ELECTROPHORETIC AND SERUM NEUTRALIZATION STUDIES OF SERA FROM CHICKENS EXPOSED TO INFECTIOUS BRONCHITIS VIRUS

by

George T. Dimopoullos

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

1952

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AN ABSTRACT

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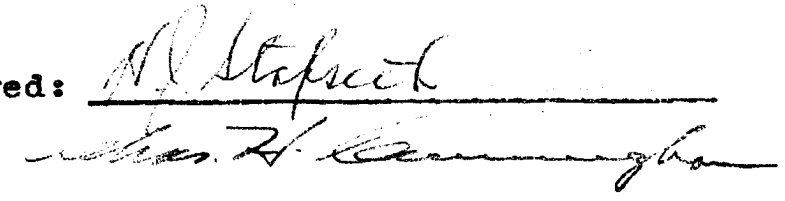
Approved: 

This study was undertaken to ascertain any possible correlations between electrophoretic and serum neutralization analyses of sera from chickens exposed to infectious bronchitis virus (IBV).

Adult Single Comb White Leghorn cockerels were divided into four groups as follows:

Group I - Controls-bled at weekly and monthly intervals.

Group II - Birds inoculated with IBV and bled immediately prior to inoculation and then at one, two, three, four, six, eight, ten, 12, 16, and 20 weeks.

Group III - Birds treated as those in Group II but challenged at the twelfth week and bled one, three, five, and seven weeks after challenge.

Group IVa - Birds inoculated with a normal lung and tracheal suspension and bled immediately prior to inoculation and then at one, two, and three weeks.

Group IVb - Birds subjected to scarification of the trachea and bled immediately prior to scarification and then at one, two, and three weeks.

Sera were diluted to a final protein concentration of two per cent (5), dialyzed (4), and analyzed electrophoretically in veronal buffer (2) at pH 8.6, 0.1 ionic strength using a potential gradient of 6.5 volts/cm^2 for 7,600 seconds. The results are expressed as relative per cent serum protein component.

For the serum neutralization test (1) equal portions of ten-fold dilutions of IBV and undiluted serum were mixed, incubated and inoculated into embryonating chicken eggs. Results are expressed as the Lethal $Dose_{50}$ Neutralization Indices (LD_{50} NIs) (3).

Sera from normal birds bled at weekly intervals showed an increase of approximately 0.20 from the initial albumin/globulin ratio of 0.85 during the first and second weeks. After this period the ratios decreased 0.40 to 0.60 of the initial value.

Sera from normal birds bled at four-week intervals showed no significant changes in the relative per cent distribution of serum protein components.

Birds exposed to IBV showed marked decreases in the albumin/globulin ratios to an average value of 0.45 during the first and second weeks. The ratios steadily increased after this period and returned to normal at the twelfth week. The normal values of 0.85 persisted for eight additional weeks.

The LD_{50} NIs increased slowly at the first and second weeks after exposure. After this period a marked increase occurred. Maximum LD_{50} NIs values of 10^6 were reached between the sixth and eighth weeks and decreased after this period to approximately 10^3 at the twentieth week.

No correlation was observed in the changes of electrophoretic patterns and changes in the LD_{50} NIs. When electrophoretic patterns had returned to normal the LD_{50} NIs were at their maximum values of 10^6.

Birds challenged at the twelfth week did not show any significant changes in their electrophoretic patterns but showed increases in the LD_{50} NIs to maximum values of 10^7.

The changes in electrophoretic patterns were not considered specific for IBV since normal birds bled at weekly intervals showed similar changes.

Sera obtained from birds inoculated with a normal lung and tracheal suspension and birds subjected to a scarification of the trachea showed varying results in the changes of albumin/globulin ratios. Results obtained did not give evidence that these treatments alone were responsible for the changes observed. There were no changes in the LD_{50} NIs.

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To

"Boss"

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Dissertation: Electrophoretic and Serum Neutralization Studies of Sera from Chickens Exposed to Infectious Bronchitis Virus

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ACKNOWLEDGEMENTS

1. The author wishes to express his gratitude to Michigan State College for awarding him an Alumni Predoctoral Fellowship for the academic year 1951-1952 and supplying other funds for materials which made it possible to conduct and complete this investigation.
2. He is also indebted to Dr. Charles H. Cunningham and to Dr. Henrik J. Stafseth for their spiritual and material aid and suggestions during the investigation and in the preparation of the manuscript.
3. To Mr. John E. Lynch, he is also indebted for his aid during the drawing of the blood samples from the experimental birds.
4. For the sincere cooperation of Dr. Erwin J. Benne and the other staff members of the Department of Agricultural Chemistry responsible for the protein nitrogen analyses, the author is deeply grateful.
5. The author also wishes to express his thanks to Dr. Nelson F. Waters and Dr. George E. Cottral for their kind cooperation in supplying the experimental birds.

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I

INTRODUCTION

The moving boundary method of electrophoresis has, in recent years, contributed greatly to a better understanding of the characteristic properties of plasma and serum and of their respective components. It has become a valuable tool in the study of the change and distribution of plasma and serum components in disease and experimental immunology. The method has been applied in the study of bacterial diseases and to a lesser degree to viral diseases. Knowledge of antiviral sera has been limited due to their comparative poverty in quantity, and in many cases, to their distinguishing characteristic of exhibiting little or no definite change even though the antiviral sera are drawn from subjects with demonstrable increased antibody activity. Results obtained in the study of the relative percentage distribution of plasma and serum components in antiviral sera have also been subjects of controversy as compared to results obtained with most antibacterial sera.

For the above reasons it was decided to conduct a physical and biological study of infectious bronchitis of chickens. This disease, which is of virus etiology, is of great importance in the poultry industry, causing great losses and morbidity in infected flocks. It was also thought that possibly a contribution to the differential diagnosis of the disease and to fundamental virus research could be given through

the combination of these studies. Therefore, the investigation was directed toward the study of the change in serum electrophoretic patterns and the change in Lethal $Dose_{50}$ Neutralization Indices (LD_{50} NIs) after primary exposure and challenge to a tissue suspension of a chicken-propagated strain of infectious bronchitis virus. Results obtained early in the study prompted further investigations on the effects of normal tissue inoculations and tracheal injuries on the serum electrophoretic patterns and LD_{50} NIs.

II

HISTORICAL REVIEW

A. Electrophoresis

Various substances in the colloidal state, when suspended in an aqueous medium, have a characteristic electric charge, the sign of which depends upon the nature of the particle and the suspending medium. Similarly, protein molecules possess charged groups on their surfaces when in the colloidal state suspended in an aqueous medium. The number of these charged groups determines the net charge density of the protein molecules. The net charge density of the protein molecules determines their mobilities in an applied electric field. Mobility, which is independent of size and shape, may be changed by varying the ionic atmosphere of the charged particles through change of the pH or ionic strength. This migration of an electrically charged particle in an applied electric field is termed electrophoresis.

The application of electrophoresis in the study and analysis of plasma and serum proteins has become a valuable tool during the past 15 years. The development by Tiselius (155) of a standard electrophoresis apparatus and its modifications and improvements (103) have directed investigators in the field of protein chemistry to a more comprehensive knowledge of plasma and serum and their respective components. It has been applied in the study of the changes of plasma and serum components in bacterial diseases and experimental immunology

(1, 13, 15, 35, 43, 45, 58, 66, 80, 91, 102, 109, 112, 114, 117, 143, 146, 147, 148, 157, 158, 161, 162, 163), in viral diseases (14, 29, 67, 69, 70, 83, 95, 116, 128, 140, 142, 144, 153, 165, 172), in rickettsial diseases (46), to nutritional deficiency in relation to antibody production (4, 11, 12, 22, 48, 50, 97, 117, 159), in the study of normal and immune plasma and serum proteins and other body fluids and tissue proteins (2, 17, 19, 21, 27, 28, 31, 41, 42, 44, 58, 60, 62, 63, 64, 68, 81, 82, 88, 90, 99, 100, 102, 104, 106, 107, 108, 109, 120, 121, 122, 126, 129, 134, 144, 150, 151, 152, 155, 156, 157, 158, 160, 167, 175), in the study of the effects of injury and traumatic shock (22, 62, 63, 64, 124, 125), in the study of nutritional deficiency in relation to serum and plasma protein depletion and regeneration (21, 23, 24, 25, 26, 49, 51, 52, 96, 141, 166, 173, 174), and in non-specific diseases (59, 66, 111, 112, 114, 140, 148, 149, 151).

In general, results obtained in the above studies show definite qualitative and quantitative changes in plasma and serum components. There are instances where little or no change is observed in the serum electrophoretic patterns in Western equine encephalomyelitis (95, 128), influenza (172), and Japanese B encephalitis, and Venezuelan equine encephalomyelitis (95). The alterations observed are not considered to be specific for any one disease since these changes only measure the current status of the subject under study. The outstanding changes of the serum and plasma proteins are a

reduction in albumin, an increase in gamma-globulin, and a moderate increase in alpha-globulins and beta-globulin (114).

In 1937 Tiselius (155) made his first attempt to analyze blood serum using his improved technique and apparatus which utilized the principle of the Foucalt-Toepler schlieren optical method. The new, flat, narrow, multiple-compartment electrophoresis cell had the advantages that higher potential gradients could be utilized. Since the cell contained more surface area, more efficient cooling in the ice-bath could also be obtained during the passage of current. Isolation of various components after separation could also be accomplished in this cell. His first analysis demonstrated that serum was composed of four components, or boundaries, which migrated at different and characteristic mobilities under specified condition of pH, ionic strength, and potential gradient. This unique feature of characteristic mobility for a specific component has aided in the identification of various fractions. The four components were designated by Tiselius as albumin, two globulins, and the remaining component was unnamed.

During the same period (156, 157), while experimenting with purified horse serum globulin in the electrophoresis apparatus, Tiselius named the components of the globulin mixture alpha-, beta-, and gamma-. These notations are in general use today.

Tiselius' method of analysis, although enabling one to observe visually and record photographically the number

and the migration of the boundaries, did not yield itself to the interpretation of quantitative data on the relative concentration and homogeneity of these boundaries. It was not until Longsworth's (103) modifications and adaptation of the Foucalt-Toepler schlieren method and the development of the schlieren technique that quantitative data could be obtained. The theoretical basis as explained by Longsworth (103) is as follows:

The angular deviation of a pencil of light in a boundary present in the glass electrophoresis cell is proportional to the refractive index gradient and the horizontal breadth of the boundary. The displacement of the schlieren diaphragm required to intercept the pencil of light deflected downward by the boundary is also proportional to the distance between the cell center and the diaphragm. Upon raising the horizontal schlieren diaphragm the first light beams to be intercepted will naturally be those which are due to the steepest refractive index gradients in the cell, i.e., the center of the boundary. When a series of photographic exposures are made while raising the diaphragm before each exposure the resulting photographic record gives an indication of the variation of the refractive index throughout the electrophoresis cell. These adjustments are accomplished mechanically in a continuous fashion in the schlieren scanning method and the resulting diagram is recorded photographically. Longsworth's excellent paper should be consulted for a more detailed description (103).

The Longsworth scanning modification of the schlieren technique is unexcelled for obtaining permanent records of electrophoretic patterns but does not allow for direct visual observation of the patterns. If a visual inspection of the entire pattern is desired, use is made of the diagonal schlieren diaphragm method of Thovert in one of its modifications, incorporating the cylindrical lens. For a detailed description of this method Longsworth's papers (104, 105) should be consulted.

In a study of various buffers for use in electrophoresis Longsworth (104) observed that an additional component in human plasma could be resolved from the albumin when employing sodium diethylbarbiturate buffer (veronal buffer) at pH 8.6 and 0.1 ionic strength. This component, a globulin, was present between the original alpha-globulin and albumin and was designated as alpha 1-globulin. The original globulin was named alpha 2-globulin. This buffer also had the advantages of causing more efficient separation of the gamma-globulin at salt-protein boundaries and also giving better symmetry between the patterns obtained in the ascending limb and those of the descending limb. The components of horse plasma do not separate as well in Longsworth's buffer as they do in phosphate buffer at pH 7.7 suggesting that the proper buffer for the analysis of a given type of plasma or serum varies with the species and should be determined experimentally.

In additional studies (72) on the effects of various buffers on the resolution of human serum components it has been

shown that phosphate buffer at pH 8.8 and 0.15 ionic strength is as good as Longsworth's buffer (104) in the resolution of the serum components. In veronal buffer, 0.1 ionic strength, at pH values between 8.2 and 8.8, there is little change in relative percentage composition of human serum (99).

A comparison of results obtained with bovine serum and plasma in veronal, veronal-sodium chloride, and veronal-citrate buffers (79) showed unsatisfactory resolution of the gamma-globulins and fibrinogen. Phosphate buffer was superior for the separation of beta-globulin from fibrinogen. Veronal and veronal-citrate buffers resolved two beta-globulins. Veronal-sodium chloride buffer was found more efficient for the separation of total alpha-globulins from albumin. The most satisfactory resolution of alpha 1- and alpha 2-globulins was found in veronal and veronal-citrate buffers.

Resolution of the beta-globulin, fibrinogen, and gamma-globulin of swine plasma in phosphate buffer, 0.2 ionic strength was superior to veronal buffer. The resolution of the alpha-globulins and albumin was superior in veronal buffer. Also, in veronal buffer, two alpha-globulins and two beta-globulins were resolved in comparison to one alpha-globulin and one beta-globulin in phosphate buffer (92).

It is evident that the composition of the buffer (79, 92, 104), its pH (2, 92, 104), and ionic strength (2, 92, 104) affect the relative percentage distribution and resolution of the individual components under study. In addition, the degree

of hemolysis (126, 149), storage conditions (126), the method of area measurement (73, 126), and the period of dialysis of the diluted sample (126) also affect the relative percentage distribution of the serum and plasma components.

Mobility values of the individual components are also affected by the length of dialysis (59), the concentration of protein (34, 101, 132), the pH (34, 59, 99), the ionic strength (34, 93, 136), and the composition of the buffer (59).

Chicken serum and plasma and their respective fractions have been extensively studied (17, 27, 41, 43, 100, 115, 121, 122, 129, 143, 144).

In a study of the effect of age on the electrophoretic patterns of chicken serum and plasma it was shown that there is a relative decrease in albumin and an increase in globulins as the bird matures (17, 122). Prior to sexual maturity chickens were shown to possess low gamma-globulin levels and total serum proteins (17, 144). As the birds matured gamma-globulin levels and total serum proteins increased with no significant changes in the beta-globulins (17, 144). The explanation given by the authors (17) is that this decrease in albumin/globulin ratio may have been the result of a normal development as the bird matured. An additional explanation (43) states that this change may have possibly been due to foreign antigen contacts which stimulated

the production of antibody globulins which in turn was observed as an increase in total gamma-globulin as measured by electrophoresis.

Antibodies (43, 129, 143) and possibly antibody-related substances (144) have been shown to be associated with serum gamma-globulin in chickens. Antibodies have also been shown to be associated with gamma-globulins in other studies (147, 150, 153, 157, 161, 162, 163). All gamma-globulin is not antibody (87, 91) neither are all antibodies contained exclusively in the gamma-globulin fraction (35, 53, 128, 158, 162). The electrophoretic increase or decrease in antibody in chickens immunized with human serum gamma-globulin parallels the increase or decrease in gamma-globulin (43). This parallelism has also been confirmed in other studies (1, 13, 15, 45, 83, 163, 172). Still other studies have shown that this is not necessarily the case (97, 117, 172).

Very characteristic sex differences have been shown to appear in the electrophoretic patterns of chicken serum after sexual maturity at about the fourth or fifth month of life (121, 122). Prior to this time sex differences as observed electrophoretically are insignificant.

One case is cited in which normal, adult male and female White Leghorn chickens only showed significant changes in the alpha-globulin and gamma-globulin (41). Neither the exact age nor the state of egg production of the birds was mentioned. It has been shown that laying hens possess a greater quantity of gamma-globulin than non-laying hens (17, 100, 122) and adult males (17, 122).

The effect of contra-sex hormones in chickens and the resulting electrophoretic patterns have been studied (27, 122). It has been found that electrophoretic patterns of sera change into patterns typical of the opposite sex upon administration of these hormones.

From the above data it may be seen that many factors will affect the relative composition and mobilities of chicken and other animal sera and plasma and their respective components, depending upon the sex and age of the animal and the general composition and properties of the buffer used as the solvent.

Normal electrophoretic values for chicken serum and plasma studied under a variety of conditions have also been reported. Using phosphate buffer at pH 7.7, 0.2 ionic strength, San Clemente (143) demonstrated that normal chicken serum had the following relative percentage composition: albumin - 35 per cent, alpha-globulin - 15 per cent, beta-globulin - five per cent, and gamma-globulin - 45 per cent. The average albumin/globulin ratio was 0.54. A beta-globulin anomaly was present in the pattern of the descending limb. The presence of this anomaly has also been reported elsewhere by Deutsch and Goodloe (41). Sex or age of the birds were not mentioned. Electrophoretic mobilities (cm/sec/volt/cm x 10^{-5}) of the components were calculated as follows: albumin - 5.3, alpha-globulin - 3.9, beta-globulin - 3.0, and gamma-globulin - 2.0. These values agreed essentially with the results obtained by Moore (122).

The electrophoretic analysis of mature, male chicken serum in phosphate buffer at pH 7.4 by Moore (122) showed an average relative percentage composition as follows: albumin - 43 per cent, alpha-globulin - 21 per cent, beta-globulin - 7.1 per cent, and gamma-globulin - 28.9 per cent in contrast to San Clemente's study (143).

Veronal-citrate buffer at pH 8.6 has been used for the analysis of the plasma of male chickens of undetermined age. The average relative percentage composition of the plasma samples was as follows: albumin - 38.2 $\pm$ 1.3 per cent, alpha 1-globulin - 15.8 $\pm$ 0.6 per cent, alpha 2-globulin - 7.7 $\pm$ 0.5 per cent, beta-globulin + fibrinogen + gamma-globulin + 37.5 $\pm$ 1.3 per cent. Separation of the three components of lowest mobility was incomplete. Electrophoretic mobilities were also extremely high in this buffer (41).

In the analysis of sera from chickens of undetermined sex and age, in veronal buffer at pH 8.6, 0.1 ionic strength, the average relative percentage composition was as follows: albumin - 46 per cent, alpha-globulins - 22 per cent, beta-globulin - 8 per cent, and gamma-globulin - 24 per cent (43).

An excellent study of the electrophoretic distribution of normal serum and plasma components of 15 to 18 week old Single Comb White Leghorn chickens of unknown sex was conducted by Sanders, et al (144) during a study of leucosis. Veronal buffer at pH 8.6, 0.1 ionic strength was used with a potential gradient of 6 to 7 volts/cm^2. These conditions gave excellent

resolution of the serum and plasma components. The following average values for mobilities (cm/sec/volt/cm x 10^{-5}, calculated from the descending limb) were found:

Component	Average	Range
albumin	5.9	5.7-6.0
alpha-globulins	4.7	4.5-5.0
beta-globulin	3.5	3.5- 3.6
fibrinogen	2.5	2.5
gamma-globulin	2.0	1.9-2.1

Average relative percentage composition values, calculated from the descending limb were as follows:

Component	Average (per cent)	Range (ascending limb, uncorrected) (per cent)
albumin	46.8	41.7-47.2
alpha-globulins	17.9	9.0-16.3
beta-globulin	11.3	10.9-14.8
fibrinogen	13.5	14.1
gamma-globulin	19.4	13.7-32.5
albumin/globulin ratio	1.00	0.72-1.34

Total normal serum protein values were found by Sanders, et al (144) to vary from 2.19 to 3.74 gm/100 ml of serum in these 15 to 18 week old birds. Brandt, et al (17) have shown values of 4.63 ± 0.29 for four month old cockerels and 4.49 ± 0.37 for four month old pullets. In general, total serum protein values increase with maturity.

B. Infectious Bronchitis of Chickens

Infectious bronchitis of chickens was first described by Schalk and Hawn (145) in 1931 as an acute respiratory disease of chicks prevalent in the Midwestern States. Since that time the disease has been reported throughout the United States (5, 8, 9, 18, 61) and has also been observed in England (3, 9), Holland (154), and Canada (8, 9).

The virus etiology of the disease has been established by filtration experiments (7, 10, 18, 39, 145) and electron microscopy (136, 137).

The disease was originally thought to be confined solely to chicks (18, 145) but it has been reported and is recognized in chickens of all ages (5, 10, 39, 55, 86).

The morbidity and mortality rates may be as high as 90 per cent in infected chicks (18, 39, 145). In mature chickens the mortality rate is negligible (164), although the morbidity rate may be high (164). In laying flocks there may be a temporary cessation of egg production (39, 164). After production returns the first few eggs are abnormal in quality (65, 164).

Symptoms in chickens of all ages are similar but in adult chickens the symptoms are less severe than those observed in chicks (164). Characteristic symptoms of sneezing, gasping, tracheal rales, and anorexia are observed (7, 18, 145, 164).

The incubation period varies from 24 to 48 hours (8, 9, 164), although in some cases symptoms have not been

observed for as long as six days after exposure (164). The symptoms persist for approximately one week (164).

The histopathologic alterations resulting from infectious bronchitis in chickens have been described (74). These alterations consist of leucocytic infiltration and edema of the mucous membranes and submucosa of the trachea. The liver, spleen, and kidneys do not show any significant lesions which can be ascribed to the disease (74).

Gross lesions include accumulation of mucus in the trachea and bronchi, a congestion of the lungs, and clouding of the air sac membranes (5, 8, 9, 36, 37, 47, 61, 74, 145, 164).

The virus is found most abundantly in tracheal exudates and in the lungs (94, 164) and can be transmitted by infected tissue suspensions to chickens by the intranasal and intratracheal routes (7) and by the subcutaneous and intraperitoneal routes (39).

The carrier problem has also been extensively studied. Chickens recovered from the disease may continue to discharge virus from the upper respiratory tract and serve as potential reservoirs of infection (75, 77, 94).

Birds recovered from the disease develop a specific immunity to subsequent infection with the virus (7, 10, 36, 39, 78, 86). Birds that have become infected naturally or experimentally are capable of producing neutralizing antibodies (7, 57, 130). Naturally acquired, passive immunity in chicks has also been demonstrated (78, 86).

The serum neutralization test has been the only satisfactory serological test used to demonstrate the presence of neutralizing antibodies to infectious bronchitis virus (32, 57, 130, 164). The virus is incapable of agglutinating red blood cells as is characteristic with Newcastle disease virus (6, 16, 54, 76, 113) and fowl plague virus (113). Clinical history, lesions, symptoms, and isolation of the virus with the production of characteristic pathologic alterations of embryonating chicken eggs may also be used as a method of diagnosis (5, 7, 18, 36, 39, 56, 74, 164).

The cultivation of infectious bronchitis virus on the chorio-allantoic membranes of embryonating chicken eggs was first reported by Beaudette and Hudson (10). Early passages of the virus produced no noticeable changes in the embryo, although succeeding virus transfers produced definite mortality (10, 39, 40). The virus became increasingly virulent for the embryo and less virulent for the chicken. After the ninetieth passage the virus had lost all its virulence for the chicken and became incapable of inciting the production of antibodies, while becoming fatal for all inoculated embryos (10, 40). This egg-adapted virus is in general use today as the antigen in the serum neutralization test (33, 57, 130).

The virus produces definite alterations in embryonating chicken eggs (110), although Hitchner, et al (71) have shown similar changes in embryos inoculated with the B 1 strain of Newcastle disease virus. The changes produced

by infectious bronchitis virus inoculated via the chorio-allantoic cavity of embryonating chicken eggs have been discussed by Delaplane (40), Fabricant (55), and Loomis, et al (110).

Jones (85) observed that the highest chicken embryo mortality could be produced by amniotic inoculation followed in decreasing order by chorio-allantoic cavity and chorio-allantoic membrane inoculations. Low mortality followed yolk inoculations. Chorio-allantoic cavity inoculations were the most desirable because of their great convenience and simplicity.

III

MATERIALS AND EXPERIMENTAL PROCEDURES

Incomplete knowledge of certain physical and biological properties of antiviral sera prompted this investigation. The study was directed toward an analysis of the changes in serum electrophoretic patterns and the changes in LD_{50} NIs in chickens after exposure to infectious bronchitis virus.

A. Experimental Birds

Twenty-four Single Comb White Leghorn cockerels ranging in age from five months, seven days to seven months, twenty-four days were obtained from the U. S. Regional Poultry Research Laboratory, East Lansing, Michigan on November 15, 1951. These birds were originally used for genetic studies and were from healthy flocks of lines, some resistant and some susceptible to lymphomatosis. They were maintained in batteries under strict sanitary and quarantine conditions in previously unused isolation quarters.

The susceptibility and resistance of the various lines to lymphomatosis were as follows:

Line 7 - susceptible to lymphomatosis
Line 9 - susceptible to lymphomatosis
Line 10 - resistant to lymphomatosis
Line 14 - resistant to lymphomatosis
Line 15 - susceptible to lymphomatosis

The birds were divided into four groups as follows: The letter and number designations of the birds are those which were given to each bird by the U.S. Regional Poultry Research Laboratory. The number following the hyphen was given to each bird for convenience in conducting the experiment.

Group I - Control birds

M727S -1	Line 15
M541B2 -2	Line 10
M400D2 -4*	Line 9
M362V -5	Line 7
M347B2 -6*	Line 7

Group II - Birds receiving a primary inoculation of infectious bronchitis virus

M716G2 -7	Line 15
M347C2 -8	Line 7
M735R -9	Line 15
M349T -10	Line 7
M538H -11	Line 10
M4320 -12	Line 9

Group III - Birds receiving a primary inoculation of infectious bronchitis virus and challenged in the twelfth week after primary inoculation

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* Inoculated with a normal tissue suspension during the seventeenth week of this study.

M414Q -13 Line 9

M400U -14 Line 9

M589U -15 Line 10

M727B2 -16 Line 15

M361Q -17 Line 7

M369N -18 Line 7

M541C2 -19 Line 10

Group IVa - Birds inoculated with a normal lung and tracheal suspension

M346B -21 Line 7

M673V -22 Line 14

M645H -23 Line 14

Group IVb - Birds subjected to a tracheal injury, virus not introduced

M542A -24 Line 10

M333H -25 Line 7

M673U -26 Line 14

The birds were maintained on a commercial growing mash* containing not less than 20 per cent protein, not less than 3.50 per cent fat, and not more than 5.50 per cent fiber. The mash was top-dressed two or three times weekly with a commercial

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* Michigan State Growing Mash, manufactured by A. K. Zinn & Co., Battle Creek, Michigan

scratch feed* containing not less than 9 per cent protein, not less than 2 per cent fat, and not more than 5 per cent fiber. Water was allowed ad libitum.

B. Virus Antigens

Two different strains of infectious bronchitis virus were used. Strain V114D**, a chicken-embryo-adapted strain, was capable of killing all embryos inoculated via the allantoic cavity within 48 hours and was used as the antigen in the serum neutralization tests. This strain is capable of entering into specific combination with antibodies against infectious bronchitis virus (32, 33, 57, 130). It is prepared by inoculating nine-to 11-day-old embryonating chicken eggs with undiluted, virus-infected allantoic fluid via the allantoic cavity, incubating for 24 to 30 hours, and harvesting the allantoic fluid of living embryos (32).

Strain VR (Lot 285)*** was a chicken-propagated strain which was supplied as lyophilized, infected tracheal washings. This strain was used for all inoculations of the experimentally-infected groups.

In order to have a large volume of inoculum and to check the virulence of the virus for chickens this preparation was resuspended to volume with Difco nutrient broth.

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* Zinn's Climax Scratch Feed, manufactured by A. K. Zinn & Co., Battle Creek, Michigan

** Strain V114D has been maintained in the laboratory for at least 150 passages in embryonating chicken eggs.

*** Supplied by Dr. Henry Van Roekel, Department of Veterinary Science, University of Massachusetts

The resulting suspension was instilled in 0.2 ml amounts intratracheally and 0.05 ml amounts intranasally in three normal six-week-old chickens.

Six-week-old chickens were used because it has been shown (78, 86) that naturally acquired, passive antibodies against infectious bronchitis virus are at a low or negligible level at this age and are incapable of protecting young birds against a subsequent inoculation with the virus.

The tracheas were scraped with sterile cotton-tipped applicator sticks to facilitate more intimate contact of the virus with the tracheal epithelium (32). Characteristic symptoms of infectious bronchitis (164) were observed in these birds 24 hours after inoculation. The birds were killed 72 hours after inoculation at a period in which symptoms were at their greatest. The tracheas and lungs were harvested, pooled, ground with sand using a mortar and pestle, and made up to a 20 per cent suspension with Difco nutrient broth. The suspension was then treated with 10, 000 units each of penicillin and streptomycin per ml (32, 38) and centrifuged to sediment sand and tissue debris. The supernatant fluid was stored at -40 C for further use.

This process of inoculating six-week-old chickens with the chicken-propagated strain of infectious bronchitis virus was repeated and the resulting highly potent virus was used as the antigen for the experimentally-infected groups.

One-tenth ml of this suspension was inoculated into five nine-day-old embryonating chicken eggs via the allantoic

cavity. The inoculum produced characteristic gross lesions of dwarfing and curling of the embryos (110) by the third and fourth post-inoculation days. Hitchner, et al (71) showed that the B 1 strain of Newcastle disease virus also may produce lesions, dwarfing, and curling which are similar to those produced by infectious bronchitis virus in the chicken embryo. Therefore, an hemagglutination test was conducted on the harvested allantoic fluid to determine the presence or absence of Newcastle disease virus. Lack of agglutination of red blood cells indicated the absence of Newcastle disease virus. Abnormal embryos were not observed.

C. Serum Neutralization Test (32)

Nine-day-old embryonating chicken eggs were used in the serum neutralization tests. The eggs were maintained in an electric, forced-draft incubator* at 99.5 F (88 F wet-bulb thermometer). The site for inoculation via the allantoic cavity was determined by trans-illumination of the egg. An area devoid of large blood vessels, approximately two mm below the base of the air cell, and at a side opposite to the embryo was selected. A small hole was drilled through the shell without piercing the shell membrane by means of an electrically-driven drill. An additional hole was drilled directly above the air cell to serve as an air vent in equilizing the pressure produced by the injection of the inoculum into the egg. Both holes were painted with tincture of metaphen and the shell membrane above the air cell was pierced with a sterile teasing needle.

*Model 252, manufactured by Jamesway Manufacturing Company Fort Atkinson, Wisconsin

Serial ten-fold dilutions of V114D virus-infected allantoic fluid were prepared with Difco nutrient broth in the proportions of 0.5 ml of virus-infected allantoic fluid to 4.5 ml of diluent. Serum-virus mixtures were prepared separately by mixing equal portions of each virus dilution and test serum. The sera were usually used undiluted, although occassionally it was necessary to dilute sera 1:10 or more with Difco nutrient broth to give sufficient volume for the test and for the electrophoretic analysis. In some instances the sera completely neutralized undiluted virus and in order to establish an endpoint for calculation of the LD_{50} NIs it was necessary to dilute the sera 1:10 or more. The virus dilutions were mixed with equal portions of Difco nutrient broth to obtain a quantitative estimation of the virus titer. Three-tenths ml of the virus dilutions were mixed with 0.3 ml of the serum preparations. All mixtures were incubated in an ice-bath at 4 C for 30 minutes. Dilution of sera in the serum neutralization test for infectious bronchitis does not significantly affect the LD_{50} NIs (131).

Five eggs were used per dilution and each egg received an inoculum of 0.1 ml using a one-ml B-D Yale tuberculin syringe fitted with a 27 gauge, one-half inch needle. The eggs for the quantitative virus titrations were inoculated last to make provisions for any possible deleterious effect of incubation of virus. Page (131) showed that the infectious bronchitis virus LD_{50} titer does not significantly change up to 16 hours of incubation at 4 C. After inoculation

the holes were sealed with melted paraffin and the eggs were reincubated and candled daily for five days. Death of the embryos during the first 18 hours was attributed to trauma or other non-specific causes and these eggs were not included in the calculations of the final results. The results of the serum and virus titrations were evaluated according to the 50 per cent endpoint formula of Reed and Muench (138) and expressed as the LD_{50}.

The difference between the reciprocal of the virus titer and the reciprocal of the serum titer was designated as the LD_{50} NI. The antilog of the LD_{50} NI represented the numbers of serum neutralizing doses. In cases where the sera were diluted the LD_{50} NI was calculated by multiplying the difference between the virus titer and the serum titer by the dilution factor.

The procedure for the neutralization test for infectious bronchitis has been thoroughly described by Cunningham (32) and should be consulted for further details.

D. Electrophoresis

All sera obtained were analyzed for protein nitrogen content using the macro-Kjeldahl technique (conversion factor, 6.25) previous to dilution for dialysis. A two per cent final serum protein concentration was used in all electrophoretic analyses. It has been shown that the protein concentration greatly affects the mobility (34, 101, 136) and relative percentage distribution of serum protein components (132). A high protein concentration may also produce boundary

disturbances (123). Sanders, et al (144) used a final two per cent concentration of serum protein and obtained excellent results in the resolution of chicken serum and plasma components.

A standard veronal buffer (104) of 0.1 ionic strength and pH 8.6 as measured with the glass electrode (Beckman, Model G pH meter) at 25 C was used in all dialyses and dilutions of serum samples. It was composed of 0.0152 M diethylbarbituric acid (2.797 gm/liter) and 0.1 M monosodium salt of diethylbarbituric acid (20.6 gm/liter). This buffer has been shown to be excellent for use in the electrophoretic analysis of chicken serum (43, 144).

Dialysis of serum samples previous to electrophoretic analysis is necessary to equilibrate the sample and buffer with respect to electrolytic conductivity.

The stirring dialysis method of Reiner and Fenichel (139) was used for each serum sample. The diluted serum sample was placed in a bag made of seamless cellulose tubing* which was previously tested for leaks. It was dialyzed against a one-hundred-fold volume of buffer for two hours at room temperature. These conditions gave excellent equilibration when electrolytic conductivities of both buffer and sample were measured at 0.5 C using a conductivity cell especially designed

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* Nojax-Visking Cellulose Sausage Casing (unknown pore size), manufactured by the Visking Corporation, Chicago, Illinois

for use with the Model 38, Perkin-Elmer Tiselius Electrophoresis Apparatus* and an electrolytic conductivity bridge**.

Conductivity measurements are necessary to determine equilibration in electrolytic conductivity since inadequate equilibration may result in boundary disturbances (104, 107, 133). The specific conductance of the dialyzed sample also is needed to determine potential gradients across the cell and mobilities of the individual serum components (104)

The specific conductance (Ksp) of each sample was calculated as follows:

$$Ksp = \frac{Kc}{R} \qquad (I)$$

where Kc is the conductivity cell constant and R is the measured resistance.

The Kc must be determined for each cell since the value varies with the distance between the electrodes. This was determined as follows: Using standard 0.01 N KCl, C.P. (0.7455 gm/1000 gm double-distilled, CO_2 -free water) the measured resistance of this solution in the conductivity cell was 1080 ohms (R) at 0 C. The specific conductance of this solution at 0 C is 0.0007728 mhos (γ) (98). Substituting in the formula,

$$Kc = \gamma R \qquad (II),$$

a value of 0.8346 was obtained. Substituting the value for Kc

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* Manufactured by the Perkin-Elmer Corporation, Norwalk, Conn.

** Leeds and Northrup #4960 Electrolytic Conductivity Bridge, manufactured by Leeds and Northrup Company, Philadelphia, Pa.

in formula (I), and measuring the resistance (R) of the dialyzed sample the specific conductance (Ksp) can be determined.

From previous studies in this laboratory equilibration of diluted serum samples and buffer can be attained in one and one-half hours at room temperature. Therefore, in order not to disturb the electrophoretic experiments, the samples were dialyzed for two hours at room temperature and placed at 4 C overnight. In this way the samples can be kept cold for use the following day. It was also observed that cold samples and cold electrophoresis cells minimize the formation of air bubbles in the cells during the cell-filling process. Air bubbles greatly disturb the boundaries formed in the cell during the experiments. Conductivities were then measured at the end of the day after all electrophoretic analyses were completed.

The Model 38, Tiselius Electrophoresis Apparatus was used for the electrophoretic analyses of all serum samples. The apparatus has been described by Moore and White (127) but for a more detailed description and operational procedure the reader should consult the instruction manual (133).

The Toepler schlieren method has been adopted with certain improvements such as the schlieren lens system being replaced by two separate lenses in conjunction with a smaller aperture allowing for a greater reduction in the length of the optical path. A scanning modification (103) of the schlieren system is also employed but with a two-knife-edge diaphragm which gives two similar patterns.

A two-ml capacity electrophoresis cell having a cross-sectional area of about 0.30 cm^2 is used with the apparatus (28, 127, 133).

A resumé of the operation of the instrument is given as follows:

Both sides of the ice compartment of the water-bath are filled with ice. The water-bath is filled with cold water and the stirrer is turned on until the water-bath reaches a uniform temperature of 0.5 C by the time the cell is filled.

The circulating air pump is started to remove water condensate from the internal windows and lens surfaces by passing air through a calcium sulphate drying tower. There are also two 75 watt heating elements near the external lens surfaces which heat the lenses and prevent moisture from collecting on them.

The cell is greased and assembled according to instructions and kept at 4 C for 12 to 18 hours in order to have it cold for the following day's experiments.

The cell is placed in position in the cell holder, filled, disaligned, and the two buffer bottles are connected. The electrodes are inserted and buffer is added up to the level of the side arms of the assembly. This is done as rapidly as possible to prevent the samples and cell from becoming warm. The complete assembly is placed in the water-bath, clamped into position and the level-equilizing gate is raised. The electrodes are connected to the leads and 15 ml of cold, one-third saturated KCl, C.P. is layered beneath the buffer in each buffer

bottle through the electrode capillaries by means of a syringe fitted with a four-inch needle.

The cover of the water-bath is replaced and more ice is added through the ice-chimney. When temperature equilibrium is reached (ten to 15 minutes) the level equilizing gate is lowered gently and the cell channels are aligned by means of the shifting rod.

Buffer is gently flowed into the left-hand buffer bottle to bring the boundaries into view (approximately three mm) by means of a mechanically-operated compensator*. The boundaries are originally behind the flange plates of the cell. By advancing the boundaries no more than three mm the current can be applied for a longer period of time and optimum resolution of the serum components is usually obtained. The compensator is stopped and the beginning boundaries of the ascending and descending limbs are photographed. Either Kodak M or Kodak Process Panchromatic 3¼ x 4¼ inch plates are used and are exposed for five seconds.

The current is adjusted to six milliamperes and the voltage is maintained at approximately 107 volts, giving 0.65 watt heat dissipation.

During the electrophoretic run the separation and migration of the serum components are observed by means of the

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* Manufactured by the Perkin-Elmer Corporation, Norwalk, Conn.

cylindrical lens and diagonal schlieren diaphragm attachment of Thovert (104, 105). When the fastest component has migrated approximately two-thirds of the distance across the cell (7,600 seconds) the current is stopped and scanning photographs of the ascending and descending limbs are taken. These photographs are developed in total darkness in Kodak D-19 developer for five minutes and fixed in Kodak Acid Fixer for 15 minutes. After fixing the photographs are washed in running water for 30 minutes and allowed to dry.

The pattern from the descending limb (104, 106, 107) is projected with a photographic enlarger and traced on paper at a linear enlargement of double size. The areas attributable to the various serum components are defined by the method of Tiselius and Kabat (158) in which ordinates are drawn from the lowest point between two components to the base line.

Each area is measured with a precision disc, compensating polar planimeter* in arbitrary units and the relative concentrations in per cent are determined by dividing the area of each component by the area of the entire pattern, excluding the area of the epsilon-boundary. The epsilon-boundary is a buffer salt-protein interaction complex.

Mobilities are calculated on the descending patterns as advocated by Longsworth and MacInnes (107). The distance between the beginning boundary and the ordinate dividing the

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* Model 4236, manufactured by Keuffel & Esser Company, New York

respective area in half is used as a measure of the distance migrated by each component.

The formula of Longsworth (108) is used in the calculation of mobilities and is used as follows:

$$\mu \quad (\text{cm/sec/volt/cm} \times 10^{-5}) \quad = \quad \frac{d \quad q \quad Ksp}{i \quad t \quad m}$$

where d is the distance migrated in cm, q is the cross-sectional area of the descending limb in cm^2, Ksp is the specific conductance of the sample, t is the time of migration in seconds, i is the current in amperes, and m is the enlargement factor.

The potential gradient (F) in volts/cm^2 , which averaged 6.5 volts/cm^2 is determined as follows:

$$F \quad = \quad \frac{i}{q \quad Ksp}$$

where i is the current in amperes, q is the cross-sectional area of the descending limb in cm^2, and Ksp is the specific conductance of the sample.

E. Experimental Exposure

All birds were bled by cardiac puncture one week after they were obtained. The sera collected were analyzed for antibodies specific for Newcastle disease virus by means of the hemagglutination-inhibition test (54) and for antibodies specific for infectious bronchitis virus by means of the serum neutralization test (32). Infectious bronchitis virus is incapable of

of agglutinating red blood cells and the serum neutralization test is the only method available at the present time for the detection of antibodies specific for infectious bronchitis virus. All sera tested were considered to be negative according to established standards (33, 54, 57).

On January 19, 1952 the investigation was begun. All birds were fasted for 18 to 24 hours prior to bleeding to decrease the quantity of serum lipids (134, 144). Zeldis, et al (175) showed that lipids become additive in the beta-globulin of human plasma and in the alpha-globulin in dog plasma. Birds in Groups I, II, and III were bled by cardiac puncture. Birds in Groups II and III were inoculated with the chicken-propagated strain of infectious bronchitis virus by depositing with a syringe, 0.2 ml in the upper trachea and 0.05 ml into the nares. A cotton-tipped applicator stick was used to scarify the lumen of the trachea to ensure more intimate contact of the virus with the epithelium.

Characteristic symptoms of infectious bronchitis (164) appeared in all inoculated birds within 24 to 36 hours after inoculation. The symptoms persisted for eight days and consisted of dyspnea, sneezing, tracheal rales, nasal discharge, and a slight anorexia.

