# AN ELECTROPHORETIC AND CHEMICAL FRACTIONATION STUDY OF SERA FROM RATS IMMUNIZED AGAINST THE NEMATODE, NIPPOSTRONGYLUS MURIS

By

Stanley Edward Leland, Jr.

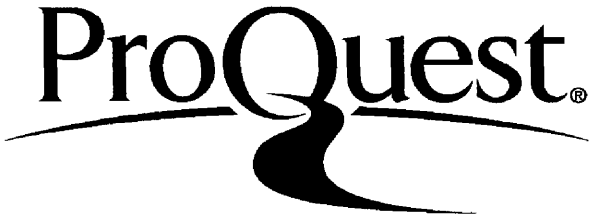
A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Bacteriology and Public Health

1953



## DEDICATION

The author wishes to dedicate this thesis to his parents, Mr. Stanley Edward Leland, Sr., and Mrs. Kathryn Miklas Leland.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. William D. Lindquist who, after the untimely death of Dr. Phillip A. Hawkins, has acted in a supervisory capacity during the remaining course of this study. The author is indebted to Dr. Hans A. Lillevik for offering his knowledge of the electrophoretic techniques and certain chemical aspects of the problem. To Mrs. Stanley E. Leland, Jr. the author is indebted for her untiring aid in preparing the manuscript. Acknowledgment is also due to Michigan State College for providing the author with the opportunity to make this study.

# VITA

Stanley Edward Leland, Jr.

candidate for the degree of

Doctor of Philosophy

Dissertation: An Electrophoretic and Chemical Fractionation Study of Sera from Rats Immunized Against the Nematode, Nippostrongylus muris.

Outline of Studies:

Major subject: Parasitology
Minor subjects: Biochemistry, Animal Pathology

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Experience: Graduate Teaching Assistant, Michigan State College, 1950-53, Member United States Navy, 1945-46

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# AN ELECTROPHORETIC AND CHEMICAL FRACTIONATION STUDY OF SERA FROM RATS IMMUNIZED AGAINST THE NEMATODE, NIPPOSTRONGYLUS MURIS

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AN ABSTRACT

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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Approved by William D. Sudgwick

The purpose of this study was: (1) to investigate the changes in the host serum protein components which occur as a result of infection with Nippostrongylus muris (2) to study these changes progressively through the hyperimmunization period and (3) to determine the conditions that cause these serum protein changes.

Hyperimmunization was attained by subjecting the rats to increasing numbers of larvae at two week intervals. The actual sequence of injections was 1,000; 2,000; 5,000; 10,000; 20,000; 50,000 larvae. Serum analysis were made on various days between the injections by means of the Perkin-Elmer Tiselius electrophoresis apparatus and fractionation with sodium sulfite. The following serum changes were noted in the infected animals when compared with uninfected litter mates:

(a) The total protein increased as the number of larvae injected was increased.

(b) The beta and total globulin increased as the number of larvae injected increased, and diminished when the number of larvae injected was reduced, or when the larval injections were stopped.

(c) In five out of six two week intervals between injections the beta globulin of the infected animals was slightly higher after two weeks than after one week following injection. The beta globulin increase was electrophoretically determined in veronal buffer at pH 8.6 and phosphate buffer pH 7.4. This minimized any anomalous effects that might occur at a particular pH or with a particular buffer.

(d) The albumin/globulin ratio decreased as the number of larvae injected was increased and continued to increase when the larval injections were halted.

(e) The most striking serum protein changes occurred around seven days after the 50,000 larval injection.

(f) The gamma globulin content showed no significant increase or decrease.

(g) The mobilities of the protein components showed a decrease in the majority of infected serum samples.

(h) Serum from rats, which had received injections of the supernatant from the larval suspension, was essentially normal. This indicated the material in the inoculum other than the larvae did not cause the observed serum protein changes.

These serum changes are interpreted as responses of the host to injury or trauma by migrating larvae, although, the possible existence of an antibody can not be ruled out. Since rats were bled only once during the course of the experiments, serum depletion changes were eliminated.

Living larvae, saline extracts of living larvae, and ground larvae, were used as antigens and exposed to immune sera. Antigen-antibody combination, as indicated by the precipitation of a serum fraction, was not observed.

Utilizing bovine albumin as a standard, a modification for determining total protein of rat sera by the biuret reaction was established. A modification for fractionating rat sera into albumin and globulin, utilizing a 24 percent sodium sulfite solution, was devised.

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# INTRODUCTION AND HISTORICAL REVIEW

The nematode, Nippostrongylus muris, was first described by Yokogawa in 1920 as Heligmosomum muris. The adult worm (3-4 mm in length and 0.085-0.1 mm thick) is a natural parasite in the small intestine of the wild rat.

In 1922 Yokogawa worked out the life cycle in detail, but he overlooked one of the larval molts which was later observed by Lucker (1936). Thus, N. muris undergoes four complete molts and does not differ in any essential respect from the development reported for other skin-penetrating nematodes.

The third stage filariform larvae actively penetrate the skin of the rat. They are then carried by the blood stream to the lungs and here, break through the alveolar epithelium. After a period of development in the lungs the larvae migrate up the trachea, down the esophagus and through the stomach to the small intestine where they develop to maturity. The females begin to lay eggs 5 to 6 days after infection. The eggs require a 4 to 5 day developmental period outside the host to reach the infective third stage.

Africa (1931) and Schwartz, Alicata, and Lucker (1931) first reported that rats, after recovery from an initial infection, are relatively resistant to subsequent infections. Furthermore, Schwartz et al. noted that many of the larvae from the second infection were retained in the lungs of the rats.

Chandler (1932) concluded from his investigations that the resistance acquired as the result of: (1) previous infections, (2) inoculation

with the living larvae of a related worm (Longistriata) and (3) inoculation with killed larvae, is similar in nature in each case. He states, "It appears not to be due to the development of a local lethal effect on the worms in the intestine, but to a more general effect, acting through the blood, which tends (1) to interfere with reproduction, (2) to cause a stunting in growth, (3) to inhibit development to the adult stage, (4) to prevent successful establishment in the intestine, and (5) to bring about the elimination of worms already established."

Graham (1934) showed that the degree of acquired resistance developed is associated with the size of the initial infection, i.e., the heavier the primary worm burden, the greater the resistance developed. Repeated exposure to increasingly large numbers of larvae at weekly intervals resulted in marked resistance as judged by egg counts.

Chandler (1935a) in one out of four experiments was able, by the injection of immune serum, to demonstrate passive immunity as evidenced by the effect on the prepatent period, number of worms established, egg output, rate of inhibition of egg production, and rate of loss of worms. Since he was unable to duplicate the passive transfer in the other three experiments he attributes the abnormally high egg and worm counts in the controls of this positive experiment to some unknown factor. These abnormally high counts in the controls, when compared with the lower counts of the rats injected with immune sera, suggested passive immunization. In experiments with parabiotic twins in which one of the pairs was immunized either before or after being joined to its mate, Chandler (loc. cit.) showed, that although proof of a good intermingling of blood

was obtained, no immunity at all was conferred on the previously uninfected member of the pair. Chandler thus states, "The conclusion is inevitable, therefore, that the immunity is local, a property of the intestinal mucosa."

Chandler (1935b) showed there was a progressive increase in the resistance to superinfection as the interval between the two infections is increased from 7 to 14 or 15 days. After the fifteenth day the resistance developed was gradually lost until by the thirtieth day there is even less resistance than was manifested after an interval of 7 days. This resistance was demonstrated by a falling off in numbers of worms acquired, a gradual decrease in the percentage of worms which succeeded in passing the final molt, and a gradual decrease in the amount of growth of the worms.

Chandler (1936) found that worms could resume egg-laying or could grow and begin egg-laying when transferred from the intestines of immune rats to those of normal rats. Chandler feels this supports the hypothesis that the immunity of white rats to N. muris is due to the development of anti-enzymes which prevent the worms from digesting and assimilating the host's protein.

When Spindler (1936) transferred young and adult worms by duodenal tube to the intestines of normal rats he found that the rats were immune to a second infection by the usual cutaneous route. Since only small numbers of larvae were recovered from the lungs he concluded that lung-migration was necessary for inhibition of development in the lungs, but that intestinal infection by itself could give a local intestinal immunity.

Sarles and Taliaferro (1936) studied the migration and development of the parasite in normal and hyperimmunized rats at short intervals during the first week of the infection. In hyperimmunized rats the above authors found the worms were retained and killed, to a small extent, in the skin, and to a larger extent, in the lungs; of those that migrated to the intestine, many were delayed, and upon their arrival, the majority not only failed to grow or produce eggs but failed to remain.

By means of intraperitoneal injections of serum from hyperimmunized rats these authors were able to demonstrate passive immunity as manifested by: a slower rise in the number of eggs passed, a smaller maximum egg count, a smaller total number of eggs passed, a smaller number of worms in the intestine and a slightly smaller size and paler color of the worms.

Sarles (1938) demonstrated in vitro antibody action as evidenced by invariable precipitate formation of four types: (1) cuticular (with infective larvae and lung-stage only), (2) excretory, (3) oral, and (4) intestinal, and sometimes the decreased activity (of infective larvae and lung-stage) and the inhibition of development (of larvae). Before the work on Nippostrongylus, Blacklock, Gordon, and Fine (1930) showed that death of the larvae of the myiasis-producing fly, Cordylobia anthropophaga, in the skin of immune guinea pigs was associated with a precipitate in the gut and around the larvae and that the precipitate in turn was due to the formation of precipitins by the immune host to the hemocoel fluid and excreta of the larvae. In addition, Fourie (1936) described hyaline masses closely applied to the surface of

Oesophagostomum columbianum which no doubt was a precipitin reaction. Similar reactions have since been noted by Otto (1940) with Ancylostoma caninum, Mauss (1940) and Oliver-González (1940) with Trichinella spiralis, Oliver-González (1943) with Ascaris lumbricoides and Hawkins and Cole (1945) with sheep strongyles.

Sarles (1939) found the degree of passive immunity produced varied with the dosage, interval after infection, and the inherent potency of the serum.

Taliaferro and Sarles (1939) in a study of the cellular reactions in the skin, lungs, and intestine of normal and immune rats after infection with N. muris concluded that immunity against the worm was largely dependent upon humoral factors with secondary cellular cooperation. The precipitins and possible other humoral factors produced the following effects on the worm: (1) immobilization (2) formation of precipitates in the alimentary canal and around the body orifices (3) stunting and (4) the occasional killing of the worm. The antibodies also localized the irritating excretions and secretions of the worm and brought about more intense inflammatory responses. The same authors pointed out that during the intestinal phase of the infection the worms pierced the epithelium and ingested antibodies (as evidenced by intestinal precipitates) and deposited antigenic secretions in the lamina propria. Thus the worm in the intestine had intimate contact with lymphoid tissue and the blood and lymph.

Thorson (1951) incubated larvae in normal rat serum. The larvae were then removed and the normal serum presumably containing their secretions and excretions was injected intraperitoneally into rats. The

rats were then infected with larvae and after 8 days the worm burden was determined. The injected rats showed a significantly lower worm burden than did uninjected controls, indicating that protective antibodies were formed against the secretions of the worm.

A few references are available which indicate changes occur in the serum proteins during parasitic infections. Ikejiani (1946) found an increase of globulin and a decrease of albumin in rats infected with Trypanosoma brucei and Trypanosoma equiperdum. Lysenko (1951) associated a high gamma-globulin with the presence of ablastic activity and a low gamma-globulin with no ablastic activity in rats infected with Trypanosoma lewisi.

In contrast to the many electrophoretic studies that have been made in the field of bacteriology, there have been relatively few in the field of parasitology; and of these studies that have been made, most seem concerned with protozoan infections such as those of Cooper (1946) Lysenko (1951) Dole and Emerson (1945).

In summarizing the review of the literature cited above the following statements can be made concerning the activities of this host-parasite relationship: (1) after recovery from an initial infection rats are relatively resistant to subsequent infections (2) acting through some medium in the blood the host influences reproduction, growth, development, maintenance of the worm (3) the degree of acquired resistance of the host is associated with the size of the initial infection (4) resistance to superinfection is highest 14 days after a previous infection (5) worms regain their egg-laying potential when they are transferred from an immune rat to a normal rat (6) resistance qualities

can be passively transferred by the serum of immune rats (7) immune sera is capable of precipitate formation when exposed to larvae and (8) the resistance of the host appears to be in response to the excretions and secretions of the worm.

The purpose of the present study was (1) to investigate the changes in the serum protein components which occur as a result of infection with N. muris (2) to study these changes progressively through the hyper-immunization period and (3) to determine the conditions that causethese serum protein changes.

# MATERIALS AND METHODS

## Experimental Procedure

In the present study certain advantages and disadvantages were encountered in regard to the host and parasite used. One disadvantage that arose in the preliminary investigations was the limited amount of serum that could be obtained from an eight week old rat. Even when rats were bled to death there was insufficient serum available for the desired determinations and, in addition, the rats were no longer available for later comparative studies. At this point another problem was encountered, namely: if the rats were partially bled, how much blood could be removed without hindering the normal physiology of the rat? A number of workers have shown that protein depletion either by restricting dietary protein or by plasmapheresis results in an altered electrophoresis pattern [Chow et al. (1945), Benditt et al. (1949), Chow, B. F. (1946), Chow et al. (1948)].

In order to assure a sufficient amount of serum the following procedure was utilized. Rats of the same strain, age, and sex were divided into two groups (control and experimental) by including half of every litter in each group. When determinations were to be made three rats from the experimental pool and the three corresponding litter mates from the control pool were bled completely and discarded. In this way sufficient serum was usually available for the various determinations and the possibility of detrimental effects due to blood depletion was eliminated.

The advantage of working with this host-parasite combination lies in relatively low cost and maintenance of the host and the ease with which large numbers of the larvae can be cultivated.

In the present work rats were subjected to increasing numbers of larvae at two week intervals with the exception of absorption experiment IV in which case the rats were subjected to larvae at one week intervals. The actual sequence was 1,000; 2,000; 5,000; 10,000; 20,000; 50,000 larvae. Thus, an eight week old rat inoculated with 1,000 larvae was eighteen weeks old when 50,000 larvae were administered. This rather long period of time in the life of the rat meant that in any conclusion drawn concerning serum protein changes would necessitate a consideration of normal physiological development during this period. There is evidence from the data on rats and cats, of which the young were studied, and from data on developing chick and pig embryos that there are major changes in the serum patterns with age and development (Moore et al. 1945b). Brandt et al. (1951) in the case of normal chickens was able to show quantitative changes in some of the serum protein components as the birds matured. The uninfected controls in the present work which were litter mates of the infected group served as a check on this point since they underwent the normal growth process in the absence of N. muris.

The rats used in the experiments were either the Wistar strain which were bought as weanlings or the Sprague-Dawley strain which were raised in this laboratory. The rats were kept in an isolated room away from other animals in order to minimize the chances of contaminating infections. They were housed individually in most cases and in wire-bottomed

cages. Food and water were available at all times. Throughout the experiments the rats were maintained on a pellet ration[1] which contained, as recorded by the manufacturer, a guaranteed minimum analysis of 22.00 percent protein, 3.00 percent fat, 4.00 percent fiber and 45.00 percent nitrogen free extract.

When serum was to be collected, the rats were anesthetized by injecting intraperitoneally 0.05 to 0.10 ml. of Halatal[2]. If anesthesia was incomplete, ether or chloroform was used in addition to the Halatal. The rats were immobilized after anesthesia by stretching them back down on a board using heavy rubber bands looped around the legs and attached to nails appropriately placed in the board. The blood was then drawn from the heart, placed in sterile plugged test tubes and the tubes slanted to give an increased surface from which a larger serum harvest was possible. The slanted tubes were left at room temperature from 12 to 18 hours. Under aseptic conditions the serum was then removed with Pasteur pipettes, placed in centrifuge tubes, and centrifuged at 2,000 R.P.M. for 30 minutes. The clear serum was removed with Pasteur pipettes and placed in tubes. Determinations were made immediately when possible to minimize any serum changes due to storage. If measurements could not be made immediately the serum was stored at 4°C.

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[1]Miller's Eaties - Manufactured by Battle Creek Dog Food Co., Battle Creek, Michigan.

[2]Halatal, Jensen-Salsbery Laboratories, Inc., Kansas City, Missouri.

## Infection Technique

From initially-infected rats 7 to 12 days after infection feces were collected in dropping pans containing moistened paper toweling to prevent the feces from drying. The feces were thoroughly mixed with water to the consistancy of a paste and an equal volume of charcoal added and again mixed. The charcoal-feces mixture was then set out in petri dishes in the form of a patty which was one-half inch from the edge of the petri dish and did not touch the lid. The petri dishes were then stored at room temperature from one to three weeks in suitable closed containers, which contained moistened paper toweling to prevent the cultures from drying out. The filariform larvae were isolated with a Baerman apparatus and repeatedly washed with sterile distilled water by centrifugation. Five hundred to one thousand units of penicillin and 0.05 gm. of streptomycin were added per cc. of suspension.

The larval concentration was determined by making serial dilutions of an aliquot of the concentrated suspension until 50 to 200 larvae were counted in 0.05 cc. of the last serial dilution in a Scott counting chamber[3]. In this manner the number of larvae in the original larval suspension could be calculated. The suspension was diluted so that the desired number of larvae for injection were contained in 0.1 to 0.25 cc. of solution. After the dilution the larval concentration was checked by making another count.

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[3]Counting Slide Scott No. 4099-A for hookworm (3x1 1/2 inches) Arthur H. Thomas Company, Philadelphia, U.S.A.

The larval suspension was injected subcutaneously by means of a tuberculin syringe. After all larval injections were made the remaining larval suspension was allowed to settle and the supernatant drawn off. This supernatant material was examined under the dissecting microscope to insure the absence of larvae. The larva-free supernatant was then injected into rats in an amount equal to the volume of the larval injection. The injection of this supernatant material provided a control on the non-larval portion of the inoculum and showed its influence on the serum proteins.

## Chemical Fractionation and Analysis

Total protein determination. The total serum protein was determined with the aid of Weichselbaum's biuret reagent prepared according to Wolfson et al. (1948). This method necessitates a rather involved standardization but allows determinations to be made on small amounts of serum and the time of analysis is much shorter than the Kjeldahl method. The standardization was carried out in the following manner. Serum from 10 to 15 rats was collected (as described above), pooled and thoroughly mixed. From this pooled sample, standards were prepared by using normal saline as a diluent. Thus four or five standards ranging from undiluted (about 6.00 grams per 100 cc. serum) to zero protein concentration were prepared. The protein concentration of the pooled sample was determined as follows: The total nitrogen was ascertained by the macro-kjeldahl method according to Gradwohl (1943). Non-protein nitrogen was determined by the method of Folin and Wu (1919) and was

subtracted from the total nitrogen. The difference represents protein nitrogen which was multiplied by the factor[4] 6.25 to obtain total protein. Therefore, knowing the total protein concentration of the pooled sample, the protein concentrations of standards could be calculated and plotted. Each of the standards (of known protein concentration) was analyzed for total protein by the following biuret method:

1. Into a 10 ml. graduated mixing cylinder, 0.4 ml. of serum (or standard) was pipetted by means of a Kahn pipette. From a burette 7.6 ml. of distilled water was added. The solution was mixed well by inversion taking care not to cause foaming.
2. To another graduated mixing chamber 4.0 ml. of the diluted sample and 4.0 ml. of biuret reagent were added and mixed well.
3. A blank was prepared with 4.0 ml. of distilled water and 4.0 ml. of biuret reagent.
4. The solutions were allowed to stand 30 minutes for complete color development and then read on the photelometer[5] at a wave length of 525 millimicrons (green filter). From the data obtained a standard curve was constructed on semi-log graph paper.

In addition to the pooled rat sera, a series of pure bovine albumin[6] solutions diluted to known protein concentrations were analyzed by the above method. It was found that the standard curve obtained from bovine albumin although not superimposible was very close to that of the pooled rat sera as is shown in Figure 1.

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[4]The conventional procedure of converting nitrogen determination to values for whole protein by multiplying by 6.25 is quite arbitrary (Jager et al. 1950). The experimental conversion factors determined for various protein fractions of human serum have been found to vary from 6.10-3.40. (Armstrong et al. 1947).

[5]Cenco-Sheard-Sanford Photelometer, Cat. No. 41000, Central Scientific Co., Chicago.

[6]The author wishes to express his thanks to Dr. M. C. Ziporyn of the Armour Laboratories, Chicago for supplying the Bovine Albumin Solution.

Since the two materials reacted so similarly to the biuret reagent and since the bovine albumin is more readily available it was used as a primary standard throughout the course of the experiments, The author felt the use of the bovine albumin curve was both justified and desirable.

Salt fractionation. In selecting a method for chemically fractionating serum the author was faced with a number of complicating factors. A few will be mentioned in order to justify the final decision. Cohn and Wolfson (1947) and Petermann et al. (1947) showed that "serum albumin" as determined by the classical Howe (1921) sodium sulfate method, actually included both albumin and alpha globulin. Certain disadvantages are encountered in some of the other methods. Globulin precipitation by methanol as devised by Pillemer and Hutchinson (1945) involved working between 0° and 1° C. with occasional erratic results. Popjak and McCarthy (1946) used magnesium sulfate to separate albumin and globulin, but this method involved a delay of twelve hours before filtration. The method of Milne (1947) required an overnight delay for precipitation of globulin. The quantitative immunochemical reaction of Chow (1947) employed biologic material of unstable titer and uncertain composition.

A method suggested by Wolfson et al. (1948) appeared to overcome these difficulties. These authors utilized sodium sulfite at a concentration of 26.88 which precipitates globulin from serum. Campbell and Hanna (1937) used sodium sulfite but their method was adjusted to give results approximating Howe's fractions.

In preliminary determinations for the present study an attempt was made to utilize the method as described by Wolfson et al. (1948). It

was soon evident that although this method was satisfactory for human serum it gave poor results in the case of rat serum when checked by electrophoretic analysis. Hence, it was found necessary to modify this method for application to rat serum analysis.

This adjustment was carried out in the following manner: a pooled sample was analyzed by the Tiselius electrophoretic method and the total protein determined by the method described previously. Using solutions of sodium sulfite ranging in concentration from 20 to 28 percent (W/V), the amount of albumin fraction was determined upon aliquots of the pooled sample according to the following procedure:

1. Into a 10 ml. graduated mixing cylinder or tube at room temperature, 0.4 ml. of pooled serum was pipetted by means of a Kahn pipette.
2. To the serum 7.6 ml. of sodium sulfite solution of appropriate concentration was added and the two components mixed by inversion.
3. After the development of maximum precipitate (about ten minutes) two ml. of Span[7]-ether reagent[8] was added and the mixture gently shaken. This was followed by centrifugation for 5-10 minutes at 2,000 R.P.M.
4. After centrifugation, a pipette was carefully inserted through the Span-ether layer and beneath the packed globulins and 4.0 ml. of clear centrifugate transferred to another cylinder. This was found to be best accomplished by slanting the tube to separate the precipitate from the wall of the cylinder.
5. To the clear centrifugate 4.0 ml. of biuret reagent was added and the components mixed well by inversion.
6. A blank was prepared with 4.0 ml. of sodium sulfite and 4.0 ml. of biuret reagent.
7. After a period of 30 minutes the protein content was determined by reading the color development on the photelometer and consulting the standard curve described above.

Since the absolute values of the pooled sample were known from the electrophoretic and total protein determinations, the proper sodium

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[7] Span 20 (Sorbitan monolaurate) Batch 488-c, Atlas Powser Co., Wilmington, Delaware.

[8] Wolfson et al. (1948).

sulfite concentration for use in rat sera was that salt concentration which yielded results in agreement with the electrophoretic analysis. It can be seen from Figure 2 that 24 percent sodium sulfite (under these conditions) appears to be the critical concentration wherein the globulins are separated from the albumin in pooled rat sera.

The total serum globulin was obtained by subtracting the quantity of serum albumin found from the total amount of serum protein determined.

The A/G ratio was obtained by dividing the serum albumin value by the total serum globulin value.

## Electrophoresis

All electrophoretic analyses were carried out with a Perkin-Elmer Model 38 Tiselius Electrophoresis Apparatus. Unless otherwise indicated the buffer used in the present study was a barbiturate (veronal and sodium veronal) solution of pH 8.6, 0.1 ionic strength and made up at 25°C. This buffer gave good resolution of the alpha globulin and revealed somewhat more total globulin than phosphate buffer. There was also better separation of the delta and epsilon boundaries from the gama globulin peak (Longsworth 1942).

