using a salt gradient technique. The H^3-valine labeled NDV from allantoic fluid of chicken

embryonated eggs was separated by a DEAE-cellulose column into three main fractions. The first fraction was eluted at 0.15M NaCl concentration, and consisted mainly of hemagglutinin and some virus particles. Most protein and radioactivity was eluted in the early stages, which means that most of the host and small fractions of the viral subunits were eluted in this lower sodium chloride concentration. However, the most active virus particles were eluted at 0.33M. This fraction exhibited peaks high in PFU, HA-units and low in protein content and radioactivity (cpm), which means that the purest virus was eluted at this concentration. By following the amount of radioactivity in these fractions, we can calculate the purity of the virus. H^3-valine is usually incorporated in the protein structure of both the complete virus particle and viral subunits as well as in the host protein.

The amount of radioactivity loaded on the column was 613,920 cpm, and the total PFU was 2.6×10^9, or a ratio of 4.3×10^4 PFU/cpm. When a comparison of this type of ratio was considered in the 0.15M (6.8×10^5 PFU/cpm) and the 0.33M (1.1×10^7 PFU/cpm) fractions, it gave an indication of the purity of the virus.

The fractionation of H^3-uridine virus from allantoic fluid gave approximately the same results as the H^3-valine virus from infected egg allantoic fluid, except that the radioactivity showed the highest peak at 0.15M. The H^3-uridine which was incorporated into nucleic acid (RNA) was eluted at this molarity. This result showed that most nucleic acid (RNA) can be eluted at lower concentrations of NaCl.

The virus from infected tissue cultures gave different chromatographic results. At the same time different tissue culture hosts gave different virus particle characteristics. Kates (1961) found that the phospholipids of the myxovirus are preformed in the host cell and incorporated directly

into the virus. Virus lipids have been shown to be similar to those of normal host membranes. Marcus (1962) reported that the lipid part of the virus came from the host cells. Gelenczei and Bordt (1960) showed that the growth and behavior of NDV in different hosts could be modified by a change of the host: Stenback and Durand (1963) reported that the density of NDV was influenced by the amount or composition of host material incorporated directly into the virus structure, or variation in packaging of virus subunits. Drake and Lay (1962) found that the type of host controlled the variation of NDV characteristics.

The interesting thing is the differences in chromatographic behavior. Such differences must be presumed to be due to intrinsic differences in the surfaces of these fractions, from the virus and host material. In this work the NDV from infected quail tissue cultures, chick tissue cultures and allantoic fluid, exhibited different behavior on the DEAE-cellulose column due to different types of host, which gave different virus characteristics.

The virus from quail tissue culture was eluted at 0.4M NaCl, whereas the virus from infected chick tissue culture was eluted principly at 0.45M NaCl. In contrast the virus from allantoic fluid passed through the column at lower NaCl concentrations. The characteristics of the allantoic membrane cells may have modified the behavior of the allantoic virus on the column. These differences in behavior can be applied to all other fractions of the virus as well as the host material.

It may be concluded that DEAE-cellulose chromatography can differentiate between individual particles with different physicochemical properties of the particle surface as described before by Sober and Peterson (1964). Hoyer, et al., (1958) suggested that the chromatographic behavior of the animal virus particles depends more upon the chemical nature of the surface agents

than upon their size.

The chromatography experiment was mainly concerned with the effect of the host composition on the behavior of the virus in a DEAE-cellulose column, as well as elimination of the contaminating host debris from the crude lysate. The chromatographic technique will contribute to the preparation of a more purified antigenic material which can serve as a better and more reliable NDV vaccine, and may be very important in reducing side effects on vaccinated animals due to the host debris.

The one-step growth curve of NDV in quail embryo tissue culture was carried out. The latent period of the virus was found to be five and one half hours, which was different from the one-step growth curve of NDV on tissue cultures of chick embryo cells of Levine and Sagik (1956). They found the latent period to be between 2 and 3 hours. This was different from the one-step growth curve of NDV in chick embryo lung epitehlium, as reported by Rubin, et al., (1957) who found that the time required for release of newly formed virus particles is about 80 minutes.