Birds in Group II were bled immediately prior to inoculation and then at one, two, three, four, six, eight, ten, 12, 16, and 20 week intervals. Birds in Group III were also bled immediately prior to inoculation and then at one, two, three, four, six, eight, ten, and 12 week intervals. At the

twelfth week the birds in Group III were challenged with the chicken-propagated strain of infectious bronchitis virus and bled again at one, three, five, and seven weeks after challenge.

The control birds in Group I were bled according to the schedule of Group II, but unfortunately many of them died due to internal hemorrhage caused by the experimental bleedings. Bird M727S -1 was bled at four, eight, 12, and 17 week intervals. Birds M541B2 -2 and M365V -5 were bled at the first bleeding period as birds in Groups II and III and then at one, two, and three week intervals. Bird M400D2-4 was also bled at the first experimental bleeding period as birds in Groups II and III and then at one, two, three, four, eight, 12, and 17 week intervals.

In order to determine if inoculations of normal tissue suspensions could cause changes in serum electrophoretic patterns or changes in LD_{50} NIs bird M400D2 -4 was inoculated with a normal lung and tracheal suspension via the intratracheal and intranasal routes during the seventeenth week and bled one and two weeks after inoculation.

Bird M347B2 -6 was also bled at the first experimental period as birds in Groups II and III and then at one, two, three, four, eight, and 17 week intervals. At the seventeenth week this bird was also inoculated with a normal lung and tracheal suspension and bled at one, two, and three weeks after inoculation.

Birds of Group IVa were bled on June 14, 1952 and inoculated with a normal lung and tracheal suspension followed

by a scarification of the trachea using a cotton-tipped applicator stick. The birds were bled at one, two, and three week intervals after inoculation. It has been shown that normal homologous tissue inoculations in rabbits produce microscopic manifestations of inflammation (118, 119). Inflammation caused by intradermal injections of turpentine or other irritants can alter the serum electrophoretic patterns (63, 64). It was thought that possibly a relationship existed between inflammation, inoculations of normal tissues, and changes in serum electrophoretic patterns.

Traumatic injury has also been shown to alter the serum electrophoretic pattern (22, 62, 124, 125). Therefore, birds in Group IVb were bled and subjected to a tracheal injury by scarification using a cotton-tipped applicator stick. These birds were then bled one, two, and three weeks after injury.

The presence of Newcastle disease virus or infectious bronchitis virus in the normal tissue suspension was eliminated by inoculating 0.1 ml into nine-day-old embryonating chicken eggs via the allantoic cavity. The embryos were candled daily for five days and the allantoic fluid was harvested. This was repeated for four passages. No alterations in the embryos were observed. The allantoic fluid also did not agglutinate red blood cells.

IV

RESULTS

A. Group I

1. Electrophoretic Analyses

Electrophoretic analyses of sera from this control group showed varied results. Bird M727S -1 was bled at the four, eight, 12, and 17 week interval. Neither the relative percentage distribution of the serum components nor the albumin/globulin ratio changed significantly during this period. (Table 1, Figure I)

Bird M541B2 -2 was bled at the first experimental period and then at the one, two, and three week interval. Unfortunately the bird died at the third week because of internal hemorrhage due to cardiac puncture. An increase in the relative percentage of albumin was observed at the first and second weeks. There was a definite decrease in albumin at the third week. Gamma-globulin levels decreased at the first week interval and increased steadily up to and including the third week. Albumin/globulin ratios followed the same general trend as the albumin values. Alpha 1-globulin values changed slightly as did the beta-globulin values. Alpha 2-globulin values decreased at the first week interval and steadily increased up to and including the third week. (Table 2)

Bird M362V -5 was bled at the first experimental period and then at the one, two, three, and four week interval.

Table 1

Bird M727S -1

Group I-Control

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
4	50.7	5.8	6.2	10.5	26.8	1.03	3.43
8	51.6	6.1	5.2	11.7	25.4	1.07	3.43
12	48.9	8.9	6.8	11.4	24.0	0.96	3.43
17	50.8	6.5	8.7	10.8	23.2	1.03	3.60

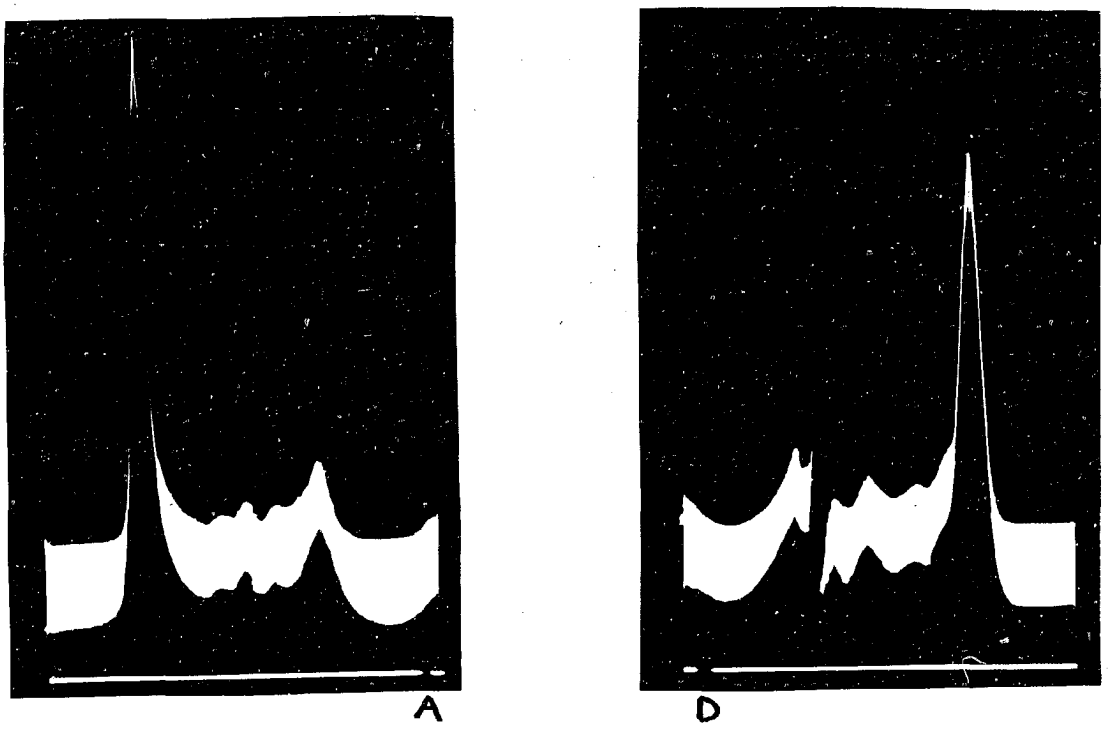
A/G Albumin/Globulin Ratio

Figure I

Bird M727S-1

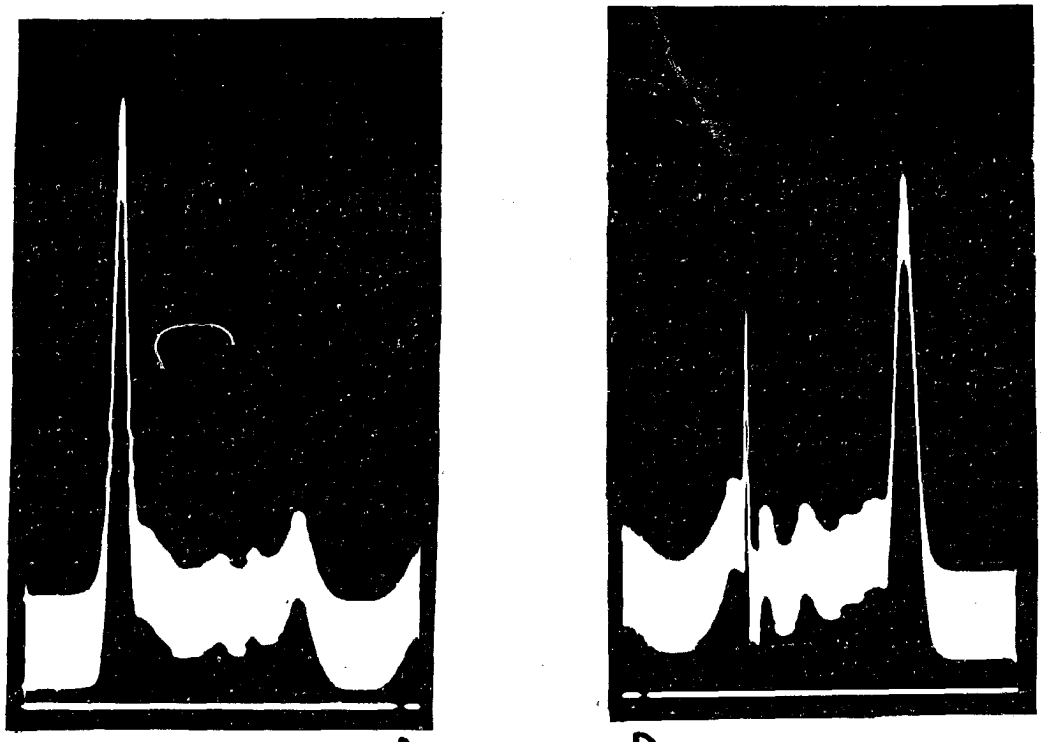
Group I-Control

Electrophoretic Patterns



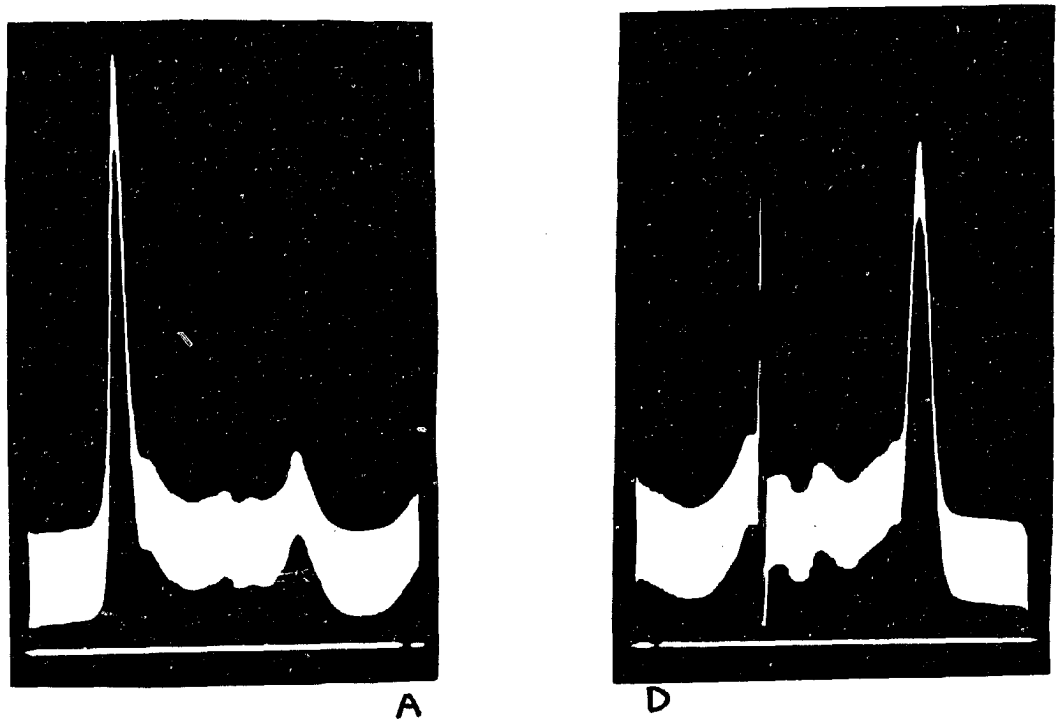
A ASCENDING LIMB
D DESCENDING LIMB

Figure I (cont.)



A D

12 WEEKS



A D

17 WEEKS

Table 2

Bird M541B2 -2

Group I-Control

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	42.3	10.6	9.0	8.5	29.6	0.73	3.73
1	47.2	12.4	4.7	10.3	25.4	0.89	3.73
2	46.7	9.6	5.3	9.6	28.8	0.86	4.04
3	39.2	12.5	6.0	10.1	32.2	0.65	3.78

* First bleeding period corresponding to pre-exposure bleeding period of infected bird

This bird died at the fourth week period because of internal hemorrhage due to cardiac puncture. A slight increase in the relative percentage of albumin was observed at the second week interval. Albumin decreased after this period up to and including the fourth week. The gamma-globulin levels decreased at the first week but steadily increased up to and including the fourth week. The albumin/globulin ratios followed the same general trend as the albumin values. Alpha 1-globulin values increased at the first week, decreased at the second week, and increased again at the third and fourth weeks. Alpha 2-globulin did not change significantly during the experimental period. Beta-globulin levels showed a slight increase at the end of the experiment. (Table 3, Figure II)

Equal portions of sera from birds M400D2 -4 and M347B2 -6 were pooled and analyzed electrophoretically as one sample at the first experimental period and then at the one, two, three, four, and 12 week interval. A slight increase in the relative percentage of albumin was observed at the first week interval. After this period there was a general decrease in albumin up to and including the twelfth week. Gamma-globulin values decreased at the first week but generally increased after this period up to and including the twelfth week. Alpha 1-globulin levels did not significantly change up to the fourth week but decreased greatly at the twelfth week. Alpha 2-globulin levels steadily increased while beta-globulin values did not change appreciably. Albumin/globulin ratios varied slightly except that there was a significant

Table 3

Bird M362V -5

Group I-Control

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	45.6	7.7	5.5	8.8	32.4	0.84	3.91
1	45.7	12.6	6.0	7.5	28.2	0.84	3.60
2	47.5	4.4	6.8	9.7	31.6	0.91	3.51
3	40.5	9.6	5.4	10.0	34.5	0.68	4.04
4	39.9	9.5	5.3	10.1	35.2	0.66	3.86

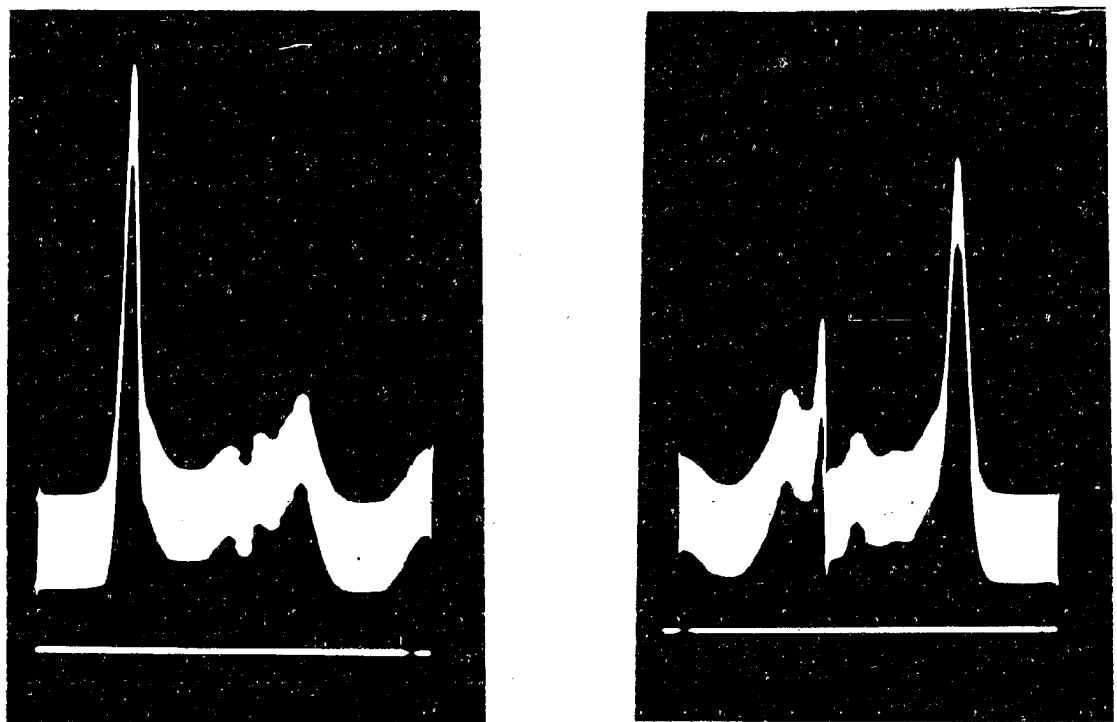
* First bleeding period corresponding to pre-exposure bleeding period of infected bird

Figure II

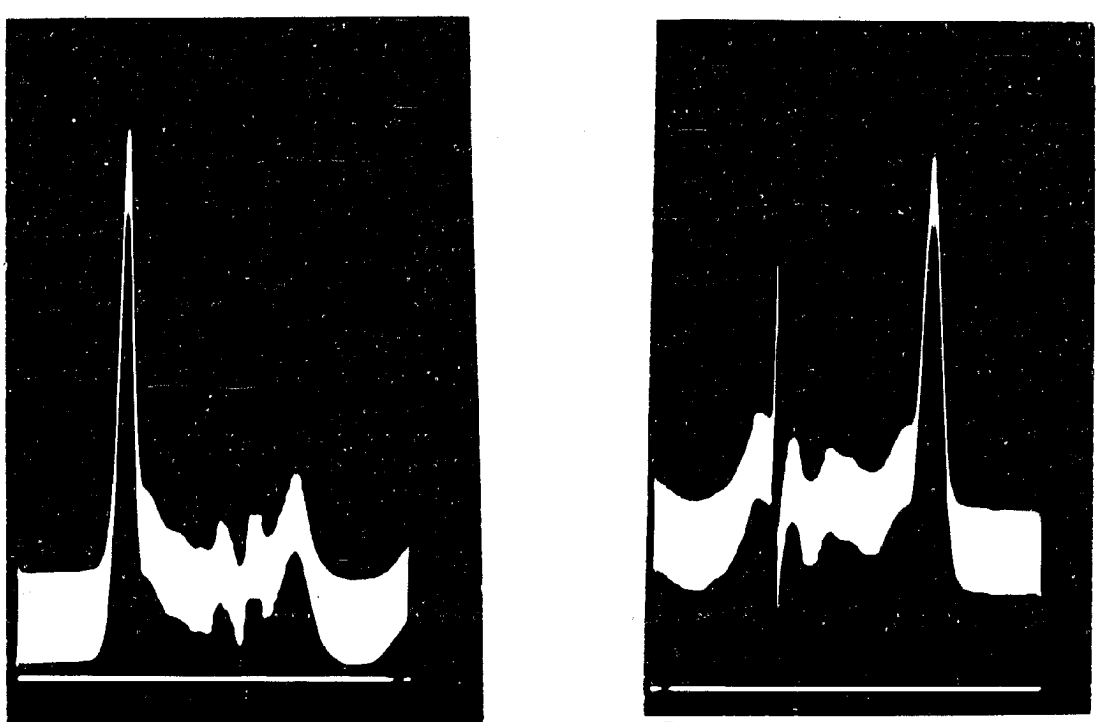
Bird M362V -5

Group I-Control

Electrophoretic Patterns

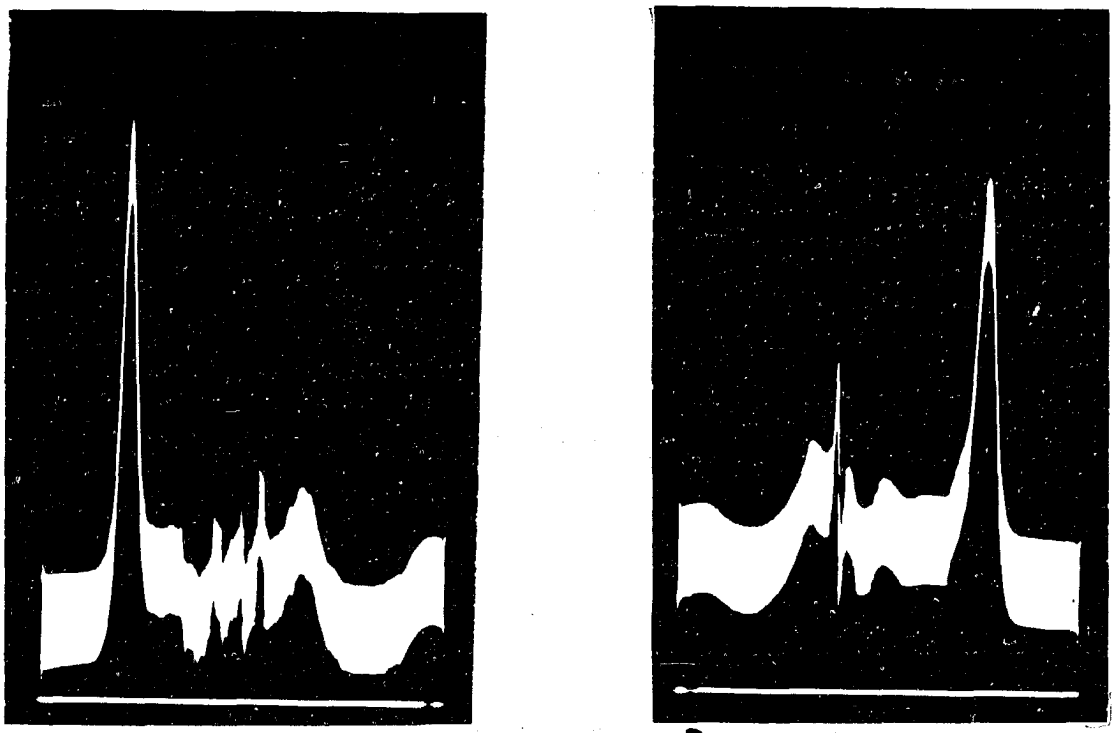


0 WEEKS
FIRST BLEEDING PERIOD



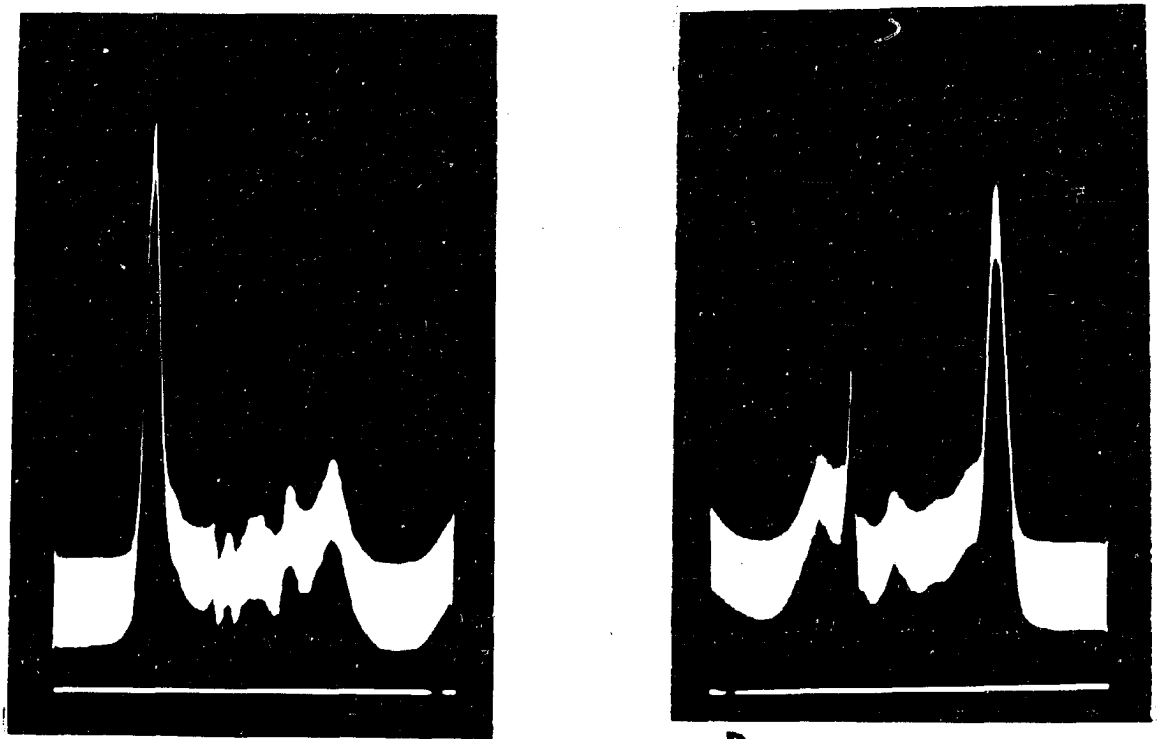
1 WEEK

Figure II (cont.)



A D

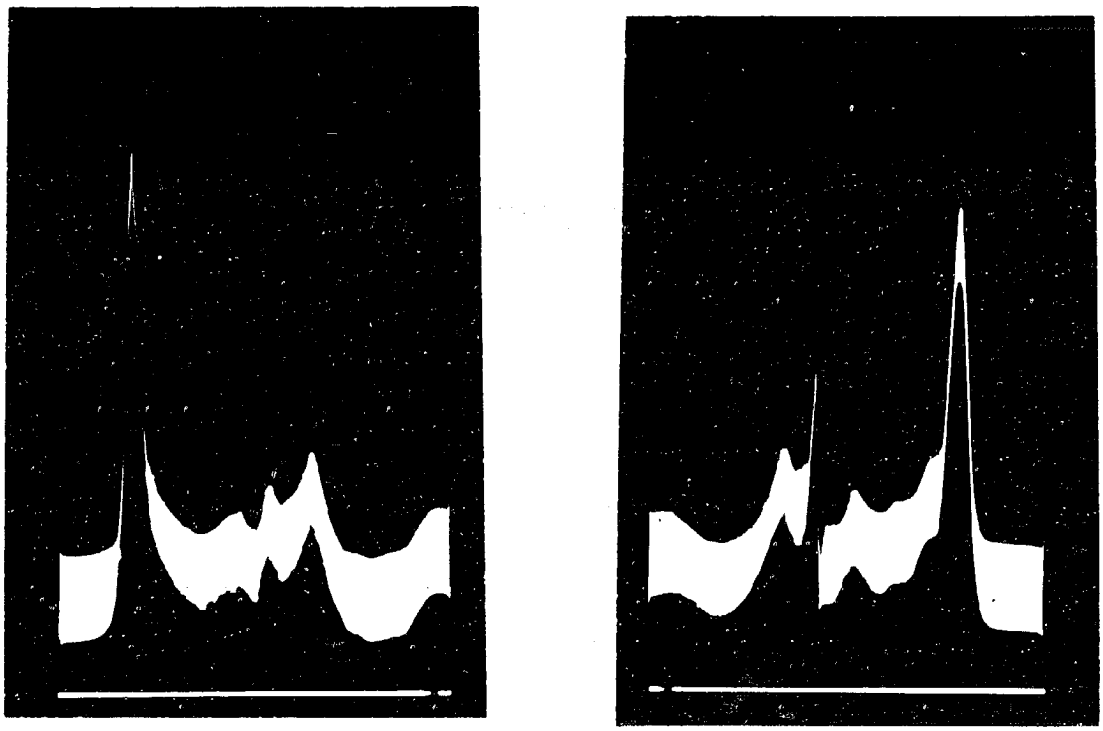
2 WEEKS



A D

3 WEEKS

Figure II (cont.)



A D

4 WEEKS

increase at the first week interval following the change in albumin. (Table 4)

At the eighth and seventeenth weeks sera from the above birds were analyzed individually. Bird M400D2 -4 showed a low albumin value and a high gamma-globulin value. The other globulins were apparently unchanged. (Table 5)

Bird M347B2 -6 showed a significant increase in albumin and a decrease in gamma-globulin at the seventeenth week interval as compared with results obtained at the eighth week. The other serum globulins did not change appreciably. (Table 6, Figure III)

In order to determine if normal tissue suspensions could alter the serum electrophoretic patterns or the LD_{50} NIs, these two birds were inoculated with a normal lung and tracheal suspension at the seventeenth week. Bird M400D2 -4 and bird M347B2 -6 were bled one and two weeks after inoculation. (Table 5) Bird M347B2 -6 was also bled three weeks after inoculation. (Table 6, Figure III)

Bird M400D2 -4 showed a slight decrease in albumin and a rise in gamma-globulin. Alpha 1-globulin values increased appreciably. Alpha 2-globulin and beta-globulin levels did not change significantly. (Table 5)

Bird M347B2 -6 showed a great reduction in albumin one week after inoculation. There was also a slight increase in gamma-globulin which decreased slightly up to and including the third week after inoculation. The relative percentage of alpha 1-globulin increased slightly throughout this period.

Table 4

Pool of Birds M400D2 -4 and M347B2 -6

Group I-Control

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	40.7	10.6	3.7	11.2	33.8	0.69	3.78
1	43.5	11.1	4.7	9.3	31.4	0.77	3.91
2	39.5	9.6	5.0	10.0	35.6	0.66	4.13
3	38.9	8.2	7.7	9.6	35.6	0.64	4.21
4	41.1	9.6	6.9	9.6	32.8	0.70	3.86
12	38.2	5.8	9.0	12.2	34.8	0.62	4.13

* First bleeding period corresponding to pre-exposure bleeding period of infected bird

Table 5

Bird M400D2 -4

Group I-Control

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
8	35.9	11.5	4.8	13.4	34.4	0.54	4.21
17*	31.9	14.7	7.8	10.8	34.8	0.42	4.65
1	29.1	16.8	8.2	10.2	35.7	0.41	4.30
2	28.9	13.6	9.7	9.7	38.1	0.40	4.65

* Inoculated with normal tissue suspension

Alpha 2-globulin increased and beta-globulin remained approximately at the same level as the pre-inoculation value. (Table 6, Figure III)

There was wide variation between different birds in the relative percentage distribution of the individual serum components even at the first bleeding period. Sanders, et al (144), Deutsch, et al (43), and Moore (122) found the same to be the case in sera of normal chickens.

The sera obtained at the first experimental period from the birds in this control group showed fair correlation with the values obtained by Sanders, et al (144). Gamma-globulin levels were lower in Sanders' study since 15- to 18-week-old chickens were used. It has been shown that gamma-globulin levels in chickens increase with maturity (17, 46, 144).

The electrophoretic mobility values of the individual serum components of this group and of the other groups closely approximate the values obtained by Sanders, et al (144), when veronal buffer at pH 8.6, 0.1 ionic strength (104) was used. The following average values were found (cm/sec/volt/cm x 10^{-5}) which were calculated from the patterns of the descending limb:

albumin	6.0
alpha 1-globulin	5.2
alpha 2-globulin	4.4
beta-globulin	3.6
gamma-globulin	2.0

Table 6

Bird M347B2 -6

Group I-Control

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
8	39.0	10.5	6.9	8.3	35.3	0.64	3.51
17*	43.1	9.0	7.6	10.0	30.3	0.76	3.51
1	33.8	11.3	10.8	10.8	33.3	0.51	3.95
2	41.5	9.3	8.2	9.8	31.2	0.71	4.13
3	41.5	11.0	8.5	10.0	29.0	0.71	3.95

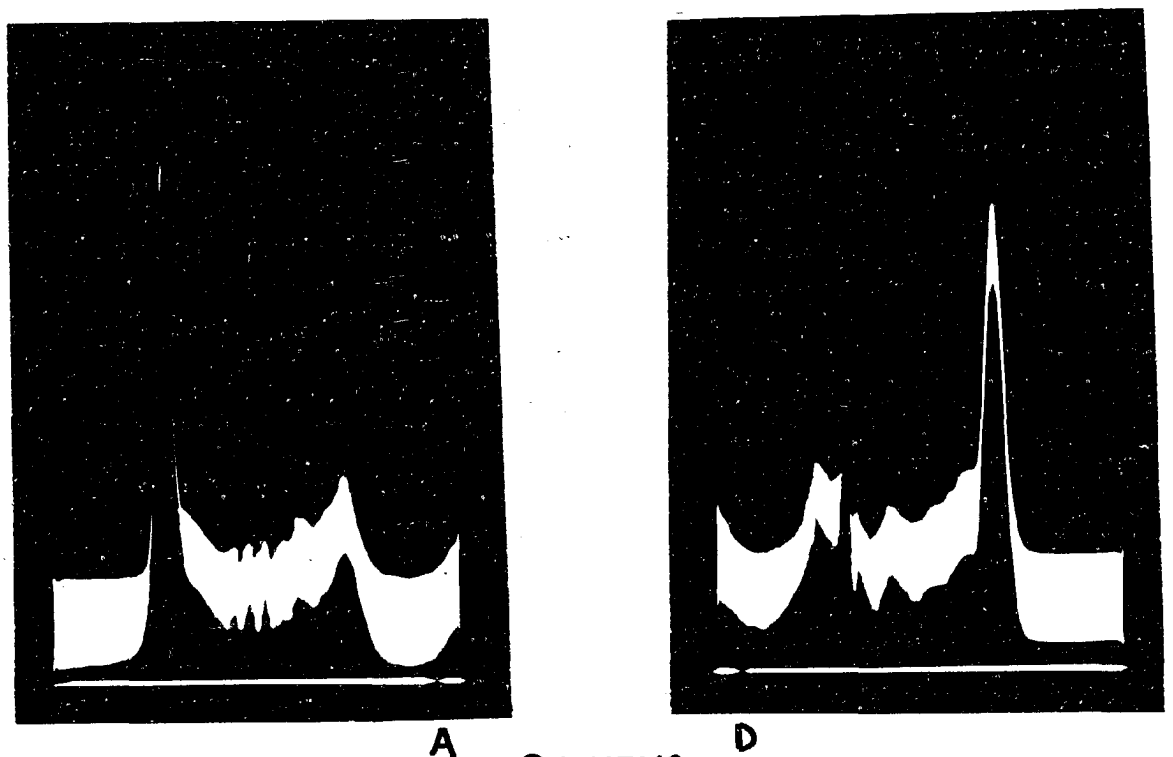
* Inoculated with normal tissue suspension

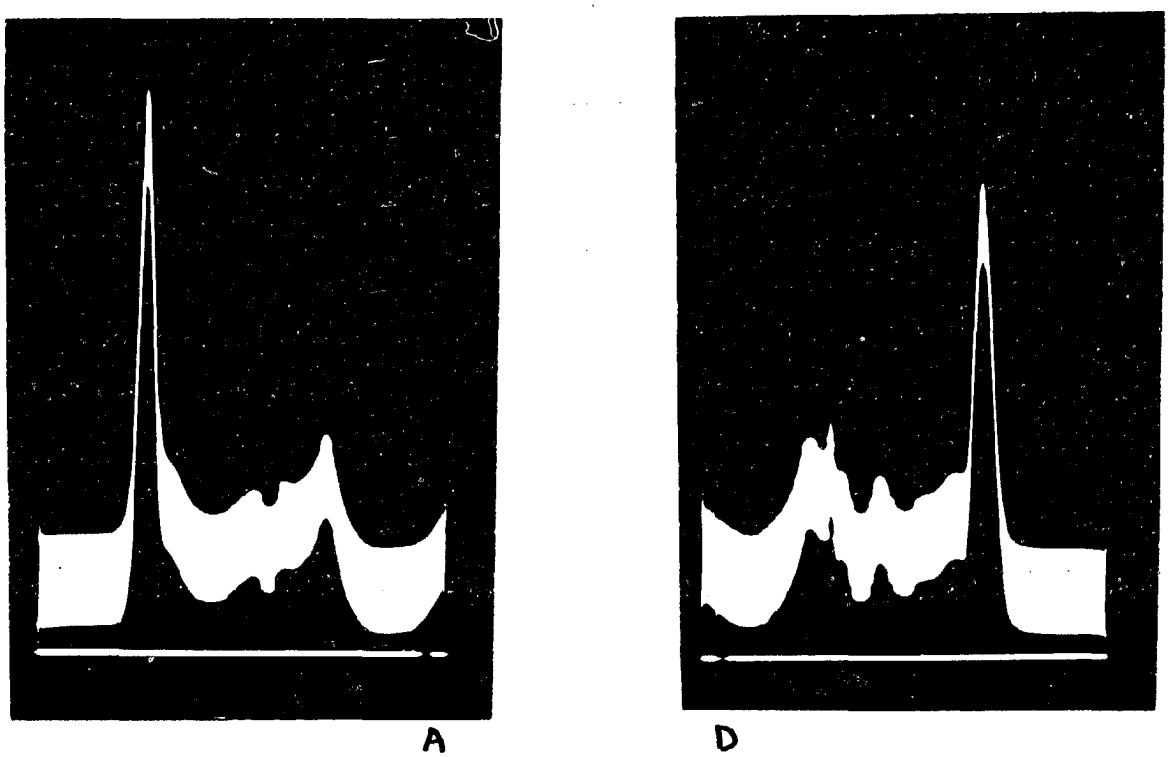
Figure III

Bird M347B2 -6

Group I-Control

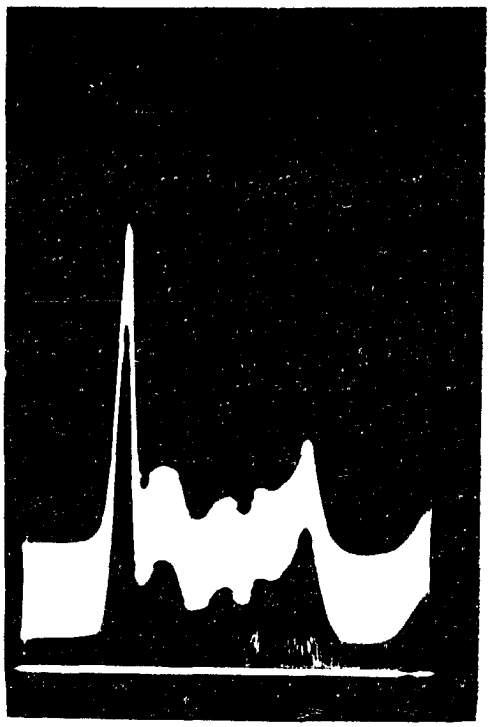
Electrophoretic Patterns



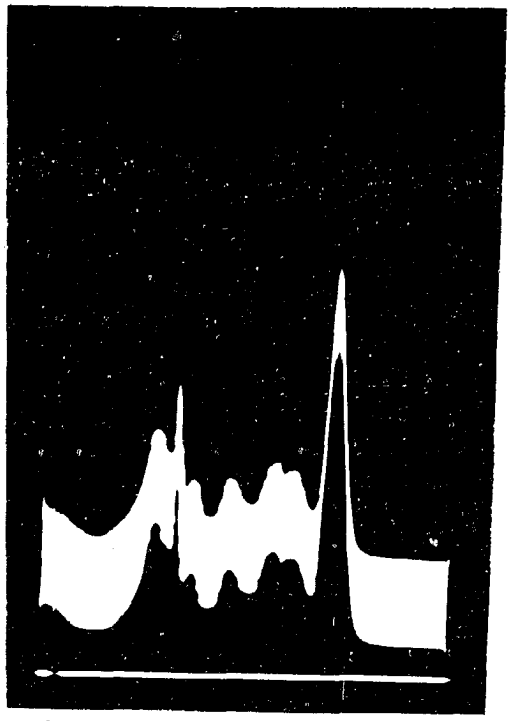


INOCULATED WITH NORMAL TISSUE SUSPENSION

Figure III (cont.)

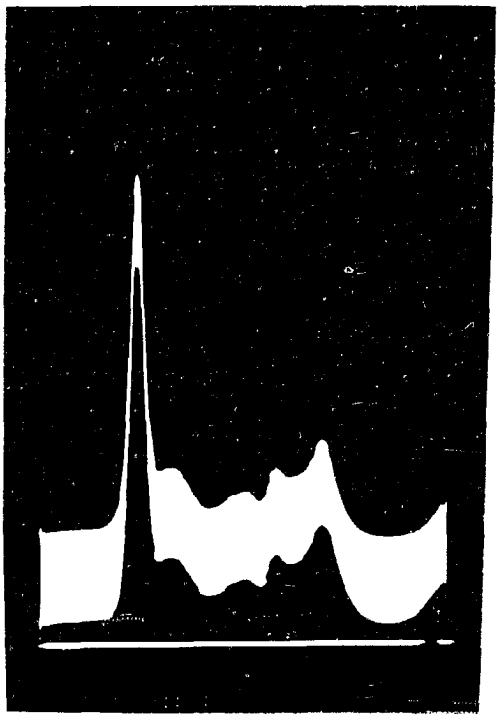


A

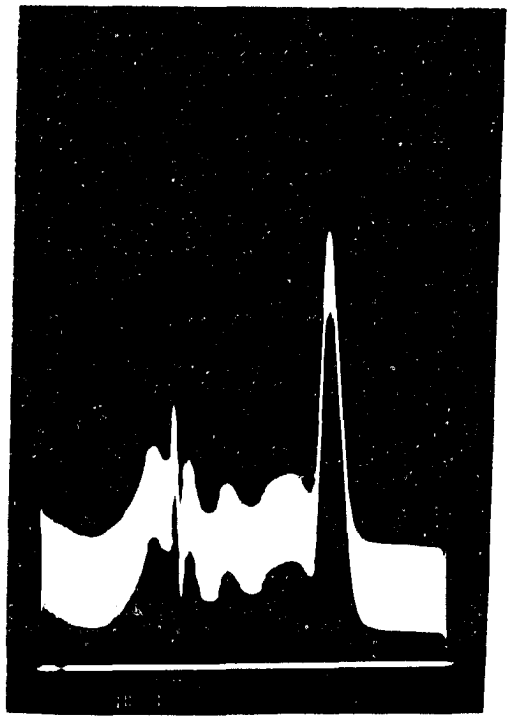


D

1 WEEK



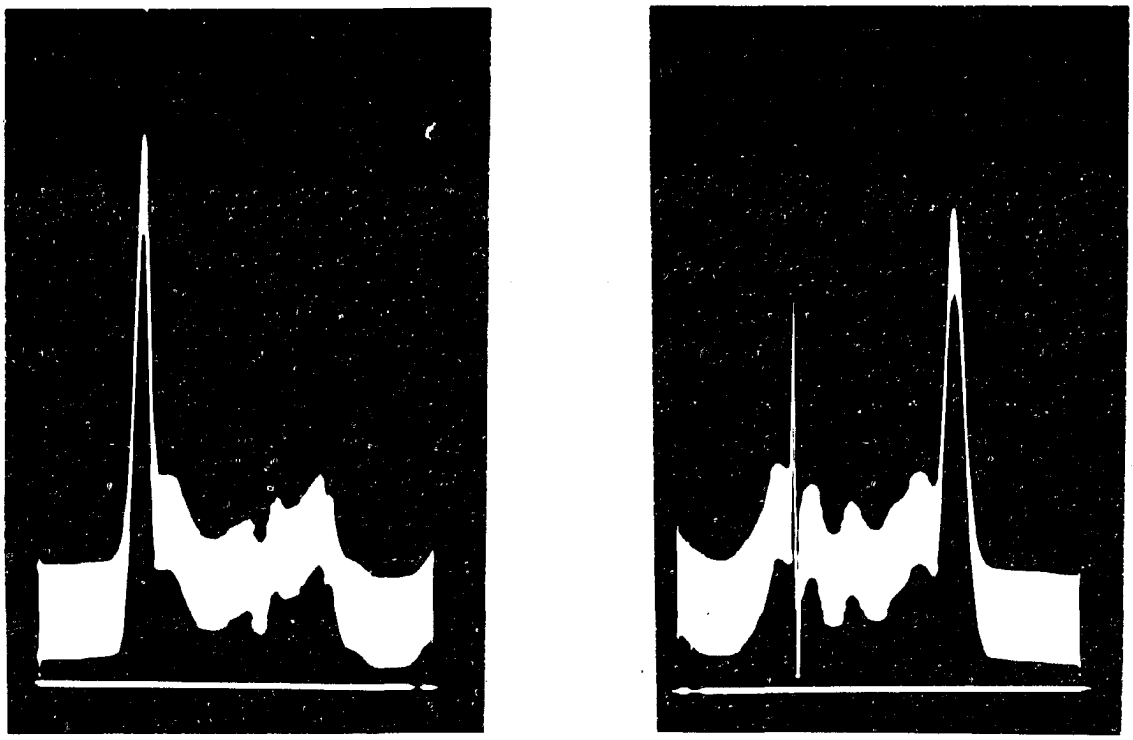
A



D

2 WEEKS

Figure III (cont.)



