

THE EFFECT OF NEWCASTLE DISEASE  
VIRUS ON SUSCEPTIBLE AND RESISTANT  
TISSUES CULTURED FROM CHICKENS  
AND RABBITS

Thesis for the Degree of Ph. D.

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Lenore Jones

1954

This is to certify that the  
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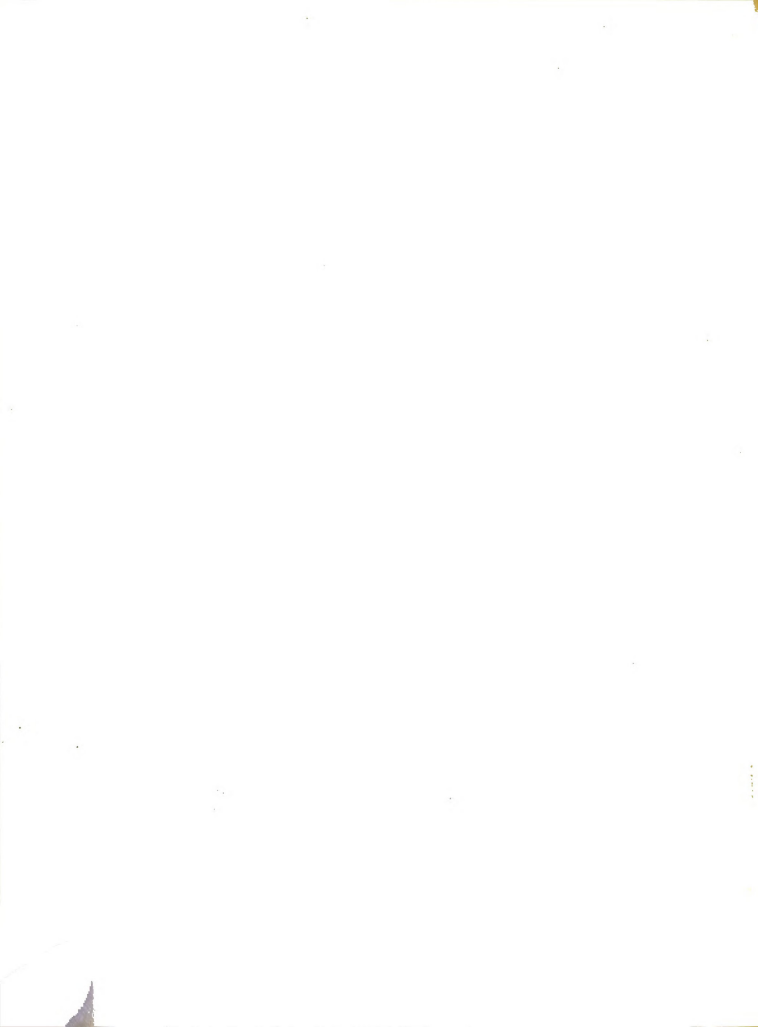
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W. J. Mack  
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Date May 21, 1954







THE EFFECT OF NEWCASTLE DISEASE VIRUS ON SUSCEPTIBLE AND  
RESISTANT TISSUES CULTURED FROM CHICKENS AND RABBITS

By

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A THESIS

Submitted to the School of Graduate Studies of Michigan  
State College of Agriculture and Applied Science  
in partial fulfillment of the requirements  
for the degree of

DOCTOR OF PHILOSOPHY

Department of Bacteriology and Public Health

1954



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Sincere appreciation is also extended to the typist, Mrs. Ardeth Frisbey and to the fellow students and members of the staff of the Departments of Bacteriology and Pathology whose cooperation and encouragement made this study possible.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry should be supported by a valid receipt or invoice. This ensures transparency and allows for easy verification of the data.

2. The second part outlines the process of reconciling the accounts. This involves comparing the internal records with the bank statements to identify any discrepancies. It is crucial to investigate these differences promptly to prevent errors from compounding.

3. The third section details the various methods used for data collection and analysis. It includes a list of key performance indicators (KPIs) that are tracked on a regular basis. These metrics provide valuable insights into the overall health and performance of the organization.

4. The final part of the document provides a summary of the findings and recommendations. It highlights the areas where the current processes are effective and identifies the key challenges that need to be addressed. The recommendations focus on improving data accuracy and streamlining the reporting process.



THE EFFECT OF NEWCASTLE DISEASE VIRUS ON SUSCEPTIBLE AND  
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AN ABSTRACT

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Approved

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In these experiments a comparative study has been made on the effect of Newcastle Disease Virus (N.D.V) on susceptible and resistant tissues cultured from chickens and rabbits.

Embryonating chicken, adult chicken lung, liver and spleen and adult rabbit lung, liver, spleen and lymph node tissues were cultured. Both normal and immune adult chickens and rabbits were used. Hemagglutination and infectivity tests were carried out to determine the growth curves of N.D.V. in embryonating chicken tissues.

The growth cycle was composed of (a) a lag phase of 24 hours, (b) a phase of rapid virus multiplication in which peak infectivity titers were reached in 36 hours and peak hemagglutination titers in 36 to 48 hours, (c) a plateau lasting 48 to 60 hours, (d) a sudden decrease in the rate of virus production 96 hours after N.D.V. inoculation.

Growth curves prepared from hemagglutination titrations on the tissues cultured from normal adult chickens and rabbits were similar to those of the embryonating chicken tissue but the peak hemagglutination titers occurred 36 to 96 hours after N.D.V. inoculation. This was also found in the immune chicken lung and spleen cultures but not in the liver cultures. In the immune chicken liver tissues and in all the tissues of the immune rabbit there was a steep decrease in infectivity titers from an LD<sub>50</sub> of  $10^{-7}$  on the day of inoculation to  $10^{-1}$  or  $10^{-2}$  48 hours later. Hemagglutination activity was recovered only in the undiluted fluids



from the immune chicken liver cultures.

Cytological degeneration was seen in the embryonating chicken cultures 15 to 48 hours after N.D.V. inoculation. The fibroblasts showed an increase in vacuolization of the cytoplasm and a tendency in the formation of giant cells which contained 3 to 5 nuclei. The only degenerative changes in the adult tissue cultures occurred in normal adult chicken liver and this did not appear until 7 or 8 days after N.D.V. inoculation. There was a gradual degeneration of the liver cells in which the cytoplasm lost its staining properties and broke up into large granules and the cell wall became fragmented. The nucleus, however, was unaltered. Neither the uninoculated cultures nor the cultures inoculated with heat-inactivated N.D.V. showed any degenerative change.



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## INTRODUCTION

The purpose of this study was to determine whether or not resistance and susceptibility of animals to Newcastle Disease Virus (N.D.V.) could be attributed to specific tissues or organs. The liver, lung and spleen of adult, normal and immunized chickens and rabbits, as well as normal chicken embryo tissue were chosen for tissue cultivation. The chicken represented the naturally susceptible, and the rabbit, the naturally resistant animals to N.D.V. As soon as these tissues attained maximal growth in tissue cultures they were infected with N.D.V. to study the morphological changes and variation in their capacity to support multiplication of the virus.

This was the specific purpose of the work but there was also the more general purpose of seeking any evidence which might contribute to a better understanding of resistance and susceptibility of animal cells to a virus.

Virologists have been limited in many of their practical studies because it has been impossible, in many cases, to detect virus activity by any means other than animal inoculation. Primary isolation of such viruses as those of hog cholera, measles and infectious hepatitis, may be accomplished in only the original host or a few large





animal species. This restricts laboratory investigations greatly due to the high cost and the time involved in maintaining these animals. Most of the recent studies pertaining to the relationship of host cells and viruses have been carried out with the purpose of finding a means of increasing their host range so that small animals may be used. In spite of persistent research, no adequate explanation has been found to account for the resistance of small laboratory animals to these viruses.

On the other hand, there exists a group of viruses which can be propagated readily in small animals as well as in the embryonating chicken egg. Consequently several methods have been developed for determining virus activity. For example, N.D.V., mumps and influenza viruses may be propagated in the embryonating chicken egg; then, the allantoic or amniotic fluids may be used as antigenic suspensions for hemagglutination, hemagglutination-inhibition and complement fixation tests. In addition, N.D.V. may be propagated in most laboratory animals which may be easily maintained for laboratory experimentation, e.g., chickens, hamsters and mice. With these viruses, therefore, the problem involves not an understanding of resistance but an explanation of the factors responsible for the susceptibility of these animals. If these factors could be determined the virologist might in turn find a means of lowering the



resistance of other small animals to the viruses of hog cholera, infectious hepatitis, etc.

### I. The Use of Tissue Culture Techniques in Studying Animal Viruses

Viruses were first propagated in tissue cultures when Parker and Nye (1925) carried vaccinia virus through a series of 11 cultures of rabbit testicular tissue. Since that time many of the viruses known to cause disease in animals have been grown in tissue cultures. This technique has been applied to many types of virus studies such as:

- (a) effects on the cells which viruses attack
- (b) site of virus multiplication
- (c) enumeration of conditions essential for virus propagation
- (d) details of the mechanisms of natural and acquired immunity caused by viruses
- (e) changes in pathogenicity which may occur as a result of cultivation of the virus in tissue cultures especially associated with the possibilities of vaccine production
- (f) determination of the effects of various chemical compounds, including antibiotic agents, on the multiplication of viruses
- (g) attempts to adapt tissue culture techniques for the purpose of rapid diagnosis of some virus diseases.

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## A. Methods of Demonstrating Virus Activity in Tissue Cultures

Two methods have been used for demonstrating the multiplication of viruses in tissue culture. The first consists of showing that the fluid withdrawn from a culture exhibits activities characteristic of the virus in question; such as, animal infectivity, hemagglutinating or complement fixing activity. The second method is the demonstration of abnormal cellular changes or an alteration in the pH of the surrounding fluid due to a decrease in tissue metabolism caused by the virus.

### 1. Animal Infectivity Tests

Animal infectivity tests have proved to be the most reliable for detecting virus multiplication and are necessary to confirm the results of serological and cytological findings. With N.D.V., for example, serial dilutions of the infected tissue culture fluids are inoculated into embryonating chicken eggs and the fifty percent mortality endpoint, LD<sub>50</sub>, is determined according to the method of Reed and Muench (1938).

### 2. Serological Tests

Hemagglutination and complement fixation tests provide more rapid means of demonstrating virus activity than do animal infectivity tests. The basic principles of the complement fixation method are the same for viruses as for bacteria. This test may be used with many viruses such as

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those of the lympho-granuloma-psittacosis group, and the mumps, influenza-N.D.V. group. The hemagglutination test has the advantage of being more rapid but is limited to a smaller number of viruses, namely the mumps-influenza-N.D.V. group.

Hemagglutination Test: Hirst (1942a) and McClelland and Hare (1941), discovered that influenza viruses agglutinate the red blood cells of fowl. Burnet (1942) reported the agglutination of fowl cells by N.D.V.-infected fluids of the embryonating chicken egg and Levens and Enders (1945) described similar agglutination of sheep red blood cells by mumps virus. The Bureau of Animal Industry (1946) standardized the hemagglutination test which is a modification of the hemagglutination procedure described by Salk (1944). This quantitative measurement has been described in the following manner:

Two-fold serial dilutions of the virus suspensions were made in physiological saline. To these dilutions a suitable concentration of a red blood cell suspension was added. After thorough shaking the cells were allowed to settle for a period of one to two hours. In the test tubes containing virus the agglutinated red blood cells deposited in a thin film covering the entire portion of the bottom of the tube, while in the tubes containing no detectable virus the cells settled out in the form of a small sharply-outlined button. The endpoint was considered to be the highest dilution of a virus producing maximal hemagglutination and the titer was expressed as the reciprocal of the final dilution of virus. This titer has also been designated as one hemagglutination unit. Thus if one hemagglutination unit were represented by a titer of 1600, two hemagglutinating units would correspond to a titer of 800 and 8 hemagglutinating units a titer of 200.





Two disadvantages in the use of the hemagglutination test for this experiment have been reported. First, when a N.D.V. suspension was treated by heating at 56° C for 40 minutes it was no longer infectious for embryonating chicken eggs but the hemagglutinating activity was only slightly decreased (Durusan, 1949). Thus the hemagglutinating titer was found to be proportional to the sum of the infections as well as the non-infectious virus particles. Secondly, measurement by hemagglutination is restricted to those suspensions in which large quantities of virus are present. An N.D.V. suspension which will infect the embryonating chicken when diluted 1,000,000,000 times will give maximal hemagglutination only up to a dilution of 1600. Gordon (1952) showed that the multiplication of N.D.V. in the embryonating chicken egg increased to a  $10^{-6}$  titer before there was any evidence of hemagglutination activity. The hemagglutinating titer only then showed a proportional increase as the infectivity titer in the embryonating chicken egg increased from  $10^{-6}$  to  $10^{-9}$ .

Hemagglutination Inhibition Test: Hemagglutination has also been widely used for antibody measurement. This adaptation of the test is called hemagglutination inhibition and is based on the principle that the hemagglutinating viruses, when mixed with homologous immune serum, lose their ability to agglutinate red blood cells. The degree of inhibition gives an estimate of the amount of antibody in



the serum. Two hemagglutination inhibition methods have been used with N.D.V. In the "alpha" procedure, serial dilutions of virus are admixed with a fixed amount of serum, whereas in the "beta" procedure a definite amount of virus suspension containing the hemagglutinating units is admixed with an equal amount of serial serum dilutions before the red blood cells are added. Brandly (1947) reported that there was a 93-percent agreement between the alpha and beta procedures.

### 3. Growth Curves Illustrating the Multiplication of Viruses in Tissue Culture

Animal infectivity tests and hemagglutination tests have been used to prepare growth curves illustrating the multiplication of viruses in tissue cultures. Of particular interest are the growth curves established for poliomyelitis virus by Salk (1952) and Syverton (1952), as well as those for influenza and mumps viruses set up by Enders (1950). Enders established growth curves for influenza and mumps viruses in embryonating chicken tissue cultures using the hemagglutination technique, whereas Salk and Syverton made their determination for poliomyelitis virus in monkey testicular tissue cultures using monkey infectivity tests.