From preliminary studies it appeared that the best resolution of rat serum proteins was obtained by operating at 7.5 milliamperes for 7,200 seconds (veronal buffer). Therefore, these conditions were chosen for standard procedure to be used for the duration of the experiments. However, since the experiments were carried on over an extended period of the rat's life in which the serum components changed with growth of

the rat, it was soon evident that these conditions would require some alteration. These alterations in the standard conditions are, therefore, indicated in the "Results" section and unless so indicated the above standard conditions were employed. Where alteration in the standard procedure was necessary, the corresponding control was altered indentically.

The Perkin-Elmer manual for the model 38 Tiselius apparatus gives the technical procedure for analysis. In the present work this was followed with the following modifications:

Dialysis. Two ml. of serum was diluted with four ml. of buffer and placed into a dialyzing membrane of seamless regenerated viscose process cellulose[9]. The sack was suspended from a clamp on a ring stand and then lowered into a cylinder of buffer revolving on a turn table. Care was taken to lower the sack into the buffer to the depth where there was just enough buoyance to insure constant mixing of the contents within the sack. In this manner the solutions inside the outside of the sack were in continual motion. The dialysis was carried out in a cold room at a temperature of 4°C. The protein solutions were dialyzed for two to four hours against 100 mls. of buffer, followed by four to six hours against 100 ml. of fresh buffer. The protein solution was then equilibrated overnight (sixteen hours) against 300 ml. of fresh buffer. The equilibrated serum was clarified by centrifuging 15 minutes at 2,000 R.P.M. in the cold room (4°C.).

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[9]Visking Corp., Chicago, Illinois.

Photographic Film. The film[10] was developed 4 minutes in D19[11] developer (total darkness) followed by 10-15 minutes in hypo and 15-20 minutes wash in running water.

Analysis of Electrophoretic Patterns. Two-fold enlargements of the descending patterns on negative film were projected from an enlarger upon plain paper and traced (Longsworth and MacInnes 1940). The base-line was drawn in such a manner as to connect the minimum values of concentration gradient. In assigning areas to the various components of the serum, the method of Tiselius and Kabat (1939) was followed by drawing an ordinate from the lowest point between adjacent maxima to the base line.

The areas were measured with a planimeter[12]. Moore et al. (1949) had discussed the importance of obtaining accuracy and precision when using the planimeter for determining areas of serum protein. Thus, each component area from a pattern was traced three times such that three readings did not differ more than five planimeter area units. The average of the three readings was used for calculation.

By relating the total area of the pattern to the total analyzed protein concentration by the biuret method, the protein concentration of each component was determined from the size of its area in terms of the fractional part of the total area.

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[10]Kodak Contrast process ortho 3 1/4 x 4 1/4 inches.

[11]Kodak Company.

[12]K&E No. 4236 Compensating polar planimeter, Keiffel and Esser Co., New York.

The serum protein component mobilities were calculated as recommended by Longsworth and MacInnes (1939) from the measured distance in centimeters between the initial boundary and the ordinate dividing the area of the component in half. This migration distance (d) was substituted into the following formula of Longsworth (1940) along with other pertinent data for the determination of mobility.

$$u = \frac{d\ q\ K_{sp}}{i\ t\ m}$$

The potential gradients were evaluated by supplying data in the following formula:

$$\text{Potential gradient} = \frac{i}{q\ K_{sp}}$$

Where: d = distance migrated in cm.
q = cross-sectional area of the cell
t = time of migration in seconds
i = current in amperes
m = enlargement factor
$K_{sp}$ = specific conductance of protein

$$= \frac{\text{conductivity cell constant}}{\text{resistance of protein in ohms}}$$

A COMPARISON OF TOTAL PROTEIN MEASUREMENTS BY THE BIURET REACTION OF BOVINE ALBUMIN AND POOLED RAT SERA

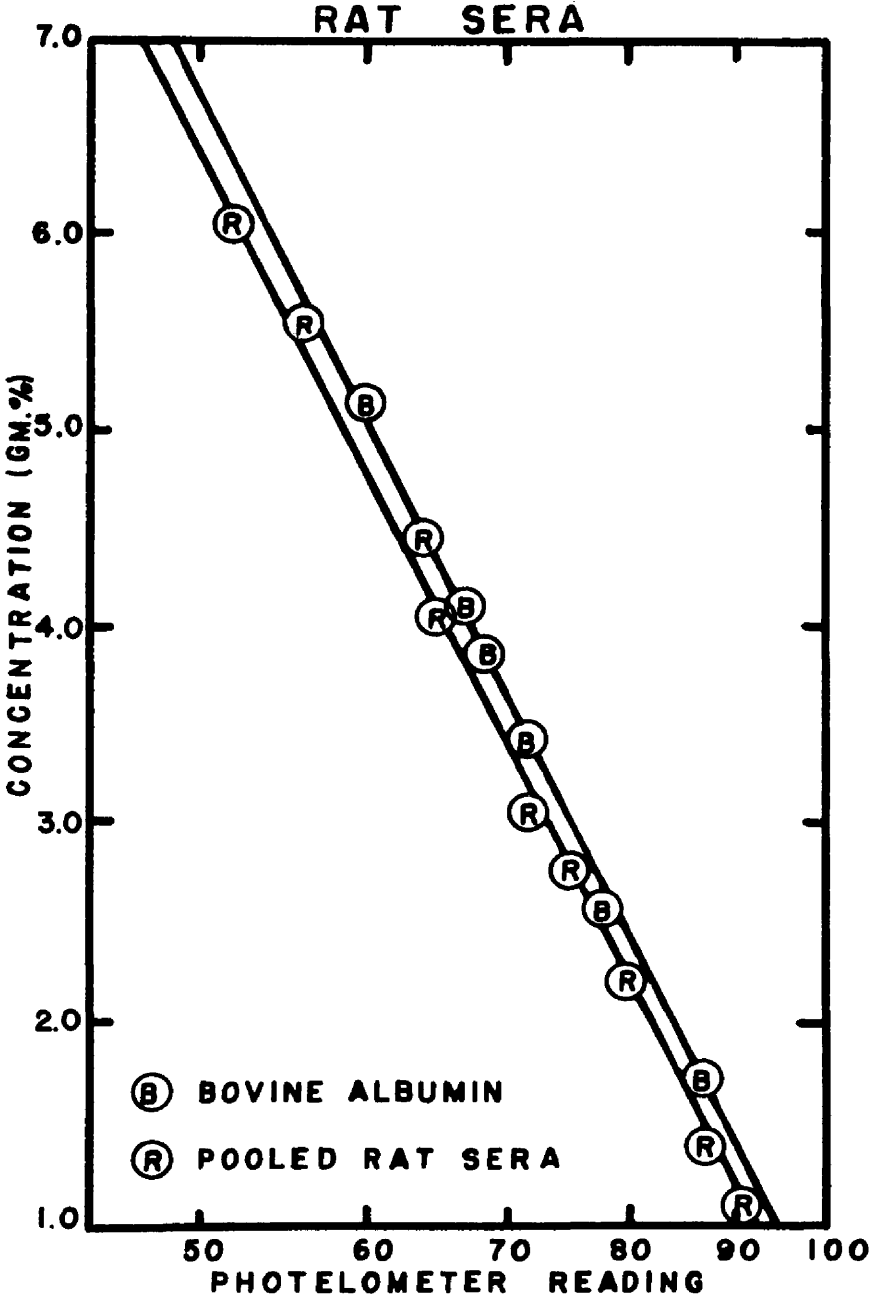
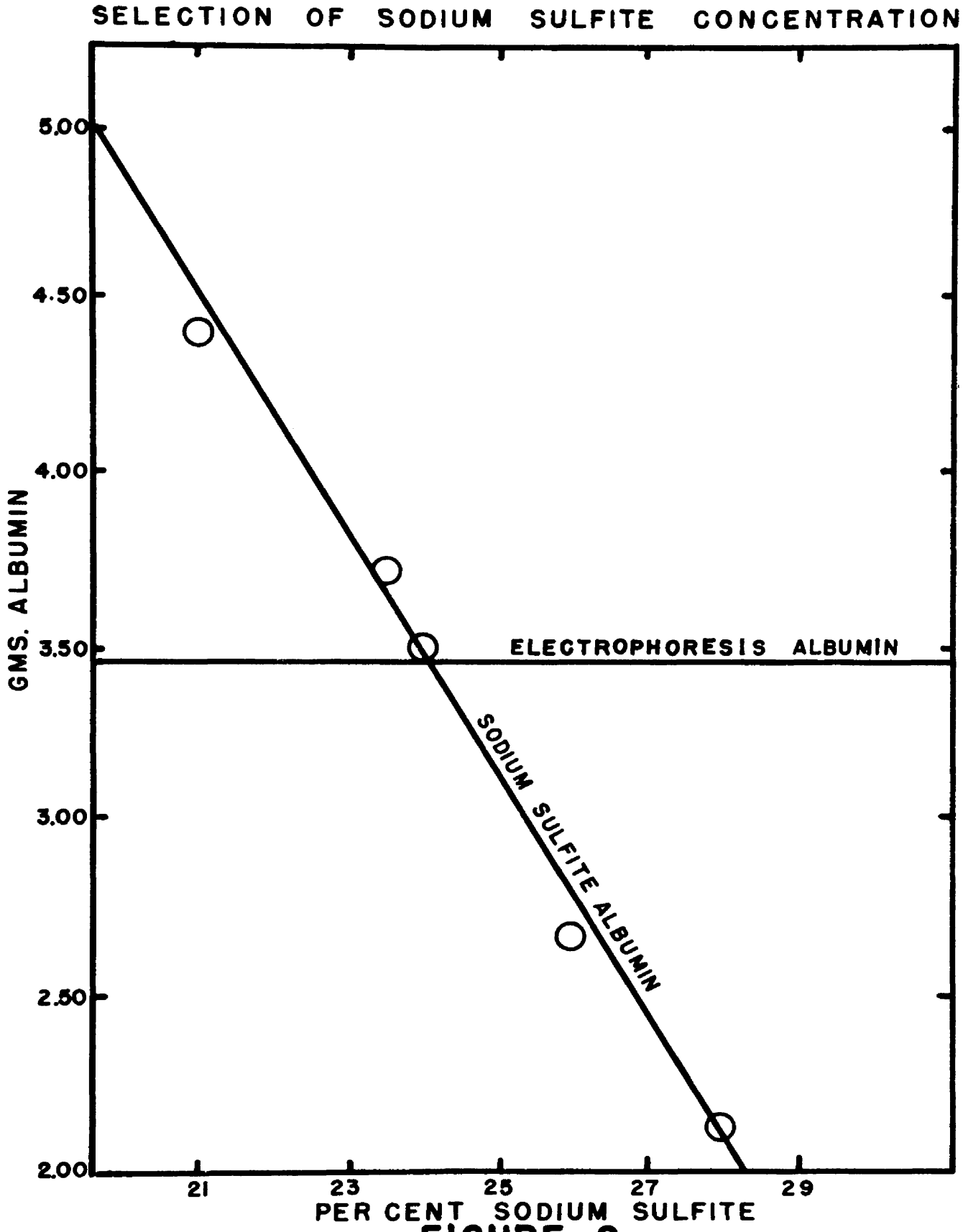



FIGURE 1


SELECTION OF SODIUM SULFITE CONCENTRATION
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GMS. ALBUMIN
ELECTROPHORESIS ALBUMIN
SODIUM SULFITE ALBUMIN
21
23
25
27
29
PER CENT SODIUM SULFITE

FIGURE 2

# RESULTS

## Preliminary Experiments

The purpose of the preliminary experiments was to establish a direct connection between host response seen by earlier investigators and the present study. Results similar to early investigations, when obtained under the conditions of the present work, render possible a more valid correlation between serum protein changes and the various manifestations of immunity observed previously. Thus, the following experiments were carried out to insure that the proposed larval sequence would duplicate the results of earlier work:

(1) The ability of an immune host to reduce the parasites' egg-production. Four rats were subjected to increasing numbers of larval injections at two week intervals until they were able to tolerate 50,000 larvae. During immunization (60 days) the egg production curve was essentially the same as recorded by other investigators [Graham (1934), Chandler (1936)]. The egg production reached a peak five to eight days after the initial infection and then decreased to less than 200 eggs per gram of feces after 16 to 18 days. Despite the repeated infections of increasing numbers of larvae, the eggs never reappeared in amounts equal to the initial response but finally decreased until quantitative measurement was no longer possible. Thus, it was assured that the sequence of larvae administered produced a resistance in the host as manifested by the reduction of eggs.

(2) The ability of immune serum to produce precipitate. Larvae were incubated in immune serum utilizing the method of Sarles (1938). In place of the 0.1 percent mercuric chloride solution employed by Sarles, 500 units of penicillin and 0.05 gm. of streptomycin per cc. of serum were used to control bacterial growth. Oral, excretory, and intestinal precipitates were observed in agreement with Sarles (1938). Figure 2a shows a filariform larva incubated in the presence of immune serum. The precipitate is visible in the excretory pore and in the vincinity of the pore. The immune serum used for incubation was obtained from a rat which had received the proposed sequence of larval injections. This assured that the sequence was adequate for a production of the antibody responsible for the precipitate. Larvae incubated in normal serum produced no precipitates.

(3) The ability of the immune host to resist reinfection. To test protection produced by the larval sequence against reinfection, two mature litter mates were injected with 50,000 larvae. One had previously received the larval sequence, while the other had never been infected and served as a control. The latter animal died the third day following the injection. Necropsy established the cause of death as verminous pneumonia. The other rat which had received the larval sequence showed no visible adverse symptoms.

## Preliminary Electrophoretic Analysis

To ascertain the optimum conditions of electrophoretic analysis the following determinations were made:

An electrophoretic analysis was made on normal and immune serum in phosphate buffer (the immune serum was produced with the proposed larval sequence). The patterns from these preliminary runs are found in Figure 3 and a comparison of the control with the infected samples shows an extrao rdinary increase in the globulin component for the latter.

Several electrophoretic runs were made on normal rat serum in veronal buffer. Somewhat better resolution of serum proteins was obtained in this buffer. Figure 4 shows two sets of patterns from normal rats in this buffer.

From this preliminary work it was established that: (1) at the selected infection sequence the egg production of the worm was decreased below quantitative measurement; (2) the immune serum from the infection sequence was able to produce the precipitates previously described by Sarles (loc. cit.); (3) rats which had received the sequence were protected against lethal effects of 50,000 larvae, whereas, controls died; and (4) the electrophoretic patterns of the immune rats showed striking increases in serum globulin production.

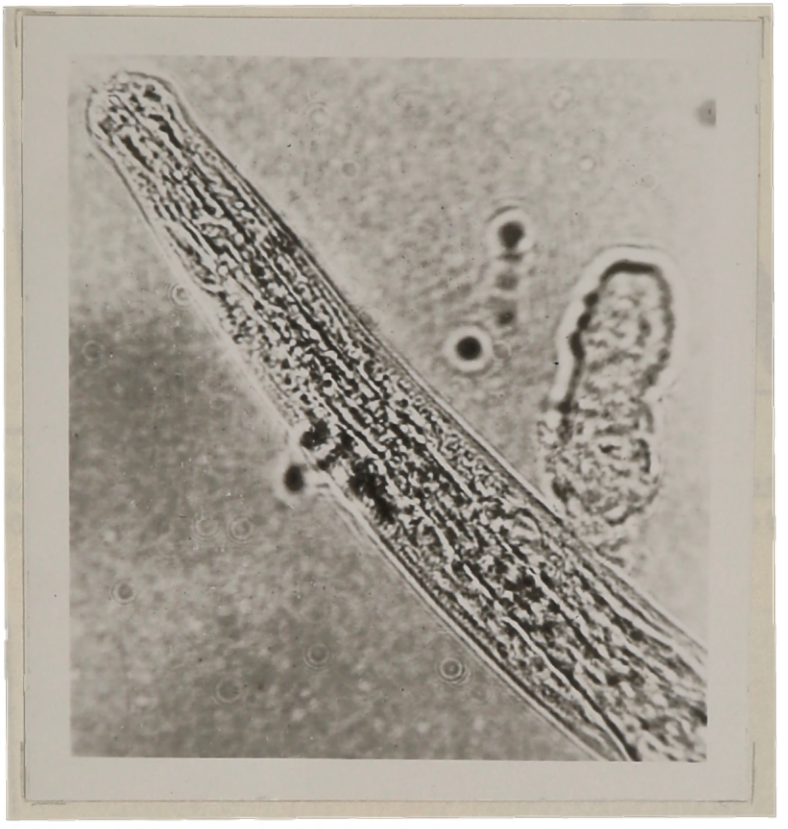
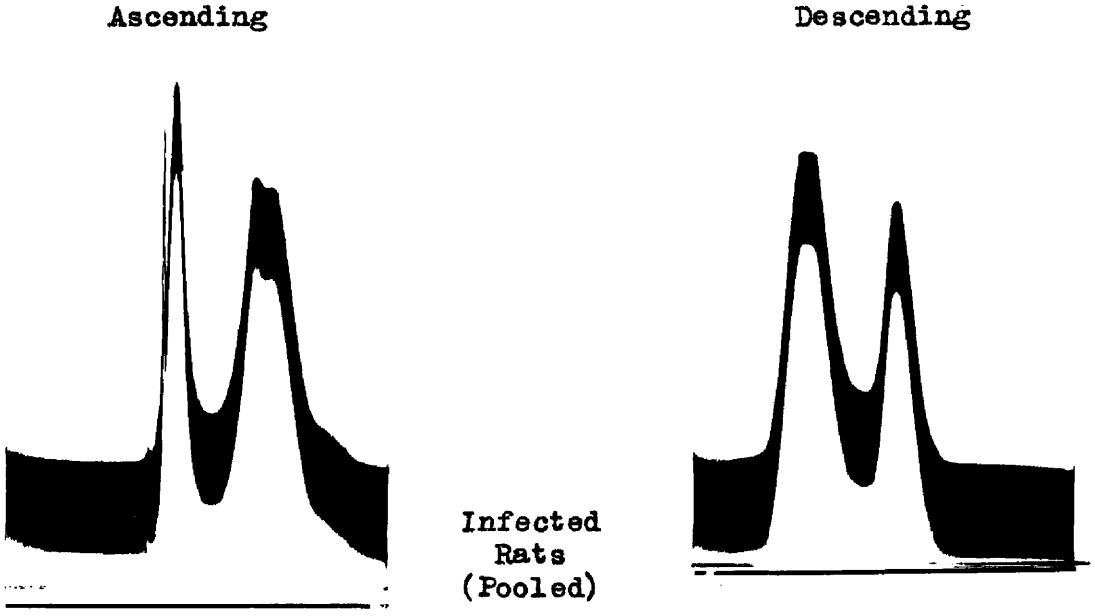


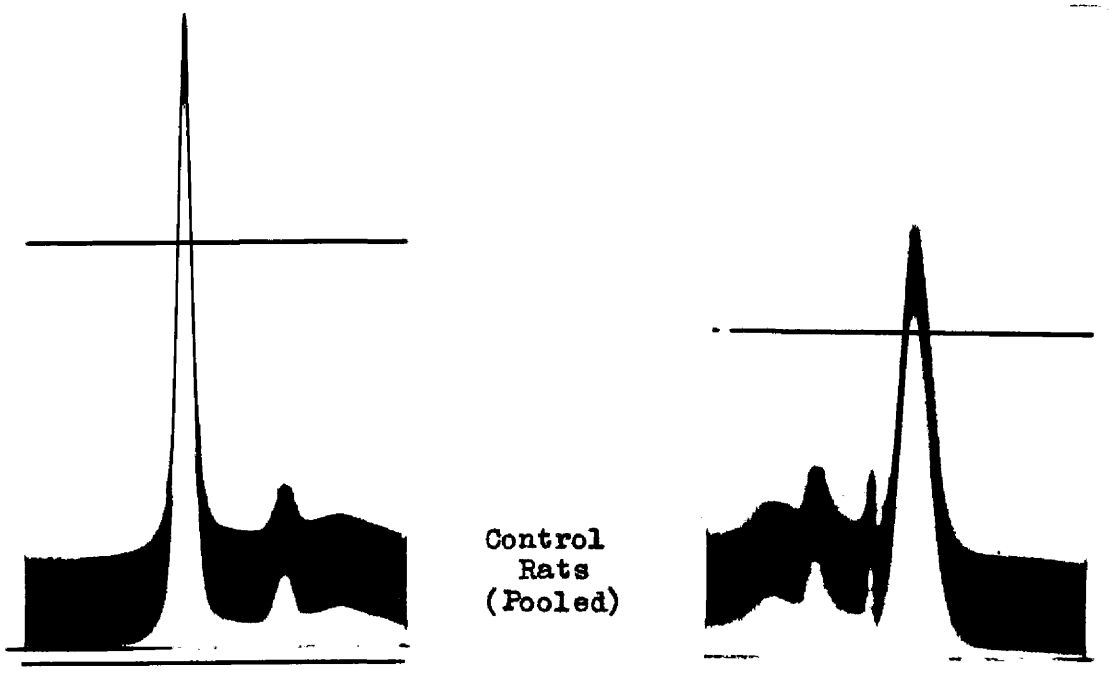
Figure 2a

Filariform larva incubated in immune serum which shows excretory precipitate

ELECTROPHORETIC PATTERNS OF RAT SERA SEVEN DAYS AFTER THE FIRST 50,000 LARVAL INJECTION COMPARED WITH CONTROL



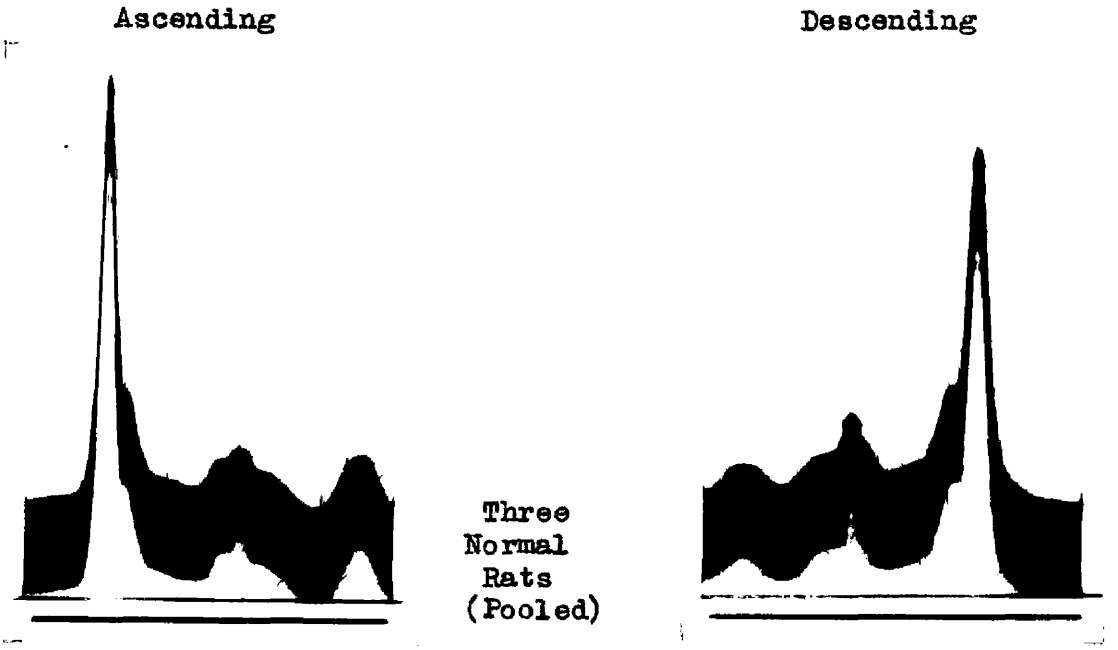

pH 7.4; Phosphate buffer (sodium phosphate 0.02M and sodium chloride 0.15M); 10,800 sec.; Pot. Grad. 5.2 volts/cm.; 2.25% protein.