A D

3 WEEKS

A significant difference in mobility values of serum components of inoculated birds and control birds was not observed.

The results obtained in this group clearly demonstrate that frequent bleeding changes the serum electrophoretic pattern by reversing the albumin/globulin ratio. Bird M727S -1, which was bled at four or more weekly intervals did not show this change (Table 1, Figure I) while the other birds in the control group showed a marked change and a decrease in the albumin/globulin ratio when they were bled at weekly intervals.

The change in per cent serum protein did not appear to be significantly important during the experiment.

2. Lethal Dose $_{50}$ Neutralization Indices

The serum LD_{50} NIs of the birds in this group showed variations between periods but remained below the accepted normal values (33, 57). (Tables 7, 8, 9, 10, 11, 12, 13, Figures IV, V, VI, VII, VIII, IX) Page has also observed this type of variation (130).

Hemagglutination-inhibition titers for Newcastle disease were negative at each period.

Upon examination at necropsy all birds were found to be normal except bird M400D2 -4 which showed a non-specific enteritis. Observations for coccidia and attempts at bacterial isolation proved to be negative.

B. Group II

1. Electrophoretic Analyses

Electrophoretic analyses of sera from birds of this group showed the same general trend as the birds in Group I

Table 7

Bird M727S -1

Group I-Control

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Serum Titer#	NI
4	5.50	5	5	4	0			4.38	1.12
8	6.32*	4	5	5	1	0		4.52	1.80
12	6.50*	5	5	4	3	0		5.00	1.50
17	6.32*	5	5	5	3	1		5.32	1.00

These footnotes apply to serum neutralization test tables for Groups I, II, and III:

* Serum diluted 1:10

** Serum diluted 1:100

\# Reciprocal of negative exponent log base 10

⁂ One embryo out of five inoculated died due to trauma

Number of embryos dead out of five inoculated per dilution. Death due to virus.

NI Neutralization index

FIGURE IV

GROUP I - CONTROL

BIRD M727S-1

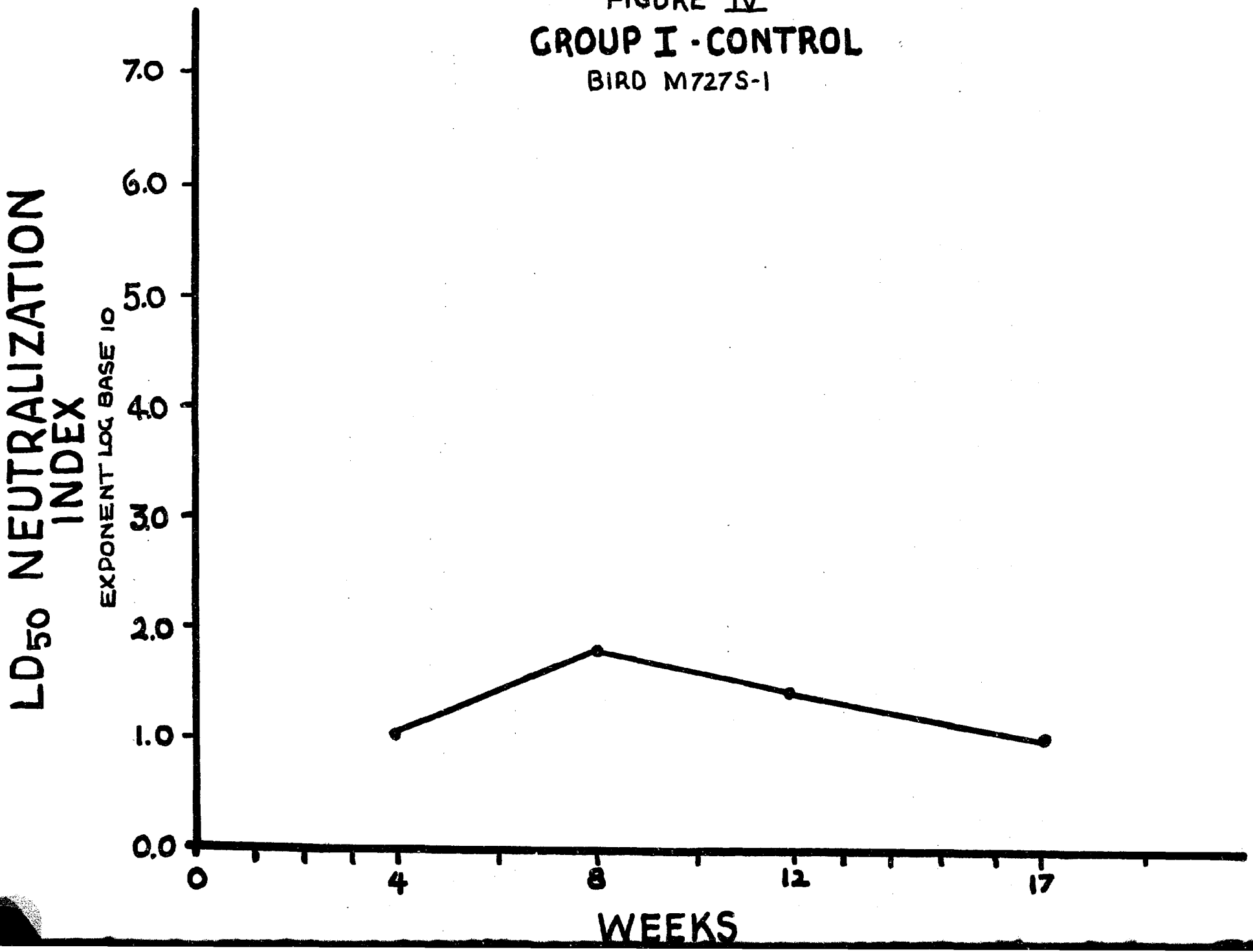


Table 8

Bird H541B2 -2

Group I-Control

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Serum Titer#	NI
0*	6.54	5	5	5	5	0		5.50	1.04
1	6.50	5	5	4	3	0		5.00	1.50
2	5.63	5	5	5	3	1		5.32	0.31
3	6.38*		5	5	4	2		4.68	1.70

* First bleeding period corresponding to pre-exposure bleeding period of infected bird

FIGURE V

GROUP I - CONTROL

BIRD M1541 B2-2

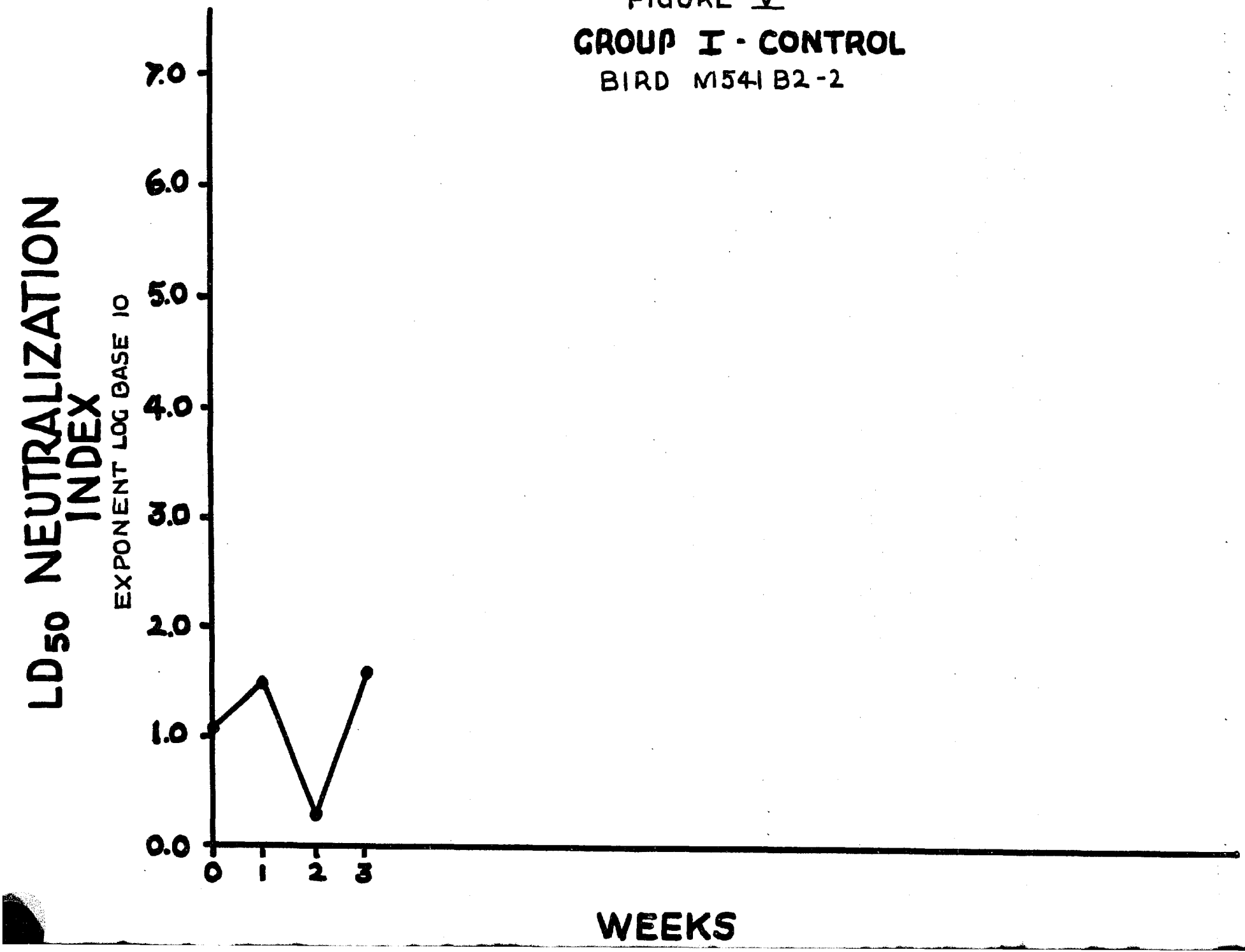


Table 9

Bird M362V -5

Group I-Control

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Serum Titer#	NI
0*	6.54	4**	5	5	4	2		5.68	0.86
1	6.50	5	5	5	4	0		5.38	1.12
2	5.63	5	4	3	0	0		4.00	1.63
3	6.38*		5	5	4	1		4.50	1.88
4	5.50*		5	5	4	3	0	5.00	0.50

* First bleeding period corresponding to pre-exposure bleeding of infected bird

FIGURE VI

GROUP I - CONTROL

BIRD M362V-5

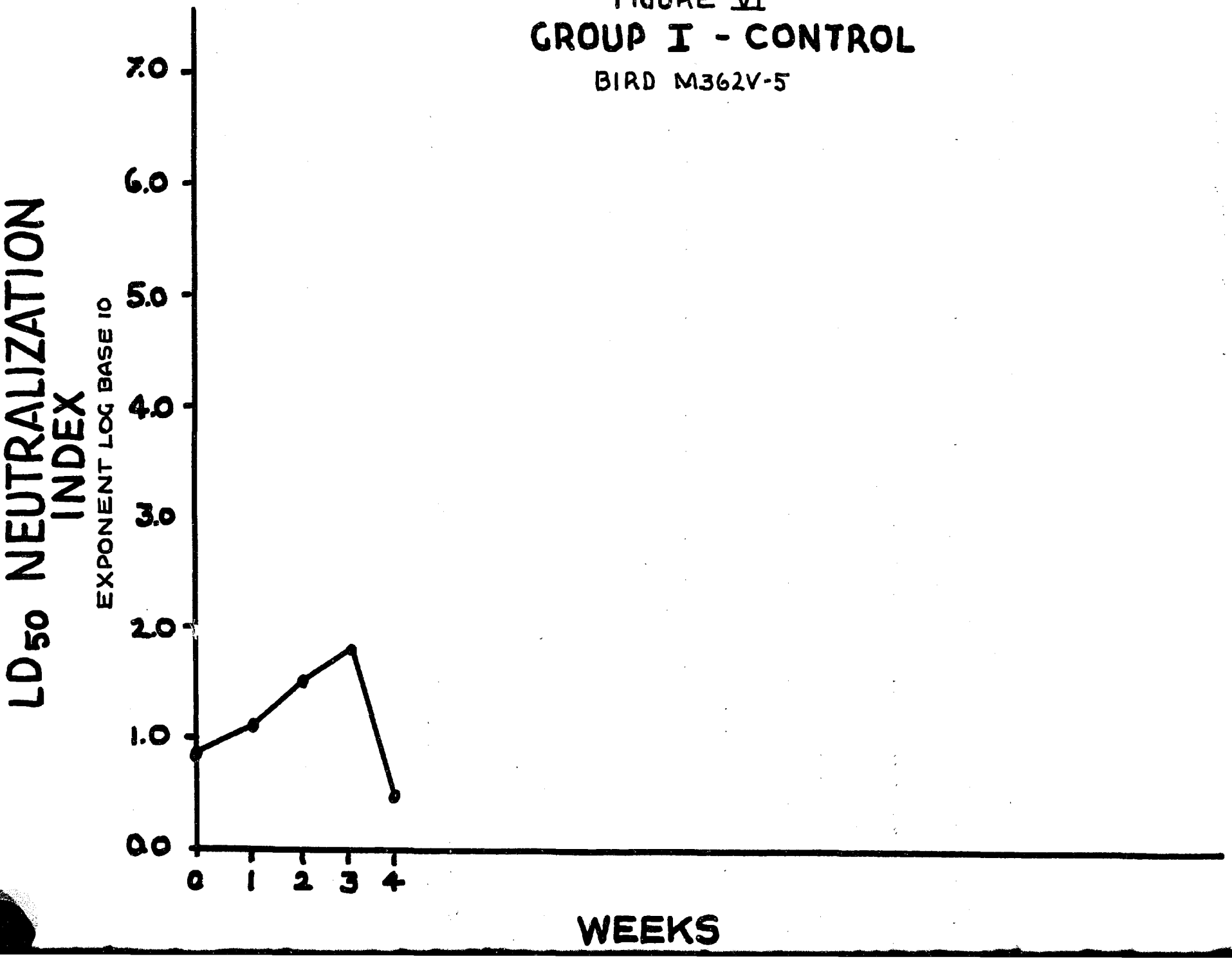


Table 10

Pool of Birds M400D2 -4 and M347B2 -6

Group I-Control

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Serum Titer#	NI
1	6.50	5	5	5	1	0		4.63	1.87
2	5.63	5	5	4	1	0		4.50	1.13
3	6.38*		5	5	5	1		4.63	1.75
4	5.50*		5	5	2	0		3.83	1.67
12	6.50*		5	5	4	3	1	5.16	1.34

FIGURE VII

GROUP I - CONTROL

POOL OF BIRDS M40002-4 AND M34782-6

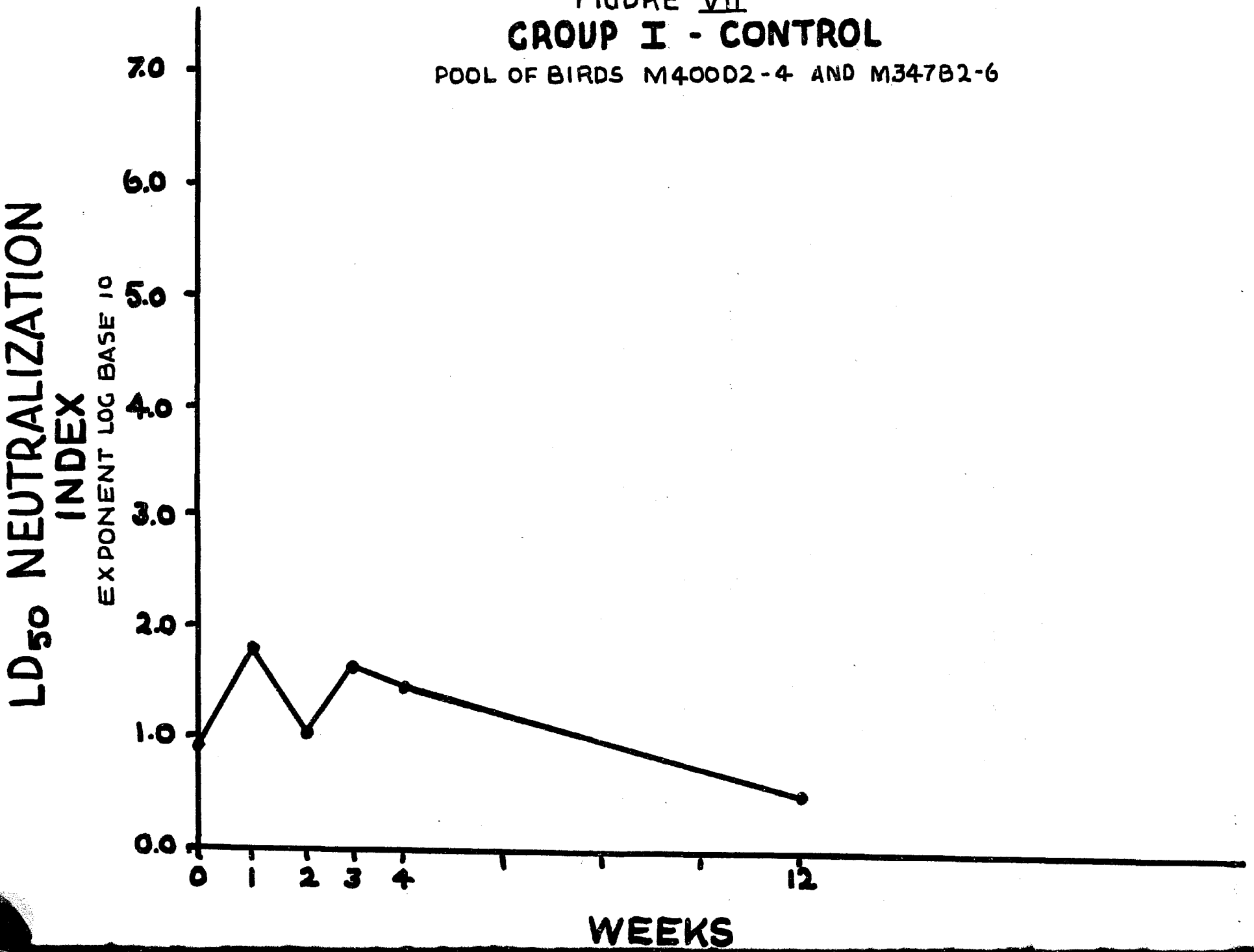


Table 11

Bird M400D2 -4

Group I-Control

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Serum Titer#	NI
8	6.32*	5	5	4	3	0		5.00	1.32
17*	6.32*	5	5	5	5	1		5.63	0.69
1	6.83*	5	5	5	4	2		5.68	1.15
2	7.17*	5	5	5	4	2		5.68	1.49

* Inoculated with normal tissue suspension

FIGURE VIII

GROUP I - CONTROL

BIRD M400D2-4

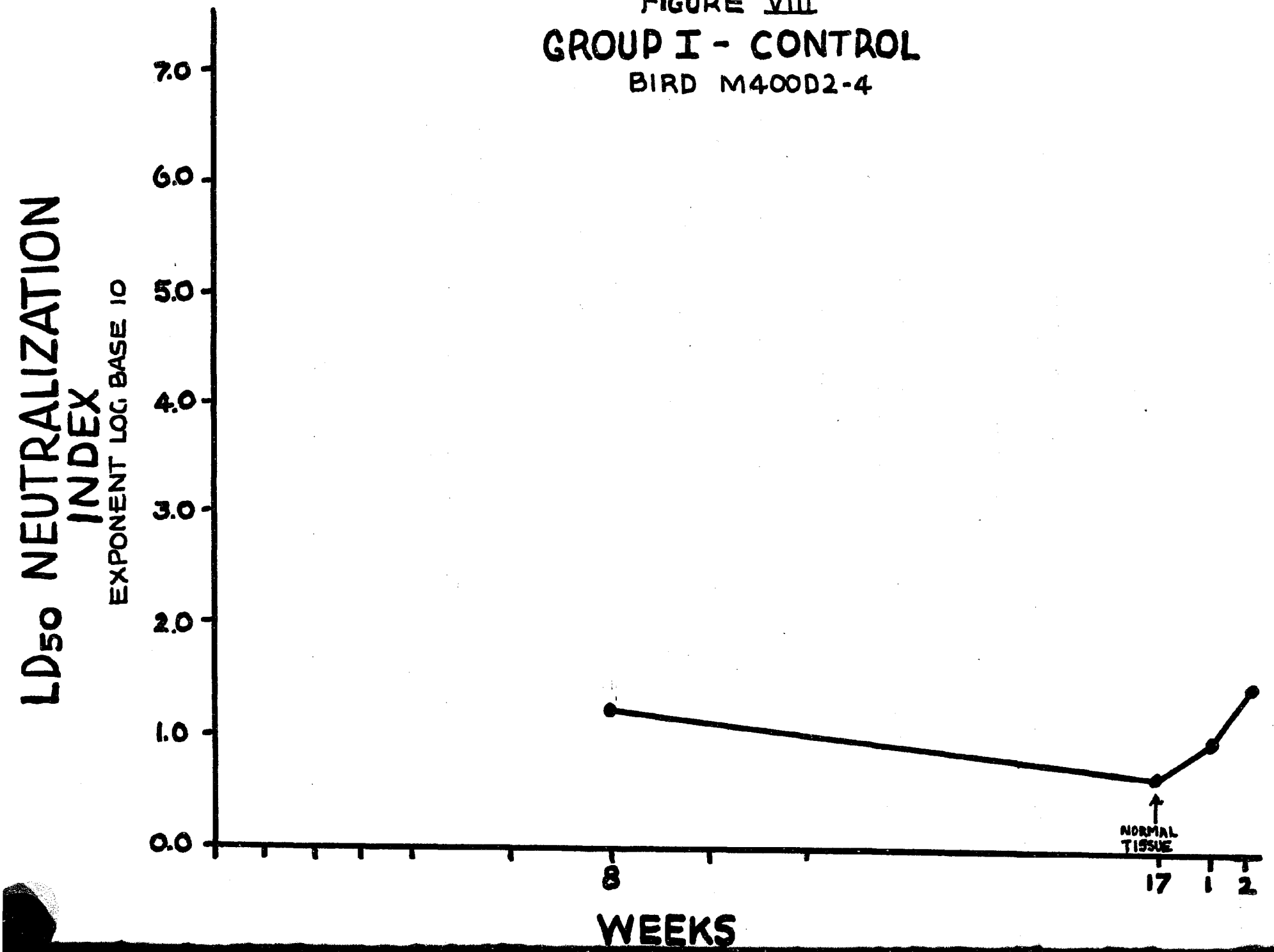


Table 12

Bird M347B2 -6

Group I-Control

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Serum Titer#	NI
8	6.32*	5	5	5	4	2		5.68	0.64
17*	6.32*	5	5	3	2	0		4.50	1.82
1	6.83*	5	5	4	3	2		5.33	1.50
2	7.17*	5	5	3	3	0		5.78	1.39
3	6.16	5	5	3	2	1		5.67	0.49

* Inoculated with normal tissue suspension

FIGURE IX

GROUP I - CONTROL

BIRD M347B2-6

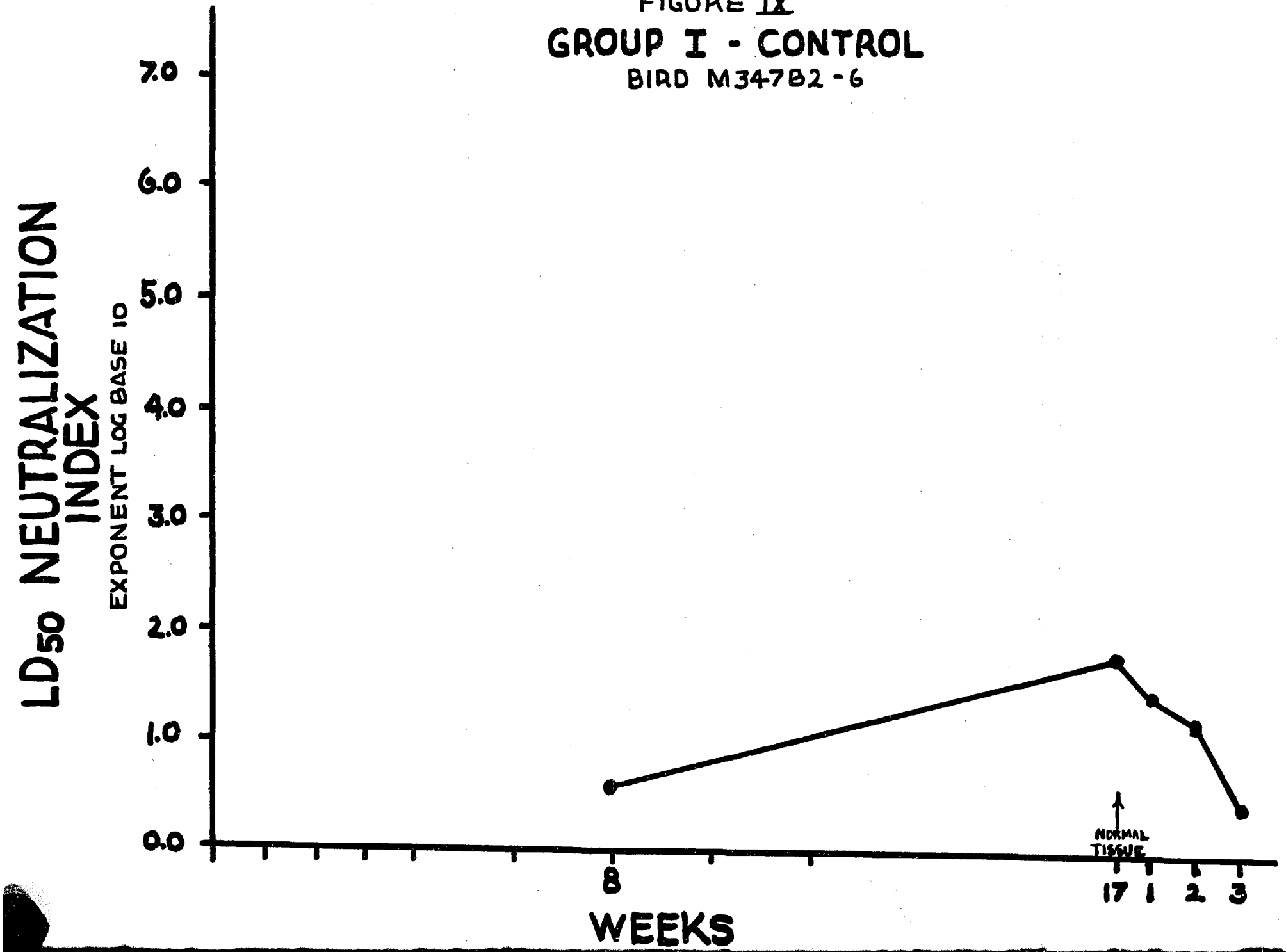


Table 13

Summation of LD_{50} NIs

Group I-Control

Period Weeks	LD_{50} NI				
	M727S -1	M541B2- 2	M400D2 -4	M362V -5	M347B2 -6	Pool M400D2 -4 M347B2 -6
0*#		1.04	0.86		0.91
1		1.50	1.12		1.87
2		0.31	1.63		1.13
3		1.70	1.88		1.75
4	1.12		0.50		1.67
6					
8	1.80	1.32		0.64	
10					
12	1.50				1.34
16					
17*	1.00	0.69		1.82	
1		1.15		1.50	
2		1.49		1.39	
3				0.49	

*# First bleeding period corresponding to pre-exposure bleeding of infected bird

* Inoculated with normal tissue suspension

which were bled at weekly intervals. The change varied depending upon the pre-exposure levels of the individual serum components.

Bird M347C2 -8 was bled immediately prior to inoculation and then at the one, two, three, four, six, eight, ten, 12, 16, and 20 week interval. The relative percentage of albumin decreased greatly one week after inoculation with infectious bronchitis virus while the gamma-globulin level increased. From this point on, and up to and including the twentieth week, the albumin generally increased and the gamma-globulin decreased. There were varying changes in the other globulins with a slight decrease in the alpha 1-globulin. The albumin/globulin ratio followed the same general trend as the change in albumin. (Table 14, Figure X)

Bird M349T -10 was bled according to the schedule of bird M347C2 -8 with essentially the same results although the albumin level was lower at the pre-exposure period. The final albumin level was higher than the pre-exposure level while the gamma-globulin level was slightly lower than the pre-exposure value. (Table 15).

Bird M538H -11 was bled immediately prior to inoculation and then at the one, two, three, four, and six week interval. It died at the six week period from internal hemorrhage due to cardiac puncture. Electrophoretic analysis of pre-exposure serum samples from this bird showed an extremely low albumin level and a high gamma-globulin level. This trend increased even more following the period after inoculation with virus. (Table 16)

Table 14

Bird M347C2 -8

Group II-Primary Exposure

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	43.3	11.2	4.8	9.1	31.6	0.77	4.17
1	32.4	13.0	8.5	9.7	36.4	0.48	3.86
2	36.6	9.5	4.7	10.4	38.8	0.58	4.83
3	36.7	10.3	6.4	9.4	37.2	0.58	4.65
4	40.1	10.3	4.8	9.5	35.3	0.67	4.21
6	36.9	9.3	4.4	8.9	40.5	0.59	4.48
8	39.9	10.8	3.9	10.8	34.6	0.66	4.21
10	40.4	12.2	5.9	10.1	31.4	0.68	4.39
12	42.0	9.3	6.5	10.5	31.5	0.73	4.21
16	42.5	9.9	6.4	11.0	30.2	0.74	5.00
20	42.1	9.5	6.8	10.0	31.6	0.73	4.21

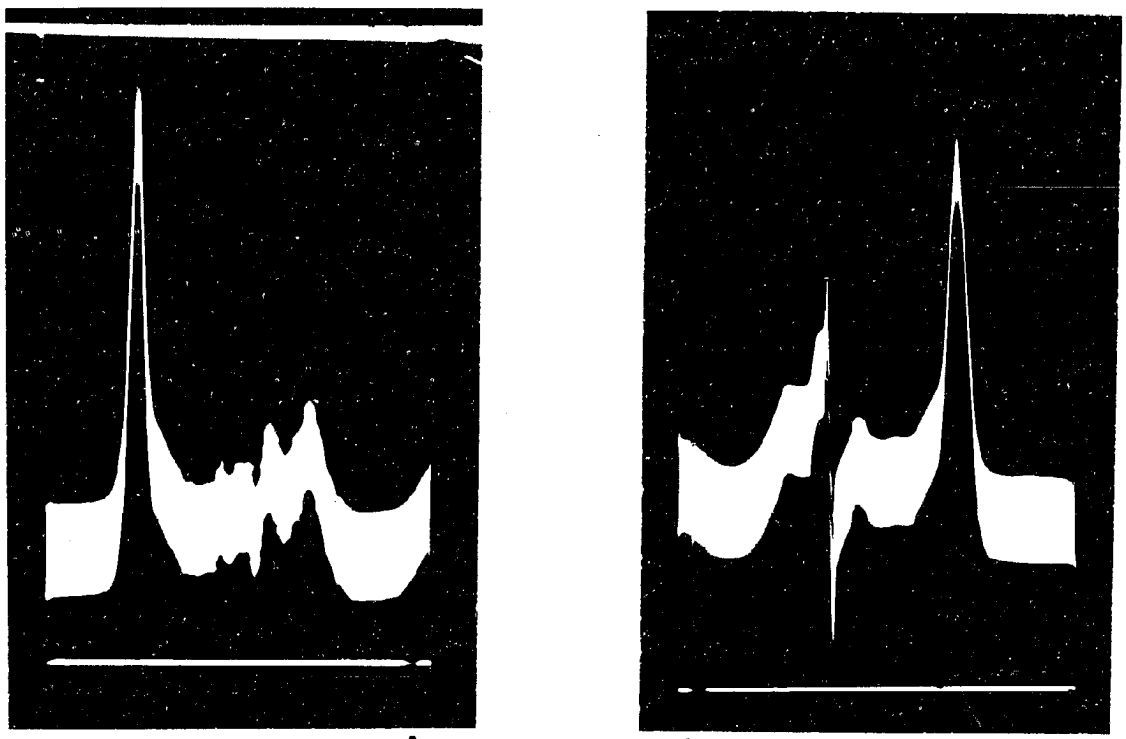
* Pre-exposure bleeding, inoculated with virus

Figure X

Bird M347C2 -8

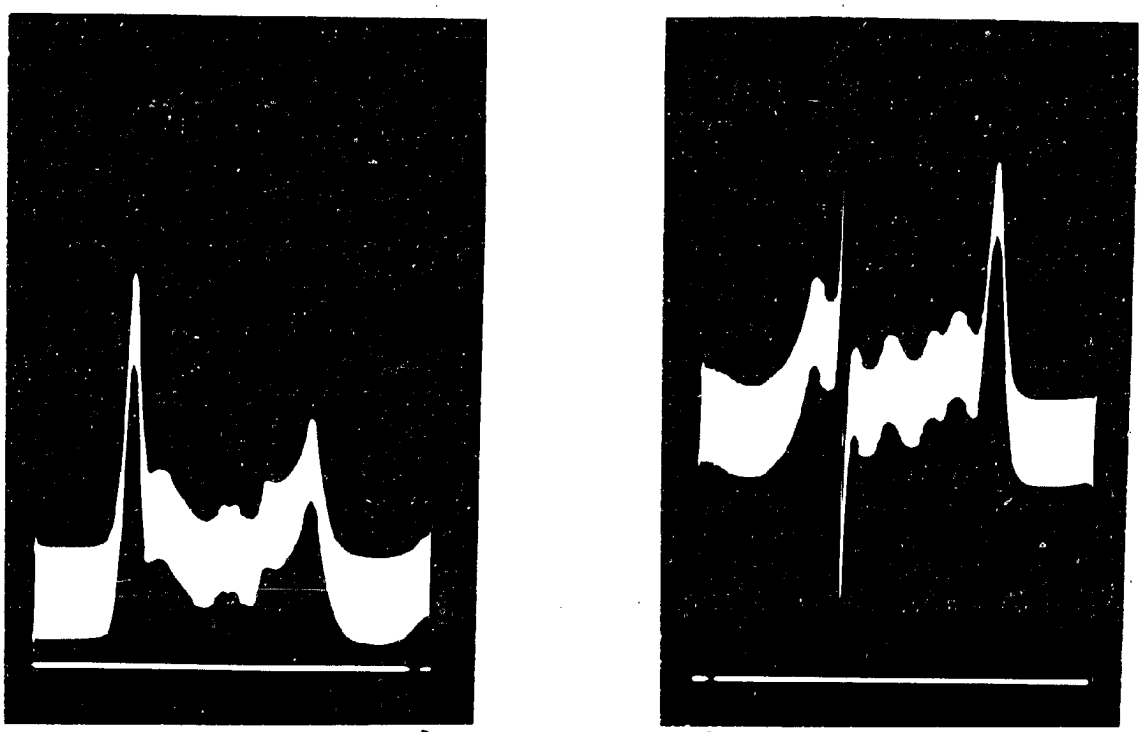
Group II-Primary Exposure

Electrophoretic Patterns



A D

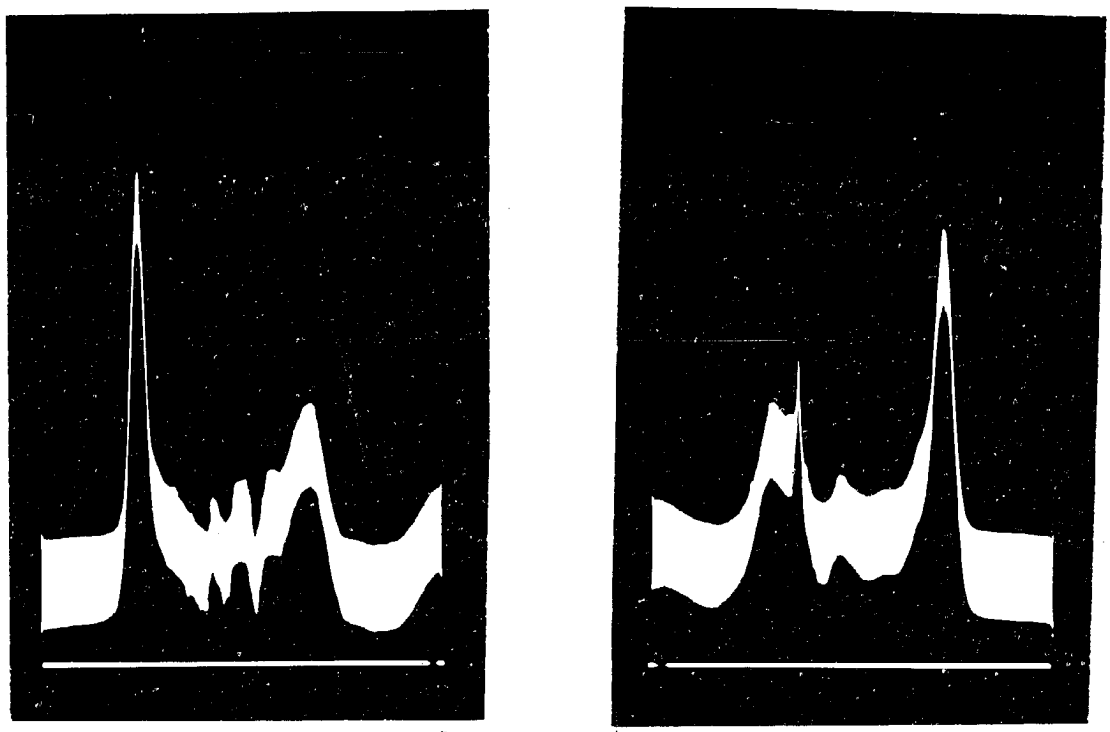
0 WEEKS
PRE-EXPOSURE



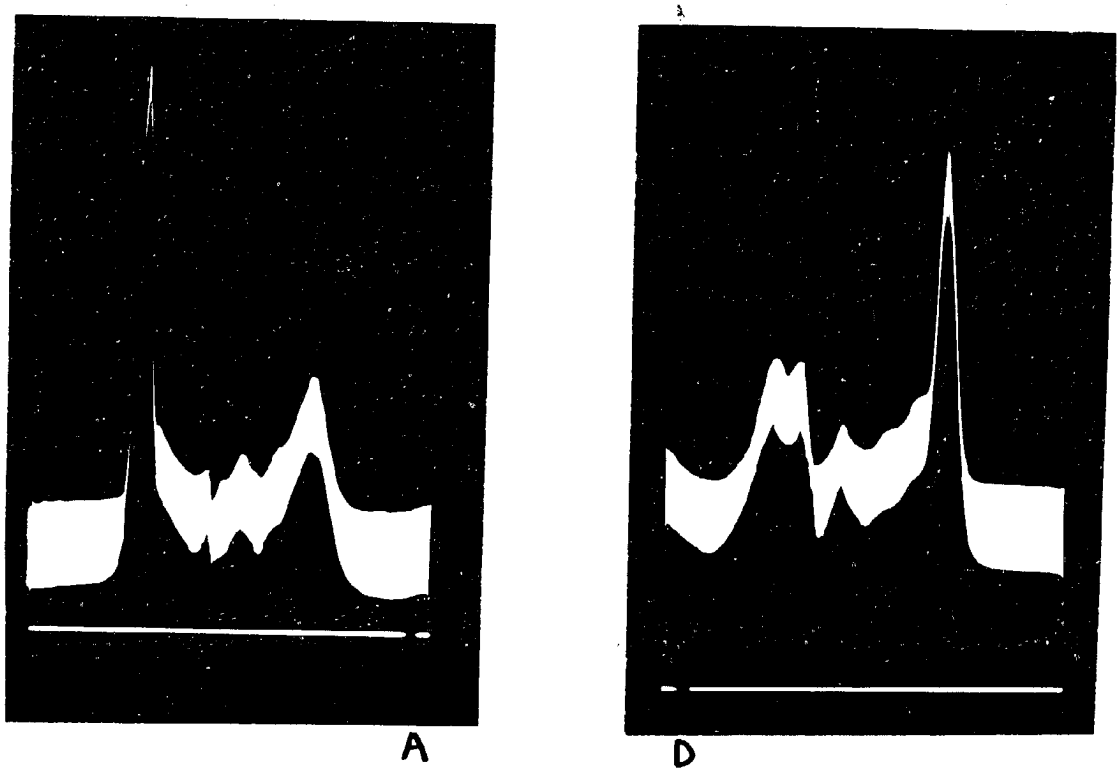
A D

1 WEEK

Figure X (cont.)