Growth of these viruses occurred in a discrete cycle characterized by:

- (a) a lag phase - in which the virus required time for infection and multiplication in the cell

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- (b) a phase of rapid virus multiplication which reflects the release of virus from within infected cells to the extracellular fluid
- (c) a plateau phase which represented a decrease in virus production
- (d) a stage in which the virus production was reduced to zero.

Influenza virus showed a very short lag phase, then increased rapidly attaining a peak hemagglutination titer at 48 to 72 hours. Thereafter relatively little additional virus was produced. Mumps virus increased relatively slowly usually reaching maximum concentration in the fluid phase on the twelfth day of virus cultivation. There was no visual evidence of cytological changes in the tissue cultures in which either mumps or influenza virus were propagated.

The duration of the growth cycle of poliomyelitis virus was approximately 10 days. Significant amounts of virus were not released into the liquid phase until the fifth to seventh day. Occasionally, two successive cycles of poliomyelitis virus production were observed in a single culture. This was shown by a secondary rise in the quantity of the virus released into the liquid from the eighth to the eighteenth day of cultivation. By 30 days virus was not detectable either in the tissue or liquid phase. The first cycle was correlated with the destruction of cells which

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extended from explants while the second cycle occurred only if there were a sufficient number of surviving host cells within the explants.

#### 4. Abnormal Changes in Tissue Culture Caused by Viruses

Viruses have been shown to cause degenerative changes in tissue culture cells as well as the formation of characteristic intracellular inclusion bodies. Huang (1943) showed that the virus of equine encephalomyelitis completely destroyed embryonating chicken cells within 72 hours. Salk (1952) described a slower type of degeneration which was produced in the fibrocytes of monkey testicular tissue infected with poliomyelitis virus. First, there occurred various granular changes and vacuolization in the cytoplasm, then a varying degree of cytolysis and a decrease in the development of new fibrocytes surrounding the tissue explants took place. Most of the nuclei were shrunken and pyknotic, but a few appeared swollen and were stained less intensely. Bland and Canti (1935), using the tissue culture technique, found that the elementary bodies of psittacosis virus pass through a definite cycle of development and that the plaque or inclusion body is essentially a colony of elementary bodies.

The effect of a virus on tissue metabolism may be indicated by a decreased acid production in the tissue culture. Huang (1943) noted that chicken embryonating tissues from 48-hour cultures of equine encephalomyelitis virus were no longer capable of reducing methylene blue.



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10. The tenth part of the document discusses the importance of the financial system in the overall economy. It notes that the financial system is a key component of the economy, and that it is essential for the economy to be able to grow and prosper.

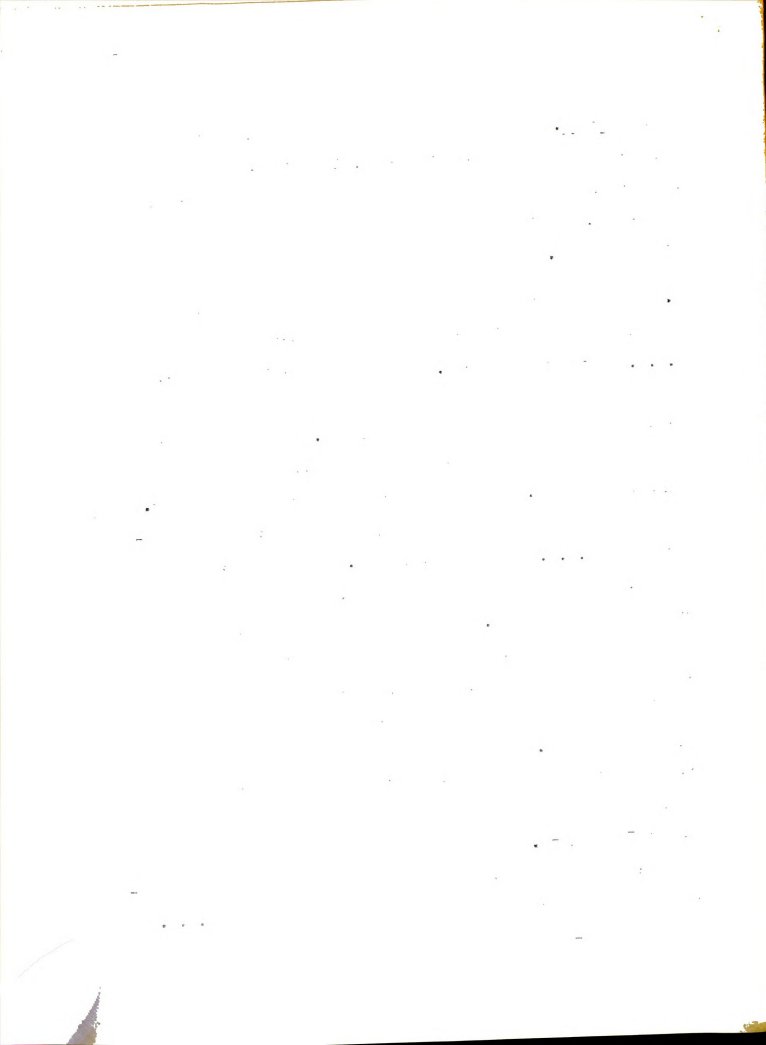
Enders et al. (1949) found that acid production in flask cultures of human embryonic brain, intestine, and skin-muscle inoculated with poliomyelitis virus after variable intervals, declined more rapidly than in control cultures without virus.

#### 5. Propagation of Newcastle Disease Virus in Tissue Cultures

Topaccio (1934) reported the first attempt to propagate N.D.V. in tissue cultures. Embryonating chicken tissue cultures were inoculated with a virus suspension prepared from the spleen of an infected chicken. Subcultures were made every three to five days and 31 successive passages were carried out. Virus was recovered from all passages.

Bankowski and Boynton (1948) also reported the propagation of N.D.V. in tissue cultures. Embryonic chicken livers, infected 36 hours previously, were cultured by the suspended cell method. After each tissue culture passage portions of the suspended cells were used for embryonating chicken egg inoculation, so that every other passage was an egg passage and not an uninterrupted series of tissue culture passages. Subcultures were made every 72 hours from the tissue culture passage to the embryonating egg and at this time the LD<sub>50</sub> of the infected tissue culture ranged from 10<sup>-4</sup> to 10<sup>-7</sup>.

Scott (1954) described a simplified tissue culture procedure for the propagation of the Roakin strain of N.D.V. in the chorio-allantoic membrane of the embryonating



chicken. Growth curves were established using infectivity tests as well as hemagglutination tests. It was found that a peak LD<sub>50</sub> titer of 10<sup>-8</sup> appeared in 24 hours and a peak hemagglutination titer of 128 appeared in 36 hours.

## II. Choice of Virus

Newcastle Disease Virus (N.D.V.) was chosen in this experiment for the following reasons. First, N.D.V. activity may be demonstrated by several laboratory procedures: by infecting the embryonating chicken egg, by the complement fixation and the hemagglutination tests. Secondly, this virus is relatively well known because of its relationship to the mumps and influenza group of viruses. A large part of recent investigations on host-cell-virus relationships have been carried out with the mumps-influenza-N.D.V. group of viruses, and, therefore, any additional findings may be more easily fitted into an explanation of the mechanisms of resistance and susceptibility. A third reason for choosing N.D.V. is the fact that it is a relatively thermolabile virus. Durusan (1949) found that N.D.V.-infected allantoic fluids were no longer infectious for the embryonating chicken egg after an incubation period of five days at 37° C. This is a significant factor in tissue culture studies because if the virus is not decreased by a temperature of 37° C (the temperature of incubation) it is difficult to prove that virus multiplication, not passive



transfer of the virus, has occurred. It follows, therefore, that, if the fluids of the N.D.V.-infected cultures still prove infectious after being incubated five days at 37° C, multiplication of the virus must have taken place.

#### A. Description of Newcastle Disease Virus

##### 1. Etiology of Newcastle Disease (synonym: avian pneumo-encephalitis)

Newcastle disease is an epornitic infection of poultry characterized by a viremia and signs of involvement of the respiratory, central nervous system, and gastro-intestinal organs. Outbreaks of Newcastle disease due to immunologically identical strains have occurred in many parts of the world; for example, in England, U.S.A., Asia, Africa and Australia. Newcastle disease primarily affects chickens and turkeys.

The etiological agent of Newcastle disease is a virus of medium size. Burnet and Ferry (1933) showed that the particle size of N.D.V. was 80-120 mu. The virus particle is filamentous (Bang, 1946) or spermlike (Cunha, et al., 1947). It is filterable through Berkefeld V. N and W candles, Chamberland L<sub>3</sub> and L<sub>5</sub> filters, and Seitz pads (ST-3). The virus may be preserved in 50 percent glycerine, in the frozen state, or by lyophilization (Burnet and Ferry, 1934). Inactivation of the virus has been accomplished by ultraviolet light when a clear suspension was irradiated by



1600-1800 A for 0.8-1.08 seconds. It may also be inactivated by a 1/5000 dilution of formalin and by N/50 sodium hydroxide in one hour (Brandly, et al., 1946a).

The maximal stability of N.D.V. lies between pH 4 to pH 11 (Brandly, Moses and Jones, 1946d). Miller and Stanley (1944) found that the pH of the medium had only a minor effect on the hemagglutinating activity of the virus in a range of pH 6 to pH 8.

Burnet (1945) found that, if the allanto-amniotic fluid suspension of N.D.V. was held at 65° C for 15 minutes, there was complete loss of infectivity but the hemagglutinating activity was still 50 percent of the original titer. Durusan (1949) also found that the hemagglutinating activity of the N.D.V.-infected allantoic fluid was more thermostable than the infectivity of the virus. The initial LD<sub>50</sub> of the virus was 10<sup>-9.32</sup> and its hemagglutinating titer was 1280. When the virus was stored for 13 weeks at 4° C the infectivity titer decreased to 10<sup>-1.62</sup> whereas the hemagglutinating titer remained constant. When held at room temperature (22°-27° C) for 30 days the infectivity decreased to less than 10° but the hemagglutinating titer decreased only from 1280 to 640. Durusan also found that when the virus suspension was held at 62° C there was complete loss of infectivity after 15 minutes but hemagglutinating activity was not lost until after 25 minutes.



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5. The fifth part of the document describes the process of preparing the annual financial statements. It requires that the statements be prepared in accordance with the relevant accounting standards and regulations.

6. The sixth section outlines the process of presenting the financial statements to the board of directors. It states that the board is responsible for reviewing the statements and approving them for publication.

7. The seventh part of the document discusses the importance of maintaining the confidentiality of the financial information. It requires that all financial records be stored securely and that access be restricted to authorized personnel only.

8. The eighth section outlines the process of archiving the financial records. It states that all records must be retained for a minimum of seven years after the end of the financial year.

9. The ninth part of the document discusses the process of conducting an external audit. It requires that the external auditors be independent and qualified to audit the financial statements.

10. The tenth and final section of the document discusses the process of responding to the findings of the external audit. It states that the company must take prompt action to address any deficiencies identified by the auditors.

The correlation of this study and Durusan's experiment on the thermolability of N.D.V. at 37° C has already been mentioned. He found that when the virus suspension was incubated at 37° C the initial LD<sub>50</sub> of 10<sup>-9.32</sup> dropped to 10<sup>-5.5</sup> after one day, to 10<sup>-3.62</sup> after two days, to less than 10<sup>-3</sup> after three days and to 10<sup>0.21</sup> after four days. During the same period, however, the hemagglutination titer changed only from 1280 to 640 and remained at 640 for more than eight days. For this reason it is evident that the hemagglutination test is of value only when an increase in hemagglutination titer occurs.

## 2. Host Range of N.D.V.

Newcastle disease occurs naturally in chickens, turkeys, pheasants, guinea fowl, sparrows, crows, francolines and parrots (Beaudette, 1942). Experimental infection has been achieved in chick embryos, ducks, geese, pigeons and several varieties of wild birds. Mice, hamsters, cotton rats and monkeys may be infected experimentally with N.D.V. resulting in a meningo encephalitis. Over 300 serial passages in hamsters and 30 serial passages in mice have been carried out (Reagan, 1952a, b). Several accidental infections have occurred in human beings handling infected fowl or working with the virus (Burnet, 1943; Hungherr, et al., 1949).

Rabbits, cows, horses, pigs, sheep, ferrets and guinea pigs are considered to be resistant to N.D.V. (Beaudette,

1948

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1943). A meningo-encephalitis may occur in rabbits after intracerebral inoculation but the virus has not been transferred from rabbit to rabbit.

### 3. Description of Newcastle Disease in Chickens

The present opinion is that the pneumonic and central nervous system (CNS) symptoms deominate the American type of Newcastle disease, while respiratory and gastro-intestinal symptoms are most prevalent in the Asian and European type (Brandly, et al., 1946a). In naturally-infected chickens the incubation period is 4 to 11 days and is followed by characteristic symptoms of pneumonia or central nervous system involvement. Death may ensue after two to four days.

### 4. Distribution of N.D.V. in Animal Tissues

Karzon and Bang (1950) and Hofstad (1951) reported the following results from quantitative studies of the American type of N.D.V. in tissues of experimentally-infected chickens. When the virus was inoculated by the intranasal, intravenous or intramuscular routes it was recovered from the blood, spleen, kidneys, lung, brain, and trachial and intestinal contents within 24 hours. Virus concentrations as high as  $10^{-5}$  and  $10^{-7}$  (embryo lethal doses) were found by the fourth day then gradually decreased until the virus could no longer be isolated after the seventh day. Neutralizing antibodies against N.D.V. and hemagglutinin-inhibiting antibodies were demonstrated five to seven days after N.D.V. inoculation;

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thus, the virus disappeared as soon as the antibody concentration in the blood became relatively high. The virus neutralization and the hemagglutination-inhibition tests yielded parallel results early in the convalescent period but later (22 to 100 days) the hemagglutinin-inhibiting activity of the serum showed a steep decrease.

Newcastle disease virus has been isolated from the liver of infected chickens but never in sufficiently high concentrations to yield consistent titers.

