AN ELECTROPHORETIC STUDY OF PULLORUM IMMUNE TURKEY SERUM

By

John Edward Lynch

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

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Department of Bacteriology

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AN ABSTRACT

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Abstract

Data are presented on the results of 126 electrophoretic analyses of normal and pullorum immune turkey serum.

The relative percent composition and mobilities of the serum proteins was established. In the case of the normal serum samples these values differed somewhat from those reported for other species. Pullorum antisera revealed rather characteristic alterations from the normal.

Twenty-nine Broad Breasted Bronze turkey toms were used during the course of this study and were divided into five groups designated as Control Group, Group 10, Group 11, Group 20, and Group 11 P.

The relative percent composition of normal serum proteins was determined from an average of 23 serum samples as follows: Albumin 66.5 percent, alpha globulin 7.9 percent, beta globulin 14.4 percent, and gamma globulin 11.2 percent.

The mobility values for the serum proteins were quite consistent for all serum samples. These, expressed in the order of 10^{-5} $Cm^2Sec^{-1}volt^{-1}$, were as follows: albumin 5.60, alpha globulin 3.76, beta globulin 2.40, and gamma globulin 1.60.

Three standard strains of Salmonella pullorum, obtained from the Bureau of Animal Industry, were employed in this study. These strains were designated as strains 10, 11 and 20. The groups mentioned above were numbered to correspond to the strain of S. pullorum used in the production of pullorum antisera. Suspensions of the various strains were inoculated into the turkeys either intraveneously or subcutaneously.

The turkeys in Group 11 P were used to study the course of immunization over an extended period of time. After preexposure electrophoretic patterns were established the turkeys were inoculated with S. pullorum intraveneously. They were subsequently bled one, two, three, five, seven and ten weeks after primary inoculation. At the eleventh week, four of the birds received another inoculation of the organism. Their electrophoretic patterns were subsequently determined at the twelfth, fourteenth, and sixteenth weeks post-exposure.

The tube agglutination test was carried out in conjunction with electrophoretic analyses for each serum sample. A somewhat definite relation was shown to exist between the degree of immunization and the relative percent of serum gamma globulin. In most instances where high agglutinin titers existed, there was also observed a high relative percent of gamma globulin. As antibody titers decreased there was a corresponding decrease in the gamma globulin fraction.

In addition to the quantitative alterations observed in the serum proteins there was also noted, in some instances, qualitative alterations especially in those serum samples where the alpha globulin separated into two distinct peaks.

The constant occurrence of gelling serum during the earlier phase of the experiment greatly hampered the attainment of normal yields. This condition, however, was fully aleviated by increasing the concentration of alfalfa leaf meal in the ration.

The appearance of weak, if any, prozones did not add to our knowledge of this particular phenomenon.

Two of the experimental turkeys suffered traumatic injuries with the result that their electrophoretic patterns were markedly varied from those of all other experimental birds.

All electrophoretic analyses were carried out in a Perkin Elmer Model 38 Tiselius Electrophoresis apparatus.

Veronal buffer of 0.1 ionic strength at pH 8.6 was employed throughout the experiment.

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INTRODUCTION

The present study was undertaken for the purpose of contributing to our knowledge of certain immunological reactions of turkeys affected with pullorum disease.

As no references were available pertaining to any earlier electrophoretic studies on turkey serum, the primary objective of this work is to establish normal electrophoretic patterns for turkey sera.

It has frequently been observed that immune pullorum sera showing high agglutinin titers fail to agglutinate the homologous bacteria in low dilutions. The portion in which agglutination failed to occur has been designated as the prozone or proagglutinoid zone, and this phenomenon is referred to in the literature as zone reactions, prozones, prezones, zone of inhibition and proagglutinoid zones. Many workers have attempted to determine the exact nature of the prozone. The importance of this phenomenon, in regard to pullorum disease in turkeys, is that it may at times be the cause of false negative results in the tube agglutination test. To a great extent the eradication of pullorum disease in turkeys is based upon the successful detection of carriers in breeding flocks. A comprehensive blood testing program employing the tube agglutination test is carried out for this purpose. In this regard it is hoped that an electrophoretic study of pullorum immune turkey sera reacting negatively to, or showing zone reactions in the tube agglutination

test, may contribute further information as to the exact nature of the prozone phenomenon.

It was also desired to study electrophoretically the course of immunization in a group of turkeys affected with pullorum disease over an extended period of time in conjunction with the tube agglutination test to ascertain whether the immunization reflected by the tube agglutination test can be correlated with electrophoretic analyses.

Furthermore, this work includes a study of the electrophoretic responses of the serum proteins of turkeys injected with various strains of Salmonella pullorum and the effect that the route of inoculation may have on the electrophoretic pattern.

HISTORICAL REVIEW

The etiological agent of pullorum disease was first described by Rettger (53) who referred to the disease as "Fatal Septicemia of Young Chicks". In 1899, he had occasion to observe the symptoms of pullorum disease and also to describe the postmortem appearances.

Rettger and Stoneburn (54) suggested that the term "Bacillary White Diarrhea" be applied to the disease and that the causative organism be designated Bacterium pullorum. Today the disease is quite generally referred to as "Pullorum Disease" and the causative agent as Salmonella pullorum. The term pullorum is the genitive plural form of pullus, the Latin word for a young fowl.

Rettger and Plastridge (55) presented, in a monograph on pullorum disease, many of the important contributions of workers in the field. The monograph includes several abstracts of studies on the mode of transmission, pathology and eradication of the disease.

Pullorum disease has been of economic importance to the turkey industry especially since the development of commercial hatching of turkey eggs. Hewitt (24) was the first worker to describe the disease in turkeys. He observed pullorum infection in young turkey poults which were hatched in an incubator that had previously been employed for incubating hen's eggs. Burnet (6) isolated an organism from the ovary of mature turkeys which he identified as Bacterium pullorum.

Tittsler (68), Johnson and Anderson (30), and Hinshaw (25) made extensive literature reviews on pullorum disease in turkeys.

The observation has frequently been made that adult turkeys, hatched and reared on poultry farms where pullorum disease occurred in epizootic form, gave positive agglutination reactions with S. pullorum antigen, and that the ability of the blood to react might be persistent or relatively permanent. A turkey showing such a reaction, may be a carrier. Pullorum carriers are important means of transmission and must be eliminated if the disease is to be eradicated. Carriers must not be kept in breeding flocks.

Corpron, Bivins and Stafseth (9), while working with the tube agglutination test for pullorum disease in turkeys, observed that sera of certain turkeys which gave doubtful reactions in 1:25 and 1:50 serum-antigen dilutions, gave maximum agglutination reactions in higher dilutions. This phenomenon, known generally as a prozone reaction, is sometimes observed in antigen-antibody reactions.

A rather extensive amount of research has been conducted to determine the exact nature of the prozone phenomenon. The workers concerned generally agreed that the prozone is due to some inhibitive substance which combines with the antigen and as a result inhibits the formation of the specific antigen-antibody complex. Hewes and Stafseth (23), presented a rather extensive review of the literature concerning the nature of the inhibitive factor responsible for this phenomenon, which is still a matter of much speculation.

Electrophoresis is defined as the migration of charged particles through an elective field. When the size of the particles is too small to be observed individually under the microscope, optical methods,

utilizing properties of groups of particles or molecules, such as light absorption, fluorescence or refraction, are usually used to establish the position of such a group of particles.

The method used in electrophoretic analysis is based on the measurement, by various optical methods, of the progress of boundaries formed between colloidal and buffer systems. Studies on the behavior of colloidal systems, such as proteins, date back to the first half of the Nineteenth Century, but the last fifteen years have shown an unprecedented development of the field of electrophoresis. This rapid advancement is due largely to the fundamental research of Tiselius (65) who equipped his apparatus with an optical system which renders the moving boundaries visible. In his apparatus, Tiselius used the schlieren optical system, with which he was able to record images which showed the position of each boundary as a dark band. As a group of molecules having a like net charge migrated under the influence of the elective field, shadows were observed that reflected the progress of migration. The word schlieren denotes an inhomogeneity in an optical medium, such as water, and is caused by refractive index gradients arising out of density or concentration changes.

The moving boundary method is designed essentially for material in the colloidal state, and, as stated by Hardt (19), this method has the following advantages:

(1) It is applicable to a wide variety of high molecular weight substances in solution in both their native and denatured forms.

(2) It is applicable to mixtures of these substances and, when applied to such mixtures, yields information as to: (a) the number of electrically separable components in the mixture, (b) the degree of electrical homogeneity of each component, (c) the concentration and (d) the mobility of each component.

(3) It may be used to separate, in a pure state, the components of a mixture.

(4) The mobilities of a given material as a function of pH may be studied over the entire aqueous pH scale.

(5) It may be used to study the interaction between the solvent and the substance and between the particles of the substance itself since the method is applicable over a wide range of concentrations of the substance in a given solvent.

Tiselius further showed with his apparatus that it was possible to isolate the serum constituents of blood without chemical reactions, and thus enable workers to show that these fractions were individual proteins.

Since the work of Tiselius, numerous improvements have been made in the equipment, the most important ones in the method of recording the boundaries. While the technique employed by Tiselius allowed the visual observation and photographic recording of the migration of colloids, such as the serum protein during electrophoresis, and also the detection of the number of individual components in the substance, it did not yield quantitative data on the relative

concentration and homogeneity of each component. Longsworth (36) adapted the schlieren method so that quantitative information could be obtained regarding the various components. His schlieren-scanning mechanism gives a more detailed diagram in the form of a succession of peaks and valleys with each peak representing the the position of a boundary in the moving column. The area under each peak, for example, in the case of the serum proteins, indicates the concentration of each of the serum proteins responsible for the boundary.

The schlieren-scanning mechanism is essentially used for obtaining permanent records of electrophoretic analyses. However, it does not allow for direct observation of the progress of migration of the pattern on the ground glass screen of the apparatus.

The cylindrical-lens method developed by Thovert and modified by Svensson and Philpot has been described by Longsworth (39)(40). In this method the entire pattern of the material in one limb of the cell is visible on the ground glass screen and therefore allows for direct observation of the progress of migration of the pattern.

During the past ten years several hundred publications have appeared dealing with electrophoresis in its different aspects. It is, therefore, necessary to limit the scope of the present review to a discussion of some of the more significant contributions to problems of predominantly immunological interest. Some references

regarding electrophoretic studies on normal and pathological blood sera and plasmae will be discussed. Studies of immunological interest dealing with immune bodies and antigen will be considered. Species differences in regard to mobilities and relative composition of serum and plasma proteins will be considered in the discussion. Many of the more important factors which govern the character of the electrophoretic patterns will be discussed initially.

Tiselius (66) reported in 1937 that horse serum showed five boundaries of different electrophoretic mobilities. The fastest boundary he identified as that of serum albumin; the next three boundaries were recognized as due to three different globulin components which were designated as alpha, beta and gamma globulin in decreasing order of mobility. The fifth, a stationary boundary, was attributed to a delta component. Horsfall (27) and Stenhagen (62) pointed out that plasma exhibited, in addition to the boundaries just mentioned, a sixth component located between the gamma and delta boundaries which was shown to be due to fibrinogen. Later the stationary delta boundary observed in the ascending limb of the apparatus and the corresponding epsilon boundary in the descending limb were recognized by Tiselius and Kabat (67) as boundary anomalies due largely to the transport of buffer ions by the proteins during electrophoresis. Longsworth (39) stated that these anomalies were also due to a superimposed protein gradient rather than an additional protein component.

The number of boundaries observed in electrophoretic patterns of serum and plasma depends on the type of buffer used and on the species studied. Longsworth (39) showed that, by using a sodium diethylbarbiturate buffer of pH 8.6 and an ionic strength of 0.1, human plasma was resolved into six well-defined components, namely albumin, alpha$_1$, alpha$_2$, and gamma globulin in addition to fibrinogen and the stationary anomalous boundaries. Phosphate buffer of pH 7.7 and ionic strength of 0.2 or lithium veronal buffer of pH 7.9 and ionic strength of 0.05 did not facilitate the separation of the alpha$_1$ globulin from the albumin nor the separation of the gamma globulin from the delta or epsilon boundaries.

The serum and plasma proteins, when separated by the moving boundary method, are electrophoretically homogeneous but are not to be interpreted as being chemically homogeneous. Zeldis et al. (74), revealed that all serum protein fractions obtained by electrophoretic separation contain some cholesterol and phosphatids in bound form, although the alpha and beta globulin fractions are richer in lipids than either albumin or gamma globulin; likewise all fractions were found to contain carbohydrate, with the alpha and beta globulins again showing the highest percentage. However, 50 percent or more of the total lipids and carbohydrates in serum are contained in the albumin and gamma globulin fractions since alpha and beta globulin constitute a minor part of the serum proteins.

Perlmann and Kaufman (50) pointed out the effects of the ionic strength of the buffer and the protein concentration on the values obtained for relative concentrations of various protein components as computed from electrophoretic data. In a two percent protein solution and with veronal buffer of pH 8.6 the apparent albumin concentration decreased from 57 to 54 percent while the gamma globulin value rose from 10 to 13 percent upon increasing the ionic strength from 0.1 to 0.3. These workers also observed similar variations when the protein concentration was varied and the ionic strength of the buffer was maintained at a constant value.

The chief value of determining the mobilities of the various serum or plasma proteins recorded during electrophoretic migration lies in their use for correlation of the individual maxima with the corresponding components in those instances where the entire type of the electrophoretic diagram is changed. The values for electrophoretic mobility as computed from observations on the raising and falling boundaries in the apparatus vary somewhat. The areas under the individual maxima are likewise not identical in all instances. Mobility determinations are equally important in instances where additional peaks are observed, as is the case in certain pathological sera. For this reason the emphasis in the electrophoretic analysis of serum and plasma under a variety of patho-physiological conditions centers at present on the shifts observable in the relative concentration of the individual components of serum or plasma.

The changes which occur in the electrophoretic pattern of serum during immunization and after reaction with the specific antigen offer a clear picture of the appearance and removal of antibody. These studies are important in establishing the relationship of antibody to plasma proteins and in addition, such studies are important in the understanding of the electrophoretic patterns. Tiselius and Kabat showed that circulating antibodies appeared in the gamma globulin fraction of rabbit plasma (67). Several other workers have reported confirming results in many other species (10) (13) (14) (31) (33) (59) (60) (69) (73).

Tiselius and Kabat (67) and Van der Scheer et al. (70), among others, have shown that an increased globulin after immunization is not necessarily identical with normal gamma globulin. These workers described a new serum component formed between the beta and gamma globulins. The antibodies produced are associated with the new component and as immunization increased, the component became larger. The latter authors called the component the T component and stated that despite the association of antitoxic activity with the T component, the areas under it cannot be taken as proportional to the increased antitoxic activity.

Enders (13), Kekwick and Record (33), and Fell et al. (14), demonstrated that some antibody is formed in globulins other than the gamma fraction. Stern and Reiner (64) published an extensive review of studies on pathological sera and body fluids. In their

paper, the authors cite additional references regarding the association of antibodies with serum proteins other than gamma globulin.

Leutscher (42) presented an exhaustive review on the biological and medical applications of electrophoresis. He reported that electrophoretic patterns, for the most part, have proved to be characteristic not of the specific disease but of the host's reaction to infection or injury. The various changes are frequently proportional to the severity of the physiological disturbance and may vary with the duration or stage of the disease, with nutritional factors, with loss of plasma proteins, and with the involvement of certain organs, such as the liver (Moore (46) and Seibert et al. (61)).

The common denominator of almost every pathological state is a relative or absolute decrease in the serum albumin. The electrophoretic technique demonstrates this with greater sensitivity than the salting-out methods. The reduction of serum albumin is often associated with two factors common to many diseases -- a deficiency of protein and a general reaction of the body to injury and infection.

MATERIALS AND METHODS

Twenty-nine, four to six-month-old, Broad Breasted Bronze turkey toms were obtained from a U. S. Pullorum-Clean source. The turkeys were housed in a new and previously unused pen during the entire experiment.

The turkeys were fed pellets and coarse scratch grain. The pellets contained not less than 22 percent protein, 3.50 percent fat, and not more than 5.50 percent fiber. The ingredients contained in the pellets are listed below in Table I.

TABLE I

INGREDIENTS CONTAINED IN THE PELLET RATION

Concentrate Soy bean oil meal Dehydrated alfalfa meal	1%
Ground yellow corn Corn gluten meal Pulverized oats Wheat bran Wheat middlings	
D-activated animal sterol	0.15%
Steamed bone meal	2%
Ground limestone	2%
Salt	0.5%
Manganese sulfate	0.05%
Potassium iodide	0.0007%
Iron sulfate	0.04%
Copper sulfate	0.002%
Cobalt sulfate	0.0005%
Zinc sulfate	0.0006%
Riboflavin	2 mg./lb.
Choline chloride	0.025%
Niacin	6 mg./lb.
Pantothenic acid	4 mg./lb.
Vitamin B_{12} supplement containing 3.5 mg. of vitamin B_{12}/lb.	0.15%
Antibiotic feed supplement containing 1000 mg. of procaine penicillin and 900 mg. of aureomycin/lb.	0.2%

The scratch feed contained a guaranteed analysis of not less than 9.0 percent protein, 2.0 percent fat, and not more than 5.0 percent fiber. The ingredients were cracked corn, wheat, milo, buckwheat and barley.

The pullorum antigens used in the experimental stimulation of antibody production in the turkeys, and in the tube agglutination test, consisted of three standard strains of S. pullorum obtained from the U. S. Bureau of Animal Industry. These strains, designated as strains 10, 11 and 20 were classified as S. pullorum on the basis of morphology, motility and biochemical reactions. Hewes and Stafseth (23) were able to obtain wide zones of inhibition in the tube agglutination test with serum from turkeys that were inoculated with strain 11.

The antigens for the tube agglutination tests were prepared by seeding a layer of nutrient agar (Difco) in 32-ounce, screw-cap bottles with a 24-hour culture of the respective strain followed by incubation at 37°C. for 48 hours. The cultures were washed off the agar with a small amount of physiological saline (0.85 percent NaCl). This suspension was then diluted with physiological saline to an optical density corresponding to tube one of a McFarland Nephelometer (44). Phenol was used as a preservative and was added to the suspension making a final concentration of 0.5 percent. The resulting antigens were adjusted to pH 7.8 with 0.1 N NaOH. The three antigens were designated as 10, 11 and 20 corresponding to the three strains of S. pullorum used in the preparation.

The antigens used for the experimental stimulation of antibody production in the turkeys were prepared by seeding nutrient agar (Difco) slants with the three respective strains of S. pullorum. After 24 hours incubation at 37°C. the cultures were washed off the slants with physiological saline. The suspensions were diluted further with physiological saline to an optical density corresponding to tube three of a McFarland Nephelometer.

To measure the degree of antibody production the standard tube agglutination test, using constant amounts of antigen (1 ml.) and two-fold serial dilutions of antiserum, was used throughout the experiment. The serum-antigen mixtures were incubated at 37°C. for 24 hours. The results of the tube agglutination test are expressed in terms of the degree of agglutination as follows:

Complete agglutination - - - - 4

Marked agglutination - - - - - 3

Moderate agglutination - - - - 2

Slight agglutination - - - - - 1

No agglutination - - - - - - - -

All electrophoretic analyses were carried out in a Perkin-Elmer Model 38 Tiselius Electrophoresis Apparatus. The construction and mechanical operation of the instrument are presented in detail in a manual published by the manufacturers.[1] Moore and White (47) also described this electrophoretic apparatus in some detail.

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[1]Perkin-Elmer Corporation, Norwalk, Connecticut.

The buffer used throughout the experiment was the standard barbiturate (veronal and sodium veronal) buffer solution of 0.1 ionic strength. It contained 2.79 gms. of diethylbarbituric acid and 20.6 gms. of the monosodium salt of diethylbarbituric acid per liter of solution with a pH of 8.6 at 25°C. Longsworth (39) introduced the diethylbarbiturate buffer of pH 8.6 and 0.1 ionic strength. This buffer, according to Longsworth, gives a more complete separation of alpha-globulin and reveals somewhat more total globulin. Also, the delta and epsilon boundaries separate from the peak due to the gamma globulin.

EXPERIMENTAL PROCEDURE

The turkeys were divided into five groups, designated as Group 10, Group 11, Group 20, Group 11P and Control Group, the numbers corresponding to the strain of S. pullorum used in immunizing the birds.

Group 10

Turkey 54193
Turkey 54197
Turkey 54198
Turkey 54195
Turkey 54199

Group 11

Turkey 54180
Turkey 54181
Turkey 54185
Turkey 54184
Turkey 54186

Group 20

Turkey 54191
Turkey 54192
Turkey 54194
Turkey 54196
Turkey 54200

Group 11P

Turkey 85193
Turkey 85194
Turkey 85195
Turkey 85196
Turkey 85197
Turkey 85198
Turkey 85199
Turkey 85200
Turkey 54177

Control Group

Turkey 54182
Turkey 54183
Turkey 54188
Turkey 54189
Turkey 54190

The turkeys in Group 10, Group 11, Group 20 and Control Group were procured on September 21, 1951 from a flock hatched on June 21, 1951. The turkeys in Group 11P were obtained December 26, 1951. These birds were from the same hatch as those in the other four groups.

On October 20, 1951, the turkeys in Group 10, 11 and 20 were inoculated as follows:

Group 10

Turkey 54193 - Intravenously
Turkey 54197 - Intravenously
Turkey 54198 - Intravenously
Turkey 54195 - Subcutaneously
Turkey 54199 - Subcutaneously

Group 11

Turkey 54180 - Intravenously
Turkey 54181 - Intravenously
Turkey 54185 - Intravenously
Turkey 54184 - Subcutaneously
Turkey 54186 - Subcutaneously

Group 20

Turkey 54191 - Intravenously
Turkey 54192 - Intravenously
Turkey 54194 - Subcutaneously
Turkey 54196 - Subcutaneously
Turkey 54200 - Subcutaneously

The inoculum in each instance consisted of 1.0 ml. of the saline suspension of S. pullorum, the strain number corresponding to the number of the group. These turkeys were injected with the same inoculum and by the same route seven days and fourteen days after the initial inoculation. The Control Group was not inoculated. One week after the final inoculation, the turkeys in these three experimental groups and the control group were bled from the brachial vein. Twenty ml. of blood was drawn from each bird. These samples were collected in large test tubes to create a greater surface area,

and allowed to clot at room temperature. After clotting appeared to be complete, the samples were placed in a refrigerator at 5°C. for approximately 12 hours, allowing the serum to separate. In all instances the cells settled out and the serum formed a firm gel. The gel also formed when the cells were allowed to settle completely at room temperature. It was observed that when gelling occurred, the blood samples had failed to clot properly, indicating that some factor was lacking in the coagulating mechanism. The clots were musky.

The gelled sera were broken up, and by recentrifuging, what appeared to be normal sera were obtained along with a cloudy, stringy sediment. The final serum yields were very small, averaging not much more than two ml. in most instances. Some samples required more rigorous treatment as the consistency of the gel varied.

The condition prevailed when bleedings were attempted up to the latter part of December, 1951. As stated earlier, the turkeys in Group 11P were obtained on December 26, 1951. In this group, gelling serum also occurred when the birds were bled so that pre-exposure patterns could be obtained.

Since alfalfa leaf meal is an excellent source of vitamin K, a factor in coagulation of blood, it was suggested that its concentration in the ration be raised to five percent. Two of the experimental turkeys were isolated and placed on a ration containing the

increased amount of alfalfa meal for a period of three days. They were then bled from the brachial vein and for the first time in the course of these studies, normal clot formation occurred with excellent yields of serum. The remaining turkeys were then placed on this ration. The pellets were ground in order that the alfalfa could be more evenly mixed. The serum samples obtained after feeding the ration for one week were normal as to consistency and yield. No further difficulty was encountered with gelling sera. Through the remainder of the experiment, the turkeys were maintained on the ration with the increased percentage of alfalfa leaf meal.

The dehydrated alfalfa leaf meal contained the following analysis: crude protein, not less than 17 percent; crude fat, not less than 1.5 percent; crude fiber, not more than 27 percent.

The sera from the turkeys in Group 10 contained antistrain 10 S. pullorum antibodies and those from Group 11 and Group 20 contained their corresponding antibodies.

The tube agglutination test was run in conjunction with the electrophoretic analysis of each serum sample. The results of these tests were compared to those of the same tests conducted on the samples from the Control Group. These findings served as a basis for further studies.