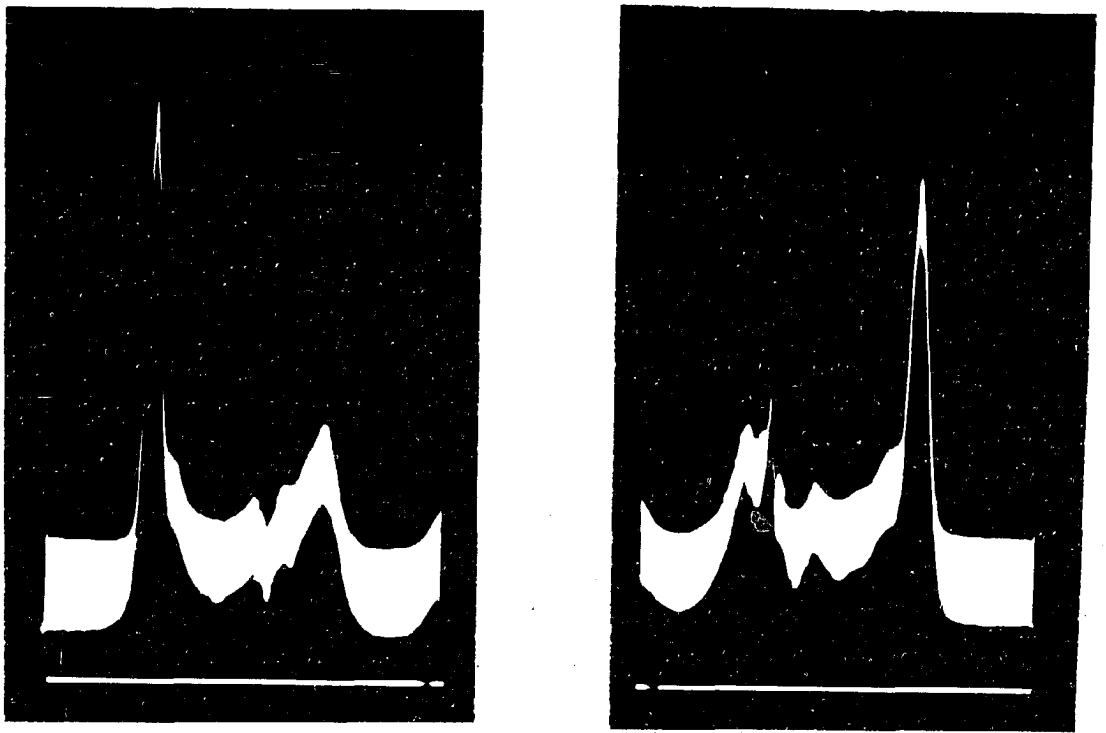


2 WEEKS



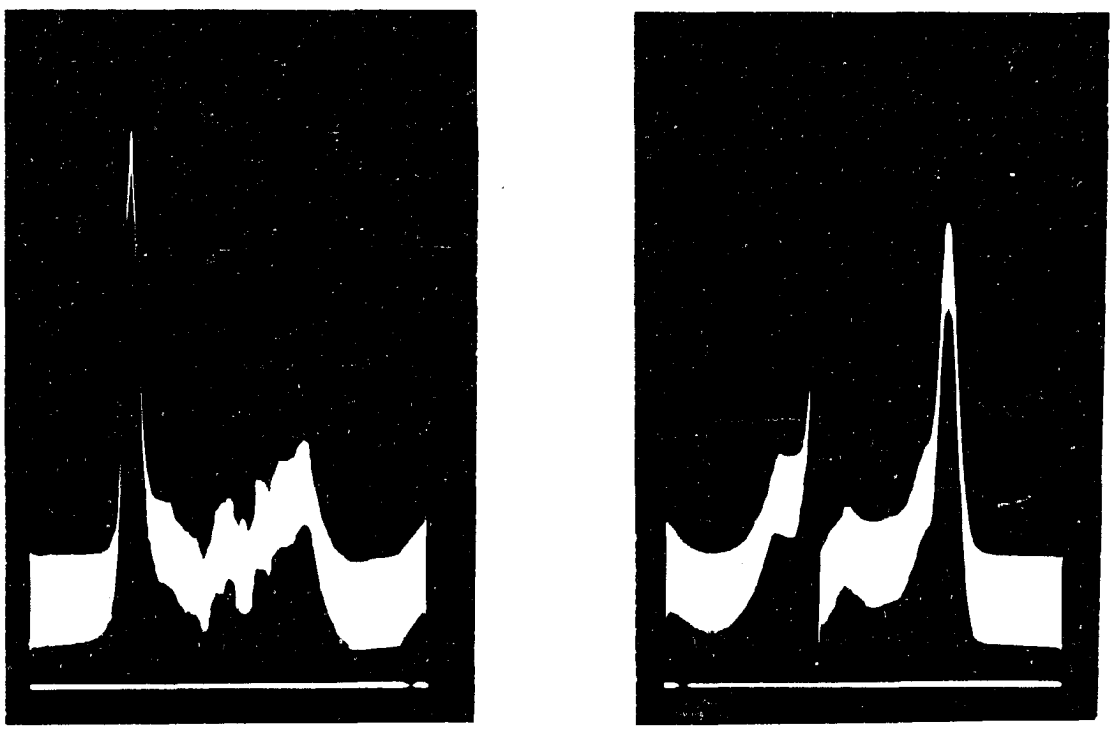
3 WEEKS

Figure X (cont.)



A D

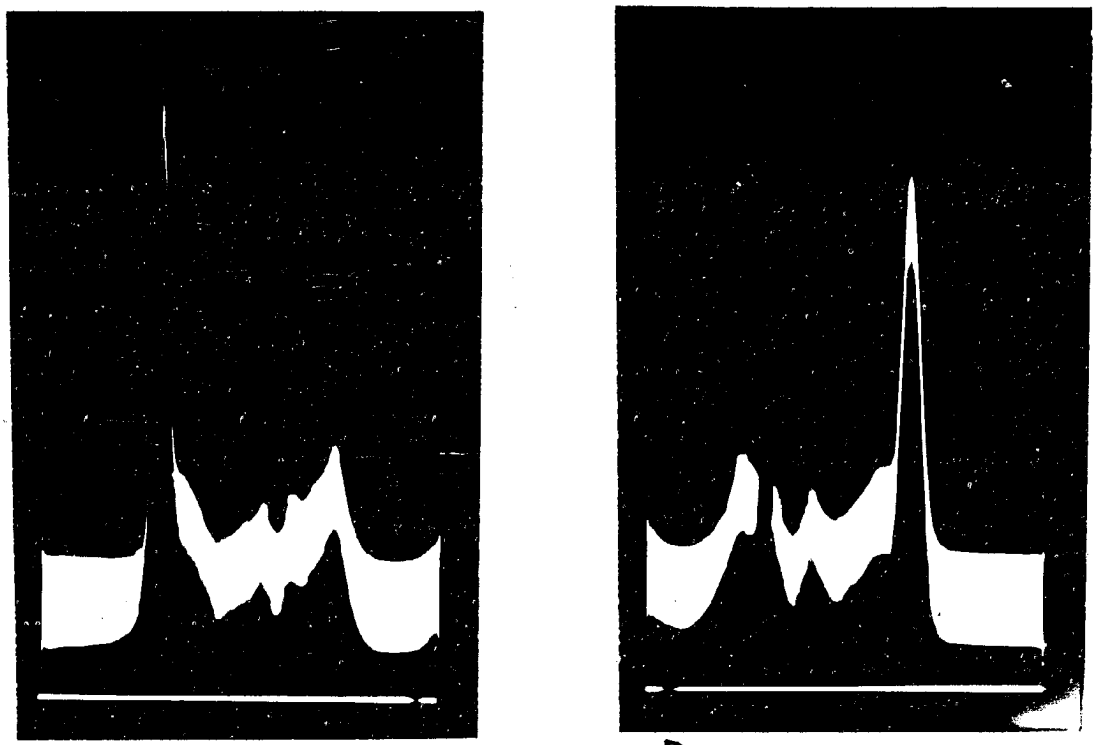
4 WEEKS



A D

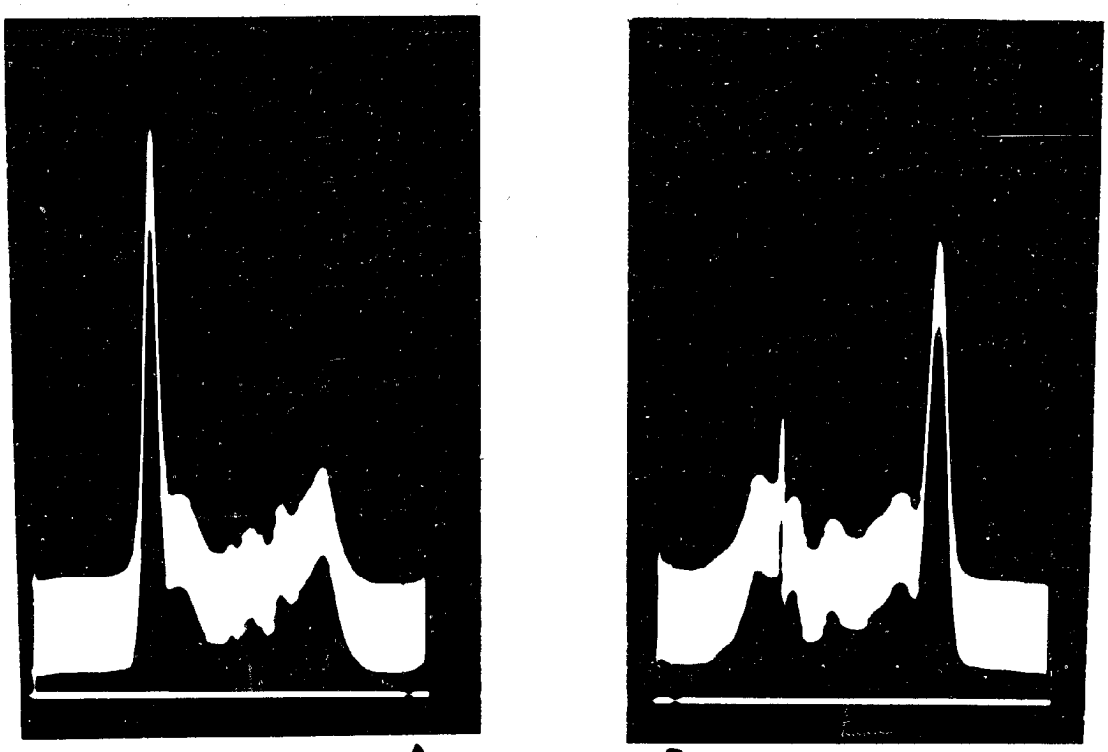
6 WEEKS

Figure X (cont.)



A D

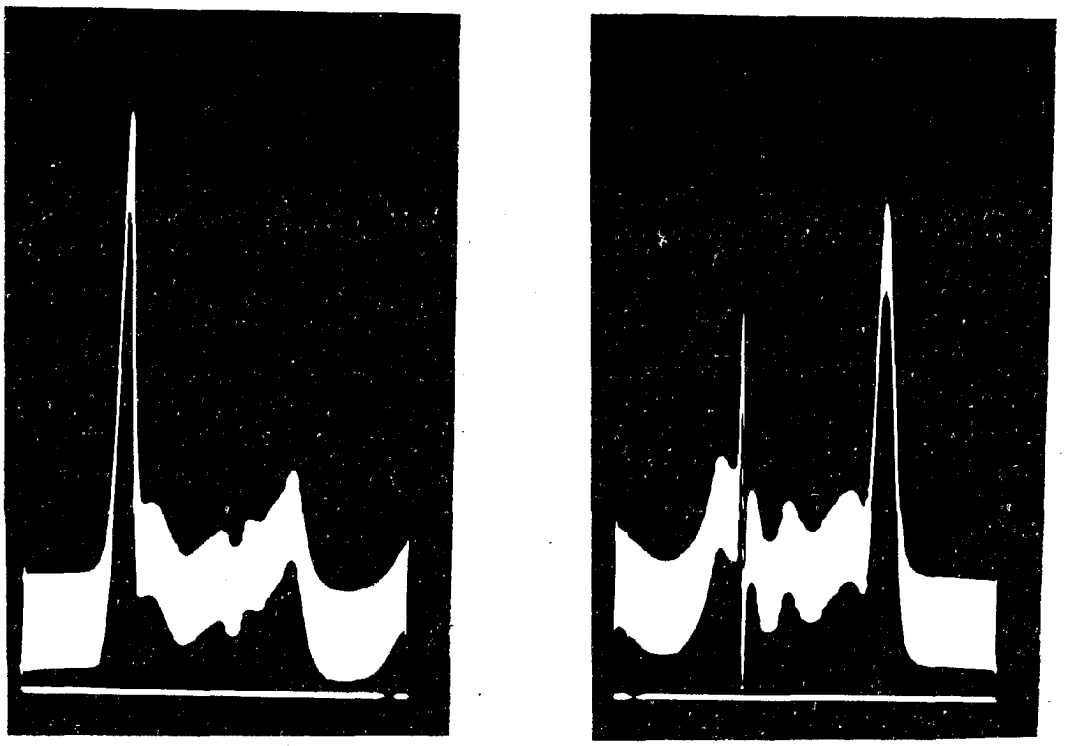
8 WEEKS



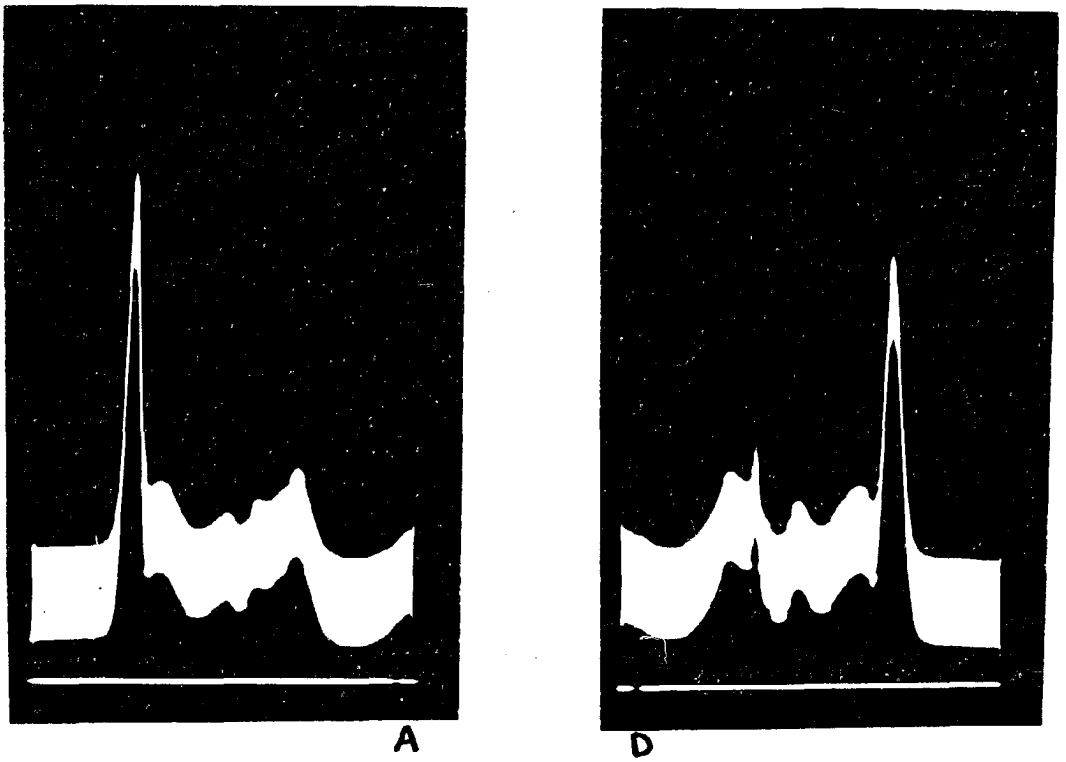
A D

10 WEEKS

Figure X (cont.)

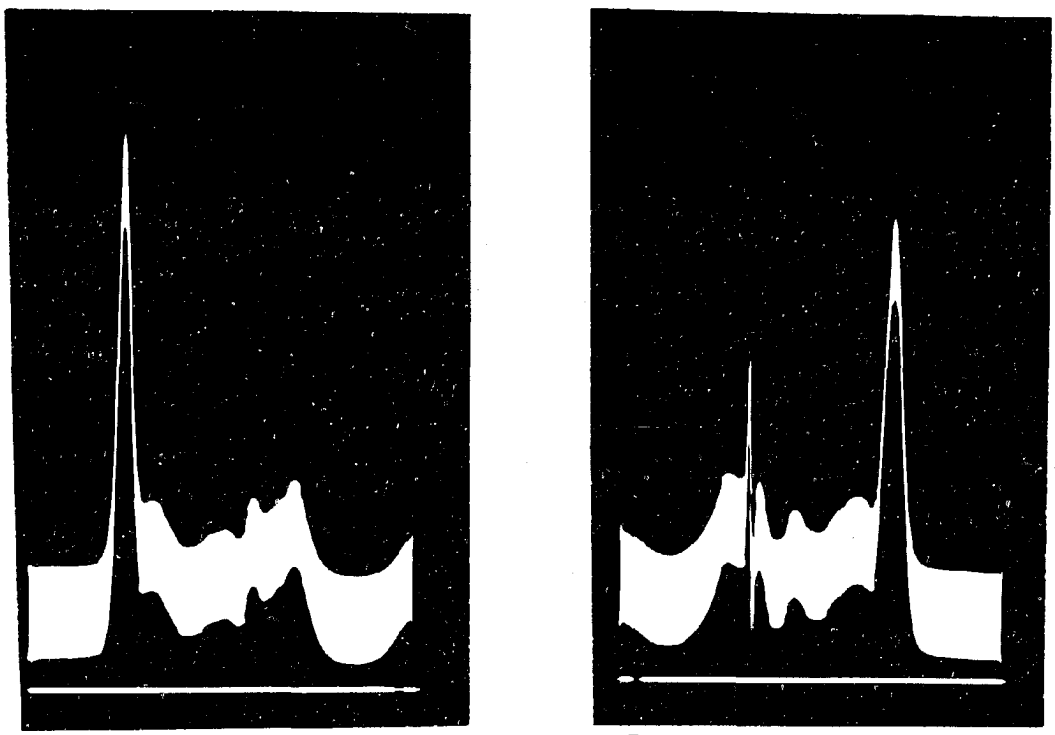


12 WEEKS



16 WEEKS

Figure X (cont.)



A D

20 WEEKS

Table 15

Bird M349T -10

Group II-Primary Exposure

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	38.2	10.9	8.1	8.1	34.7	0.62	4.30
1	26.7	12.3	7.7	11.8	41.5	0.36	4.83
2	32.6	10.9	7.5	9.0	40.0	0.48	5.00
3	34.1	12.6	5.3	8.1	39.9	0.52	4.65
4	39.5	7.5	5.8	9.8	37.4	0.65	4.39
6	37.1	9.7	7.8	8.3	37.1	0.59	3.86
8	39.4	10.5	7.5	11.0	31.6	0.65	4.04
10	40.6	10.8	5.4	11.3	31.9	0.68	4.13
12	41.3	18.0		9.0	31.7	0.71	3.86
16	43.4	7.4	10.8	8.4	30.0	0.77	4.13
20	42.2	18.2		8.6	31.0	0.73	3.16

* Primary bleeding, pre-exposure, inoculated with virus

Table 16

Bird M538H -11

Group II-Primary Exposure

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	31.4	10.5	8.7	11.6	37.8	0.46	5.08
1	31.3	8.6	9.6	14.7	35.8	0.46	4.83
2	18.1	14.4	6.9	13.4	47.2	0.22	5.35
3	23.8	13.6	7.7	11.1	43.8	0.31	4.56
4	25.8	14.4	6.1	11.8	41.9	0.35	5.00
6	25.6	11.6	5.8	11.6	45.4	0.34	4.65

* Pre-exposure bleeding, inoculated with virus

Equal portions of sera from birds M716G2 -7, M735R -9, and M4320 -12 were pooled and analyzed as an individual sample immediately prior to inoculation and then at the one, two, and three week interval. (Table 17) At the third week period bird M716G2 -7 died because of hemorrhage due to cardiac puncture. Therefore, samples from birds M735R -9 and M4320 -12 were pooled and analyzed at the four, six eight, ten, 12, 16, and 20 week interval. (Table 18) Individual samples comprising this pool were also analyzed separately as follows: bird M735R -9 - six, eight, ten, 16, and 20 week interval; (Table 19) bird M4320 -12 - ten, 16, and 20 week interval. (Table 20).

There was a decrease in the relative percentage of albumin at the first and second weeks after inoculation with the virus. After this period a general increase in the albumin followed until the end of the experimental period when a general maximum was reached. Gamma-globulin levels reached a maximum at the second week after exposure and decreased generally until a minimum was reached at the end of the experiment. The other globulins showed varying changes although alpha 1-globulin increased at the first week and remained at an elevated level throughout the experiment.

The values obtained in pre-exposure serum electrophoretic analyses closely approximated the results obtained by Sanders, et al (144), although the gamma-globulin values were lower in Sanders' study. Birds M349T -10 (Table 15) and M538H -11 (Table 16) showed low pre-exposure albumin values.

Table 17

Pool of Birds M716G2 -7, M735R -9, and M4320 -12

Group II-Primary Exposure

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	45.3	9.5	7.9	8.9	28.4	0.83	3.47
1	34.0	16.7	7.1	13.2	29.0	0.52	3.95
2	37.7	10.8	6.4	10.3	34.8	0.61	4.04
3	40.0	9.8	5.6	11.2	33.4	0.67	4.04

* Pre-exposure bleeding, inoculated with virus

Table 18

Pool of Birds M735R -9 and M4320 -12

Group II-Primary Exposure

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
4	40.0	12.2	6.6	9.6	31.6	0.67	3.69
6	43.6	7.3	6.5	11.6	31.0	0.77	3.51
8	41.2	14.0	5.1	11.7	28.0	0.70	3.69
10	42.0	13.0	8.0	11.0	26.0	0.72	3.43
12	44.5	9.3	6.7	9.9	29.6	0.80	3.78
16	42.4	11.9	4.2	10.1	31.4	0.74	4.39
20	44.4	12.3	6.6	9.2	27.6	0.80	3.95

Table 19

Bird M735R -9

Group II-Primary Exposure

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
6	43.2	11.7	5.4	9.2	30.5	0.76	3.07
8	44.7	7.8	6.5	12.9	28.1	0.81	3.07
10	49.6	12.4	5.2	11.4	21.4	0.98	2.89
16	50.6	10.0	6.3	11.2	21.9	1.04	3.78
20	52.8	9.6	6.5	10.5	20.6	1.12	3.34

Table 20

Bird M4320 -12

Group II-Primary Exposure

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
10	37.8		24.2	7.6	30.4	0.61	3.86
16	38.7	14.1	6.5	8.5	32.2	0.63	4.13
20	42.9		21.2	7.6	28.3	0.75	4.13

Results obtained on the relative percentage distribution of serum components of pooled serum samples and samples comprising the pools were favorably comparable. In some instances there were slight differences which were not significant.

A study of the changes of per cent serum protein did not reveal any significant findings.

2. Lethal Dose$_{50}$ Neutralization Indices

The serum LD_{50} NIs of this group showed a general pattern as follows: There was a slight increase in the LD_{50} NIs of all sera between one and two weeks after inoculation. After this period a marked increase occurred. The maximum LD_{50} NIs were reached between the sixth and tenth weeks, decreasing after this period until the end of the experiment. (Tables 21, 22, 23, 24, 25, 26, 27, 28, Figures XI, XII, XIII, XIV, XV, XVI, XVII) Variations in individual neutralizing antibody responses were also observed (130, 131).

Pre-exposure LD_{50} NIs were considered normal according to accepted standards (33, 57).

Upon examination at necropsy, all birds were normal except birds M349T -10, and M538H -11 which showed visceral lymphomatosis and neural lymphomatosis, respectively.

Sanders, et al (144) observed a new component, which was designated as the L component, in sera from chickens inoculated with leucosis-containing tissue suspensions. The L component was not observed in the serum electrophoretic patterns of birds showing manifestations of lymphomatosis in this study.

Table 21

Bird M347C2 -8

Group II-Primary Exposure

Serum Neutralization Tests

Period	Virus#	Virus Dilutions							Serum	
Weeks	Titer	10^0 ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Titer#	NI
0*	6.54			4*	5	5	4	2	5.68	0.86
1	6.50			5	5	5	1	0	4.63	1.87
2	6.32*			5	4	4	1	0	3.37	2.95
3	6.32*			5	4	2	0		2.68	3.64
4	5.50*		5	4	0	0	0*		1.38	4.12
6	6.72*		5	4	2	0	1		1.84	4.88
8	6.32*		5	3	1	0	0		1.32	5.00
10	6.17*	5	3	2	0	0	0		0.50	5.67
12	6.50*		5	5	3	0	0		2.17	4.33
16	6.68*		5	5	1	0	0		2.63	4.05
20	6.83*	5	5	3	3	0	0		2.78	4.05

* Pre-exposure bleeding, inoculated with virus

FIGURE XI

GROUP II - PRIMARY EXPOSURE

BIRD 347C2-8

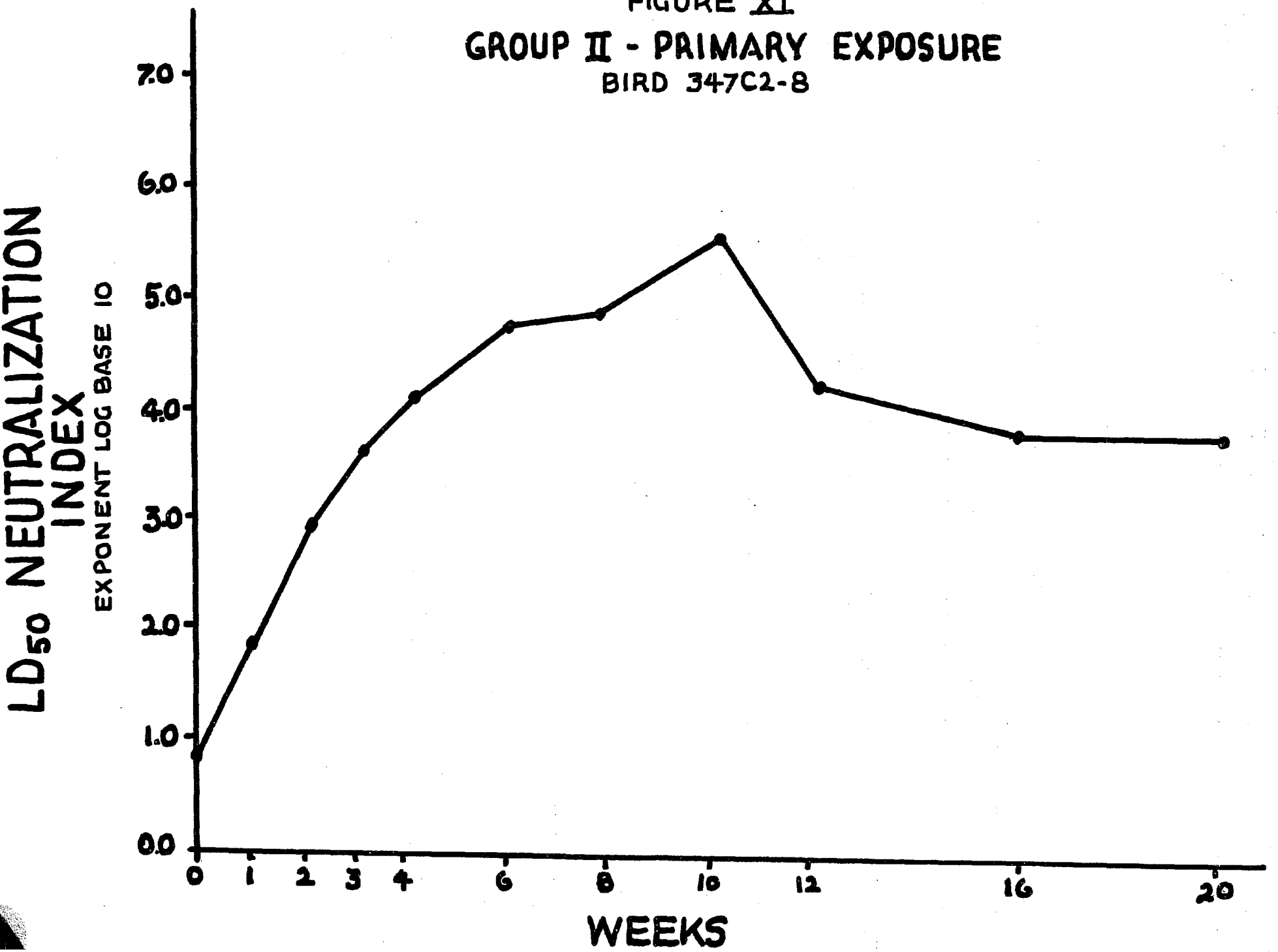


Table 22

Bird 349T -10

Group II-Primary Exposure

Serum Neutralization Tests

Period	Virus#	Virus Dilutions							Serum	
Weeks	Titer	10^{0} ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Titer#	NI
0*	6.54			5	5	4	5	1	5.52	1.02
1	6.50			5	5	5	4	1	5.50	1.00
2	6.32*			5	5	5	1	0	3.63	2.69
3	6.32*			5	5	3	1*		3.33	2.99
4	7.32*		5	5	5	4	3	0	4.00	3.32
6	6.72*		4	5	3	1	0		2.16	4.56
8	6.32*		5	5	0	2	0*		1.70	4.62
10	6.17*	5	5	2	1	0*	0		1.00	5.17
12	6.50*		5	5	3	1	0		2.32	4.18
16	6.68*			5	5	1	1	0	2.75	3.93
20	6.68*	5	5	5	4	3	2		3.33	3.35

* Pre-exposure bleeding, inoculated with virus

FIGURE XII

GROUP II - PRIMARY EXPOSURE

BIRD M349T-10

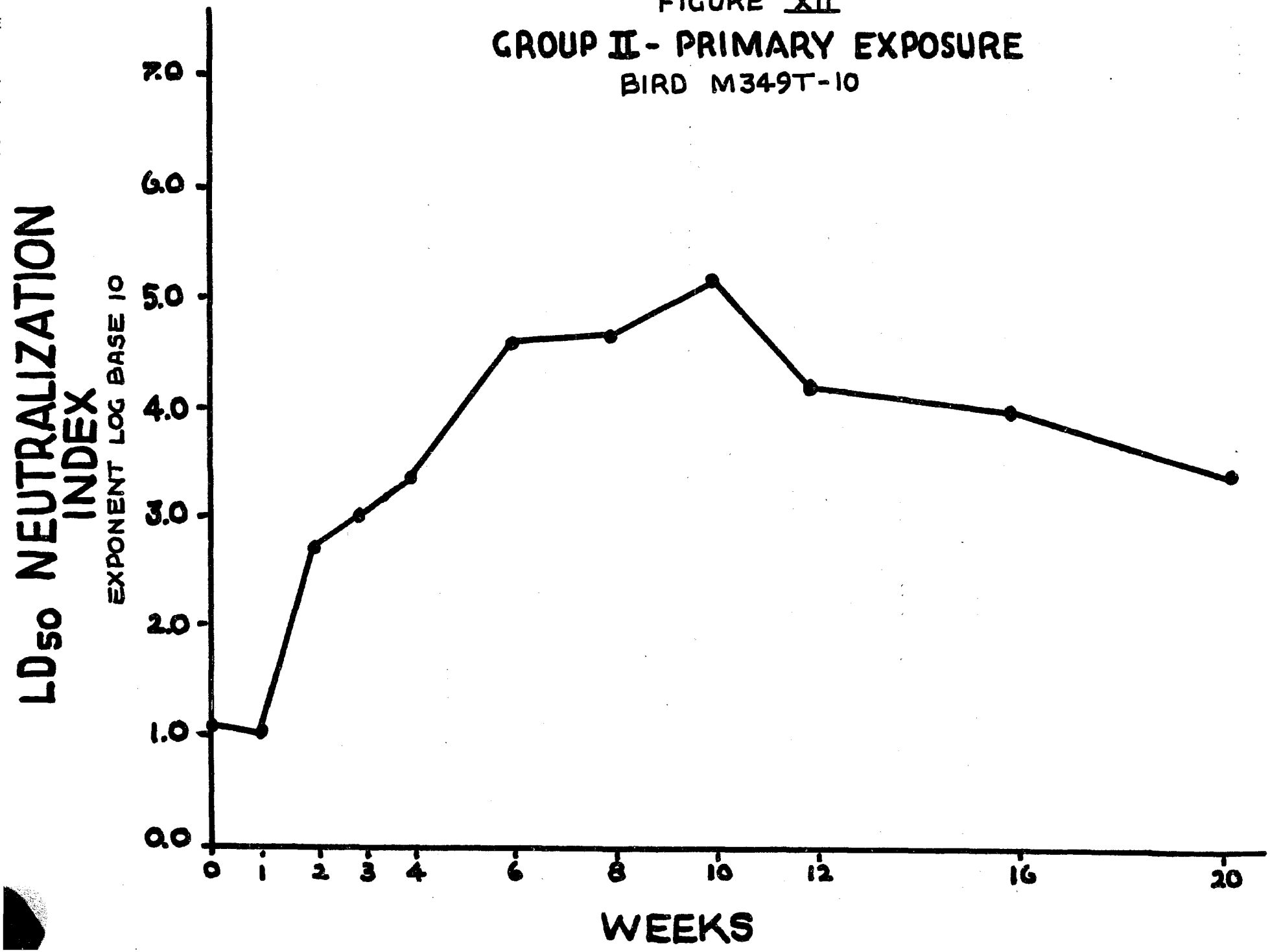


FIGURE XII

GROUP II - PRIMARY EXPOSURE

BIRD M349T-10

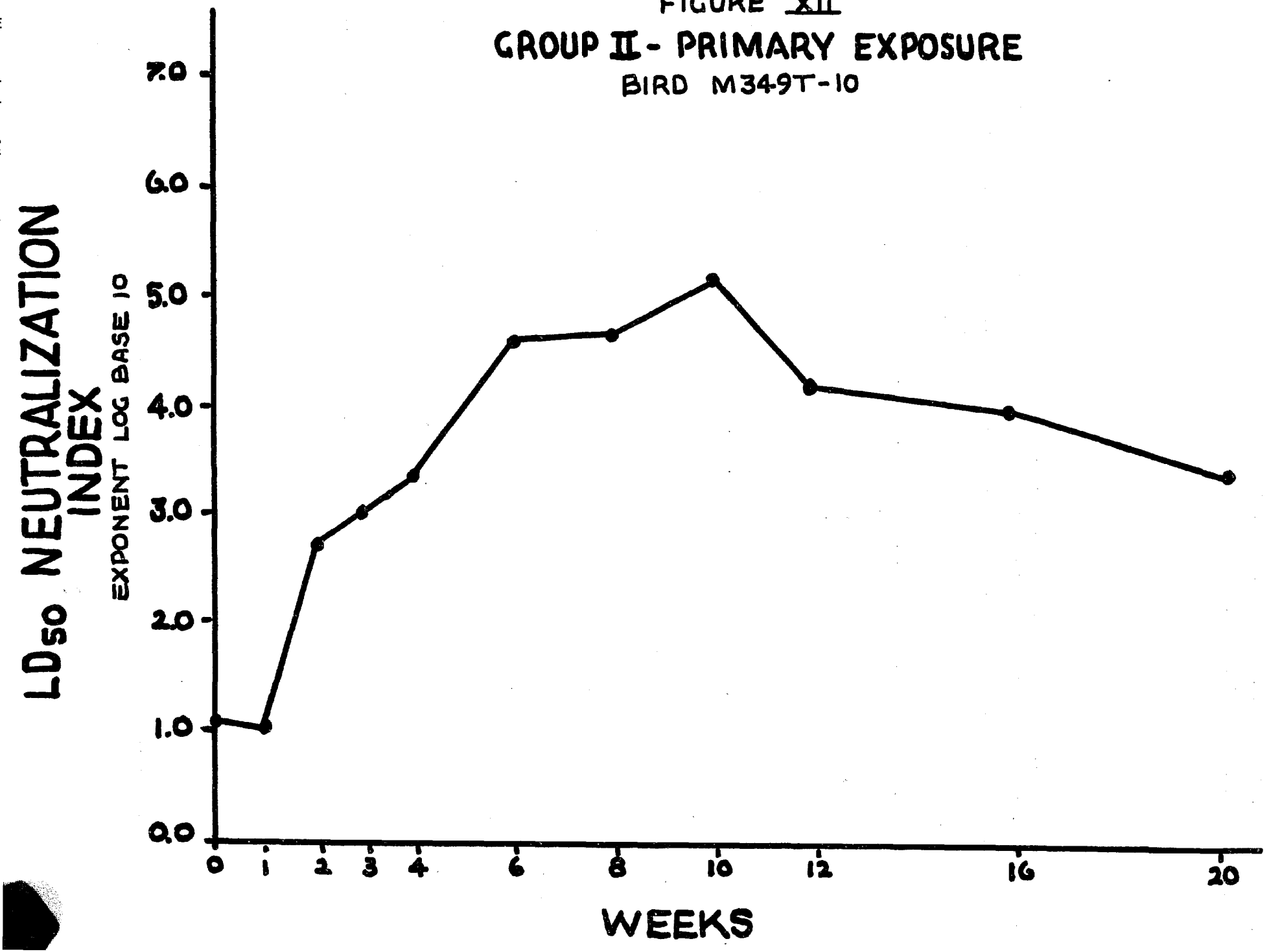


Table 23

Bird M538H -11

Group II-Primary Exposure

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-1} ##	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Serum Titer#	NI
0*	6.54		5	5	5	3	0	5.17	1.37
1	6.50		5	5	4	2	0	4.68	1.82
2	5.63	5	3	0	1	0	0	2.31	3.32
3	6.32*		5	3	1	0		2.32	4.00
4	7.32*	5	4*	5	2	0	0	2.83	4.49
6	6.72*	5	5	2	1	0		1.00	5.72

* Pre-exposure bleeding, inoculated with virus

FIGURE XIII

GROUP II - PRIMARY EXPOSURE

BIRD M538H - 11

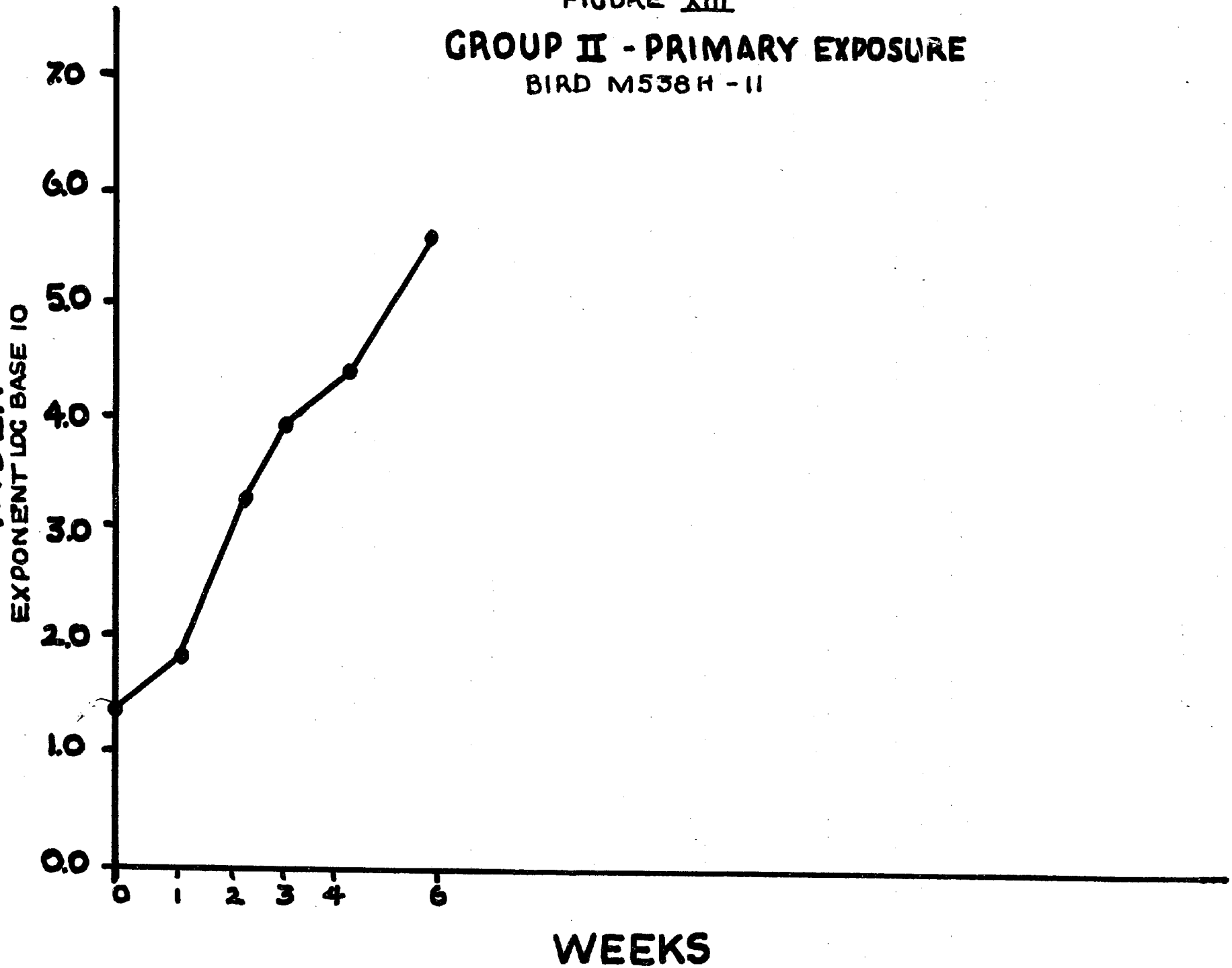


Table 24

Pool of Birds M716G2 -7, M735R -9, and M4320 -12

Group II-Primary Exposure

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Serum Titer#	NI
0*	6.54	5	5	5	4	0	5.38	1.16
1	6.50	5	5	4	1	0	4.50	2.00
2	6.32*	5	5	5	1	0	3.63	2.69
3	6.32*	5	5	5	1*		3.67	2.65