#### 5. Growth of N.D.V. in the Embryonating Egg

When N.D.V. was inoculated into the allantoic cavity of the embryonating chicken egg it was rapidly adsorbed by the cells of the allantoic membrane, (Gordon, 1952). Within the first hour the  $LD_{50}$  titer decreased from  $10^{-4.5}$  to  $10^{-1.6}$  and the hemagglutinating activity titer was lost. The infectivity titer increased when tested every four hours, until it reached  $LD_{50}$  titers of  $10^{-6}$  at 12 hours and  $10^{-8.3}$  at 16 hours. The hemagglutinating activity did return after 12 hours; then, the titer increased from 10 to 1280 by the 24th hour. Meanwhile, the infectivity titer showed no increase in the 16 to 48 hour period.

#### 6. Histopathology of Newcastle Disease in Adult Chickens

Grossly the disease in fowl produced multiple focal necrosis in the viscera, and hemorrhages, especially in the respiratory and alimentary tracts; at times, an interstitial pneumonitis was observed (Brandly, et al., 1946a, b).

1. The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for the integrity of the financial system and for the ability to detect and prevent fraud.

2. The second part of the document outlines the various methods used to collect and analyze data. It describes the use of statistical techniques to identify trends and anomalies in the data, and the importance of using reliable sources of information.

3. The third part of the document discusses the role of the auditor in the process. It explains that the auditor's primary responsibility is to provide an independent and objective assessment of the financial statements. This involves a thorough review of the records and a comparison of the results with the applicable accounting standards.

4. The fourth part of the document discusses the importance of transparency and accountability in the financial system. It explains that transparency allows stakeholders to make informed decisions based on the available information, and accountability ensures that those responsible for the financial system are held to a high standard of performance.

5. The fifth part of the document discusses the role of the government in the financial system. It explains that the government has a responsibility to ensure that the financial system is stable and sound, and that it is able to provide the services that are needed to support the economy.

6. The sixth part of the document discusses the importance of risk management in the financial system. It explains that risk management is the process of identifying, measuring, and managing the risks that are associated with the financial system. This is essential for ensuring the stability and soundness of the financial system.

7. The seventh part of the document discusses the importance of international cooperation in the financial system. It explains that the financial system is a global system, and that it is essential for countries to work together to ensure its stability and soundness.

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10. The tenth part of the document discusses the importance of regulation in the financial system. It explains that regulation is essential for the financial system to function properly, and that it is important to ensure that the financial system is subject to a high standard of regulation.

Cytologically, the exudative lesions in the lung consisted of cellular and serous accumulations in the peribronchial alveoli with extension into the lumen of the regional tertiary branches. The exudate was composed of various types of desquamated pulmonary cells including swollen, vacuolated (foam) cells, and disintegrating heterophiles and erythrocytes. The central nervous system showed perivascular infiltration, a honey-combed appearance of degenerated neurons and gliosis near the altered neurons in the lumbar cord, medulla and cerebellar nuclei (Brandly, et al., 1946b). In addition to focal necrosis in the liver, pericarditis, and secondary inflammatory changes in the abdominal air sacs Fukushima (1932) found hyperplasia of the intestinal lymphoid patches with occasional necrosis and hyaline degeneration of the splenic follicles with development of network-like homogeneous masses.

#### 7. Histological Changes in Embryonating Chickens due to N.D.V.

Cytological changes in embryonating chicken eggs due to N.D.V. were first described by Burnet (1942). When the virus was inoculated into the amniotic sac, multiple chorio-allantoic hemorrhages, hemorrhagic encephalitis and respiratory lesions appeared within 36 hours. In sections of the lung cellular changes were seen to be widespread. The earliest change recognizable was a distortion of the nucleus of epithelial cells giving a clear oval area within which



the chromatin was piled up into masses of various shapes and distribution. In tracheal smears large vacuolated cells were found arranged in agglutinated masses in which it was impossible to pick out the outlines of individual cells. Some cells showed a nucleus broken into many heavily stained circular fragments as well as a great degree of cytoplasmic degeneration.

#### B. The G.B. Strain of Newcastle Disease Virus

The G.B. strain of N.D.V. was chosen for this work because it is a well-known field and laboratory strain and it is highly pathogenic for chickens. This strain was isolated in May, 1948 by Boney and Grumbles from an outbreak of a severe nervous and respiratory infection of chickens (Boney, 1948). Chickens infected experimentally with the virus show different symptoms depending upon the route of inoculation. Those exposed by intramuscular injection develop a profuse diarrhea, weakness, and general paralysis with tonic-clonic symptoms. The same symptoms occur in chickens exposed by the intranasal route but there is usually an interstitial pneumonia as well. Morbidity and mortality up to 100 percent may occur in young susceptible chickens. The  $LD_{50}$  for 10-day old embryonating chicken eggs is  $10^{-9}$ .

## MATERIAL AND METHODS

### A. Description of Procedure of the Experiment

#### 1. Choice of Tissue Culture Technique for Virus Propagation

The simplest procedure which could be readily adapted to large-scale tissue cultivation was chosen. The tissues were minced, then embedded in a plasma matrix in the bottom of a Petri dish (9 cm x 1.5 cm). When the tissues became fixed in the plasma they were flooded with Hank's nutrient solution. In this way it was possible to prepare and maintain several hundred Petri dish cultures at one time.

This method provided an additional advantage in that representative cultures could be examined microscopically at a magnification of 100x to 970x. This was accomplished by simply removing the nutrient fluid, covering the tissue explant with a glass cover slip and lowering the objective of the microscope into position directly over the tissue growth. For comparative studies and photomicrographs permanent preparations were made of the Petri dish cultures by staining the tissue with Giemsa stain.

#### 2. Preparations for Tissue Cultures

Heparinized plasma was used for the mat in which the tissues were embedded. The plasma was filtered through Seitz filter (St-3), heated in a water bath at 56° C for

30 minutes and tested for bacterial sterility as well as hemagglutinating-inhibiting properties. All the plasma used showed a hemagglutinating-inhibition titer of less than  $1/40$  which according to the standards of the Bureau of Animal Husbandry is considered to be within the normal range. A quantity of 0.5 to 1.0 ml of plasma was sufficient to cover the bottom of a Petri dish.

Hank's nutrient medium, a balanced physiological solution, recommended by Enders (1950) as a tissue culture nutrient, was used for all cultures. Ten milliliters of the nutrient solution was sufficient to cover each Petri dish culture and to sustain growth of the cells for a period of four to seven days. Phenol red, an indicator with a color range between pH 6.8-8.4 was incorporated in this solution. The hydrogen ion concentration of the nutrient was adjusted to a pH 7.2-7.4 before it was added to the cultures. The G.B. strain of N.D.V. was used for both tissue culture inoculation and animal immunization. The cultures were inoculated by replacing the fluids with the N.D.V.-infected allantoic fluid, diluted  $1/200$  in Hank's nutrient solution. This dilution represented eight hemagglutinating N.D.V. units. The succeeding fluid changes were made with Hank's nutrient solution alone.

In the first two experiments all fluids were checked for bacterial sterility using blood plates and N.I.H. thioglycollate sterility-broth, but this was discontinued when

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it was found that the Petri dish tissue cultures provided a more sensitive test for bacterial growth. Often the broth and blood plates failed to show bacterial contamination while the stained Petri dish cultures showed a few bacteria.

Bacterial and mycotic contamination was reduced to less than five percent by incorporating 100 units of penicillin, 500 micrograms of streptomycin and two Candida albicans units of endomycin\* in each milliliter of Hank's nutrient solution. Endomycin, a new antibiotic, was first prepared by Gottlieb (1951). It inhibits the growth of a wide variety of fungi pathogenic to plants and animals, many gram positive and a few gram negative bacteria.

Chemically clean glassware was obtained by washing with the detergent Tetra D and then rinsing twice in tap water and twice in distilled water.

### 3. Outline of the Experiment

Ten to twenty Petri dish cultures were prepared for each "tissue culture group" in order to provide a sufficiently large number of representative cultures for each virus inoculum (Table I). The term "tissue culture group" was coined for convenience to designate five to ten Petri dish cultures of one type of tissue embedded in either rabbit or chicken plasma. Each group consisted of tissues

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\*The author is grateful to the Upjohn Company for supplying the endomycin utilized in this experiment.

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2. The second part of the document outlines the specific procedures that should be followed when recording transactions. This includes the use of standardized forms and the requirement that all entries be supported by appropriate documentation, such as invoices and receipts.

3. The third part of the document discusses the importance of regular audits and reviews of the financial records. It notes that these activities are necessary to identify any errors or discrepancies and to ensure that the records are accurate and complete.

4. The fourth part of the document provides a detailed description of the accounting system that will be used by the organization. This includes information about the software that will be used and the specific accounts that will be maintained.

5. The fifth part of the document discusses the responsibilities of the various personnel involved in the financial management process. It outlines the roles and duties of the accounting staff and the management, and provides guidance on how they should interact with each other.

6. The sixth part of the document provides a summary of the key points discussed in the document and offers some final thoughts on the importance of maintaining accurate financial records.

## FIGURE I

### OUTLINE OF THE EXPERIMENT

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The following is an outline of one complete experiment which comprised 27 "tissue culture groups". Each tissue culture group consisted of all the cultures prepared from one type of tissue embedded in either chicken plasma or in rabbit plasma, for example, group 1 represented all the embryonating chicken tissues embedded in chicken plasma, which included tissue receiving (a) heat-inactivated N.D.V., (b) active N.D.V., and (c) control cultures which were Hank's nutrient solution instead of N.D.V. solution.

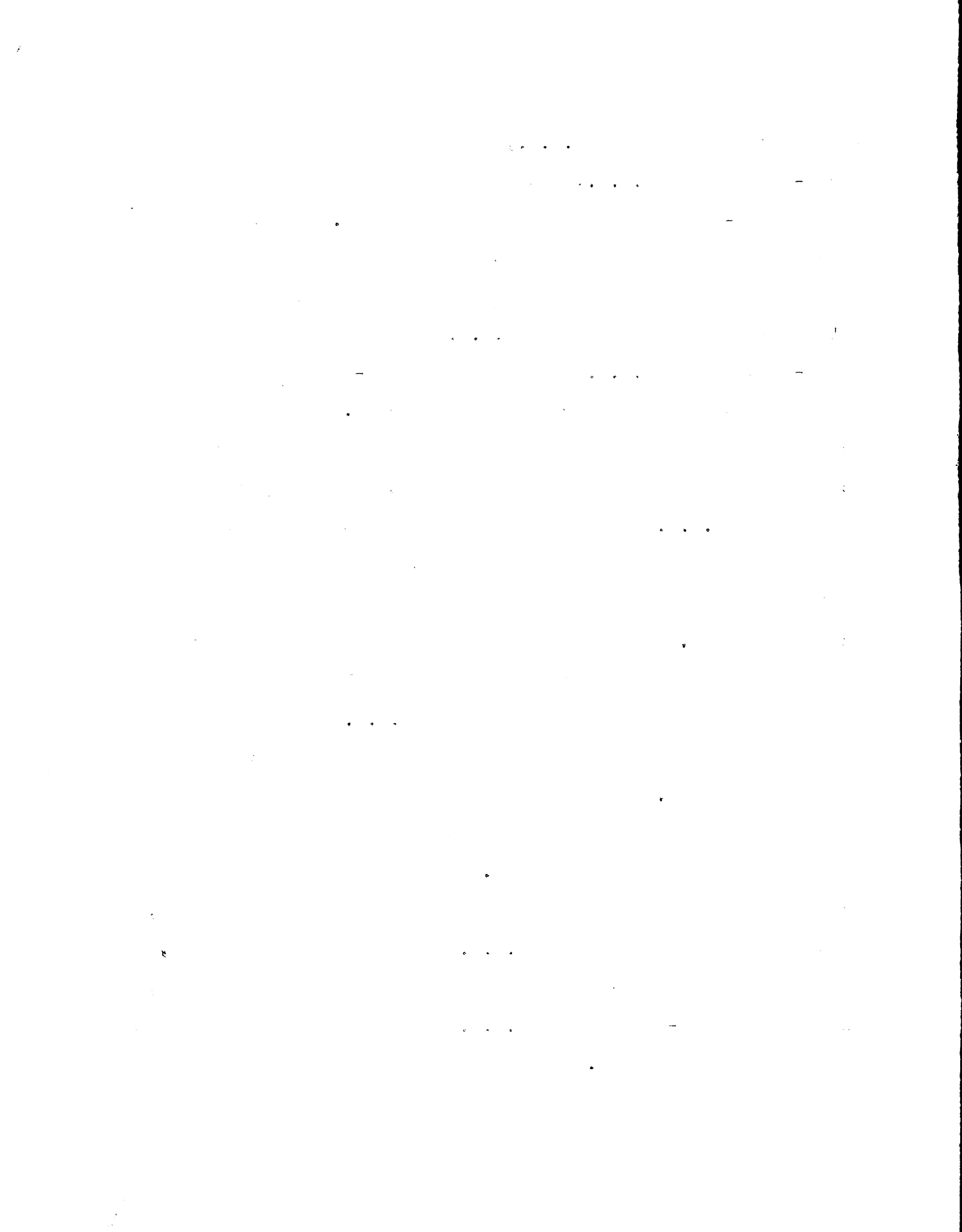
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- A. Cultures of tissues from embryonating chicken egg
    - 1. Embryonating chicken tissue embedded in normal chicken plasma
    - 2. Embryonating chicken tissue embedded in normal rabbit plasma
  
  - B. Cultures of tissues from normal adult chickens
    - 3. Lung tissue embedded in normal chicken plasma
    - 4. Lung tissue embedded in normal rabbit plasma
    - 5. Liver tissue embedded in normal chicken plasma
    - 6. Liver tissue embedded in normal rabbit plasma
    - 7. Spleen tissue embedded in normal chicken plasma
    - 8. Spleen tissue embedded in normal rabbit plasma
  
  - C. Cultures of tissues from immune adult chickens
    - 9. Lung tissue embedded in normal chicken plasma
    - 10. Lung tissue embedded in normal rabbit plasma
    - 11. Liver tissue embedded in normal chicken plasma
    - 12. Liver tissue embedded in normal rabbit plasma
    - 13. Spleen tissue embedded in normal chicken plasma
    - 14. Spleen tissue embedded in normal rabbit plasma
  
  - D. Cultures of tissues from normal adult rabbits
    - 15. Lung tissue embedded in normal chicken plasma
    - 16. Lung tissue embedded in normal rabbit plasma
    - 17. Liver tissue embedded in normal chicken plasma
    - 18. Liver tissue embedded in normal rabbit plasma
    - 19. Spleen tissue embedded in normal chicken plasma
    - 20. Spleen tissue embedded in normal rabbit plasma
  
  - E. Cultures of tissues from immune adult rabbits
    - 21. Lung tissue embedded in normal chicken plasma
    - 22. Lung tissue embedded in normal rabbit plasma
    - 23. Liver tissue embedded in normal chicken plasma
    - 24. Liver tissue embedded in normal rabbit plasma
    - 25. Spleen tissue embedded in normal chicken plasma
    - 26. Spleen tissue embedded in normal rabbit plasma
    - 27. Lymph node tissue embedded in normal rabbit plasma
-

inoculated with active N.D.V., plus tissues inoculated with heat-inactivated N.D.V., plus five to ten Petri dish cultures of the non-infected tissue used for controls. Thus, the first tissue culture group listed in Table I included the cultures of normal adult chicken lung embedded in chicken plasma which received active N.D.V. and those which received heat-inactivated N.D.V. as well as the non-infected lung tissue cultures which were used as controls. Five to ten cultures were prepared for each tissue so that representative cultures could be withdrawn at different intervals, to be tested for N.D.V. activity and the tissue stained by the Giemsa method for microscopic examination and enough cultures would be left for observation until the end of the experiment. The tissues were embedded in both rabbit and chicken plasma to determine if either plasma would enhance or suppress multiplication of N.D.V., or if the ability to support growth of the virus was a property of the tissue alone.