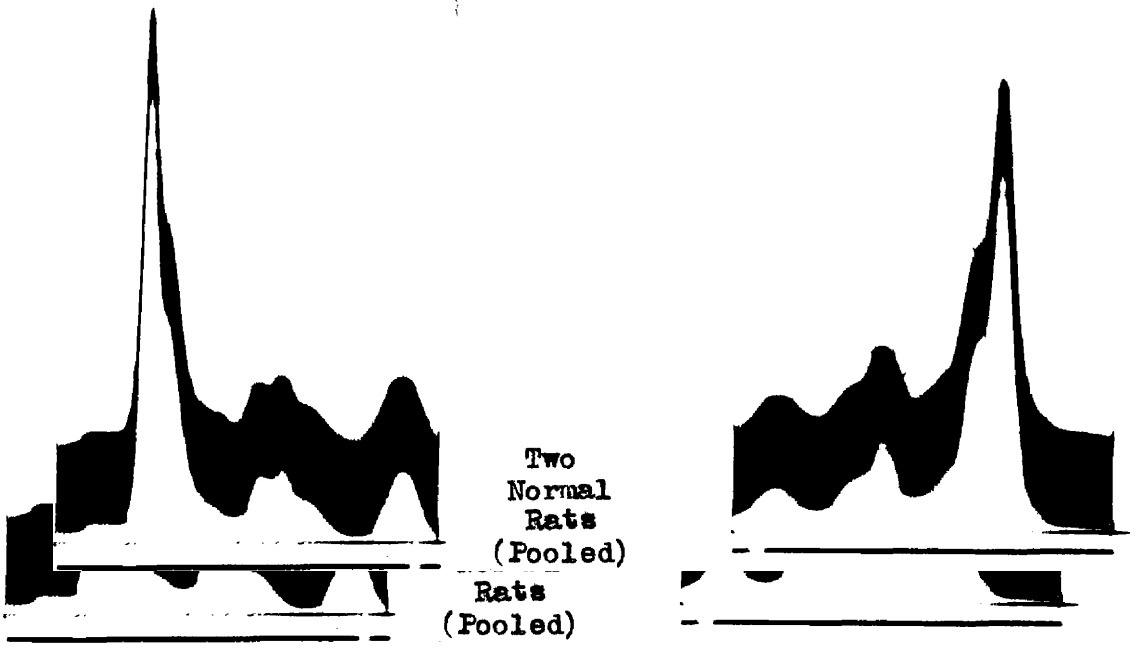

pH 7.4; Phosphate buffer (sodium phosphate 0.02M and sodium chloride 0.15M); 10,800 sec.; Pot. Grad. 4.9 volts/cm.; 1.78% protein.

FIGURE 3

ELECTROPHORETIC PATTERNS OF NORMAL RAT SERA




Veronal buffer at pH 8.6, ionic strength 0.1, for 7,200 sec.; Pot. Grad. 7.3 volts/cm.




Veronal buffer at pH 8.6, ionic strength 0.1, for 7,200 sec.; Pot. Grad. 7.8 volts/cm.

FIGURE 4

## The Analysis of Sera Two Weeks After Each Infection

Experiment I. From the work of Chandler (1935b) it was demonstrated that resistance to superinfection increased as the interval between infections was increased during 7 to 14 days. From the fifteenth day on, resistance gradually decreased. Thus, the fourteenth day after the last infection represented the time of maximum resistance. This was manifested by a falling off in numbers of worms acquired, a gradual decrease in the percentage of worms which succeeded in passing the final molt, and a decrease in worm growth. Since the immunity was strongest 14 days after infection, it seemed logical to examine the serum at this time. Consequently the various determinations in Experiment I were made, in most cases, 14 days after the last larval injection.

Tables I and II are composites of the data obtained from electrophoretic and chemical fractionation analyses of sera in Experiment I. Figures 5, 6, and 7 show graphically the pertinent data of Table I.

Examination of Figure 5 indicates, with the exception of the determinations following the infection of 1,000 larvae, the total protein was consistently higher than the corresponding control. Tiselius and Kabat (1939) also observed that on immunization an increase generally occurs in total serum protein as well as total globulin and antibody fractions. An interesting significant fact was that the highest total protein concentration occurred seven days after the last 50,000 larval injection. It suggested that although the host's resistance was highest 14 days after infection, the greatest serum protein alterations were occurring earlier. This observation led to Experiments II and III.

From Figure 5 it may be noted that rats injected with the supernatant material from the larval suspension fell well within the normal range. This indicated the material in the inoculum other than the larvae did not cause the increased total protein.

Figure 6 shows a marked increase in the total globulin as the number of larvae injected was increased; and a decrease of total globulin following a decrease in the number of larvae injected. The highest total serum globulin value was noted, again, seven days following the last administration of 50,000 larvae. However, the total serum globulin of rats injected with the supernatant of the larval suspension was within the normal range.

Figure 7 shows the beta globulin fraction (electrophoretically determined) was higher than the corresponding control in every case. The gamma globulin component showed no significant increase (Table I).

Figures 8, 9, 10, and 11 represent electrophoretic patterns from which the corresponding calculations of Table I were made. They show the variation in serum protein components with the number of larvae injected. The patterns of Figure 8 indicate there is little difference between the infected animals after administration of 1,000 larvae and the control animals. (This was also found to be the case in Experiment Ia which followed).

In Figure 9 the difference between the infected and control animals thirteen days after the 20,000 larval infection is quite marked. It may also be noted that in the infected animals the mobility of the protein components was distinctly slower than that of the controls. Table II contains the calculated mobility values from Experiment I. The most striking difference between the control and infected animals was the increase in the component area which corresponds to the beta globulin. With the increase of the beta globulin, the gamma globulin tended to become less resolvable from the former at the standard time of 7,200 seconds. This plus the decreased mobilities in the infected animals

tended to produce poor resolution of the globulins. To improve resolution photographs were also taken at 10,000 seconds.

Figure 10 shows the patterns as they appeared four days after the second injection of 50,000 larvae taken at both 7,200 and 10,000 seconds (see Table I for sequence of injections). At 10,000 seconds the gamma globulin separated better from the beta globulin and was distinguishable as the slowest migrating component. Rat serum contains little gamma globulin (Moore 1945). In veronal buffer at pH 8.6 the gamma globulin electrophoretically separates from the salt boundary so that it is possible to more accurately determine total globulin. But in this buffer the beta and gamma globulins do not separate as sharply and are sometimes not as well defined as in phosphate buffer at 7.4 (Moore 1945). Thus, in veronal buffer the gamma globulin appeared as a slight shoulder on the beta globulin peak, which is in agreement with the patterns of Moore et al. (1944). According to this description of the gamma globulin, the globulin increase may be attributed to the beta component. Later in Experiment III, serum electrophoretically analysed in both veronal and phosphate buffers corroborated this statement (Figures 19 and 20).

The pattern in Figure 11 from pooled sera of rats number 25a and 27a shows the changes that occurred seven days after the last injection. (See Table I for injection sequence). In these rats the total protein, total globulin and specifically the beta globulin reached the highest level of all measurements taken in Experiment I.

The rats which received injections of supernatant from the larval suspensions showed normal patterns. An example of such may be seen in

the pattern from rat 45 in Figure 11. Inspection of Table I also shows that the various serum components were normal in these rats.

Body weights were measured throughout Experiment I and Table III shows the hyperimmunization process produced a decrease in body weight when compared with the uninfected controls.

Summarizing the results of Experiment I it may be noted that measurements, taken 14 days after the last injection, showed a steady increase in the total protein and total globulin as the number of larvae injected was increased. More specifically the beta globulin was consistently higher in the infected animals. Of the determinations made at four, seven, and fourteen days after the last infection of 50,000 larvae, the greatest changes were noted seven days after infection. As mentioned, Experiments II and III were the result of this finding.

Experiment Ia. From the results of Experiment I it was found that after the 1,000 and 2,000 larval injection, there was little or no change in the serum components when compared with the controls. Since after the administration of 1,000 larvae the host exhibits a rather strong resistance to reinfection as manifested by reduced egg production, it was decided to repeat the serum analysis 14 days after the 1,000 and 2,000 larval injections. The results are reported in Table IV. The findings substantiate the results of Experiment I in that there was no significant difference in the serum protein components at this level of infection between control and infected animals.

TABLE I. ELECTROPHORETIC AND SALT

| Rat No. | Age (Days) Last Infected | Bled | No. of Larvae | By Salt Fractionation (Gm. %) Total Protein | Alb. | Glob. | A/G |
|---|---|---|---|---|---|---|---|
| 1,2,3 | 45 | 58 | 1,000 | 5.10 | 2.97 | 2.13 | 1.39 |
| 4,5,6 | --- | 58 | Control | 5.52 | 3.51 | 2.01 | 1.75 |
| 7,8,9 | 59 | 72 | 2,000 | 6.47 | 3.73 | 2.74 | 1.36 |
| 10,11,12 | -- | 72 | Control | 6.03 | 3.90 | 2.13 | 1.83 |
| 37,38,39 | 73 | 86 | 5,000 | 7.67 | 4.31 | 3.36 | 1.28 |
| 40,41,42 | -- | 86 | Control | 7.03 | 4.90 | 2.13 | 2.30 |
| 25,49,27 | 87 | 100 | 10,000 | 7.70 | 3.67 | 4.03 | .91 |
| 28,29,30 | -- | 100 | Control | 6.75 | 4.19 | 2.56 | 1.64 |
| 13,14,15 | 101 | 114 | 20,000 | 7.96 | 3.50 | 4.46 | .78 |
| 16,17,18 | -- | 114 | Control | 6.47 | 3.61 | 2.86 | 1.26 |
| 19,20,21 | 118 | 128 | 50,000 | 7.70 | 3.30 | 4.40 | .75 |
| 22,24,47 | -- | 128 | Control | 7.22 | 4.03 | 3.19 | 1.26 |
| 31,32,33 | 132 | 142 | 20,000 | 7.57 | 3.92 | 3.65 | 1.07 |
| 34,35,36 | -- | 142 | Control | 6.75 | 4.57 | 2.18 | 2.09 |
| 5a,51 | 146 | 150 | 50,000 Control | 7.54 | -- | -- | -- |
| 25a,27a | 165 | 172 | 50,000 | 8.21 | 3.30 | 4.91 | .67 |
| 43,44 | -- | 172 | S | 6.65 | 3.85 | 2.80 | 1.38 |
| 46 | -- | 181 | --- | 6.20 | -- | -- | -- |
| 45 | -- | 181 | S | 6.82 | 4.10 | 2.72 | 1.51 |

S = Supernatant

FRACTIONATION ANALYSIS OF EXPERIMENT I

| By Electrophoresis (Gm. %) | | | | | | |
|---|---|---|---|---|---|---|
| Alb. | Total Glob. | Globulins | | | | |
| | | $Alpha_1$ | $Alpha_2$ | Beta | Gamma | A/G |
| 2.97 | 2.09 | .44 | .40 | .96 | .32 | 1.42 |
| 3.58 | 1.98 | .65 | .39 | .73 | .26 | 1.81 |
| 3.57 | 2.91 | .71 | .83 | 1.17 | .20 | 1.25 |
| 3.52 | 2.52 | .91 | .48 | .69 | .52 | 1.40 |
| 4.31 | 3.39 | .80 | .80 | 1.57 | .18 | 1.27 |
| 4.98 | 2.07 | .79 | .42 | .72 | .15 | 2.40 |
| 4.06 | 3.64 | .64 | .86 | 1.62 | .53 | 1.11 |
| 4.23 | 2.52 | .54 | .58 | 1.05 | .31 | 1.68 |
| 4.40 | 3.65 | .57 | .57 | 2.02 | .48 | 1.25 |
| 3.53 | 2.90 | .73 | .62 | 1.13 | .40 | 1.22 |
| 3.59 | 4.14 | .74 | .70 | 2.23 | .43 | .87 |
| 4.07 | 3.18 | .63 | .67 | 1.41 | .44 | 1.28 |
| 3.78 | 3.78 | 1.14 | .77 | 1.62 | .40 | 1.00 |
| 4.08 | 2.67 | .76 | .53 | .84 | .50 | 1.53 |
| 3.25 | 4.24 | 1.06 | .98 | 1.57 | .71 | .76 |
| 3.51 | 4.70 | .92 | .80 | 2.79 | .19 | .75 |
| 3.97 | 2.68 | .74 | .37 | 1.25 | .33 | 1.48 |
| 3.84 | 2.36 | .71 | .31 | 1.09 | .25 | 1.59 |
| 3.41 | 3.37 | 1.28 | .60 | .96 | .64 | 1.01 |

TABLE II. MOBILITY CALCULATIONS OF EXPERIMENT I

| Rat No. | MOBILITIES* | | | | |
|---|---|---|---|---|---|
| | Alb. | Globulins | | | |
| | | $Alpha_1$ | $Alpha_2$ | Beta | Gamma |
| 1,2,3, | -- | -- | -- | -- | -- |
| 4,5,6 | 6.10 | 5.51 | 4.83 | 3.31 | 2.63 |
| 7,8,9 | 5.37 | 4.71 | 3.95 | 3.04 | 2.38 |
| 10,11,12 | 6.22 | 5.74 | 4.88 | 3.54 | 2.96 |
| 37,38,39 | 5.04 | 4.35 | 3.62 | 2.57 | 1.74 |
| 40,41,42 | 5.10 | 4.16 | 3.59 | 2.83 | 2.08 |
| 25,49,27 | 6.03 | 5.24 | 4.55 | 3.48 | 2.47 |
| 28,29,30 | 4.36 | 3.99 | 3.34 | 2.51 | 1.81 |
| 12,14,15 | 4.21 | 3.73 | 2.97 | 1.93 | 1.32 |
| 16,17,18 | 4.85 | 4.35 | 3.73 | 2.94 | 1.91 |
| 19,20,21 | 3.56 | 3.02 | 2.53 | 1.78 | 1.12 |
| 22,24,47 | 4.64 | 4.06 | 3.56 | 2.86 | 2.24 |
| 31,32,33 | 3.59 | 3.18 | 2.82 | 2.34 | 2.08 |
| 34,35,36 | 5.17 | 4.60 | 4.06 | 3.31 | 2.76 |
| 5a,51 | 3.26 | 2.79 | 2.18 | 1.54 | .90 |
| 25a,27a | 4.17 | 3.68 | 3.06 | 2.23 | 1.49 |
| 43,44 | 3.73 | 3.02 | 2.56 | 2.05 | 1.47 |
| 46 | -- | -- | -- | -- | -- |
| 45 | -- | -- | -- | -- | -- |

*Table figure X $10^{-5}$ $cm^2$ $volt^{-1}$ $sec^{-1}$

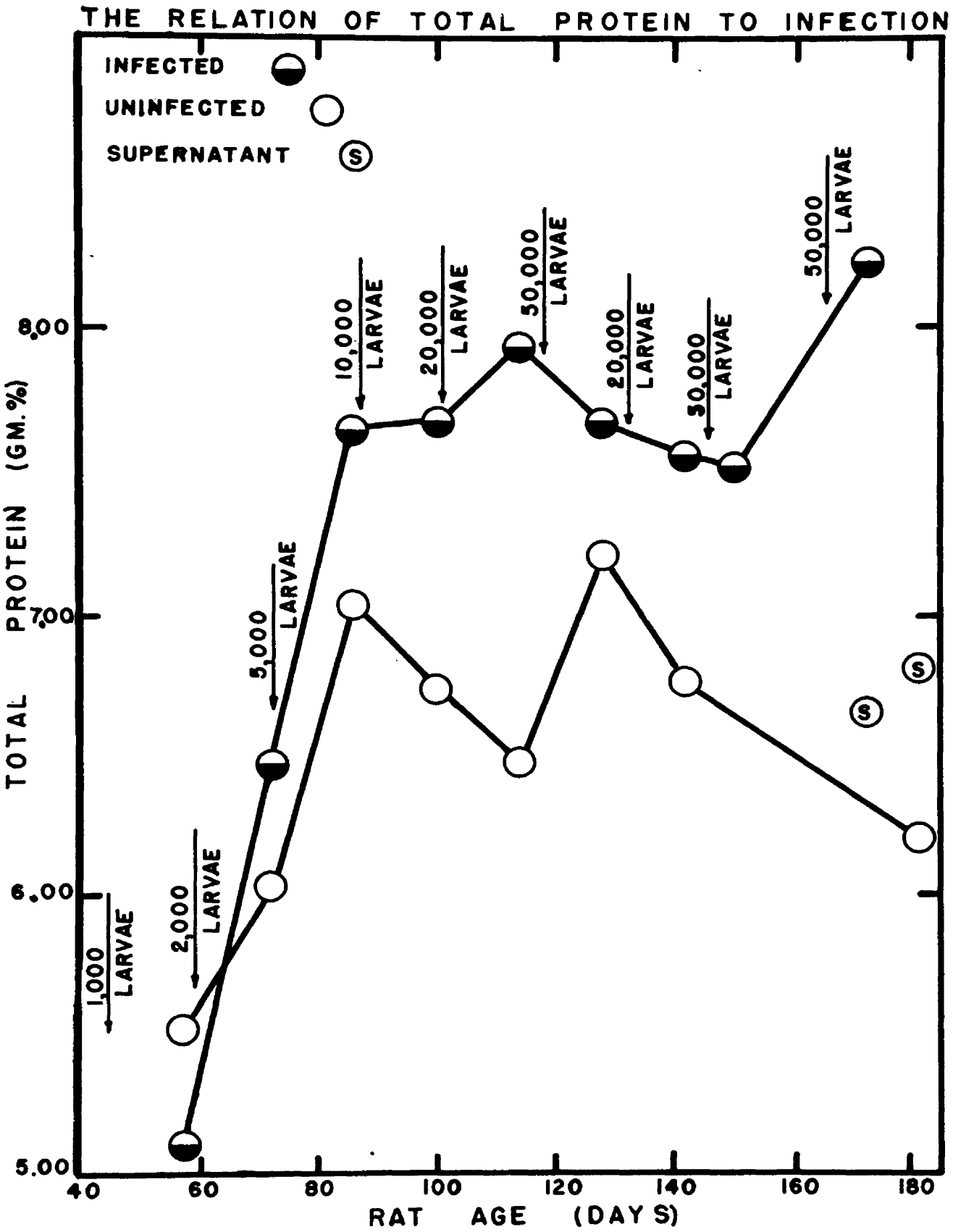

THE RELATION OF TOTAL PROTEIN TO INFECTION
INFECTED
UNINFECTED
SUPERNATANT
S
TOTAL PROTEIN (GM.%)
8.00
7.00
6.00
5.00
1,000 LARVAE
2,000 LARVAE
5,000 LARVAE
10,000 LARVAE
20,000 LARVAE
30,000 LARVAE
20,000 LARVAE
30,000 LARVAE
50,000 LARVAE
40
60
80
100
120
140
160
180
RAT AGE (DAYS)

FIGURE 5

THE RELATION OF TOTAL GLOBULIN TO INFECTION

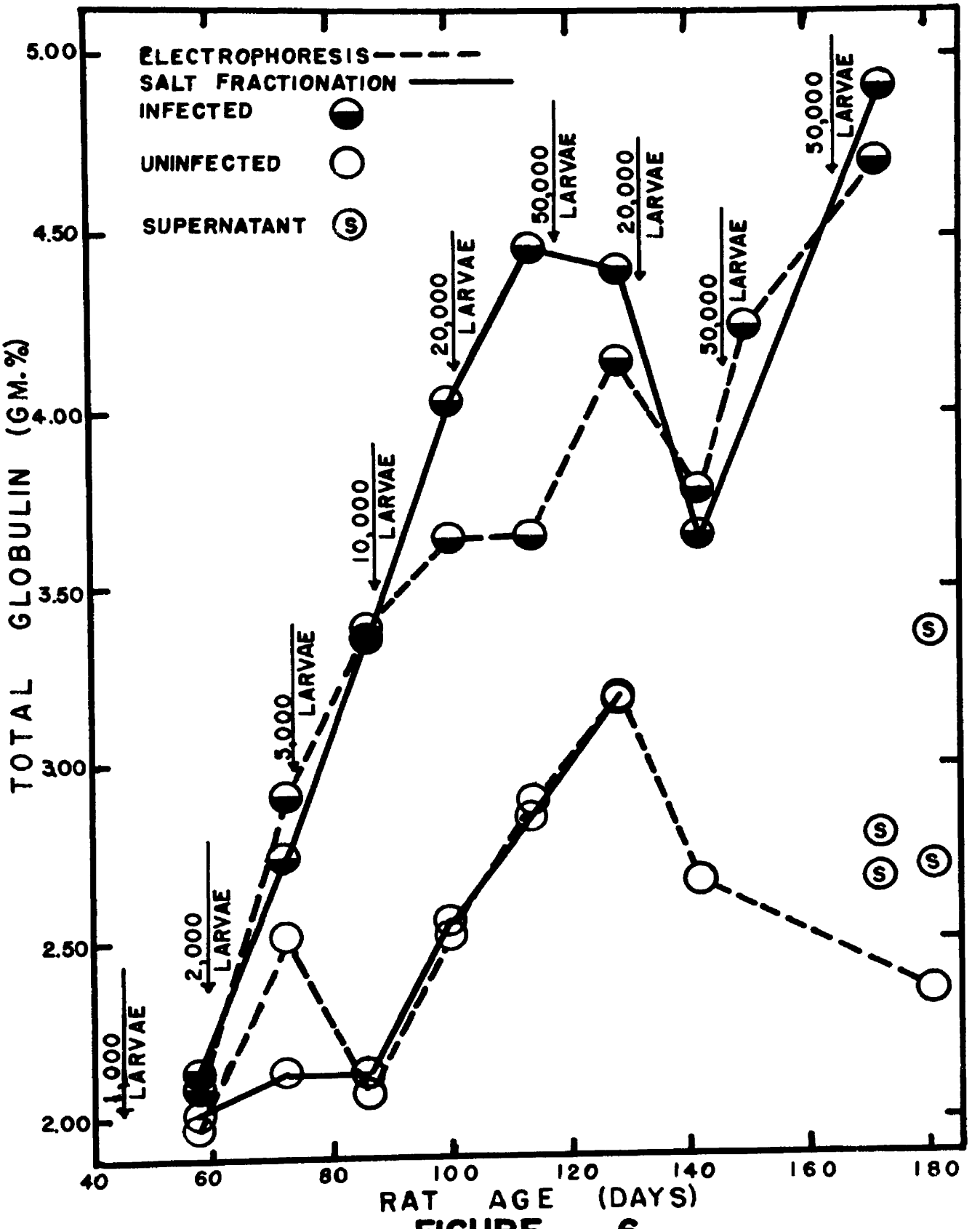



FIGURE 6

THE RELATION OF BETA GLOBULIN TO INFECTION

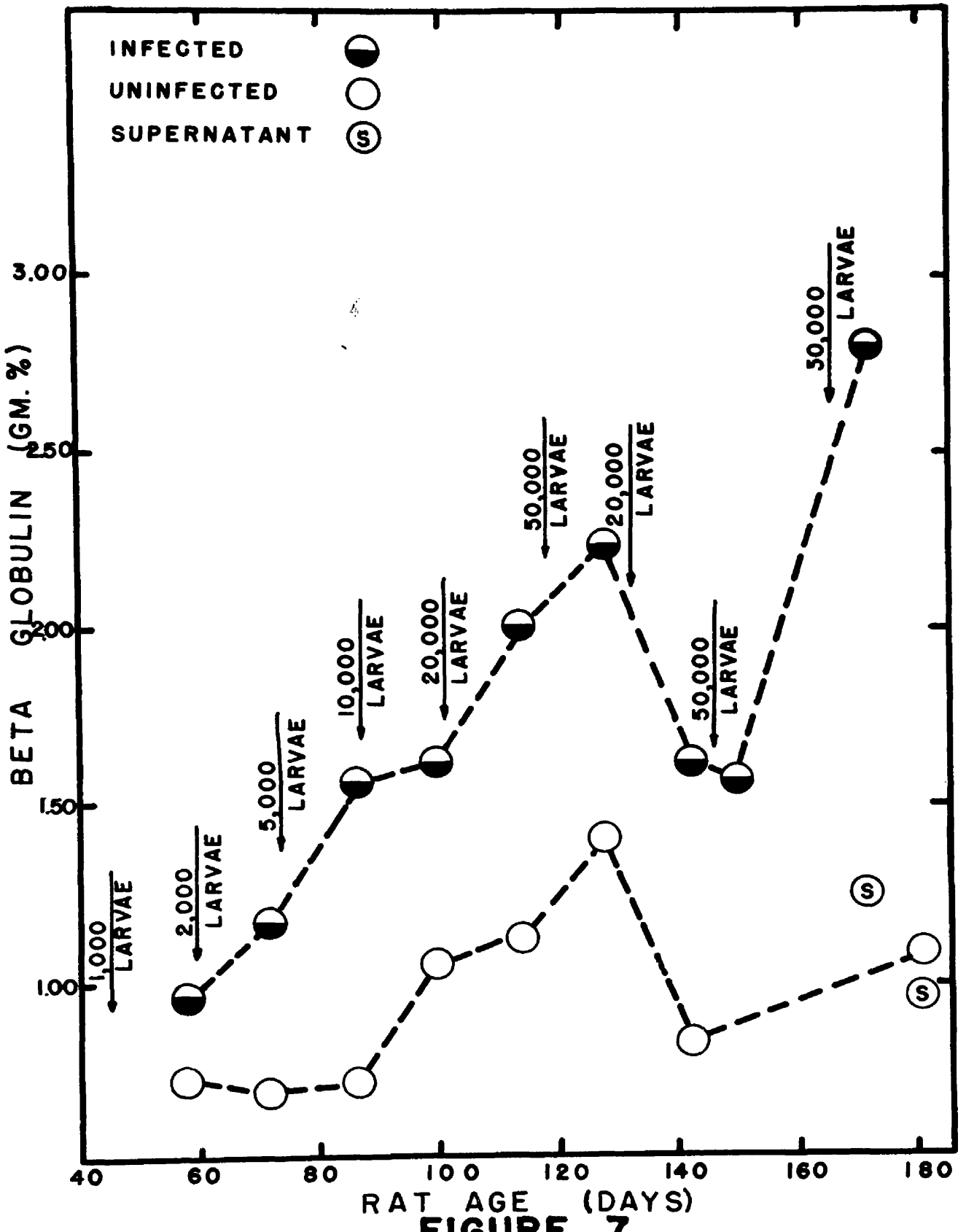
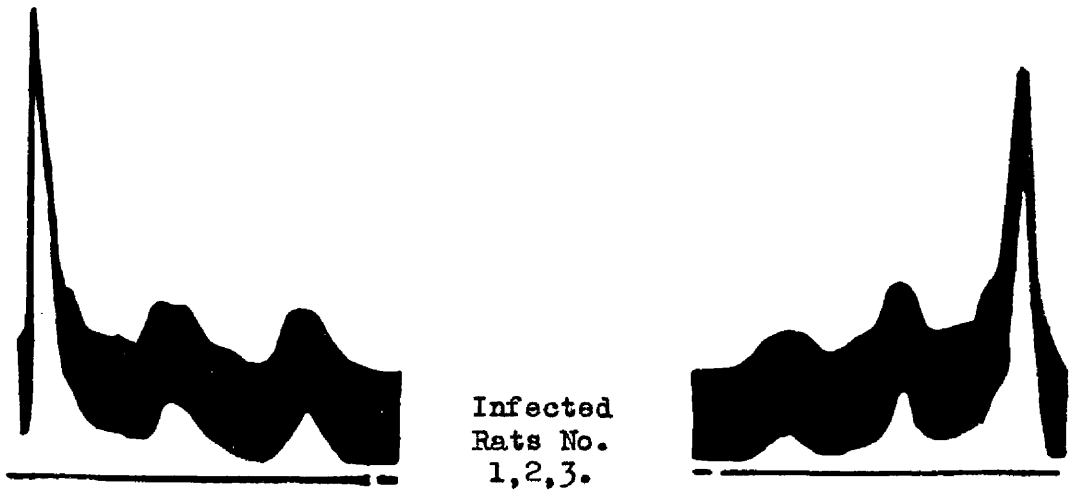