* Pre-exposure bleeding, inoculated with virus

FIGURE XIV

CROUP II - PRIMARY EXPOSURE

POOL OF BIRDS M716G2-7, M735R-9, and M4320-12

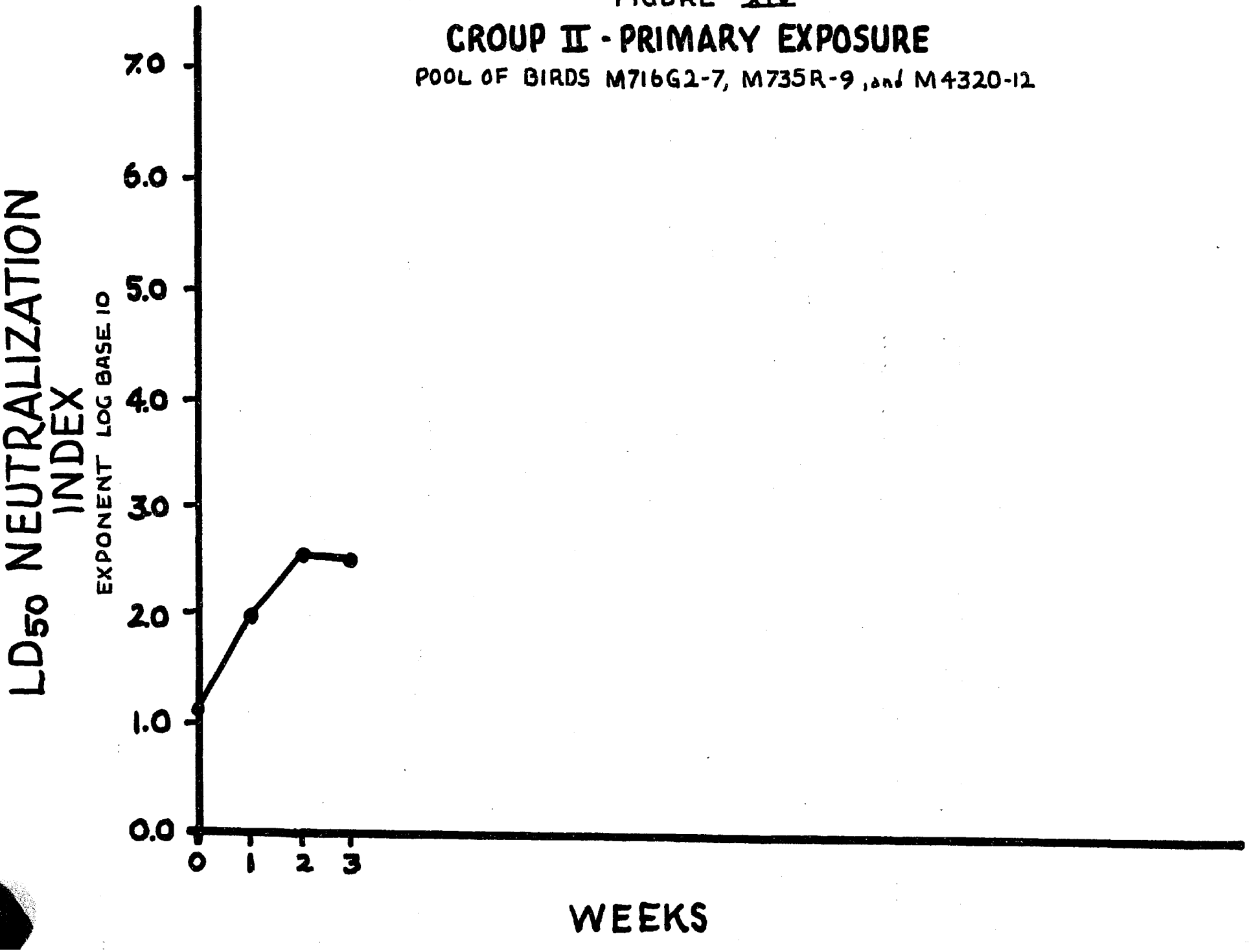


Table 25

Pool of Birds M735R -9 and M4320 -12

Group II-Primary Exposure

Serum Neutralization Tests

Period	Virus#	Virus Dilutions							Serum	
Weeks	Titer	10^{0} ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Titer#	NI
4	7.32*		5	5	4*	5	4	1*	4.53	2.79
6	6.72*		5	5	5	3	0		3.17	3.55
8	6.32*		5	5	2	1	1		1.20	5.12
10	6.17*		5	4	3	0	0		2.00	4.17
12	6.50*		5	5	5	1	0		2.63	3.87
16	6.68*			5	4	2	1	0	2.84	3.84
20	6.68*	5	5	5	4	3	0		3.00	3.68

FIGURE XV

GROUP II - PRIMARY EXPOSURE

POOL OF BIRDS M735R-9 AND M4320-12

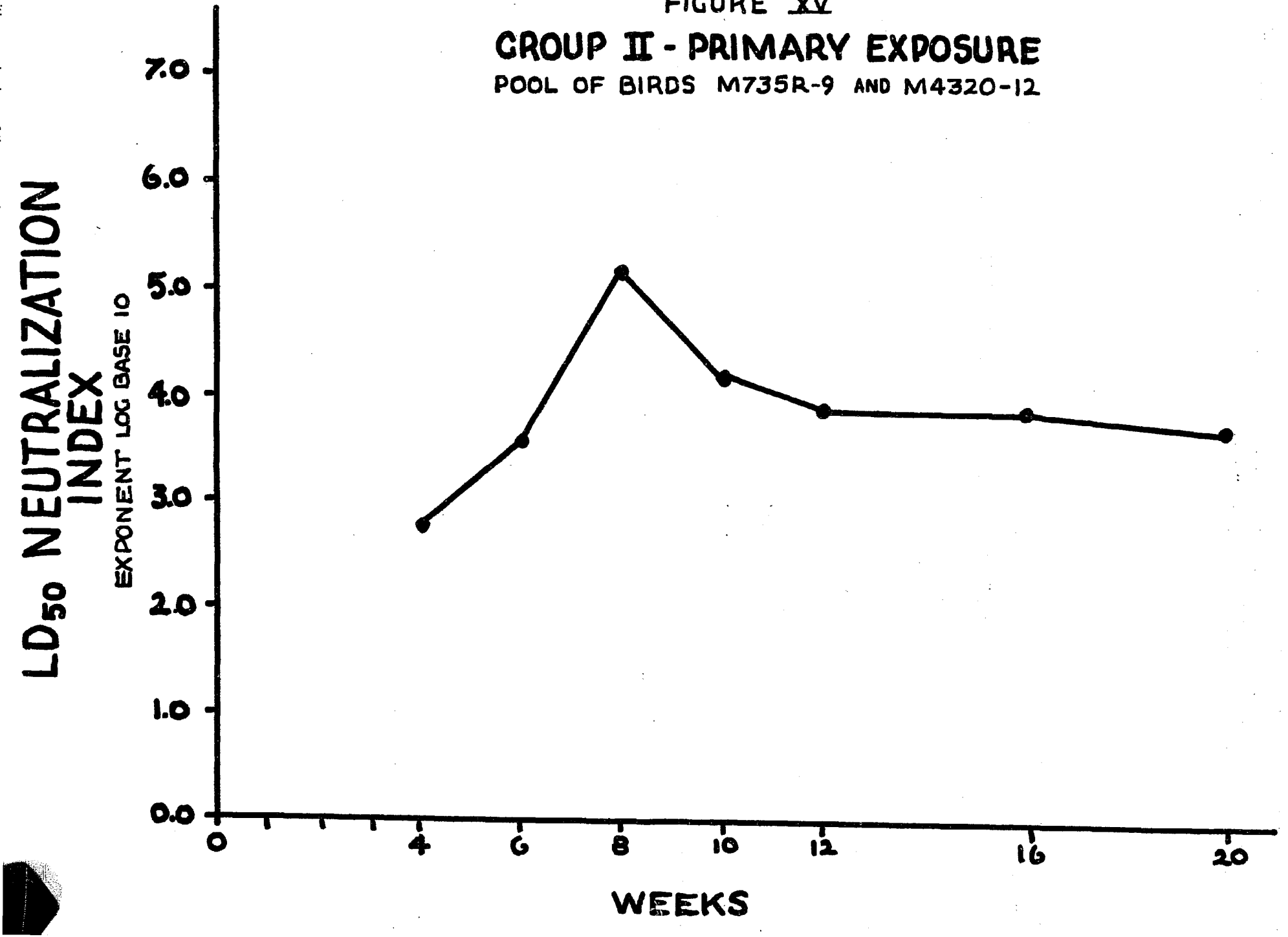


Table 26

Bird M735R -9

Group II-Primary Exposure

Serum Neutralization Tests

Period	Virus#	Virus Dilutions							Serum	
Weeks	Titer	10^{-1} ##	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Titer#	NI
6	6.72*	5	5	5	3	2			3.50	3.22
8	6.32*	5	5	5	5	0			3.50	2.82
10	6.17*	5	5	5	4	1			3.50	2.67
16	6.68*			5	4	3	0	0	4.00	2.68
20	6.68*		5	5	4	3	1		4.16	2.52

FIGURE XVI

GROUP II - PRIMARY EXPOSURE

BIRD M735R-9

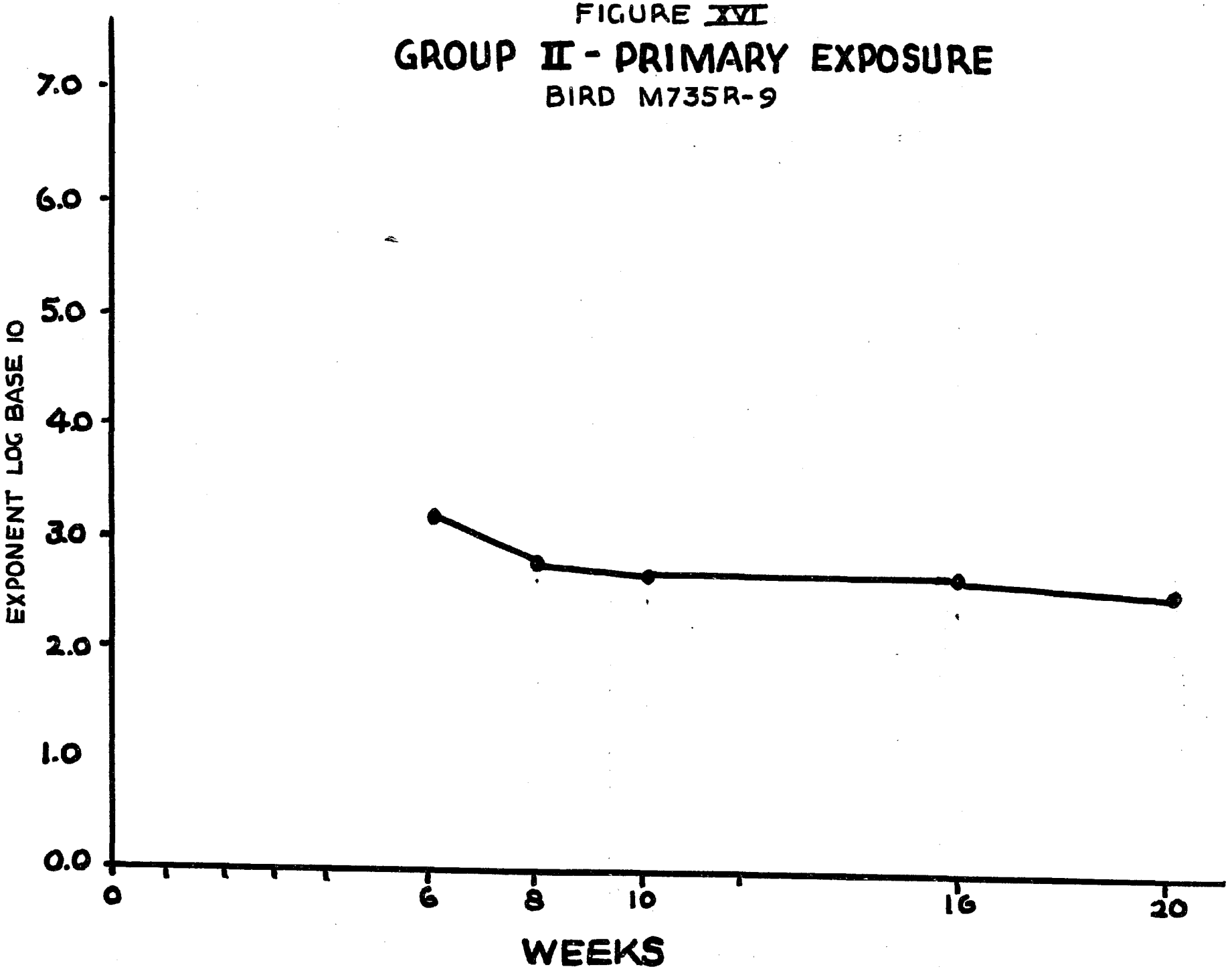


Table 27

Bird M4320 -12

Group II-Primary Exposure

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{0} ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Serum Titer#	NI
10	6.17*		5	5	3	1	0	2.32	3.85
16	6.68*		5	3	3	0	0	2.78	3.90
20	6.68*	5	5	5	5	1	0	2.63	4.05

FIGURE XVII

GROUP II - PRIMARY EXPOSURE

BIRD M4320-12

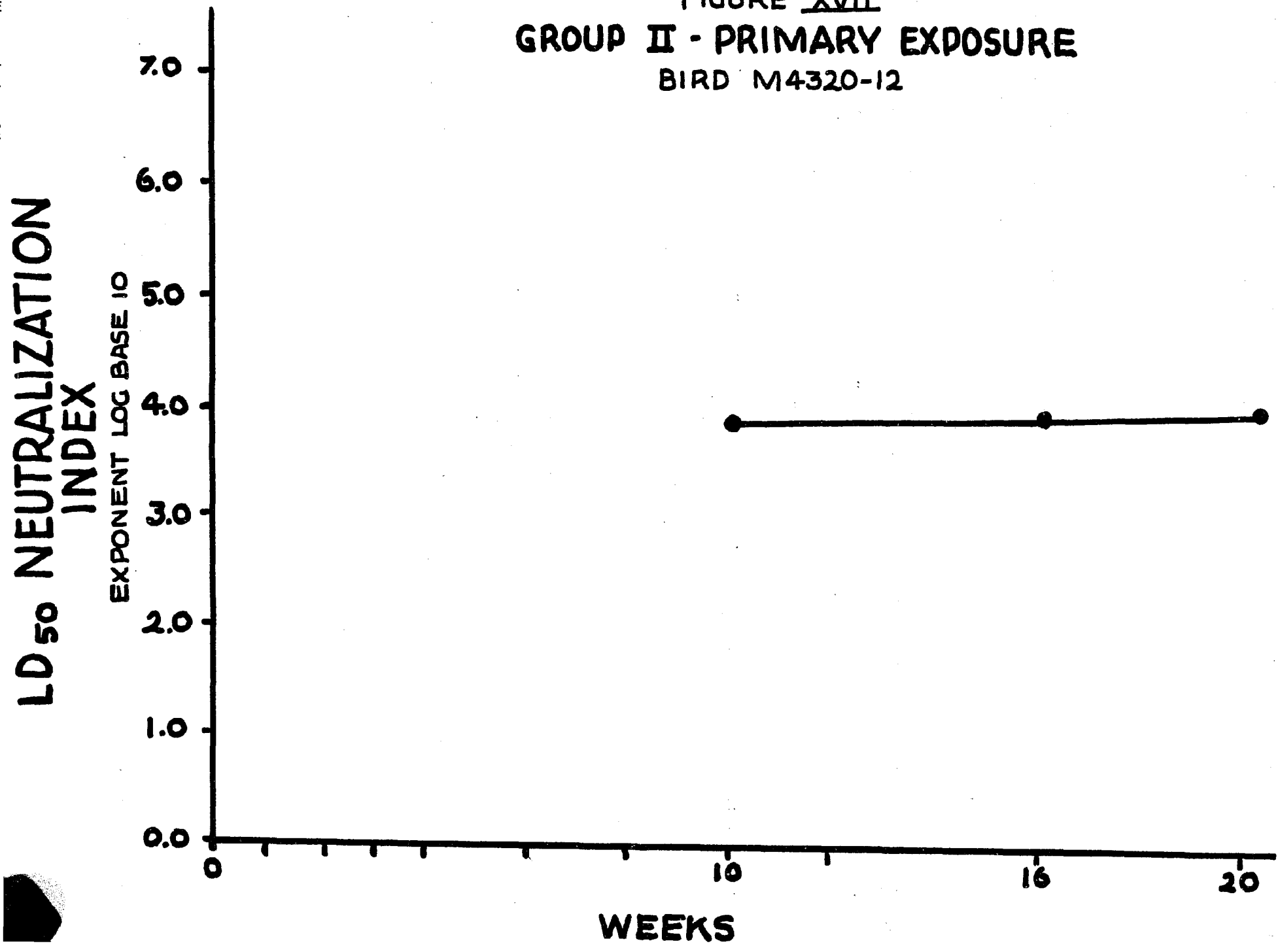


Table 28

Summation of LD_{50} NIs

Group II-Primary Exposure

Period	LD_{50} NI						
Weeks						Pools	
	M347C2-8	M735R-9	M349T-10	M538H-11	M4320-12	M716G2-7 M735R-9 M4320-12	M735R- M4320-1
0*	0.86		1.02	1.37		1.16	
1	1.87		1.00	1.82		2.00	
2	2.95		2.69	3.32		2.69	
3	3.64		2.99	4.00		2.65	
4	4.12		3.32	4.49			2.79
6	4.88	3.22	4.56	5.72			3.55
8	5.00	2.82	4.62				5.12
10	5.67	2.67	5.17		3.85		4.17
12	4.33		4.18				3.87
16	4.05	2.68	3.93		3.90		3.84
20	4.05	2.53	3.35		4.05		3.68

* Pre-exposure bleeding, inoculated with virus

The LD_{50} NIs of the pooled sera varied approximately 0.4 to 1.0 log unit from the LD_{50} NIs of the average of the individual serum samples comprising the pooled samples.

Hemagglutination-inhibition titers for Newcastle disease were negative at each period.

C. Group III

1. Electrophoretic Analyses

This group of birds was challenged with infectious bronchitis virus at the twelfth week after initial inoculation. The birds were bled at the following time schedule: birds M414Q -13 and M400U -14 - one, three, five, and seven weeks after challenge; M539U -15 - immediately prior to primary inoculation, one, two, and three weeks (died at third week due to internal hemorrhage); M727B2 -16 - four, six, eight, ten, and 12 weeks after primary inoculation (challenged but not tested); pool of sera from birds M361O -17 and M369N -18 - immediately prior to primary inoculation, one two, three, four, six, eight, and ten weeks; M361C -17 - six, ten, and 12 weeks; then at one, three, five, and seven weeks after challenge; M369N -18 - six and ten weeks; M541C2 -19 - immediately prior to primary inoculation, one, two, three, four, six, eight, ten, and 12 weeks; then at one, three, five, and seven weeks after challenge.

The relative percentage of albumin decreased slightly one and three weeks after challenge in bird M414Q -13. Gamma-globulin levels increased slightly and then decreased. The other globulins varied slightly during the experiment. (Table 29)

Table 29

Bird M414Q -13

Group III - Challenge

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
12*	47.8	12.2	5.8	9.7	24.5	0.92	3.78
1	44.5	14.6	5.6	8.5	26.8	0.80	3.69
3	43.4	13.2	7.4	6.9	29.1	0.77	3.86
5	42.8	12.8	8.4	8.9	27.1	0.75	4.13
7	48.1	12.1	5.3	7.3	27.2	0.93	3.86

* Challenge with virus

Sera from bird M400U -14 showed a slight decrease in albumin at the first week after challenge but increased again after this interval. Gamma-globulin increased slightly during the experimental period. Other globulins did not change significantly. (Table 30)

Sera from bird M539U -15 showed a marked decrease in albumin one week after primary inoculation and began to increase at the second week. The gamma-globulin level increased greatly at the first week and began to decrease after this period. (Table 31)

Sera from bird M727B2 -16 showed a definite increase in albumin during the experimental period. There was a slight decrease in the gamma-globulin level. Alpha 1-globulin decreased greatly at the twelfth week, while alpha 2-globulin and beta-globulin remained relatively unchanged. (Table 32)

Sera from birds M3610 -17 and M369N -18 were pooled and tested as one sample immediately prior to primary inoculation and then at the one, two, three, four, six, eight, and ten week interval. (Table 33) The sera of these birds were also tested individually as follows: bird M3610 -17 - six, ten, and 12 weeks, and then at one, three, five, and seven week intervals after challenge; (Table 34) bird M369N -18 - six and ten weeks. (Table 35)

The electrophoretic analyses of pooled serum samples from birds M3610 -17 and M369N -18 showed the characteristic pattern as before. A sharp decrease in albumin and an increase in the relative percentage of gamma-globulin was observed one

Table 30

Bird M400U -14

Group III - Challenge

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
12*	45.3	8.2	8.2	10.7	27.6	0.83	3.86
1	43.0	10.2	7.8	11.2	27.8	0.75	3.43
3	45.4	9.6	6.0	11.0	28.0	0.84	3.69
5	43.4	9.8	7.4	9.8	29.6	0.77	3.86
7	47.0	5.0	8.5	9.0	30.5	0.89	3.78

* Challenge with virus

Table 31

Bird M539U -15

Group III - Challenge

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	48.0	7.2	7.2	10.4	27.2	0.92	4.13
1	30.3	9.5	7.9	11.8	40.5	0.44	4.56
2	35.0	6.0	12.5	10.8	35.7	0.54	4.48
3	37.8	10.8	7.9	10.3	33.2	0.61	4.30

* Pre-exposure bleeding, primary inoculation with virus

Table 32

Bird M727B2 -16

Group III - Challenge

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
4	46.7	8.0	6.2	10.7	28.4	0.88	3.69
6	42.5	11.9	6.0	9.4	30.2	0.74	3.51
8	46.4	9.9	5.4	11.3	27.0	0.87	3.60
10	48.3	10.2	7.8	9.3	24.4	0.93	3.51
12*	51.1	5.1	6.5	11.3	26.0	1.05	3.60

* Challenge with virus

Table 32

Bird M727B2 -16

Group III - Challenge

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
4	46.7	8.0	6.2	10.7	28.4	0.88	3.69
6	42.5	11.9	6.0	9.4	30.2	0.74	3.51
8	46.4	9.9	5.4	11.3	27.0	0.87	3.60
10	48.3	10.2	7.8	9.3	24.4	0.93	3.51
12*	51.1	5.1	6.5	11.3	26.0	1.05	3.60

* Challenge with virus

Table 33

Pool of Birds M3610 -17 and M369N -18

Group III - Challenge

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	40.8	10.9	4.8	10.5	33.0	0.69	3.73
1	34.9	11.5	9.2	9.6	34.8	0.54	3.73
2	33.2	12.4	6.0	9.7	38.7	0.51	4.04
3	39.0	7.5	6.1	10.1	37.3	0.64	4.39
4	38.5	9.5	6.3	9.5	36.2	0.63	3.78
6	38.9	8.8	6.9	9.3	36.1	0.64	4.13
8	36.4	11.7	6.0	10.4	35.5	0.57	3.86
10	41.3	12.3	6.6	10.7	29.1	0.70	4.04

* Pre-exposure bleeding, primary inoculation with virus

Table 34

Bird M3610 -17

Group III - Challenge

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
6	38.6	12.4	6.4	8.4	34.6	0.63	4.04
10	41.4	11.7	6.1	11.2	29.6	0.71	3.95
12*	38.9	10.7	7.9	9.7	32.8	0.64	3.78
1	40.9	7.9	6.4	11.3	33.5	0.69	4.13
3	35.6	8.6	6.7	11.0	38.1	0.55	4.21
5	38.3	8.3	8.7	9.7	35.0	0.62	4.48
7	38.2	11.8	8.2	8.2	34.6	0.62	3.78

* Challenge with virus

Table 35

Bird M369N -18

Group III - Challenge

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
6*	35.6	10.4	7.4	10.0	36.6	0.55	3.95
10	43.1	9.7	7.6	8.6	31.0	0.76	4.04

* After Primary Inoculation

and two weeks after primary exposure. An increase in albumin occurred at the third week and at the same time gamma-globulin levels began to decrease. This continued until the end of the experimental period of ten weeks. Beta-globulin levels did not change significantly during the experiment. Alpha 1-globulin decreased early during the experiment but increased again at the tenth week. Alpha 2-globulin changed at the first week but remained constant during the rest of the experiment. (Table 33)

The electrophoretic analyses of sera from bird M3610 -17 showed a decrease in albumin and an increase in gamma-globulin during the third week after challenge.This condition was reversed after this period up to and including the seventh week. Alpha 2-globulin levels did not change significantly during the experimental period. Alpha 1-globulin decreased one week after challenge but increased steadily to a final high value. Beta-globulin decreased during the challenge period. (Table 34)

The analyses of sera from bird M369N -18 during the sixth and tenth weeks showed a great increase in albumin and a decrease in gamma-globulin. Values for the other globulins were relatively unchanged. (Table 35)

The same general trend followed in the electrophoretic analyses of sera from bird M541C2 -19. There was a sharp decrease in the relative percentage of albumin at the first week after primary exposure. Gamma-globulin levels increased at this period, decreased at the fourth week and were at a

minimum value at the twelfth week. There was a general increase in alpha 1-globulin at the first and second weeks with a decrease in this fraction up to and including the twelfth week. Alpha 2-globulin decreased slightly during the twelve weeks, while beta-globulin remained relatively unchanged except that a rise was observed at the first week which remained at this level for twelve weeks.

During the challenge period there was little change in the relative percentage distribution of the serum components. (Table 36, Figure XVIII)

The changes in serum electrophoretic patterns after challenge with infectious bronchitis virus was not as marked as the changes observed in the electrophoretic patterns after primary exposure.

The same general trend in the change of serum electrophoretic patterns in Group II was observed in Group III prior to challenge. After challenge there was relatively little change in the relative percentage distribution of the serum components.

A comparison of results obtained with pooled serum samples and individual serum samples of the pool showed very close correlation in the relative percentage distribution of the serum components.

The changes in percent serum protein were not considered significant.

Table 36

Bird M541C2 -19

Group III - Challenge

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0#	39.6	8.4	9.0	8.4	34.6	0.66	3.99
1	27.8	12.1	6.6	13.6	39.9	0.39	4.04
2	34.0	11.5	5.0	11.9	37.6	0.52	4.30
3	40.9	7.4	6.1	10.0	35.6	0.69	4.04
4	39.8	9.5	4.1	11.8	34.8	0.66	4.13
6	38.0	9.8	6.1	11.0	35.1	0.61	3.69
8	41.8	6.8	7.3	10.5	33.6	0.72	3.95
10	43.0	10.5	6.0	12.0	28.5	0.75	3.69
12*	44.0	8.0	6.7	11.7	29.6	0.79	3.69
1	43.2	8.3	6.8	11.5	30.5	0.76	3.86
3	46.4	7.8	5.6	11.2	29.0	0.87	3.34
5	44.0	8.9	7.3	10.5	29.3	0.79	3.95
7	43.0	10.7	7.3	9.8	29.2	0.76	3.51

Pre-exposure Bleeding, Inoculation with Virus

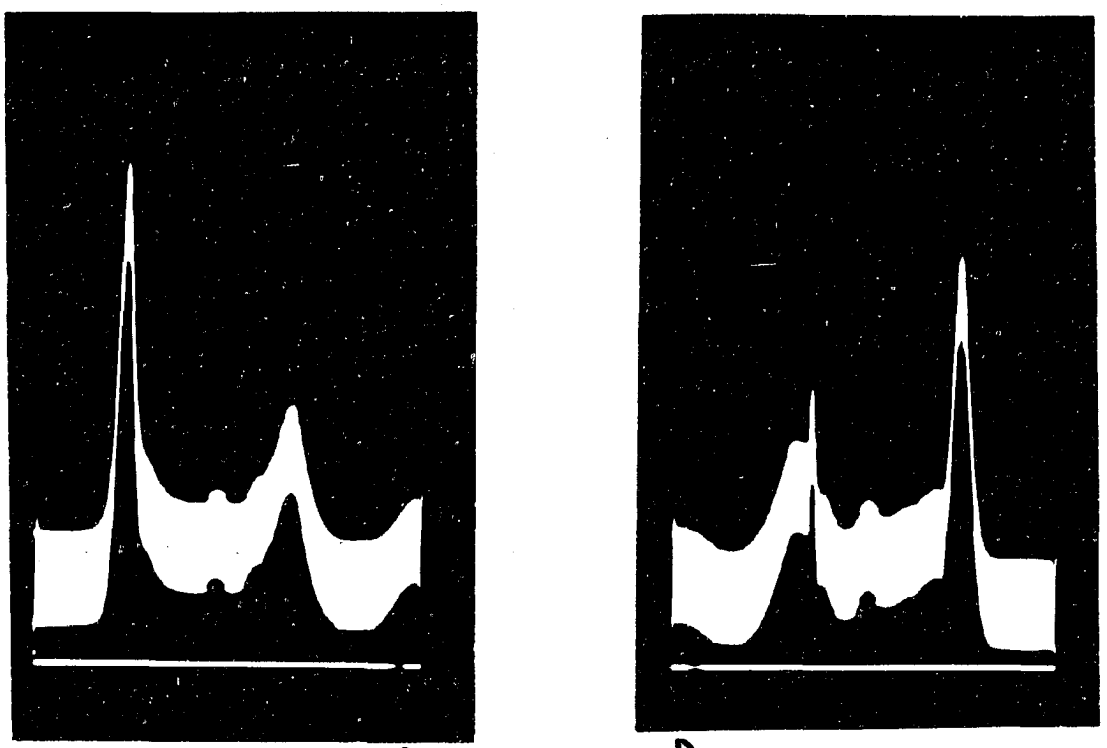
* Challenged with virus

Figure XVIII

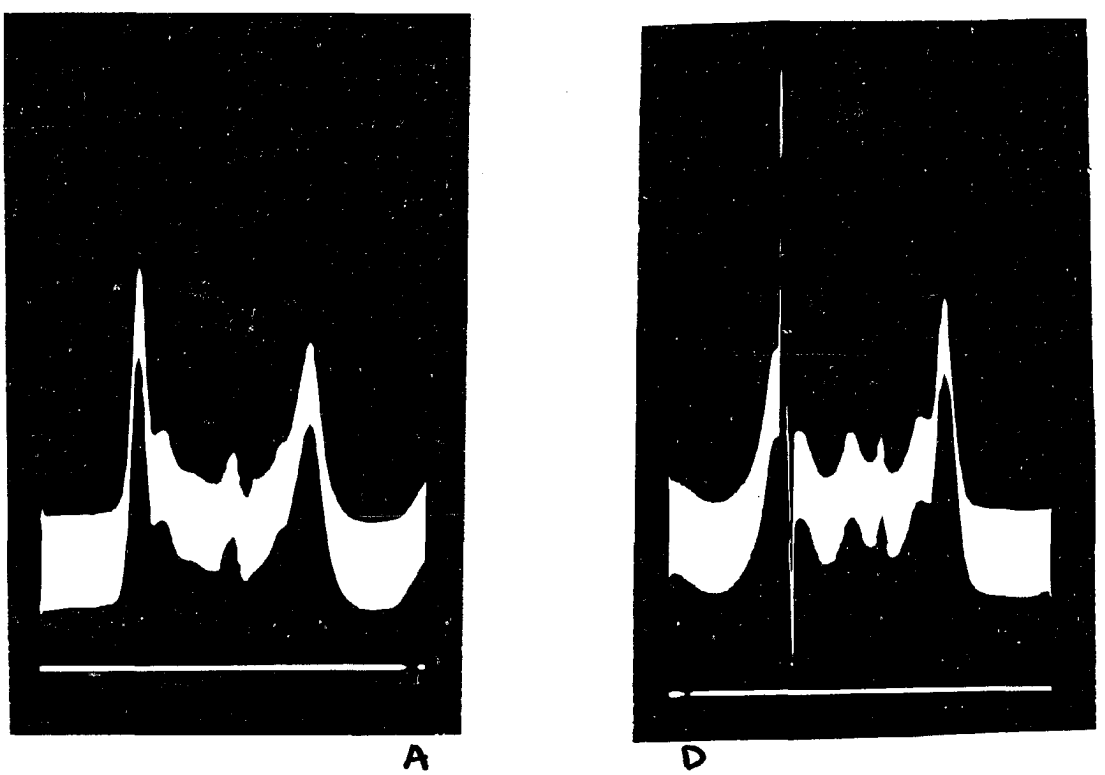
Bird M541C2 -19

Group III-Challenge

Electrophoretic Patterns

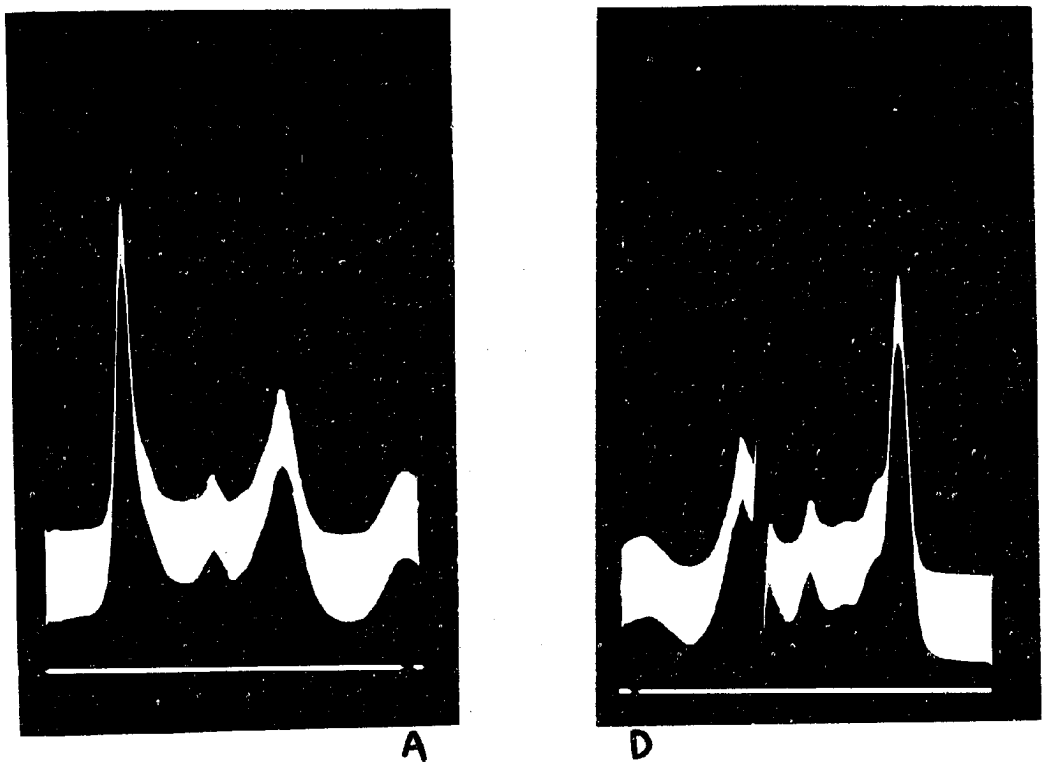


0 WEEKS
PRE-EXPOSURE



1 WEEK

Figure XVIII (cont.)



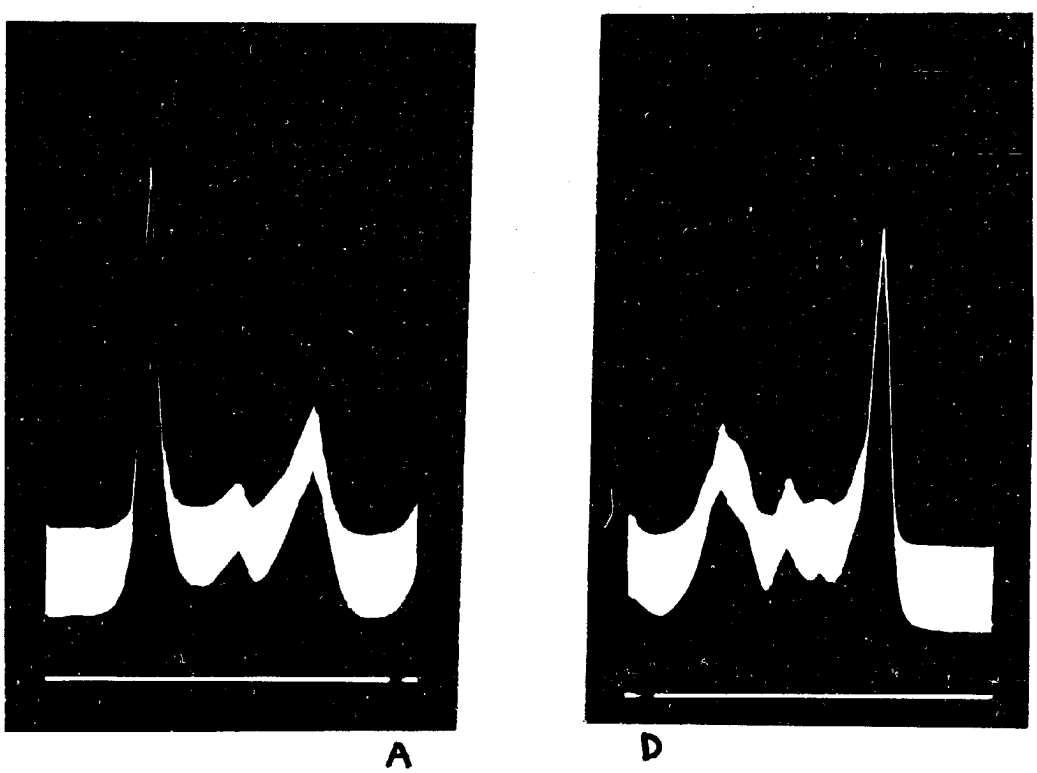
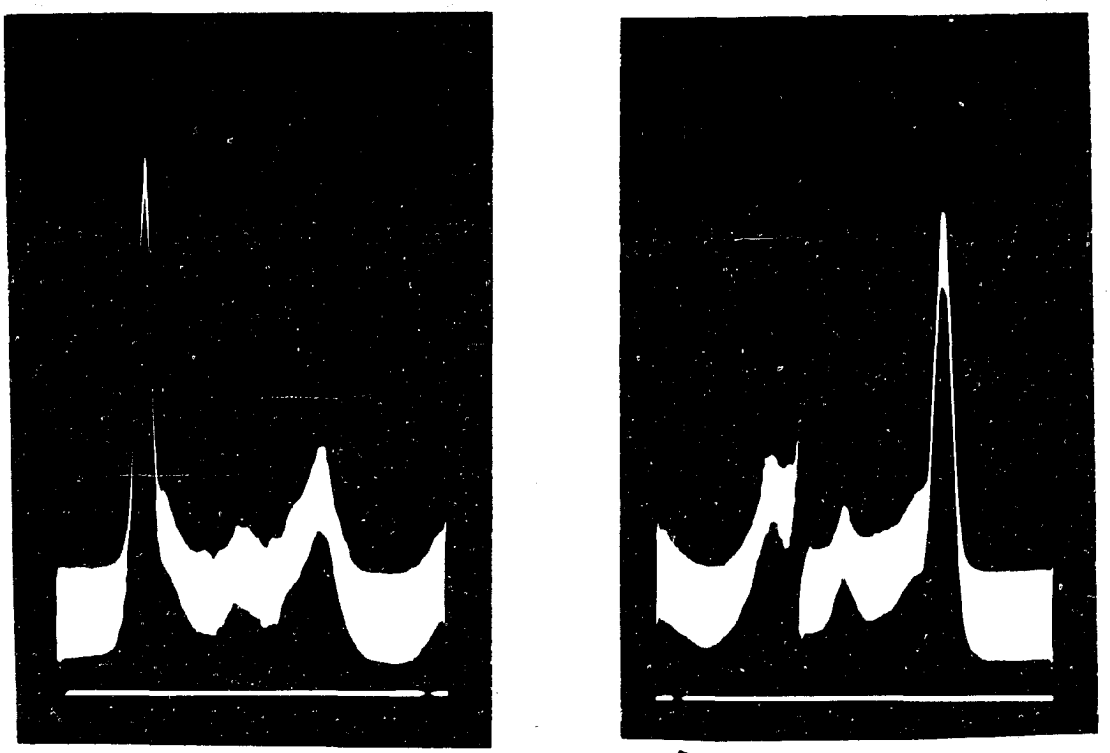
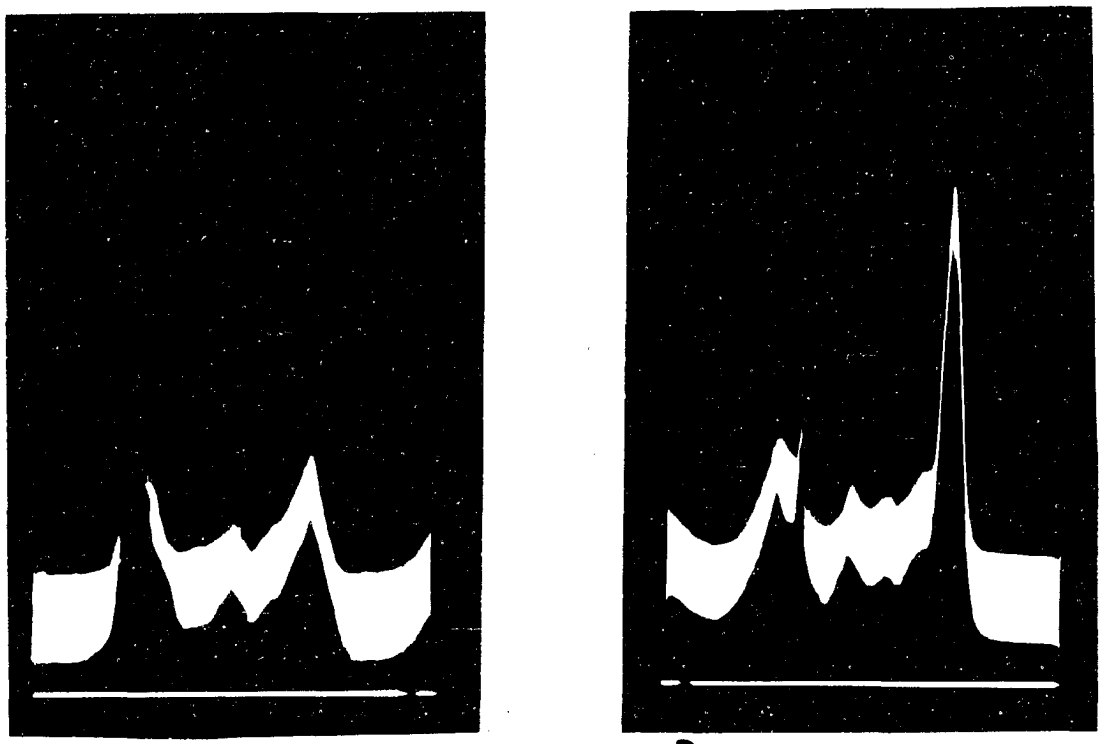


Figure XVIII (cont.)