An equal number of tissue cultures was maintained as controls during the same period. These cultures were subjected to the same conditions, and the same fluid changes, with the exception that the N.D.V. suspension was not added. In one experiment an additional set of controls was included, in which a heat-inactivated N.D.V. suspension was added to each type of tissue culture. This virus suspension was incubated for seven days at 37° C so that it was no longer infectious





for the embryonating chicken egg but retained a hemagglutinating titer of 1/800.

#### 4. Description of Tissue Culture Technique

The adult tissues used consisted of lung, liver and spleen from normal, as well as N.D.V.-immune rabbits and chickens. Lymph node tissue from normal and immune rabbits was also cultured. Embryonic tissue, consisting of chorio-allantoic membrane, brain and torso of 11-day embryonating chicken eggs were used for all preliminary studies because they were most easily obtained and gave consistently uniform growth of fibrocytes and round cells.

Fragments of the tissues from animals were minced within one hour after removal. Each tissue was placed in a Petri dish and flooded with warm nutrient solution, washed free of extraneous blood and tissue fluids and then transferred to a fresh nutrient solution where it was cut with a fine, curved scissors. The average fragment size approximated 0.1 mm in diameter.

These small fragments were embedded in the thin layer of normal chicken or rabbit plasma covering the bottom of a Petri dish and allowed to become fixed before Hank's nutrient solution was added. Then the tissue cultures were incubated for four days at 37° C. The nutrient fluid was not changed during this period unless the medium became acid. This occurred usually with embryonic and splenic

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4. The fourth section discusses the role of the auditor in verifying the accuracy of the records. It notes that the auditor has the right to request any supporting documents and to conduct a physical count of the cash on hand.

5. The fifth part of the document addresses the consequences of failing to maintain proper records. It states that any individual found to be deliberately falsifying records may be subject to disciplinary action, including suspension or termination.

6. The sixth section provides information on the penalties for non-compliance with the financial reporting requirements. It mentions that individuals who fail to provide accurate records may face fines or legal action.

7. The seventh part of the document discusses the importance of confidentiality in handling financial information. It requires that all records be stored securely and that access be restricted to authorized personnel only.

8. The eighth section outlines the process for archiving old records. It states that records that are no longer needed for operational purposes should be properly stored in a secure location for a specified period.

9. The ninth part of the document discusses the importance of regular audits. It notes that regular audits help to identify potential areas of improvement and to ensure that the financial reporting process remains effective.

10. The tenth and final section of the document provides a summary of the key points discussed. It reiterates the importance of accuracy, transparency, and accountability in all financial transactions.

tissue. The four-day incubation period was sufficient to assure the growth of even the most slowly developing adult tissues so that all cultures could be maintained simultaneously, be given the same virus inoculum, and be subjected to the same experimental conditions.

On the fourth day the nutrient fluid was withdrawn aseptically using separate pipettes for each culture to avoid cross infection. The cultures were then flooded with Hank's nutrient solution containing eight hemagglutinating units of N.D.V. On the fourth, eighth, twelfth, and eighteenth days after virus infection, the nutrient solution was removed and replaced with fresh Hank's nutrient fluid. Samples of the fluid from each culture were withdrawn at intervals of 2, 6, 12, 24, 36, 48, 72 and 96 hours after the N.D.V.-infected suspension was added, as well as just before and two hours after each subsequent fluid change. Samples from each type of tissue culture were pooled for each incubation period and stored at  $-20^{\circ}$  C until they could be tested for virus activity. Petri dish cultures representing similar incubation periods were stained by the Giemsa method for microscopic examination.

## B. Methods Used for Determining N.D.V. Activity in Infected Tissue Cultures

### 1. Infectivity Tests Using Embryonating Chicken Eggs

All samples of the tissue culture fluids were tested for N.D.V. activity by inoculating 0.2 ml of the fluid into



the allantoic cavity of 10-day embryonating chicken eggs. Infectivity tests were also carried out on suspensions of each type of tissue which were prepared at the time they were removed from the animals.

If N.D.V. was present in the tissue culture fluids or in the suspensions of the animal tissues, death of the embryo occurred within two to five days. At the time of death the eggs were placed in the refrigerator ( $+4^{\circ}$  C) for six hours and then the allantoic fluids were withdrawn and tested for bacterial sterility as well as hemagglutinating activity.

Quantitative infectivity tests were carried out with the samples of tissue culture fluids which showed positive infectivity in order to determine the 50 percent mortality endpoint ( $LD_{50}$ ). Samples from culture fluids of embryonating chicken tissue were tested at intervals of 2, 6, 12, 24, 48, 72 and 96 hours after N.D.V. inoculation to determine the titer of virus. The  $LD_{50}$  titers of the fluids removed from rabbit and chicken tissue cultures 96 hours after virus inoculation were also determined.

The eggs which were used were received from W. S. Hannah and Sons of Grand Rapids, Michigan. These eggs were collected from flocks which had not received N.D.V. vaccine.

In order to confirm the work of Durusan (1949), concerning the thermolability of N.D.V. at  $+37^{\circ}$  C, infectivity tests were carried out with samples of an undiluted



N.D.V.-infected allantoic suspension incubated at  $+37^{\circ}$  C from one to seven days. Serial dilutions were also set up before incubation to determine if the diluted virus would be more thermolabile than the undiluted virus. Hemagglutination tests as well as quantitative infectivity tests were carried out with samples of these solutions at 24-hour intervals up to seven days.

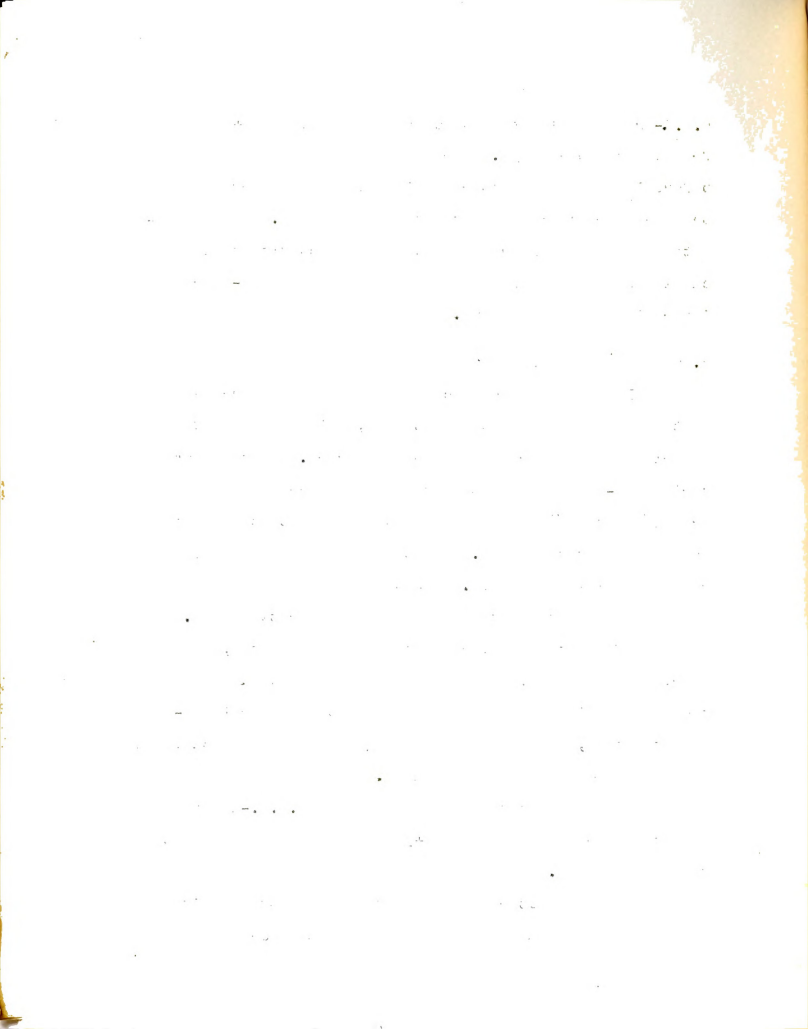
## 2. Hemagglutination Tests

Pooled samples from each type of tissue culture were tested for hemagglutinating activity, using the procedure described by the Bureau of Animal Industry. In this method serial two-fold dilutions of the virus suspension ranging from 1/100 to 1/6400 were prepared and to each test tube an equal quantity of a 0.25 percent suspension of chicken red blood cells was added. The test tubes were shaken and allowed to stand at room temperature for two hours. Results of the test were recorded after 30 minutes, one hour, and two hours. The endpoint was considered to be the highest dilution of the virus producing maximal hemagglutination, and the titer was expressed as the reciprocal of the final dilution of the virus.

The hemagglutinating activity of the N.D.V.-infected allantoic fluids incubated at  $+37^{\circ}$  C from one to ten days were also tested.

In order to determine if the tissues of the rabbit and chicken possessed either hemagglutinating or





hemagglutination-inhibiting activity before they were used for tissue cultures, ten percent suspensions of each type of tissue were prepared, centrifuged and the supernatant fluids were withdrawn for these tests. In the hemagglutination-inhibition test a modified B-procedure was used, two units of N.D.V. suspension admixed with serial two-fold dilutions of the tissue fluids ranging from the undiluted fluid to a  $1/64$  dilution.

### 3. Methods Used for Animal Immunization

Young adult rabbits and chickens were given four 0.5 milliliter doses of N.D.V.-infected allantoic fluid intranasally at weekly intervals. For the first and second inoculations, heat-inactivated virus was given. Active N.D.V.-infected allantoic fluid was then used for the third and fourth inoculations as well as for the challenging dose which was given three weeks later. Three months after the first inoculation the animals were bled and their sera were tested for hemagglutinin-inhibiting activity. Those animals showing a titer of at least  $1/320$  were killed and their tissues removed for tissue cultivation. In this case the standard Beta procedure for the hemagglutination-inhibition test was used. A suspension containing 10 units of N.D.V. was mixed with an equal quantity of serial two-fold dilutions of serum, and incubated at room temperature for 10 minutes before adding 0.25 percent suspension of chicken red blood cells.

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry should be clearly documented, including the date, amount, and purpose of the transaction. This ensures transparency and allows for easy reconciliation of accounts.

In the second section, the author outlines the various methods used to collect and analyze data. These methods include direct observation, interviews, and the use of specialized software tools. Each method is described in detail, highlighting its strengths and limitations.

The third section focuses on the results of the data analysis. It presents a series of tables and graphs that illustrate the trends and patterns observed in the data. The author provides a detailed interpretation of these results, explaining their significance and how they relate to the overall objectives of the study.

Finally, the document concludes with a summary of the findings and a discussion of the implications for future research. The author suggests several areas for further investigation and provides recommendations for how the findings can be applied in practice.

## RESULTS

### A. Thermolability of the Virus

The undiluted solution of the G.B. strain of N.D.V. showed a similar thermolability when incubated at  $+37^{\circ}$  C as did the strain of N.D.V. used by Durusan. All of the G.B. solutions which were diluted beyond  $10^{-1}$  showed a sharper decrease in the  $LD_{50}$  titers within four days. After two days the  $LD_{50}$  titers of the diluted solutions decreased to  $10^{-4}$ , after three days to  $10^{-1}$  and after four days the virus was completely inactivated. The hemagglutination titers decreased from 1600 to 800 during the first 24 hours, then remained at this titer for the seven-day incubation period. Since the virus inoculum for the tissue cultures consisted of a 1/200 solution these hemagglutination titers would correspond to 16 and 8 respectively. It follows, therefore, that if virus was recovered from the tissue culture solution which showed an infectivity titer higher than  $10^0$  or a hemagglutination titer higher than 8, multiplication of the virus had taken place in the tissues.

Similarly, if the hemagglutination titer of the undiluted N.D.V.-infected allantoic fluid was 1600, this fluid, when diluted to 1/200 to contain eight hemagglutinating units represented a hemagglutinating titer of eight.

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For example, the N.D.V. inoculum containing eight hemagglutinating units could be diluted serially 1/2, 1/4, 1/8 and still produce maximal hemagglutination. It follows, then, that when a titer higher than eight could be recovered from a tissue culture fluid, that tissue had supported growth of the virus.