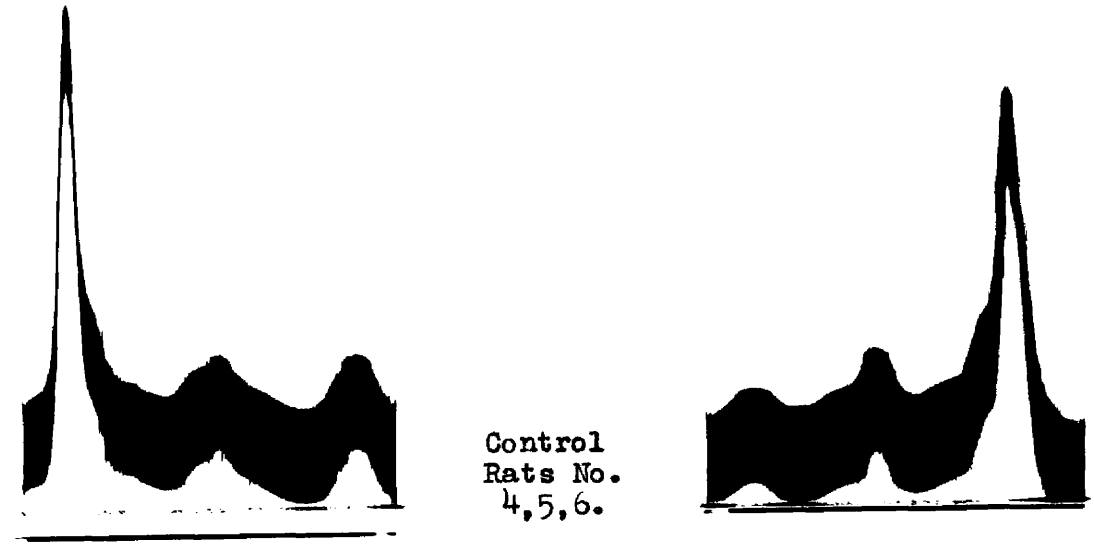

FIGURE 7

ELECTROPHORETIC PATTERNS OF RAT SERA 14 DAYS AFTER THE 1,000 LARVAL INJECTION COMPARED WITH CONTROL

Ascending Descending



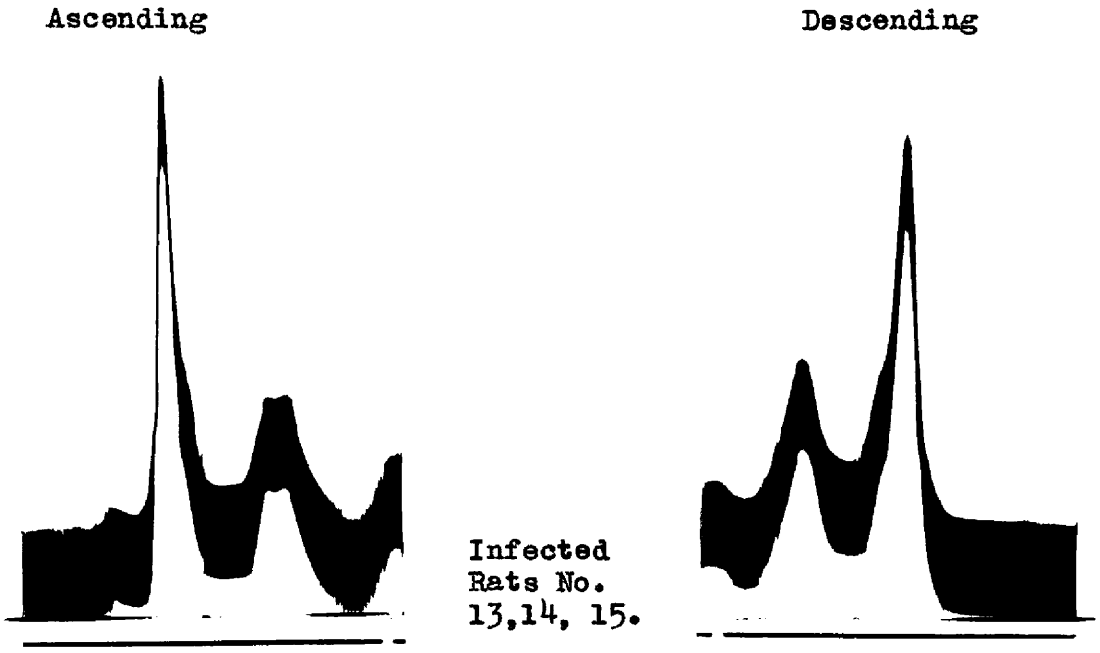

pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,200 sec.; Pot. Grad. not available; 1.70% protein.



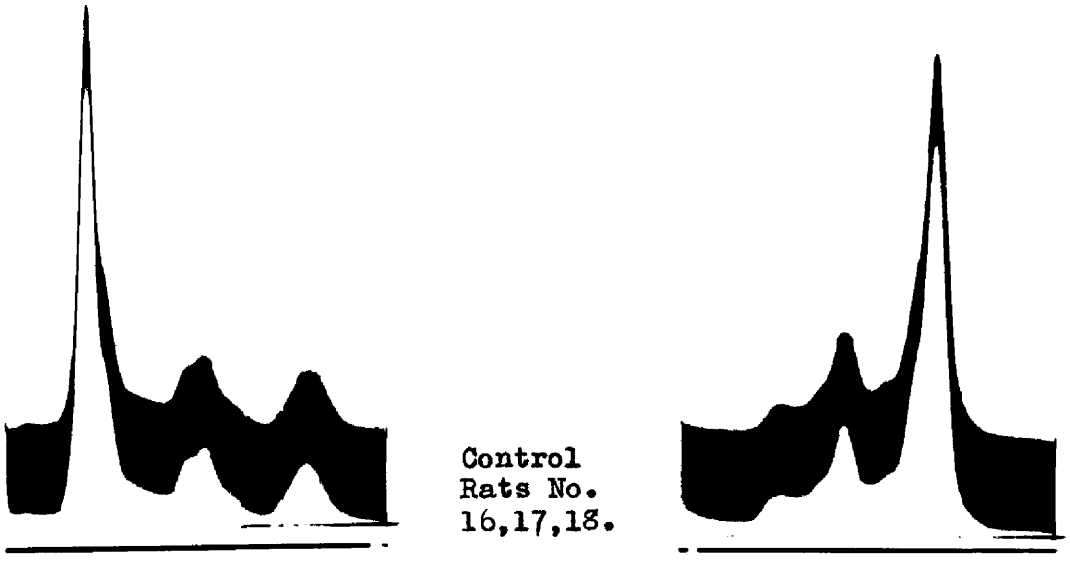

pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,000 sec.; Pot. Grad. 8.4 volts/cm.; 1.84% protein.

FIGURE 8

ELECTROPHORETIC PATTERNS OF RAT SERA 13 DAYS AFTER THE FIRST 20,000 LARVAL INJECTION COMPARED WITH CONTROL



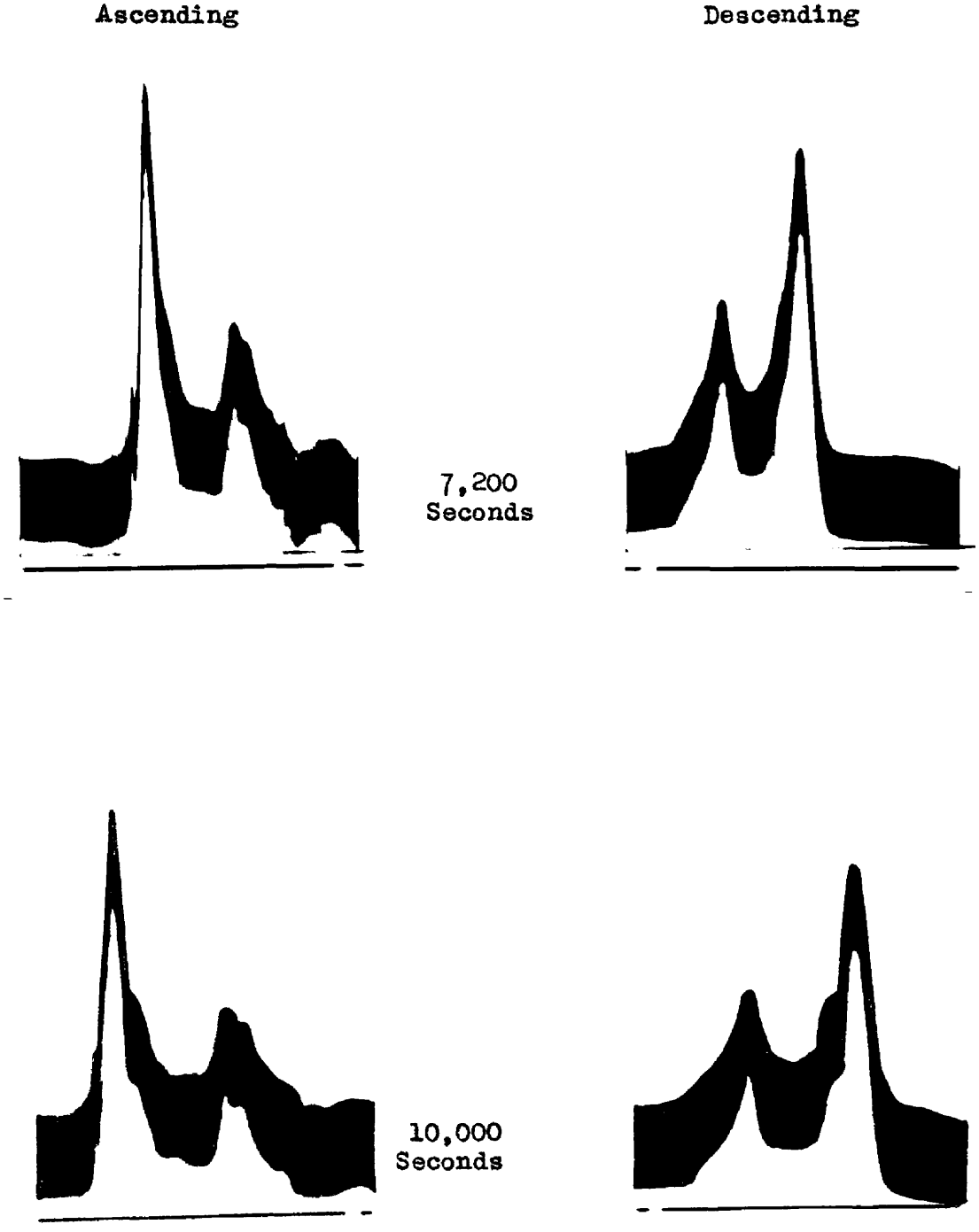

pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,200 sec.; Pot. Grad. 7.4 volts/cm.; 2.98% protein.




pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,200 sec.; Pot. Grad. 8.4 volts/cm.; 2.18% protein.

FIGURE 9

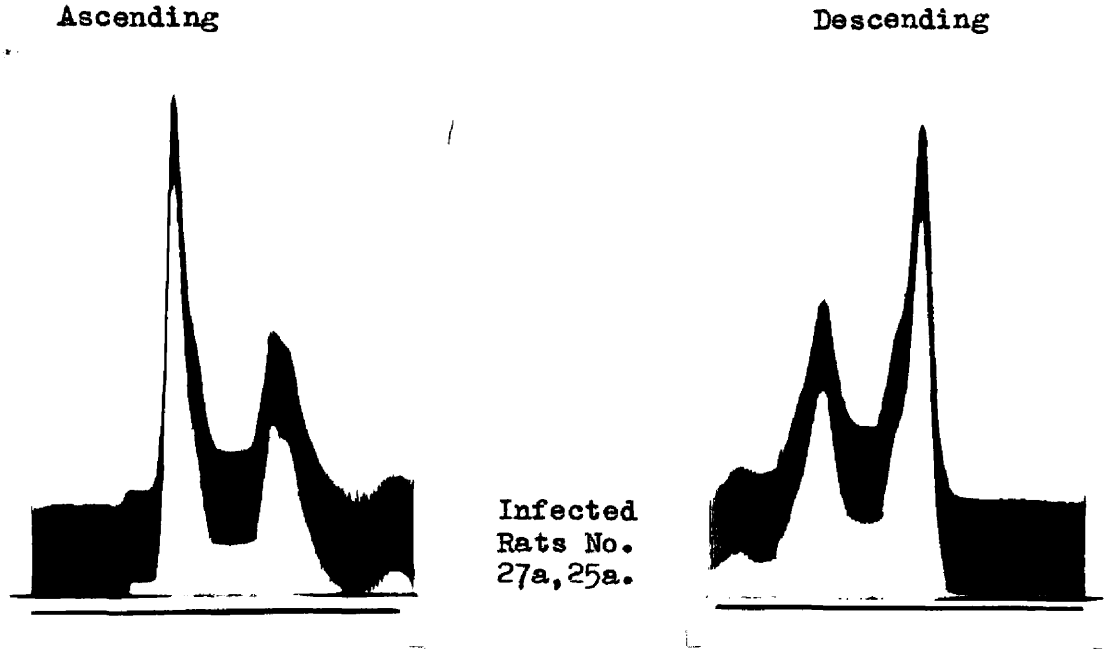
ELECTROPHORETIC PATTERNS OF RAT SERA FOUR DAYS AFTER THE SECOND 50,000 LARVAL INJECTION COMPARED AT 7,200 AND 10,000 SEC.



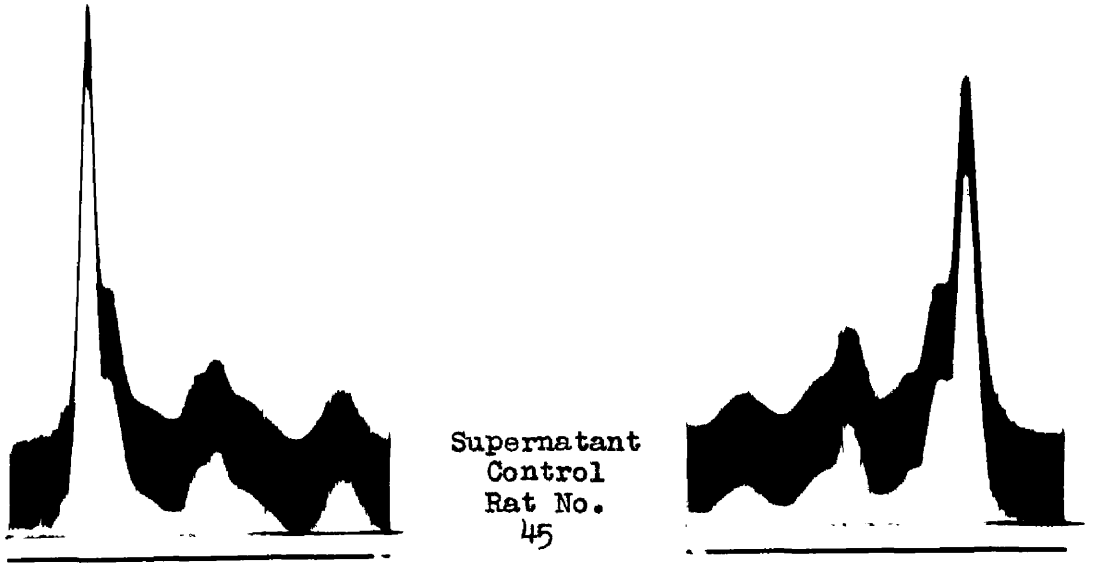

pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; Pot. Grad. 8.6 volts/cm.; 2.77% protein; rats No. 5a and 51.

FIGURE 10

ELECTROPHORETIC PATTERNS OF RAT SERA SEVEN DAYS AFTER THE LAST 50,000 LARVAL INJECTION COMPARED WITH SERA FROM A RAT WHICH RECEIVED INJECTION OF SUPERNATANT FROM THE LARVAL SUSPENSIONS




pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7214 sec.; Pot Grad. 8.4 volts/cm.; 2.74% protein




pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,200 sec.; Pot. Grad. not available; 2.22% protein.

FIGURE 11

TABLE III. THE RELATION OF BODY WEIGHT TO INFECTION (EXPERIMENT I)

| Level of Infection | Age (Days) When Weighed | Number of Rats Weighed | Average Body Weight |
|---|---|---|---|
| Uninfected | 44 | 21 | 87.9 |
| Control | 44 | 21 | 89.4 |
| 1,000 | 58 | 21 | 129.1 |
| Control | 58 | 21 | 137.9 |
| 2,000 | 72 | 17 | 152.8 |
| Control | 72 | 18 | 177.3 |
| 5,000 | 86 | 14 | 206.9 |
| Control | 86 | 15 | 214.9 |
| 10,000 | 100 | 11 | 225.8 |
| Control | 100 | 12 | 239.1 |
| 20,000 | 114 | 9 | 248.2 |
| Control | 114 | 9 | 261.2 |
| 50,000 | 128 | 6 | 252.7 |
| Control | 128 | 5 | 270.0 |
| 20,000 | 142 | 3 | 238.7 |
| Control | 142 | 3 | 269.0 |

TABLE IV. ELECTROPHORETIC AND SALT FRACTIONATION ANALYSIS OF EXPERIMENT Ia

| Rat No. | Age (Days) Last Infected | Age (Days) Bled | Level of Infection | By Salt Fractionation (Gm. %) Total Protein | Alb. | Glob. | A/G |
|---|---|---|---|---|---|---|---|
| 55,56 | 88 | 103 | 1,000 | 5.53 | 3.40 | 2.13 | 1.60 |
| 59,60 | -- | 103 | Control | 6.37 | 3.45 | 2.92 | 1.18 |
| 53,54 | 104 | 117 | 2,000 | 6.28 | 3.39 | 2.89 | 1.17 |
| 57,58 | -- | 117 | Control | 7.04 | 4.31 | 2.73 | 1.58 |

| Rat No. | By Electrophoresis (Gm. %) Alb. | Total Glob. | Globulin $Alpha_1$ | $Alpha_2$ | Beta | Gamma | A/G |
|---|---|---|---|---|---|---|---|
| 55,56 | 3.36 | 2.17 | .62 | .59 | .73 | .14 | 1.55 |
| 59,60 | 3.30 | 3.07 | 1.36 | .50 | .79 | .46 | 1.07 |
| 53,54 | 3.56 | 2.73 | .89 | .43 | .92 | .49 | 1.03 |
| 57,58 | 3.61 | 3.43 | .90 | .64 | 1.46 | .34 | 1.65 |

| Rat No. | MOBILITIES* Alb. | Globulins $Alpha_1$ | $Alpha_2$ | Beta | Gamma |
|---|---|---|---|---|---|
| 55,56 | 5.25 | 4.61 | 4.06 | 3.27 | 2.67 |
| 59,60 | 5.27 | 4.56 | 3.94 | 2.83 | 2.17 |
| 53,54 | 3.62 | 2.97 | 2.55 | 1.95 | 1.25 |
| 57,58 | 4.82 | 4.11 | 3.49 | 2.64 | 1.89 |

*Table figure x $10^{-5}$ $cm^2$ $volt^{-1}$ $sec^{-1}$

## The Analysis of Sera From Older Rats One Week After Infection

Experiment II. Results of Experiment I suggested that the serum changes might be more pronounced seven days after injection although resistance was highest 14 days following injection. Therefore, the purpose of this experiment was to study the serum protein changes seven days after each larval injection.

At the time this experiment was set up, the only rats readily available were those considerably older than used in Experiment I. The rats in Experiment I were initially infected with 1,000 larvae at 45 days of age while those of Experiment II received the same initial infection at 120 days of age. As a result of this wide age difference it is difficult to make direct comparisons between the two experiments. Therefore, the results of Experiment II may be considered as an isolated investigation and an amalgamation with the other experiments will be included in the "Discussion" portion of this thesis.

Tables V and VI contain a composite of the electrophoretic and chemical fractionation analysis for Experiment II. Figures 12 and 13 illustrate graphically portions of Table V.

Table V demonstrates that although the differences were not large, the total protein was higher in the infected animal than in the corresponding control in every instance.

Figure 12 graphically shows that the total globulin increased markedly as the number of larvae is increased.

In Figure 13 the beta globulin concentrations resulting from larval injections are plotted. Only points which were obtained from the analysis of

the best resolved patterns are included in the graph. The beta globulin concentration was increased in the infected animal in every case with the highest point following the 50,000 larval injection.

Figures 14 and 15 contain electrophoretic patterns which compare the early part of the hyperimmunization process with the latter part. The pattern from the 2,000 larval injection showed little difference in the concentrations of the various components when compared with the corresponding control (Figure 14). Again it was noted that the component mobilities of the infected animals decreased markedly when compared with the control. Examination of the patterns in Figure 15 shows the infected animals (bled seven days following the only 50,000 larvae) exhibited a marked increase in the beta globulin peak and a decrease in mobility.

It has been shown (Luetscher 1947) that certain pathological changes affect electrophoretic patterns. In the necropsy examinations of Experiment I it was noted that the lungs of the infected animals were greatly enlarged. Therefore, in Experiment II it was decided to take weight measurements of the lungs throughout the course of hyperimmunization. The results of these measurements are recorded in Table VII. When the lung weight is converted to per cent of the total body weight[13] the increase in the infected animals was noted in some cases to be four-fold (Rats 68 and 78, Table VII).