The turkeys in Group 11P were used to study the course of immunization over an extended period of time. With this thought in mind, pre-exposure electrophoretic patterns were obtained for

the nine turkeys in the group. The birds were then inoculated intravenously with 1.0 ml. of a heat-killed (60°C for one hour) saline suspension of strain 11 S. pullorum, corresponding to tube three of a McFarland Nephelometer. The turkeys were subsequently inoculated with the same amount and type of inoculum seven days and fourteen days after the initial inoculation. The birds were bled from the brachial vein one, two, three, five, seven and ten weeks after the first inoculation. Electrophoretic analyses were conducted in conjunction with the tube agglutination test on the serum of each turkey at the times listed.

At the eleventh post-exposure week, turkeys 85194 and 85197 received an inoculation of 1.0 ml. of the heat-killed suspension of the organisms. Turkeys 85198 and 54177 were challenged with a 1.0 ml. suspension of living S. pullorum antigen of strain 11.

At the twelfth, fourteenth and sixteenth weeks post-exposure, or first, third and fifth weeks post-challenge the four turkeys were bled at which times the tube agglutination test and the electrophoretic analyses were carried out.

Samples of serum were submitted to the Department of Agricultural Chemistry for the purpose of determining the protein nitrogen content by the Kjeldahl method. Total protein in each sample of serum was calculated by applying the factor 6.25 to the value for protein nitrogen. The samples were then prepared for dialysis.

For dialysis serum samples were diluted to a final concentration of two percent in the barbiturate buffer of 0.1 ionic strength and pH 8.6. Moore and Abramson (49) showed that the colloid concentration for the moving boundary method should not be too great as boundary anomalies become appreciable. They also stated that the protein concentration should not be too small, for small components become invisible. For best results, the concentration may range from 0.5 - 2.5 percent depending on the number of components present. Lippman and Banowitz (34) studied the influence of protein concentration upon electrophoretic mobility of serum proteins. They observed that the mobility of all serum components in the descending limb increased about 33 percent as the protein concentration fell from 5.5 to 0.5 percent and that the relationship of mobility to concentration was linear below three percent. The effects may be the result of interaction between protein molecules in concentrated solution which would reduce the effective charge.

The diluted samples were then placed into dialyzing sacs made of seamless regenerated viscose process cellulose (18/32" diameter) and equilibriated with mechanical stirring against standard barbiturate buffer at room temperature for a period of two to three hours. Reiner and Fenichel (51) showed that it is possible to equilibriate protein solutions against a buffer within two hours, using cellophane tubing and a simple mechanical dialyzer. This

procedure greatly reduces the time required for electrophoretic examination of clinical materials such as serum.

The specific resistance of equilibriated serum samples was measured at 0.6°C., at the completion of each electrophoretic run. A Leeds and Northrop No. 4960 electrolytic conductivity bridge[1] and a conductivity cell, designed especially for use with the Perkin-Elmer Model 38 Tiselius Electrophoresis Apparatus were employed in determining the specific conductance of the samples.

A brief outline of the steps involved in the operation of the Tiselius Electrophoresis Apparatus is to be presented here. For a more detailed description, the reader should consult the instruction manual for the Tiselius Electrophoresis Apparatus.

The ice compartment of the tank was filled with ice cubes. Distilled water was then added to the point where the water commenced to overflow and drip out through the overflow line. The stirrer was turned on to have the water bath reach a uniform temperature at or near 0.6°C. Morre (45) demonstrated the importance of carrying out experiments at a temperature where the change in density with temperature is small. A bath temperature of 0° - 2° C. is ideal for general analysis, for, at such temperatures, we have the maximum density of the protein solution and therefore the greatest resolution of components.

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[1]Manufactured by Leeds and Northrop Co., Phila., Pa.

While the water bath temperature equilibrated, the cells to be used were assembled. All ground glass surfaces of the cells were greased, using a mixture of three parts vasoline and one part mineral oil for this purpose. The assembled cells were then placed into the proper holders.

The cell was then filled with the serum sample to be analyzed. The buffer bottles were assembled and the Ag-AgCl electrodes were placed into the buffer bottles after first rinsing them in buffer solution. Buffer was added to the bottles leaving enough space for the addition of 15 ml. of concentrated K Cl to each bottle. The center gate of the top section is in the raised position during this procedure. The salt solution was gradually introduced through the electrode capillaries to the bottom of each buffer bottle. It was found that 15 ml. of the salt solution was necessary to immerse completely the coiled ball of silver chloride coated silver wire.

The loaded cell was placed in the bath and clamped into position. The plastic tank cover was placed into position after alligator clamps were clipped to the electrodes protruding through the wall of the tank. The center gate of the top section was then lowered.

Five drops of buffer were added to the buffer bottle adjacent to the ascending limb to compensate for the difference in weight of the serum and buffer in the two limbs of the cell. If the buffer were not added, the serum could move toward the ascending side when the cell sections are aligned, producing a disturbance of the initial boundary.

The center section of the cell was then brought into alignment followed immediately by the start of the compensator which allowed the buffer to fall gently into the left-hand buffer bottle. The compensator was allowed to run until the initial boundaries were brought into full view. When this was accomplished, the Schlieren slit was so set that all the light reached the photographic screen except that which passed through the boundaries. The masking diaphragm was then adjusted so that its aperture showed only the left-hand cell image. A film holder was then inserted into the camera back and the film was exposed. The same procedure was used to obtain the starting boundary for the right-hand cell image.

The current was then turned on for the electrophoretic run. Unless otherwise indicated, electrophoretic runs were carried out at eight milliamperes and approximately 145 volts for a period of 6000 seconds. The field strength under these conditions averaged 8.7 volts/cm. During the early stages of the experiment the time, current and voltage were varied to determine whether these factors would influence the resolution of the samples.

When the electrophoretic pattern appeared to exhibit full resolution into all individual components present in the system, the current was turned off. The total duration of the run in seconds was noted and the current strength, in milliamperes, was observed.

Immediately after the current was turned off, the electrophoretic patterns present in both limbs of the cell were recorded with the schlieren-scanning system of Longsworth (36). Eastman Contrast Process Panchromatic and "M" Process plates, (3 1/4" x 4 1/4") were used for recording the electrophoretic diagrams. Eastman D-19 Developer and Eastman F-5 Fixer were used for processing the photographic plates. An acid stop batch containing four drops of glacial acetic acid per 100 ml. water was used between developing and fixing. A developing time of five minutes, stop bath time of thirty seconds, fixing time of twenty minutes and a washing time of thirty minutes were used with good results.

From the electrophoretic diagrams recorded on the photographic plates, the mobilities of the various serum proteins, in terms of $Cm^2\ sec^{-1}\ volts^{-1}$, were obtained from the following formula (Longsworth (37)): $\mu = \frac{h\ K_p\ A}{I\ t\ 2}$ where K_p is the specific conductance of the protein, A is the cross-sectional area of the cell, h is the distance the boundary has moved from the starting point, I is the current in amperes, t is the time and 2 is the linear enlargement of the electrophoretic pattern. Using the beginning boundary that was recorded on the photographic plates before the current was applied, as the starting point, the distance moved, h, for each component is found by measuring in centimeters, the distance from the center of the starting to the position of the boundary on

the electrophoretic diagram to the various maxima of the gradient curve as recorded after the period of electrophoresis.

Tracings were made of the descending patterns, for according to Longsworth and MacInnes (38), the descending boundaries yield correct values of the mobility although in general, more diffuse than the ascending boundaries.

The relative percent composition of the serum components was determined as follows: The electrophoretic diagram, as recorded on the photographic plate by the schlieren-scanning method, was enlarged two-fold by projection and a tracing of the outline of the pattern was made on plain paper. The base-line was drawn in such a way that the extreme lower portions of the gradient curve were connected. The procedures employed in assigning areas to the various components of the serum are taken from the method of Longsworth (39) which consists of drawing an ordinate from the lowest point between adjacent maxima.

The measurement of the areas involved was accomplished with the aid of a planimeter which is a tool which automatically integrates areas by tracing their outline with the stylus of the instrument.[1] The stylus was guided around the envelope of the entire electrophoretic pattern including the base-line. The value of this area is noted in the planimeter units by reading the drum of the instrument

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[1]The planimeter used was manufactured by Keiffel and Esser Co., New York.

before and after the tracing operation and computing the difference between the two readings. The areas of the individual components are next circumscribed with the planimeter stylus and the values for them were noted. Moore et al. (48), pointed out the importance of obtaining accuracy when using the planimeter for determining areas of serum proteins. Three different people traced the same pattern with the same planimeter with the result that deviations were obtained. With this observation in mind, the author took the average of three readings for each component for each pattern as the true reading.

The relative concentration of each component was determined from the size of its area in terms of percentage of the total area, as measured by the planimeter.

RESULTS

Control Group:

Typical electrophoretic patterns of normal turkeys are illustrated in Figures 1 and 2. A total of 14 analyses were carried out on the Control Group. Serum from each of the turkeys, except No. 54182, was electrophoretically analyzed at three intervals during the course of the experiment which was carried on through a period of nine months. Turkey No. 54182 died prior to the time the third analysis was to be carried out. Necropsy revealed that the cause of death was trauma.

Table II is a composite of the data obtained in these electrophoretic analyses. The average relative percent composition for each of the serum proteins is as follows:

albumin	=	65.8
alpha globulin	=	8.2
beta globulin	=	14.4
gamma globulin	=	11.6

The pre-exposure patterns obtained from each of the birds in Group 11P may also be considered as normal or control pictures. The average relative percent composition for each of the serum proteins in this group was as follows:

albumin = 67.2

alpha globulin = 7.6

beta globulin = 14.4

gamma globulin = 10.8

Considering the data on the relative percent composition of the serum samples as a unit of normal turkey samples, we have a total of 23 electrophoretic analyses which average out for each component as follows:

albumin = 66.5

alpha globulin = 7.9

beta globulin = 14.4

gamma globulin = 11.2

The mobility calculations for the serum proteins were quite consistent for all electrophoretic analyses, both in the control and exposed groups. The serum proteins of turkey toms revealed the following mobilities, expressed in the order of 10^{-5} cm^2 sec^{-1} $volt^{-1}$.

albumin = 5.60

alpha globulin = 3.76

beta globulin = 2.40

gamma globulin = 1.60

Rhian et al. (56) observed when studying the composition of blood of normal turkeys that the absolute percent plasma protein ranged from 4.28 to 5.44 percent. In the present study, the serum proteins of turkeys was under consideration, and the absolute percent serum protein ranged from 3.96 to 4.91 percent for normal samples. These figures are to be expected when we consider the absence of the fibrinogen component in the serum samples. The values for the nine pre-exposure samples from the turkeys in Group 11P also fell into this range.

The tube agglutination test was also conducted in conjunction with electrophoretic analyses. The results of the former tests consistently showed that the birds in the Control Group had not acquired any immunity detectable to pullorum disease.

The patterns given by the ascending and descending boundaries are not identical. In general, the rising albumin boundary is better defined than is the falling albumin boundary, and the beta peak in the descending limb in the apparatus shows a peculiar anomaly which has been ascribed to an instability of this component after electrophoretic reparation from the accompanying serum proteins (Stern and Reiner (64)).

Longsworth et al. (37) stated that the differences in the patterns are due to the movement of the boundaries producing changes in the conductance of the solutions between the boundaries, and these changes, in turn, affect the distribution of protein and buffer.

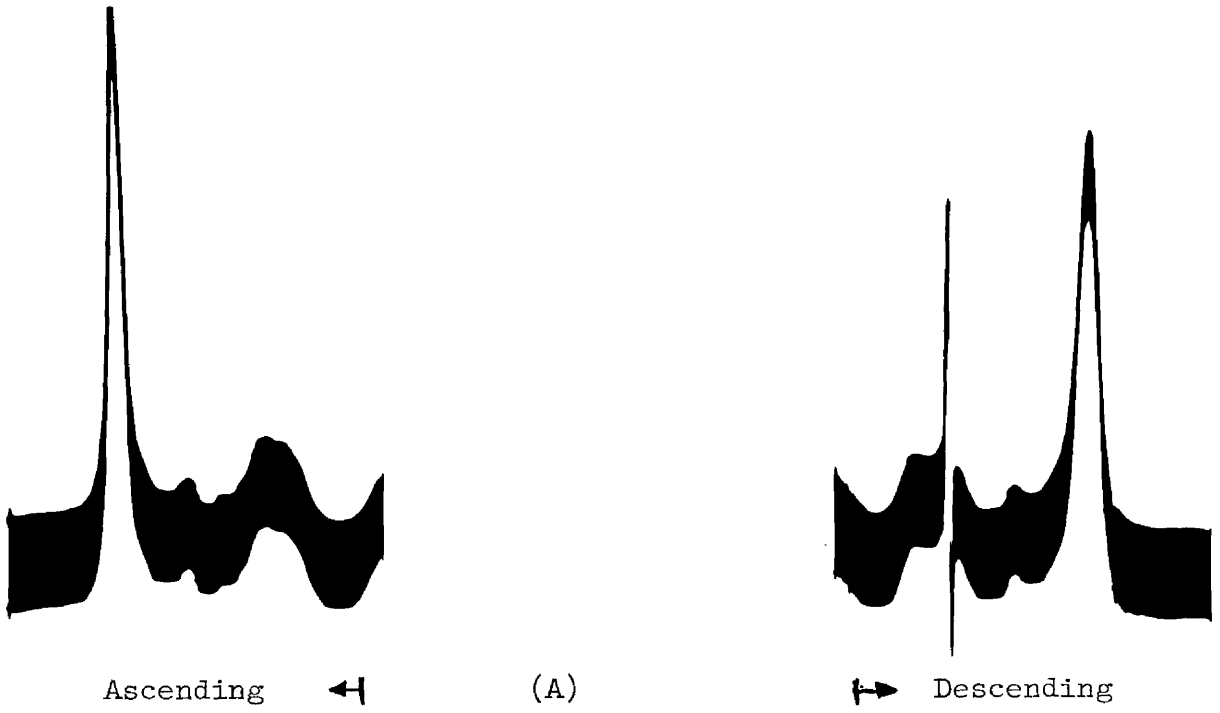
These workers further state that the beta globulin disturbance, almost always is observed in the patterns of the descending boundaries and that this is probably due to convection, resulting from a reaction in the neighborhood of the boundary following the electrophoretic separation of the constituents. It was noted that the beta globulin in the descending boundary migrates in the absence of albumin and alpha globulin, whereas in the ascending boundaries this is not the case. Turbidity occasionally develops at the site of this beta disturbance.

The time of electrophoresis was varied in order to ascertain whether the resolution of the serum components would be influenced by this factor. Figure 2 illustrates the patterns obtained from the serum of a Control turkey. The time of electrophoresis in one instance (Fig. 2a) was 5400 seconds and in the other it was 6000 seconds (Fig. 2b). The resolution of the patterns, in each instance is approximately identical.

TABLE II

ELECTROPHORETIC ANALYSES OF CONTROL GROUP TURKEY SERA

Turkey No.	% Protein	Time of Electrophoresis (In Seconds)	Relative Percent Composition			
			Albumin	Alpha	Beta	Gamma
54182	4.01	5400	70.24	6.55	13.09	10.12
	4.13	6000	63.27	8.85	15.93	11.95
54183	3.96	6000	72.08	6.49	12.99	8.44
	4.30	6000	66.99	7.77	14.07	11.17
	4.91	6000	64.58	8.76	12.50	14.16
54188	4.83	6300	67.06	8.82	13.53	10.59
	4.65	6000	61.91	9.53	16.66	11.90
	4.91	6000	61.95	8.85	15.93	13.27
54189	4.30	5400	66.50	8.12	14.21	11.17
	4.13	6000	61.40	7.89	17.54	13.17
	4.13	6000	65.22	7.25	16.42	11.11
54190	4.58	4800	69.23	9.23	10.77	10.77
	4.39	6000	60.24	7.53	18.01	14.22
	4.21	6000	65.50	7.50	14.50	12.50



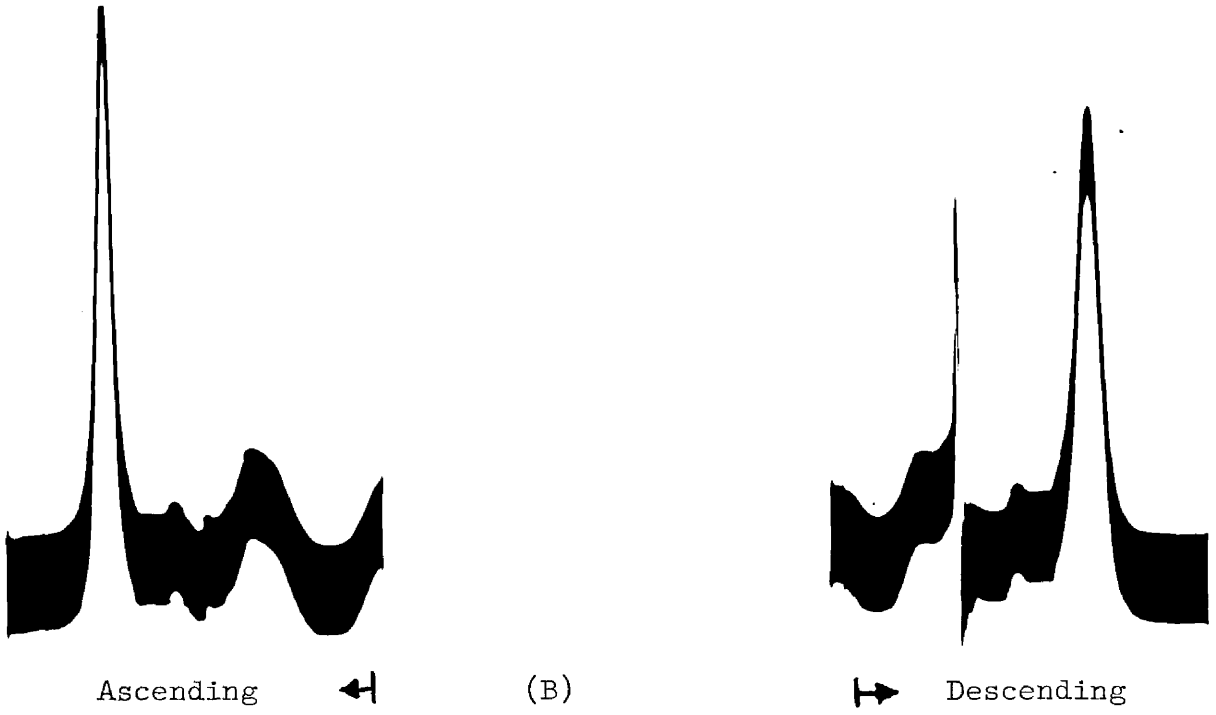


Fig. 1. Electrophoretic patterns of control turkeys.

A = 54190
B = 54183

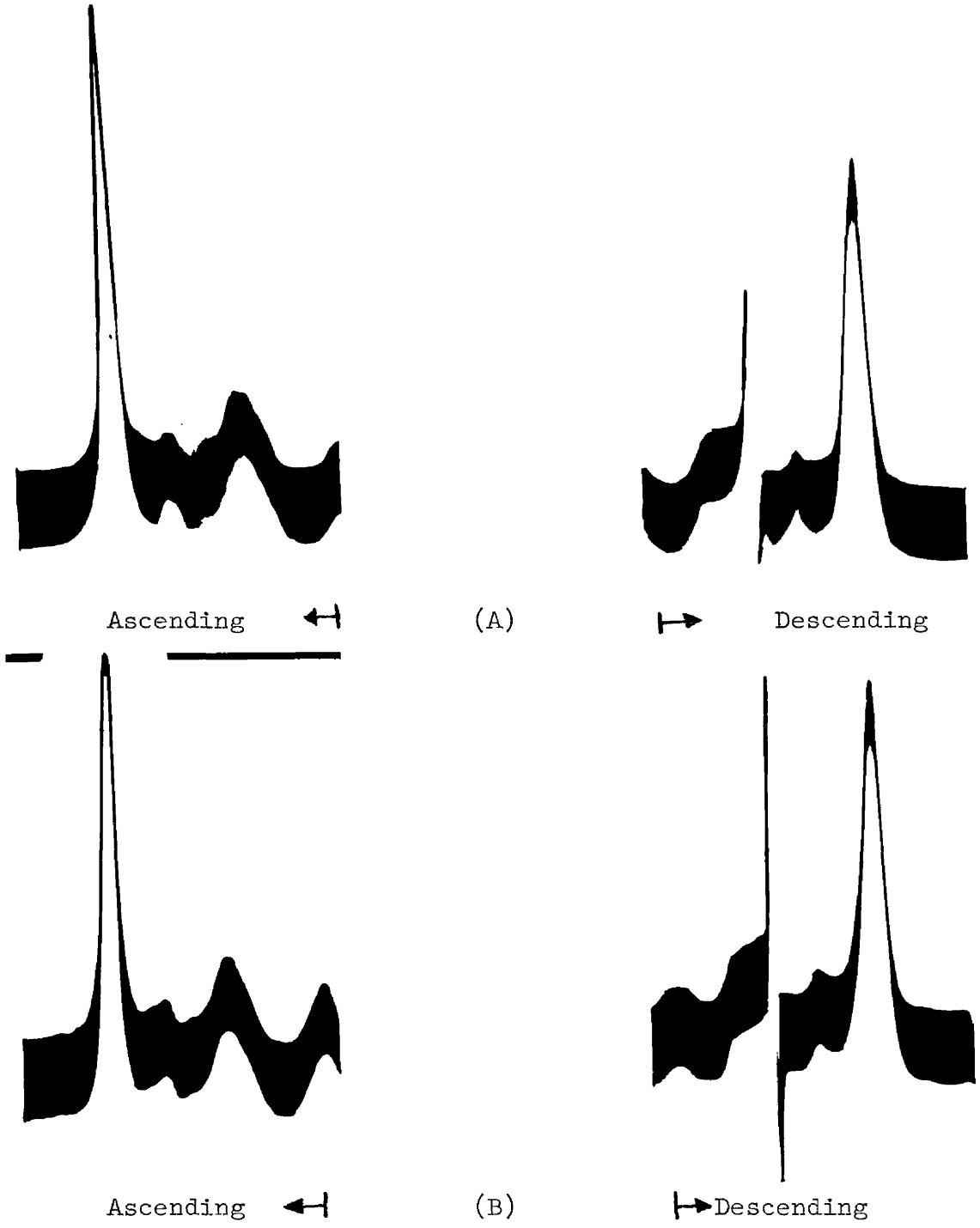


Fig. 2. Electrophoretic patterns of control turkey No. 54189.

Group 10:

The primary serum titrations carried out three weeks after the initial inoculation (Table III) indicated that antibodies homologous to antigen 10 developed in significant quantities. The effect of the route of inoculation on the extent of antibody production is demonstrated, in that those birds inoculated intravenously developed higher antibody titers. The serum titrations carried out at the eleventh week post-exposure showed only a slight decrease in antibody titers.

Typical electrophoretic patterns for this group are presented in Figures 3 and 4. Figure 3a is typical of those turkeys in the group which were inoculated intravenously and whose serum samples were electrophoretically studied at the third post-exposure week. The serum samples taken from the group at this time period were analyzed at eight milliamperes. Figure 3b is a pattern of the serum of the same bird analyzed at six milliamperes at the eleventh post-infection. The resolution of the serum components appears to be unchanged when the samples were analyzed at the lower amperage. Figure 4a is a typical example of serum from a turkey which had been subcutaneously inoculated. Figure 4b is a follow-up of the same turkey at the eleventh week post-exposure.

The relative percent composition of the serum proteins vary only slightly from that of the controls as revealed by electrophoretic analyses during the third week post-exposure. No significant

alterations are to be detected in the serum patterns even though all turkeys in the group exhibited significant agglutination titers. The absolute percent protein concentration for the turkeys in this group fell within the range of that for normal turkeys.

When the electrophoretic patterns were repeated at the eleventh week post-exposure, more significant changes were observed, as is illustrated in Table IV. All the turkeys in the group showed an increase in serum gamma globulin above the normal with the exception of turkey No. 54199. Turkeys 54193 and 54198 showed a significant decrease in serum albumin. The route of inoculation appears to have influenced the changes which occurred in the electrophoretic patterns.