### B. Results of Hemagglutination and Infectivity Tests

Infectivity and hemagglutination tests were negative on all the control tissues and tissue cultures, i.e., in samples of all the tissue removed from the animal and in all cultures of these tissues which were not given the N.D.V. inoculation. Infectivity tests were negative also on all tissues which received heat-inactivated N.D.V. None of the tissue suspensions from the immune chicken or rabbit showed hemagglutination-inhibiting activity.

#### 1. Hemagglutination Tests with Tissue Cultures Given Heat-Inactivated N.D.V.

When the heat-inactivated N.D.V. suspension was added to the tissue cultures the initial hemagglutination titer remained constant in all the cultures with the exception of those of the lung and liver of the immune chicken and the tissues cultured from the immune rabbit. The titer of the fluids from the lung and liver of the immune chicken and the lung and spleen of the immune rabbit dropped to a titer of four within two hours, and remained constant



during the next 94 hours. The titer of the fluids removed from the liver cultures of the immune rabbit also decreased to four then to zero within 24 hours. None of the fluids withdrawn from any of the tissue cultures after the first nutrient change showed any hemagglutination activity (Figs. II-VI).

## 2. Hemagglutination and Infectivity Tests with Embryonating Chicken Tissue Culture

Embryonating chicken tissue cultures were used for all the preliminary tests to determine the growth curve of N.D.V. in tissue cultures (Fig. II). Within two hours after virus inoculation the fluids from these tissue cultures showed a rapid decrease of hemagglutinating activity from eight to two due to adsorption of the virus by the tissue. Within six hours hemagglutination occurred only in the undiluted fluids and did not increase in titer until after 24 hours. Fluids from embryonating chicken tissues which were embedded in chicken plasma thereafter showed a rapid increase in hemagglutination titer reaching a peak titer of 64 in 36 hours. This titer was constant from 36 to 96 hours, then disappeared for two to six hours after the first fluid change. After eight days hemagglutinating activity appeared in the undiluted fluids but was lost completely after the second fluid change.

In the fluids from the embryonating chicken tissues which were embedded in rabbit plasma the hemagglutinating



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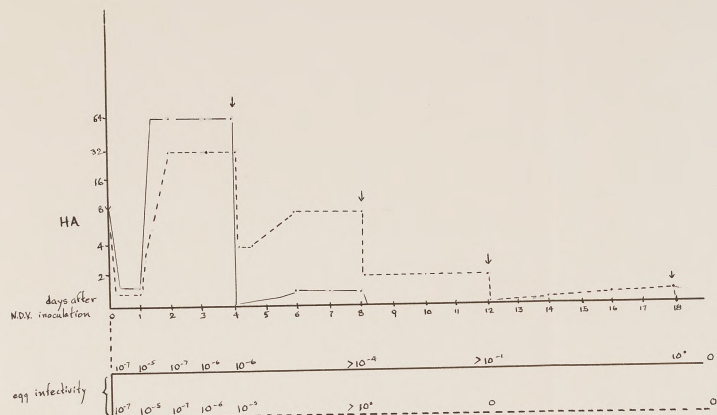
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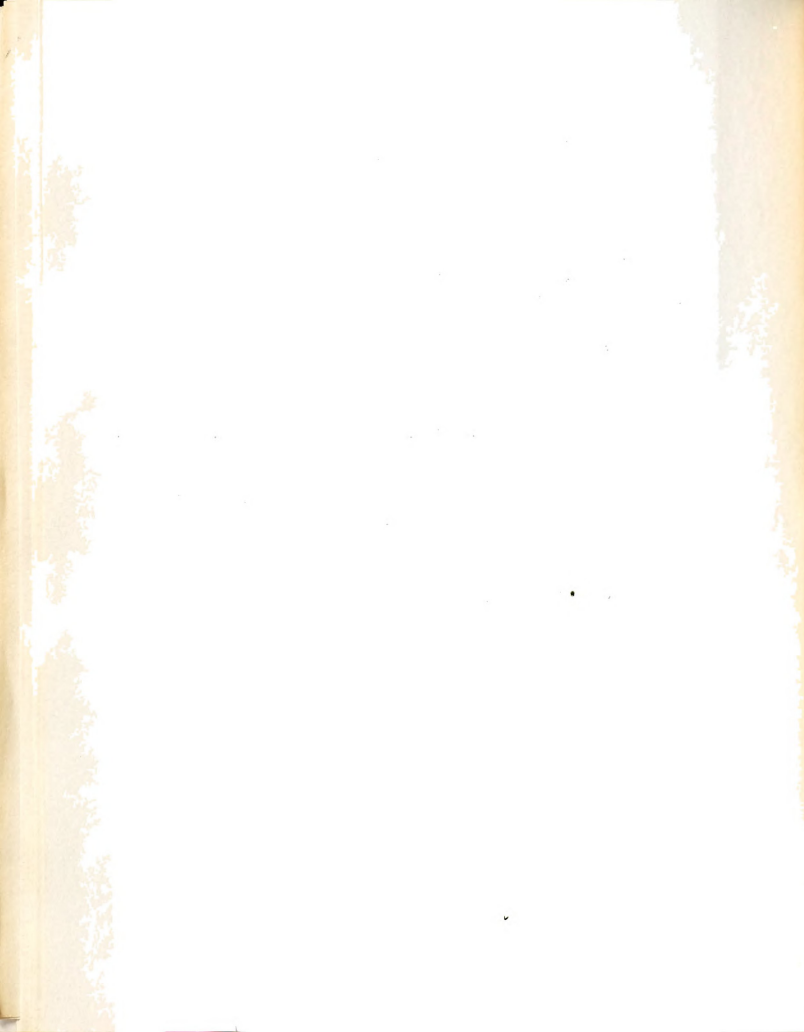
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HEMAGGLUTINATION AND INFECTIVITY TITERS OF N. D. V. IN EMBRYONATING CHICKEN TISSUE CULTURES EMBEDDED IN CHICKEN AND IN RABBIT PLASMA

FIG. II

———— N. D. V. TITERS IN CULTURES EMBEDDED IN CHICKEN PLASMA  
 - - - - - N. D. V. TITERS IN CULTURES EMBEDDED IN RABBIT PLASMA



activity increased more slowly reaching a peak titer of 32 within 48 hours. After the first fluid change this titer decreased from 32 to four but then rose again to eight on the sixth day and remained at this titer until the eighth day. After the third fluid change hemagglutinating activity could still be recovered from the unutiluted fluids of the embryonating chicken tissues which had been cultured in a rabbit plasma matrix.

Fluids were withdrawn from these tissue cultures at intervals of 2, 24, 48, 72 and 96 hours after N.D.V. inoculation for infectivity and hemagglutination tests. Since the virus inoculum consisted of a 1/200 solution the LD<sub>50</sub> of the initial solution was 10<sup>-7</sup>. This titer dropped to 10<sup>-5.82</sup> within 24 hours but rose to an LD<sub>50</sub> of 10<sup>-7</sup> within 36 hours, then decreased to 10<sup>-6.39</sup> after 72 and 96 hours. Fluids from the embryonating chicken tissue embedded in chicken plasma showed infectivity up to eight days while those embedded in rabbit plasma were infectious for 12 days.

### 3. Hemagglutination and Infectivity Tests with Rabbit and Chicken Tissue Culture

There was little difference between the hemagglutination titers of the tissues which were embedded in a rabbit plasma and in chicken plasma matrix (Figs. III-VI).

Hemagglutination Tests: When active N.D.V. was added to the tissue cultures of the rabbit and chicken their hemagglutination titer decreased from eight to four or one



within two to six hours (Figs. III-VI). This was followed by a period of rapid virus production in which the titers rose to 32 or 64 within 36 to 96 hours in all the tissue cultures except those of the liver (Figs. IIIb, IVb) and spleen (Figs. IIIc, IVc) of the immune chicken and in the tissues from the immune rabbit (Figs. V, VI). Fluids from tissue cultures of spleen from the immune chicken showed a hemagglutination titer of 16 within 48 hours but did not rise above this titer (Fig. IIIc, IVc). The most remarkable loss of hemagglutinating activity was found in the fluids removed from the immune chicken liver cultures (Figs. IIIb, IVb) and in all the tissues cultured of the immune rabbit (Figs. V, VI). In the period within two to 96 hours following N.D.V. inoculation, hemagglutinating activity was found only in the undiluted fluids from the immune chicken liver cultures, and disappeared after the first fluid change. In the immune rabbit tissue cultures the hemagglutination titer decreased to one after two hours but disappeared completely within 24 hours.

It was found that the greatest variation in hemagglutinating activity in the tissue cultures occurred during the period after the first fluid change, i.e., from four to eight days after N.D.V. inoculation (Figs. III-VII). By the end of this period all the normal rabbit and chicken tissue cultures, with the exception of the normal chicken lung, showed a hemagglutination titer of four. In the normal chicken lung the hemagglutination titer increased

1. The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for the integrity of the financial system and for the ability to detect and prevent fraud.

2. The second part of the document outlines the various methods used to collect and analyze data. It describes the use of statistical techniques to identify trends and anomalies in the data, and the importance of using reliable sources of information.

3. The third part of the document discusses the role of the courts in resolving disputes. It explains how the courts use the evidence presented to them to determine the outcome of a case, and the importance of presenting clear and convincing evidence.

4. The fourth part of the document discusses the role of the government in regulating the financial system. It describes the various laws and regulations that govern the financial industry, and the importance of enforcing these laws to protect the interests of the public.

5. The fifth part of the document discusses the role of the public in the financial system. It explains how the public can protect its interests by staying informed about the financial system and by reporting any suspicious activity to the appropriate authorities.

6. The sixth part of the document discusses the role of the media in the financial system. It describes how the media can help to educate the public about the financial system and to expose any wrongdoing.

7. The seventh part of the document discusses the role of the private sector in the financial system. It describes how the private sector can contribute to the growth and stability of the financial system through innovation and investment.

8. The eighth part of the document discusses the role of the international community in the financial system. It describes how the international community can work together to address global financial issues and to promote the stability of the global financial system.

9. The ninth part of the document discusses the role of the future in the financial system. It describes the challenges that the financial system will face in the future and the steps that need to be taken to address these challenges.

10. The tenth part of the document discusses the role of the conclusion in the financial system. It describes the importance of having a clear and concise conclusion to the document and the steps that need to be taken to ensure that the conclusion is accurate and reflective of the content of the document.

from four to 16 in the cultures embedded in chicken plasma (Fig. IIIa), and from four to eight in the cultures embedded in rabbit plasma (Fig. IVa). In the immune chicken lung cultures there was also a four-fold increase in the hemagglutination titers. The immune chicken spleen cultures showed hemagglutinating activity only in the undiluted fluids (Figs. IIIc, IVc).

After the second and third fluid changes hemagglutinating activity could not be found in the normal tissues of the rabbit and chicken but reappeared within 48 to 96 hours in the undiluted fluids. Hemagglutinating activity disappeared in most of the tissue cultures after the fourth fluid change, i.e., 18 days after N.D.V. inoculation.

Infectivity Tests: Quantitative infectivity tests were carried out with the fluids of all the rabbit and chicken tissue cultures 72 hours after N.D.V. inoculation. In the rabbit and chicken tissue cultures which showed hemagglutination titers of 32 to 64 the LD<sub>50</sub> titers ranged from 10<sup>-5</sup> to 10<sup>-7</sup> (Figs. III-VII). In the immune chicken liver cultures the hemagglutinating activity appeared only in the undiluted fluids but an LD<sub>50</sub> titer of 10<sup>-3</sup> was found (Fig. IIIb). Although the immune rabbit tissue cultures did not show hemagglutinating activity, LD<sub>50</sub> titers of 10<sup>-1</sup> and 10<sup>-2</sup> appeared within 72 hours after N.D.V. inoculation (Figs. V-VII).





Infectivity tests on the fluids of the normal rabbit and chicken tissue cultures were positive up to 18 to 24 days after N.D.V. inoculation. The immune chicken lung and spleen cultures also showed N.D.V. infectivity during this period but the immune chicken liver and all the tissue cultures of the immune rabbit failed to show this activity after the first fluid change, i.e., four days after N.D.V. inoculation.\*

#### C. Changes in the Tissue Cultures Which Were Infected with N.D.V.

The presence of phenol red in the nutrient medium made it possible to check the pH of the fluids visually. The hydrogen ion concentration of the fluids bathing the tissue cultures did not change after N.D.V. inoculation except in the spleen tissue cultures. The N.D.V.-infected spleen

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\*Fluid samples from all the tissue cultures were removed each day for four days after N.D.V. inoculation and every other day for the next 14 days for quantitative infectivity tests. These samples were stored at  $-20^{\circ}$  C but before the experiment was completed the freezing unit went out of commission and the infectious activity of the fluids was decreased so that quantitative results could not be obtained. It was necessary, therefore, to repeat the experiment and run only the quantitative infectivity tests which were necessary to confirm the results of the hemagglutination tests. Thus quantitative infectivity tests were carried out on the fluids removed from the embryonating chicken cultures at intervals of 2, 24, 48, 72 and 96 hours after N.D.V. inoculation and on all the other tissue cultures 72 hours after N.D.V. inoculation. The undiluted fluids removed from these cultures 72 hours after each nutrient change were tested for infectious activity but quantitative tests were not carried out.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions. This includes not only sales and purchases but also the flow of cash and the status of accounts receivable and payable.

2. The second part of the document details the various methods used to collect and analyze financial data. This involves the use of specialized software and the application of statistical techniques to identify trends and anomalies.

3. The third part of the document focuses on the implementation of internal controls to prevent fraud and ensure the integrity of the financial reporting process. This includes the establishment of clear policies and procedures, as well as the regular monitoring and auditing of these controls.

4. The fourth part of the document addresses the challenges of managing financial risk in a volatile market environment. This involves the use of derivative instruments and other risk management strategies to protect the company's assets and ensure its long-term viability.

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5. The fifth part of the document discusses the role of the board of directors in overseeing the company's financial performance and ensuring that management is acting in the best interests of the shareholders. This involves the regular review of financial reports and the approval of major financial decisions.