The conclusion was that the quail tissue culture cells were potentially capable of producing the virus. The anatomical structure and physiological behavior of these different host cells may be the factor which causes these differences in the latent period of the NDV.

The NDV was also propagated in serial passage on a line of BHK-21 cells in tissue cultures, which proved the susceptibility of this line of cells for NDV. The virus titer increased during each passage due to the adaptation of the virus to these types of host cells.

SUMMARY

Techniques of column chromatography with DEAE-cellulose have been successfully applied to the Roakin strain of Newcastle disease virus. Recovery of virus was excellent. An appreciable purification of the virus, separation of the host material, and virus hemagglutinin have been demonstrated.

Elution characteristics of the virus from infected allantoic fluid of embryonated chicken eggs labeled with H^3-uridine and H^3-valine were similar. The virus grown on the quail tissue culture cells and chick embryo tissue culture cells was different in elution behavior, and different from the virus grown on egg allantoic fluid.

The chromatographic behavior of NDV may depend more upon the chemical nature of the surfaces of these infectious agents, than upon their size. This chromatographic procedure may become useful for the removal of unwanted host material for further studies of the biochemical and immunological properties of the virus particle and its subunits, as well as in preparing a better vaccine.

A one-step growth curve of NDV on quail cells was studied and the latent period was found to be different from that of NDV grown on chick tissue culture cells and chick embryo lung epithelium cells. These differences may be due to the differences in the anatomical structure and physiological behavior of these cells.

NDV was successfully propagated in BHK-21 tissue culture. The BHK-21 cell cultured virus produced a good titer after several passages, which proved that these cells were susceptible to the Newcastle disease virus.

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ELUTION PROPERTIES OF NEWCASTLE DISEASE VIRUS FROM DEAE-CELLULOSE

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ABSTRACT

The purification and effect of different host strains on the behavior of the Roakin strain of Newcastle disease virus (NDV) was studied by fractionation on a DEAE-cellulose (N,N-diethylaminoethy-) column. The hosts were, egg allantoic membrane, quail tissue culture, and chick tissue culture.

The labeled NDV (H^3-uridine and H^3-valine) from infected chick embryo allantoic fluid was eluted at 0.33M NaCl in 0.01M tris-acetate buffer (pH 7.2). The greatest number of active virus particles were eluted in this fraction, which was high in PFU and HA-units, and low in protein content and radioactivity (cpm). Thus the virus eluted at this concentration was relatively free of extraneous protein. Most of the protein and radioactivity was eluted at 0.1M and the amounts decreased with increasing NaCl concentration. The H^3-uridine which was incorporated in the nucleic acid (RNA) was eluted mainly in the 0.15M fraction.

Results different from the above were obtained when the NDV from infected quail tissue culture was chromatographed. The greatest number of infective virus particles were eluted at 0.4M. The hemagglutinin activity was eluted at 0.18 and 0.2M and at these concentrations the protein content and the number of HA units of the eluate was high while the number of PFU found was low.

The same procedure was applied to NDV from infected chick embryo tissue culture. Most infectious virus was eluted at 0.45M, which was different from the above previous result.

These differences in chromatographic behavior may be due to the quail host cells, chick host cells and allantoic membrane cells, which may have modified the elution behavior of the NDV. Such differences in behavior must

be presumed to be due to intrinsic differences in the chemical nature of the surfaces of these particles.

The one-step growth curve of NDV in quail embryo tissue culture was carried out. The latent period was 5.5 hours as compared to 2 and 3 hours in chick embryo cells and 1.33 hours in chick epithelium lung embryo cells tested under similar conditions. These differences in the latent period of NDV in different types of host cells may be due to the differences in the anatomical structure and physiological behavior of each host cell.

BHK-21 cells showed susceptibility to this strain of NDV, and the titer of this virus increases after several passages, presumably due to the adaptation of the virus to this type of host cell.