TABLE III

SERUM TITRATIONS, TURKEYS IN GROUP 10

Preliminary Titrations: 3 Weeks Post-exposure

Source of Antisera	Antigen	Route of Inoculation	Serum Dilutions											
			10	20	40	80	160	320	640	1280	2560	5120	10240	C
54193	10	I.V.	4	4	3	3	2	2	2	1	-	-	-	-
54197	10	I.V.	4	4	4	3	2	2	2	1	1	-	-	-
54198	10	I.V.	4	4	4	3	3	3	3	2	1	-	-	-
54195	10	S.Q.	4	4	3	3	2	1	-	-	-	-	-	-
54199	10	S.Q.	4	4	3	3	2	2	1	-	-	-	-	-

Titrations at 11 Weeks Post-exposure:

54193	10	I.V.	4	4	4	4	3	2	1	1	-	-	-	-
54197	10	I.V.	4	4	4	3	2	2	2	1	-	-	-	-
54198	10	I.V.	4	4	4	3	2	2	2	1	-	-	-	-
54195	10	S.Q.	3	3	3	2	1	1	-	-	-	-	-	-
54199	10	S.Q.	3	3	3	2	1	1	-	-	-	-	-	-

I.V. = Intravenous
S.Q. = Subcutaneous

TABLE IV

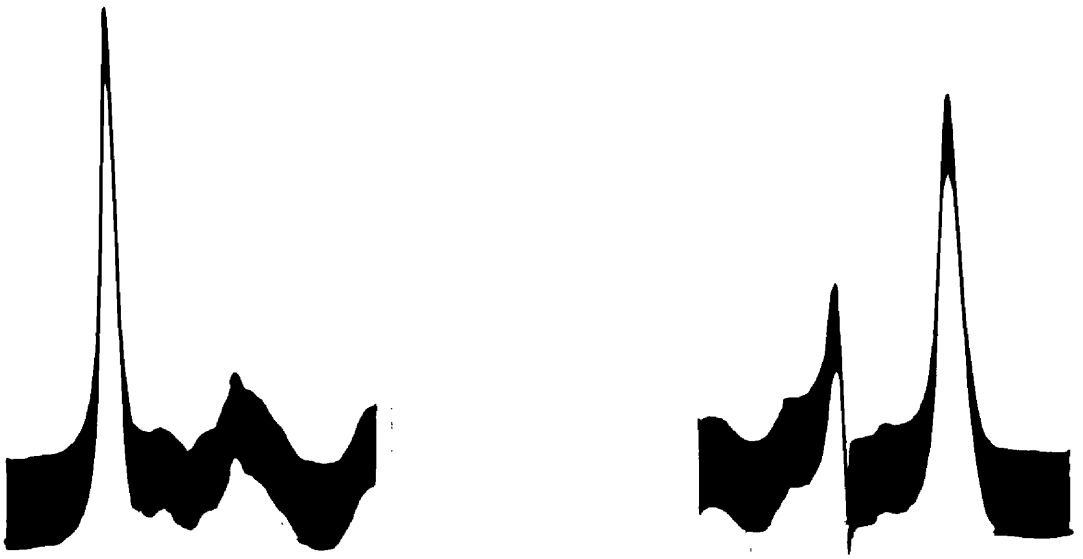
ELECTROPHORETIC ANALYSES, GROUP 10

Third Week Post-exposure: Electrophoresis at 8 milliamperes for 6000 seconds. Field strength 8.7 volts/cm

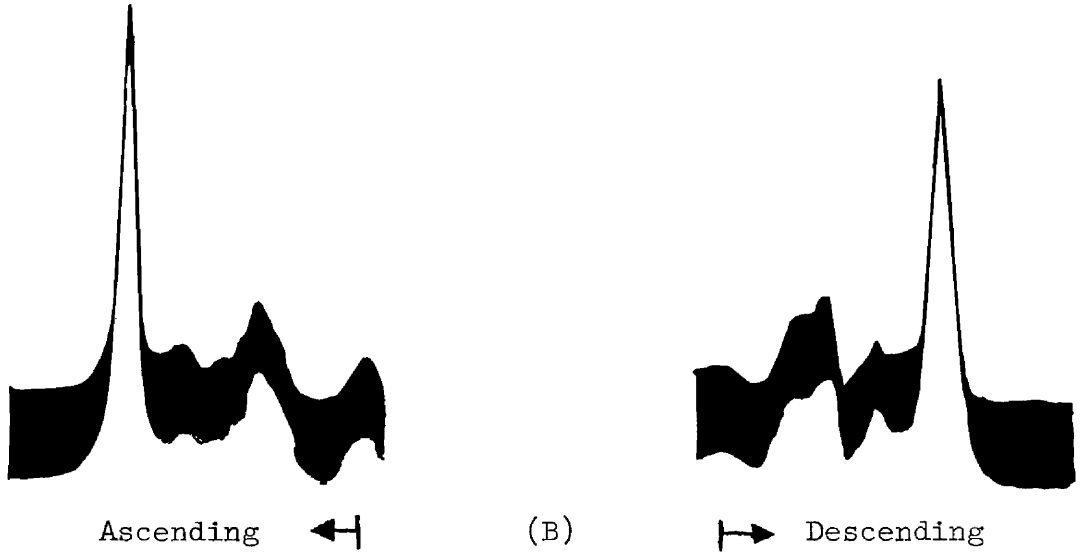
Turkey No.	% Protein	Relative Percent Composition			
		Albumin	Alpha	Beta	Gamma
54193	4.30	65.54	10.17	12.99	11.30
54197	4.50	60.27	8.04	19.19	12.50
54198	4.67	63.81	9.05	17.62	9.52
54195	4.41	66.49	6.39	17.55	9.57
54199	4.83	66.66	6.96	15.93	10.45

Eleventh Week Post-exposure: Electrophoresis at 6 milliamperes for 7200 seconds. Field strength 6.5 volts/cm

54193	5.97	55.28	14.90	16.78	13.04
54197	4.91	64.35	8.26	11.30	16.09
54198	5.79	56.18	10.11	18.54	15.17
54195	5.18	62.78	10.56	14.44	12.22
54199	5.53	65.22	9.78	14.67	10.33

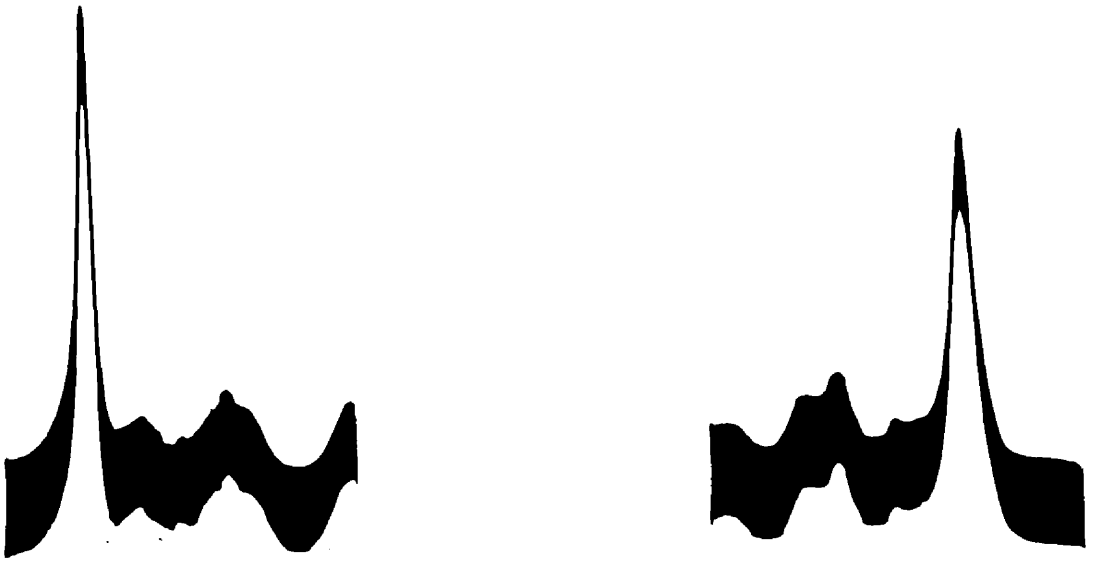


Electrophoresis at 8 milliamperes, field strength of 8.7 volts/cm for 6000 seconds. Three weeks post-exposure.



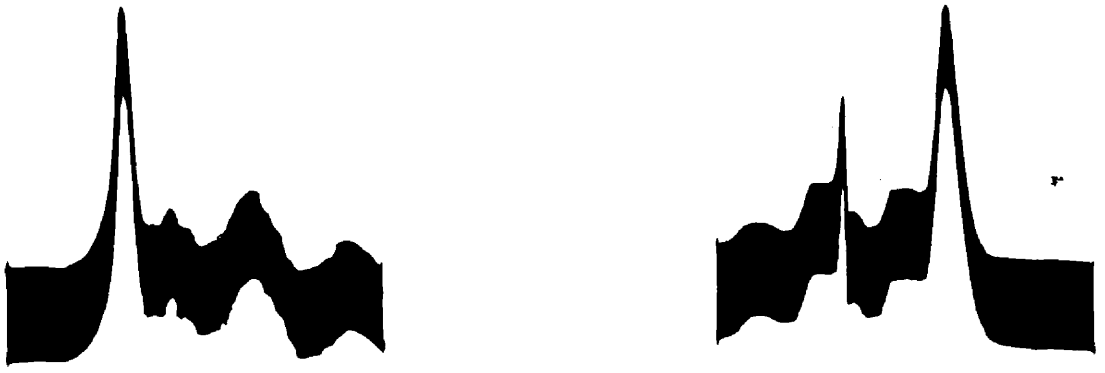
Electrophoresis at 6 milliamperes, field strenth of 6.5 volts/cm for 7200 seconds. Eleven weeks post-exposure.

Fig. 4. Electrophoretic patterns of turkey No. 54195.



Ascending ◄┤ (A) ├► Descending

Electrophoresis at 8 milliamperes, field strength of 8.7 volts/cm for 6000 seconds. Three weeks post-exposure.



Ascending ◄┤ (B) ├► Descending

Electrophoresis at 6 milliamperes, field strength of 6.5 volts/cm for 7200 seconds. Eleven weeks post-exposure.

Fig. 3. Electrophoretic patterns of turkey No. 54193.

Group 11:

The data from the electrophoretic analyses of Group 11 are presented in Table VI. When compared to similar analyses for the turkeys in the Control Group, some striking alterations are observed.

The initial patterns for Group 11, which were determined one week after the third inoculation of strain 11 S. pullorum, will be treated in some detail.

Prior to the time that the group was to be bled for the initial electrophoretic analyses it was noticed that turkey No. 54185 was in an extremely emaciated condition. Further examination revealed that the bird had sustained a fractured left femur. The turkey continued to lose weight as it was unable to get to its feed. After the bird was bled from the heart, it was sacrificed and autopsied. Typical lesions of pullorum disease were observed in the heart musculature, liver and lungs. The left femur showed a definite fracture. S. pullorum was not isolated from the organs of this turkey. The electrophoretic pattern for this bird is presented in Figure 8. It is interesting to note that this turkey's pattern was entirely different from any others obtained throughout the course of the experiment. There appears to have been a great depletion of serum protein in this turkey due, perhaps, mainly to the fact that the bird was unable to eat or drink over a period of approximately one week. The serum titer of this turkey was similar to those of other birds in the group which were inoculated intravenously.

The electrophoretic patterns of turkeys No. 54180 (Figure 5a) and No. 54181 (Figure 6a) are different from those of Control birds in that their patterns show the presence of an $alpha_1$ and an $alpha_2$ globulin. Furthermore the gamma globulin in each birds' serum shows the appearance of what might be two gamma globulin peaks. At the eleventh week post-exposure the serum of turkey No. 54180 (Figure 5b) showed a definite decrease in gamma globulin and the appearance of this component in a single peak. However, the alpha globulin continued to resolve itself into two distinct components. The serum of turkey No. 54181 when electrophoretically studied at the eleventh week post-exposure showed a definite tendency toward the normal. A marked increase in the relative percent serum albumin along with a decrease in serum globulin is readily observed. The alpha globulin in this instance resolved itself into a single peak.

The patterns of turkeys No. 54184 and 54186 (Figures 7a and 7b respectively) showed no such drastic alterations. These birds were inoculated subcutaneously. These patterns differed only slightly from those of normal turkeys.

The data for this group would seem to indicate that the route of inoculation is a major factor in the response of the serum proteins to pullorum disease. This point is substantiated when we consider Table V in which we observe the difference in the quantities of antibodies produced by the turkeys inoculated intravenously and subcutaneously.

The serum titrations at the eleventh week post-exposure show a decrease in the amount of antibodies. This decrease seems to be reflected in the electrophoretic patterns.

TABLE V

SERUM TITRATIONS, TURKEYS IN GROUP 11

Preliminary Titrations: 3 Weeks Post-exposure

Source of Antisera	Antigen	Route of Inoculation	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
54180	11	I.V.	4	4	4	4	4	3	3	2	2	2	1	-
54181	11	I.V.	4	4	4	4	3	3	2	2	2	1	-	-
54185	11	I.V.	4	4	4	3	3	2	2	2	2	1	-	-
54184	11	S.Q.	4	4	3	2	2	1	1	-	-	-	-	-
54186	11	S.Q.	4	4	3	2	2	1	1	-	-	-	-	-

Titrations at 11 Weeks Post-exposure:

54180	11	I.V.	4	4	4	4	4	3	2	2	2	1	-	-
54181	11	I.V.	4	4	4	3	2	2	2	2	1	-	-	-
54184	11	S.Q.	4	3	2	1	1	-	-	-	-	-	-	-
54186	11	S.Q.	4	3	2	1	1	-	-	-	-	-	-	-

TABLE VI

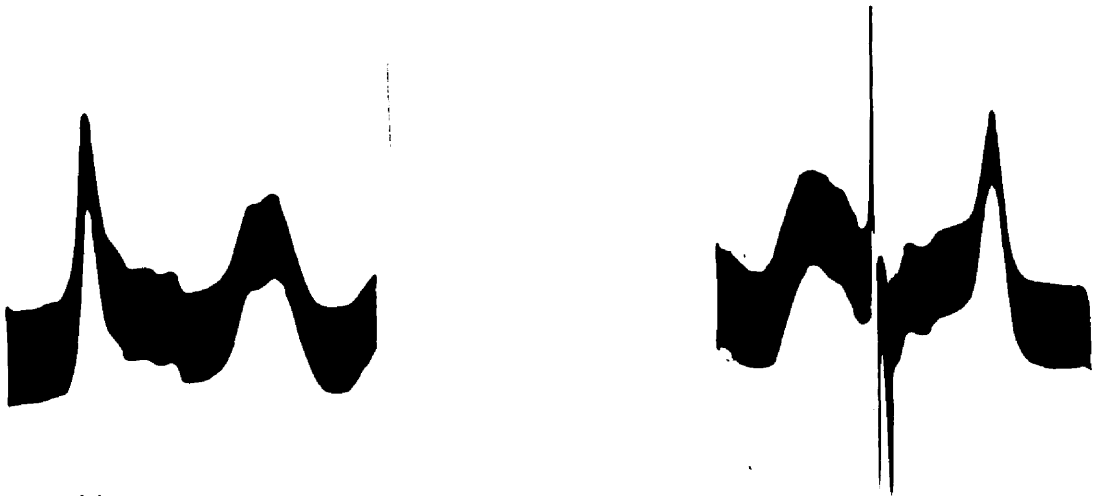
ELECTROPHORETIC ANALYSES, GROUP 11

Third Week Post-exposure: Electrophoresis at 8 milliamperes for 6000 seconds. Field strength 8.7 volts/cm.

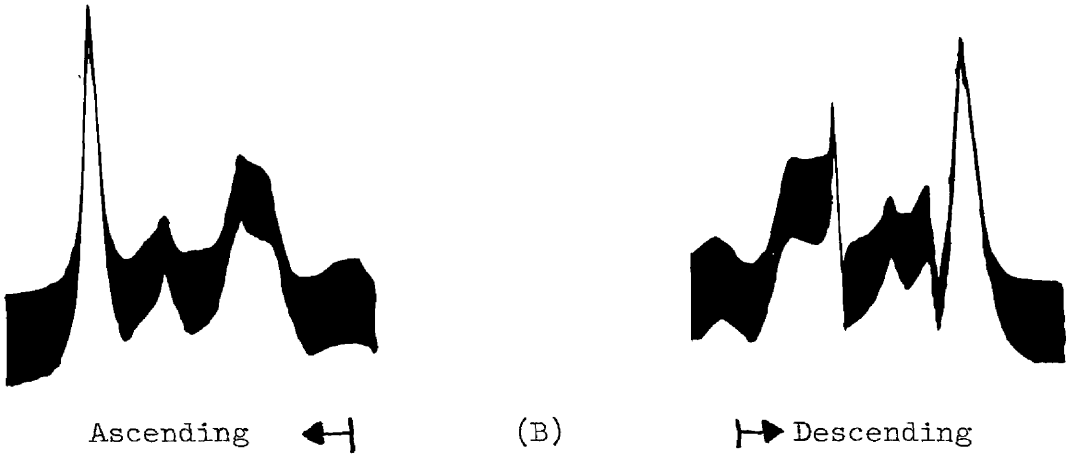
Turkey No.	% Protein	Relative Percent Composition				
		Albumin	Alpha 1	Alpha 2	Beta	Gamma
54180	6.75	38.32	11.38	5.98	8.38	35.94
54181	5.59	43.68	12.10	6.85	5.79	31.58
54185	4.36	30.59	28.24		20.00	21.17
54184	4.86	65.55	6.66		18.33	9.46
54186	5.11	65.05	7.76		11.66	15.53

Eleventh Week Post-exposure: Electrophoresis at 8 milliamperes for 6000 seconds. Field strength 8.7 volts/cm

54180	5.97	36.29	11.39	12.24	17.72	22.36
54181	4.21	59.66	8.52		13.07	18.75
54184	4.56	62.33	8.07		16.15	13.45
54186	4.13	67.26	8.97		13.90	9.87

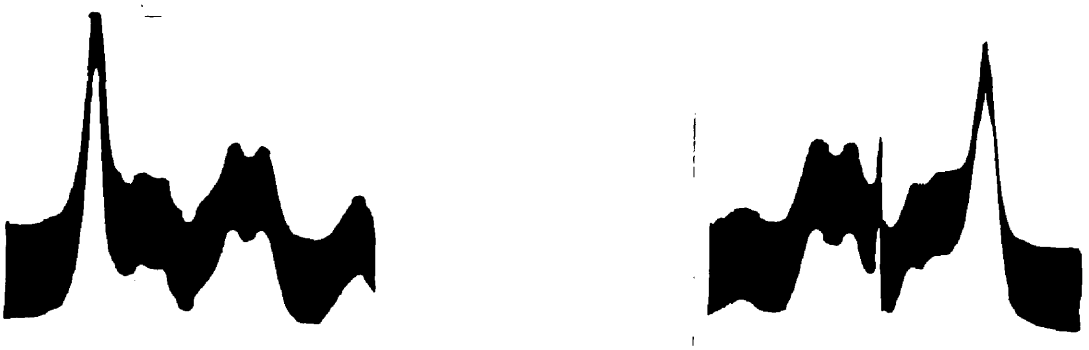


Third week post-exposure.



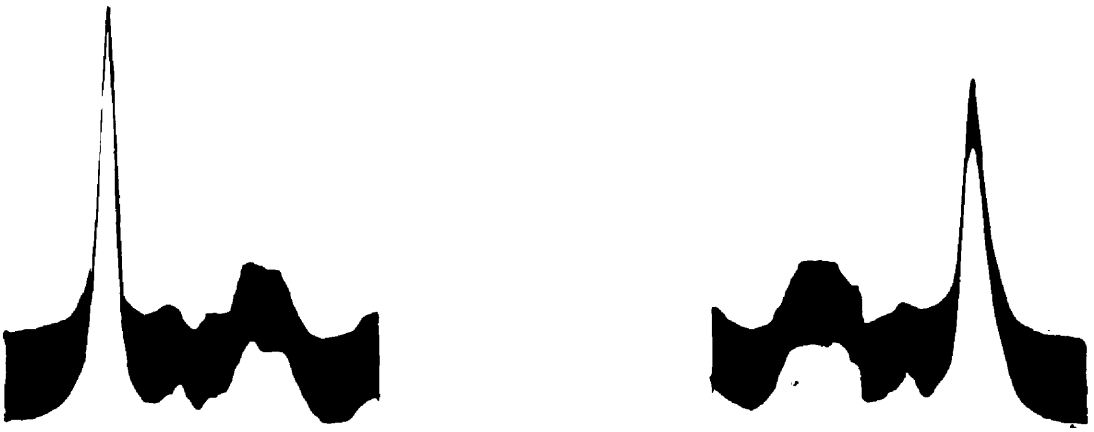
Eleventh week post-exposure.

Fig. 5. Electrophoretic patterns for turkey No. 54180.



Ascending ◄┤ (A) ├► Descending

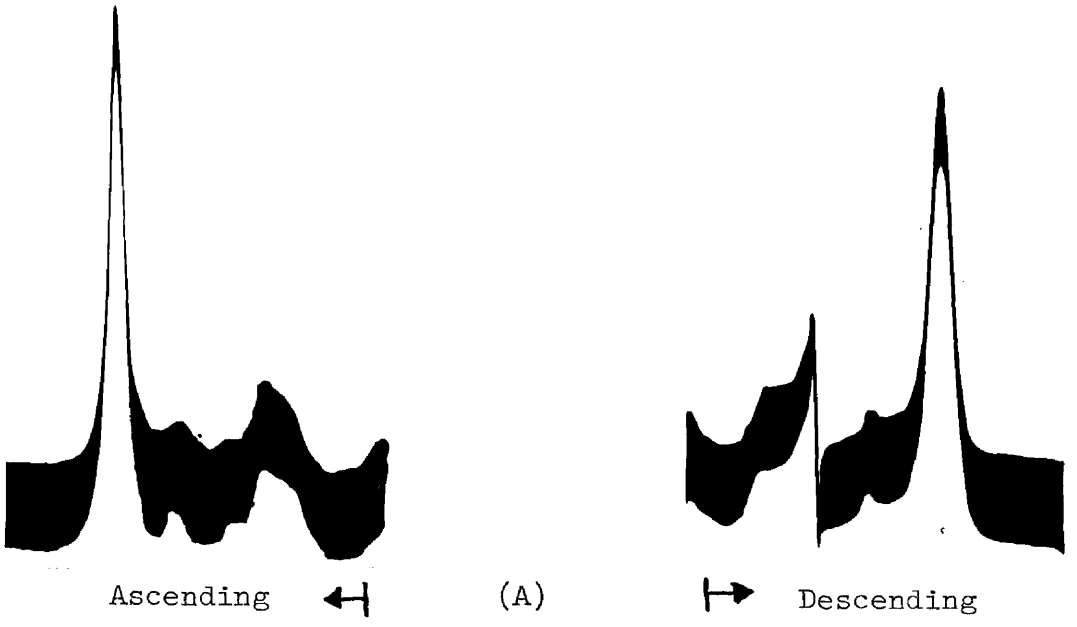
Third week post-exposure.



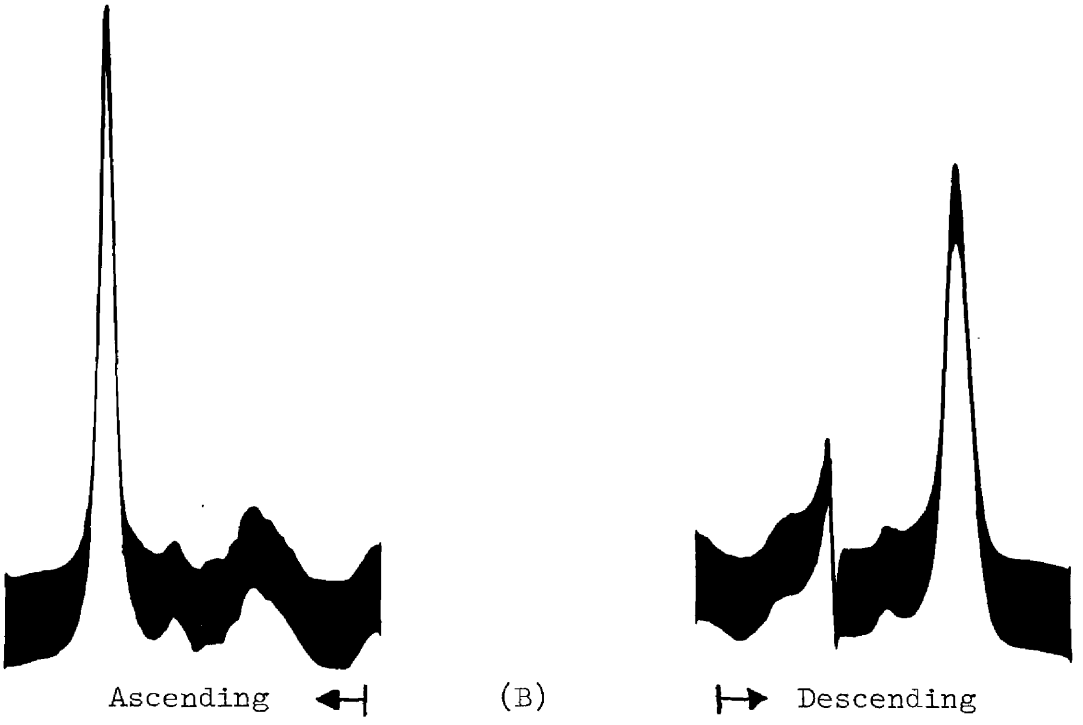
Ascending ◄┤ (B) ├► Descending

Eleventh week post-exposure.