6. The sixth part of the document focuses on the importance of transparency and communication in financial reporting. This involves the timely and accurate disclosure of financial information to investors and other stakeholders, as well as the use of clear and concise language to explain complex financial data.

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10. The tenth part of the document addresses the challenges of managing financial risk in a volatile market environment. This involves the use of derivative instruments and other risk management strategies to protect the company's assets and ensure its long-term viability.

11. The eleventh part of the document discusses the role of the board of directors in overseeing the company's financial performance and ensuring that management is acting in the best interests of the shareholders. This involves the regular review of financial reports and the approval of major financial decisions.

12. The twelfth part of the document focuses on the importance of transparency and communication in financial reporting. This involves the timely and accurate disclosure of financial information to investors and other stakeholders, as well as the use of clear and concise language to explain complex financial data.

cultures showed acid production even after two fluid changes but they were less acid than the non-infected spleen controls.

The embryonating chicken tissue cultures showed a decided cytological destruction after N.D.V. inoculation (Plates I-II). The only cytological change in the adult rabbit and chicken tissue cultures occurred in the liver cultures of the normal chicken which were embedded in chicken plasma and this did not take place until seven or eight days after the N.D.V. inoculation. There was a gradual degenerative change in these liver cells in which there was a breaking up of the cytoplasm into large granules and a fragmenting of the cell wall. The entire cytoplasm lost its staining properties but the nucleus remained unaltered. The liver tissues of the normal chicken which were embedded in rabbit plasma and the liver tissues of the immune chicken as well as those of the normal and immune rabbit failed to show any degeneration after N.D.V. inoculation (Plate IV).

None of the control tissue cultures or the tissue cultures inoculated with heat-inactivated N.D.V. showed any degenerative change.

In the embryonating chicken cultures which were embedded in chicken plasma the new cells surrounding the tissue transplant showed definite cytological changes within 15 hours after N.D.V. inoculation. There was an increase in the vacuolization of the cytoplasm of the fibroblasts and



a tendency toward the formation of giant cells which contained three to five nuclei (Pl. I, Figs. 2-3). Sometimes it was difficult to determine whether these multinucleated cells were giant cells or an agglomeration of fibroblasts which had lost their individual cell boundaries and become fused. It was necessary, therefore, to examine these particular cells microscopically every few hours. When several tissue culture fields were marked and examined in this manner it could be seen that the multiple nuclei appeared within a single cell. By 48 hours a pronounced degeneration occurred in the fibroblasts surrounding the tissue culture explants (Pl. II, Figs. 5-6). The cytoplasm became more vacuolated and filled with fatty globules, the cell membrane lost its continuity and most of the fibroblasts almost lost their staining properties. In some areas only shrunken pyknotic nuclei were left. Within four or five days degeneration of all the fibroblasts surrounding the tissue explant occurred leaving only a fringe-like halo of faintly stained ghost cells. A few scattered round cells remained.

The infected embryonating tissue cultures which were embedded in rabbit plasma showed the same type of degeneration but this process took place at a slower rate, lagging 24 to 48 hours behind the degenerative changes shown by the tissues embedded in chicken plasma (Pl. II, Figs. 4, 6). The embryonating tissues which were embedded

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1. The first part of the report deals with the general situation in the country. It is noted that the economy is in a state of depression, and that the government is unable to meet its obligations. The report also mentions that the population is suffering from a lack of food and clothing, and that there is a high level of unemployment.

2. The second part of the report discusses the political situation. It is noted that the government is weak and corrupt, and that there is a lack of political stability. The report also mentions that there is a high level of corruption and that the government is unable to carry out its duties.

3. The third part of the report discusses the social situation. It is noted that there is a high level of poverty and that the population is suffering from a lack of education and health care. The report also mentions that there is a high level of crime and that the government is unable to maintain law and order.

4. The fourth part of the report discusses the economic situation. It is noted that the economy is in a state of depression and that there is a high level of unemployment. The report also mentions that there is a high level of inflation and that the government is unable to meet its obligations.

5. The fifth part of the report discusses the international situation. It is noted that the country is isolated and that there is a high level of international isolation. The report also mentions that there is a high level of international isolation and that the country is unable to carry out its duties.

6. The sixth part of the report discusses the future of the country. It is noted that the country is in a state of crisis and that there is a high level of international isolation. The report also mentions that there is a high level of international isolation and that the country is unable to carry out its duties.

in rabbit plasma seemed to be protected in some manner by the rabbit plasma. Only about 50 percent of the fibroblasts surrounding the tissue explant showed degeneration, and many of the remaining cells looked normal even 14 days after N.D.V. inoculation. There was a higher proportion of round cells produced by the embryonating tissues embedded in rabbit plasma and these cells showed no cytological change after exposure to the virus (Pl. II, Fig. 6). Multinucleated giant cells appeared also in these tissues (Pl. II, Fig. 4). There were never more than two nuclei found in any of the fibroblasts arising from the embryonating chicken cultures which did not receive the N.D.V. inoculum, or in the tissues which received the heat-inactivated N.D.V. suspension.





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EXPLANATION OF FIGURES III - VII

H.A = hemagglutination titers

↓ = nutrient fluid change

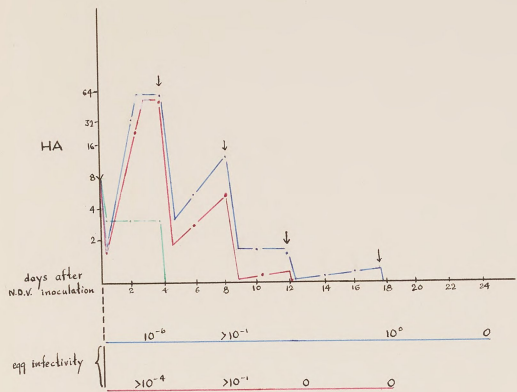
--- = period of hemagglutination titration

— = hemagglutination or infectivity titers of active N.D.V. in normal chicken or rabbit tissue cultures

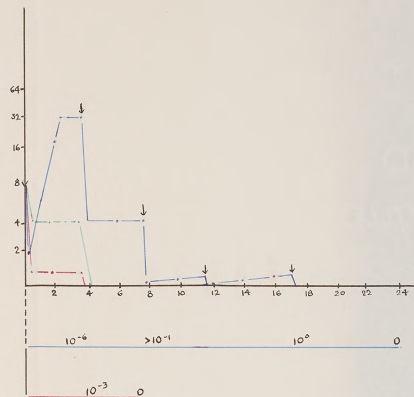
— = hemagglutination or infectivity titers of active N.D.V. in immune chicken or rabbit tissue cultures

— = hemagglutination titers of heat-inactivated N.D.V. in immune chicken or rabbit tissue cultures

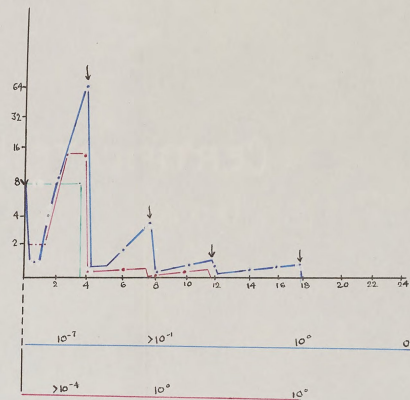
Note: H.A. of heat-inactivated N.D.V. in normal chicken and normal rabbit tissues were omitted in the Figures because the titer remained constant at 8 and decreased to zero after the first nutrient fluid change



IIIa. NORMAL AND IMMUNE CHICKEN LUNG CULTURES



IIIb. NORMAL AND IMMUNE CHICKEN LIVER CULTURES

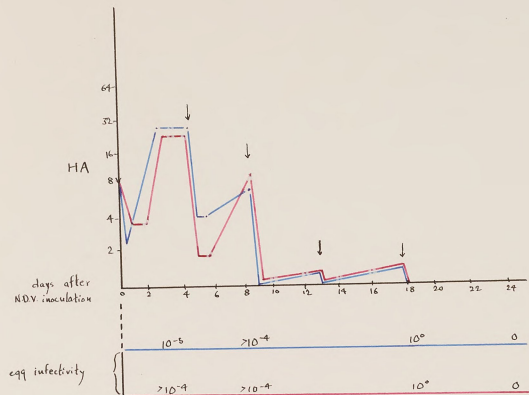


IIIc. NORMAL AND IMMUNE CHICKEN SPLEEN CULTURES

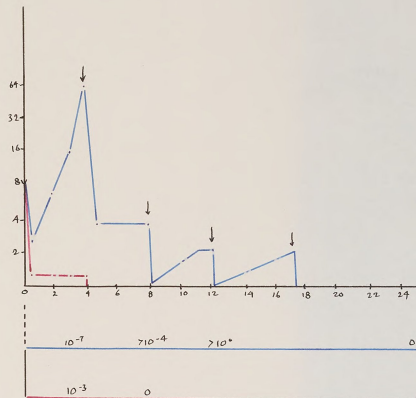
HEMAGGLUTINATION AND INFECTIVITY TITERS OF ACTIVE AND INACTIVE N. D. V. IN NORMAL AND IMMUNE CHICKEN TISSUES EMBEDDED IN CHICKEN PLASMA

FIG. III

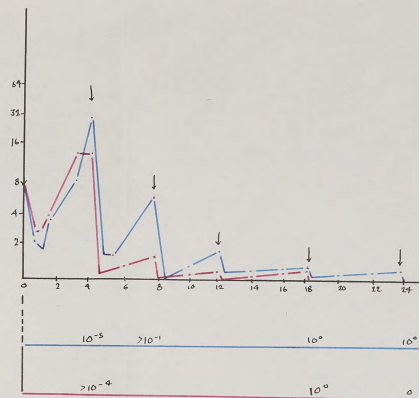




IV a NORMAL AND IMMUNE CHICKEN LUNG CULTURES



IV b NORMAL AND IMMUNE CHICKEN LIVER CULTURES

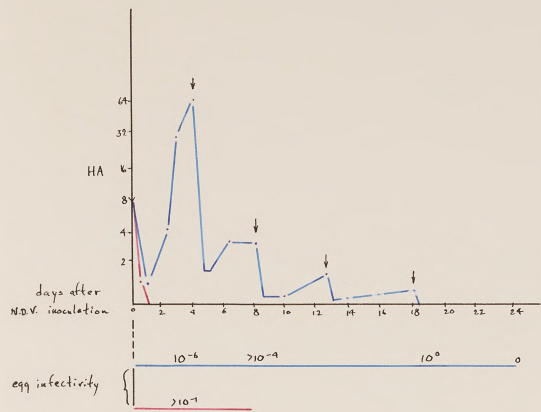


IV c NORMAL AND IMMUNE CHICKEN SPLEEN CULTURES

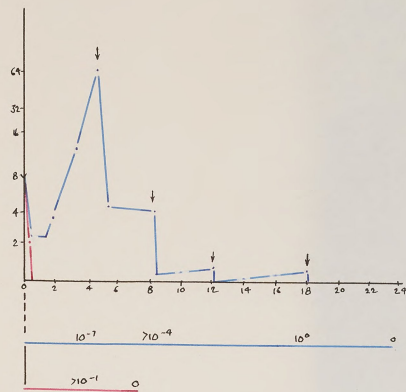
HEMAGGLUTINATION AND INFECTIVITY TITERS OF ACTIVE AND INACTIVE N. D. V. IN NORMAL AND IMMUNE CHICKEN TISSUES  
EMBEDDED IN RABBIT PLASMA

FIG. IV

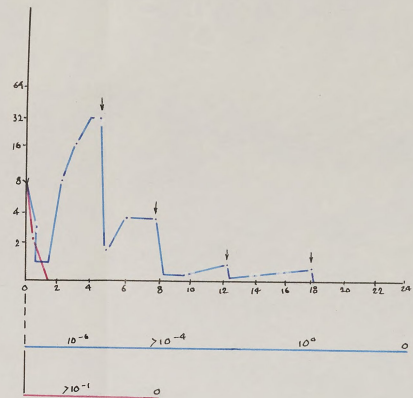
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Ⅴa NORMAL AND IMMUNE RABBIT LUNG CULTURES



Ⅴb NORMAL AND IMMUNE RABBIT LIVER CULTURES



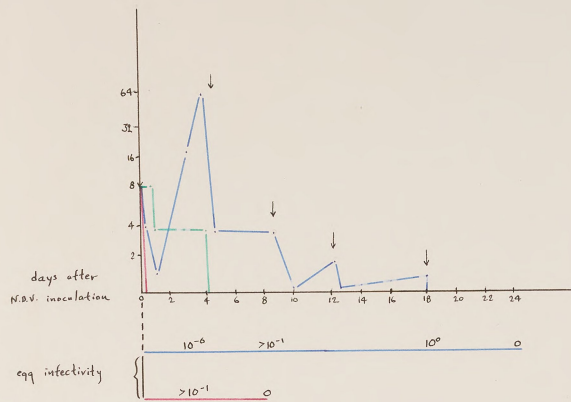
Ⅴc NORMAL AND IMMUNE RABBIT SPLEEN CULTURES

HEMAGGLUTINATION AND INFECTIVITY TITERS OF ACTIVE AND INACTIVE N. D. V. IN NORMAL AND IMMUNE RABBIT TISSUES  
EMBEDDED IN RABBIT PLASMA

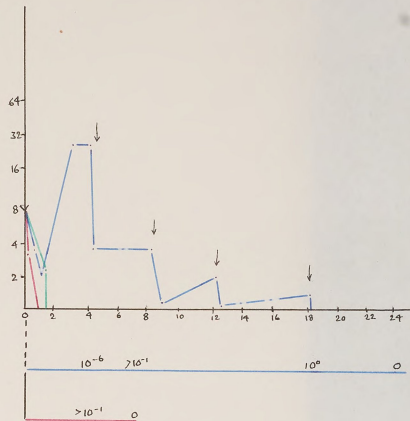
FIG. Ⅴ



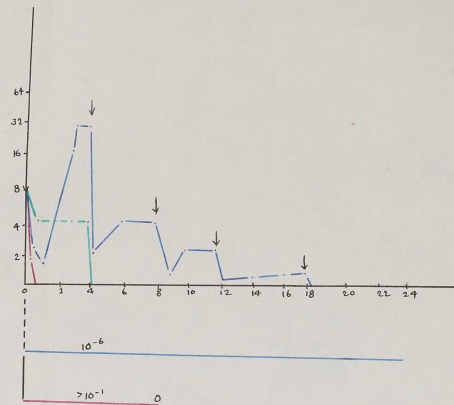
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VIa NORMAL AND IMMUNE RABBIT LUNG CULTURES