Grossly the lungs of the infected animals were enlarged, highly infiltrated with scar tissue and contained a considerable number of petechial hemorrhages.

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[13] $\frac{\text{lung weight}}{\text{body weight}} \times 100$ = per cent of body weight attributable to the lungs.

On microscopical examination the air sacs were found generally to contain an exudate composed of (in order of their prevalence) erythocytes, polymorphonuclear leucocytes, lymphocytes, and monocytes. This pathology cannot be totally disregarded in evaluation of electrophoretic patterns.

Summarizing the results of Experiment II it was noted that in the infected animals the total protein, total globulin, and specifically the beta globulin were increased when compared with the control. During hyperimmunization the lungs increased as much as four-fold by weight (percent of total body weight).

TABLE V. ELECTROPHORETIC AND SALT

| Rat No. | Age (Days) Last Infected | Bled | No. of Larvae | By Salt Fractionation (Gm. %) Total Protein | Alb. | Glob. | A/G |
|---|---|---|---|---|---|---|---|
| 61,62 | 133 | 144 | 2,000 | 6.65 | 4.10 | 2.55 | 1.61 |
| 71,76 | -- | 144 | Control | 6.38 | 4.18 | 2.20 | 1.90 |
| 63,64 | 148 | 155 | 5,000 | 6.55 | 3.74 | 2.81 | 1.33 |
| 72,77 | -- | 155 | Control | 5.74 | 3.58 | 2.16 | 1.66 |
| 65,66 | 161 | 169 | 10,000 | 6.85 | 3.78 | 3.07 | 1.22 |
| 73,74 | -- | 169 | Control | 6.39 | 4.12 | 2.27 | 1.83 |
| 67,68 | 176 | 183 | 20,000 | 6.55 | 3.10 | 3.45 | .90 |
| 78,79 | -- | 183 | Control | 6.47 | 4.10 | 2.37 | 1.73 |
| 69,70 | 190 | 197 | 50,000 | 6.74 | 2.73 | 4.01 | .68 |
| 75 | -- | 197 | Control | 6.22 | 3.56 | 2.66 | 1.34 |

FRACTIONATION ANALYSIS OF EXPERIMENT II

| By Electrophoresis | | | | | | |
|---|---|---|---|---|---|---|
| Alb. | Total Glob. | Globulins | | | | |
| | | $Alpha_1$ | $Alpha_2$ | Beta | Gamma | A/G |
| 3.52 | 3.13 | .91 | .49 | 1.39 | .35 | 1.12 |
| 3.50 | 2.88 | 1.28 | .50 | .90 | .27 | 1.21 |
| 3.76 | 2.79 | -- | -- | -- | -- | 1.34 |
| 3.19 | 2.55 | 1.14 | .40 | .86 | .21 | 1.25 |
| 3.36 | 3.49 | .94 | .62 | 1.49 | .35 | .96 |
| 3.73 | 2.66 | 1.13 | .31 | 1.10 | .19 | 1.40 |
| 3.14 | 3.41 | -- | -- | -- | -- | .92 |
| 3.82 | 2.64 | -- | -- | -- | -- | 1.45 |
| 2.90 | 3.84 | 1.03 | .42 | 2.06 | .39 | .76 |
| 3.40 | 2.82 | 1.33 | .42 | .80 | .38 | 1.20 |

TABLE VI. MOBILITY CALCULATIONS OF EXPERIMENT II

| Rat No. | Alb. | MOBILITIES* Globulins | | | |
|---|---|---|---|---|---|
| | | $Alpha_1$ | $Alpha_2$ | Beta | Gamma |
| 61,62 | 3.64 | 3.06 | 2.65 | 1.99 | 1.37 |
| 71,76 | 5.92 | 5.17 | 4.43 | 3.31 | 2.65 |
| 63,64 | 3.01 | -- | -- | -- | -- |
| 72,77 | 5.90 | 5.00 | 4.10 | 3.05 | 2.15 |
| 65,66 | 3.84 | 3.27 | 2.61 | 1.88 | 1.23 |
| 73,74 | 3.69 | 3.95 | 2.42 | 1.64 | .94 |
| 67,68 | 4.72 | 4.27 | — | -- | -- |
| 78,79 | 3.72 | 3.20 | 2.89 | 2.46 | — |
| 69,70 | 3.48 | 3.81 | 2.31 | 1.74 | 1.09 |
| 75 | 4.33 | 3.69 | 3.18 | 2.59 | 1.99 |

* Table figure x $10^{-5}$ $cm^2$ $volt^{-1}$ $sec^{-1}$

# THE RELATION OF TOTAL GLOBULIN TO INFECTION

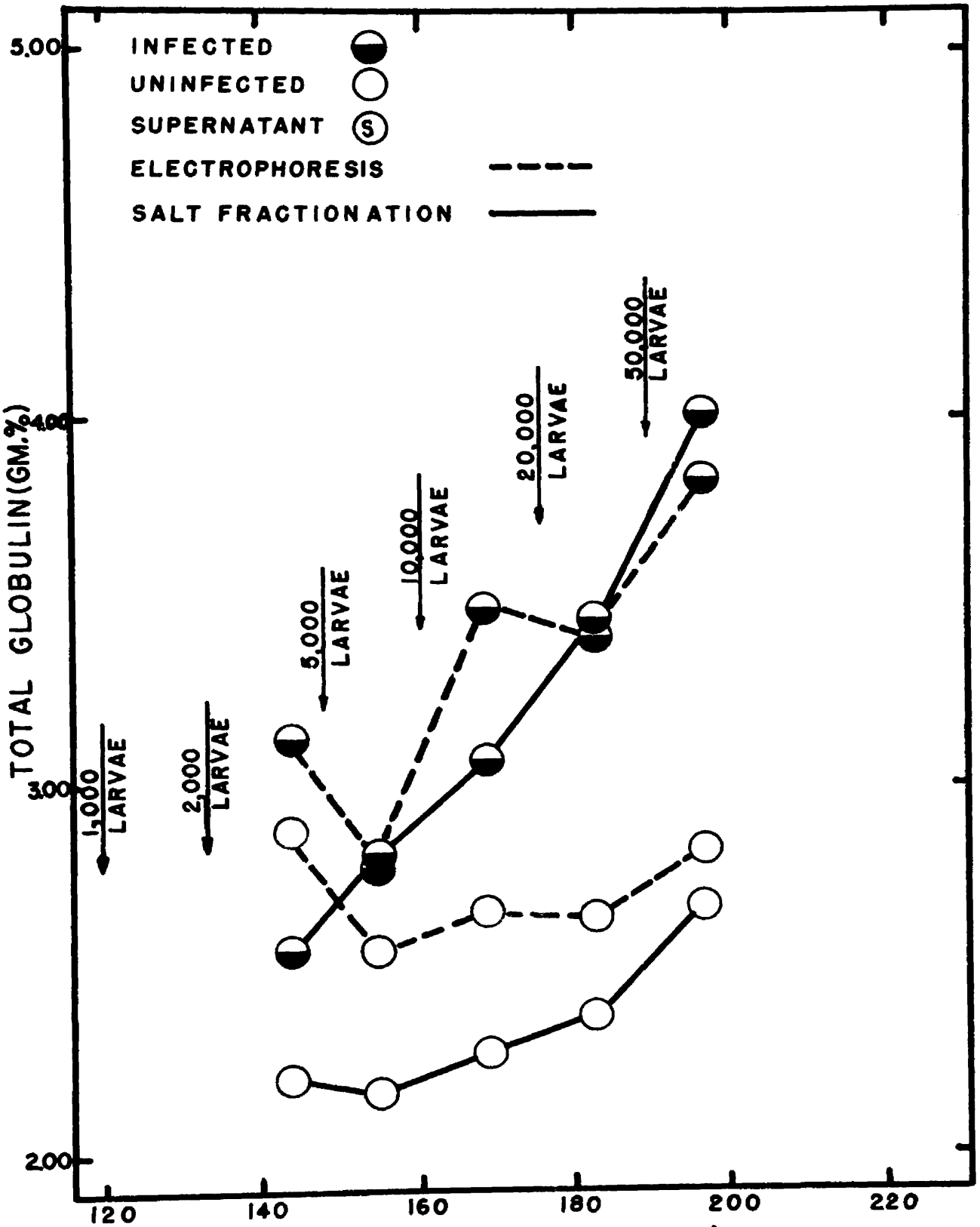



**FIGURE 12**

# THE RELATION OF BETA GLOBULIN TO INFECTION

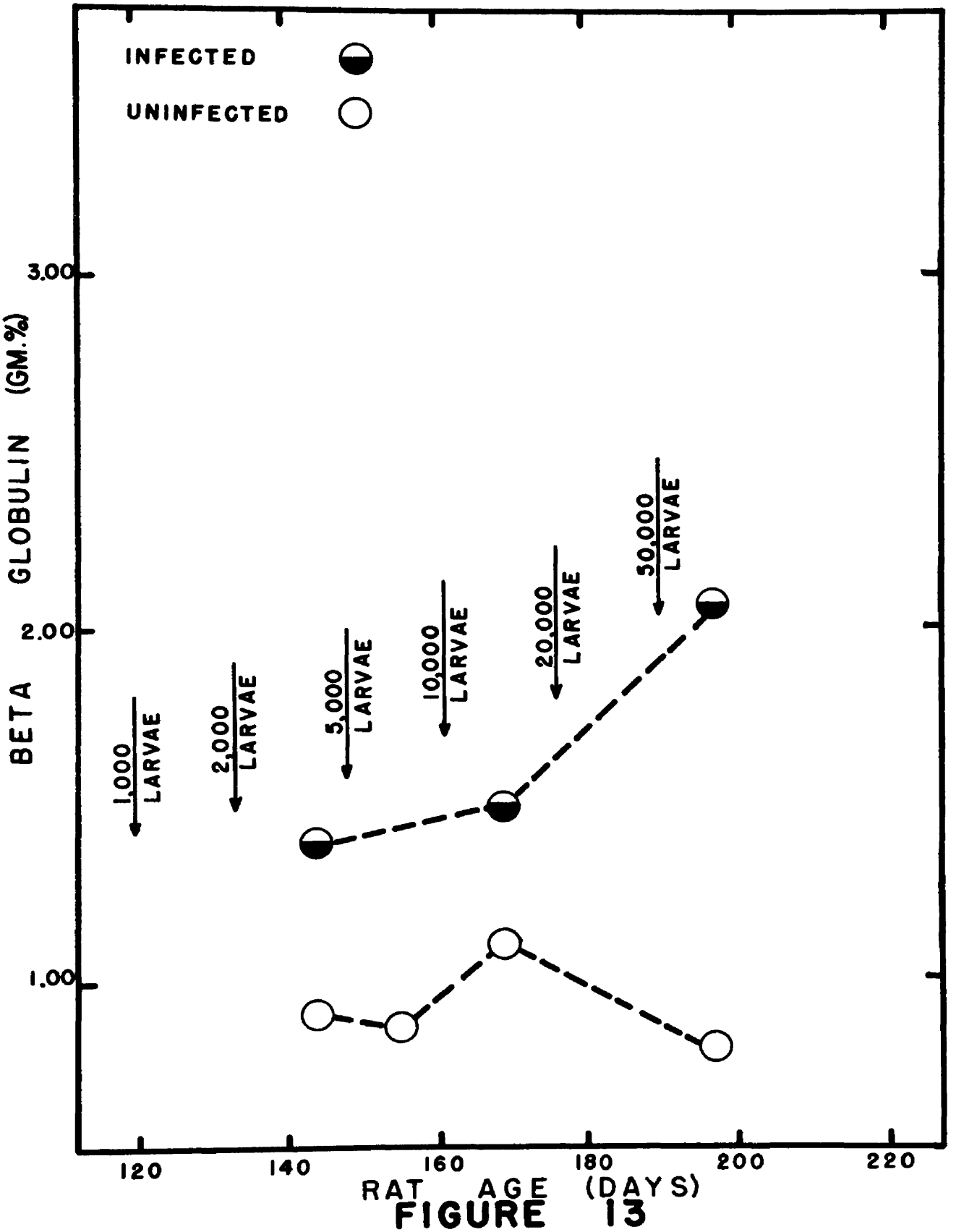
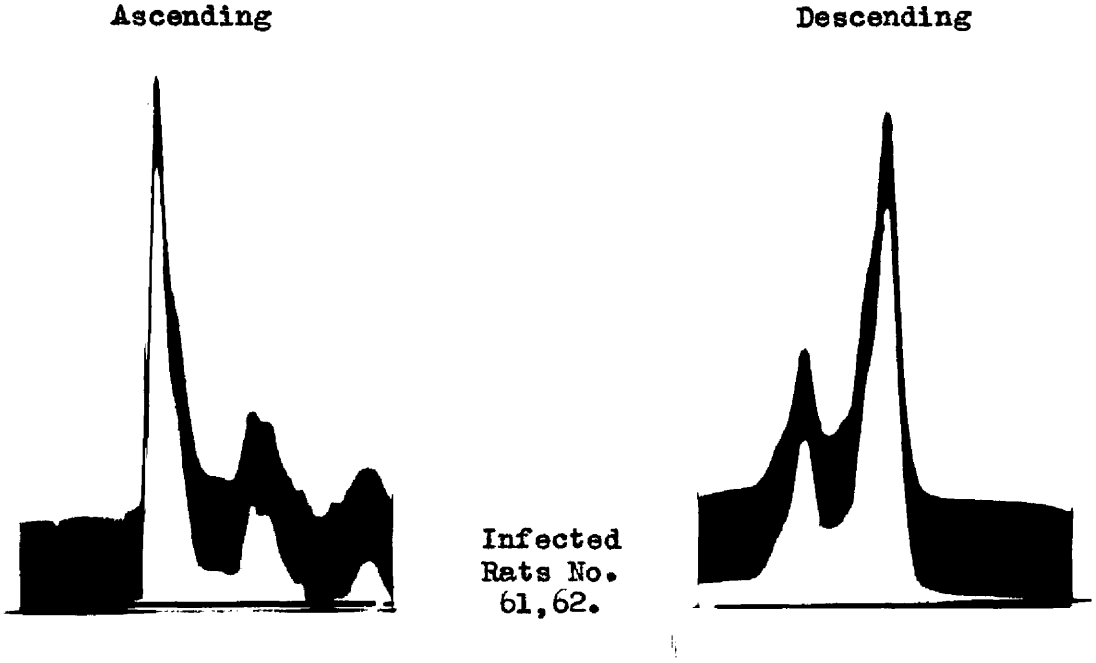
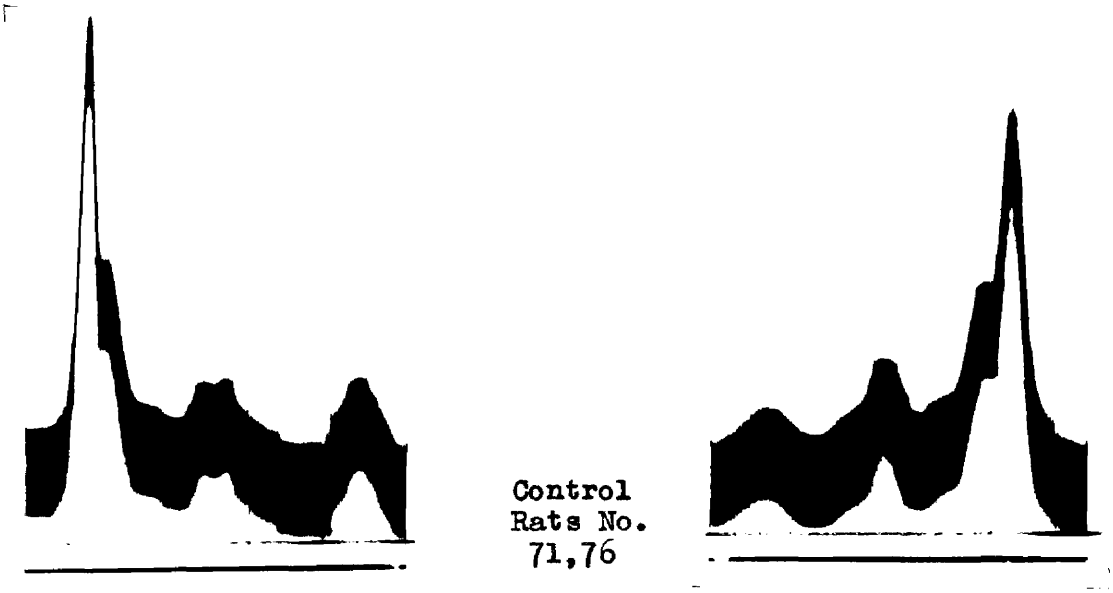



FIGURE 13

ELECTROPHORETIC PATTERNS OF RAT SERA 11 DAYS AFTER THE 2,000 LARVAL INJECTION COMPARED WITH CONTROL




pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,200 sec.; Pot. Grad. 8.4 volts/cm.; 2.22% protein.

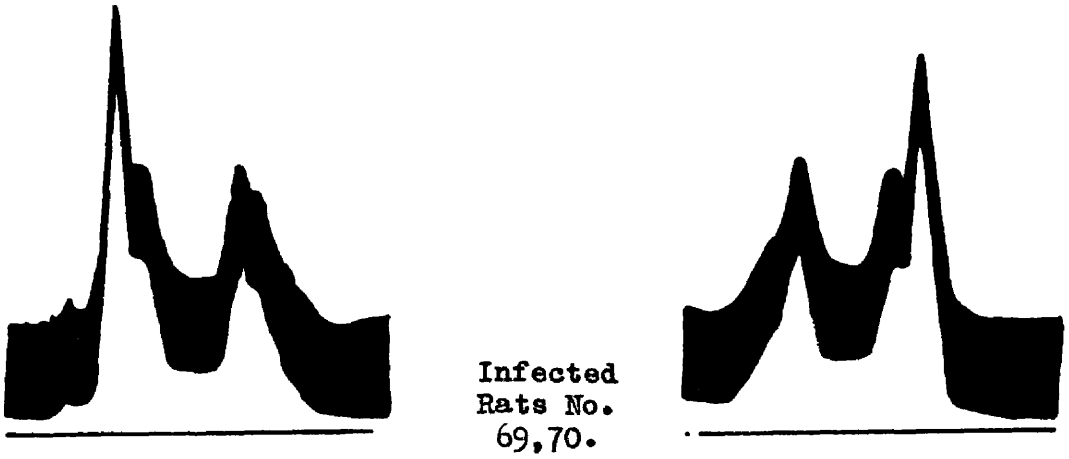



pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,200 sec.; Pot. Grad. 8.4 volts/cm.; 2.13% protein.

FIGURE 14

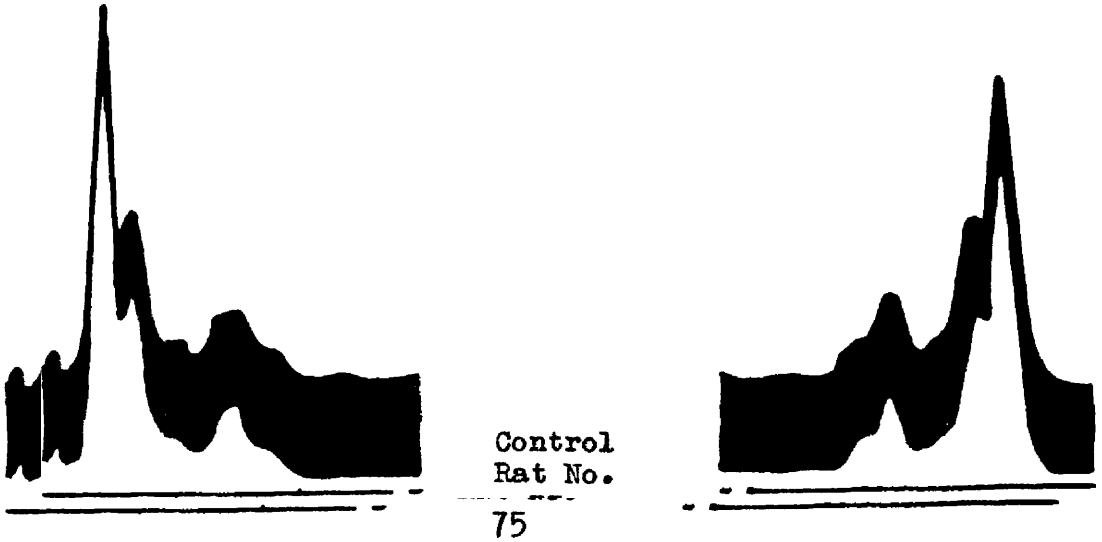
ELECTROPHORETIC PATTERNS OF RAT SERA SEVEN DAYS AFTER THE ONLY 50,000 LARVAL INJECTION COMPARED WITH CONTROL

Ascending Descending



Infected Rats No. 69,70.

pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 10,000 sec.; Pot. Grad. 8.6 volts/cm.; 2.27% protein.



Control Rat No. 75

pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 10,000 sec.; Pot. Grad. 8.2 volts/cm.; 2.07% protein.

FIGURE 15

TABLE VII. THE RELATION OF LUNG WEIGHT TO INFECTION (EXPERIMENT II)

| | Rat No. | Level of Infection | Body Weight | Lung Weight | $\frac{\text{Lung wgt.}}{\text{Body wgt.}} \times 100$ |
|---|---|---|---|---|---|
| Infected | 61 | 2,000 | 259 | 3.05 | 1.18 |
| Infected | 62 | 2,000 | 246 | 2.09 | .85 |
| Control | 71 | -- | 271 | 1.71 | .63 |
| Control | 76 | -- | 278 | 1.34 | .48 |
| Infected | 63 | 5,000 | 237 | 2.60 | 1.10 |
| Infected | 64 | 5,000 | 248 | 3.10 | 1.25 |
| Control | 72 | -- | 208 | 1.28 | .61 |
| Control | 77 | -- | 256 | 1.59 | .62 |
| Infected | 65 | 10,000 | 266 | 4.10 | 1.54 |
| Infected | 66 | 10,000 | 264 | 4.80 | 1.82 |
| Control | 73 | -- | 272 | 1.80 | .66 |
| Control | 74 | -- | 287 | 1.50 | .52 |
| Infected | 67 | 20,000 | 292 | 4.15 | 1.42 |
| Infected | 68 | 20,000 | 293 | 5.36 | 1.83 |
| Control | 78 | -- | 295 | 1.17 | .40 |
| Control | 79 | -- | 317 | 1.38 | .44 |
| Infected | 70 | 50,000 | 215 | * | |
| Infected | 69 | 50,000 | 277 | * | |
| Control | 75 | -- | 267 | * | |

* Weights not available.

## The Analysis of Sera From Young Rats One Week After Each Infection

Experiment III. Results from Experiment I showed the greatest serum protein changes to occur seven days after the last injection of 50,000 larvae (see Table I for injection sequence). The purpose of Experiment III was to determine the serum protein changes that occurred seven days after the last injection in order to compare these changes with those observed 14 days after infection. When Experiment III was initiated, the rats were approximately the same age as those used in Experiment I. Therefore, a better comparison between the two experiments was possible.

Tables VIII and IX are composites of the results obtained by the electrophoretic and chemical fractionation analysis of Experiment III. Pertinent data of Table VIII are presented graphically in Figures 16, 17, and 18.

Consistent with the findings of the previous experiments, the total serum globulin showed a progressive increase as the number of larvae was increased (Figure 16). The most significant increase in total globulin was again noted seven days after the only injection of 50,000 larvae. Figure 16 shows that from a determination made 21 days after this 50,000 larval injection, the total globulin had dropped considerably when compared with the determination made seven days after the injection of the same number of larvae.

The beta globulin was higher in the infected animal than in the corresponding control in every case (Figure 17). The most outstanding increase occurred seven days after the 50,000 larval injection.

Twenty-one days after this injection of 50,000 larvae the beta globulin had decreased considerably from the concentration it had reached seven days after injection.

In Figure 18 the albumin to globulin (A/G) ratios for Experiment III are plotted. The A/G ratio gradually decreased as the number of larvae increased. This was not surprising since the globulins were already shown to increase in the infected animal. In addition, in almost every pathological state there is a relative or absolute decrease in serum albumin (Luetscher 1947). Examination of Table VIII substantiates this observation. The influence of these two developments would result in an A/G ratio decrease.