Fig. 6. Electrophoretic patterns for turkey No. 54181.



Turkey No. 54184



Turkey No. 54186

Fig. 7. Electrophoretic patterns for turkeys No. 54184 and No. 54186.

Fig. 8. Electrophoretic pattern for turkey No. 54185.

Group 20:

Turkey No. 54192 was found dead just prior to the time of collecting blood samples from the group for electrophoretic studies. Necropsy and subsequent bacteriological examination revealed that the turkey had died of pullorum disease. Its organs showed the lesions characteristic of this disease.

The serum of turkey No. 54191, the other bird in the group that was inoculated intravenously, on primary electrophoretic analysis, revealed a pattern somewhat similar to those of turkeys No. 54180 and No. 54181 of Group 11. Figure 9a illustrates this observation. The patterns obtained during the eleventh post-exposure week for the same bird are shown in Figure 9b. An increase in the quantity of serum albumin is evident. The amount of alpha globulin was decreased. However, the gamma globulin showed an increase over the amount contained in the previous analysis. The absolute percent protein in each sample taken from this turkey was identical and above the range cited for normal turkeys.

The serum titrations presented in Table VII are similar to those for the two previous groups, and, as was the case in the previous groups, the turkeys which were subcutaneously inoculated consistently showed lower agglutinin titers and slight, if any, alterations in their electrophoretic patterns. Figures 10a and 10b illustrate typical patterns of turkeys in this group which were inoculated subcutaneously.

Table VIII is a composite of the data obtained from electrophoretic analysis of this group. The initial analyses were conducted one week after the third inoculation of strain 20 S. pullorum. These studies were made at eight milliamperes, under a field strength of 8.7 volts/cm and for a period of 6000 seconds. The serum samples collected at the eleventh post-exposure week were run, as were those sample repeats for Group 10, at six milliamperes, under a field strength of 6.5 volts/cm, and for 7200 seconds. Here too, the lower amperage gave the same resolution of serum components.

TABLE VII

SERUM TITRATIONS, TURKEYS IN GROUP 20

Preliminary Titrations: 3 Weeks Post-exposure

Source of Antisera	Antigen	Route of Inoculation	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
54191	20	I.V.	4	4	4	4	4	4	3	3	2	2	1	-
54192	20	I.V.	(Died on Nov. 12, 1951 - S. pullorum was recovered.)											
54194	20	S.Q.	4	4	3	3	2	2	1	-	-	-	-	-
54196	20	S.Q.	4	4	3	2	2	1	1	-	-	-	-	-
54200	20	S.Q.	4	4	4	3	2	1	-	-	-	-	-	-

Titrations at 11 Weeks Post-exposure:

54191	20	I.V.	4	4	4	4	3	3	2	2	1	1	-	-
54194	20	S.Q.	4	4	3	2	1	1	-	-	-	-	-	-
54196	20	S.Q.	3	3	2	2	1	1	-	-	-	-	-	-
54200	20	S.Q.	4	4	2	1	1	1	-	-	-	-	-	-

TABLE VIII

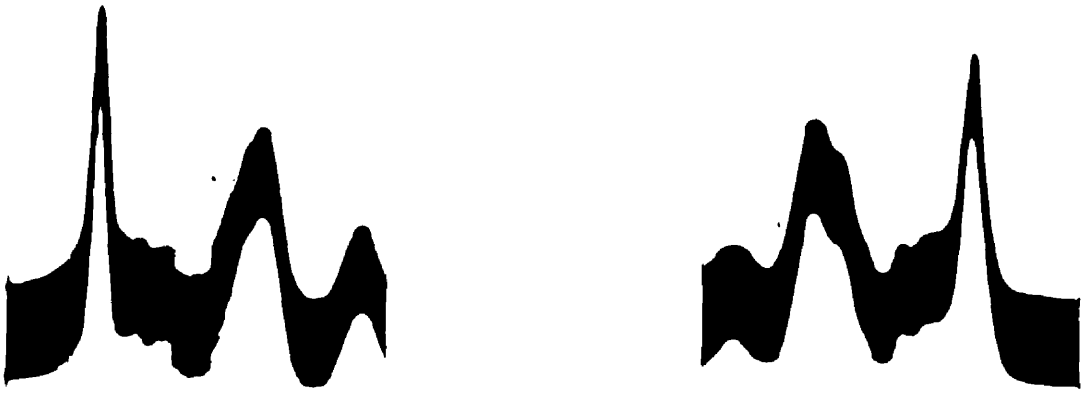
ELECTROPHORETIC ANALYSES, GROUP 20

Third Week Post-exposure: Electrophoresis at 8 milliamperes for 6000 seconds. Field strength 8.7 volts/cm

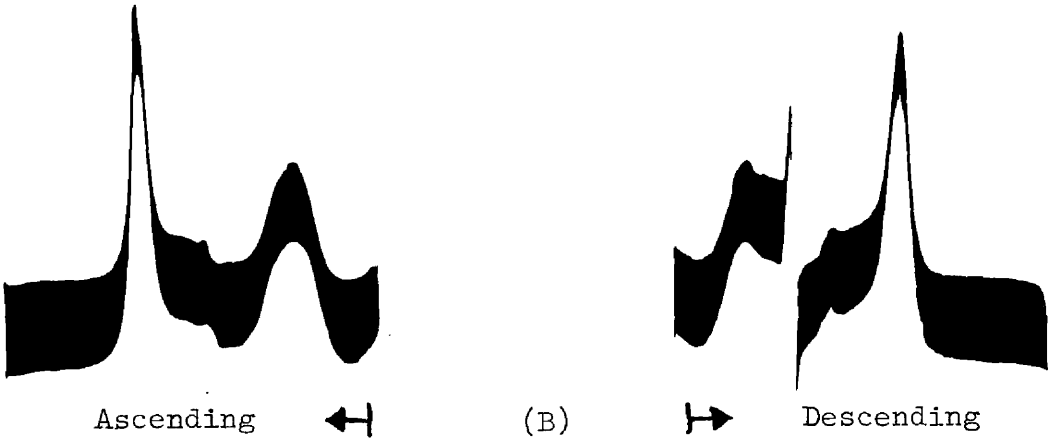
Turkey No.	% Protein	Relative Percent Composition			
		Albumin	Alpha 1	Alpha 2	Beta	Gamma
54191	5.97	41.85	6.52	5.44	19.02	27.17
54194	4.65	61.29	9.14		15.05	14.52
54196	5.44	63.18	7.97		14.92	13.93
54200	4.35	65.63	9.89		14.06	10.42

Eleventh Week Post-exposure: Electrophoresis at 6 milliamperes for 7200 seconds. Field strength 6.5 volts/cm

54191	5.97	48.99	9.09		10.60	31.32
54194	5.03	64.40	9.42		14.66	11.52
54196	5.71	66.84	5.88		15.51	11.77
54200	4.65	63.45	9.65		16.75	10.15

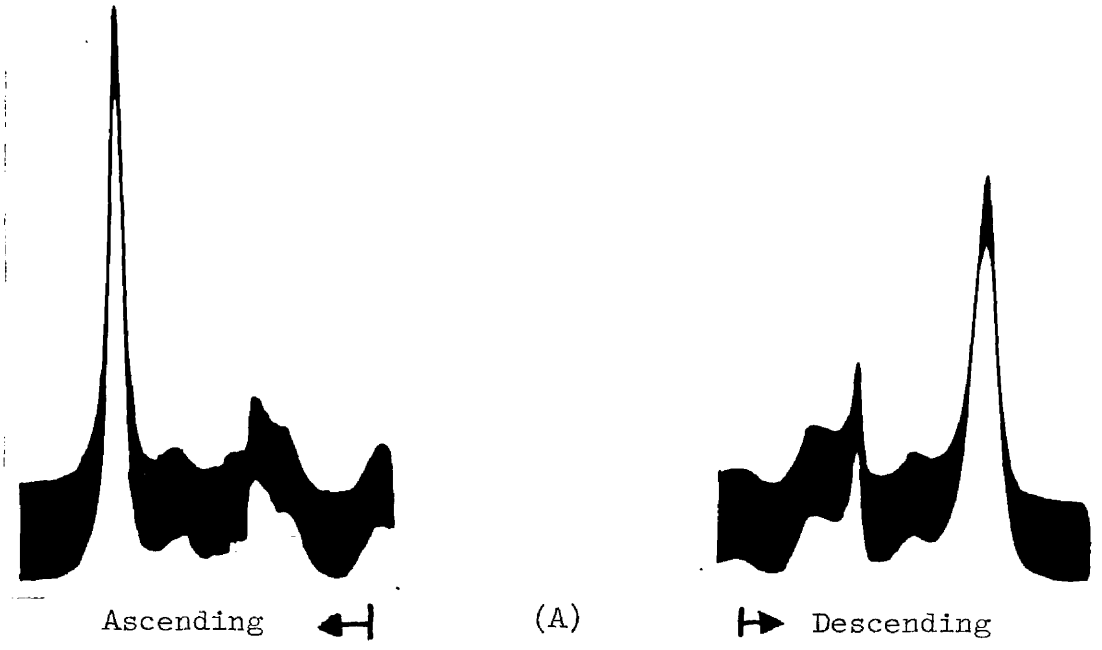


Electrophoresis at 8 milliamperes for 6000 seconds, field strength of 8.7 volts/cm. Third week post-exposure.

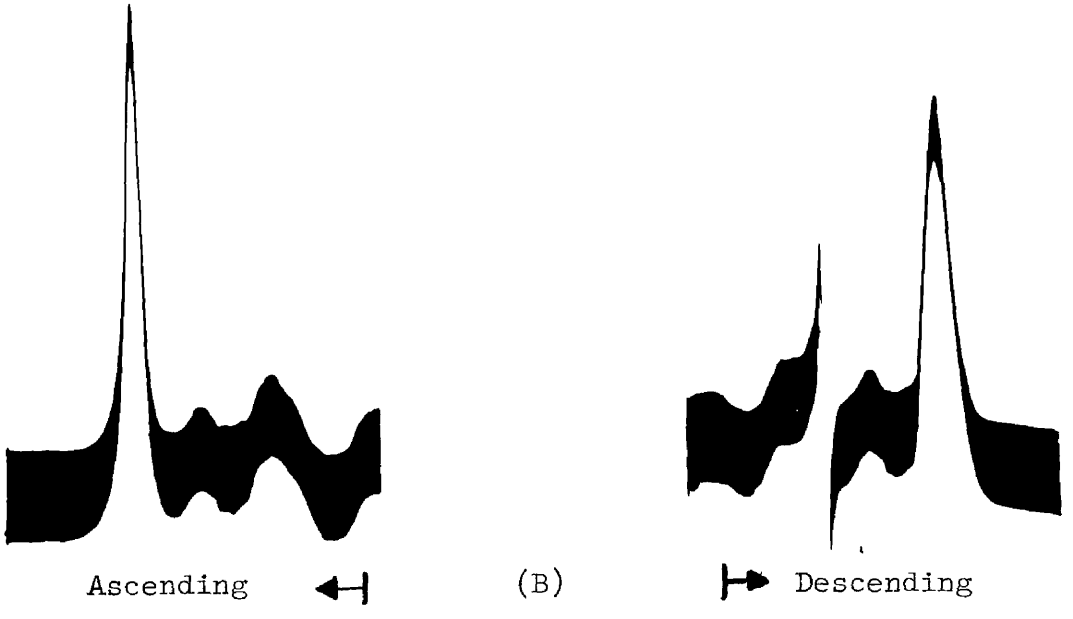


Electrophoresis at 6 milliamperes for 7200 seconds, field strength of 6.5 volts/cm. Eleventh week post-exposure.

Fig. 9. Electrophoretic patterns for turkey No. 54191.



Electrophoresis at 8 milliamperes for 6000 seconds. Field strength of 8.7 volts/cm. Third week post-exposure.



Electrophoresis at 6 milliamperes for 7200 seconds. Field strength of 6.5 volts/cm. Eleventh week post-exposure.

Fig. 10. Electrophoretic patterns for turkey No. 54200.

Group 11P:

As stated in the Experimental Procedure, there were nine turkeys in Group 11P. The serum of turkey No. 85193 consistently revealed electrophoretic patterns unlike those of the other turkeys in the group. This was due, to a great extent, to the fact that this turkey suffered a severe traumatic injury after the pre-exposure pattern had been taken. It was inoculated in the same manner as the other members in the group and its serum analyzed similarly. From the time of injury, this bird failed to gain weight even though it continued to consume a normal amount of feed and water. Because of the extreme differences in the electrophoretic analyses of this turkey's serum, the results for Group 11P will be reported on the basis of there being eight turkeys in the group. The data for turkey No. 85193 will be reported separately.

Hewes and Stafseth (23), when studying the chemical nature of the factor responsible for zone reactions in the tube agglutination test for pullorum disease in turkeys, employed the same strain 11, S. pullorum used in the present work. They were able to show wide zones of inhibition by using this antigen for the experimental production of pullorum antisera. The tables which are concerned with the weekly serum titrations illustrate that only weak, if any, prozones were formed. Both in the present study and in that presented by Hewes and Stafseth, the antigen was in the form of a

heat-killed suspension. It may be that the antigen used by Hewes and Stafseth was in a phase different from that used in the present study which enabled them to obtain such wide prozones. Nevertheless, the patterns presented in the following figures do not reveal any components or alterations of components that could be attributed to the presence of a zone of inhibition. Still, it may be that the inhibitive factor was masked by one of the serum components. Perhaps additional fractionation work would have yielded more definite information in regard to this factor. Fractionation proceedings were not carried out as it was felt that the weakness of any zones that formed did not warrant the necessity of such an investigation.

The data presented in the following tables and figures are worthy of consideration. It will be noted that for each of the eight turkeys in this group three sets of data are presented: the weekly serum titration table; the electrophoretic data; and a figure concerned with the relation of agglutinin titer to the relative percent gamma globulin over the course of immunization.

Electrophoretic patterns are presented for turkey No. 85194. It will be noted from the data sheets that this turkey was typical of this group. Only slight differences in serum titers and relative percentages of the serum components are seen from turkey to turkey. With reference to the data on turkey No. 85194, it can be seen that as the serum titer increased during immunization (Table IX), the relative percent gamma globulin increased correspondingly (Table X)

with a decrease in serum albumin. A glance at the corresponding tables for the other turkeys in the group reveals the same information although, as would be expected, some birds responded to the antigenic stimulation in a lesser or greater degree. Nevertheless, a general trend is evident. The alpha and beta globulin components were more variable during the course of immunization.

Turkeys Nos. 85194, 85197, 85198 and 54177 were given a challenging inoculation at the eleventh post-exposure week. Turkeys No. 85194 and No. 85197 received an inoculation of heat-killed S. pullorum while the other two turkeys were inoculated with live S. pullorum. The challenge dose in all four cases produced an increase in agglutinin titer and a corresponding increase in the relative percent gamma globulin. Here again we observe a decrease in serum albumin that is so evident in data on earlier electrophoretic analyses.

It will be noted that in all instances where challenge doses were given, there occurred a separation of the alpha globulin into two distinct peaks. Earlier data on the effects of routes of inoculation and the various S. pullorum strains also revealed the appearance of two alpha globulins in some instances where the agglutinin titers were relatively high. It appears then, that the separation of alpha globulin into two distinct peaks is in some way related to the degree of immunity. The $alpha_1$ peak, in this and other instances, has an average mobility of 4.59×10^{-5} cm^{-2} sec^{-1} $volt^{-1}$.

The data presented for this group also reveal that a definite trend existed between agglutinin titers and relative percentages of serum albumin and gamma globulin during the period of decreasing immunity, that is, that where we observe a decrease in the quantity of antibody we correspondingly see a decreased gamma globulin and an increased serum albumin concentration.

The challenge dose with live S. pullorum produced the approximate effect brought about by the inoculation of heat-killed S. pullorum. In the case of turkey No. 54177, however, we observe a slightly higher stimulation of gamma globulin than that brought about by the other turkey challenged with S. pullorum.

Figure 24 is a weekly average of the agglutinin titers and relative percent gamma globulin for the eight turkeys in the group over the four month period that the course of immunization was studied.

The data for turkey No. 85193 are recorded in Tables XXV and XXVI. Figures 25, 26, 27 and 28 contain the electrophoretic patterns of this turkey's serum over the course of sixteen weeks. Figure 29 is presented to show the influence that the injury had on the relation of agglutinin titer to relative percent gamma globulin during the course of immunization.

Table XXV, on the serum titrations for turkey No. 85193, indicate no significant differences from those titrations conducted on the eight other turkeys in Group 11P. This table reveals that

the injury produced no effect on the mechanism of antibody production even though the electrophoretic pattern of this bird's serum revealed drastic alterations in composition (Table XXVI). With the exception of the electrophoretic patterns for this turkey, taken before the injury occurred, two alpha globulin peaks are consistently observed. The extremely low serum albumin concentration and high gamma globulin concentration appear to be an indicator of the severity of the injury which this turkey sustained.

Figure 29 further testifies to the complete lack of correlation, over the course of the sixteen week period, between agglutinin titers and relative percent of serum proteins as a result of the injury. Similar figures for the eight other turkeys support the belief of the author that agglutinins against S. pullorum are associated mainly with the gamma globulin protein of serum.

TABLE IX

SERUM TITRATIONS, TURKEY NO. 85194

Time Interval	Antigen	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
Pre-exposure	11	2	-	-	-	-	-	-	-	-	-	-	-
1 week P.E.	11	4	1	1	2	2	2	-	-	-	-	-	-
2 week P.E.	11	2	3	4	4	4	4	3	2	1	-	-	-
3 week P.E.	11	2	3	3	4	3	4	3	3	2	1	-	-
5 week P.E.	11	3	3	4	4	4	4	4	3	2	2	1	-
7 week P.E.	11	3	3	4	4	4	3	2	2	1	-	-	-
10 week P.E.	11	3	3	4	4	3	3	1	-	-	-	-	-
12 week P.E. 1 week P.C.	11	4	4	4	4	3	3	3	2	1	-	-	-
14 week P.E. 3 week P.C.	11	4	4	4	4	4	3	3	1	-	-	-	
16 week P.E. 5 week P.C.	11	4	4	4	4	3	3	1	-	-	-	-	-

P.E. = post-exposure P.C. = post-challenge

TABLE X

ELECTROPHORETIC ANALYSES, TURKEY NO. 85194

Time Interval	% Protein	Relative Percent Composition			
		Albumin	Alpha 1	Alpha 2	Beta	Gamma
Pre-exposure	4.13	62.16	6.76		21.62	9.46
1 week P.E.	4.21	60.86	9.18		17.39	12.08
2 week P.E.	4.56	56.14	9.65		15.35	18.86
3 week P.E.	5.79	46.98	7.12	9.25	16.01	20.64
5 week P.E.	5.26	44.75	9.13		18.26	27.86
7 week P.E.	4.30	60.23	8.15		13.26	18.36
10 week P.E.	3.98	60.59	11.20		17.42	10.79
12 week P.E. 1 week P.C.	5.62	47.16	7.38	5.68	17.05	22.73
14 week P.E. 3 week P.C.	6.06	48.09	5.47	8.19	16.39	21.86
16 week P.E. 5 week P.C.	4.88	58.51	7.98		14.36	19.15

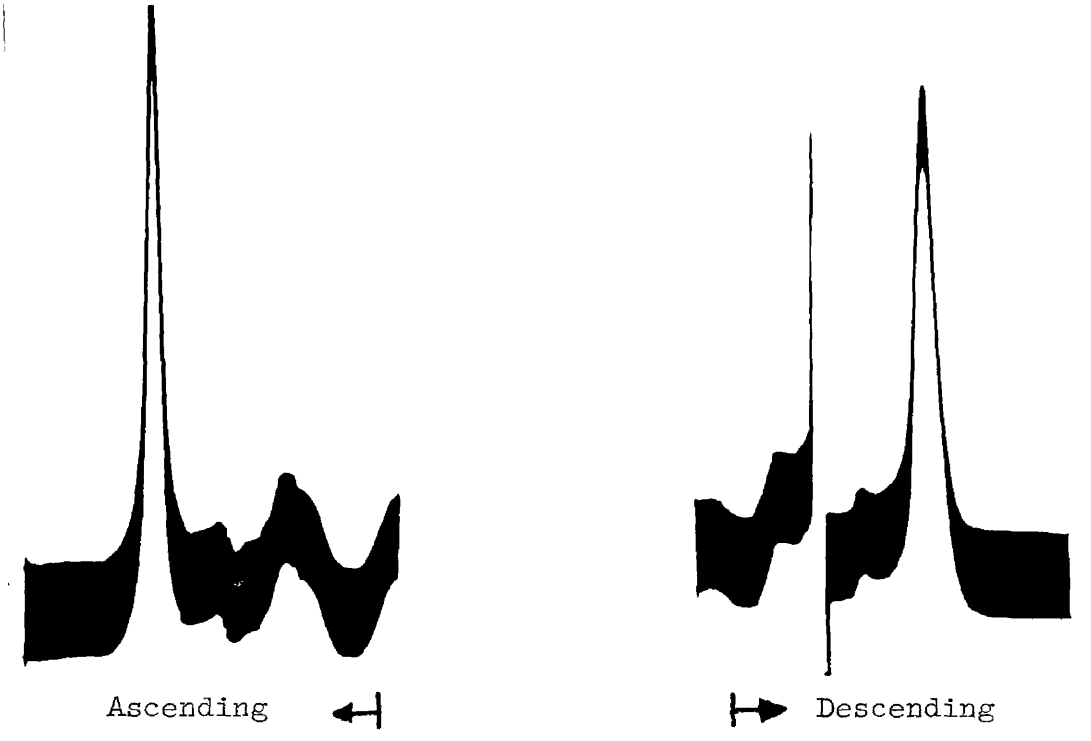
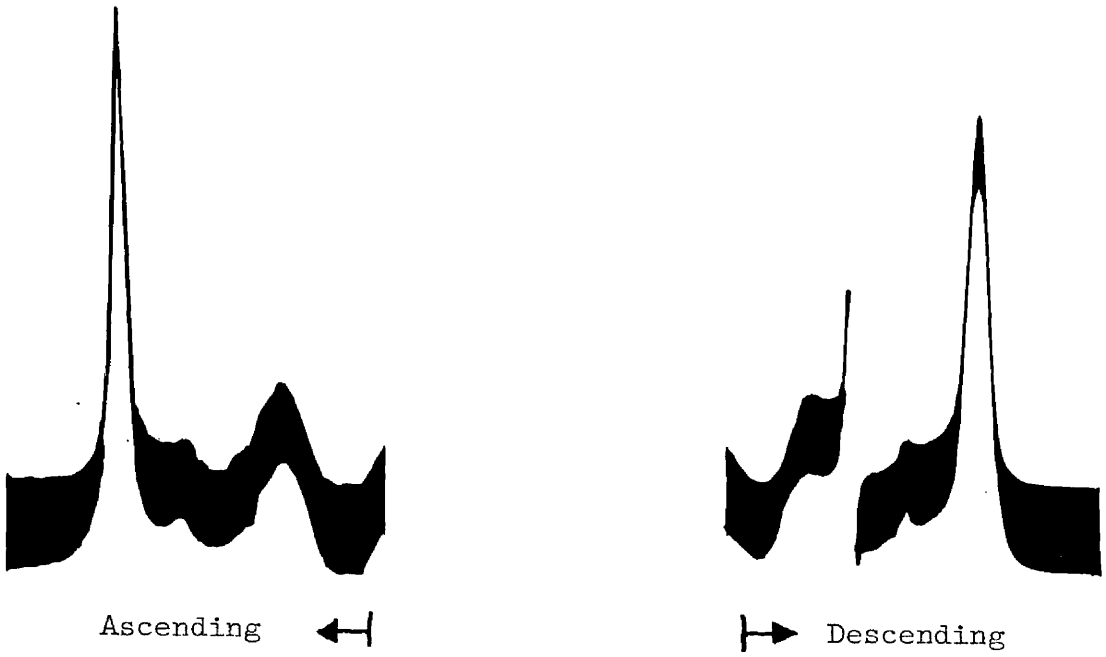
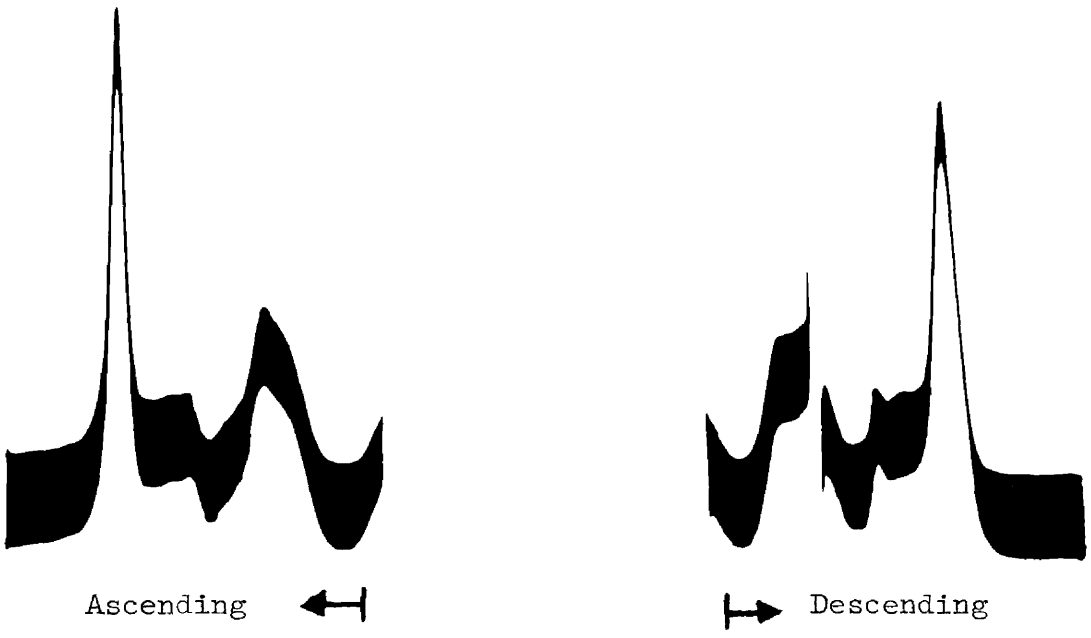


Fig. 11. Electrophoretic patterns for turkey No. 85194.