VIb NORMAL AND IMMUNE RABBIT LIVER CULTURES



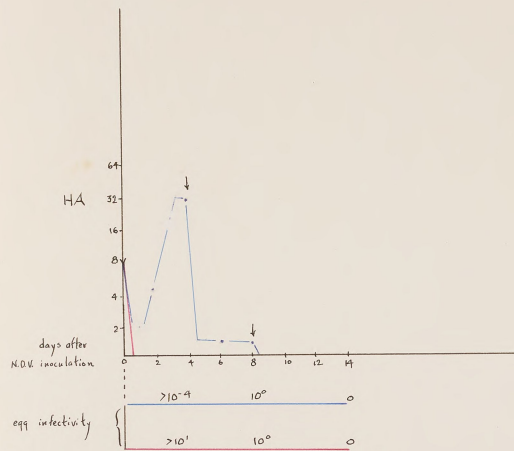
VIc NORMAL AND IMMUNE RABBIT SPLEEN CULTURES

HEMAGGLUTINATION AND INFECTIVITY TITERS OF ACTIVE AND INACTIVE N. D. V. IN NORMAL AND IMMUNE RABBIT TISSUES  
EMBEDDED IN CHICKEN PLASMA

FIG. VI

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FIG. VII



HEMAGGLUTINATION AND INFECTIVITY TITERS IN NORMAL  
 AND IMMUNE RABBIT LYMPH NODE CULTURES EMBEDDED  
 IN RABBIT PLASMA



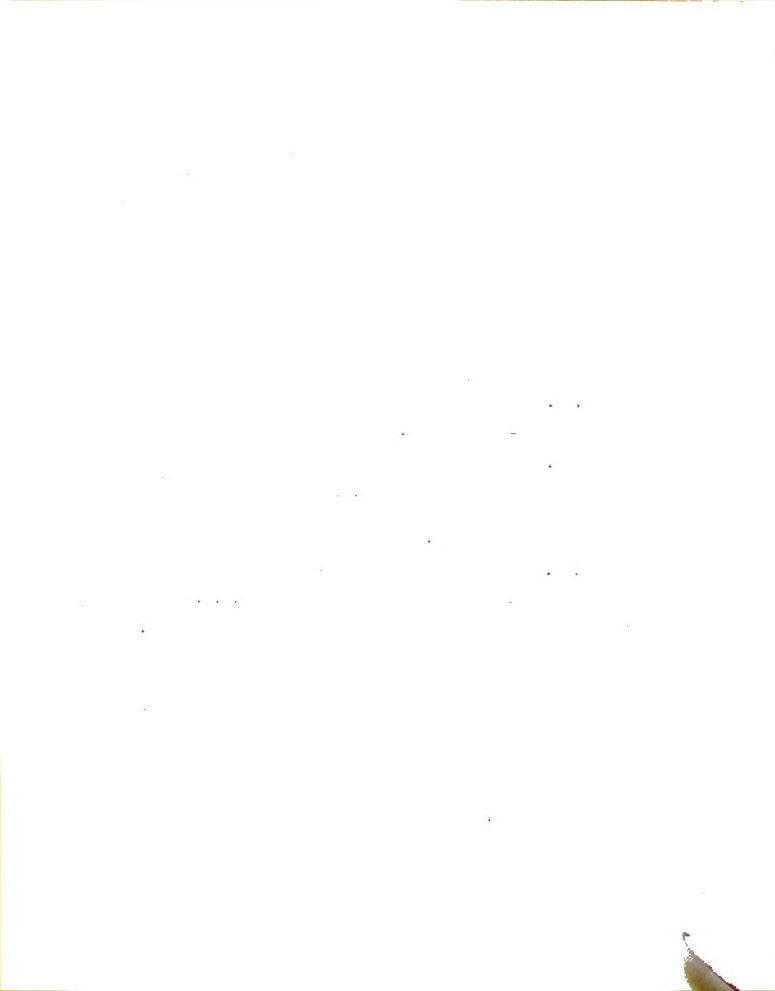


PLATE I

Results of Experiment with Embryonating Chicken Tissue Cultures

- Fig. 1. Normal embryonating chicken fibroblasts embedded in chicken plasma matrix, 6-day culture.
- Fig. 2. Embryonating chicken fibroblasts embedded in chicken plasma matrix, 5-day culture, 15 hours after N.D.V. inoculation, showing many normal fibroblasts, a few multinucleated fibroblasts and one multinucleated giant cell.
- Fig. 3. Embryonating chicken fibroblasts and round cells embedded in chicken plasma matrix, 5-day culture, 24 hours after N.D.V. inoculation, showing an increase in vacuolization in cytoplasm of fibroblasts.

Magnification: 200x

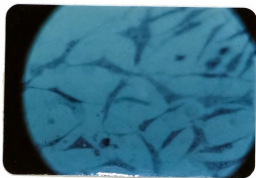


Fig. 1

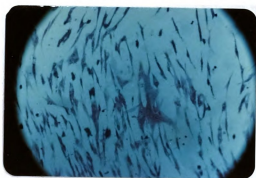


Fig. 2

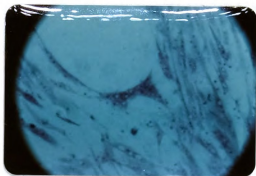
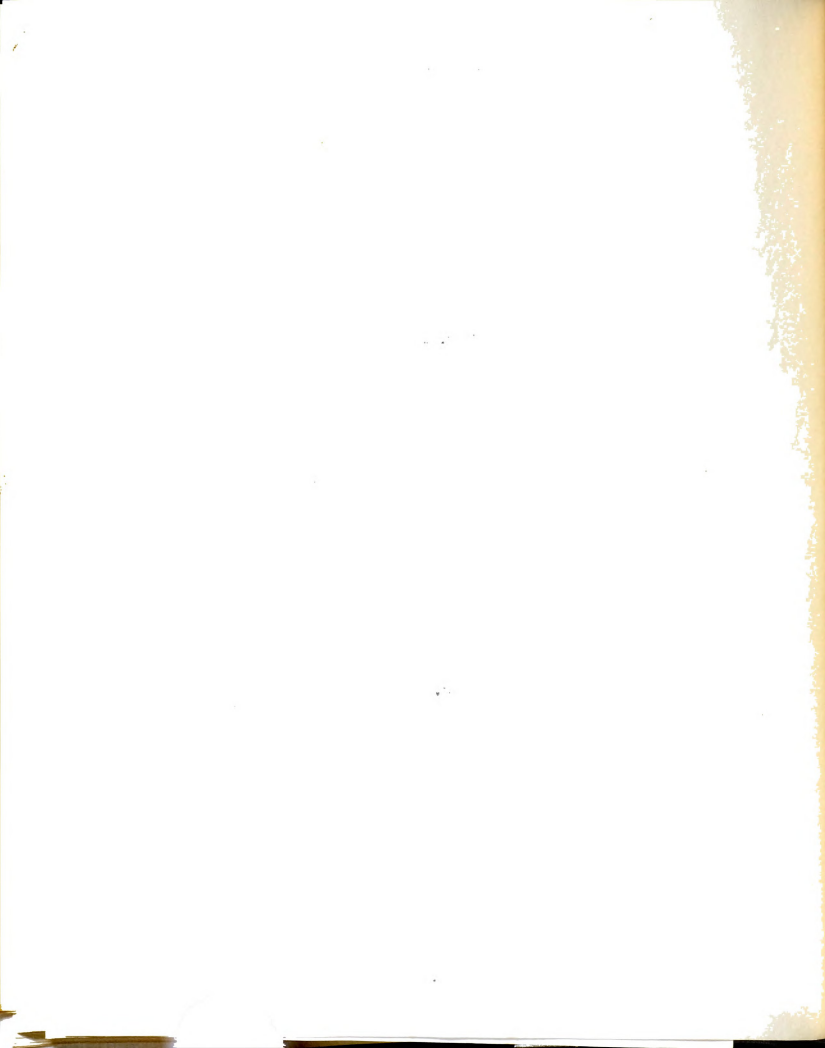


Fig. 3





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PLATE II

Results of Experiment with Embryonating Tissue Cultures

- Fig. 4. Embryonating chicken fibroblasts and round cells, embedded in rabbit plasma, 5-day culture, 24 hours after N.D.V. inoculation, showing a large multinucleated giant cell.
- Fig. 5. Embryonating chicken tissue culture, embedded in chicken plasma, 6-day culture, 48 hours after N.D.V. inoculation, showing complete degeneration of fibroblasts and abnormal round cells.
- Fig. 6. Embryonating chicken tissue culture, embedded in rabbit plasma, 8-day culture, 96 hours after N.D.V. inoculation, degeneration of fibroblasts not complete, many normal round cells.

Magnification: 200x

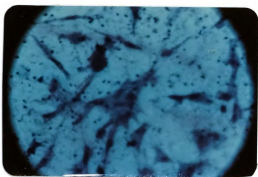


Fig. 4

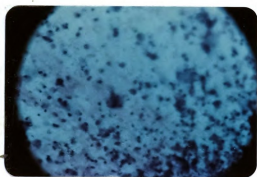


Fig. 5

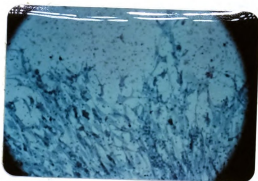


Fig. 6



PLATE III

Cultures of Adult Chicken and Rabbit Tissue

Fig. 7. Normal adult rabbit lung, 8-day culture, normal alveolar cells.

Fig. 8. Normal adult chicken lung, 8-day culture, 96 hours after N.D.V. inoculation showing normal-appearing alveolar cells.

Fig. 9. Normal adult rabbit spleen, 8-day culture, 96 hours after N.D.V. inoculation showing normal-appearing spleen cells.

Magnification: 200x

## PLATE III

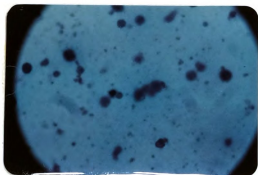


Fig. 7

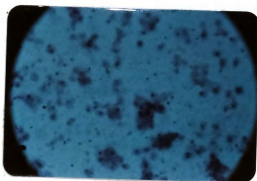


Fig. 8

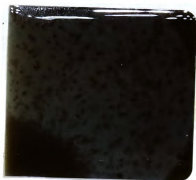


Fig. 9





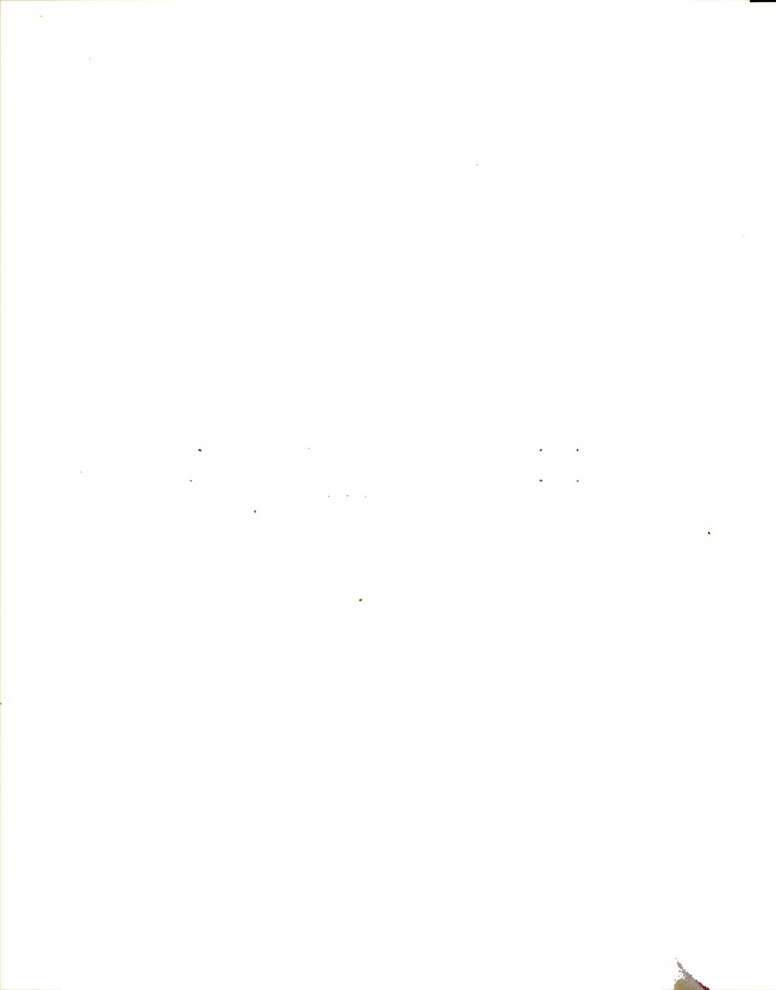


PLATE IV

Fig. 10. Normal rabbit liver, 8-day culture.

Fig. 11. Immune chicken liver, 8-day culture,  
96 hours after N.D.V. inoculation showing  
normal-appearing liver cells.