Figures 19 and 20 contain the electrophoretic patterns from infected rats 140, 141, 142 and controls 143, 144, 145. Analysis of identical samples was made in both veronal and phosphate buffers. As previously mentioned the veronal buffer separates the gamma globulin from the salt boundary, whereas the phosphate buffer shows better resolution between the beta and gamma globulins. Since the objective was to confirm the finding that the beta globulin component was increased by the infection, the use of both buffers presented the logical approach to analysis. Figure 20 shows that in phosphate buffer the beta and gamma globulins were resolved somewhat better than in veronal buffer (Figure 19). Electrophoretic data and calculations for the serum runs in both buffers are recorded in Table X. It was found that in both buffers the beta globulin was approximately doubled in the infected animal after seven days when compared with the uninfected control, whereas the gamma globulin was not significantly increased.

TABLE X. IMMUNE SERUM ANALYZED ELECTROPHORETICALLY IN VERONAL AND PHOSPHATE BUFFERS (Gm. %)

| Rats | Total Protein | Alb. | Total Glob. | Globulins | | | | A/G |
|---|---|---|---|---|---|---|---|---|
| | | | | $Alpha_1$ | $Alpha_2$ | Beta | Gamma | |
| | | | Veronal Buffer | | | | | |
| I | 7.21 | 2.47 | 4.74 | 1.43 | .59 | 2.10 | .63 | .52 |
| C | 6.90 | 3.71 | 3.19 | 1.02 | .64 | 1.02 | .53 | 1.16 |
| | | | Phosphate Buffer | | | | | |
| I | 7.21 | 3.32 | 3.89 | .94 | .47 | 1.53 | .97 | .85 |
| C | 6.90 | 3.74 | 3.16 | 1.13 | .48 | .82 | .72 | 1.18 |

I= Infected
C = Control

Figure 21 shows the serum pattern changes 21 days after the only 50,000 larval injection. It was noted that, although the beta globulin was still somewhat increased, the over-all pattern of the infected animals was approaching normalcy.

Table XI indicates that when the lung weight was converted to percent of the total body weight, the increase following the injection of the 50,000 larvae was noted to be as high as a six fold increase over the controls (Rats 144 and 289, Table XI). Twenty-one days after the 50,000 larval injection, these weights had dropped indicating the maximum change had been reached and the tendency was now toward normalcy.

Thus, in Experiment III it was ascertained that: (1) the total globulin increased as the number of larvae was increased; (2) the beta globulin specifically was increased when analysed in two different buffers; (3) changes in serum proteins were most outstanding following the injection of the 50,000 larvae; (4) the lung weight (converted to percent

of the total body weight) was increased as the number of larvae was increased and (5) after injections ceased this lung weight receded.

TABLE VIII. ELECTROPHORETIC AND SALT

| Rat No. | Age (Days) | | No. of Larvae | By Salt Fractionation (Gm. %) | | | |
|---|---|---|---|---|---|---|---|
| | Last Infected | Bled | | Total Protein | Alb. | Glob. | A/G |
| 116-17-18 | 58 | 65 | 1,000 | 5.98 | -- | -- | -- |
| 119-20-21 | -- | 65 | Control | 5.32 | -- | -- | -- |
| 134-35-36 | 73 | 80 | 2,000 | 6.32 | 3.22 | 3.10 | 1.04 |
| 137-38-39 | -- | 80 | Control | 6.05 | 3.83 | 2.22 | 1.72 |
| 152-53-54 | 87 | 94 | 5,000 | 6.20 | 3.16 | 3.04 | 1.04 |
| 155-56-57 | -- | 94 | Control | 6.03 | 3.47 | 2.56 | 1.36 |
| 128-29-30 | 101 | 108 | 10,000 | 6.77 | 3.16 | 3.61 | .88 |
| 131-32-33 | -- | 108 | Control | 7.42* | 3.85 | 3.57 | 1.08 |
| 146-47-48 | 115 | 122 | 20,000 | 5.97 | 2.65 | 3.32 | .80 |
| 149-50-51 | -- | 122 | Control | 6.04 | 3.42 | 2.62 | 1.30 |
| 140-41-42 | 129 | 136 | 50,000 | 7.21 | 2.84 | 4.37 | .65 |
| 143-44-45 | -- | 136 | Control | 6.90 | 3.88 | 3.02 | 1.28 |
| 158-59-60 | 129 | 150 | 50,000 | 7.25 | 3.38 | 3.87 | .87 |
| 161-63 | -- | 150 | Control | 6.60 | 3.85 | 2.75 | 1.40 |

* Hemolysis.

FRACTIONATION CALCULATIONS OF EXPERIMENT III

| By Electrophoresis (Gm. %) | | | | | | |
|---|---|---|---|---|---|---|
| Alb. | Total Glob. | Globulins | | | | A/G |
| | | $Alpha_1$ | $Alpha_2$ | Beta | Gamma | |
| 3.55 | 2.43 | .79 | .39 | .86 | .36 | 1.46 |
| 2.85 | 2.47 | -- | -- | -- | -- | 1.15 |
| 3.30 | 3.02 | .56 | .64 | 1.27 | .60 | 1.09 |
| 3.57 | 2.48 | .66 | .51 | .80 | .55 | 1.44 |
| 2.80 | 3.40 | 1.02 | .45 | 1.49 | .45 | .82 |
| 2.92 | 3.01 | .74 | .46 | 1.20 | .74 | .97 |
| 3.36 | 3.41 | 1.16 | .39 | 1.47 | .35 | .99 |
| 3.98 | 3.44 | -- | -- | -- | -- | 1.16 |
| 2.75 | 3.22 | .52 | .90 | 1.50 | .30 | .85 |
| 3.25 | 2.79 | -- | -- | -- | -- | 1.17 |
| 2.47 | 4.74 | 1.43 | .59 | 2.10 | .63 | .52 |
| 3.71 | 3.19 | 1.02 | .64 | 1.02 | .53 | 1.16 |
| 3.62 | 3.63 | 1.10 | .71 | 1.54 | .31 | 1.00 |
| 3.63 | 2.97 | .98 | .36 | .98 | .29 | 1.22 |

TABLE IX. MOBILITY CALCULATIONS OF EXPERIMENT III

| Rat No. | Alb. | MOBILITIES* Globulins | | | |
|---|---|---|---|---|---|
| | | $Alpha_1$ | $Alpha_2$ | Beta | Gamma |
| 116-17-18 | 3.70 | 3.09 | 2.62 | 1.93 | 1.41 |
| 119-20-21 | 3.48 | -- | -- | -- | -- |
| 134-35-36 | 4.61 | 4.04 | 3.52 | 2.63 | 1.94 |
| 137-38-39 | 4.76 | 4.17 | 3.69 | 2.98 | 2.42 |
| 152-53-54 | 3.54 | 2.70 | 2.03 | 1.19 | .36 |
| 155-56-57 | 4.38 | 3.75 | 3.24 | 2.53 | 1.78 |
| 128-29-30 | 3.56 | 2.96 | 2.56 | 1.96 | 1.48 |
| 131-32-33 | -- | -- | -- | -- | -- |
| 146-47-48 | 4.05 | 3.60 | 3.23 | 2.72 | 2.42 |
| 149-50-51 | -- | -- | -- | -- | -- |
| 140-41-42 | 3.88 | 3.35 | 2.94 | 2.21 | 1.88 |
| 143-44-45 | 5.34 | 4.77 | 4.01 | 3.03 | 2.31 |
| 158-59-60 | 4.72 | 4.06 | 3.17 | 2.07 | 1.26 |
| 161-63 | 4.72 | 4.00 | 3.32 | 2.20 | 1.52 |

*Table figure x $10^{-5}$ $cm^2$ $volt^{-1}$ $sec^{-1}$

# THE RELATION OF TOTAL GLOBULIN TO INFECTION

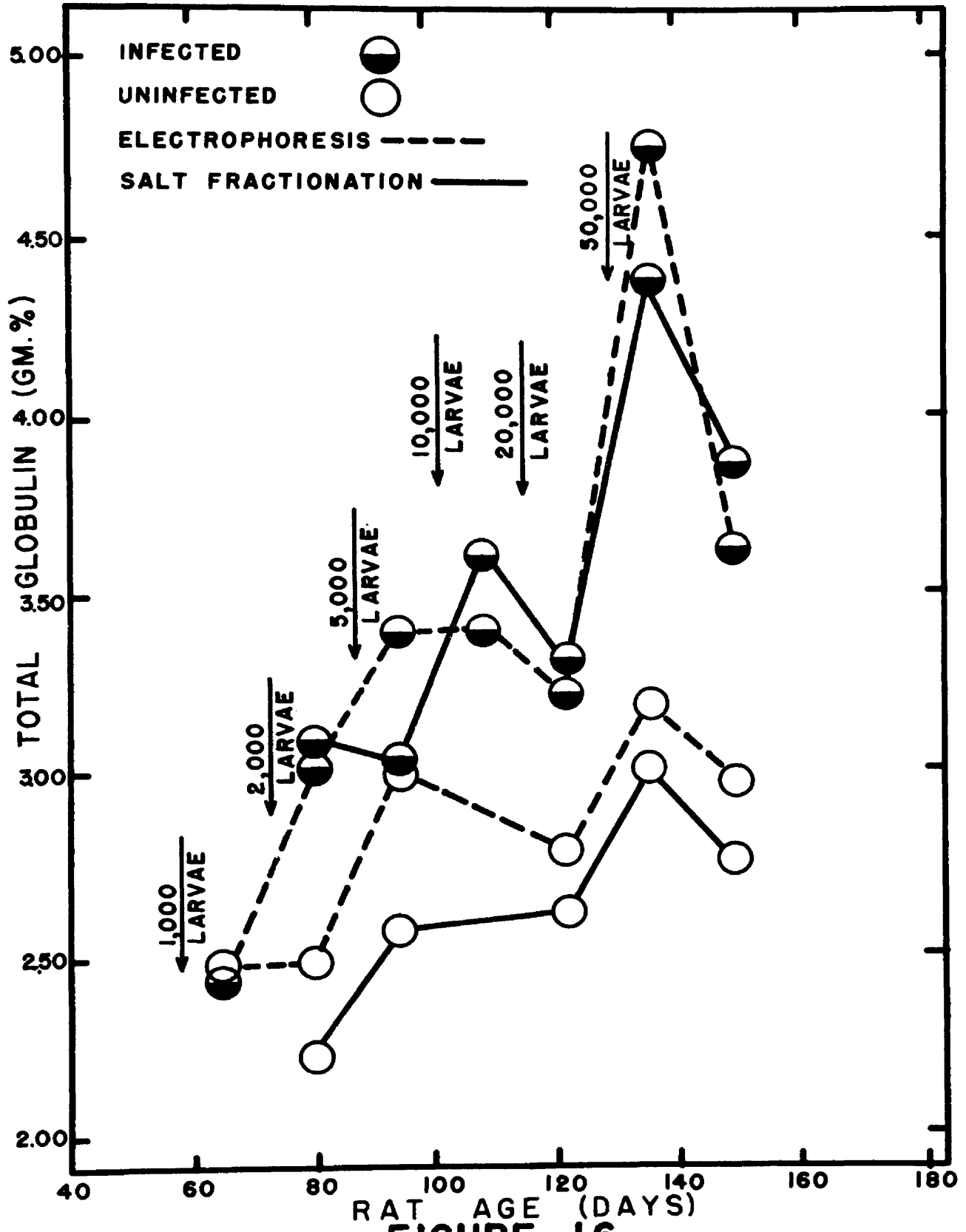



FIGURE 16

# THE RELATION OF BETA GLOBULIN TO INFECTION

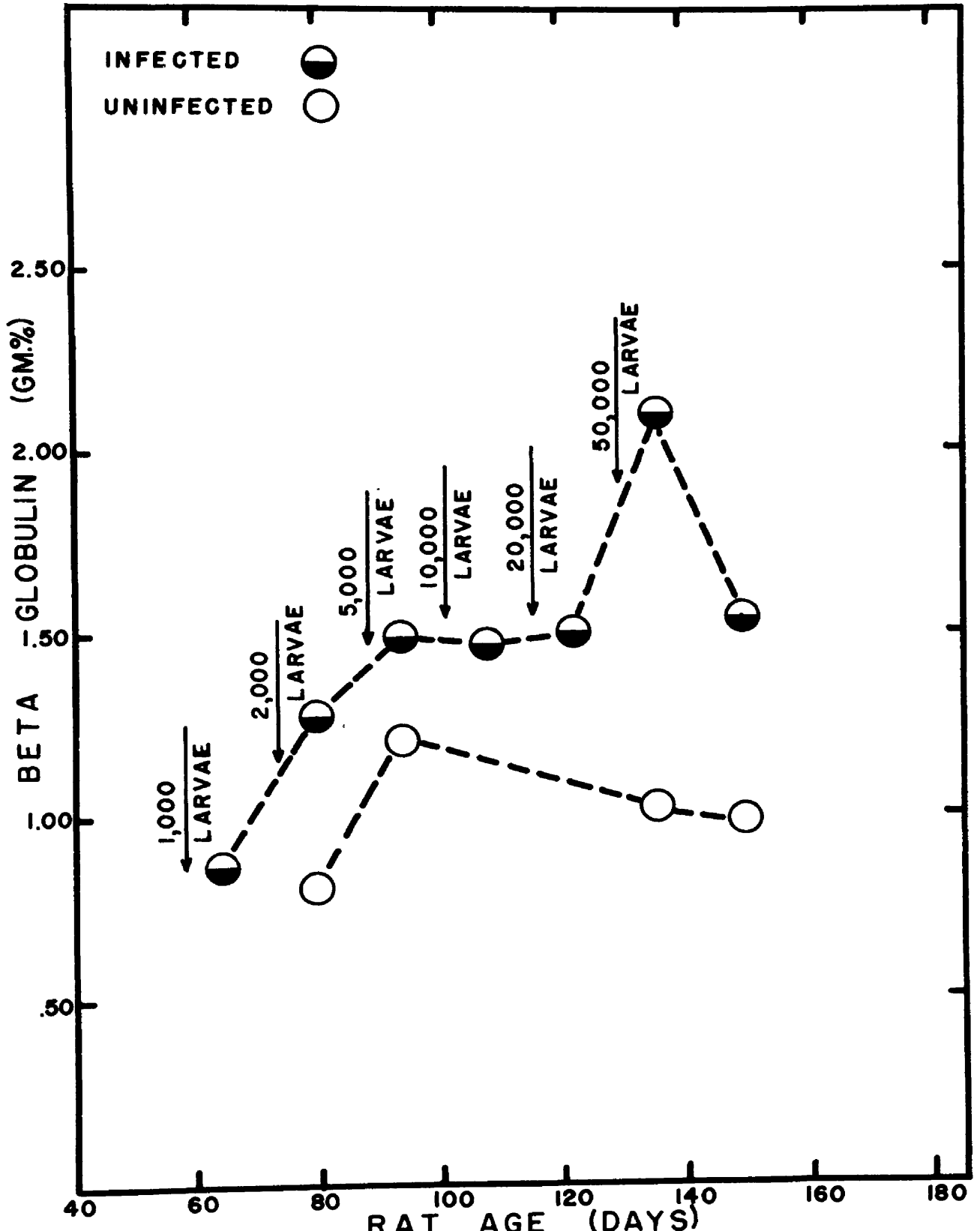



FIGURE 17

THE RELATION OF THE A/G RATIO TO INFECTION

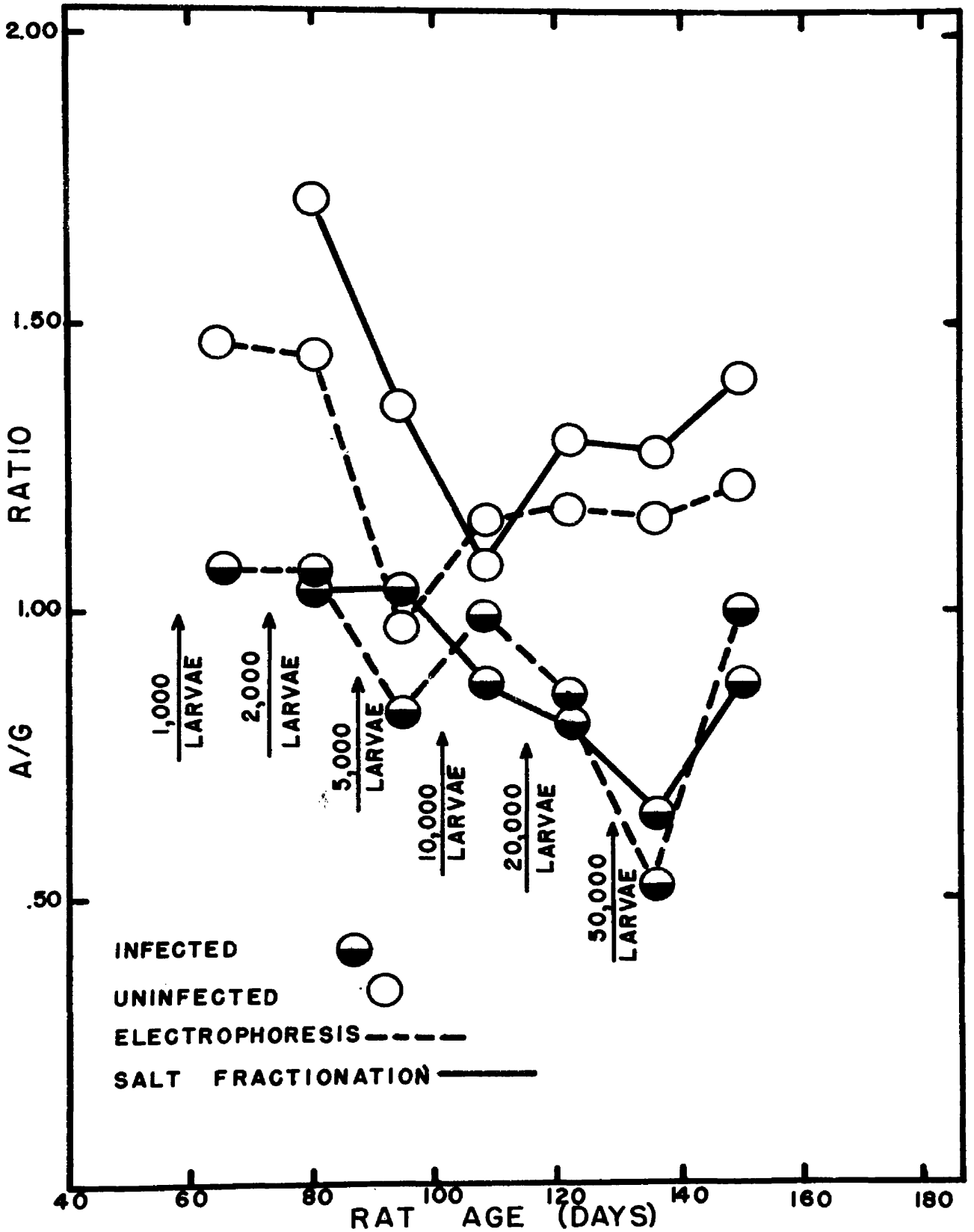
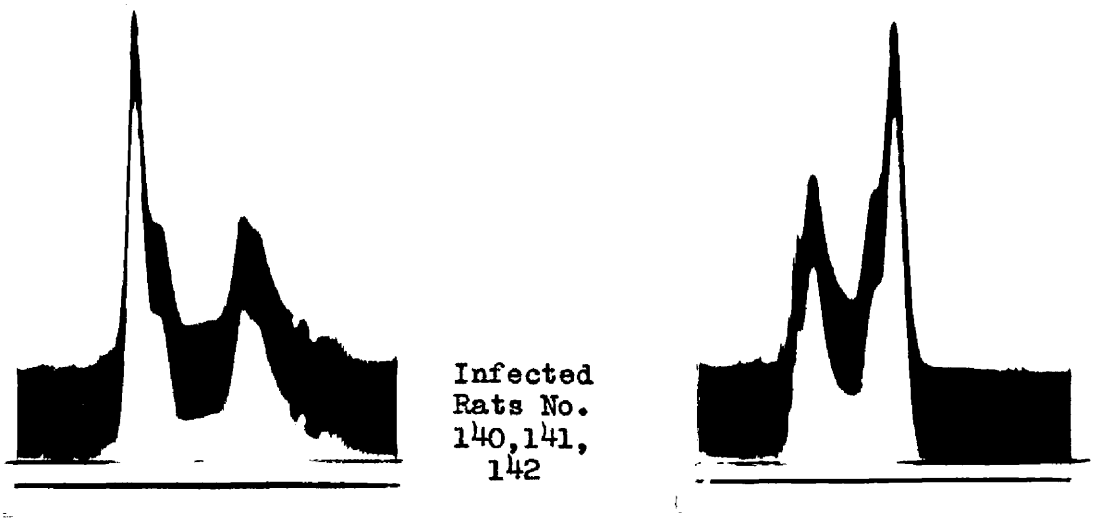



FIGURE 18

ELECTROPHORETIC PATTERNS OF RAT SERA SEVEN DAYS AFTER THE ONLY 50,000 LARVAL INJECTION COMPARED WITH CONTROL

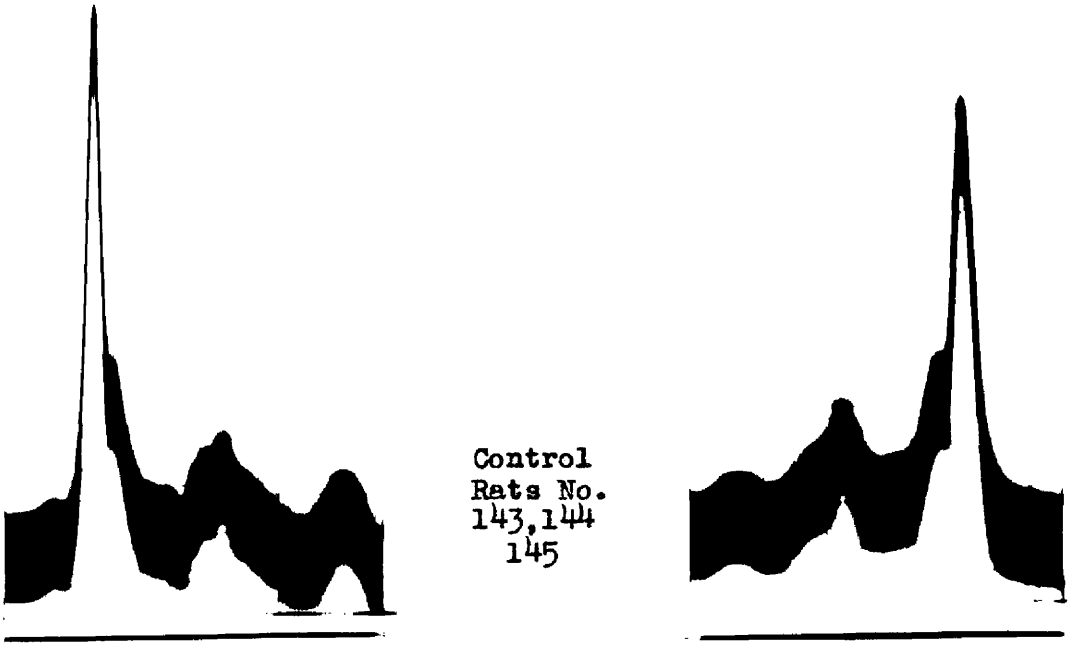
Ascending

Descending



Infected Rats No. 140,141, 142

pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,200 sec.; Pot. Grad. 8.5 volts/cm.; 2.40% protein.



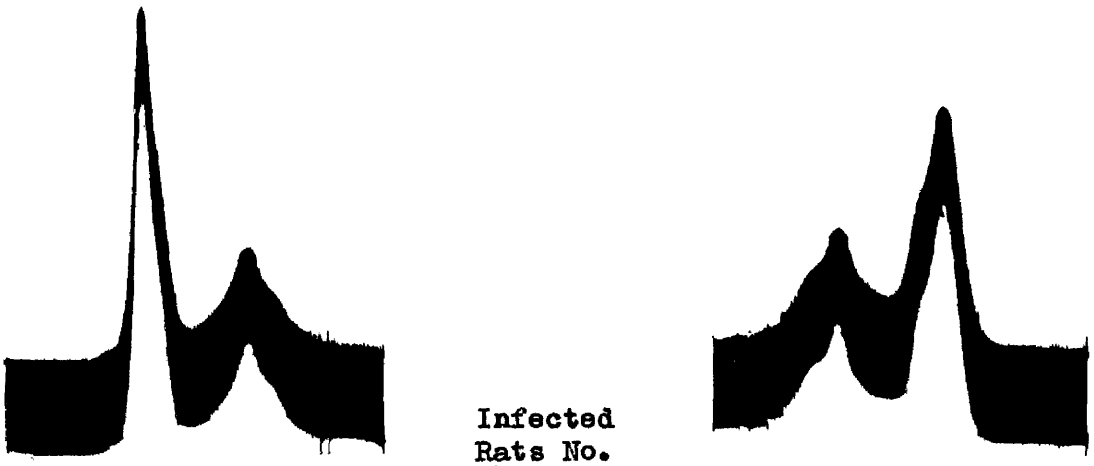
Control Rats No. 143,144 145

pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,200 sec.; Pot. Grad. 8.6 volts/cm.; 2.30% protein.