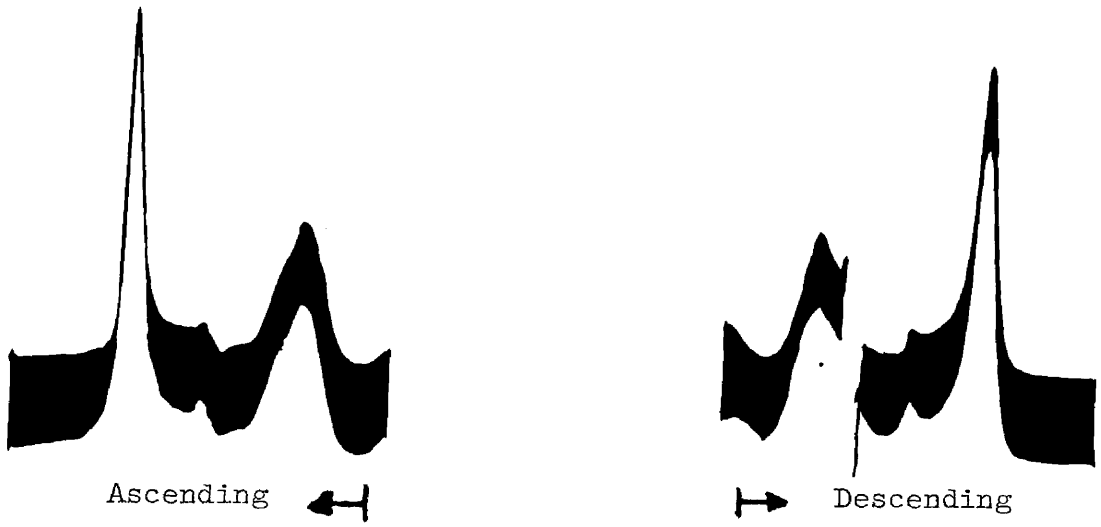


Two weeks post-exposure.

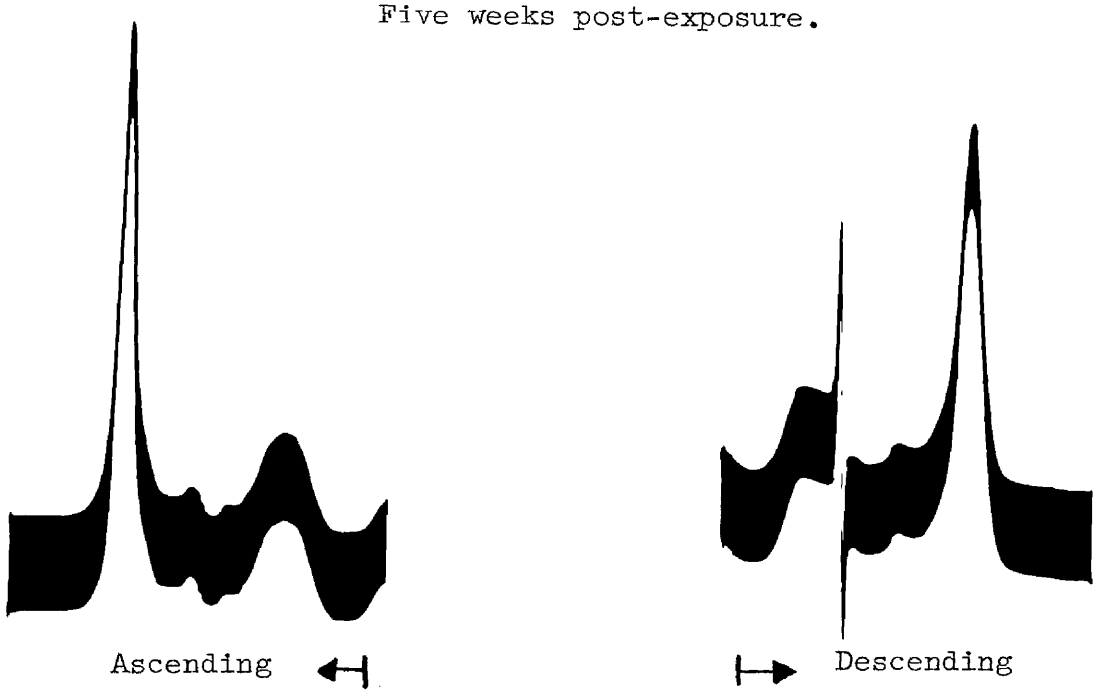


Three weeks post-exposure.

Fig. 12. Electrophoretic patterns for turkey No. 85194.

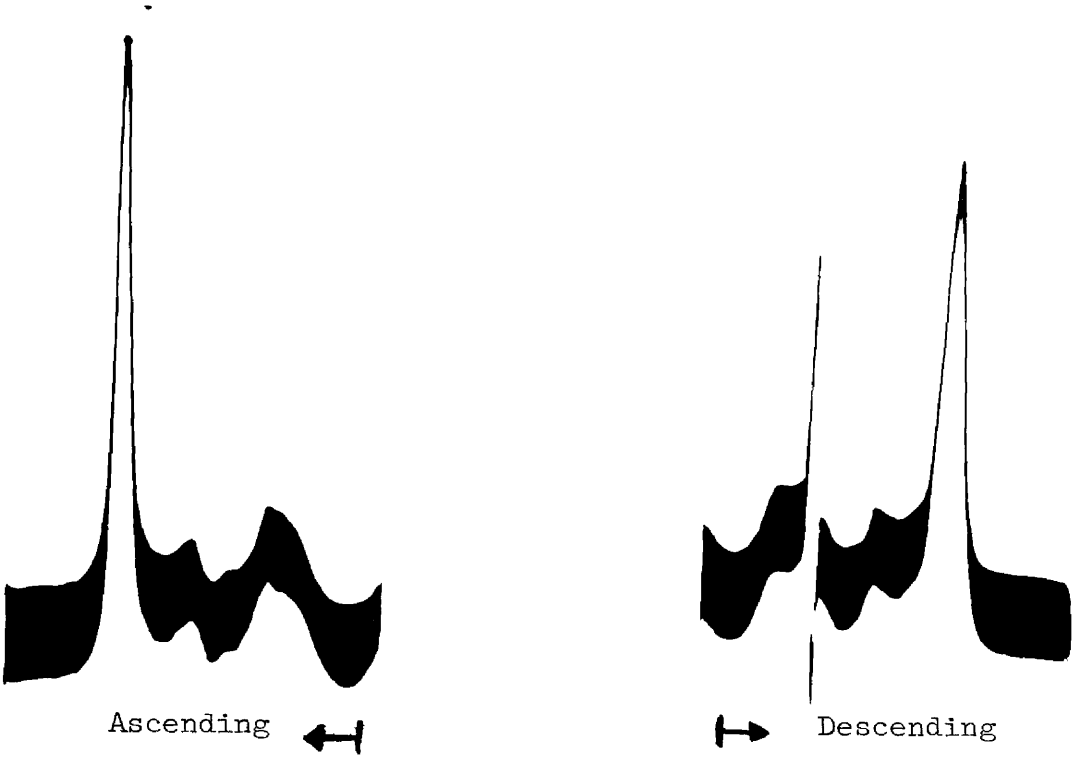


Five weeks post-exposure.

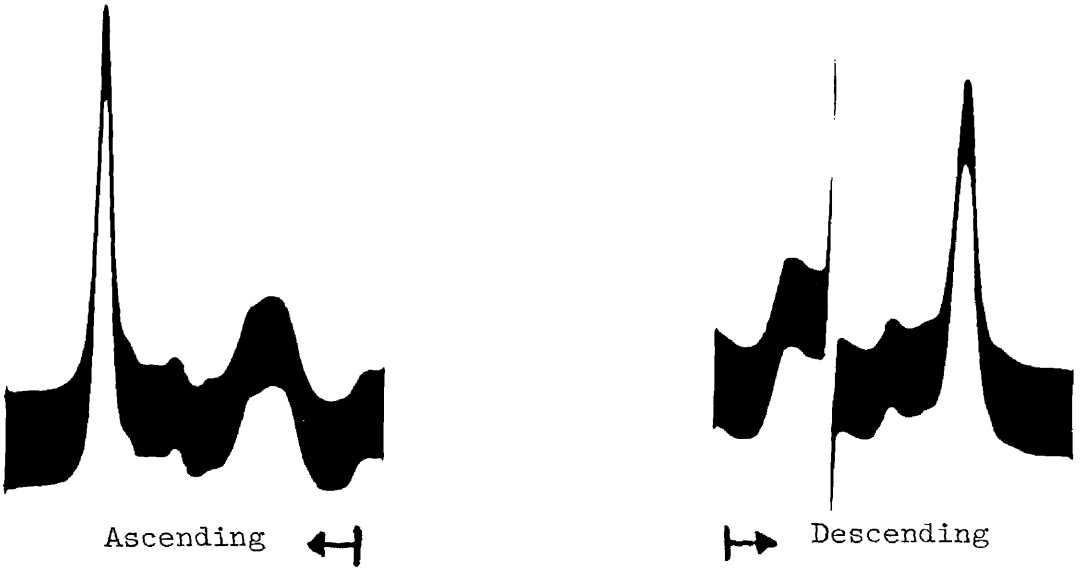


Seven weeks post-exposure.

Fig. 13. Electrophoretic patterns for turkey No. 85194.

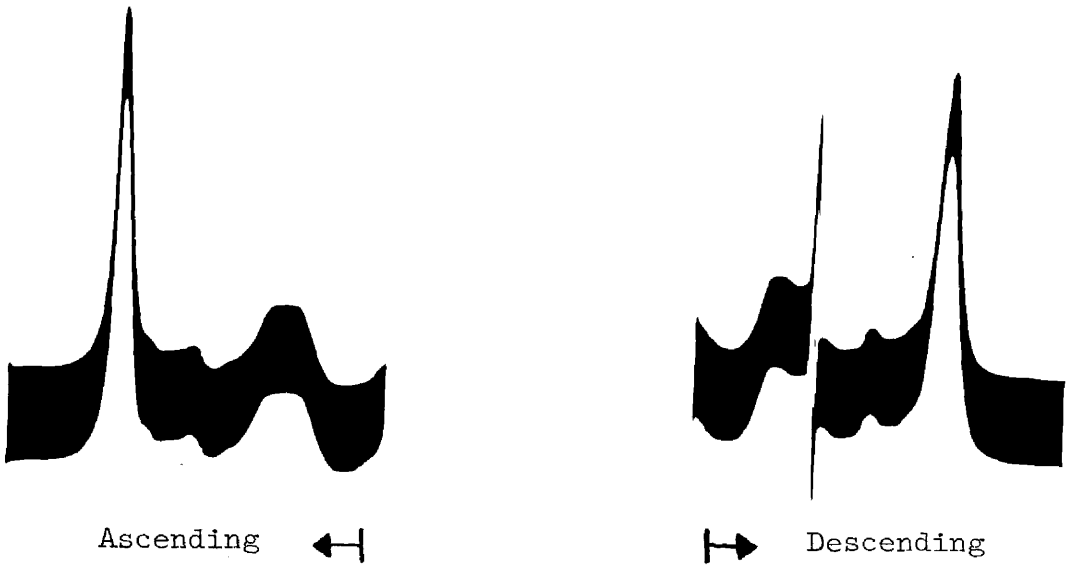


Ten weeks post-exposure.

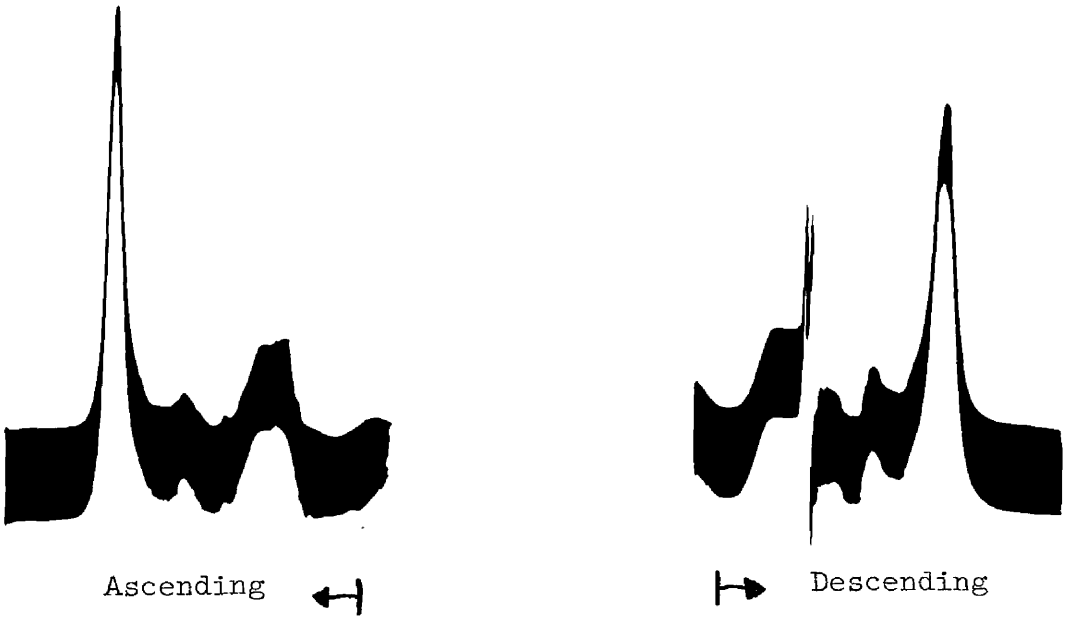


Twelve weeks post-exposure.
(One week post-challenge)

Fig. 14. Electrophoretic patterns for turkey No. 85194.



Fourteen weeks post-exposure.
(Three weeks post-challenge)



Sixteen weeks post-exposure.
(Five weeks post-challenge)

Fig. 15. Electrophoretic patterns for turkey No. 85194.

FIGURE 16.

TURKEY NO. 85 194. THE RELATION OF AGGLUTINATION TITER TO THE RELATIVE PERCENT GAMMA GLOBULIN DURING IMMUNIZATION.

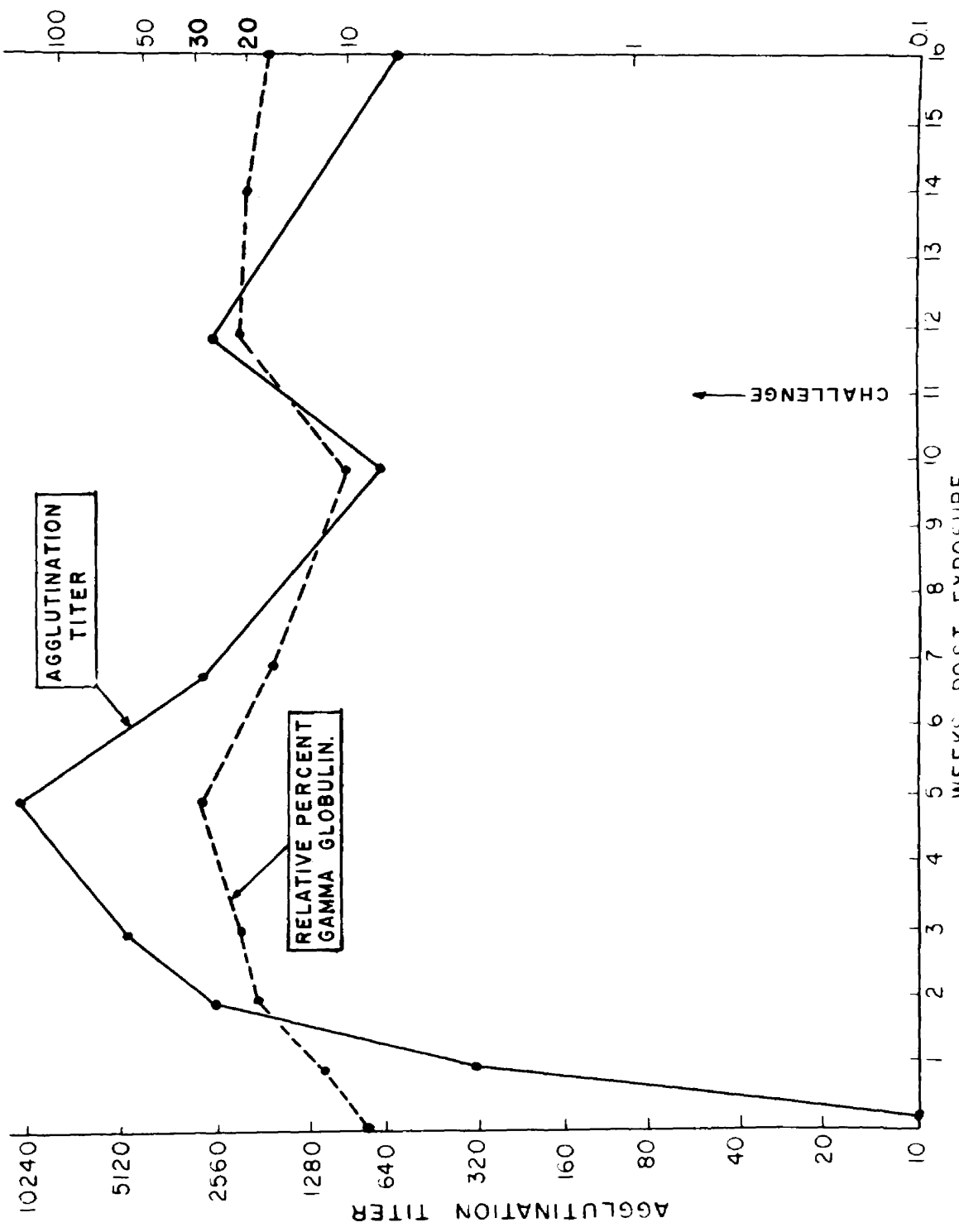


TABLE XI

SERUM TITRATIONS, TURKEY NO. 85195

Time Interval	Antigen	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
Pre-exposure	11	1	-	-	-	-	-	-	-	-	-	-	-
1 week P.E.	11	4	2	1	2	2	2	-	-	-	-	-	-
2 week P.E.	11	2	3	3	4	3	4	3	2	1	-	-	-
3 week P.E.	11	3	3	3	4	3	4	3	2	1	1	-	-
5 week P.E.	11	3	3	4	4	4	4	4	2	2	1	-	-
7 week P.E.	11	3	3	4	4	4	2	1	1	-	-	-	-
10 week P.E.	11	4	4	4	4	2	2	-	-	-	-	-	-

TABLE XII

ELECTROPHORETIC ANALYSES, TURKEY NO. 85195

Time Interval	% Protein	Relative Percent Composition			
		Albumin	Alpha	Beta	Gamma
Pre-exposure	4.21	70.65	8.15	12.50	8.69
1 week P.E.	4.56	56.82	9.09	22.73	11.36
2 week P.E.	4.74	51.43	9.52	24.76	14.29
3 week P.E.	4.30	50.22	11.56	22.72	16.00
5 week P.E.	4.39	49.10	12.61	17.57	20.72
7 week P.E.	3.86	64.46	7.83	16.26	11.45
10 week P.E.	4.39	61.18	9.13	18.27	11.42

FIGURE 17.

TURKEY NO. 85 195. THE RELATION OF AGGLUTINATION TITER TO THE RELATIVE PERCENT GAMMA GLOBULIN DURING IMMUNIZATION.