Magnification: 200x

## PLATE IV

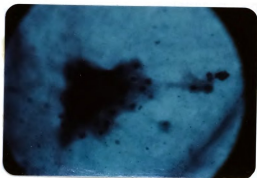


Fig. 10

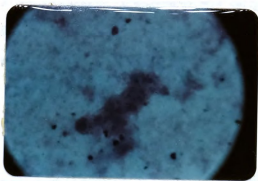


Fig. 11



## DISCUSSION

The data from this study were compiled from hemagglutination tests and microscopic studies from three experiments including cultures of embryonating chicken tissue, normal chicken and rabbit tissue and immune chicken tissues and from two experiments with cultures of immune rabbit tissue. In the first experiment quantitative infectivity tests and hemagglutination tests were carried out with the fluids removed from the embryonating chicken cultures at intervals of 2, 6, 12, 24, 36, 48, 72 and 96 hours after N.D.V. inoculation in order to prepare the N.D.V. growth curves (Fig. II). At the same time it was possible to determine the period in which the infectious activity and hemagglutinating activity appeared in the tissue culture fluids. In this experiment fluids from the cultures of tissues from the normal chicken and rabbit and the immune chicken were removed 72 hours after N.D.V. inoculation to determine their  $LD_{50}$  titers also, and to compare these titers with the hemagglutination titers. Quantitative infectivity tests were also carried out with the fluids removed 12 hours after N.D.V. inoculation from the tissues of the immune rabbit, which were embedded in chicken plasma.

From the data obtained from the hemagglutination tests and infectivity tests it was found that the growth curves



of N.D.V. in the embryonating chicken tissues cultures showed a cycle similar to that of influenza virus (Enders, 1950). This cycle is composed of (a) a lag phase which occurred within two to 24 hours after virus inoculation when the virus was adsorbed by the tissue culture cells, (b) a phase of rapid virus multiplication in which peak infectivity titers were reached by 48 hours and peak hemagglutination titers within 72 hours, (c) a narrow plateau lasting for 24 hours, and (d) a sudden decrease in virus production. These growth curves of the G.B. strain of N.D.V. were similar to those determined for the Roakin strain of N.D.V. in chorio-allantoic tissue cultures by Scott (1953). The peak infectivity and hemagglutination titers for the G.B. strain of N.D.V. in the embryonating chicken tissue cultures were slightly lower. However, lower titers were expected, for it has been shown that the chorio-allantoic membrane yields a higher N.D.V. content than embryo tissue when the embryonating chicken egg is inoculated by the intravenous route (Hanson et al., 1952). The appearance of hemagglutinating activity 24 hours later than infectious activity was also found by Gordon (1952) when the embryonating chicken egg was inoculated with N.D.V. The rate of multiplication of the virus was slower and the hemagglutination and infectivity titers were lower in the tissue cultures.

The fact that there was little difference in the peak

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hemagglutination titers and infectivity titers found in the fluids of tissue cultures embedded in rabbit and chicken plasma indicates that these body fluids are not associated with the resistance or susceptibility of the animal as far as multiplication of N.D.V. is concerned. The rabbit plasma, however, showed protection of the N.D.V.-infected liver tissues of the normal chicken and prevented extensive degeneration of the embryonating chicken fibroblasts, although it did not impair their ability to support virus growth. Once the fibroblasts were destroyed in the embryonating chicken cultures, which were embedded in chicken plasma, there was a sharp decrease in virus multiplication and the virus activity found in these tissue culture fluids eight days after N.D.V. inoculation could have arisen only from a few viable cells in the explant or the remaining round cells. Since there were a considerable number of fibroblasts and round cells found in the embryonating chicken tissues embedded in rabbit plasma 12 days after N.D.V. inoculation, the rabbit plasma apparently spared these cells so that the N.D.V. growth curve showed a slower decrease in virus multiplication as well as an extension of the period of virus multiplication. If degenerative changes did not occur in the tissue cultures of the normal chicken and rabbit their capacity to support growth of the virus was not exhausted until 12 to 18 days after N.D.V. inoculation.

The inhibition of growth of N.D.V. in the liver tissue

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry should be supported by a valid receipt or invoice. This ensures transparency and allows for easy verification of the data.

In the second section, the author outlines the various methods used to collect and analyze the data. These include direct observation, interviews with key personnel, and the use of specialized software tools. Each method has its own strengths and limitations, and they are often used in combination to provide a comprehensive view of the situation.

The third part of the report details the findings of the study. It shows that there are significant discrepancies between the reported figures and the actual data. These differences are primarily due to incomplete reporting and a lack of proper documentation. The author suggests that implementing a more rigorous record-keeping system could help to resolve these issues.

Finally, the document concludes with a series of recommendations for future work. It suggests that regular audits should be conducted to ensure the accuracy of the records. Additionally, training should be provided to staff to ensure they understand the importance of proper documentation and how to use the available tools effectively.

cultures of the immune chicken and all the tissues cultured from the immune rabbit was due evidently to a property acquired by the tissues themselves during the period of immunization because the fluids extracted from these tissues did not show inhibition of the virus at the time they were removed from the animal. The fact that the spleen tissue cultures of the immune chicken showed only slight inhibition of virus multiplication may be explained on the basis that the multiplication of the spleen cells continued to increase at a much greater rate than that of the liver cells after N.D.V. inoculation and there might have been a higher percentage of new spleen cells which had not acquired the N.D.V. inhibiting property. On the other hand, it could have been due to the fact that the spleen of the immune chicken does not play as important a role in the process of immunization as does the liver.

Since all the tissues cultured from the immune rabbit failed to support growth of N.D.V. to the same extent as the tissues cultured from the normal chicken, and rabbit as well as the lung and spleen tissues of the immune chicken tissue the resistance of the rabbit to N.D.V. disease may be due to the fact that the rabbit has many more tissues which are capable of acquiring a N.D.V.-inhibiting property when the animal is exposed to the virus although the rabbit may not be able to produce an antibody titer any higher than that of the chicken. There is also the additional



possibility that when the rabbit is first exposed to N.D.V. it may possess a plasma activity capable of protecting the tissues so that degeneration of the cells may be prevented until there are sufficient specific antibodies produced against the virus to stop the process. In this case multiplication of the virus in the rabbit tissues occurs without production of disease.

Although the growth curve of N.D.V. in tissue cultures was similar to that of influenza virus it is of interest to note that influenza produced degeneration of the cells of the embryonating chicken cultures (Enders, 1950). Mumps virus did not produce cellular changes (Enders, 1952) but this was not expected because this virus does not produce the same degree of damage in the embryonating chicken egg as do influenza virus and N.D.V. Newcastle disease virus, instead, seems to resemble poliomyelitis virus in its ability to destroy tissue culture fibroblasts. The production of multinucleated giant cells in embryonating chicken cultures inoculated with N.D.V. may be of diagnostic significance for these cells appear within 12 to 24 hours after exposure to the virus. The possibility that the tissue culture technique could provide a simple and rapid means for identifying N.D.V. warrants further investigation. The occurrence of multinucleated giant cells has often been reported in tissue cultures of malignant tumors but never in tissue cultures infected with a virus. Burnet (1942)

1. The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry should be supported by a valid receipt or invoice. This ensures transparency and allows for easy verification of the data.

2. The second section covers the process of reconciling bank statements with the company's internal records. It highlights the need to identify and investigate any discrepancies between the two sets of records. Regular reconciliation helps in detecting errors or potential fraud early on.

3. The third part of the document addresses the issue of budgeting and cost control. It suggests that setting a clear budget at the beginning of each period can help in monitoring expenses and staying within the allocated funds. This is crucial for the financial health of the organization.

4. The fourth section discusses the importance of timely payment of bills and invoices. It notes that late payments can lead to penalties, damaged relationships with suppliers, and cash flow problems. Implementing a strict payment schedule is essential for smooth operations.

5. The fifth part of the document focuses on the role of technology in financial management. It mentions that using accounting software can significantly reduce the risk of human error and streamline the reporting process. Investing in reliable technology is a key strategy for modern businesses.

6. The sixth section covers the importance of regular financial reporting. It states that providing accurate and timely reports to management and stakeholders is vital for informed decision-making. These reports should clearly show the company's financial performance and trends.

7. The seventh part of the document discusses the need for a strong internal control system. It suggests that having clear policies and procedures in place can help in preventing errors and fraud. Regular audits and reviews are also important to ensure the system remains effective.

8. The eighth section addresses the importance of staying updated with the latest financial regulations and tax laws. It notes that non-compliance can result in significant fines and legal issues. Companies should invest in professional advice to ensure they are always up-to-date.

9. The ninth part of the document focuses on the importance of maintaining a good credit rating. It suggests that paying bills on time and keeping debt levels under control are key factors in building a strong credit profile. A good credit rating can provide access to better financing options.

10. The final section of the document concludes by emphasizing the overall goal of financial management: to ensure the long-term sustainability and growth of the organization. It reiterates that a disciplined and systematic approach is essential for achieving these goals.

described the appearance of "large, vacuolated cells, agglutinated in masses in which it is impossible to pick out the outline of individual cells" in tracheal smears of the N.D.V.-infected embryonating chicken egg. It is possible that what would appear as an aggregation of superimposed cells in a smear preparation could have been one single multinucleated giant cell. Otherwise the production of multinucleated cells has not been reported in connection with N.D.V. When microscopic examination can be made on a single layer of cells which can be obtained in tissue culture preparations any changes in the isolated cells can be followed more easily.





## SUMMARY

In these experiments a comparative study has been made on the effect of N.D.V. on susceptible and resistant tissues cultured from chickens and rabbits.

Embryonating chicken, adult chicken lung, liver and spleen and adult rabbit lung, liver, spleen and lymph node tissues were cultured. Both normal and immune adult chickens and rabbits were used. Hemagglutination and infectivity tests were carried out to determine the growth curves of N.D.V. in embryonating chicken tissues.

The growth cycle was composed of (a) a lag phase of 24 hours, (b) a phase of rapid virus multiplication in which peak infectivity titers were reached in 36 hours and peak hemagglutination titers in 36 to 48 hours, (c) a plateau lasting 48 to 60 hours, (d) a sudden decrease in the rate of virus production 96 hours after N.D.V. inoculation.

Growth curves prepared from hemagglutination titrations on the tissues cultured from normal adult chickens and rabbits were similar to those of the embryonating chicken tissue but the peak hemagglutination titers occurred 36 to 96 hours after N.D.V. inoculation. This was also found in the immune chicken lung and spleen cultures but not in the liver cultures. In the immune chicken liver tissues and in all the tissues of the immune rabbit there was a steep



decrease in infectivity titers from an LD<sub>50</sub> of 10<sup>-7</sup> on the day of inoculation to 10<sup>-1</sup> or 10<sup>-2</sup> 48 hours later. Hemagglutination activity was recovered only in the undiluted fluids from the immune chicken liver cultures.

Cytological degeneration was seen in the embryonating chicken cultures 15 to 48 hours after N.D.V. inoculation. The fibroblasts showed an increase in vacuolization of the cytoplasm and a tendency in the formation of giant cells which contained three to five nuclei. The only degenerative changes in the adult tissue cultures occurred in normal adult chicken liver and this did not appear until seven or eight days after N.D.V. inoculation. There was a gradual degeneration of the liver cells in which the cytoplasm lost its staining properties and broke up into large granules and the cell wall became fragmented. The nucleus, however, was unaltered. Neither the uninoculated cultures nor the cultures inoculated with heat-inactivated N.D.V. showed any degenerative change.

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1. The first part of the report deals with the general situation of the country and the progress of the work during the year. It is divided into two main sections: the first section deals with the general situation and the second section deals with the progress of the work.

2. The second part of the report deals with the results of the work during the year. It is divided into two main sections: the first section deals with the results of the work in the field of research and the second section deals with the results of the work in the field of education.

3. The third part of the report deals with the financial situation of the institution during the year. It is divided into two main sections: the first section deals with the income and the second section deals with the expenditure.

4. The fourth part of the report deals with the general conclusions and recommendations. It is divided into two main sections: the first section deals with the general conclusions and the second section deals with the recommendations.

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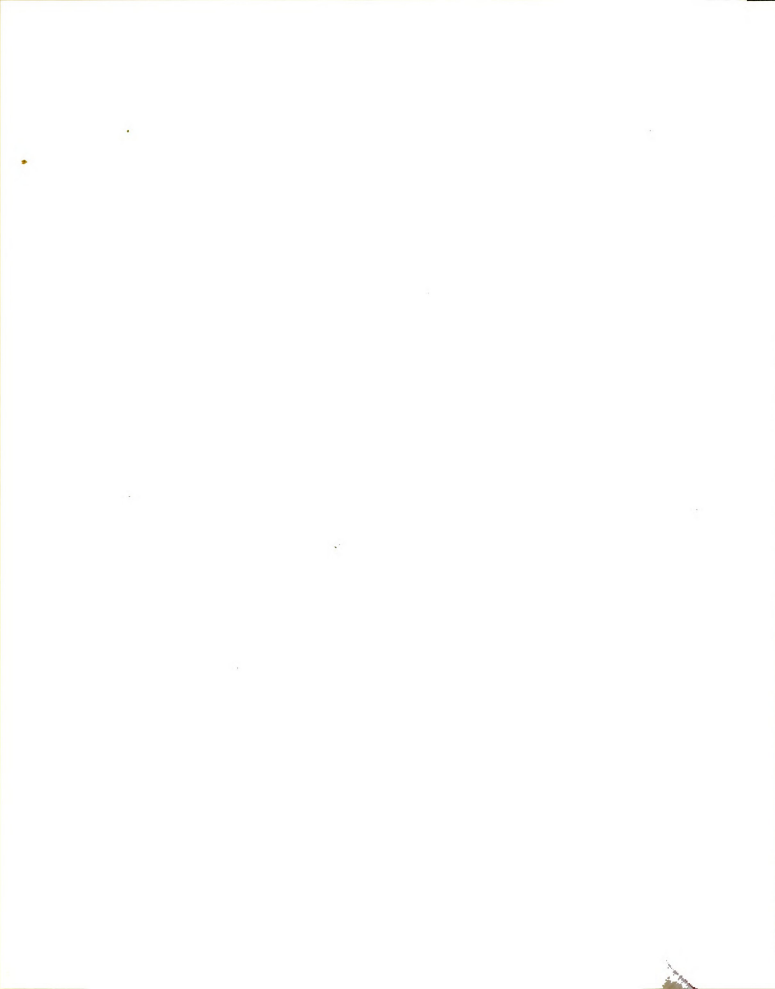
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1. The first part of the document is a list of names and addresses, which appears to be a directory or a list of contacts. The names are arranged in columns, and the addresses are listed below them. The text is somewhat faint and difficult to read, but it seems to be a list of names and addresses.

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