FIGURE 19

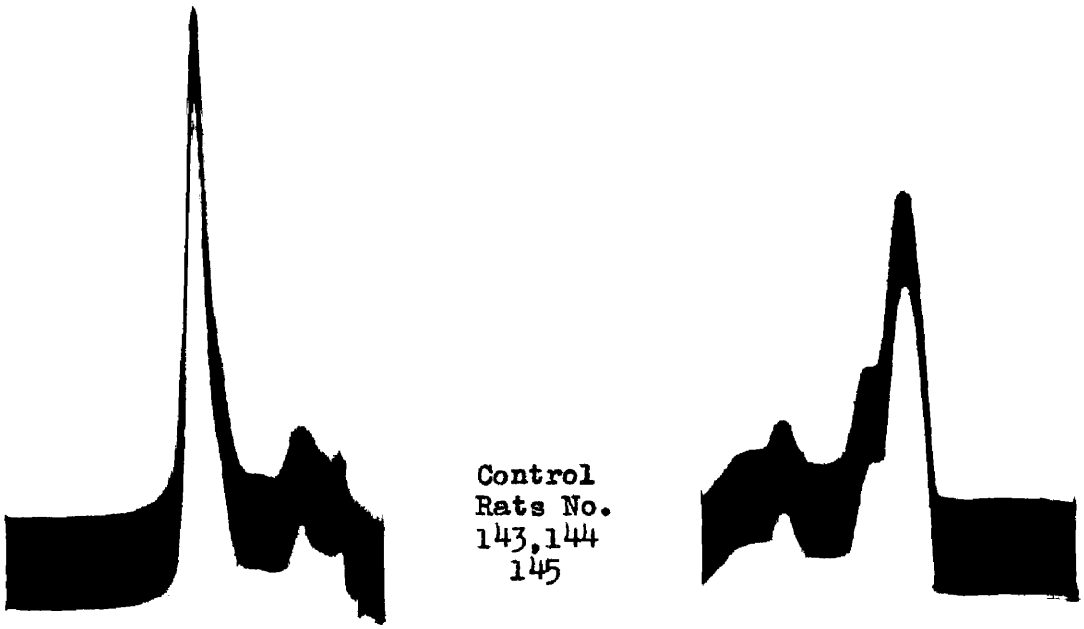
ELECTROPHORETIC PATTERNS OF RAT SERA SEVEN DAYS AFTER THE ONLY 50,000 LARVAL INJECTION COMPARED WITH CONTROL

Ascending Descending



Infected Rats No. 140,141, 142.

pH 7.4; Phosphate buffer (sodium phosphate 0.02M and sodium chloride 0.15M); 12,000 sec.; Pot. Grad. 4.9 volts/cm.; 2.40% protein



Control Rats No. 143,144 145

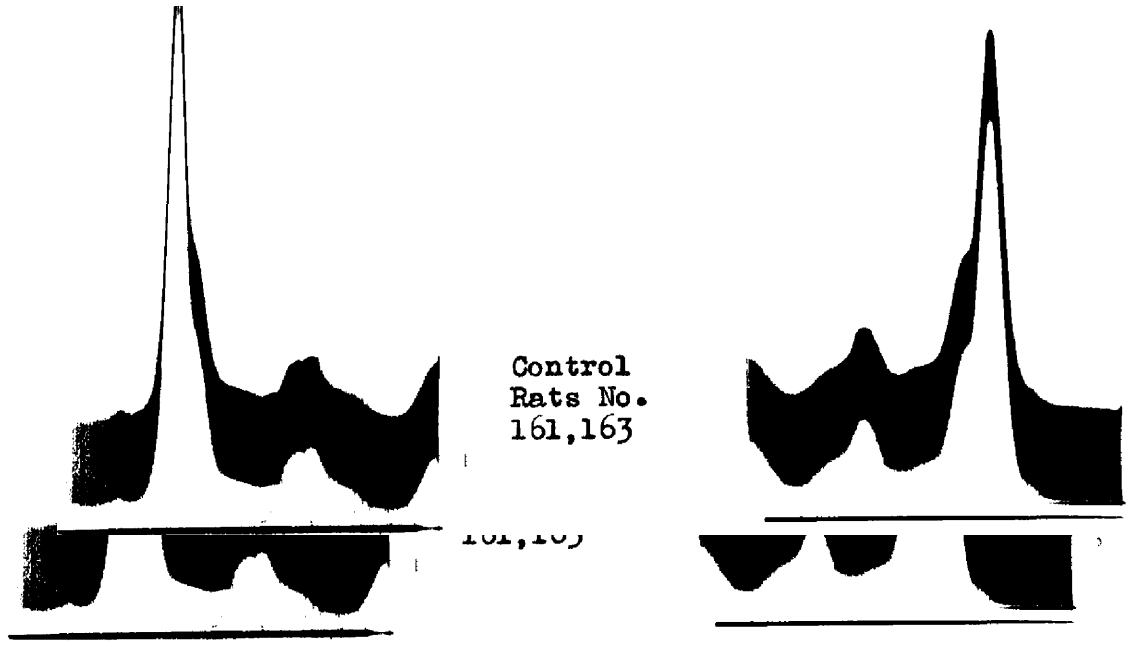
pH 7.4; Phosphate buffer (sodium phosphate 0.02M and sodium chloride 0.15M); 14,400 sec.; Pot. Grad. 4.9 volts/cm.; 2.30% protein.

FIGURE 20

ELECTROPHORETIC PATTERNS OF RAT SERA 21 DAYS AFTER THE ONLY 50,000 LARVAL INJECTION COMPARED WITH CONTROL

Ascending Descending


pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,200 sec.; Pot. Grad. 8.5 volts/cm.; 2.42% protein.




pH 8.6; 0.1M Veronal buffer; Ionic strength 0.1; 7,200 sec.; Pot. Grad. 8.7 volts/cm.; 2.20% protein.

FIGURE 21

TABLE XI. THE RELATION OF LUNG WEIGHT TO INFECTION
(EXPERIMENT III)

| | Rat No. | Level of Infection | Body Weight | Lung Weight | $\frac{\text{Lung wgt.}}{\text{Body wgt.}} \times 100$ |
|---|---|---|---|---|---|
| Control | 119 | --- | 134 | .75 | 0.56 |
| Control | 120 | --- | 174 | .95 | 0.55 |
| Control | 121 | --- | 143 | 1.38* | 0.97 |
| Infected | 116 | 1,000 | 135 | 1.05 | 0.78 |
| Infected | 117 | 1,000 | 150 | 1.00 | 0.67 |
| Infected | 118 | 1,000 | 137 | 1.07 | 0.78 |
| Control | 137 | --- | 150 | 1.00 | 0.67 |
| Control | 138 | --- | 155 | 1.01 | 0.65 |
| Control | 139 | --- | 172 | .91 | 0.53 |
| Infected | 134 | 2,000 | 144 | 1.31 | 0.91 |
| Infected | 135 | 2,000 | 166 | 1.40 | 0.84 |
| Infected | 136 | 2,000 | 181 | 1.42 | 0.78 |
| Control | 155 | --- | 186 | 1.06 | 0.57 |
| Control | 156 | --- | 187 | 1.34* | 0.72 |
| Control | 157 | --- | 237 | 1.17 | 0.49 |
| Infected | 152 | 5,000 | 169 | 1.35 | 0.80 |
| Infected | 153 | 5,000 | 223 | 1.73 | 0.78 |
| Infected | 154 | 5,000 | 228 | 2.80 | 1.23 |
| Control | 131 | --- | 250 | 1.22 | 0.49 |
| Control | 132 | --- | 248 | 1.23 | 0.50 |
| Control | 133 | --- | 242 | 1.20 | .50 |
| Infected | 128 | 10,000 | 250 | 1.67 | 0.67 |
| Infected | 129 | 10,000 | 235 | 2.38 | 1.01 |
| Infected | 130 | 10,000 | 200 | 3.53 | 1.77 |
| Control | 149 | --- | 269 | 1.30 | 0.48 |
| Control | 150 | --- | 274 | 1.10 | 0.40 |
| Control | 151 | --- | 293 | 1.50 | 0.51 |
| Infected | 146 | 20,000 | 246 | 5.00 | 2.03 |
| Infected | 147 | 20,000 | 234 | 2.10 | .90 |
| Infected | 148 | 20,000 | 266 | 8.62 | 3.24 |
| Control | 143 | --- | 326 | 1.26 | 0.39 |
| Control | 144 | --- | 265 | 1.20 | 0.45 |
| Control | 145 | --- | 230 | 1.10 | 0.48 |
| Infected | 140 | 50,000 | 300 | 3.75 | 1.25 |
| Infected | 141 | 50,000 | 275 | 7.05 | 2.56 |
| Infected | 142 | 50,000 | 256 | 7.40 | 2.89 |
| Control | 161 | --- | 255 | 1.00 | 0.39 |
| Control | 163 | --- | 216 | 1.30 | 0.61 |
| Infected | 158 | 50,000 | 264 | 2.73 | 1.03 |
| Infected | 159 | 50,000 | 283 | 3.06 | 1.08 |
| Infected | 160 | 50,000 | 284 | 3.40 | 1.20 |

*Lung showed congestion.

## Composite Results of Serum Protein Changes In Experiments I and III

Since the determinations in Experiment I were made two weeks after each injection and those of Experiment III were made one week after each injection, the serum protein changes were more completely demonstrated when the results of the two experiments were plotted on the same graph. In Figures 22 and 23 the total globulin and the beta globulin, respectively, are plotted against infection from both experiments.

The total globulin (Figure 22) of the infected animal showed a gradual increase as hyperimmunization proceeded. In the early stages no consistent pattern of changes occurred, i.e. measurements at seven days after the last injection were not consistently higher than those taken two weeks after the last injection or vice versa. However, when the four electrophoretic determinations were made 4, 7, 13, and 21 days after the 50,000 larval injection, the highest total serum globulin was found to be on the seventh day.

The composite graph of the beta globulin determinations in Figure 23 shows there was a higher concentration of this component in the infected animal throughout hyperimmunization. Of particular interest is the fact that in five out of six intervals between injections the beta globulin of infected animals was slightly higher after two weeks than one week after each infection.

Thus the composite of the two experiments, tends to substantiate the fact, that a gradual increase in total globulin and specifically, the beta component, occurs as the hyperimmunization proceeds.

# THE RELATION OF TOTAL GLOBULIN TO INFECTION (COMPOSITE)

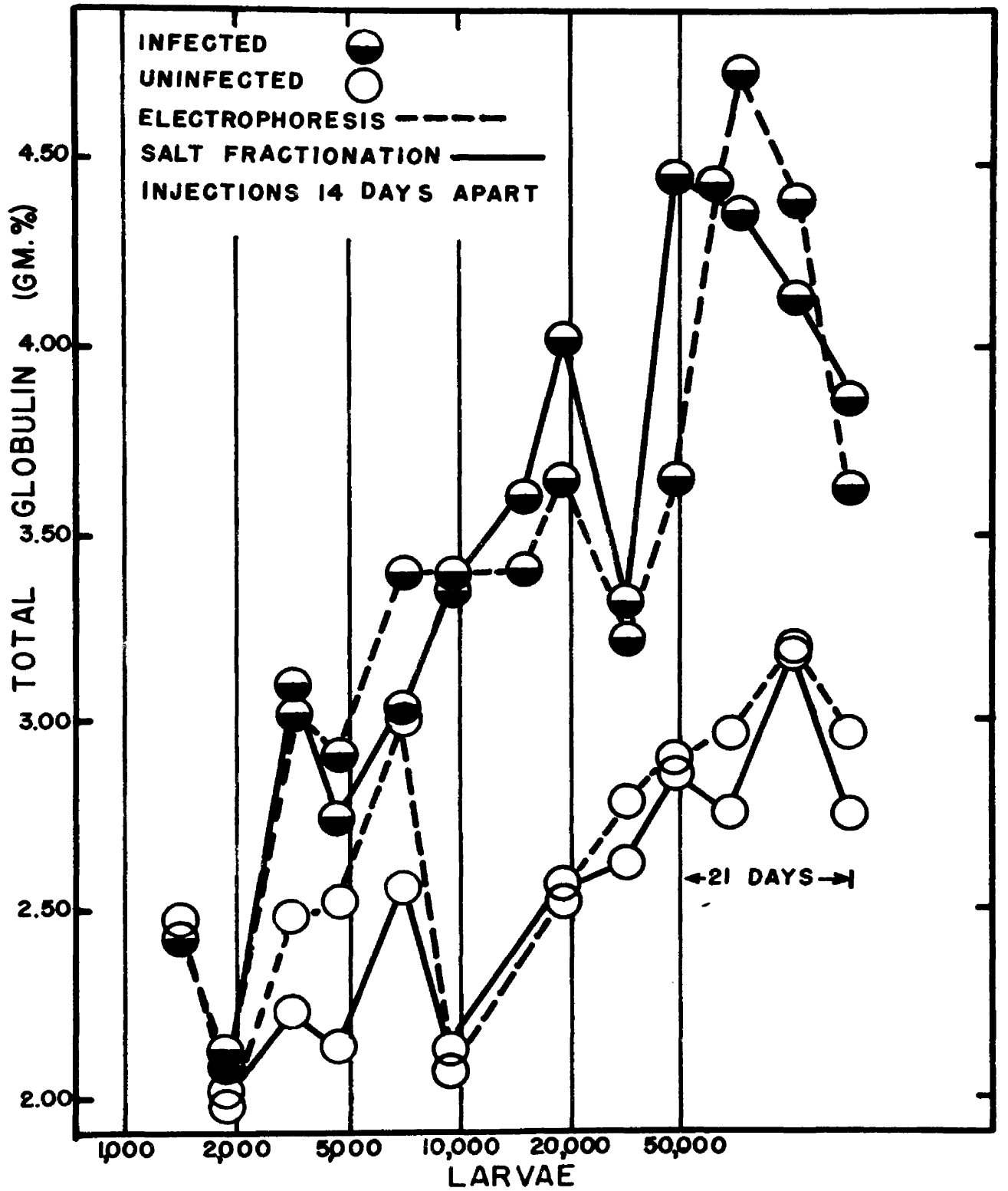



**FIGURE 22**

# THE RELATION OF BETA GLOBULIN TO INFECTION (COMPOSITE)

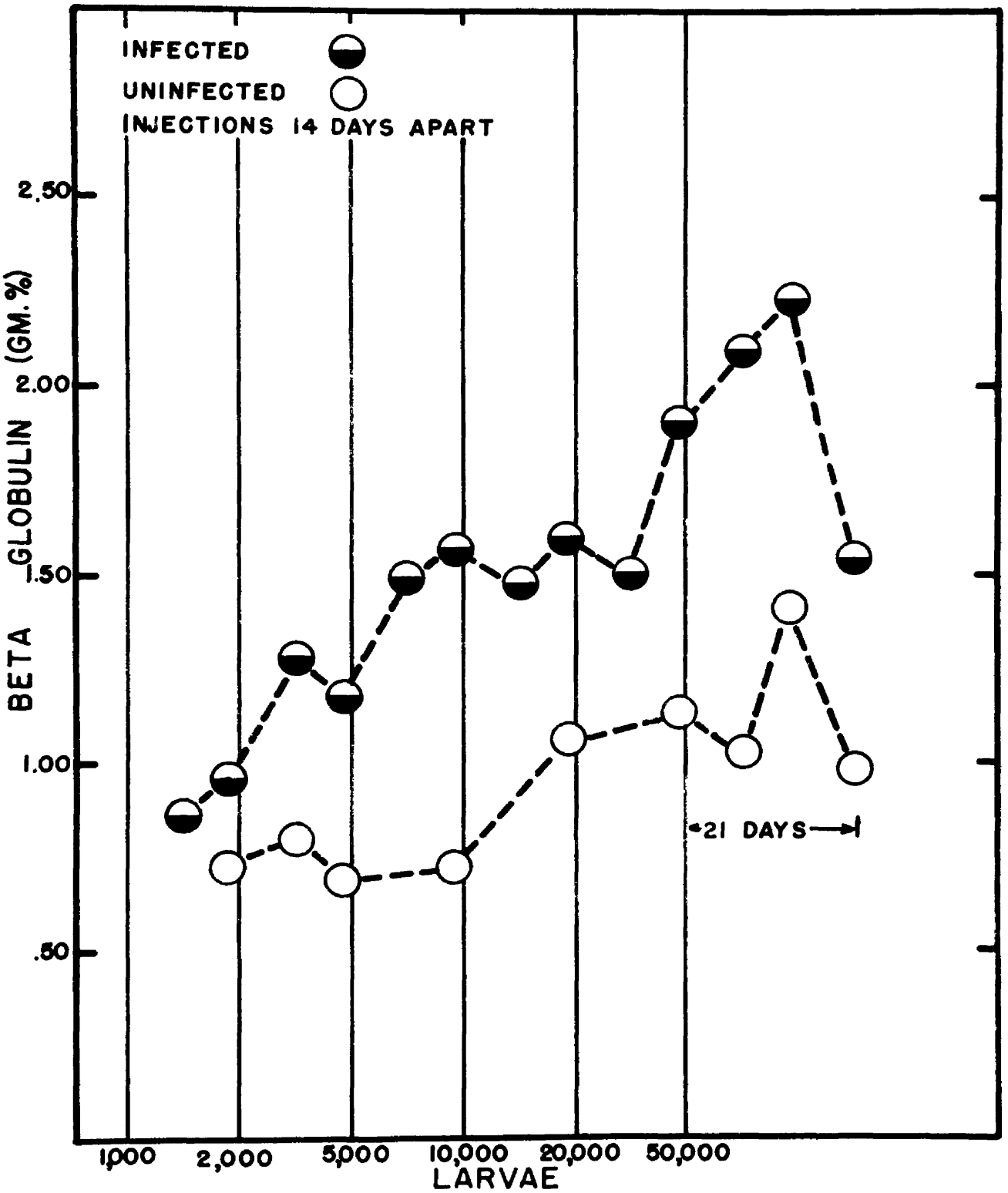



FIGURE 23

## Absorption Studies

The purpose of the absorption studies was an attempt to identify the globulin increase as antibody formation. The principle of Tiselius and Kabat (1939) was applied to the present work. The procedure consisted of comparing electrophoretic patterns of immune serum before and after exposure to prepared antigens. If the specific antibody were present in the serum and combination with the antigen occurred, the antigen-antibody complex might be removed as a precipitate. The electrophoretic pattern after exposure would reveal the absence of all or a part of a component. A component removed in this manner was considered to be antibody by these authors. Experiments of this type are important in establishing the relationship of antibody to serum proteins.

In the present studies larvae and various larval preparations were used as the antigen and were exposed to normal and immune sera. Larvae to be used as antigen were processed in the following manner: (1) the larvae were washed five times by centrifugation in tap water, (2) this was followed by two washes in distilled water (3) the larvae were then placed in a 0.5 percent mercuric chloride solution for one-half hour (4) five washings with sterile saline followed (5) five hundred units of penicillin and 0.5 gms. of streptomycin were added per cc. of larval suspension (6) this was then either ground with a tissue grinder, or incubated in the immune serum or saline.

Taliaferro and Sarles (1939) believed the secretions and excretions of the worm and mechanical damage were the effective inflammatory stimuli rather than the worm itself. These authors believed the precipitins

producing the precipitate of Sarles (1938) were in response to these secretions and excretions. The work of Thorson (1951) also seems to indicate antibodies are formed in response to the excretions and secretions of the worm. As a result of this previous work, active filariform larvae were incubated at room temperature in immune serum for six days (Absorption No. I). Inas much as samples of the same serum were used for analysis both prior and following absorption, removal of any component would be reflected as a change in the albumin/globulin ratio. Thus, an increase in the A/G ratio following exposure of antigen to serum would indicate absorption of globulin. The results of the absorption experiments are found in Table XII. No significant change in the A/G ratio was observed as a result of absorption.

Absorption experiment II was a duplication and expansion of absorption I. Immune serum was analysed electrophoretically at the time of bleeding, and after exposure to living larvae for three days. By comparing the A/G ratios (Table XII) of immune serum at the time of bleeding and after six days incubation at room temperature without larvae, it was evident little alteration occurred in relation to this time interval. A comparison of the A/G ratios of three day old immune serum and immune serum in which larvae were incubated for three days, showed no significant change.

Absorption experiment III was a duplication of absorption I with certain additions namely: normal serum was analysed before and after exposure to living larvae; and immune serum was analysed before and after exposure to saline in which larvae had been incubated one week.

This absorption experiment netted the following results: (1) no change in the A/G ratio was observed when normal serum was exposed to living larvae for six days (2) exposure to living larvae failed to absorb any protein component from immune serum in a six day incubation period. (3) a larvae free saline extract, in which larvae had previously been incubated for one week, failed to absorb any protein component from immune serum.

The results of Absorption IV are not included, because addition of veronal buffer to the serum after exposure to the antigen resulted in precipitation of much of the albumin (observed electrophoretically).

In Absorption V immune serum was exposed to ground larvae and analysis was in veronal buffer pH 8.6 and phosphate buffer pH 7.4. The addition of the phosphate buffer of pH 7.4 to the serum after exposure to ground larvae resulted in precipitation of protein while on this occasion no precipitation occurred on addition of veronal buffer of pH 8.6. Only the results of serum analysed in veronal buffer are included in Table XII.

Summarizing the results of the absorption experiments (Table XII), no significant change in the A/G ratio was observed following exposure of immune and normal sera to the various larval preparations. Thus, for the defined conditions no absorption of antibody occurred.

TABLE XII. RESULTS OF THE ABSORPTION EXPERIMENTS

| Absorption Exp. No. | *Serum Age (Days) Before Absorption | *Serum Age (Days) After Absorption | Antigen Used | A/G Ratio Before Absorption | A/G Ratio After Absorption |
|---|---|---|---|---|---|
| I-Immune | 1 | 7 | living larvae | 0.58 | 0.66 |
| II-Immune | 1 | - | none | 0.77 | — |
| Immune | 6 | 6 | living larvae | 0.70 | 0.56 |
| III-Normal | 1 | 10 | living larvae | 1.58 | 1.62 |
| Immune | 2 | 10 | living larvae | 0.61 | 0.64 |
| Immune | 2 | 2 | saline extract | 0.61 | 0.69 |
| V-Immune | 3 | 2 | ground larvae | 0.72 | 0.65 |

*These figures represent the age of the serum when determinations were made.

## DISCUSSION

Qualitative or quantitative alterations of the serum components are often encountered in diseased animals. Qualitative changes are manifested in electrophoretic patterns as new or altered peaks which have specific mobilities. Seibert and Nelson (1942) reported that the first serum protein changes in tuberculous rabbits were a rise in the alpha globulin fraction and the appearance of an unknown component designated as the "X" component. The "T" component of Tiselius and Kabat (1939) and Van der Scheer (1941) is an example of a new peak appearing in the electrophoresis patterns as immunization increases. On the other hand, numerous workers [Kedwich and Record (1940), Bjornboe (1944), Gjessing and Chanutin (1947a)] have shown quantitative increases in various serum components associated with the diseased condition.

In the present investigation no new serum components were detected in the infected animals. However, significant increases in the globulin fraction were consistently observed in the latter stages of hyperimmunization (after the 5,000 larvae injection). Increase in globulin with a decrease in albumin appears to be the characteristic serum alteration in many diseases. The rise in globulin may be caused by: (1) the possible formation of antibodies (2) alteration of the relative production and utilization of albumin and globulin, or (3) a compensatory rise in an attempt to maintain osmotic pressure (Martin 1946).

Some of the more pertinent references which support the above mentioned causes of globulin increase will be discussed.