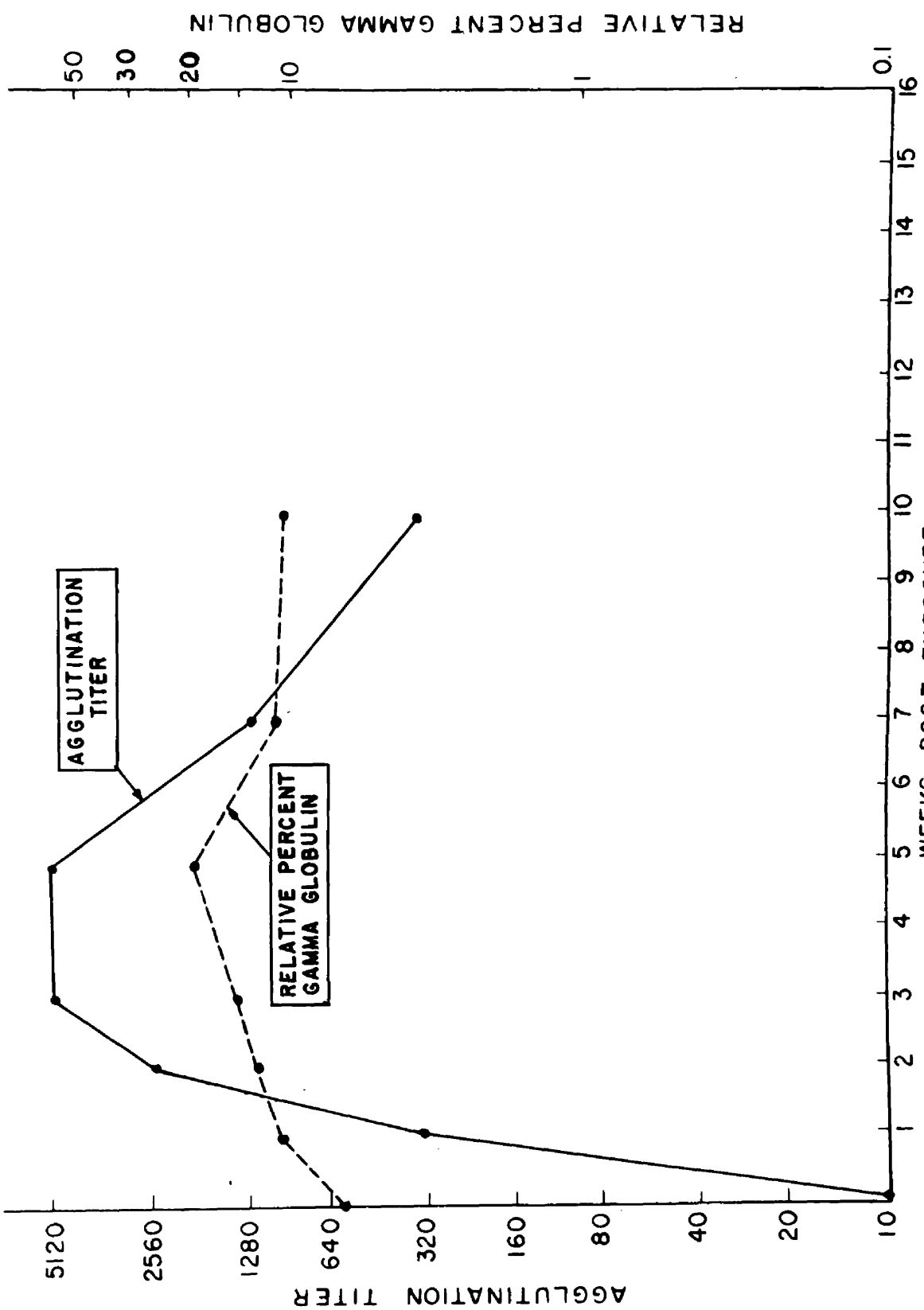


TABLE XIII

SERUM TITRATIONS, TURKEY NO. 85196

Time Interval	Antigen	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
Pre-exposure	11	1	-	-	-	-	-	-	-	-	-	-	-
1 week P.E.	11	4	3	1	2	1	1	2	-	-	-	-	-
2 week P.E.	11	2	3	4	4	4	4	3	2	1	-	-	-
3 week P.E.	11	2	3	3	4	4	4	3	2	2	1	-	-
5 week P.E.	11	3	4	4	4	4	4	4	2	2	-	-	-
7 week P.E.	11	4	4	4	4	4	2	1	-	-	-	-	-
10 week P.E.	11	4	4	4	4	3	1	-	-	-	-	-	-

TABLE XIV

ELECTROPHORETIC ANALYSES, TURKEY NO. 85196

Time Interval	% Protein	Relative Percent Composition			
		Albumin	Alpha	Beta	Gamma
Pre-exposure	4.74	66.49	6.92	13.83	12.70
1 week P.E.	4.56	55.00	10.50	19.00	15.50
2 week P.E.	4.74	50.25	10.05	17.09	22.61
3 week P.E.	5.09	47.22	10.18	12.50	30.10
5 week P.E.	4.83	47.37	13.16	14.91	24.56
7 week P.E.	4.95	61.11	7.58	15.15	16.16
10 week P.E.	3.95	64.65	8.59	15.15	11.61

FIGURE 18.

TURKEY NO. 85196. THE RELATION OF AGGLUTINATION TITER TO THE RELATIVE PERCENT GAMMA GLOBULIN DURING IMMUNIZATION.

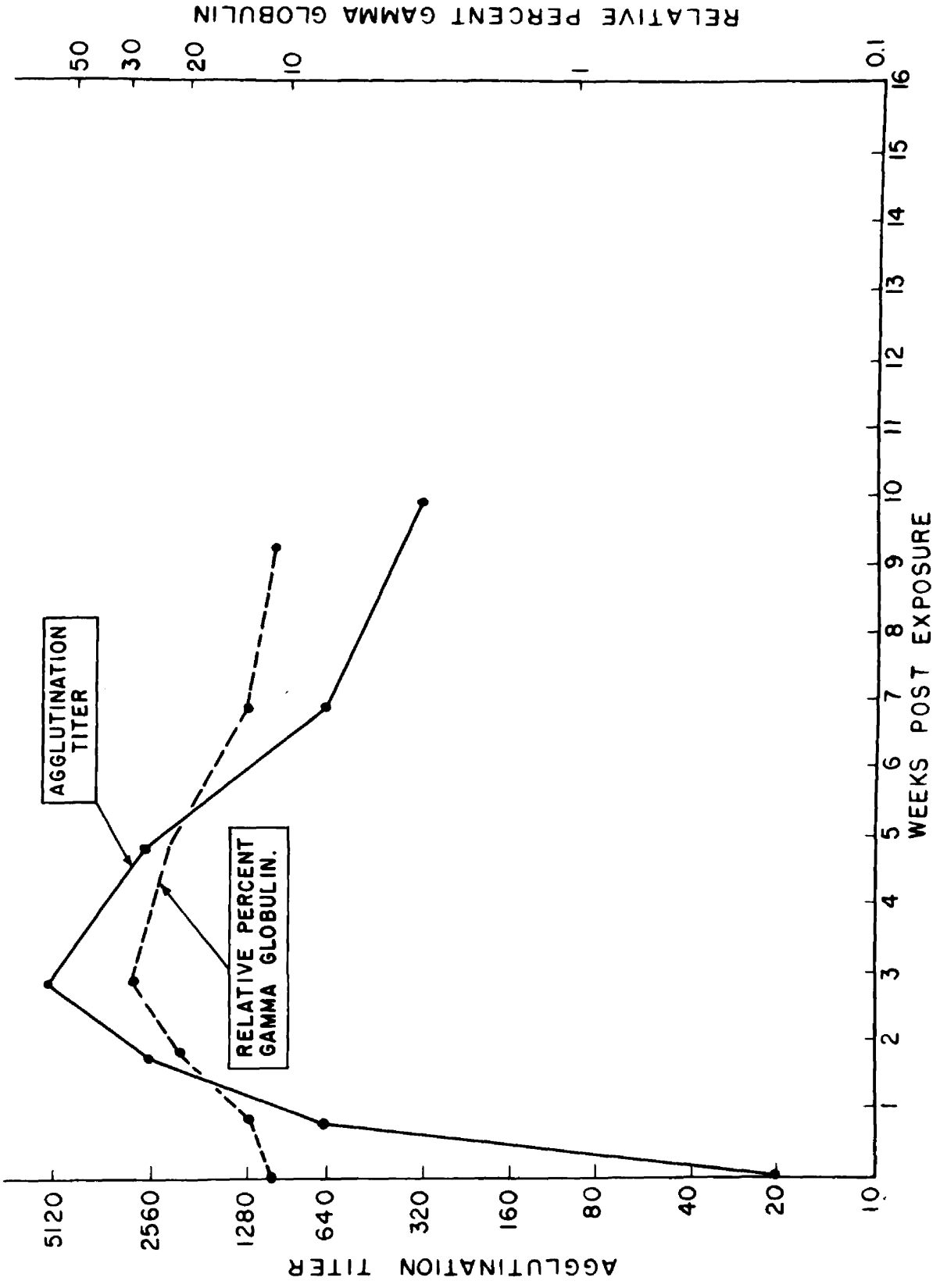


TABLE XV

SERUM TITRATIONS, TURKEY NO. 85197

Time Interval	Antigen	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
Pre-exposure	11	1	1	-	-	-	-	-	-	-	-	-	-
1 week P.E.	11	4	4	1	2	2	2	1	-	-	-	-	-
2 week P.E.	11	2	3	3	4	4	4	3	2	1	-	-	-
3 week P.E.	11	2	3	3	3	4	3	3	3	2	1	-	-
5 week P.E.	11	4	4	4	4	4	4	4	3	2	2	-	-
7 week P.E.	11	4	4	4	4	4	3	2	1	-	-	-	-
10 week P.E.	11	4	4	4	4	3	2	1	-	-	-	-	-
12 week P.E. 1 week P.C.	11	4	4	4	4	3	3	3	2	1	-	-	-
14 week P.E. 3 week P.C.	11	4	4	4	4	4	3	2	1	-	-	-	-
16 week P.E. 5 week P.C.	11	4	4	4	4	3	2	1	-	-	-		-

TABLE XVI

ELECTROPHORETIC ANALYSES, TURKEY NO. 85197

Time Interval	% Protein	Relative Percent Composition			
		Albumin	Alpha 1	Alpha 2	Beta	Gamma
Pre-exposure	4.48	67.01	6.70		13.40	12.89
1 week P.E.	4.65	58.30	9.95		16.58	15.16
2 week P.E.	4.91	55.45	10.00		14.55	20.00
3 week P.E.	5.00	53.50	8.00		13.50	25.00
5 week P.E.	5.35	46.92	11.37		17.06	24.65
7 week P.E.	4.56	63.78	8.16		12.75	15.31
10 week P.E.	3.95	60.64	7.97		17.94	13.45
12 week P.E. 1 week P.C.	5.79	48.09	4.77	7.62	13.33	29.19
14 week P.E. 3 week P.C.	5.44	49.24	4.57	11.17	14.72	20.30
16 week P.E. 5 week P.C.	5.53	59.88	7.19		16.76	16.17

FIGURE 19.

TURKEY NO. 85 197 THE RELATION OF AGGLUTINATION TITER TO THE RELATIVE PERCENT GAMMA GLOBULIN DURING IMMUNIZATION.

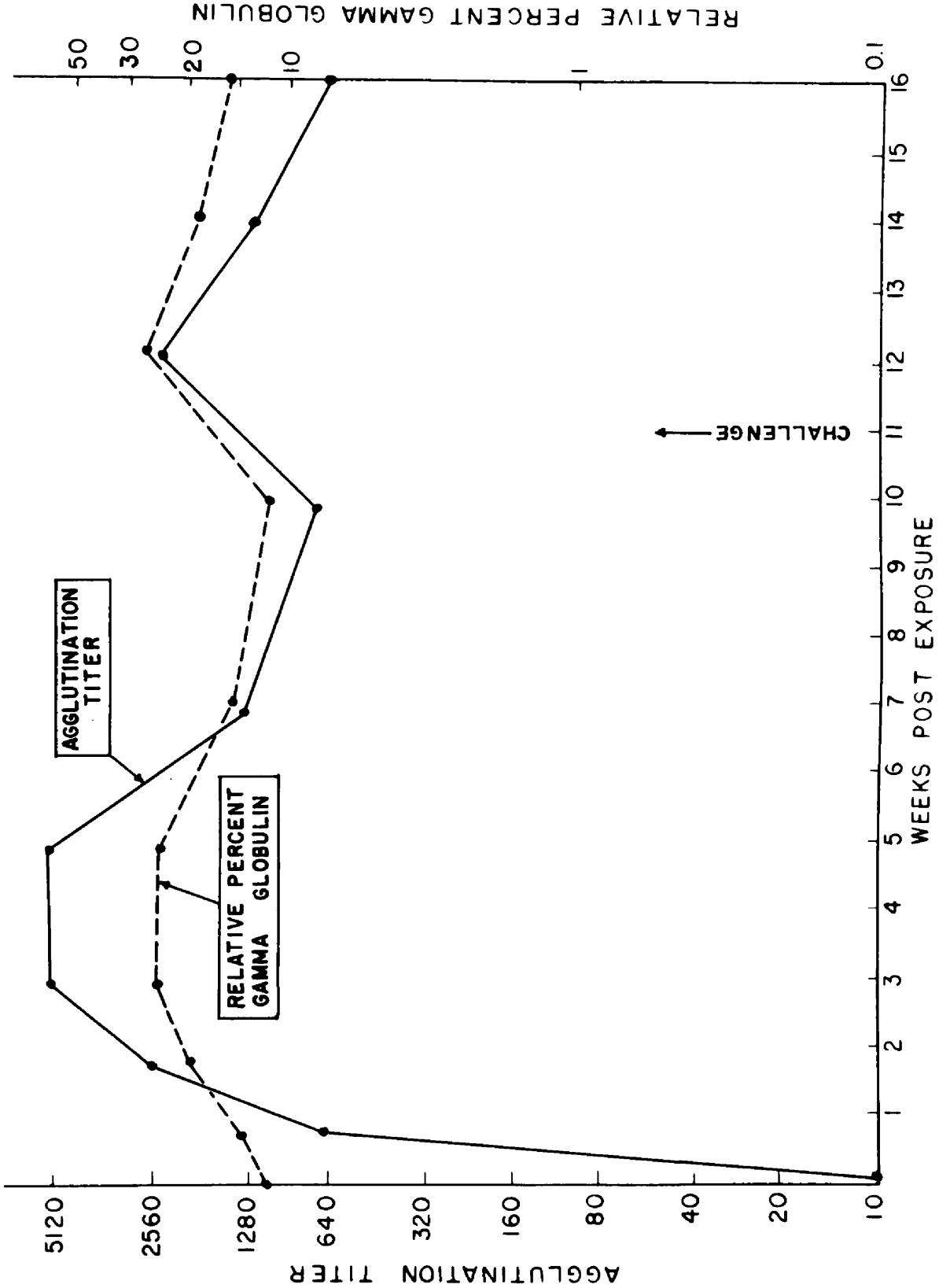


TABLE XVII

SERUM TITRATIONS, TURKEY NO. 85198

Time Interval	Antigen	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
Pre-exposure	11	1	-	-	-	-	-	-	-	-	-	-	-
1 week P.E.	11	4	3	1	2	2	2	1	-	-	-	-	-
2 week P.E.	11	2	3	3	4	4	4	3	2	1	-	-	-
3 week P.E.	11	2	3	4	4	4	4	4	2	1	1	-	-
5 week P.E.	11	3	4	4	4	4	4	4	2	2	1	-	-
7 week P.E.	11	3	4	4	4	4	2	1	1	-	-	-	-
10 week P.E.	11	4	4	4	4	3	2	1	-	-	-	-	-
12 week P.E. 1 week P.C.	11	4	4	4	4	4	4	3	2	1	-	-	-
14 week P.E. 3 week P.C.	11	4	4	4	4	4	4	2	1	-	-	-	-
16 week P.E. 5 week P.C.	11	4	4	4	4	3	3	1	-	-	-	-	-

TABLE XVIII

ELECTROPHORETIC ANALYSES, TURKEY NO. 85198

Time Interval	% Protein	Relative Percent Composition			
		Albumin	Alpha 1	Alpha 2	Beta	Gamma
Pre-exposure	4.18	65.66	7.57		14.65	12.12
1 week P.E.	4.48	56.74	9.77		20.00	13.49
2 week P.E.	5.44	56.50	9.50		12.50	21.50
3 week P.E.	5.18	52.38	9.53		15.66	22.43
5 week P.E.	4.91	55.17	10.78		14.22	19.83
7 week P.E.	4.65	64.47	7.61		14.72	13.20
10 week P.E.	4.39	65.33	8.04		16.08	10.55
12 week P.E. 1 week P.C.	5.14	49.46	6.52	8.70	17.39	17.93
14 week P.E. 3 week P.C.	5.44	55.94	9.90		16.83	17.33
16 week P.E. 5 week P.C.	5.26	59.24	4.74	7.11	13.27	15.64

FIGURE 20.

TURKEY NO. 85 198. THE RELATION OF AGGLUTINATION TITER TO THE RELATIVE PERCENT GAMMA GLOBULIN DURING IMMUNIZATION.

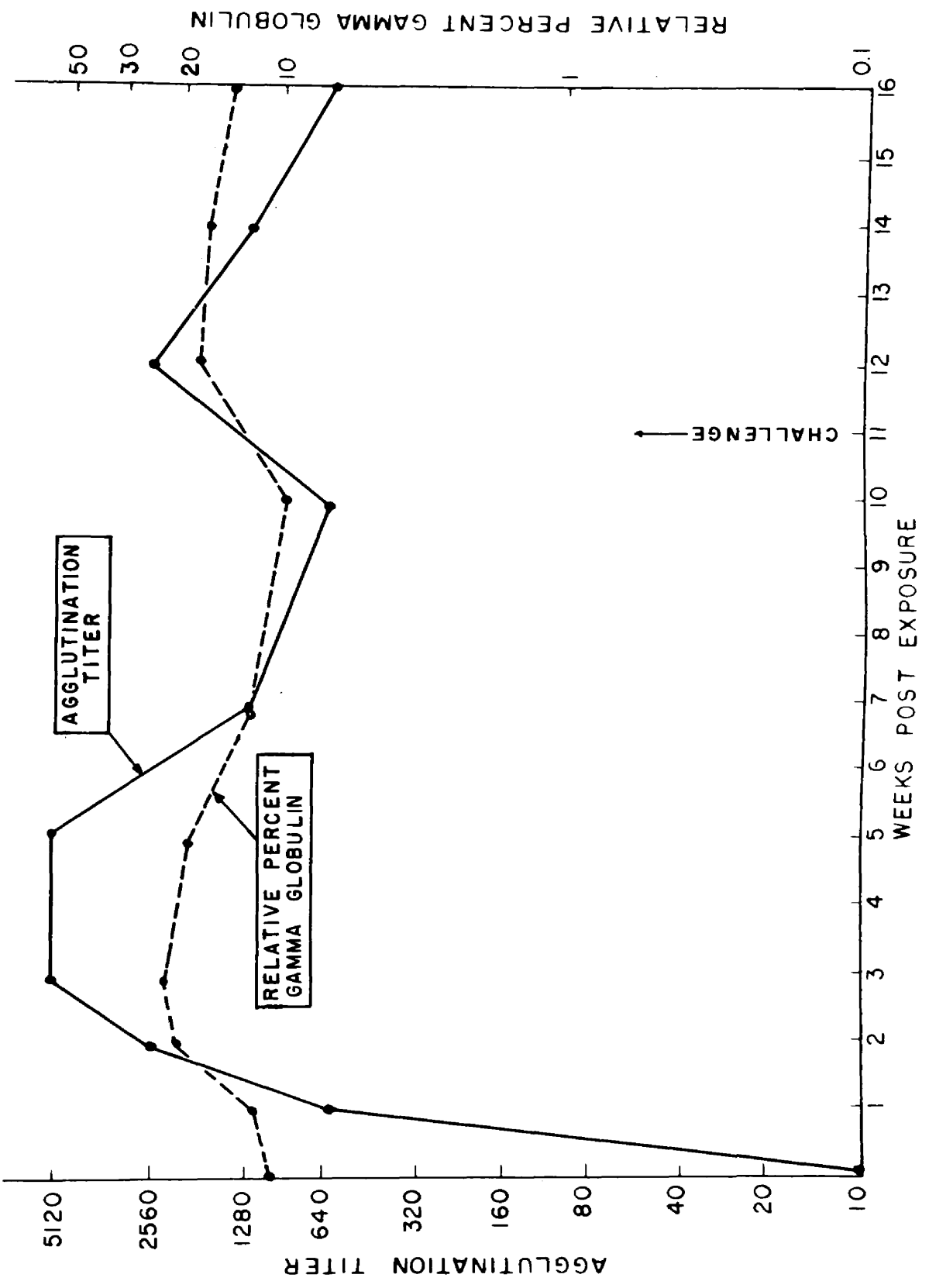


TABLE XIX

SERUM TITRATIONS, TURKEY NO. 85199

Time Interval	Antigen	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
Pre-exposure	11	1	-	-	-	-	-	-	-	-	-	-	-
1 week P.E.	11	4	3	1	2	2	2	2	-	-	-	-	-
2 week P.E.	11	2	3	4	4	4	4	4	2	-	-	-	-
3 week P.E.	11	3	3	3	4	4	4	3	3	1	-	-	-
5 week P.E.	11	3	3	4	4	4	4	4	3	2	1	-	-
7 week P.E.	11	3	4	4	4	4	3	2	1	-	-	-	-
10 week P.E.	11	4	4	4	4	3	2	-	-	-	-	-	-

TABLE XX

ELECTROPHORETIC ANALYSES, TURKEY NO. 85199

Time Interval	% Protein	Relative Percent Composition			
		Albumin	Alpha	Beta	Gamma
Pre-exposure	4.65	66.66	9.05	15:24	9.05
1 week P.E.	4.65	58.54	11.71	19.02	10.73
2 week P.E.	5.35	51.89	13.21	14.62	20.28
3 week P.E.	5.00	Void due to operational failure in apparatus.			
5 week P.E.	4.48	52.94	13.12	12.67	21.27
7 week P.E.	4.00	66.17	8.34	14.71	10.78
10 week P.E.	3.98	66.66	8.85	14.06	10.42

FIGURE 21.

TURKEY NO. 85199. THE RELATION OF AGGLUTINATION TITER TO THE RELATIVE PERCENT GAMMA GLOBULIN DURING IMMUNIZATION.

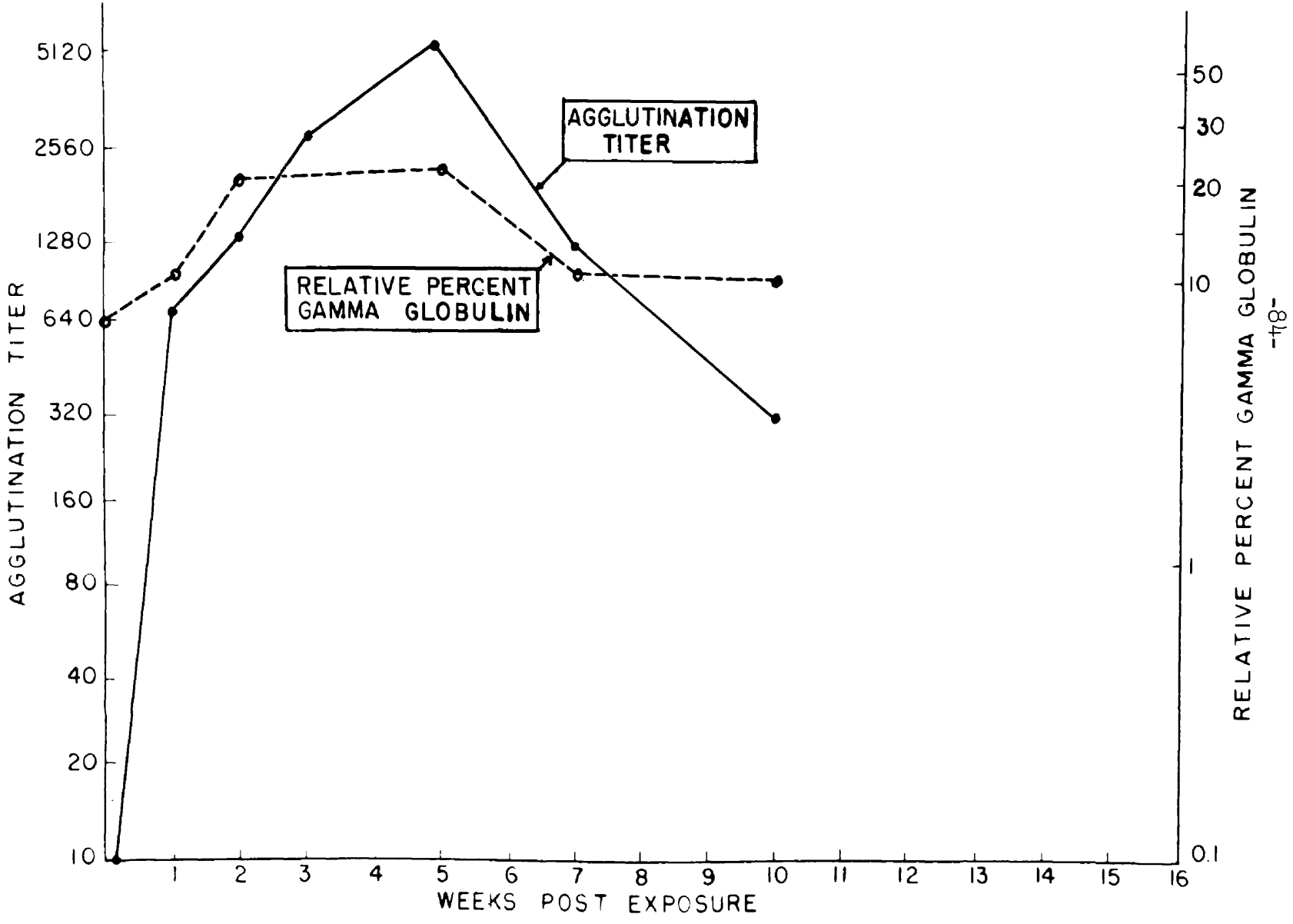


TABLE XXI

SERUM TITRATIONS, TURKEY NO. 85200

Time Interval	Antigen	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
Pre-exposure	11	1	-	-	-	-	-	-	-	-	-	-	-
1 week P.E.	11	4	4	1	2	2	2	2	-	-	-	-	-
2 week P.E.	11	3	4	4	3	3	4	4	3	1	-	-	-
3 week P.E.	11	3	4	4	4	4	4	4	2	1	1	-	-
5 week P.E.	11	4	4	4	4	4	4	4	2	2	1	1	-
7 week P.E.	11	4	4	4	4	4	3	2	1	-	-	-	-
10 week P.E.	11	4	4	4	4	3	2	1	-	-	-	-	-

TABLE XXII

ELECTROPHORETIC ANALYSES, TURKEY NO. 85200

Time Interval	% Protein	Relative Percent Composition			
		Albumin	Alpha	Beta	Gamma
Pre-exposure	4.39	72.90	6.48	12.42	8.10
1 week P.E.	5.00	59.12	9.85	19.21	11.82
2 week P.E.	4.83	50.94	10.38	14.15	24.53
3 week P.E.	5.26	52.89	8.65	12.02	26.44
5 week P.E.	5.00	53.73	9.95	11.44	24.88
7 week P.E.	4.13	60.77	9.95	13.81	15.47
10 week P.E.	4.29	65.00	8.50	15.50	11.00

FIGURE 22.