A D

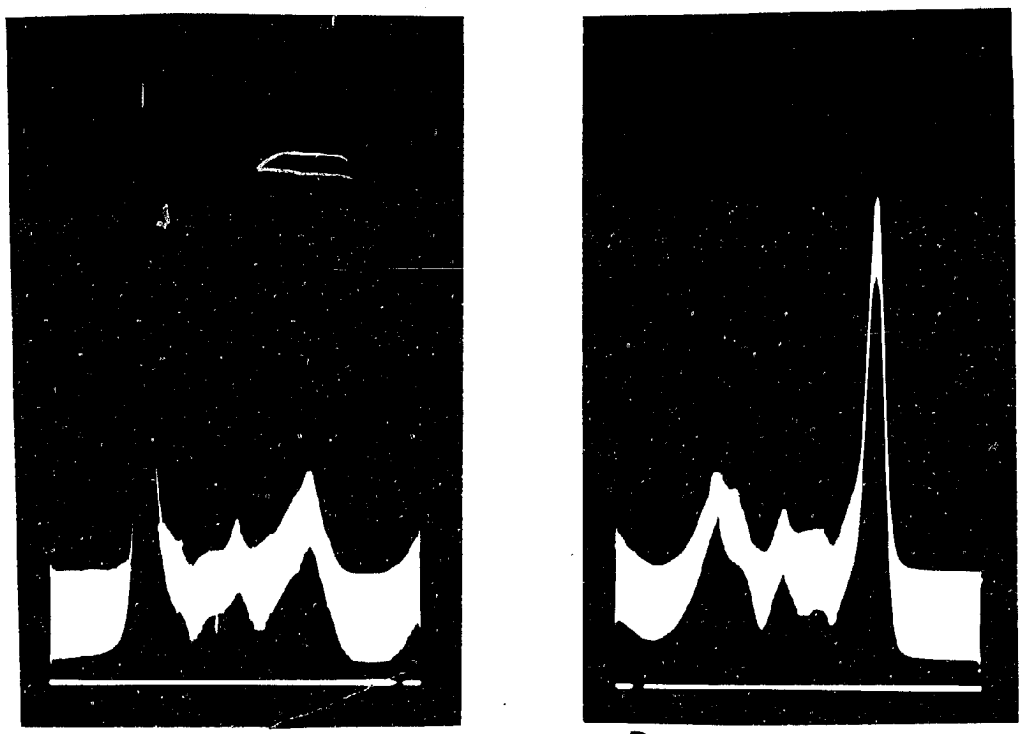
4 WEEKS



A D

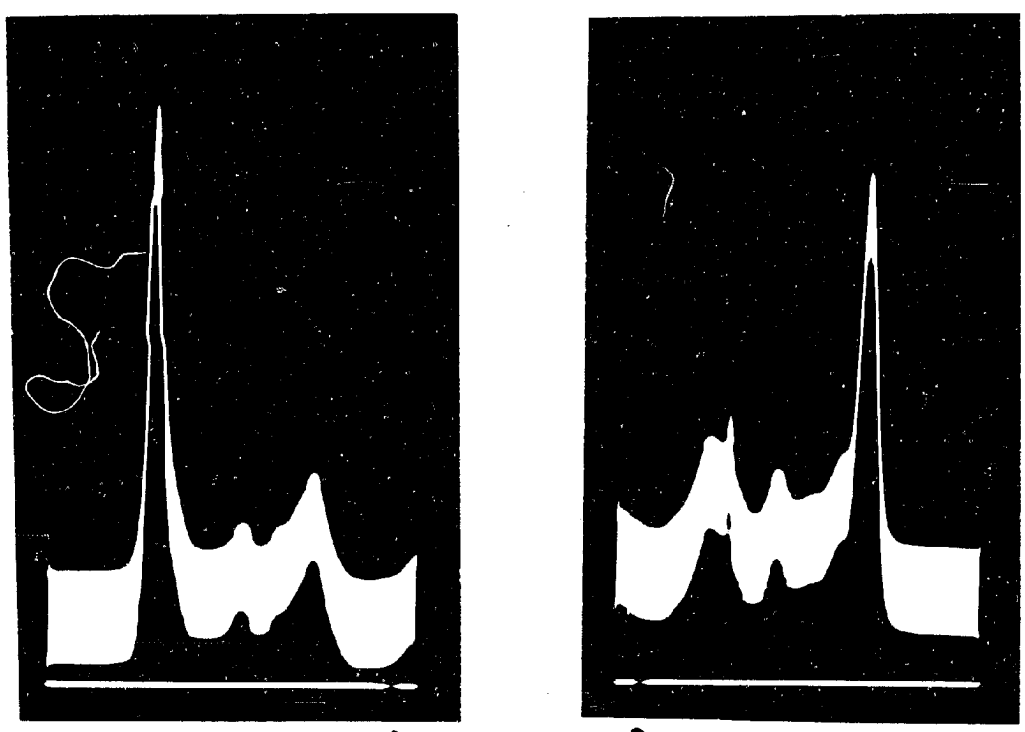
6 WEEKS

Figure XVIII (cont.)



A D

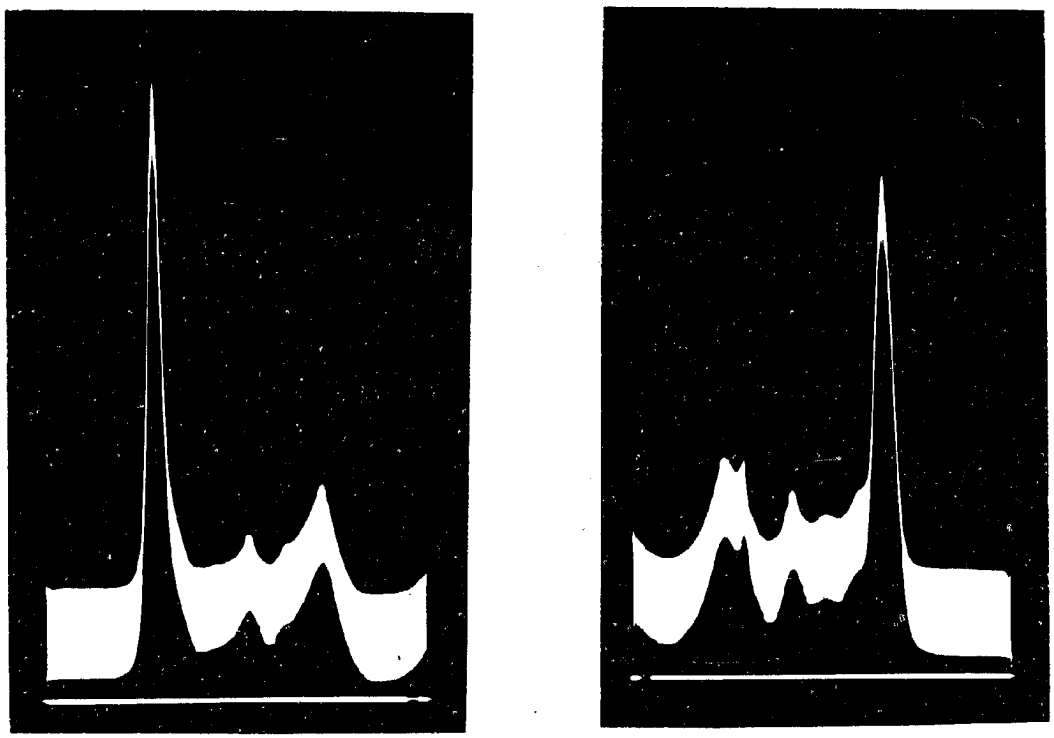
8 WEEKS



A D

10 WEEKS

Figure XVIII (cont.)



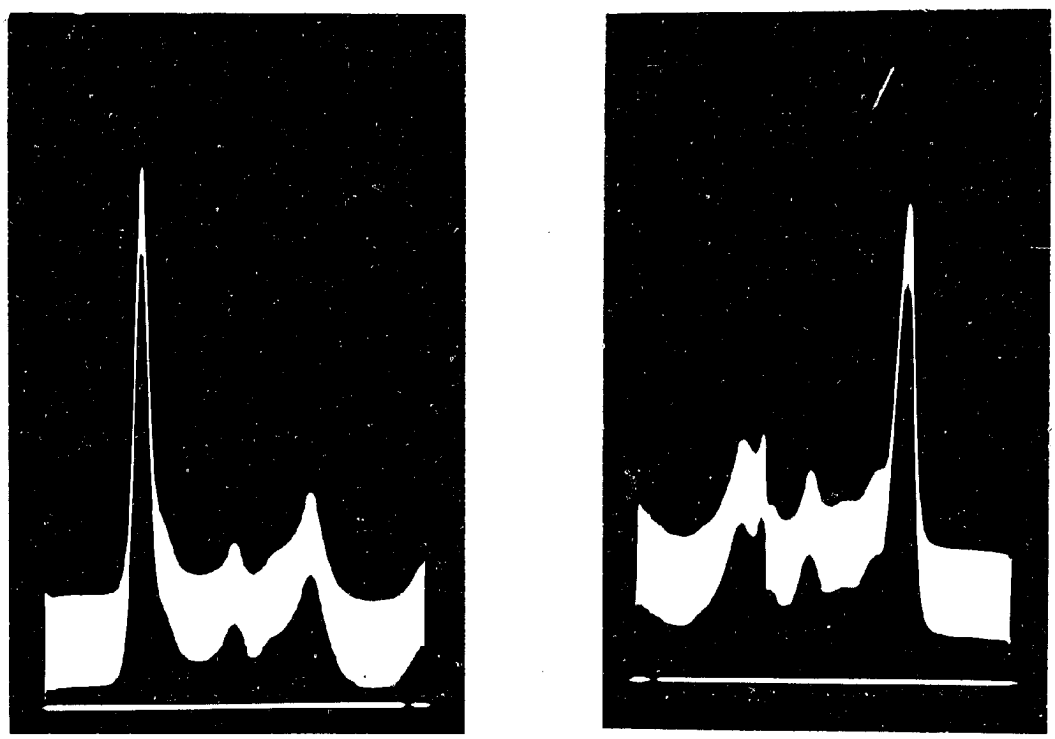
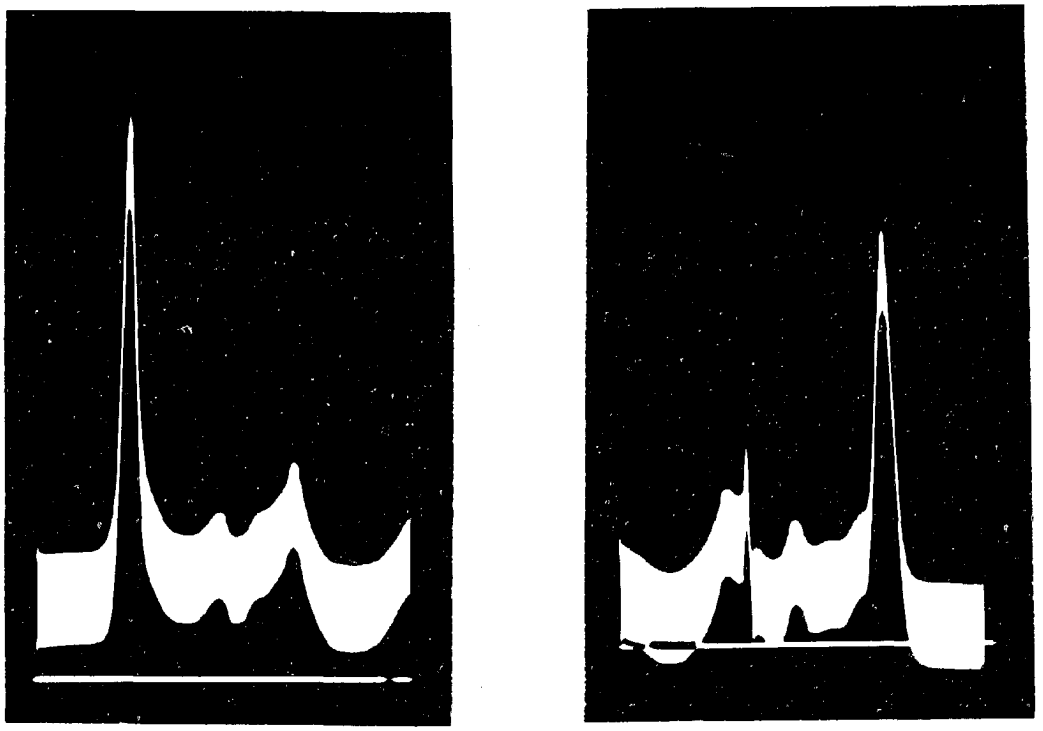
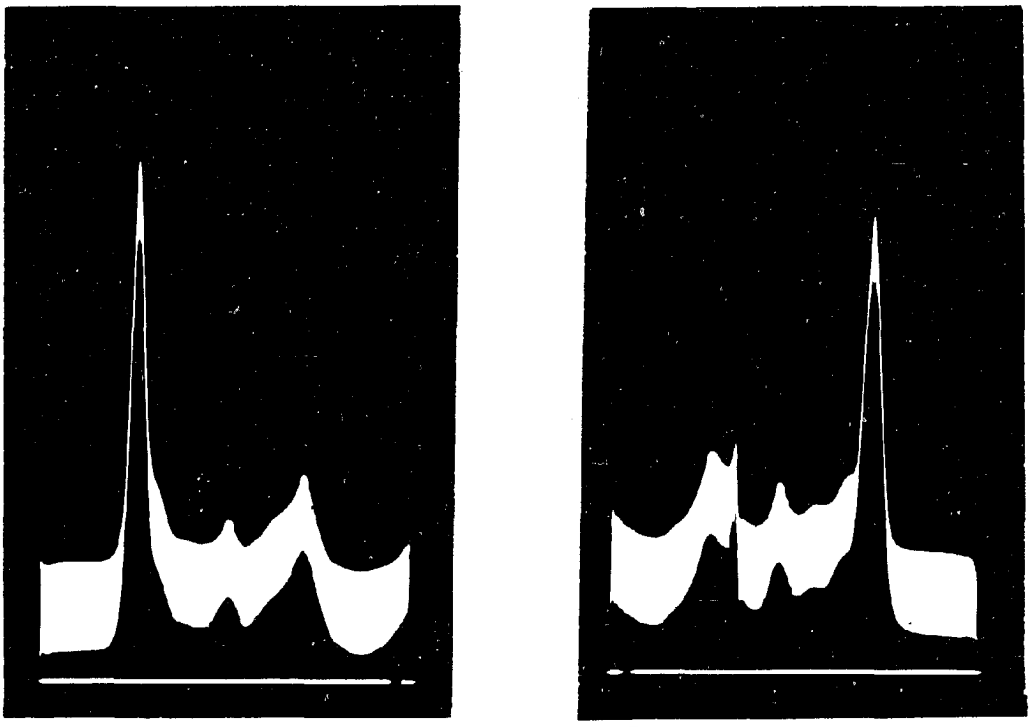


Figure XVIII (cont.)



A D

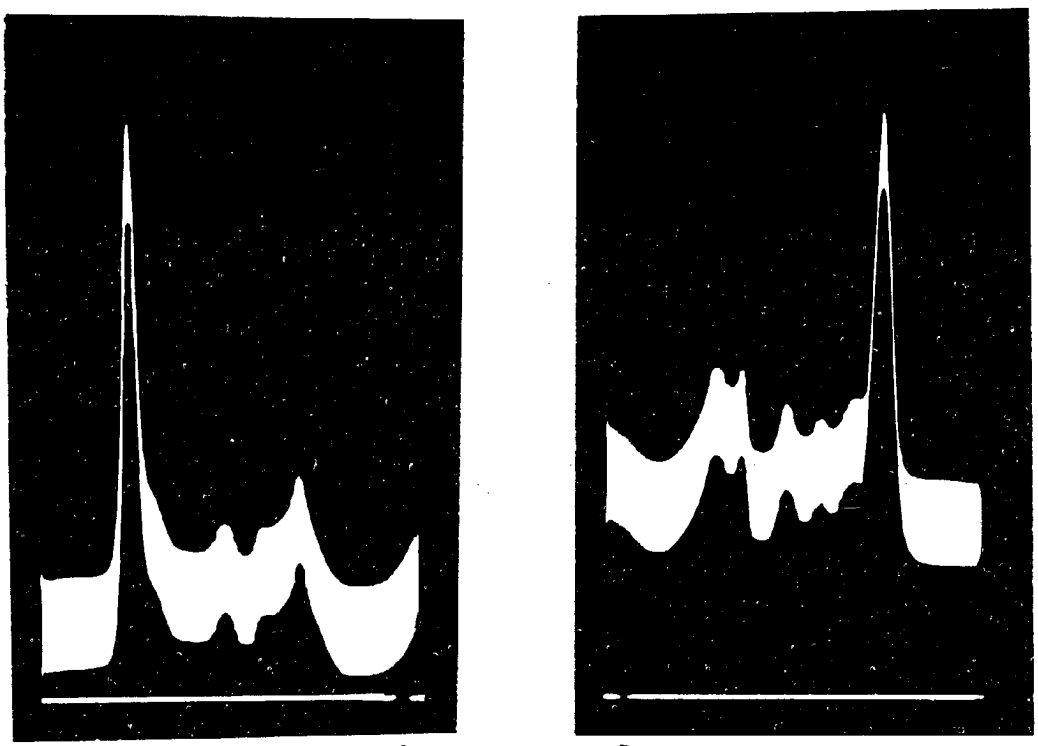
3 WEEKS



A D

5 WEEKS

Figure XVIII (cont.)



2. Lethal Dose$_{50}$ Neutralization Indices

The serum LD_{50} NIs of this group followed a general pattern as in Group II. There was a slight increase in LD_{50} NIs between the first and second weeks after primary exposure. After the second week a marked rise occurred and a maximum was reached between the third and eighth weeks. The maximum LD_{50} NIs that were reached varied with the individual birds as follows: bird M727B2 -16 - fourth week; bird M3610 -17 - sixth week; bird M369N -18 - sixth week; bird M541C2 -19 - eighth week. After challenge the LD_{50} NIs were higher than the maximum values reached after primary inoculation. The maximum LD_{50} NIs after challenge were reached between the third and fifth weeks. (Tables 37, 38, 39, 40, 41, 42, 43, 44, 45, Figures XIX, XX, XXI, XXII, XXIII, XXIV, XXV, XXVI)

Pre-exposure LD_{50} NIs were considered to be normal (33, 57).

Necropsy examination failed to reveal any abnormalities in birds of this group.

Hemagglutination-inhibition titers for Newcastle disease were negative at all times.

D. Group IVa

1. Electrophoretic Analyses

In order to determine if inoculations of a normal lung and tracheal suspension could affect the serum electrophoretic patterns or LD_{50} NIs birds M346B -21 and M673V -22 were bled immediately prior to inoculation and then at the one, two, and three week interval. Sera from bird M645H -23

Table 37

Bird M414Q -13

Group III-Challenge

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{0} ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Serum Titer#	Serum NI
12*	6.50*		5	5	3	2	1	2.67	3.83
1	6.38*	5	5	5	4	0		2.38	4.00
3	6.00*	5	4	1	0	0		0.50	5.50
5	6.32*	5	3	3	1	0	1	1.14	5.18
7	6.50*	5	4	3	2	0		1.33	5.17

* Challenge with virus

FIGURE XIX

GROUP III - CHALLENGE

BIRD M414Q-13

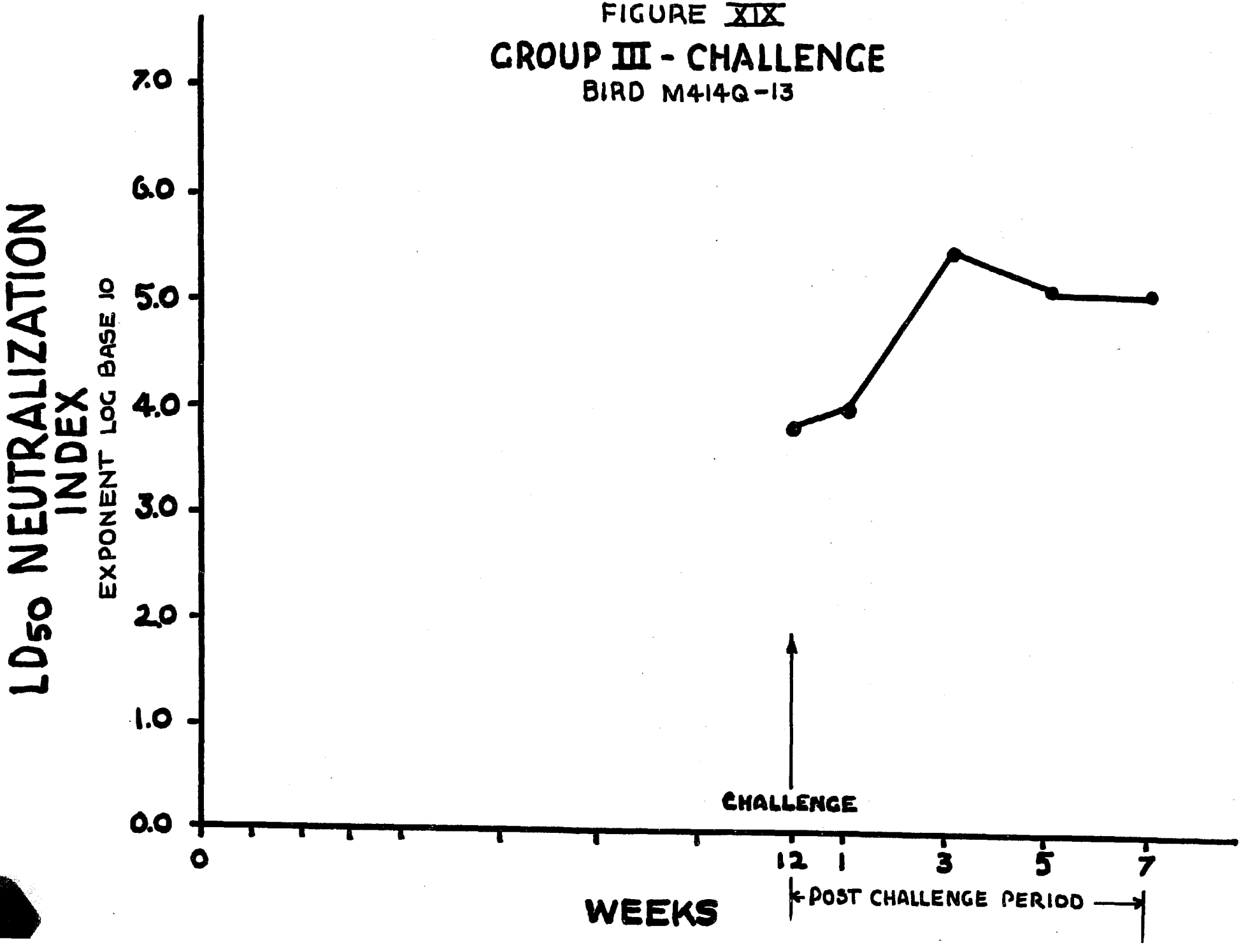


Table 38

Bird M400U -14

Group III-Challenge

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{0} ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Serum Titer#	Serum NI
12*	6.50*		5	5	3	0	1	2.17	4.33
1	6.38*	5	5	3	0	1		1.31	5.07
3	7.00*#	5	4	2	0	0		0.68	6.32
5	6.32*	5	3	0	0	0		0.17	6.15
7	6.50*	5	3	2	0	0		0.50	6.00

* Challenge with virus

\# Endpoint not reached when serum was diluted 1:100. Virus titer arbitrarily fixed at 10^{6} to calculate LD_{50} NI

FIGURE XX

GROUP III - CHALLENGE

BIRD M400V-14

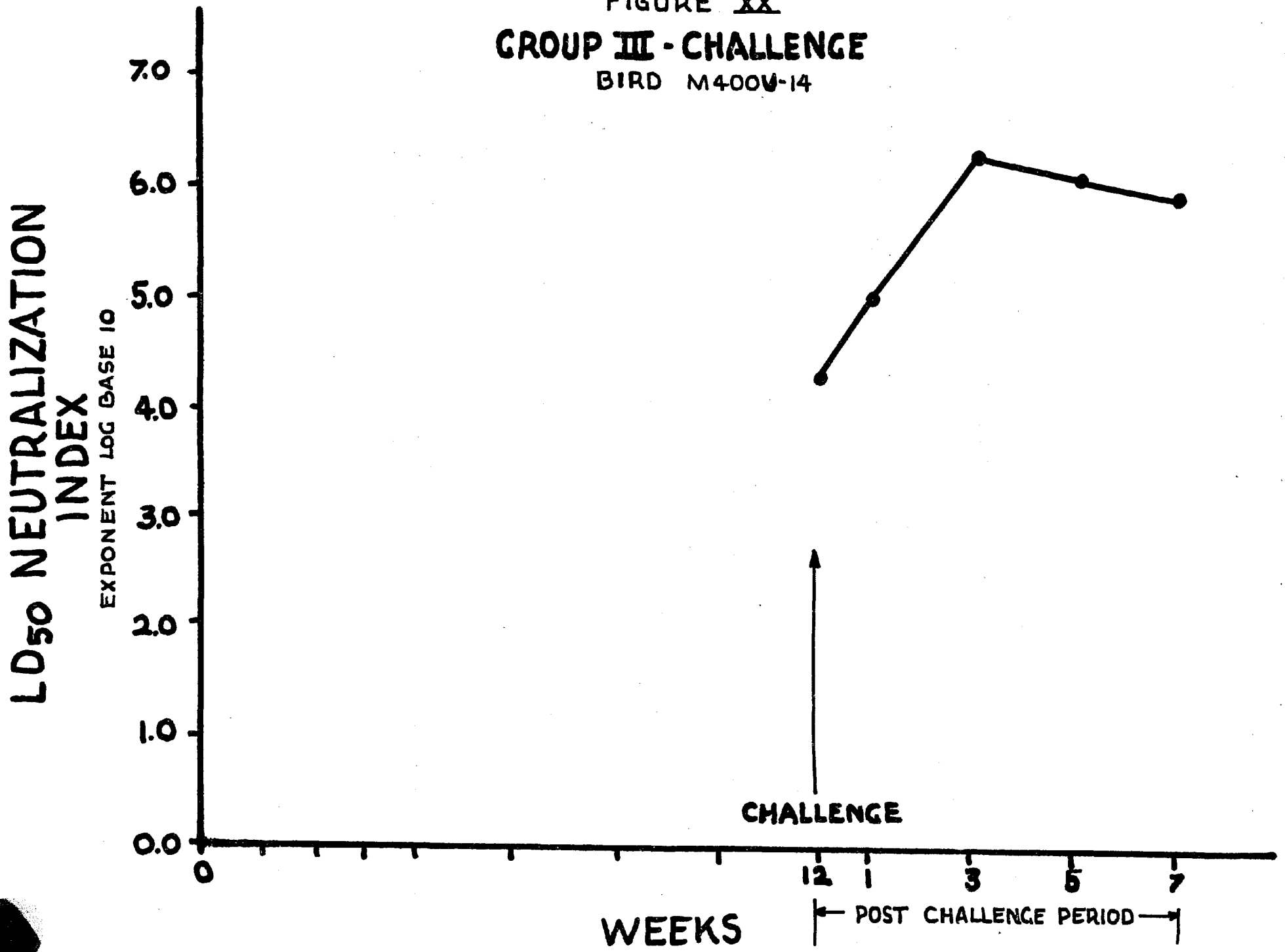


Table 39

Bird M539U -15

Group III-Challenge

Serum Neutralization Tests

Period Week	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Serum Titer#	NI
0*	6.54	5	5	5	3	2	5.50	1.04
1	6.50	5	5	4	1	0	4.50	2.00
2	5.63	5	3	0	0	0	3.17	2.46
3	6.32*	5	5	5	0		3.50	2.82

* Pre-exposure bleeding, inoculated with virus

FIGURE XXI

GROUP III - CHALLENGE

BIRD M539V-15

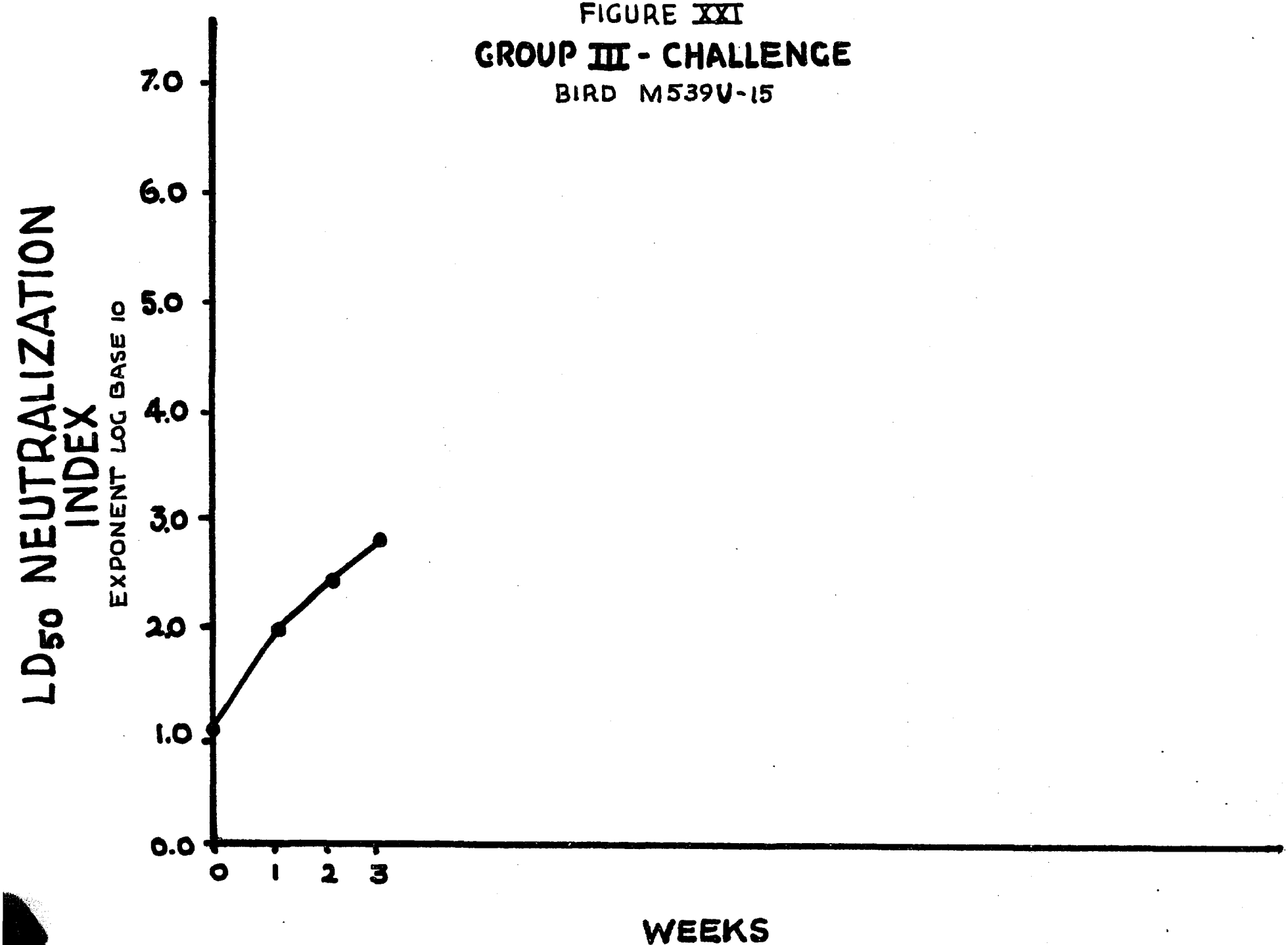


Table 40

Bird M727B2-16

Group III-Challenge

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-1} ##	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Serum Titer#	NI
4	7.32*	5	5	4	5	0	1	3.50	3.82
6	6.72*	5	5	5	4	1		3.50	3.22
8	6.32*	5	5	5	5	0		3.50	2.82
10	6.17*	5	5	5	3	2		3.50	2.67
12*	6.50*	5	5	3	3	0		3.78	2.72

* Challenge with virus

FIGURE XXII

GROUP III - CHALLENGE

BIRD M727 B2-16

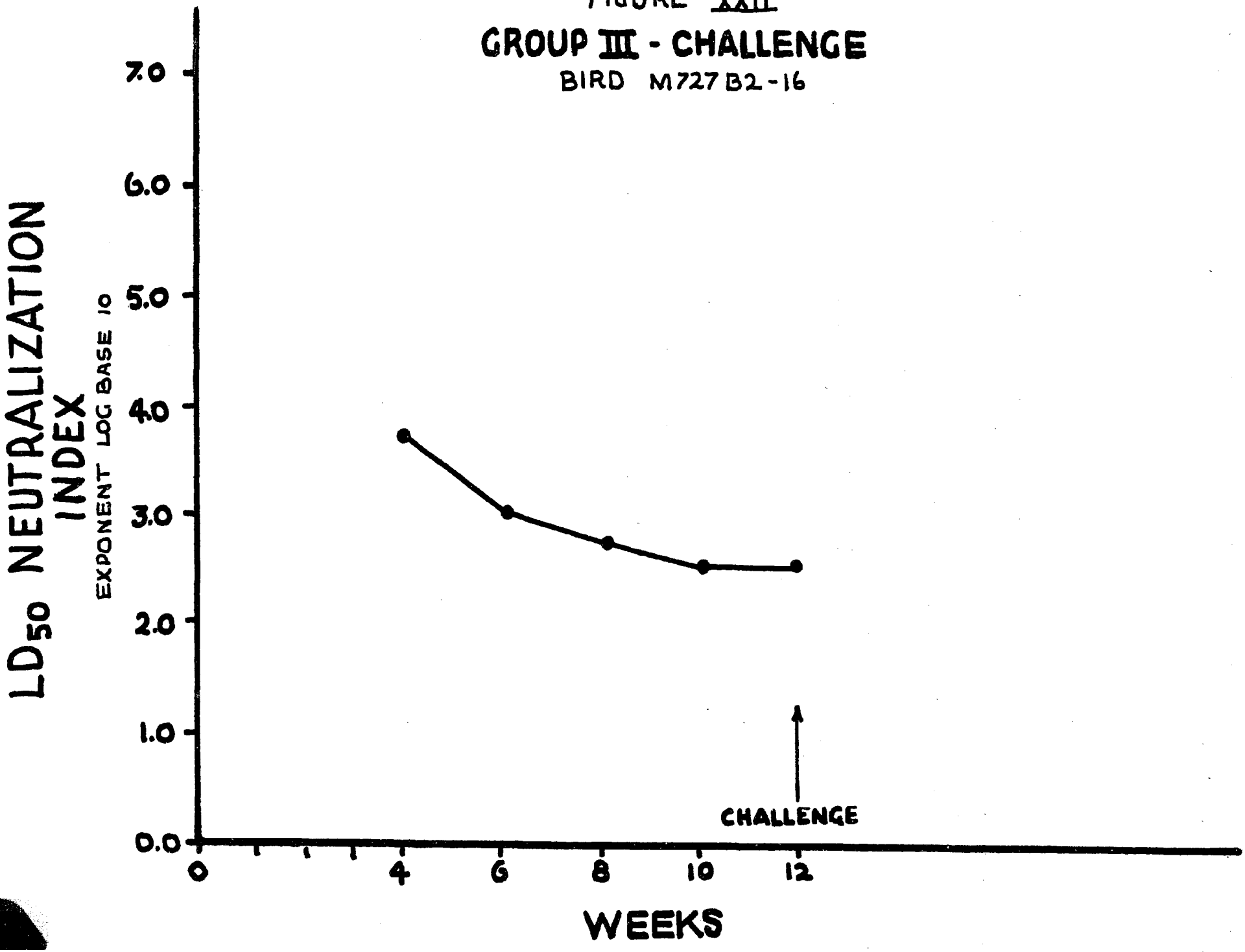


Table 41

Pool of Birds M3610 -17 and M369N -18

Group III-Challenge

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{0} ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Serum Titer#	NI
0*	6.54			5	5	5	3	1	5.32	1.22
1	6.50			5	5	2	1	1	3.20	3.30
2	6.32*			5	4	2	0	0	2.68	3.64
3	6.32*			5	5	1	1		2.75	3.57
4	7.32*		2‡‡	5	4	1	0	0	2.50	4.82
6	6.72*		5	5	1	0	0		1.63	5.09
8	6.32*		5	5	1	0	0		1.63	4.69
10	6.17*	5	4	4	2	1	0		1.69	4.48

‡‡ Three embryos died out of five inoculated due to trauma

* Pre-exposure bleeding, inoculated with virus

FIGURE XXIII

GROUP III - CHALLENGE

POOL OF BIRDS M3610-17 AND M369N-18

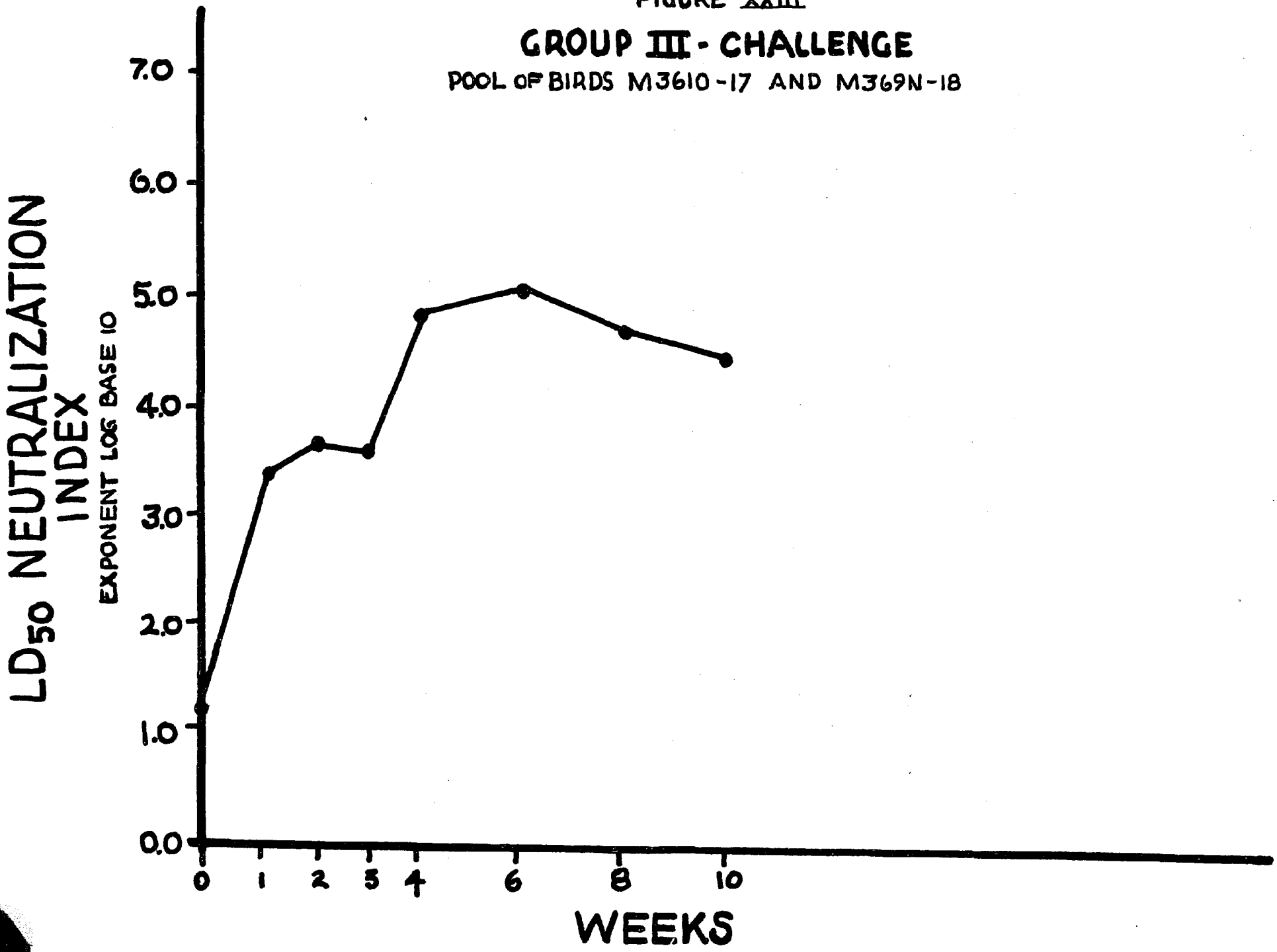


Table 42

Bird M3610 -17

Group III-Challenge

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{0} ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Serum Titer#	NI
6	6.72*		5	4	1	0	0	0	1.50	5.22
10	6.17*		5	4	0	0	0	0	1.38	4.79
12*	6.50*		5	5	3	0	0		2.17	4.33
1	6.38*	5	5	1	0	0			0.63	5.75
3	7.00*#	5	2	1	0				0.00	7.00
5	6.32*	5	3	1	1				0.56	5.76
7	6.50*	5	5	3	0	1			1.34	5.16