Tiselius and Kabat (1939) showed conclusively that the gamma globulin increase was due to antibody formation. These authors removed the increased gamma component of immune serum by exposure of the serum to the specific antigen. Other workers have established relationships of antibody to plasma proteins, Wyckoff and Rhian (1945) Seibert and Nelson (1943), Bjørnboe (1944), Boyd and Bernard (1937), Heidelberger (1939) proposed that antibodies are serum globulins modified in response to the presence of an antigen.

Chow et al. (1948) found in dogs depleted of protein by a protein-free diet and by plasmapheresis, that albumin and gamma globulin were decreased, whereas, alpha and "other globulins" remained unchanged.

Zeldis et al. (1945) showed, following plasmapheresis, that both alpha and beta globulins continued relatively elevated during the early portion of the recovery period. They suggested a rapid formation of these components from tissue reserves as well as a rapid synthesis from dietary protein.

A compensatory mechanism may be in operation which results in the maintenance of constant serum osmotic pressure. Cohn (1942) states, "Although albumin comprises but 60 per cent of the plasma proteins, it accounts for approximately 80 per cent of the osmotic pressure." Since osmotic pressure depends upon the number of particles and not upon their weight, it has been proposed that the globulin concentration compensates for any decrease in albumin. Bjørnboe (1944) suggested there was a

relation between the albumin and globulin concentrations which kept the colloid osmotic pressure constant or varying only within certain limits.

For the conditions as defined in this investigation, no absorption of antibody was detected when immune serum was exposed to the various larval preparations. The absorption studies presented here have not necessarily exhausted all the possible conditions for maximum antigen-antibody combination. If the increased beta peak of the immune serum could be reduced by exposure of the serum to some larval preparation, it would be highly indicative of an antigen-antibody combination and would tend to establish the beta globulin increase as due to antibody formation. This was not established. An increase in beta globulin in the diseased animal has been noted by other workers [Seibert and Nelson (1942), Bjørnboe (1944), Stern and Reiner (1946), and Martin (1946)]. Fell et al. (1940), Enders (1944) and Kekwick and Record (1940), have demonstrated that some antibody is formed in globulins other than the gamma fraction. In fact, the latter authors found that immunization with diphtheria toxoid leads to the formation of two antitoxins which are associated with the beta and gamma globulin fractions respectively. From the above references, it seems plausible that antibody can also be associated with the beta globulin fraction. Before one excludes the increase as due to antibody production, a thorough study of the relative amounts of immune serum and antigen necessary for a antigen-antibody reaction to occur would be necessary. This would tend to eliminate possible negative results due to zone effects.

Furthermore, the electrophoretic method used was not capable of resolving protein concentrations much below 0.002 percent in the presence of and relative to another protein in a concentration of approximately two percent. Therefore, absorption of antibody at a concentration below this limit would not be detected. Thus, the antibody absorption phase of the problem awaits further study and perhaps refinement of technical methods.

It does not seem likely that the rise in beta globulin noted could be attributed to an alteration of the relative production and utilization of albumin and globulin. Zeldis et al. (1945) found, following plasmapheresis, all plasma proteins entered the blood stream even when food was not given during the first 24 hours after depletion. In addition these authors noted that both alpha and beta globulins continued relatively elevated during the early portion of the recovery period. This suggested a rapid formation of these two globulins from tissue reserves as well as a rapid synthesis from dietary protein. To achieve these results removal of from 50 to 190 percent of the blood volume and its continuous replacement with a non-protein physiological solution and the washed cells was necessary. It would appear that any condition which resulted in an extensive and prolonged loss of plasma protein might reflect itself as a beta globulin increase. During the migration of the larvae through the lungs, there is capillary damage and hemorrhage. It seems doubtful, however, that the amount of serum lost, as a result of larval damage, reaches the proportions of plasmapheresis. In addition, four to seven days after the last larval injection when

the larvae are most active in the lung and when serum protein loss would be the greatest, the total protein was not decreased, but showed a significant increase. This would seem to indicate that plasma loss was minimal. The histological studies of Taliaferro and Sarles (1939) would lead one to believe that blood loss due to larval damage is not of the proportions encountered in plasmapheresis. The acceptance of these facts tends to rule out the observed serum changes as due to an alteration of the relative production and utilization of the serum proteins.

Since the animals in the present work were subjected to only one bleeding for analysis, changes in serum patterns due to blood depletion can be eliminated. Dimopoullos (1952) showed in chickens, which were bled weekly and from which one third of the total blood volume was removed, that the albumin/globulin ratio decreased steadily.

Thus, from the information available at the present time, one must conclude that the increase of globulin in the present work was due to injury or shock by the larvae to the host. The injury to the host was considerable when one considers the large numbers of larvae that had the opportunity of migration through the tissues. The data concerning lung enlargement reported in the "Results" section indicates that considerable pathology existed. It is entirely possible that the observed globulin increase was from a reaction by the host to the disturbance in the lungs produced by the larvae and that this increase for the most part was not due to antibody production.

Further evidence in favor of the globulin increase being due to trauma from the larvae and not antibody production, lies in the fact

that early in the hyperimmunization process little increase in globulin was noted. Yet these animals at the same time exhibited strong immunity as demonstrated by the decrease of the parasites' egg production.

References that follow substantiate the fact that serum components are altered due to shock or injury.

Moore and Fox (1950) demonstrated that mice traumatized by tourniquet for two hours produced a large quantity of a serum protein component having a mobility similar to gamma globulin.

Perlmann et al. (1943) compared electrophoretic patterns of normal serum from calves with those obtained after the animals were subjected to burns. The serum of the burned animals showed a slight decrease in the albumin/globulin ratio with an increase in the alpha globulin fraction.

Gjessing and Chanutin (1947a) dipped one-third of the clipped body of rats into hot water at 75°C for 40 seconds. The serum changes were an increase in the alpha and beta globulins and decreases of albumin and gamma globulin.

Gjessing et al. (1947b), using animals injured with sulfur mustard, turpentine, and heat, found an increase in the percentage distribution of the alpha globulins and a decrease in the albumin. The lipid combined with alpha and beta globulins also increased in injured animals. However, Gjessing et al. (1948) reported severe injury in goats produced relatively small changes in the distribution of protein components of whole serum or its fractions.

Leutscher (1947) states that electrophoretic patterns 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 involvement of certain organs such as the liver. An examination of the results obtained in the present work reveals that serum and lung changes are, indeed, proportional to the number of larvae injected which in turn is probably dependent on the severity of the physiological disturbance. That a specific organ was involved in the disturbance.was demonstrated by the increased lung/body weight ratio and microscopical changes in the lung.

Before one attributes the larval activity in the lung as solely producing the traumatic response, one must consider the lung as a possible site of antibody formation. Banks et al. (1948) noted when phosphovitellin containing $P^{32}$ was injected into rabbits, that it was rapidly bound by the liver and the lung. Haurowitz (1950) believes this finding supports the view that these organs are fundamentally involved in the process of antibody formation. The fact that large numbers of lymphocytes are found in lungs of the infected animals can not be completely ignored. Antibodies have been extracted from lymphatic cells and it has been proposed that a portion of the antibodies arise in these cells [White and Dougherty (1946), Ehrich and Harris (1942)]. Taliaferro and Sarles (1939) point out that one of the differences between the immune rat and the initially infected rat is that in the former many worms do not reach the intestine but are retained in the skin and lungs. This would seem to indicate that these stations (skin and lungs)

are actively involved in the resistance displayed by the immune rat. From the foregoing information one might conclude that if antibody is involved, the lungs may be the site of antibody production or be one of the locations wherein the antigen-antibody combination occurs. It would, in view of the great response found, seem unlikely that the beta globulin increase was due to antibody formation by the lungs. A more tenable explanation would be to attribute the globulin increase to trauma due to the massive larval doses whereby the lung is the focus of maximum shock. If the trauma explanation is conceded, the existence of an antibody still cannot be eliminated. It is conceivable that the antibody is present in the serum in minute quantities which cannot be detected by the limited mthods used herein, or on the other hand the antibody may be masked or incorporated in the greatly increased beta globulin peak. The answer to these questions await further study.

One might profitably utilize the electrophoresis-convection apparatus in establishing the presence of an antibody. Cann et al. (1949) separated bovine gamma globulin into eight fractions using this apparatus. It might be possible with the aid of this apparatus to obtain a beta globulin subfraction which might be predominantly antibody. Absorption studies would thereby be rendered less complicated.

## SUMMARY

1. Electrophoretic and salt fractionation determinations on normal and nematode immune rat sera were performed.

2. Utilizing bovine albumin as a standard, a new modification for determining total serum protein of rat sera by the biuret reaction was developed.

3. A new modification for fractionating rat sera into albumin and globulin, utilizing a 24 percent sodium sulfite solution, was devised.

4. The hyperimmunization process produced a slight decrease in body weight when compared with the uninfected controls.

5. The lung weight when converted to percent of total body weight was found to increase as much as six fold in the infected animal when compared with the control. This lung involvement is considered as the traumatic center which produces, at least for the most part, the observed serum changes.

6. Living larvae, saline extracts of living larvae, and ground larvae, were used as antigens and exposed to immune sera. Antigen-antibody combination, as indicated by the removal of a serum fraction, was not observed.

7. Rats were injected with increasing numbers of N. muris larvae at two week intervals. Serum determinations were made on various days between the injections. The following serum changes were noted in the infected animals when compared with uninfected litter mates:

(a) The total protein was increased as the number of larvae injected was increased.

(b) The beta and total globulin increased as the number of larvae injected increased, and diminished when the number of larvae injected was reduced, or when the larval injections were stopped.

(c) In five out of six intervals between injections the beta globulin of the infected animals was slightly higher in two weeks than one week after each injection. The beta globulin increase was electrophoretically analysed in veronal buffer at pH 8.6 and phosphate buffer pH 7.4. This minimized any anomalous effects that might occur at a particular pH or with a particular buffer.

(d) The albumin/globulin ratio decreased as the number of larvae injected was increased and also increased when the larval injections were halted.

(e) When rats were injected with 1,000; 2,000; 5,000; 10,000; 20,000; and 50,000 larvae at two week intervals, the most striking changes occurred seven days after the 50,000 larval level.

(f) The gamma globulin showed no significant increase or decrease.

(g) The mobilities of the protein components were decreased in the majority of infected serum samples.

(h) Serum from rats, which had received injections of the supernatant of the larval suspension, was essentially normal. This indicated the material in the inoculum

other than the larvae did not cause the observed serum protein changes.

These serum changes are interpreted as responses of the host to injury or trauma by migrating larvae, although, the possible existance of an antibody can not be ruled out. Since rats were bled only once during the course of the experiments, serum depletion changes were eliminated.

# BIBLIOGRAPHY


Africa, C. M.
1931 - Studies on the host relations of Nippostrongylus muris, with special reference to age resistance and acquired immunity. J. Parasitol. 18:1-13.

Armstrong, S. H., Jr., Budka, M. J. E., and Morrison, K. C.
1947 - Preparation and properties of serum and plasma proteins: XI. Quantitative interpretation of electrophoretic schlieren diagrams of normal human plasma proteins. J. Am. Chem. Soc., 69:416-429.

Banks, T., Boursnell, J. C., Dewey, H. M., Francis, G. E., Tupper, R., Wormall
1948 - The use of radioactive isotopes in immunological investigations. Biochem. J. 43:518-523.

Benditt, E. P., Wessler, R. W., Woolridge, R. L., Rowley, D. A., Steffee, C. H.
1949 - Loss of body protein and antibody production by rats on low protein diets. Proc. Soc. Exper. Biol. and Med. 70:240-243.

Boyd, W. C., and Bernard, H.
1937 - Quantitative changes in antibodies and globulin fractions in sera of rabbits injected with several antigens. J. Immunol. 33:111-122.

Brandt, L. W., Clegg, R. E., Andrews, A. C.
1951 - The effect of age and degree of maturity on the serum proteins of the chicken. J. Biol. Chem. 191:105-111.

Bjørnboe, M.
1944 - Serum proteins during immunization. Acta Pathol. Microbiol. Scand. 20:221-239.

Ibid.
1946 - Studies on the serum proteins in Hepatitis. I. The relation between serum albumin and serum globulin. Acta Med. Scand. 123:393-401.

Campbell, W. R., Hanna, M. I.
1937 - The albumin, globulins, and fibrinogen of serum and plasma. J. Biol. Chem. 119:15-33.

Cann, J. R., Brown, R. A., Kirkwood, J. G.
1949 - Application of electrophoresis convection to the fractionation of bovine gamma-globulin.
J. Biol. Chem. 181:161-170.

Chandler, A. C.
1932 - Experiments on resistance of rats to superinfection with the nematode, Nippostrongylus muris.
Am. J. Hyg. 16(3):750-782.

Ibid.
1935a - Studies on the nature of immunity of intestinal helminths. I. The local nature of the immunity of white rats to Nippostrongylus infection.
Am. J. Hyg. 22(1):157-168.

Ibid.
1935b - Studies on the nature of immunity to intestinal infections. II. A study of the correlations between degree of resistance of white rats to Nippostrongylus and interval between infections.
Am. J. Hyg. 22(1):243-256.

Ibid.
1936 - Studies on the nature of immunity to intestinal helminths. III. Renewal of growth and egg production in Nippostrongylus after transfer from immune to non-immune rats.
Am. J. Hyg. 23(1):46-54.

Chow, B. J.
1947 - The determination of plasma or serum albumin by means of precipitin reaction.
J. Biol. Chem. 167:757-763.

Chow, B. J., Seeley, R. D., Allison, J. B., Cole, W. H.
1948 - The effect of repletion on the plasma proteins in the dog measured by electrophoretic analysis.
Arch. Biochem. 16:69-78.

Chow, B. F.
1946 - The electrophoretic studies on the effect of protein depletion on plasma proteins and the regeneration of plasma proteins after oral administration of hydrolysates prepared from casein and lactoalbumin.
Ann. N. Y. Acad. Sci. 47:297-316.

Chow, B. F., Allison, J. B., Cole, W. H., Seeley, R. D.
1945 - Effect of protein depletion on plasma proteins in the dog measured by electrophoretic analysis.
Proc. Soc. Exper. Biol. and Med. 60:14-17.

Cohen, P. P., Thompson, F. L.
1948 - A comparative study of micro- and macroelectrophoretic analysis of human and rat serum.
J. Lab. and Clin. Med. 33:75-80.

Cohn, E. J.
1942 - The plasma proteins: their properties and functions.
Trans. & Studies Coll. Physicians, Philadelphia 10:149.

Cohn, C., Wolfson, W. Q.
1947 - Studies in serum proteins. I. The chemical estimation of albumin and of the globulin fractions in serum.
J. Lab. and Clin. Med. 32:1203-1207.

Cooper, G. R., Rein, C. R., Beard, J. W.
1946 - Electrophoretic analysis of Kala-azar human serum. Hypergamma-globulinemia associated with seronegative reactions for syphilis.
Proc. Soc. Exper. Biol. and Med. 61:179-183.

Dimopoullos, G. T.
1952 - Electrophoretic and serum neutralization studies of sera from chickens exposed to infectious bronchitis virus.
Unpublished Ph.D. Thesis. Michigan State College, 93 numb. leaves, 35 figures.

Dole, V. P., Emerson, K. E.
1945 - Electrophoretic changes in the plasma protein patterns of patients with relapsing malaria.
J. Clin. Invest. 24:644-647.

Ehrich, W. E., Harris, T. N.
1942 - The formation of antibodies in the popliteal lymph node in rabbits.
J. Exper. Med. 76:335-348.

Enders, J. F.
1944 - Chemical, clinical, and immunological studies on the products of human plasma fractionation. X. The concentration of certain antibodies in globulin fractions derived from human blood plasma.
J. Clin. Invest. 23:510-530.

Fell, N., Stern, K. G., Goghill, R. D.
1940 - A physical-chemical study of normal and immune horse serum.
J. Immunol. 39:223-246.

Folin, O., Wu, H.
1919 - A system of blood analysis.
J. Biol. Chem. 38:81-110.

Gradwohl, R. B. H.
1943 - Clinical Laboratory Methods and Diagnosis.
Third Ed., C. V. Mosby Co., St. Louis
Vol. I. 215-217.

Graham, G. L.
1934 - Resistance Studies with the nematode, Nippostrongylus muris, in laboratory rats.
Am. J. Hyg. 20(2):352-372.

Gjessing, E. C., Chanutin, A.
1947a - An electrophoretic study of plasma and plasma fractions of normal and injured rats.
J. Biol. Chem. 169:657-665.

Gjessing, E. C., Ludewig, S., Chanutin, A.
1947b - Fractionation, electrophoresis, and chemical studies of proteins in sera of control and injured dogs.
J. Biol. Chem. 170:551-569.

Ibid.
1948 - Fractionation, electrophoresis, and chemical studies of proteins in sera of control and injured goats.
J. Biol. Chem. 174:683-696

Hardt, C. R.
1943 - Operation and Application of Tiselius Electrophoresis apparatus.
Unpublished Ph.D. thesis. Michigan State College. 129 numb. leaves, 101 figures.

Haurowitz, F.
1950 - Chemistry and Biology of Proteins, First Ed.
Academic Press, Inc., New York, pp. 287.

Heidelberger, M.
1939 - Chemical aspects of the precipitin and agglutination reactions.
Chem. Rev. 24:323-343.

Howe, P. E.
1921 - The use of sodium sulfate as the globulin precipitant in the determination of proteins in blood.
J. Biol. Chem. 49:93-108.

Ikejiani, O.
1946 - Studies in Trypanosomiasis. I. The plasma proteins and sedimentation rates of erythrocytes of rats infected with pathogenic trypanosomes.
J. Parisitol. 32:369-373.

Jager, B. V., Schwartz, T. B., Smith, E. L., Nickerson, M., Brown, D. M.
1950 - Comparative electrophoretic and chemical estimations of human serum albumin; an evaluation of six methods.
J. Lab. and Clin. Med. 35:76-86.

Kekwick, R. A., Record, B. R.
1940 - Some physical properties of diphtheria antitoxic horse sera.
Brit. J. Exper. Path. 22:29-44.

Longsworth, L. G.
1942 - Recent advances in the study of proteins by electrophoresis.
Chem. Rev. 30:323-339.

Longsworth, L. G., Mac Innes, D. A.
1940 - The interpretation of simple electrophoretic patterns.
J. Amer. Chem. Soc. 62:705-711.

Longsworth, L. G., Shedlovsky, L., Mac Innes, D. A.
1939 - Electrophoretic patterns of normal and pathological human blood serum and plasma.
J. Exper. Med. 70:399-413.

Luetscher, J. A., Jr.
1947 - Biological and medical applications of electrophoresis.
Physiol. Rev. 27:621-642.

Lysenko, M. G.
1951 - Concerning salicylate inhibition of ablastic activity in Trypanosoma lewisi infection.
J. Parasitol. 37:535-544.

Martin, N. H.
1946 - The components of the serum proteins in infective hepatites and in homologous serum jaundice. An electrophoretic study.
Brit. J. Exper. Path. 27:363-368.

Milne, J.
1947 - Serum protein fractionation: A comparison of sodium sulfate precipitation and electrophoresis.
J. Biol. Chem. 169:595-599.

McMaster, P. D., Hudack, S. S.
1935 - The formation of agglutinins within lymph nodes.
J. Exper. Med. 61:783-805.

Moore, D. H.
1945 - Species differences in serum patterns.
J. Biol. Chem. 161:21-32.

Moore, D. H., Fox, C. L.
1950 - Correlation of electrophoretic studies and other factors in the syndrome of secondary shock.
Nature 165:872-876.

Moore, D. H., Levin, L. and Leathem, J. H.
1944 - The alpha globulin fraction of the serum of normal and hypophysectomized rats.
J. Biol. Chem. 153:349-353.

Moore, D. H., Levin, L. and Smelser, G. K.
1945a - Electrophoretic and salt fractionation of the serum proteins of normal and hypothyroid rats.
J. Biol. Chem. 157:723-730.

Moore, D. H., Roberts, J. B., Salonberger, L. W.
1949 - Factors influencing the electrophoretic analysis of human serum.
J. Biol. Chem. 180:1147-1158.

Moore, D. H., Shen, S. C., Alexander, C. S.
1945b - The plasma of developing chick and pig embryos.
Proc. Soc. Exper. Biol. and Med. 58:307-310.

Perlmann, G. E., Glenn, W. W. L., Kaufman, D.
1943 - Changes in the Electrophoretic pattern in lymph and serum in experimental burns.
J. Clin. Invest. 22:627-633.

Petermann, M. L., Young, N. F., Hoagness, K. R., Rhoads, C. P.
1947 - The determination of plasma albumin by chemical and electrophoretic methods.
Proc. Amer. Federation Clin. Research 3:75.

Pillemer, L., Hutchinson, M. C.
1945 - The determination of the albumin and globulin contents of human serum by methanol precipitation.
J. Biol. Chem. 158:299-301.

Popjak, G., McCarthy, E. F.
1946 - Osmotic pressures of experimental and human lipaemic sera. Evaluation of albumin-globulin ratios with the aid of electrophoresis.
Biochem. J. 40:789-803.

Sarles, Merritt P., Taliaferro, W. H.
1936 - The local points of defense and the passive transfer of acquired immunity to Nippostrongylus muris in rats.
J. Infect. Dis. 59:207-220.

Sarles, M. P.
1938 - The *in vitro* action of immune rat serum on the nematode, *Nippostrongylus muris*.
J. Infect. Dis. 62:337-348.

Ibid.,
1939 - Protective and curative action of immune serum against *Nippostrongylus muris* in the rat.
J. Infect. Dis. 65:183-195.

Schwartz, B., Alicata, J. E., Lucker, J. T.
1931 - Resistance of rats to superinfection with a nematode, *Nippostrongylus muris*, and an apparently similar resistance of horses to superinfection with nematodes.
J. Wash. Acad. Sc. 21(12):259-261.

Seibert, F. B., Nelson, J. W.
1942 - Electrophoretic study of blood protein response in tuberculosis.
J. Biol. Chem. 143:29-38.

Ibid.,
1943 - Proteins of tuberculin.
J. Amer. Chem. Soc. 65:272-278.

Spindler, L. A.
1936 - Resistance of rats to infection with *Nippostrongylus muris* following administration of the worms by duodenal tube.
Am. J. Hyg. 23(2):237-242.

Stern, K. G., Reiner, M.
1946 - Electrophoresis in medicine.
Yale J. Biol. Med. 19:67-99.

Taliaferro, W. H., Sarles, M. P.
1939 - The cellular reactions in the skin, lungs and intestine of normal and immune rats after infection with *Nippostrongylus muris*.
J. Infect. Dis. 64:157-192.

Thorson, R. E.
1951 - The relation of the secretions and excretions of the larvae of *Nippostrongylus muris* to the production of protective antibodies.
J. Parasitol. Suppl. Vol. 37, No. 5, Sec. 2, pp. 18.

Tiselius, A., Kabat, E. A.
1939 - An electrophoretic study of immune sera and purified antibody preparations.
J. Exper. Med. 69:119-131.

van der Scheer, J. Wyckoff, W. G., Clarke, J. H.
1940 - The electrophoretic analysis of several hyperimmune horse sera.
J. Immunol. 39:65-71.

Ibid.
1941 - The electrophoretic analysis of tetanal antitoxic horse sera.
J. Immunol. 40:173-177.

White, A., Dougherty, T. F.
1946 - The role of lymphocytes in normal and immune globulin production, and the mode of release of globulin from lymphocyles.
Ann. N. Y. Acad. Sci. 46:859-882.

Wolfson, W. Q., Cohn, C., Calvary, E., Ichiba, F.
1948 - Studies in serum proteins. V. A rapid procedure for the estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin and gamma globulin in 1.0 ml. of serum.
A. J. Clin. Pathol. 18:723-730.

Wyckoff, W. G., Rhian, M.
1945 - An electrophoretic study of an anti-influenzal horse serum.
J. Immunol. 51:359-363.

Yokogawa, S.
1922 - The development of Heligmosomum muris Yokogawa, a nematode from the intestine of the wild rat.
Parasitology 14(2):127-166.

Ibid.
1920 - A new nematode from the rat.
J. Parasitol. 7:29-33.

Zeldis, L. J., Alling, E. L., McCoord, A. B., Kulka, J. P.
1945 - Plasma protein metabolism-Electrophoretic studies-Restoration of circulating proteins following acute depletion by plasmapheresis.
J. Exper. Med. 81:515-537.