TURKEY NO. 85 200. THE RELATION OF AGGLUTINATION TITER TO THE RELATIVE PERCENT GAMMA GLOBULIN DURING IMMUNIZATION.

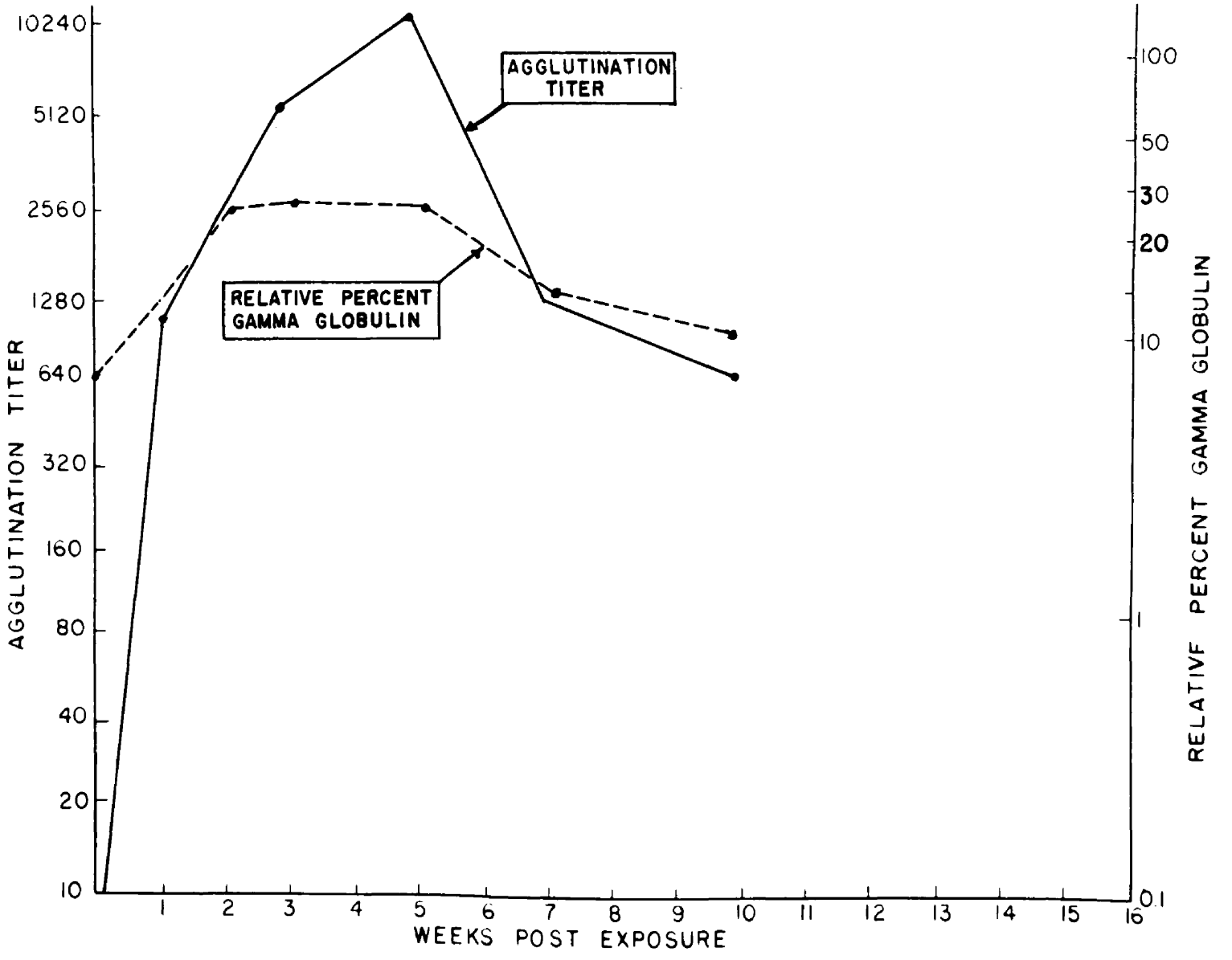


TABLE XXIII

SERUM TITRATIONS, TURKEY NO. 54177

Time Interval	Antigen	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
Pre-exposure	11	1	-	-	-	-	-	-	-	-	-	-	-
1 week P.E.	11	4	2	1	2	2	2	1	-	-	-	-	-
2 week P.E.	11	2	3	3	4	4	3	4	4	2	-	-	-
3 week P.E.	11	3	4	3	3	4	3	3	4	2	1	-	-
5 week P.E.	11	4	4	4	4	4	4	4	3	2	2	-	-
7 week P.E.	11	4	4	4	4	4	4	3	2	1	-	-	-
10 week P.E.	11	4	4	4	4	3	3	2	-	-	-	-	-
12 week P.E. 1 week P.C.	11	4	4	4	4	4	4	3	3	3	1	-	-
14 week P.E. 3 week P.C.	11	4	4	4	4	4	4	3	2	2	-	-	-
16 week P.E. 5 week P.C.	11	4	4	4	4	3	3	2	1	-	-	-	-

TABLE XXIV

ELECTROPHORETIC ANALYSES, TURKEY NO. 54177

Time Interval	% Protein	Relative Percent Composition			
		Albumin	Alpha 1	Alpha 2	Beta	Gamma
Pre-exposure	4.48	67.39	7.14		13.74	11.54
1 week P.E.	4.74	57.08	11.32		19.28	12.32
2 week P.E.	5.18	42.68	13.39		18.83	25.10
3 week P.E.	5.09	45.50	10.58		17.46	26.46
5 week P.E.	5.44	47.70	11.47		17.43	23.40
7 week P.E.	4.21	56.61	4.76	5.29	15.34	17.99
10 week P.E.	3.60	62.64	9.34		17.03	10.99
12 week P.E. 1 week P.C.	5.35	51.46	11.11		11.70	25.73
14 week P.E. 3 week P.C.	4.74	56.91	4.42	6.08	9.94	22.65
16 week P.E. 5 week P.C.	4.65	61.50	8.02		14.44	16.04

FIGURE 23.

TURKEY NO. 54177. THE RELATION OF AGGLUTINATION TITER TO THE RELATIVE PERCENT GAMMA GLOBULIN DURING IMMUNIZATION.

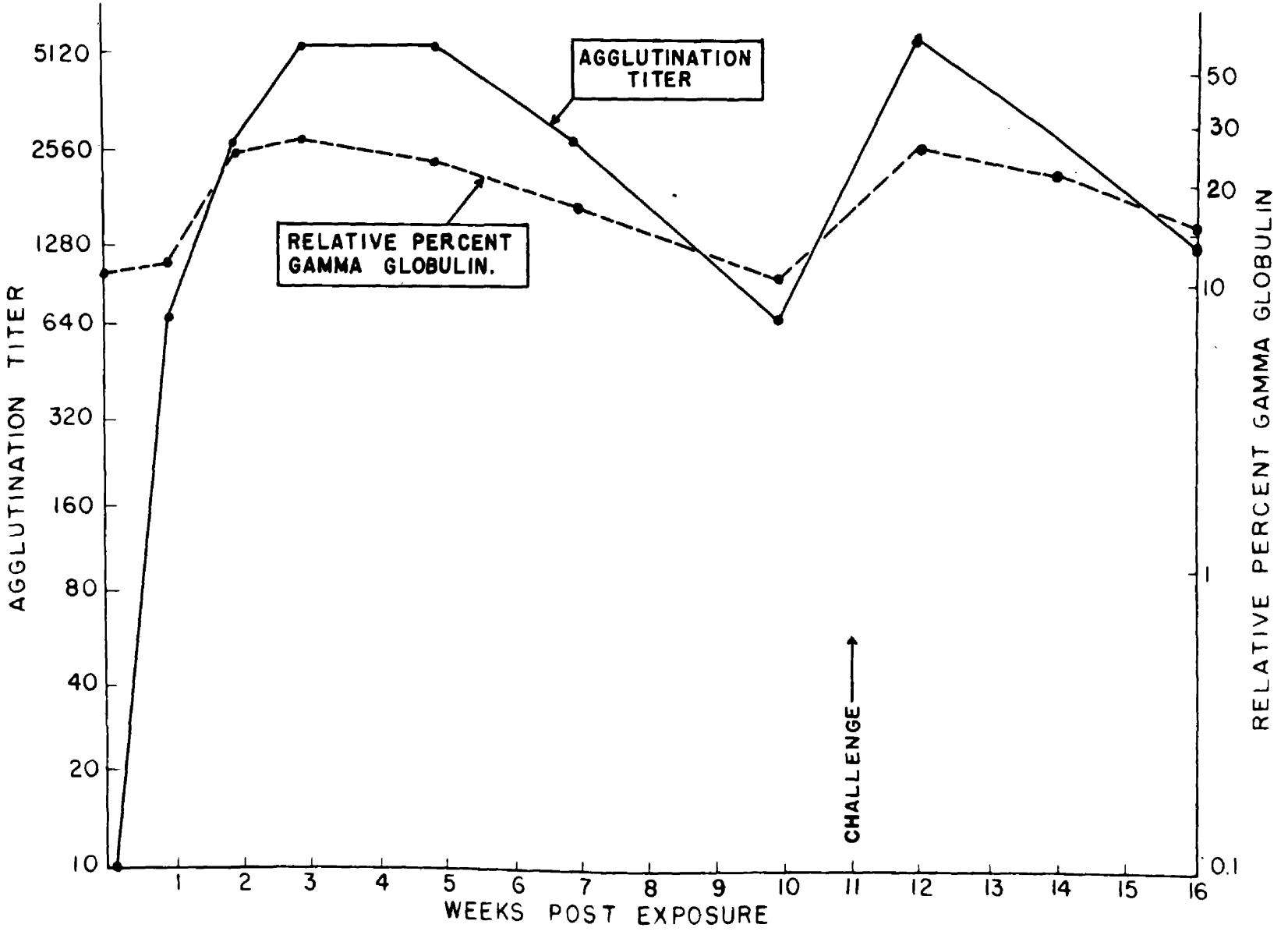


FIGURE 24

THE RELATION OF AGGLUTINATION TITER TO THE RELATIVE PERCENT GAMMA GLOBULIN DURING IMMUNIZATION. (AVERAGE WEEKLY VALUES FOR GROUP IIP 8 TURKEYS.)

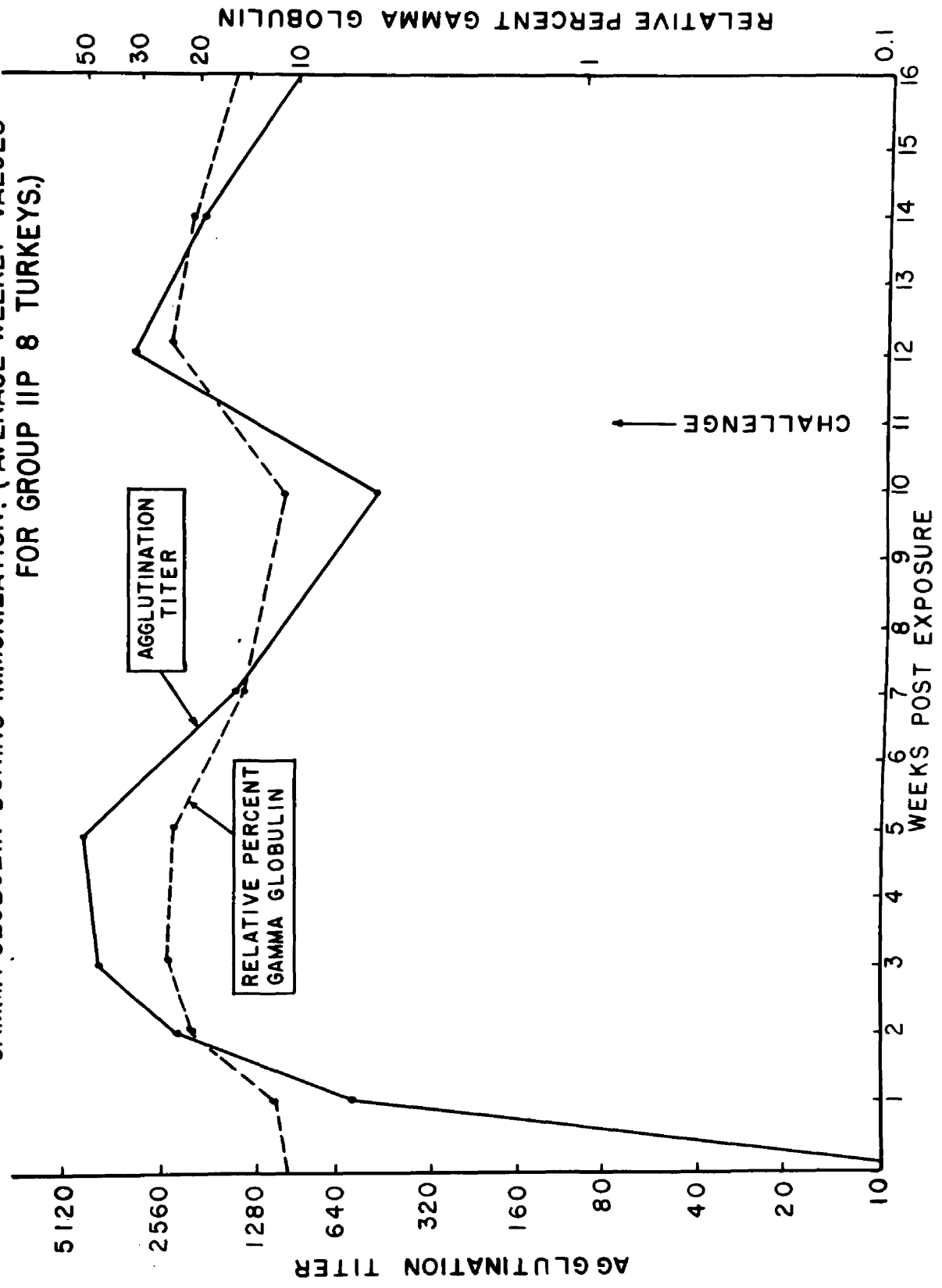


TABLE XXV

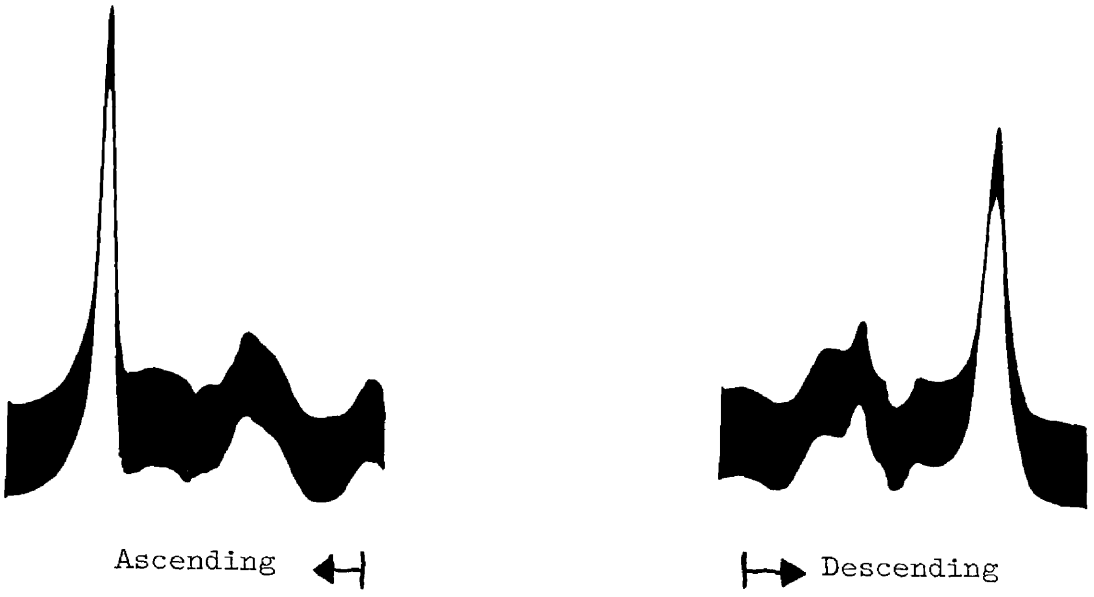
SERUM TITRATIONS, TURKEY NO. 85193

Time Interval	Antigen	Serum Dilutions 10	20	40	80	160	320	640	1280	2560	5120	10240	C
Pre-exposure	11	1	-	-	-	-	-	-	-	-	-	-	-
1 week P.E.	11	4	3	1	2	2	2	1	-	-	-	-	-
2 week P.E.	11	3	3	3	4	4	4	3	2	1	-	-	-
3 week P.E.	11	3	3	4	4	3	4	4	2	1	1	-	-
5 week P.E.	11	4	4	4	4	4	4	4	3	2	1	1	-
7 week P.E.	11	4	4	4	4	4	4	3	2	1	-	-	-
10 week P.E.	11	4	4	4	4	4	2	2	-	-	-	-	-
16 week P.E.	11	4	4	4	3	2	-	-	-	-	-	-	-

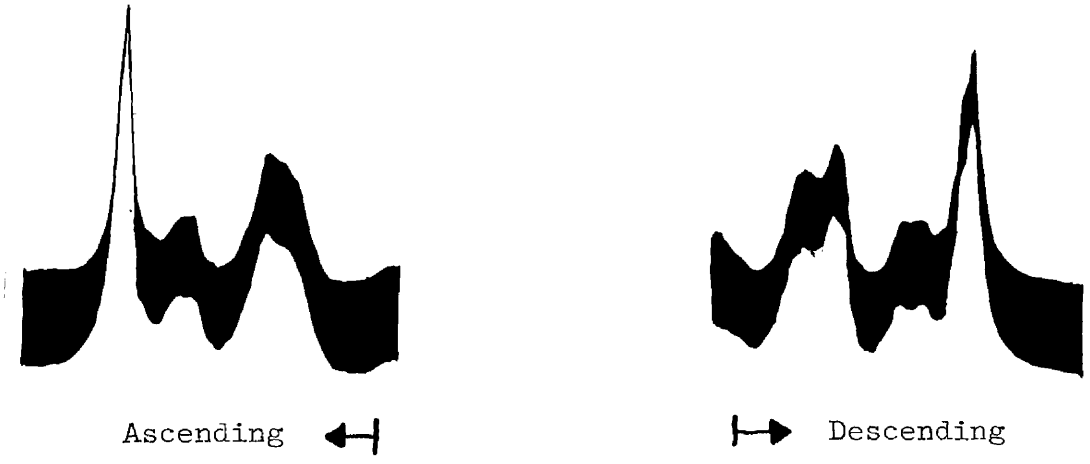
TABLE XXVI

ELECTROPHORETIC ANALYSES, TURKEY NO. 85193

Time Interval	% Protein	Relative Percent Composition				
		Albumin	Alpha 1	Alpha 2	Beta	Gamma
Pre-exposure	4.56	69.03	7.79		12.99	10.39
1 week P.E.	5.88	46.70	5.99	7.18	23.35	16.76
2 week P.E.	5.71	40.85	5.29	7.21	11.54	35.10
3 week P.E.	6.69	30.58	8.74	7.76	24.75	28.15
5 week P.E.	8.00	19.10	7.53	7.53	8.05	57.79
7 week P.E.	7.81	17.58	9.89	6.04	17.03	49.40
10 week P.E.	7.75	19.11	8.92	9.55	12.74	49.68
16 week P.E.	7.81	26.01	5.20	6.36	15.61	46.82

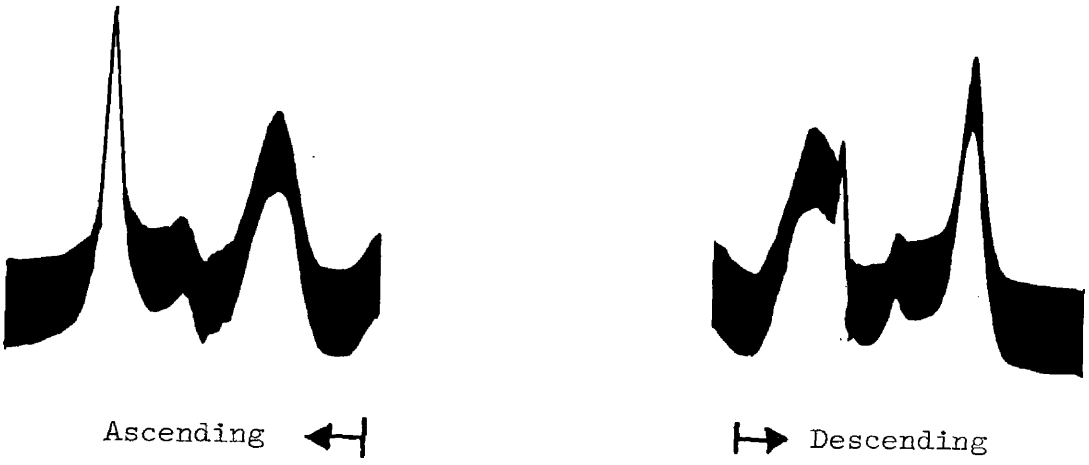


Pre-exposure.

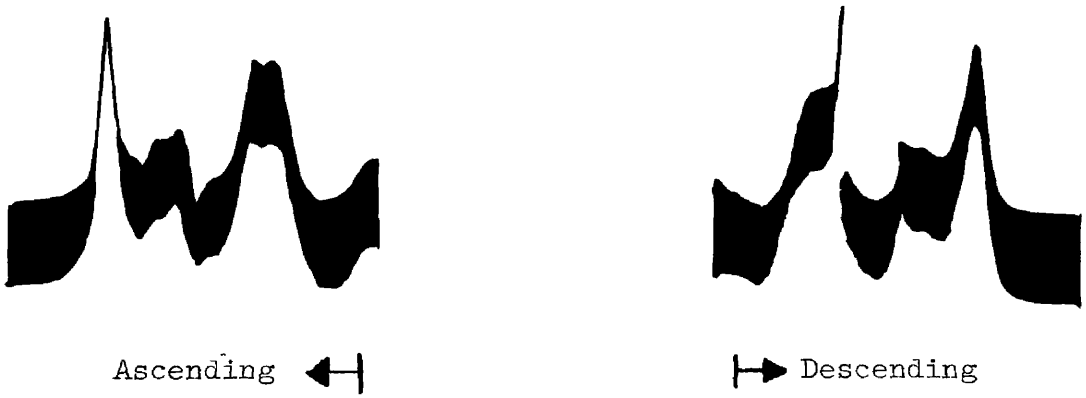


One week post-exposure

Fig. 25. Electrophoretic patterns for turkey No. 85193.

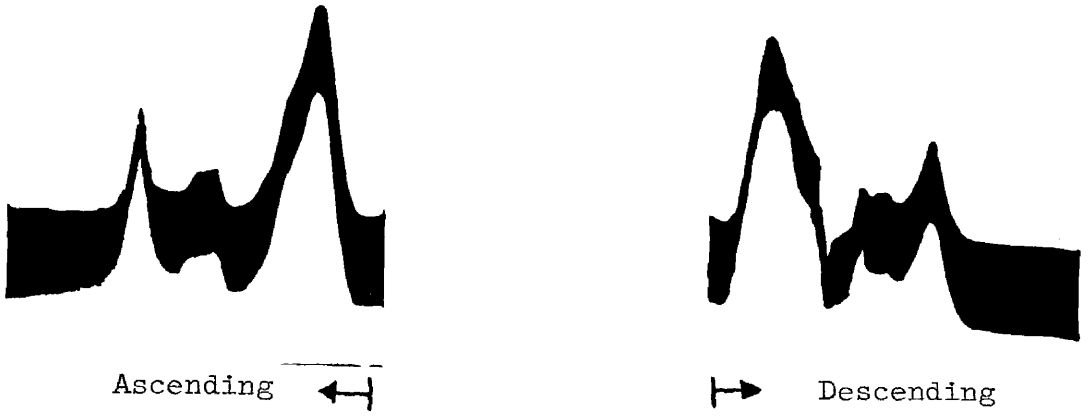


Two weeks post-exposure.