* Challenge with virus

\# Endpoint not reached when serum was diluted 1:10. Virus titer arbitrarily fixed at 10^{6} to calculate the LD_{50} NI

FIGURE XXIV

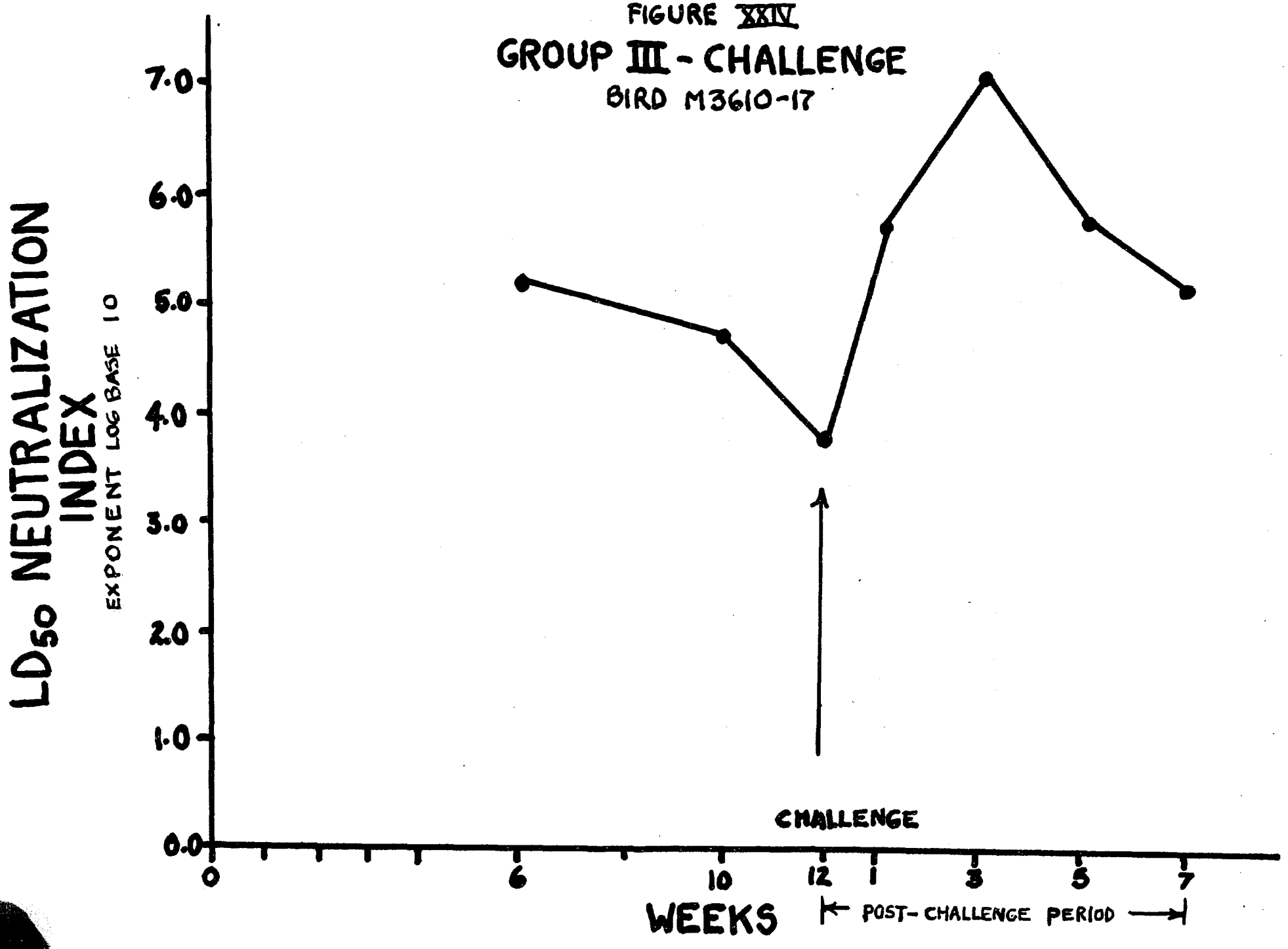


Table 43

Bird M369N -18

Group III-Challenge

Serum Neutralization Tests

Period	Virus#	Virus Dilutions						Serum	
Weeks	Titer	10^{0} ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Titer#	NI
6*	6.72*	5	5	2	1	0	0	0.00	6.72
10	6.17*	5	4	1	0	0	0	0.50	5.67

* After primary inoculation

FIGURE XXV

GROUP III - CHALLENGE

BIRD M369N-18

Table 44

Bird M541C2 -19

Group III-Challenge

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{0} ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Serum Titer#	Serum NI
0#*	6.54			5	5	5	5	3	0	6.17	0.37
1	6.50			5	5	4	3	0		5.00	1.50
2	5.63			5	1	0	0	0		2.63	3.00
3	7.32*		5	5	5	5	3	0		4.17	3.15
4	7.32*		5	5	5	4	3	0		4.00	3.32
6	6.72*		4	5	4	3	1			3.00	3.72
8	6.32*		5	5	2	1	0			1.00	5.32
10	6.17*		5	5	1	1	0			1.75	4.42
12*	6.50*		5	5	4	0	0			2.38	4.12
1	6.38*	5	5	3	1	0				1.32	5.06
3	6.00*	5	4	0	0	0				0.38	5.62
5	6.32*	5	4	1	0	0	0			0.50	5.82
7	6.50*	5	5	4	1	0				1.50	5.00

#* Pre-exposure bleeding, inoculated with virus

* Challenge with virus

FIGURE XXVI

GROUP III

BIRD M541C2 - 19

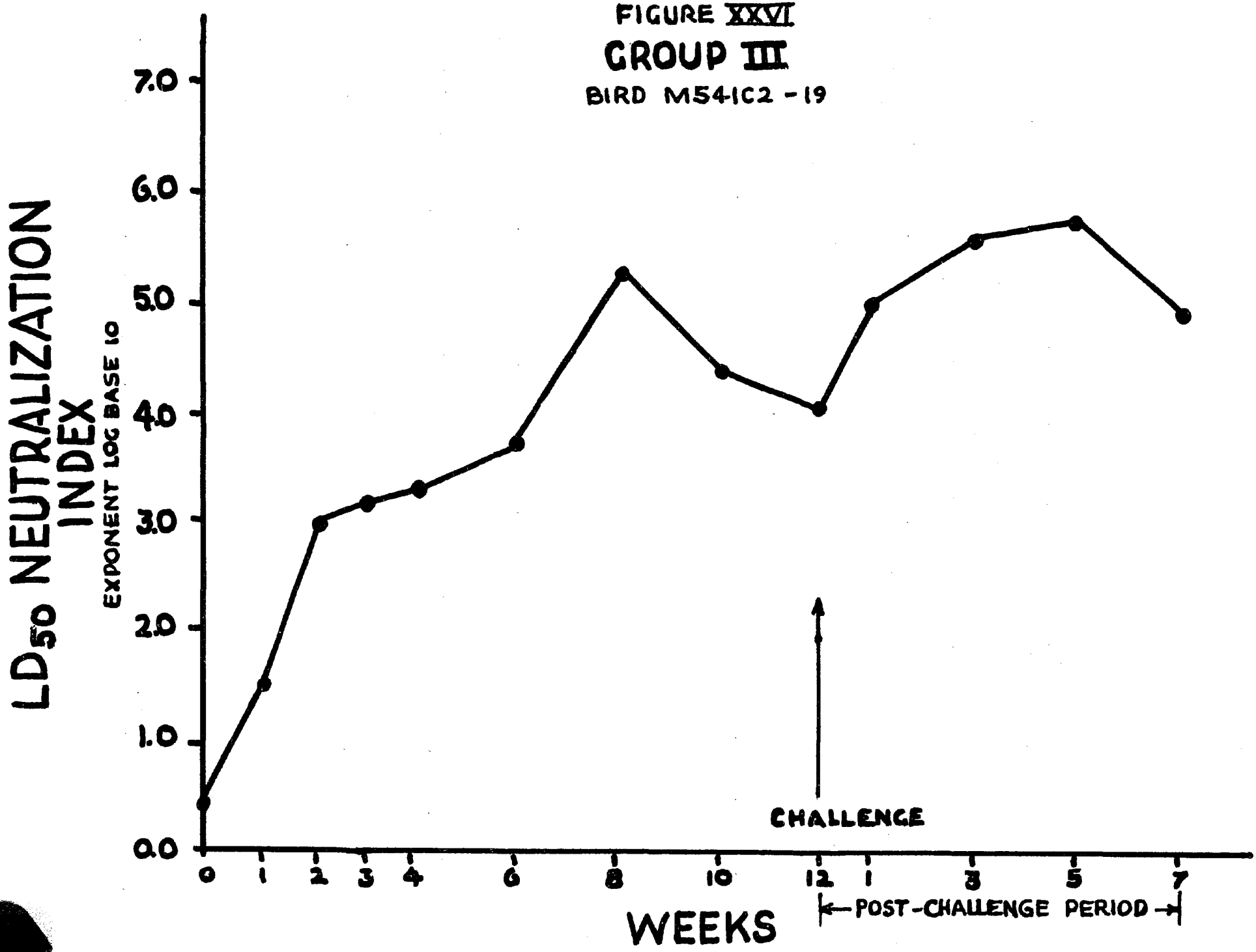


Table 45

Summation of LD_{50} NIs

Group III-Challenge

Period Weeks	LD_{50} NI						
	Pool M3610-17 M369N-18	M400U-14	M539U-15	M727B2-16	M3610-17	M369N-18	M541C2-1
0#*	1.22		1.04				0.37
1	3.30		2.00				1.50
2	3.64		2.46				3.00
3	3.57		2.82				3.15
4	4.82			3.82			3.32
6	5.09			3.22	5.22	6.72	3.72
8	4.69			2.82			5.32
10	4.48			2.67	4.79	5.67	4.42
	M4140-13						
12*	3.83	4.33		2.72	4.33		4.12
1	4.00	5.07			5.75		5.06
3	5.50	6.32			7.00		5.62
5	5.18	6.15			5.76		5.82
7	5.17	6.00			5.16		5.00

#* Pre-exposure bleeding, inoculated with virus

* Challenge with virus

were obtained immediately prior to inoculation and then at the one and two week interval since this bird died because of internal hemorrhage at the second week interval.

The results obtained in this study are shown in Tables 46, 47, 48, and Figures XXVII, XVIII.

The relative percentage distribution of serum protein components of bird M346 -21 showed a great decrease in the albumin fraction one week after the normal tissue inoculation. Gamma-globulin increased throughout the experimental period up to and including the third week. Albumin values began to increase after the second week. Alpha 1-globulin increased during the first week but returned to an essentially normal value after this period. Alpha 2-globulin varied inconsistently, while beta-globulin values remained approximately constant. (Table 46, Figure XXVII)

The electrophoretic analyses of sera from bird M673V -22 showed a rise in the albumin fraction and a general decrease in the gamma-globulin throughout the experimental period. Total alpha-globulins and beta-globulin did not change significantly. (Table 47, Figure XXVIII)

Sera from bird M645H -23 showed little change in the relative percentage distribution of serum protein components during the experimental period. (Table 48)

The results obtained in this study varied with the individual birds. There was little change in the sera of bird M645H -23 during the experiment. The analyses of sera from bird M346B2 -21 showed a sharp decrease in the albumin/globulin

Table 46

Bird M346 -21

Group IVa-Normal Tissue Suspension

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	39.4	9.8	7.1	10.4	33.3	0.65	4.91
1	29.6	13.0	5.1	12.5	39.8	0.42	4.56
2	30.1	8.2	7.7	11.7	42.3	0.43	3.69
3	32.2	8.5	4.8	11.1	43.4	0.48	3.78

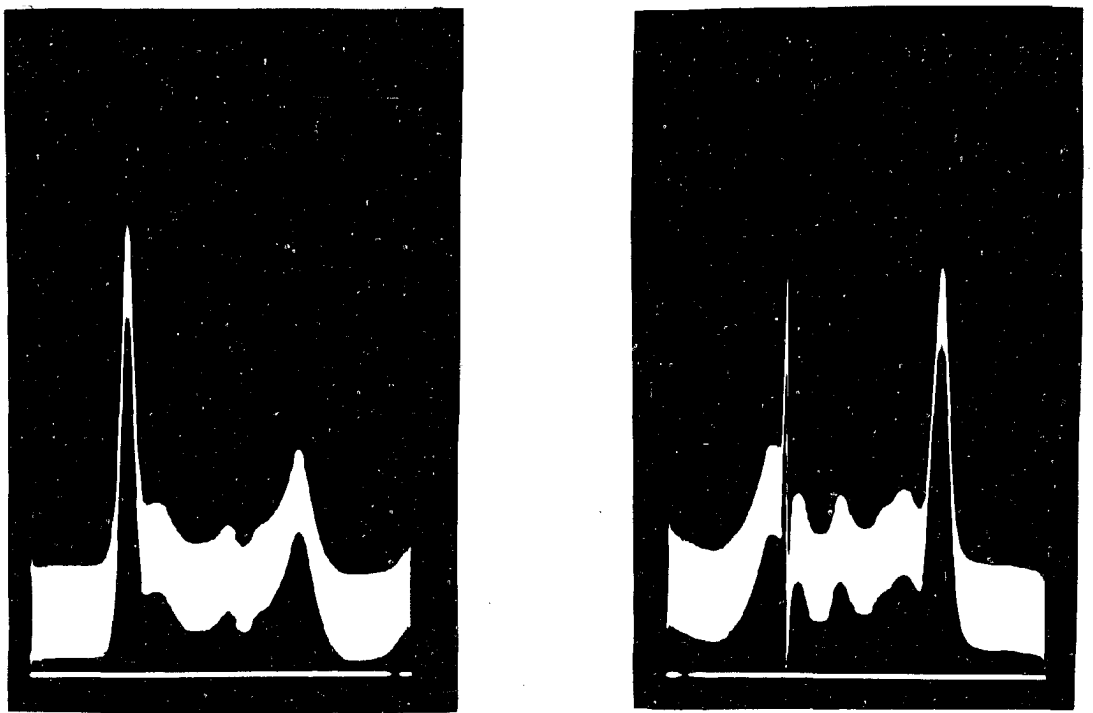
* Pre-inoculation bleeding, inoculated with normal tissue suspension

Figure XXVII

Bird M346B -21

Group IVa-Normal Tissue Suspension

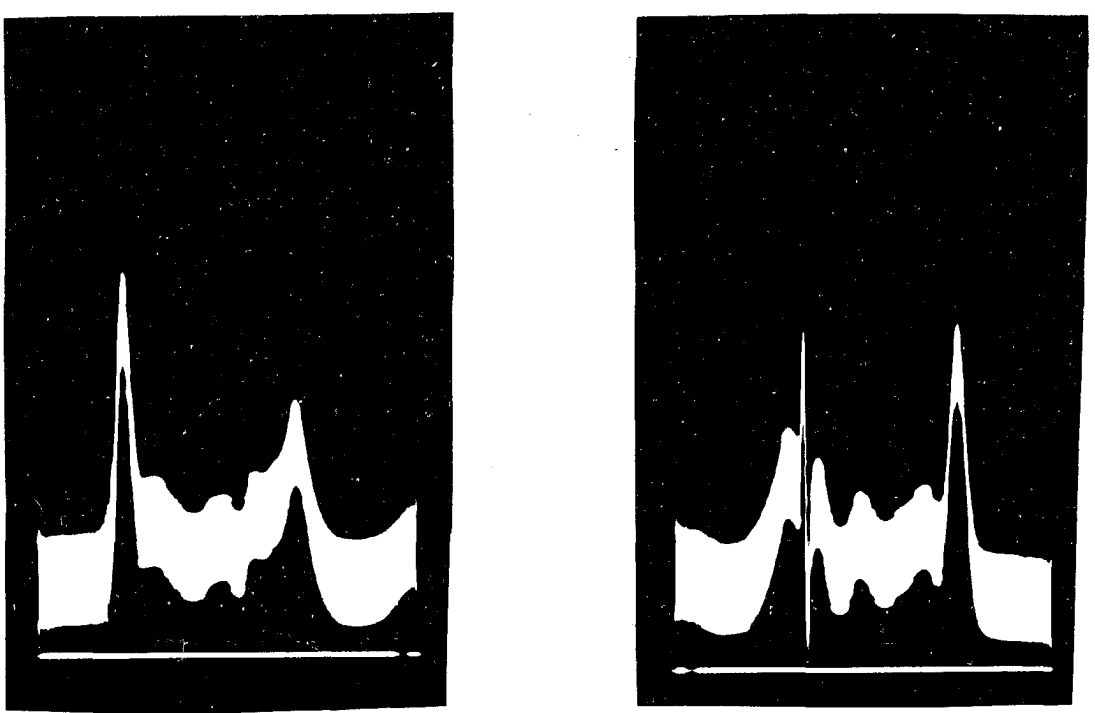
Electrophoretic Patterns



A D

0 WEEKS

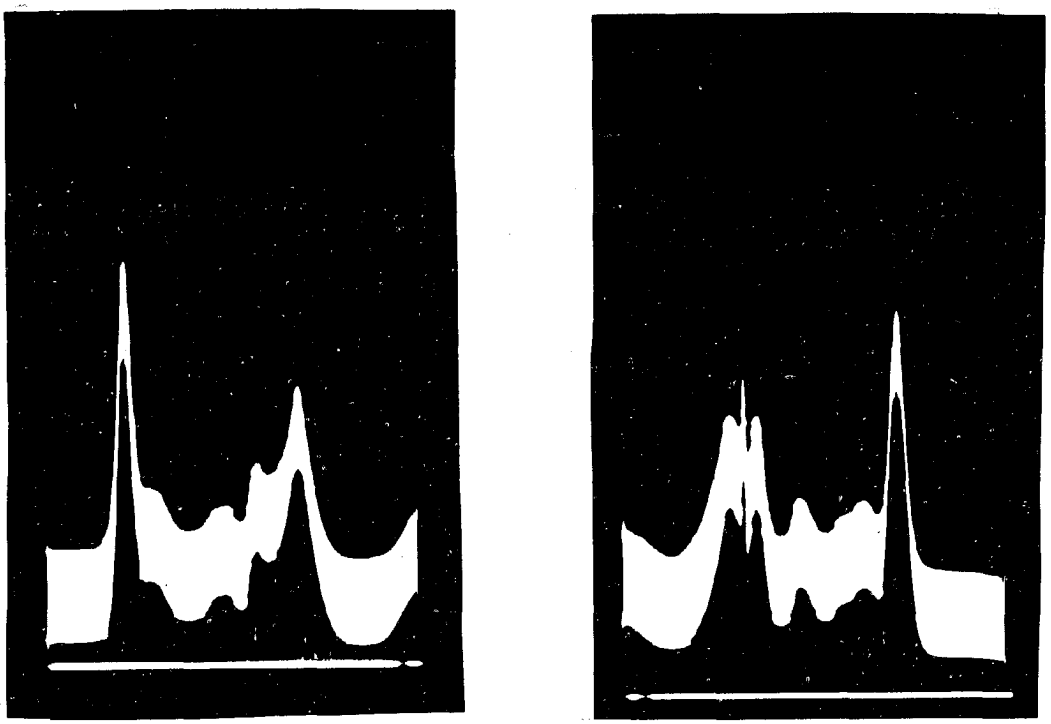
INOCULATED WITH NORMAL TISSUE SUSPENSION



A D

1 WEEK

Figure XXVII (cont.)



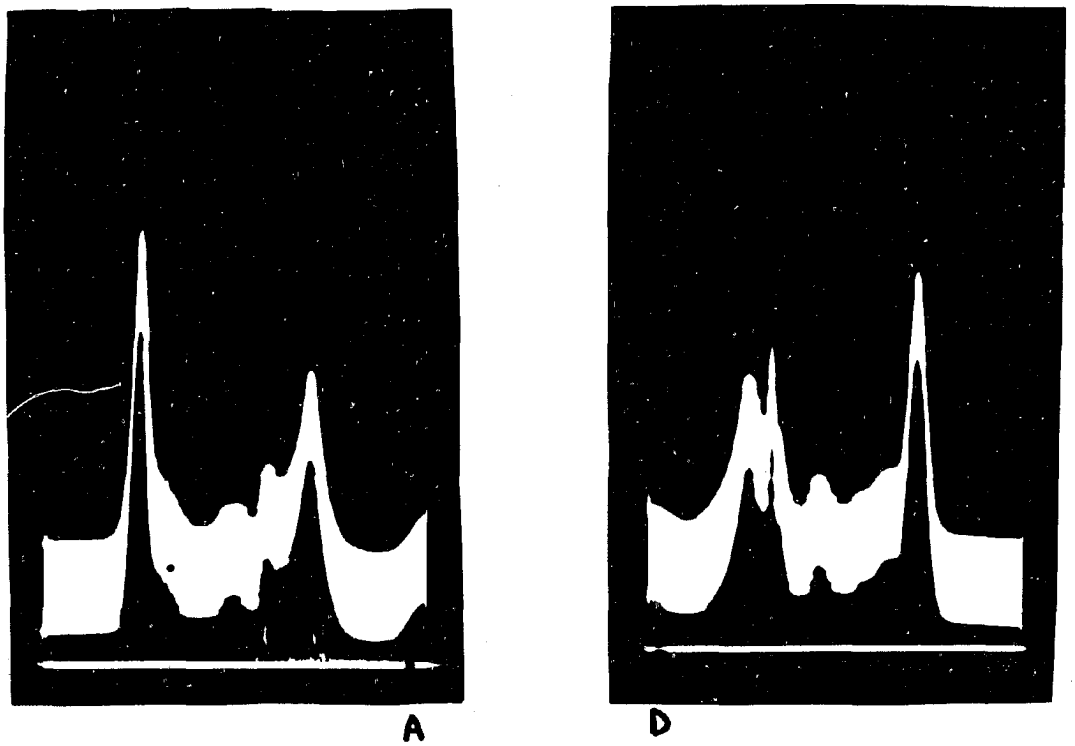


Table 47

Bird M673V -22

Group IVa-Normal Tissue Suspension

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	36.8	12.1	7.4	9.0	34.7	0.58	4.21
1	37.4	11.1	6.5	8.6	36.4	0.60	3.86
2	41.0	17.1		9.3	32.6	0.70	3.86
3	40.8	19.4		8.0	31.8	0.69	4.00

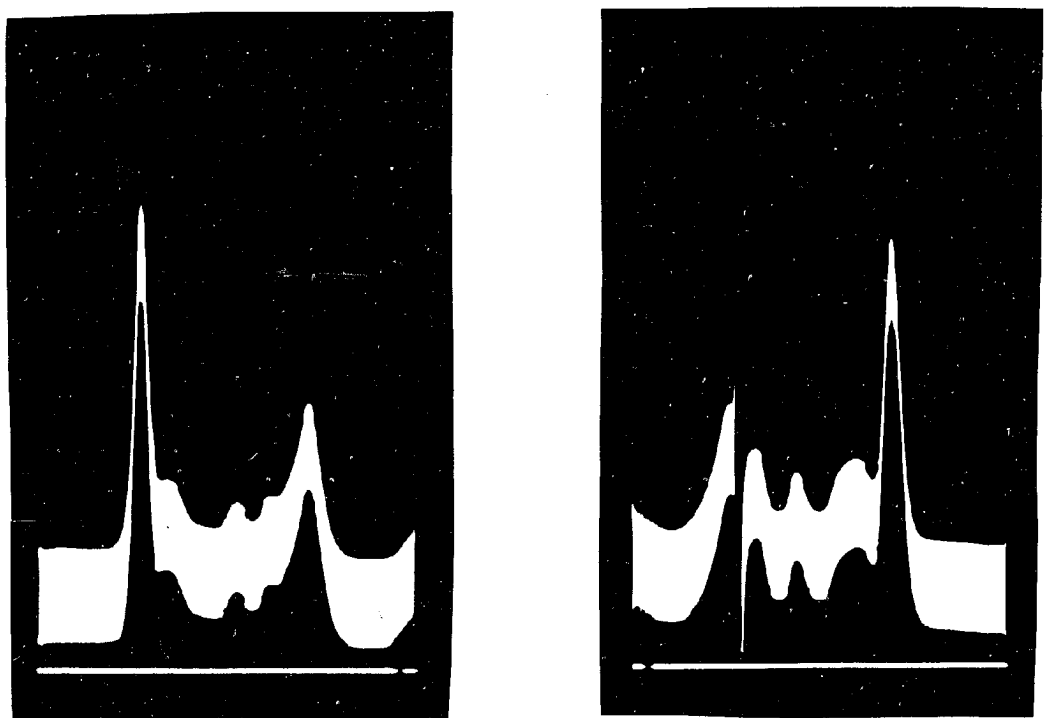
* Pre-inoculation bleeding, inoculated with normal tissue suspension

Figure XXVIII

Bird M673V -22

Group IVa-Normal Tissue Suspension

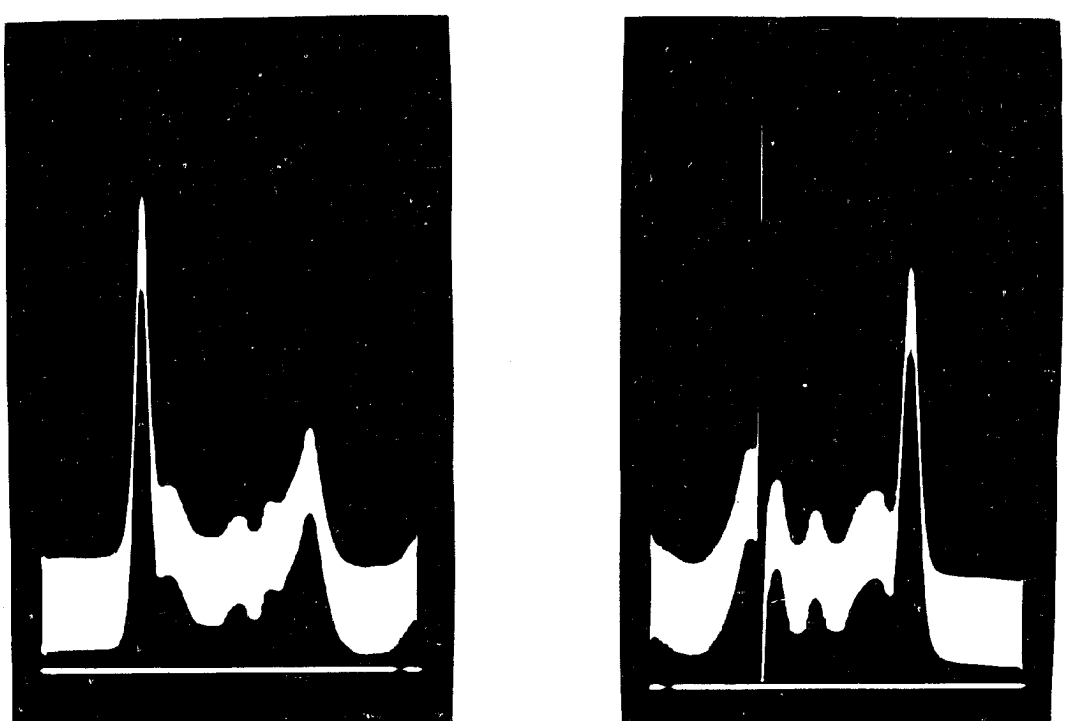
Electrophoretic Patterns



A D

0 WEEKS

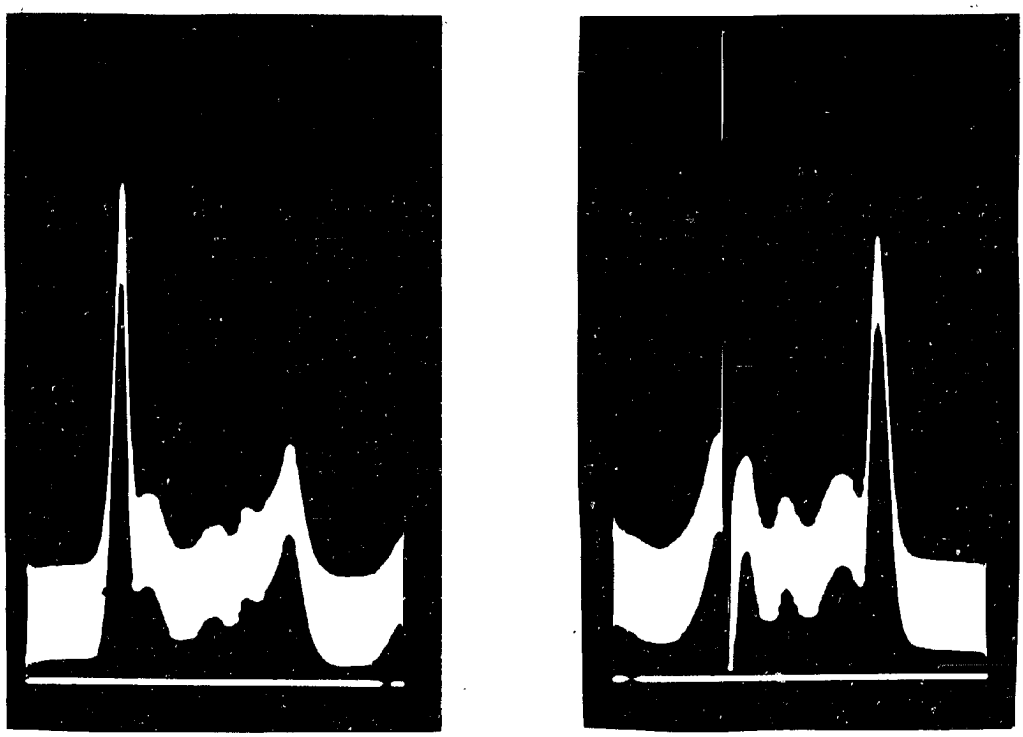
INOCULATED WITH NORMAL TISSUE SUSPENSION



A D

1 WEEK

Figure XXVIII (cont.)



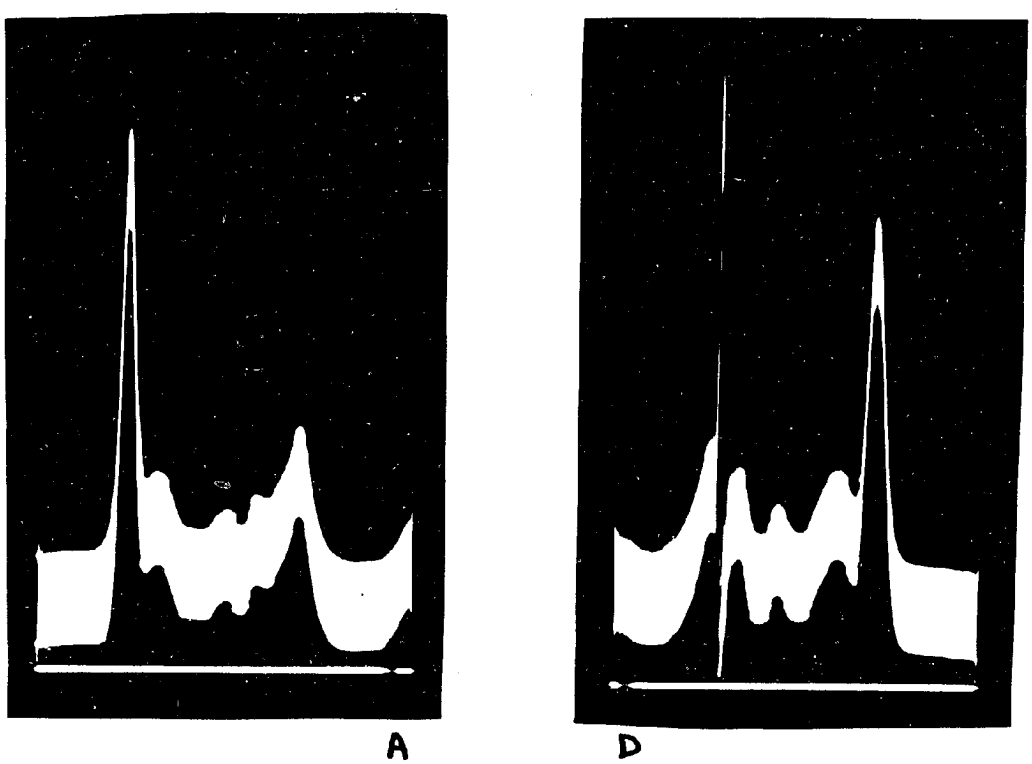


Table 48

Bird M645H -23

Group IVa-Normal Tissue Suspension

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component				A/G	Per Cent Serum Protein
		Globulins				
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-	
0*	44.0	18.6	8.5	29.2	0.79	4.13
1	42.3	17.2	8.9	31.6	0.73	3.78
2	43.6	18.8	8.5	29.1	0.77	3.78

* Pre-inoculation bleeding, inoculated with normal tissue suspension

ratio one week after inoculation such as was observed in sera of bird M347B2 -6 in Group I which was also inoculated with a normal tissue suspension. The same trend followed in the sera of bird M400D2 -4 in Group I which was inoculated in the same manner.

Sera from bird M673V -22 showed a steady increase in the albumin/globulin ratio after a normal tissue inoculation. Upon examination at necropsy a non-specific enteritis was observed which was not of demonstrable bacterial or parasitic origin.

Electrophoretic analyses of pre-inoculation sera from birds of this group closely approximate the results obtained by Sanders, et al (144) although gamma-globulin levels were elevated. In one case (M673V -22) the albumin/globulin ratio was low.

There was no significance in the change of per cent serum protein during the study.

2. Lethal Dose$_{50}$ Neutralization Indices

There was no significant response in LD_{50} NIs after inoculation with a normal tissue suspension. All LD_{50} NIs were normal (33, 57). (Tables 49, 50, 51, 52, Figures XXIX, XXX, XXXI)

Birds M346B -21 and M645H -23 were normal at necropsy.

Hemagglutination-inhibition titers for Newcastle disease were negative at all times.

Table 49

Bird M346B -21

Group IVa-Normal Tissue Suspension

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Serum Titer#	NI
0*	5.84	5	5	5	2	1	5.00	0.84
1	6.32	5	5	4	2	1	4.84	1.48
2	6.50	5	5	5	5	0	5.50	1.00
3	6.38	5	5	4	3	2	5.33	1.05

* Pre-inoculation bleeding, inoculated with normal tissue suspension

FIGURE XXIX

GROUP IV a- NORMAL TISSUE SUSPENSION

BIRD M346 B-21

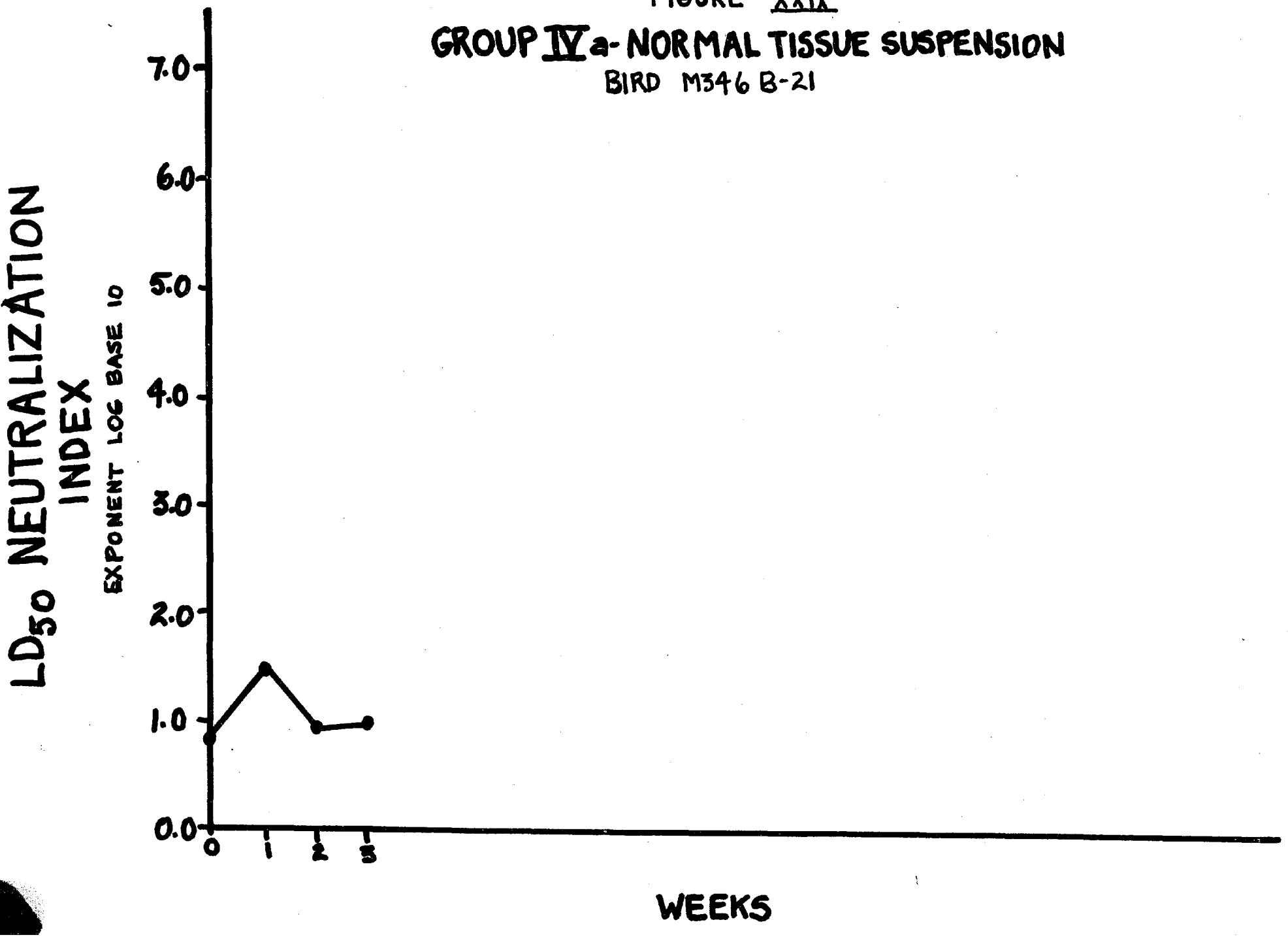


Table 50

Bird M673V -22

Group IVa-Normal Tissue Suspension

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Serum Titer#	NI
0*	5.84	5	5	4	2	0	4.83	1.01
1	6.32	5	5	5	1	1	4.75	1.57
2	6.50	5	5	4	3	2	5.33	1.17
3	6.38	5	5	4	4	2	5.54	0.84

* Pre-inoculation bleeding, inoculated with normal tissue suspension

FIGURE XXX

GROUP IVa - NORMAL TISSUE SUSPENSION

BIRD M673V-22

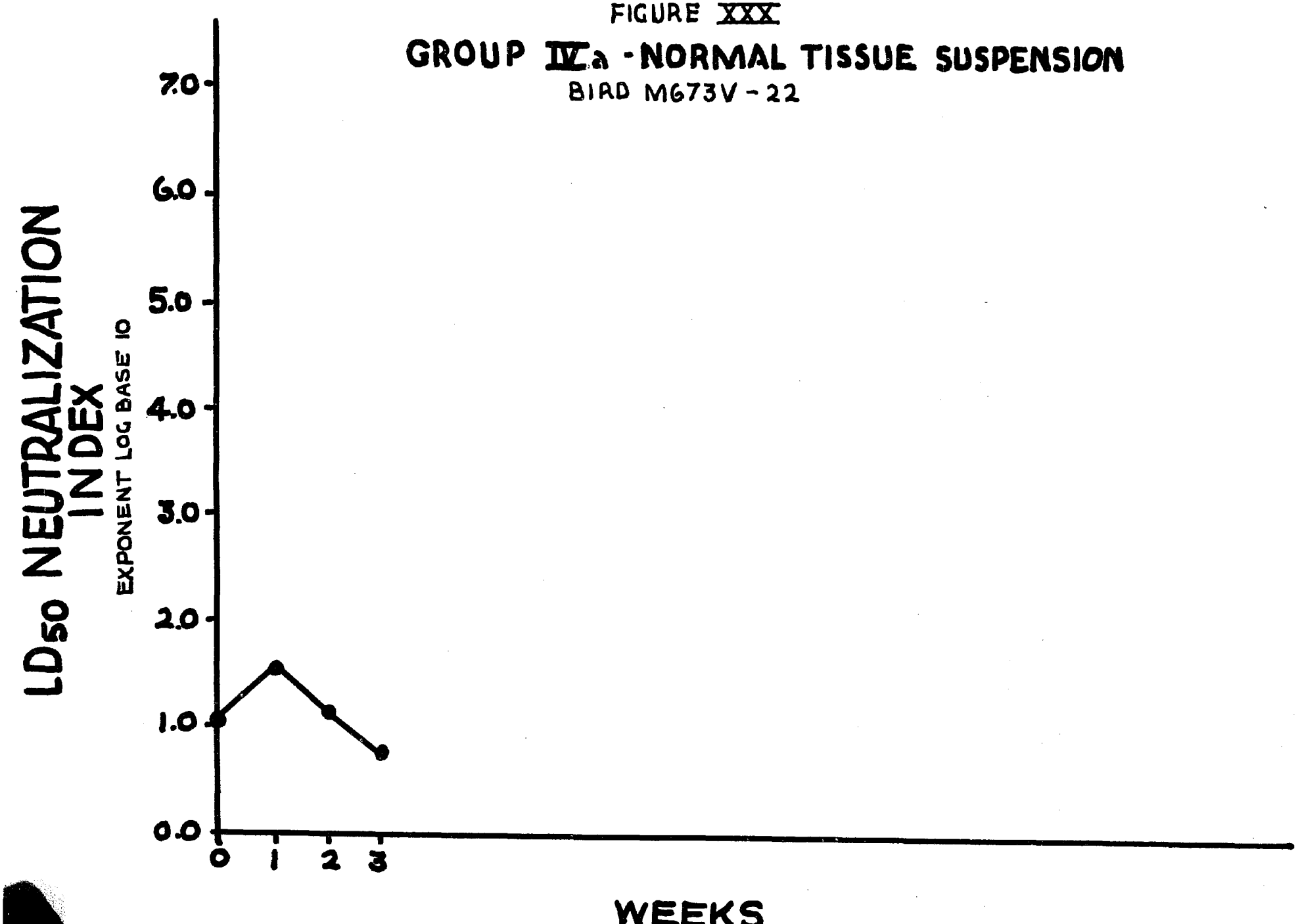


Table 51

Bird M645H -23

Group IVa-Normal Tissue Suspension

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{0} ##	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Serum Titer#	NI
0*	5.84	5	5	5	5	5	2	1	5.00	0.84
1	6.32			5	5	5	2	0	4.83	1.49
2	6.50			5	5	5	4	0	5.38	1.12

* Pre-inoculation bleeding, inoculated with normal tissue suspension

FIGURE XXXI

GROUP IVa - NORMAL TISSUE SUSPENSION

BIRD M645H-23

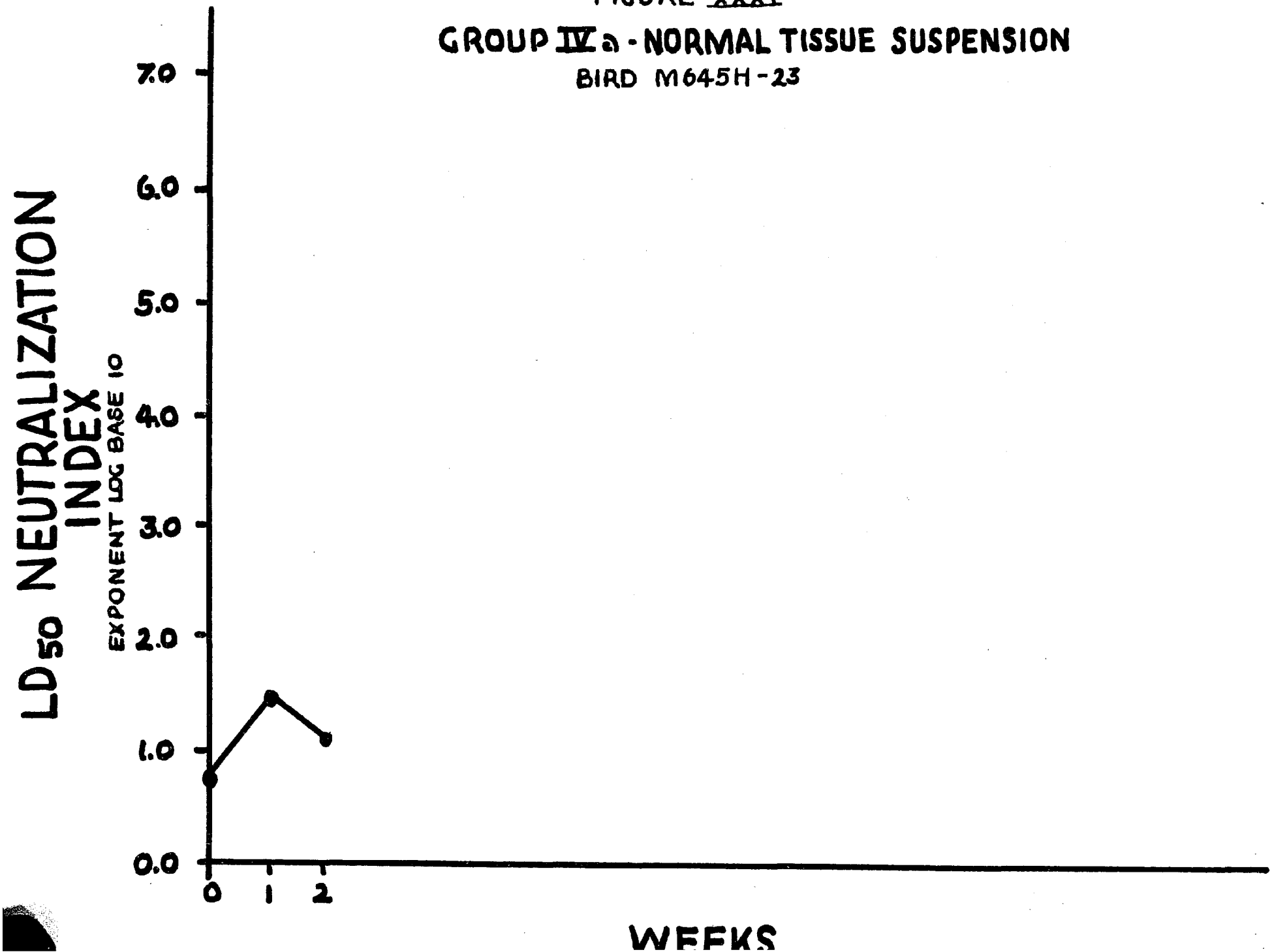


Table 52

Summation of LD_{50} NIs

Group IVa-Normal Tissue Suspension

Period Weeks	LD_{50} NI		
	M346B -21	M673V -22	M645H -23
0*	0.84	1.01	0.84
1	1.48	1.57	1.49
2	1.00	1.17	1.12
3	1.05	0.84	

* Pre-inoculation bleeding, inoculated with normal tissue suspension

E. Group IVb

1. Electrophoretic Analyses

In order to determine if an injury such as a tracheal scarification with a cotton-tipped applicator stick could alter the serum electrophoretic patterns or change the LD_{50} NIs significantly, birds M542A -24, M333H -25, and M673U -26 were injured in this manner. The birds were bled immediately prior to injury and then at the one, two, and three week interval. The results obtained on the relative percentage distribution of the serum proteins are shown in Tables 53, 54, 55, and Figure XXXII.