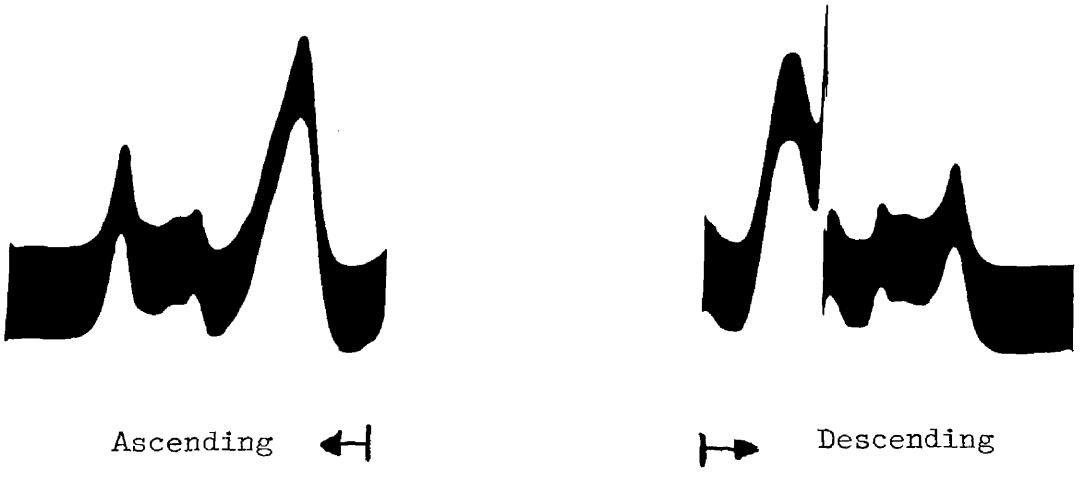


Three weeks post-exposure.

Fig. 26. Electrophoretic patterns for turkey No. 85193.



Five weeks post-exposure.

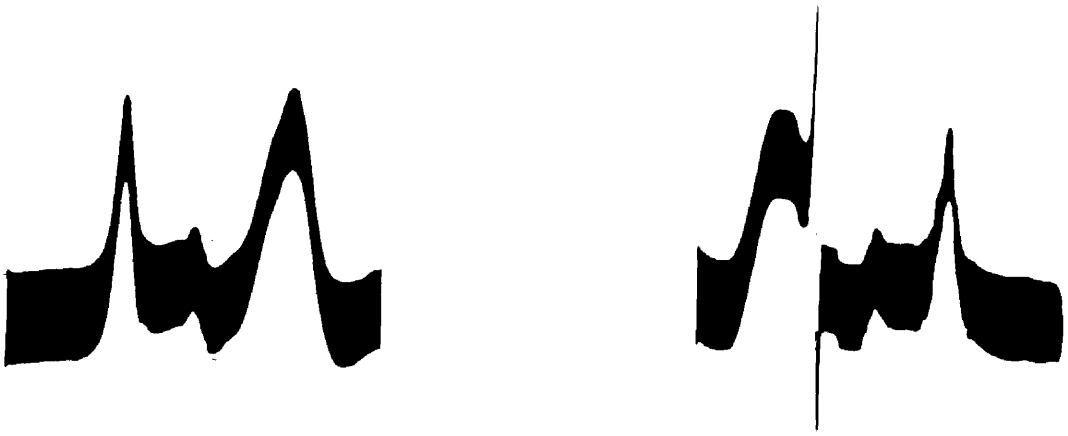


Seven weeks post-exposure.

Fig. 27. Electrophoretic patterns for turkey No. 85193.

Ascending ← → Descending

Ten weeks post-exposure.



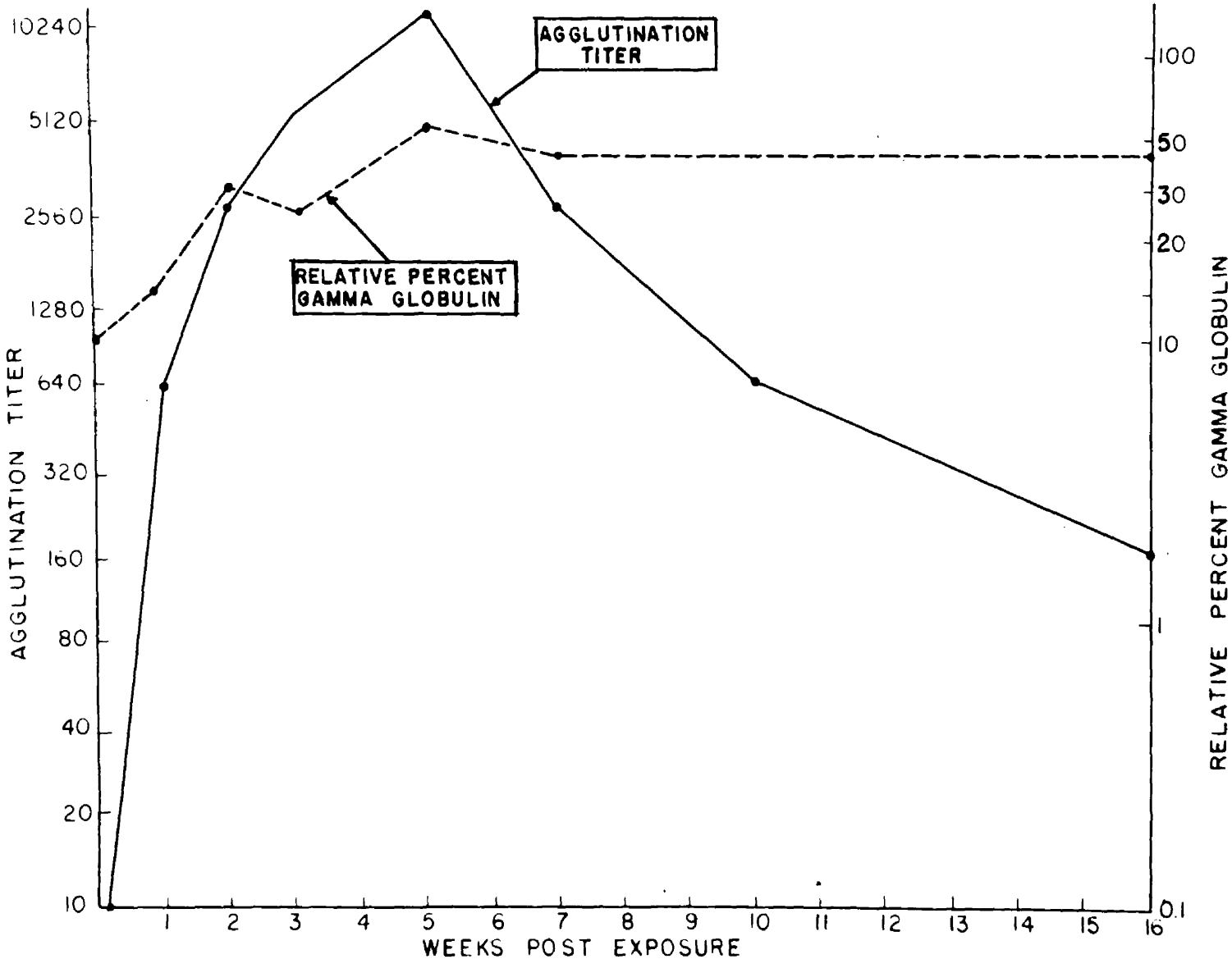
Ascending ← → Descending

Sixteen weeks post-exposure.

Fig. 28. Electrophoretic patterns for turkey No. 85193.

FIGURE 29.

TURKEY NO. 85 193. THE RELATION OF AGGLUTINATION TITER TO THE RELATIVE PERCENT GAMMA GLOBULIN DURING IMMUNIZATION.



DISCUSSION

The movement of protein ions in an electric field in recent years has become an effective means of separation and analysis of mixed proteins through the improvements in the electrophoretic method. In the moving boundary method, different species of proteins are separated because of differences in the speed with which the species moves in the electric field. Because of these different rates of movement, several boundaries are formed, each representing the end of a column of protein of different mobility. The position of each boundary and the number of such boundaries is revealed by changes in the refractive index which in turn is brought about by changes in protein concentration.

The concentrations of the plasma and serum proteins are often altered by disease. Such alterations may be seen qualitatively or quantitatively. The results of the present study have, to a certain extent, shown that such alterations occurred as the result of the antigenic stimulus by S. pullorum. Reference is made to the tables and electrophoretic patterns presented. In these data we observe that definite quantitative changes occurred in certain sera. The marked increase in serum globulins, especially gamma globulin, support the findings of earlier investigators who attached great significance to increases in gamma globulin as a result of inoculation

of bacterial antigens into various species of animals. The results of the present study also revealed in some instances, qualitative alterations, especially in those serum samples where the alpha globulin separated into two peaks on electrophoretic analysis.

The turkeys in Group 11P that were given a challenging inoculation of the test organism revealed the presence of two alpha globulin components when their sera were analyzed. In this regard, the relative degree of immunization seems to have influenced the appearance of the $alpha_1$ peak. The data presented for Groups 11 and 20 show that in those turkeys which revealed a high serum titer, the electrophoretic patterns resolved two alpha globulin peaks.

Some of the more pertinent references which support the observation that pullorum-agglutinating antibodies appear to be associated mainly with the gamma globulin will be discussed.

The data presented in this report indicate that antibodies homologous to S. pullorum appear mainly in the gamma globulin fraction. The results of the tube agglutination tests seem to add support to this observation. With the exception of the initial electrophoretic analyses on Group 10, there exists a direct relation of agglutinin titers to the relative percent gamma globulin. The results of the initial analyses on Group 10 serum samples point out that various strains of the same organism may vary in their ability to stimulate the production of antibodies. Even though rather high agglutinin titers were recorded for this group, they were not as

was the case in the results of the other experimental groups, reflected in the elctrophoretic patterns. It may have been that the technique used by the author in exposing the group was faulty. The serum titers developed in this group of birds were lower than those of the other two groups which were inoculated with living S. pullorum. It would seem more probable therefore, that the antigenicity of strain 10 was of poorer quality than that of the others.

Enders (13) pointed out that all antibodies are not exclusively contained in the gamma globulin fraction. Human gamma globulin on immunological assay was found to contain antibodies reacting with diphtheria toxin, streptococcal erythrogenic toxin, influenza B virus, mumps virus and the H antigen of E. typhosa.

Fell et al., (14) revealed that immune horse sera possessed an increase in the mass of either gamma or beta globulin which was due to the presence of specific antibody within this fraction rather than to an increase of the corresponding normal globulin component. No extra protein component appeared in the serum following immunization.

van der Scheer et al. (69) as a result of electrophoretic analyses of several hyperimmune horse sera concluded that in some of these sera, the antibody was represented by an increase in the amount of the normally present gamma globulin while in others its appearance was accompanied by the development of a new "T" component.

Kedwich and Record (33) while studying some physical properties of diphtheria antitoxic horse sera pointed out that during immunization the immediate reaction in the horse was the production of the gamma globulin antitoxin. Further injections brought about an increased quantity of beta globulin.

The electrophoretic analysis and agglutination-inhibition titrations of anti-influenzal horse serum and its fractions indicate that the influenzal antibodies in it are associated with the gamma globulin (Wyckoff and Rhian (73)).

Kabat (31) stated that antibodies have been known to be associated with the globulin fraction of serum and that antibodies produced in certain animal species have recently been shown to be gamma globulin, although all gamma globulin is not antibody.

Seibert and Nelson (59) presented data on an electrophoretic study of the blood protein response in tuberculosis. These workers observed that in all cases the percent of albumin was lower than in the normal rabbits and progressively decreased in the progression of the disease, while the globulins increased. The first changes which occurred were usually a rise in the alpha globulin fraction and the appearance of an unknown component which was designated as the "X" component. The beta globulin in all cases increased as the disease reached the terminal state. There was also a rise in gamma globulin.

Boyd and Barnard (5) reported results similar in many respects to those presented in this report. While studying the quantitative changes in antibodies and globulin fractions in sera of rabbits injected with several antigen, there was an indication that the amount of antibody produced, when immunization is steadily continued, eventually reaches a maximum and declines. The globulin produced tended to follow the same trend. These workers suggested that increase in globulin with immunization over and above what is known to be antibody may be made up largely of two fractions, first, to other antigens to which the animal had previously reacted (anomnastic reaction) and second, poor quality antibody that fails to be demonstrable.

Bjørnboe (3) reported that both globulin and antibody concentrations showed a tendency to run parallel. Antibody formation is entirely an extra production of globulin. These observations were made while studying pneumococcal and Salmonella vaccines.

Heidelberger (21) stated that antibodies are serum globulins modified in response to the presence of an antigen.

Loeb (35), when studying plasma proteins in health and disease, remarked that the disturbances of serum albumin and globulins in diseased conditions can be classified into two categories -- hypoalbumin and hyperalbumin -- as predominant factors. Abnormalities in albumin always occur in the direction of a decrease whereas

changes in the globulins are associated with increases. High albumin indicates dehydration or abnormally soluble globulin in salt-fractionation. Furthermore, it must be recognized that a disturbance in one fraction is often accompanied by a disturbance in another.

Sam Clemente (57) provided the only available reference concerning electrophoretic studies of pullorum disease. This worker has reported the results of an electrophoretic study of pullorum-agglutinating chicken serum. The percentage concentrations of the protein fractions were relatively irregular for the doubtful and positive serum samples. He also expressed the belief that the pullorum antibody is associated with the gamma globulin fraction.

The preceding references were mentioned to support the observations presented in this report. The references cited all point to the fact that the serum proteins are markedly altered during the course of disease. A decrease in the percentage composition of albumin and an increase in globulin fraction appear to be characteristic of such states. In many instances antibody and globulin formation during immunization appear to be parallel. The gamma globulin and antibody formation reach their peak as the albumin falls, dropping again as the albumin recovers, but not returning to normal in all instances. The rise in gamma globulin may be caused by: (1) a compensatory rise in an attempt to maintain osmotic pressure, (2) the possible formation of antibodies, or

(3) alteration of the relative production and utilization of albumin and globulin (Martin (43)).

The results of this electrophoretic study of pullorum immune turkey serum are broadly in line with results of other authors. They are not peculiar to pullorum disease and are not an absolute diagnostic aid.

Many other workers have reported similar findings with the electrophoretic technique. Many have supported their observations through the use of serological procedures. Further references may be cited (2) (4) (8) (11) (12) (18) (20) (22) (28) (63) (71) which report observations on the alteration of the serum proteins during immunization. Here too, the evidence points to the observations already presented.

The consistent occurrence of gelled sera during the earlier phase of the experiment greatly hampered the attainment of normal amounts of serum. Whether the gelled sera were due to a deficiency of vitamin K only, or partly to the antibiotic in the feed which may have inhibited or destroyed the enteric organisms that synthesize this vitamin, is a matter of speculation which can only be answered by further experimental work, using rations with varying antibiotic and alfalfa concentrations. The fact remains, however, that the increase in alfalfa leaf meal concentration made it possible to obtain normal serum samples.

Gelled and normal serum samples showed no appreciable differences in the relative percentage concentration of the proteins as revealed by electrophoretic analyses of serum samples from the same turkey.

Lucey et al., (41) presented the history of a patient who suffered from purpura with changes in the bone that did not resemble those of myelomatosis. This patient's serum formed a gel on cooling and contained an abnormally large amount of globulin with the mobility of beta globulin. When the protein that formed a gel was removed, this component was much reduced. This protein was shown to cross-react with normal gamma globulin antiserum.

Antibiotics in feed rations exert their influence by altering bacterial conditions in the intestine. Woods (72) expressed the possible ways in which this occurs. She included, (a) inhibition of certain bacteria which tend to destroy or utilize for their own needs, essential nutrients supplied by the food, (b) stimulation of the growth of favorable bacterial types over other types resulting in a greater synthesis of useful nutrients by the former, and (c) less injury to the animals by small amounts of toxic products normally found in the intestinal tract. This worker concluded her remarks by stating that in order to make an accurate evaluation of the possible injurious effects of antibiotics in feeds upon the livestock as well as the human consumer, more long term studies must be made.

Almquist (1) showed that numerous organisms, such as, Escherichia coli, Streptococcus lacticus and Aerobacter aerogenes synthesize vitamin K when cultured on a vitamin K free nutrient medium.

The electrophoretic diagrams of a number of normal animal as well as human sera have been presented by many investigators. These data have been used mainly for comparative purposes. When comparing the patterns of various species it is noted that they vary widely from each other both qualitatively, as regards to the number of distinct components, and quantitatively in relation to the relative percentages of each component. It is observed that all these sera contain the same main components, that is, serum albumin, alpha globulin, beta globulin and gamma globulin. Furthermore, it has been shown that even these proteins are not single components.

Janssen (29) observed that in the sera of normal man, cattle, sheep, horse and rat, two peaks of alpha globulin are present, which may also be present in the donkey and the chicken. The relative amount of the serum components in normal animals of different types may vary considerably. A comparative study of the serum composition of these animals shows clearly that a common plan of production of serum proteins is present. Janssen employed the standard phosphate buffer for the suspending medium and the results

of fourteen human serum analyses showed the following average relative amounts of serum proteins measured under these conditions: 60 percent serum albumin; 7 percent $alpha_2$ globulin; 12 percent beta globulin and 21 percent gamma globulin. The report of Kedwick (32) on the electrophoretic analysis of normal human serum reveals values in accord with Janssen on the relative percent composition of serum proteins.

Hoch (26) made a comparison of electrophoretic patterns of human sera obtained in phosphate and diethylbarbiturate buffer. The differences in the apparent relative proportions of the components of serum in phosphate buffer at pH 8.8 and in diethylbarbiturate buffer of pH 8.6 were less than three percent of the total area in 33 and less than 2.1 percent in 27 out of 34 cases. The differences are not of clinical significance and phosphate at pH 8.8 and an ionic strength equal to 0.15 may be used in place of diethylbarbiturate buffer at pH 8.6 and ionic strength one-tenth.

The figures obtained by Reiner et al. (52), for the relative protein distribution in normal human serum are as follows: albumin 56.8 ± 3.0 percent, $alpha_1$ globulin 7.2 ± 1.2 percent, $alpha_2$ globulin 8.7 ± 1.5 percent, beta globulin 12.8 ± 2.3 percent, and gamma globulin 14.4 ± 2.4 percent. These values were obtained from electrophoretic studies of serum samples from 20 professional blood donors and 60 family group donors.

The relative percent composition of normal turkey serum differs, as would be expected, from these values given for human serum. There are numerous other references in the literature concerning the subject of normal electrophoretic analyses and relative percent composition of human serum. The references cited are typical and as pointed out earlier, these data are used mainly for comparative purposes, as in this instance, the comparison of the values given for human serum with those of the turkey.

It should be pointed out that there is advantage in precision, in using the average of the values for relative composition as determined from the ascending and descending patterns. However, Foster et al. (15) showed that the advantage of this procedure is not too great and in general, the added work of tracing and planimetering both sides would hardly appear worthwhile. These workers also stated that often-times the average is less precise than either the values for the ascending or descending side.

Sanders et al. (58) obtained values for the relative percent composition of normal chicken plasma, which are: albumin 46.8 percent, $alpha_1$ and $alpha_2$ globulins 17.9 percent, beta globulin 11.3 percent, fibrinogen 13.5 percent and gamma globulin 19.4 percent. These figures are widely separated from those given in the results for turkey serum. These workers reported that the total protein of normal chicken serum varied from 2.19 to 3.74 grams

per 100 ml. The values presented in this report for total protein of normal turkey sera ranged from 3.96 to 4.91 gms/100 ml.

A comparison of the mobilities of normal chicken and turkey serum proteins further demonstrates the differences in the proteins of these avian species. The mobility of turkey serum albumin has been established in the present work as 5.6 and for the chicken the same serum component has 5.9 as its mobility. Turkey alpha globulin has a value of 3.8 while the counterpart in chicken serum has a mobility of 4.7. The mobility of beta globulin in turkey serum was found to be 2.4, while that for the chicken is 3.5. Finally, gamma globulin has a mobility of 1.6 for turkey serum, and 2.0 in the chicken serum.

The abnormal serum patterns of turkeys No. 54185 and No. 85193, due to the effects of injuries along with immunization are of particular interest. Some references were found in the literature concerning electrophoretic studies of qualitative and quantitative changes in the serum protein as a result of various types of injury. Chanutin and Gjessing (7) reported the results of analyses of serum of injured dogs. Injuries were produced by heat, cold, turpentine and bone fracture. Hot water caused a decrease in serum albumin and a rise in alpha globulin. Dry carbon dioxide produced a similar effect. After the subcutaneous administration of turpentine on backs of dogs, a decrease in albumin and an increase in alpha globulin

and gamma globulin was pronounced during the experimental period of seven days. The greatest changes were seen on the third day after injection. These workers aseptically produced a fracture of the tibia and then applied a cast. Trauma was minimal. During a 17-day observation period the albumin concentration did not change; however, both $alpha_1$ and $alpha_2$ globulins increased. The combined beta and gamma fractions remained fairly constant.

Injuries were produced in dogs by using vesicants, heat and turpentine in an attempt to determine the type of protein responsible for the abnormal patterns seen in dogs after these injuries occurred. Gjessing et al. (16), in reporting the results of these experiments, found an alpha globulin fraction containing amounts of lipid in the serum of dogs subjected to subcutaneous inoculation with turpentine. A carbohydrate-rich fraction with the mobility of alpha globulin was present in the animals injured by turpentine. The data further showed that there was a large difference in both the quality and quantity of the lipids associated with protein fractions as a result of injury. These changes in the lipid constituents may indicate a redistribution of the lipid components as well as the introduction of new lipoproteins.

In experiments with goats of the same nature as those just discussed, Gjessing et al. (17) reported that only a slight change in the serum proteins was brought about. These workers, in conclusion, noted that results obtained for the proteins of goat plasma

cannot be applied to other species. This conclusion appears valid in light of the results observed in the present study on the effects of injuries. Although there was a marked decrease in serum albumin in both cases under consideration with an increase of serum alpha globulin, the most outstanding observation was the tremendous increase in the gamma globulin fraction. This was especially true in the case of turkey No. 85193 which had suffered a traumatic injury. The great increase in gamma globulin persisted over the sixteen-week observation period.

The diagnostic value of the Tiselius electrophoresis method does not lie in the absolute specificity of electrophoretic patterns for specified diseases. The protein spectrum in plasma and serum is the resultant of a host of factors concerned with the formation, the interaction, and the destruction of the individual components. There exists a close correspondance between the blood, the protein system and the physiological state of the individual as a whole, rather than to a given pathological system.

SUMMARY

Studies are presented on electrophoretic analyses of normal and pullorum immune turkey serum. The relative percent composition and mobilities of the normal serum proteins were established. These values differed somewhat from those reported for other species. Pullorum antisera revealed rather characteristic alterations from the normal.

The tube agglutination test was carried out in conjunction with electrophoretic analyses for each serum sample. A somewhat definite relation was shown to exist between the degree of immunization and the relative percent of serum gamma globulin. In most instances where high agglutinin titers existed, there also was observed a high relative percent of gamma globulin. As antibody titers decreased there was a decrease in gamma globulin fraction.

The influence of strains of S. pullorum on the quantity of antibody production was considered, along with the effect of the route of inoculation on the development of antibody.

The occurrence of gelling serum during the earlier phase of the experiment greatly interfered with the collection of normal yields of serum.

The appearance of weak, if any, prozones did not add to our knowledge of this phenomenon.

CONCLUSIONS

1. S. pullorum antigens are capable of bringing about qualitative and quantitative alterations in the electrophoretic pattern of previously normal turkey serum.

2. The antibodies specific for S. pullorum appear to be associated with the serum globulins, especially gamma globulin fraction.

3. Agglutinin titers exhibit a close relation to the relative percent gamma globulin.

4. Secondary inoculations with S. pullorum can cause an accelerated secondary response in serum gamma globulin.

5. Gelling of turkey serum may be eliminated by an increased concentration of alfalfa leaf meal in the ration.

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