The serum sample obtained from bird M542A -24 immediately prior to tracheal injury showed a very low albumin/globulin ratio which increased markedly until the end of the experiment. Although low albumin values and high gamma-globulin values were found, this bird was normal at gross necropsy examination. (Table 53, Figure XXXII)

The electrophoretic analyses of sera from bird M333H -25 showed no significant changes although a trend of increasing albumin/globulin ratics was evident. (Table 54)

Sera from bird M673U -26 showed a slight decrease in the relative percentage distribution of albumin during the first week and a slight increase in the gamma-globulin fraction during the first and second weeks after tracheal injury. The other globulins did not change significantly during the experimental period, although during the second week total alpha-globulins decreased. (Table 55)

Table 53

Bird M542A -24

Group IVb-Tracheal Injury

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	20.5	11.9	7.5	14.4	45.7	0.26	4.91
1	21.8	8.5	7.5	15.2	47.0	0.28	4.30
2	25.0	12.5	5.2	13.6	43.7	0.33	4.39
3	30.1	11.2	6.7	11.2	40.8	0.43	4.21

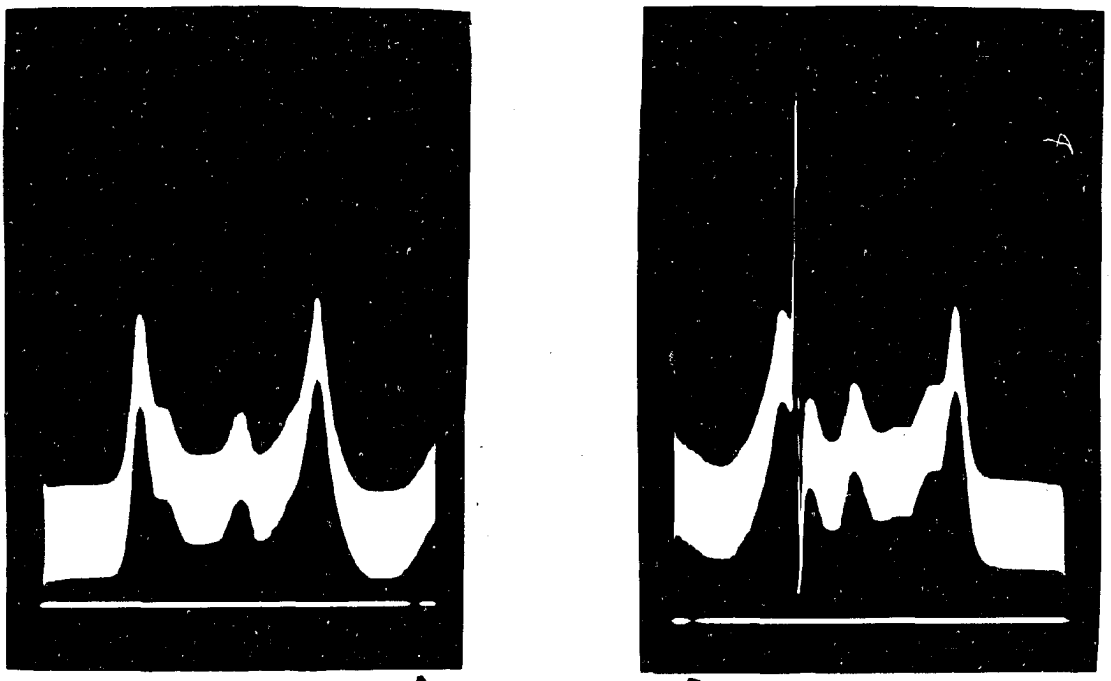
* Pre-injury bleeding, subjected to tracheal injury

Figure XXXII

Bird M542A-24

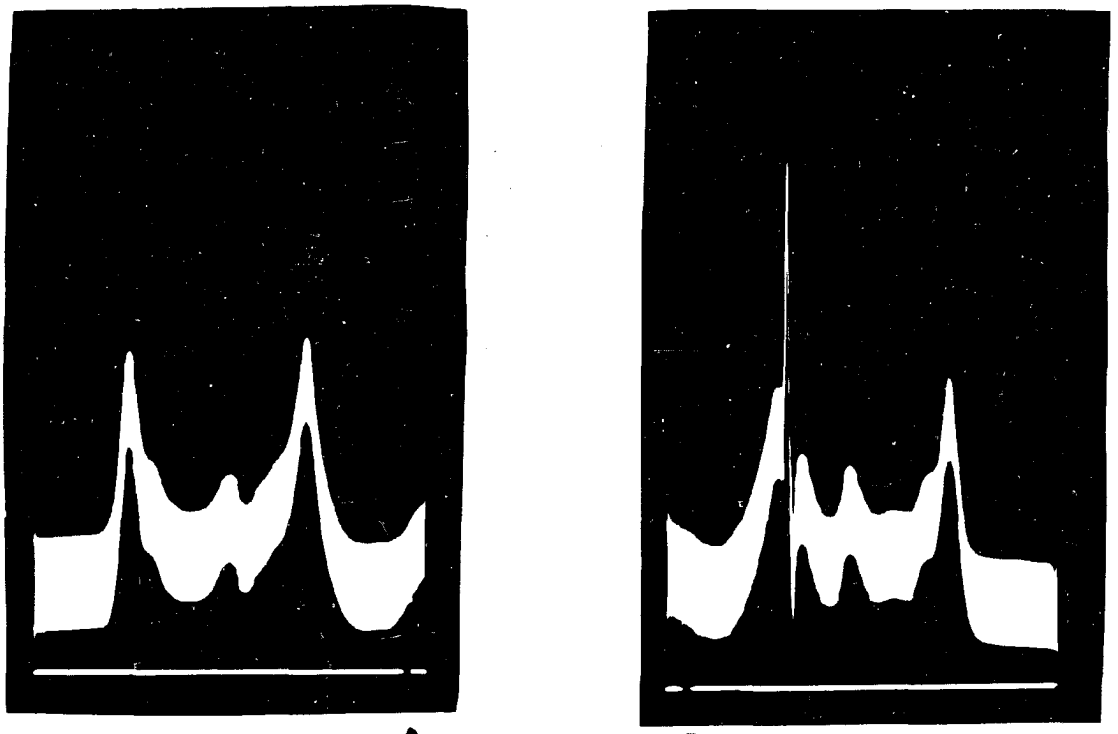
Group IVb-Tracheal Injury

Electrophoretic Patterns



A D

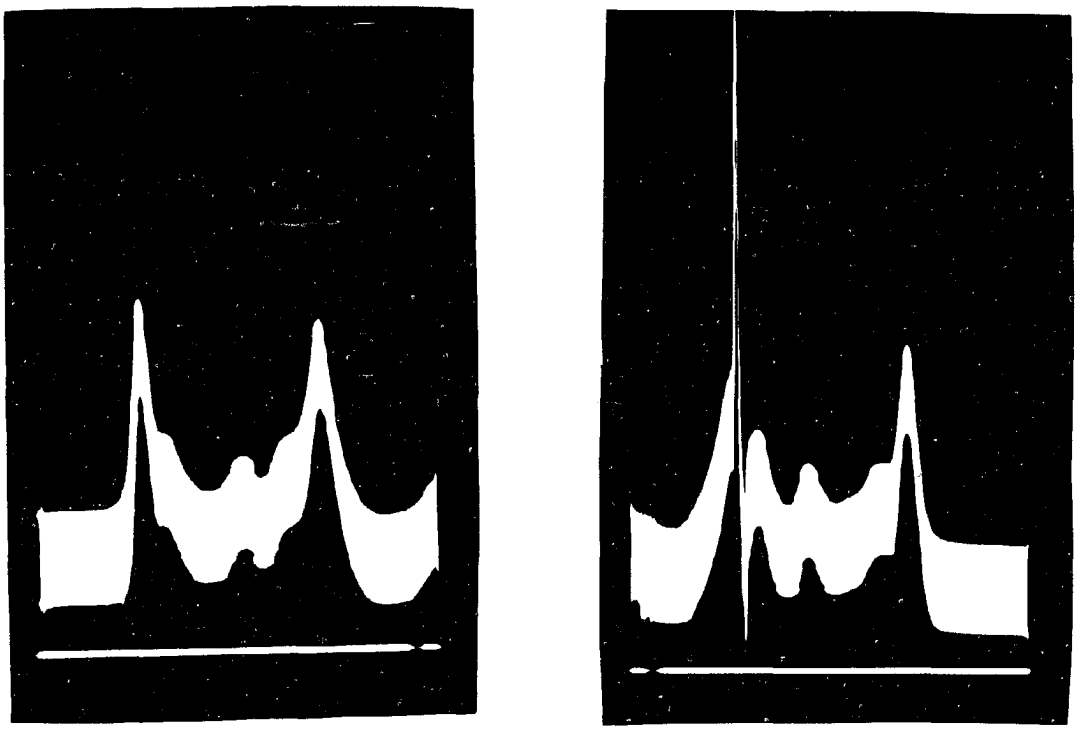
0 WEEKS
SUBJECTED TO TRACHEAL INJURY



A D

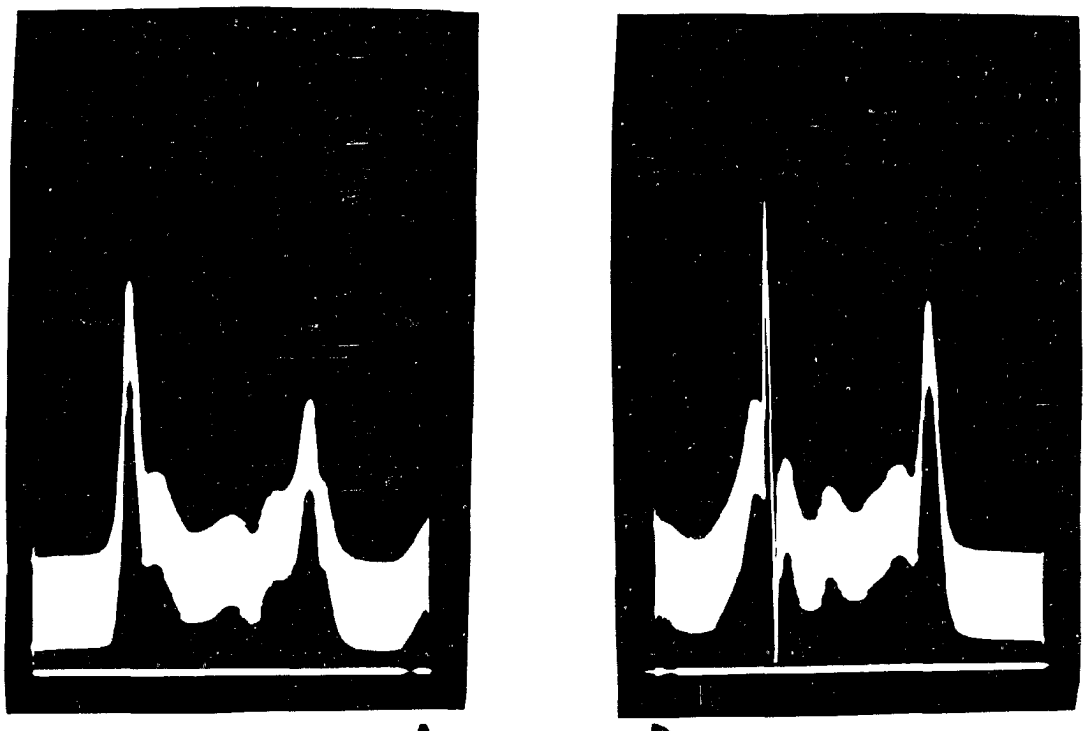
1 WEEK

Figure XXXII (cont.)



A D

2 WEEKS



A D

3 WEEKS

Table 54

Bird M333H -25

Group IVb-Tracheal Injury

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	40.4	10.8	6.9	9.9	32.0	0.68	4.04
1	38.0	21.1		9.2	31.7	0.61	4.65
2	41.4	17.1		9.6	31.9	0.71	3.60
3	41.9	20.0		8.1	30.0	0.72	3.78

* Pre-injury bleeding, subjected to tracheal injury

Table 55

Bird M673U -26

Group IVb-Tracheal Injury

Electrophoretic Analyses

Period Weeks	Relative Per Cent Serum Component					A/G	Per Cent Serum Protein
		Globulins					
	Albumin	Alpha 1-	Alpha 2-	Beta-	Gamma-		
0*	40.4	10.1	7.5	9.6	32.4	0.68	4.74
1	37.2	19.1		9.3	34.4	0.59	3.95
2	40.9	6.8	7.7	10.5	34.1	0.69	3.69
3	39.3	17.9		9.4	33.4	0.65	3.69

* Pre-injury bleeding, subjected to tracheal injury

The values obtained in the electrophoretic analyses of pre-injury serum samples from birds of this group were found to approximate the values obtained by Sanders, et al (144) in birds M333H -25 and M673U -26 but not in bird M542A -24 as stated above. Gamma-globulin levels were also higher than in Sanders' study.

There were no significant findings in the changes of per cent serum protein.

2. Lethal Dose$_{50}$ Neutralization Indices

There were no changes in LD_{50} NIs which could be attributed to the effects of tracheal injury. All LD_{50} NIs were normal (33, 57). (Tables 56, 57, 58, 59, Figures XXXIII, XXXIV, XXXV)

Birds M333H -25 and M673U -26 were found to be normal at necropsy.

Hemagglutination-inhibition titers for Newcastle disease were negative at all times.

Table 56

Bird M542A -24

Group IVb-Tracheal Injury

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Serum Titer#	NI
0*	5.83	5	4**	5	3**	0**	5.33	0.50
1	6.32	5	5	5	1	0	4.63	1.69
2	6.50	5	4	4	2	0	4.54	1.96
3	6.38	5	5	5	4	0	5.38	1.00

* Pre-injury bleeding, subjected to tracheal injury

** One embryo out of five inoculated died due to trauma

FIGURE XXXIII

GROUP IV b - TRACHEAL INJURY

BIRD M542A-24

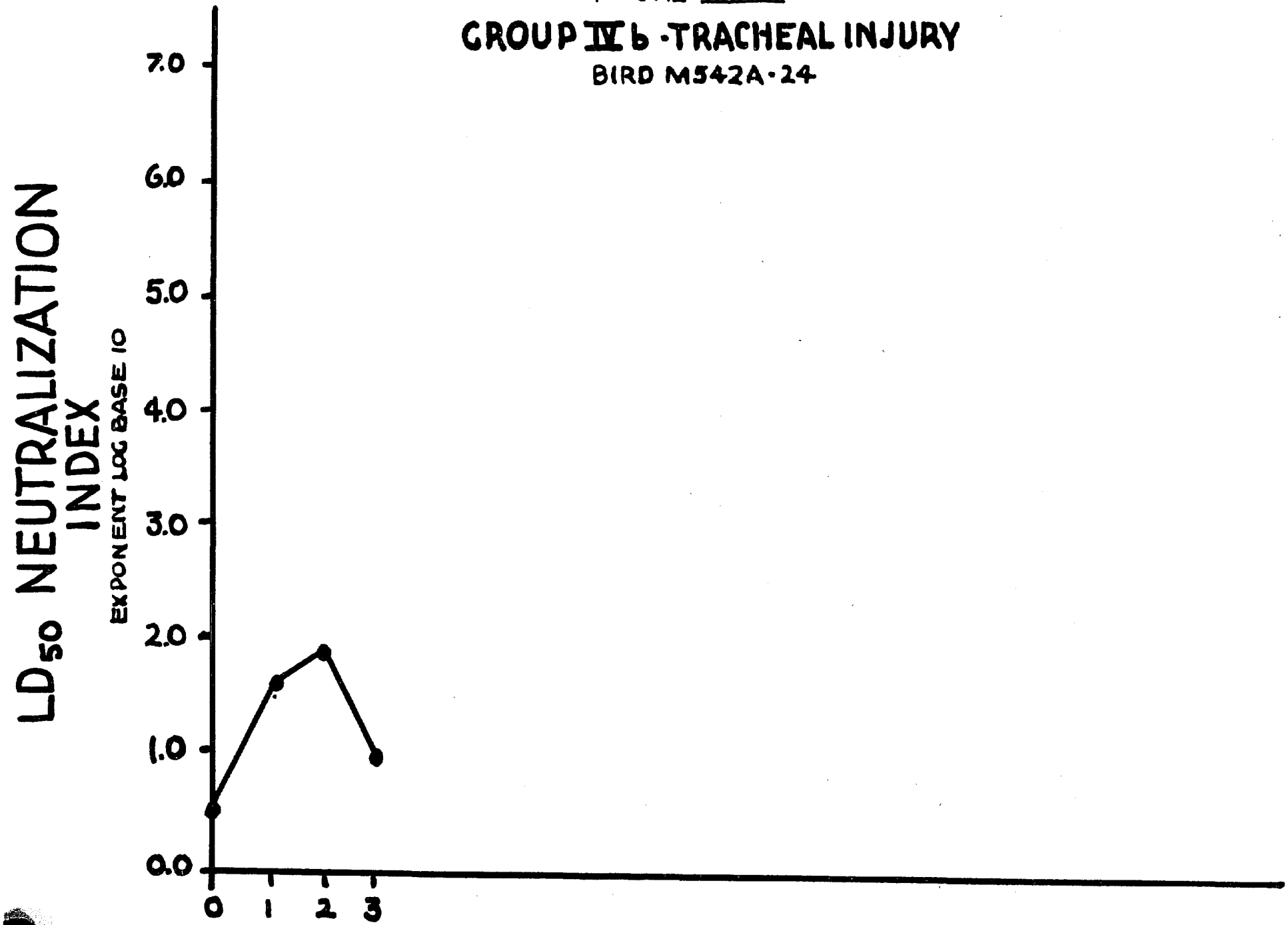


Table 57

Bird M333H -25

Group IVb-Tracheal Injury

Serum Neutralization Tests

Period Weeks	Virus# Titer	Virus Dilutions 10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-5}	Serum Titer#	NI
0*	5.84	5	5	4	0	0	4.38	1.46
1	6.32	5	5	5	3	2	5.50	0.82
2	6.50	5	5	5	3	2	5.50	1.00
3	6.38	5	5	4	2	1	4.84	1.54

* Pre-injury bleeding, subjected to tracheal injury

FIGURE XXXIV

GROUP IVb - TRACHEAL INJURY

BIRD M333H-25

Table 58

Bird M673U -26

Group IVb-Tracheal Injury

Serum Neutralization Tests

Period	Virus#	Virus Dilutions					Serum	
Weeks	Titer	10^{-2} ##	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Titer#	NI
0*	5.83	5	5	5	5	1	5.63	0.20
1	6.32	5	5	5	5	2	5.83	0.49
2	6.50		5	5	3	1	5.32	1.18
3	6.38	5	5	5	5	0	5.50	0.88

* Pre-injury bleeding, subjected to tracheal injury

FIGURE XXXV

GROUP IVb - TRACHEAL INJURY

BIRD M673V-26

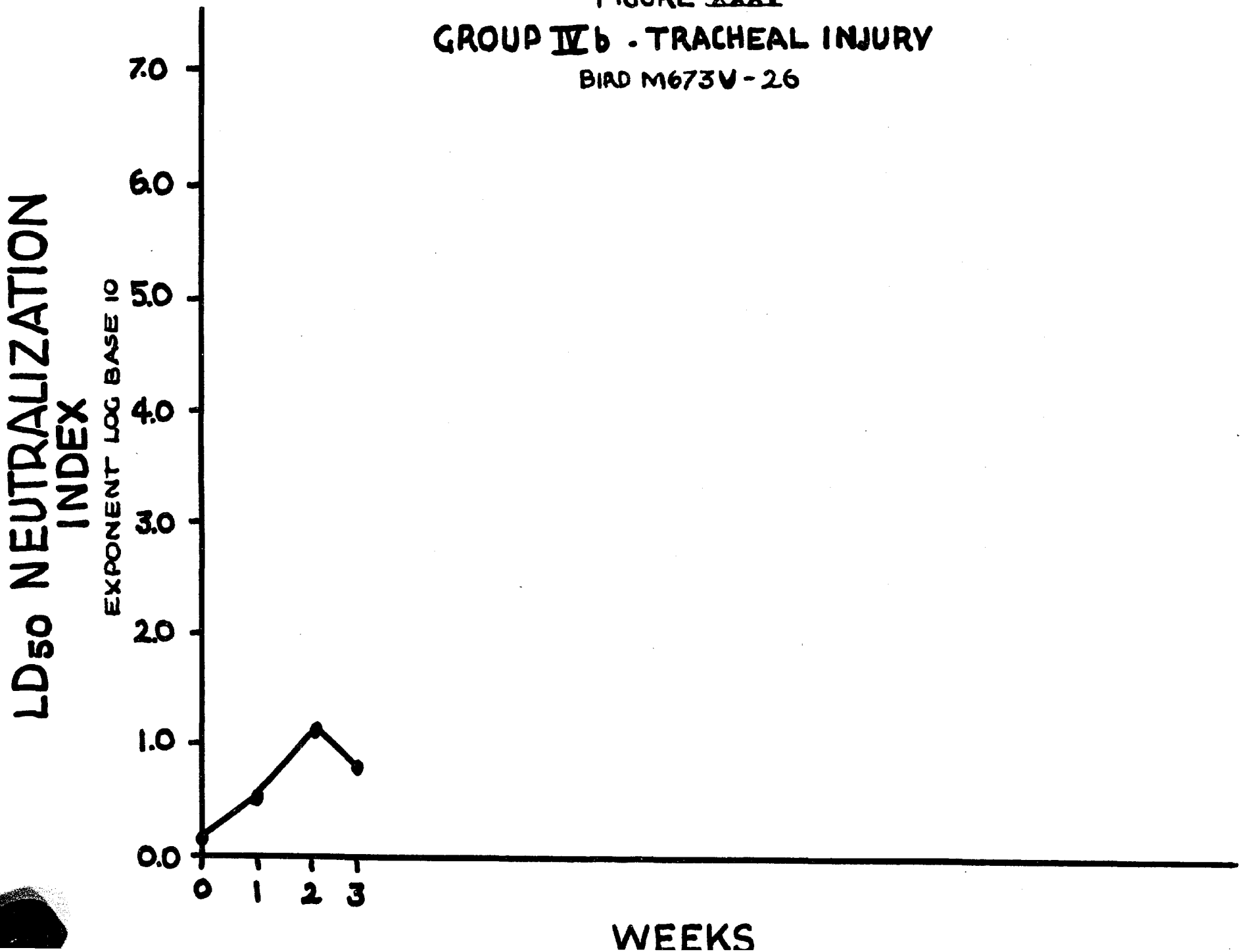


Table 59

Summation of LD_{50} NIs

Group IVb-Tracheal Injury

Period Weeks	LD_{50} NI		
	M542A -24	M333H -25	M673U -26
0*	0.50	1.46	0.20
1	1.69	0.82	0.49
2	1.96	1.00	1.18
3	1.00	1.54	0.88

* Pre-injury bleeding, subjected to tracheal injury

V

DISCUSSION

The effect of frequent bleedings and the subsequent serum protein depletion is evident in the electrophoretic patterns obtained in Group I. Bird M727S -1, which was bled at monthly intervals, did not show the decrease in the albumin/globulin ratio as did the other birds which were bled at weekly intervals. The serum electrophoretic patterns of bird M727S -1 did not change to any significant extent during the experimental period. Serum protein depletion produced by either feeding low protein diets or by plasmapheresis has been shown to change the serum electrophoretic patterns by decreasing the albumin/globulin ratio (12, 21, 23, 24, 26, 117, 174). Many investigators have observed that antibody fabricating mechanisms are impaired (11,20, 22, 117, 159, 168, 169, 170, 171) while others (4, 12) have demonstrated that there is no difference in antibody fabricating mechanisms of normal and hypoproteinemic subjects. One must consider that in the present study one-third of the total blood volume was removed from each bird at each bleeding period.

Birds M541B2 -2, M400D2 -4, M362V -5, and M347B2 -6, which were bled at weekly intervals, showed an increase in the albumin/globulin ratio during the first or second weeks of the experiment. A decrease in the ratio occurred after this period which persisted to the end of the experiment. The

increase seems to be the result of a stimulation in the production of albumin early in the study. The stimulation of albumin may possibly be due to a mechanism activated in the birds to compensate for the mechanical loss of albumin during the first bleeding period. This compensatory mechanism may be necessary for maintenance of a constant serum osmotic pressure which is dependent upon albumin (30). After the albumin is decreased following the second week interval the globulins increase. It is possible that the increase in globulins is necessary for maintenance of the normal physiological osmotic pressure after the decrease in albumin. Since osmotic pressure depends upon the number of particles and not upon their size it may be possible that the globulin molecules must increase to a quantity sufficient to compensate for the loss in albumin molecules. The globulin molecules are larger in size than albumin molecules and therefore, a greater number of globulin molecules may be needed to make up for the decrease in the osmotic pressure.

Bird M400D2 -4 was bled and tested as an individual sample at the eighth and seventeenth weeks. It was also bled at the twelfth week but the sample was used as part of a pooled serum sample. There was a marked decrease in the albumin/globulin ratio during these periods. At necropsy this bird showed a non-specific enteritis. This condition may, in part, be responsible for the decrease in the albumin/globulin ratio in that a modification of the normal physiology of the bird occurred which was reflected in a change of the relative percentage distribution of the serum protein components.

Bird M400D2 -4 was inoculated with a normal lung and tracheal suspension at the seventeenth week and bled weekly. The albumin/globulin ratio continued to decrease. This may have possibly been due to a combination of the effects of the weekly bleedings resulting in serum protein depletion and a low albumin level and as a result of the non-specific enteritis.

Bird M347B2 -6 also showed an increase in the albumin/globulin ratio prior to inoculation with a normal tissue suspension at the seventeenth week. This bird was bled five weeks previously. It also showed a sharp decrease in the albumin/globulin ratio one week after inoculation with normal tissue but an increase after this period. The decrease in albumin/globulin ratio was possibly due to the effects of the weekly bleedings after inoculation. Further investigations into the possibility of changes being produced in serum electrophoretic patterns as a result of normal tissue inoculations are needed.

Antibodies against infectious bronchitis virus were not produced following inoculations of normal tissue. The ID_{50} NIs of sera from birds of this group were considered to be negative for infectious bronchitis (33, 57).

Birds in Group II showed the same general type of change in their electrophoretic patterns. There was a sharp decrease in the albumin/globulin ratio early in the experiment and an increase during the latter portion of the experiment. These changes in the albumin/globulin ratios were

more marked in the sera of these birds than in sera of birds in Group I. It seems probable that the changes in serum electrophoretic patterns of birds in Group II were due to a combination of the effects of the frequent weekly bleedings and to the action of the virus. The decrease in the albumin/globulin ratio was most marked one and two weeks after inoculation when the LD_{50} NIs were low. This fact is contrary to other studies (15, 80, 172) which demonstrated that increases in globulins were associated with increases in antibody titers.

A compensatory stimulation in the production of albumin was not observed in the serum electrophoretic patterns of birds in Group II.

Birds M349T -10 and M538H -11 showed visceral and neural lymphomatosis, respectively, at necropsy. The L component, as described by Sanders, et al (144) was not observed in the electrophoretic patterns of these birds. There is a possibility that the L component may be an artifact as observed in the patterns of the ascending limb since it seems to be comparable with the gamma-anomaly of the pattern of the descending limb. The L component has been observed in the ascending patterns of sera from birds that did not show manifestations of lymphomatosis.

Karzon and Bang (89) have hypothesized that Newcastle disease virus antibodies in chickens are not demonstrable prior to the sixth day after inoculation because antibodies formed may have been bound to the antigen still present.

Antibodies detected after the sixth day may have been indicative of an excess of antibodies. The same may be true of infectious bronchitis virus antibodies even though they cannot be detected as early after inoculation as antibodies against Newcastle disease virus.

The serum LD_{50} NIs of birds in Group II began to increase slowly during the first and second weeks after inoculation with the virus. After this period a marked increase was observed. The maximum LD_{50} NIs were reached between the sixth and eighth weeks and decreased after this period. The results compare favorably with those obtained by Page (130).

It is interesting to note that sera possessing high LD_{50} NIs show normal electrophoretic patterns.

The changes in the LD_{50} NIs of the pooled serum samples varied approximately 0.4 to 1.0 log unit from the average of the LD_{50} NIs of the individual serum samples comprising the pooled samples. Additional work on this problem is needed to explain this phenomenon.

Bjørnboe (13) has shown that upon immunization of animals with various bacteria there is a definite change in the serum electrophoretic pattern, represented by an increase in gamma-globulin and sometimes an increase and a marked change in the alpha- and beta-globulins. Albumin is usually decreased. Many investigators have shown a direct correlation between the rise in gamma-globulin and increase in the antibody titer (1, 13, 15, 43, 45, 83, 163), while others have not shown any correlation (80, 97, 117, 172). It seems possible

that viruses do not behave in the same manner as bacteria do in the formation of antibodies. Animals immunized with Western and Venezuelan equine encephalomyelitis (95, 128), Japanese B encephalitis (95), and influenza viruses (172) have shown no definite changes in serum electrophoretic patterns. This phenomenon has also been observed in this investigation. Sera from the birds with high LD_{50} NIs have shown normal electrophoretic patterns. A chemical fractionation of sera from birds exposed to infectious bronchitis virus would possibly be of aid in determining the neutralizing antibody content of each fraction. It is possible that in virus diseases there may be no change in the relative percentage of serum components but there may be a modification of normal globulin which functions as antibody without reflection in the total globulin.

The serum electrophoretic patterns obtained in this study did not seem to be specific for infectious bronchitis since normal birds that were bled at weekly intervals also showed the same general type of pattern.

Many investigators (11, 12, 22, 159, 169, 170, 171) have shown that serum protein depletion produced as a result of feeding low protein diets or of plasmapheresis inhibit the production of bacterial antibodies. Apparently the antibody response was not inhibited in the birds used in the present study although serum protein depletion occurred due to the frequent bleedings. It is possible that a threshold was reached where antibody fabrication was not inhibited but serum electrophoretic patterns could be altered.

A study of the change in antibody response and change in serum electrophoretic patterns of birds bled at monthly intervals is needed since it is evident that protein depletion does not occur in birds that are bled at monthly intervals.

Birds in Group III were challenged at the twelfth week. Very little change occurred in the serum electrophoretic patterns although the sera possessed high LD_{50} NIs.

Antibodies have been shown to be produced in greater quantities upon secondary inoculations (103). This was confirmed with the birds of Gruop III which were challenged. There was a marked increase in the LD_{50} NIs following inoculation with the challenge virus. The LD_{50} NIs reached maximum values between the third and fifth weeks after challenge and were above those values obtained as a result of the primary inoculation.

A further explanation for the fact that serum electrophoretic patterns are normal when LD_{50} NIs are at their peak may be that the electrophoretic technique is not capable of detecting neutralizing antibodies to infectious bronchitis virus which may be present in extremely minute quantities. The Model 38, Perkin-Elmer Tiselius Electrophoresis Apparatus used in this study is not able to resolve protein concentrations much below 0.002 per cent in the presence of and relative to another protein in a concentration of approximately two per cent. However, it is possible to measure concentrations of 0.01 per cent protein in the presence of and relative to another protein in a concentration of approximately two per cent.

Therefore, it is hypothesized that the infectious bronchitis virus neutralizing antibodies may have been present in such low quantities as not to be detected by electrophoresis but could be determined by the serum neutralization test.

The fact that serum electrophoretic patterns appear normal when the LD_{50} NIs are high may point to the pathogenic processes concerning viruses in general. Viruses are intracellular parasites and viral antibodies may be produced differently from bacterial antibodies. Possibly during the production of neutralizing antibodies total globulins do not change or increase but there may be a modification of the existing normal globulins into antibody globulins. This may, in part, account for the fact that there is no increase in any of the serum components in hyperimmune serum. This hypothesis can be explored further by serum fractionation studies and the analysis of each fraction for antibody content.

Birds in Group IVa which were inoculated with a normal tissue suspension showed variable results. Bird M346B -21 showed a decrease in the albumin/globulin ratio one week after inoculation. The ratio slowly began to increase after this period. It seems possible that in this instance also, serum protein depletion is responsible for the decreases in the albumin/globulin ratios.

Bird M673V -22 did not show the same type of change as bird M346 -21. A steady increase in the albumin/globulin ratio occurred throughout the experimental period. This bird showed a non-specific enteritis at necropsy as did bird M400D2 -4 in Group I which was also inoculated with a normal tissue

suspension. An explanation for this difference is difficult to give at the present time. Further work is needed on the effects of combinations of serum protein depletion and normal tissue inoculations upon the serum electrophoretic patterns in chickens. There is a possibility that the different genetic lines are responsible for the different serum electrophoretic patterns obtained during the experiments. Another possibility may be that bird M673V -22 possessed a larger blood volume and did not show the decrease in albumin/globulin ratio up to the third week post-inoculation. It may be possible that the albumin/globulin ratio would have changed after this period but experimental data are not available due to the termination of the investigation at the third week after inoculation.

Bird M645H -23 showed approximately the same changes in serum electrophoretic patterns as did bird M673V -22. Possibly the same explanations may be used for bird M645H -23 as those given for bird M673V -22.

LD_{50} NIs did not increase in sera from birds of this group.

Birds in Group IVb, which were subjected to a tracheal injury using a cotton-tipped applicator stick also showed variable results.

Bird M542A -24 showed an extremely low albumin/globulin ratio at the first bleeding period. The ratio increased steadily after this period. Although this bird possessed quantities of serum protein components far below accepted

standards (144) it was normal at necropsy. A compensatory stimulation in the production of albumin seems responsible for the increase in the albumin/globulin ratio in this bird even though it was bled at weekly intervals. A continued increase in the ratio may have occurred as with birds in Group I. Possibly the original osmotic pressure present was reduced to a point where additional globulins could not be produced to increase the osmotic pressure. Therefore, more albumin was produced to compensate for this deficiency. Sera from birds M333H -25 and M673U -26 showed no significant changes in the relative percentage distribution of serum protein components. Serum protein depletion did not seem to be evident in these two birds. It may be possible that marked changes in the serum electrophoretic patterns could occur after the third week post-injury. This is not known since experimental data are not available due to termination of the experiment at this period. A stimulatory effect in the production of albumin was not observed.

VI

SUMMARY

1. Normal, adult Single Comb White Leghorn cockerels were exposed to a chicken-propagated strain of infectious bronchitis virus. Sera were collected at certain time intervals after exposure and were analyzed by electrophoresis and by the serum neutralization test. Results are expressed as relative percentages of serum components and as the Lethal $Dose_{50}$ Neutralization Indices (LD_{50} NIs).

2. Selected birds were challenged with virus at the twelfth week. Post-challenge sera were analyzed by electrophoresis and by the serum neutralization test.

3. The effects of normal tissue inoculations and tracheal injuries upon the serum electrophoretic patterns and LD_{50} NIs were also studied.

4. Sera from normal birds that were bled at intervals of four weeks or more did not show any significant changes in the relative percentage distribution of serum protein components. In some cases there was an increase in the albumin/globulin ratio.

5. Sera from normal birds that were bled at weekly intervals showed an increase in the albumin/globulin ratios early in the experiment. After this period the ratios decreased. These changes were possibly due to serum protein depletion caused by frequent bleeding. A possible explanation of this phenomenon is discussed.

6. Birds that were exposed to infectious bronchitis virus showed marked decreases in the albumin/globulin ratios during the first and second weeks. The ratios steadily increased after this period and had returned to normal or were higher than pre-exposure levels at the twelfth week. These values persisted for eight additional weeks.

7. The LD_{50} NIs increased slowly beginning at the first and second weeks after exposure. After this period a marked increase was observed. The maximum LD_{50} NIs were reached between the sixth and eighth weeks and decreased after this period.

8. No correlation was observed in the changes of serum electrophoretic patterns and increase in the LD_{50} NIs. When serum electrophoretic patterns had returned to normal the LD_{50} NIs were at their maximum values.

9. Birds that were challenged at the twelfth week after primary inoculation with virus did not show significant changes in their serum electrophoretic patterns but there was an increase in the LD_{50} NIs. A possible hypothesis for the results obtained in this study is given.

10. The changes in serum electrophoretic patterns were not specific for infectious bronchitis virus.

11. Sera obtained from birds that were inoculated with a normal tissue suspension and that were subjected to a tracheal injury showed varying results in the changes of albumin/globulin ratios. Factors possibly responsible for these changes are discussed. Results obtained in this study do not give substantial

evidence that these treatments alone were responsible for the changes observed as they were possibly associated with patterns of serum protein depletion or with patterns of albumin stimulation.

12. No increase in LD_{50} NIs was observed in the sera of birds inoculated with a normal tissue suspension or subjected to a tracheal injury.

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