AN ELECTROPHORETIC STUDY OF SERA FROM RATS ARTIFICIALLY INFECTED WITH AND IMMUNIZED AGAINST THE LARVAL CESTODE, *CYSTICERCUS FASCIOLARIS*

By

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INTRODUCTION

Following the improvements in the moving boundary electrophoresis apparatus introduced by Tiselius (1937), there ensued a rapid and large number of applications of electrophoresis to biological, chemical, and physical problems. The extensive Electrophoresis Bibliography of Henley and Schuettler (1953) attests to the magnitude and diversity of electrophoretic studies. Reviews by Stern and Reiner (1946), Luetscher (1947), Gutman (1948), Lewis (1950), Antweiler (1952), and Fisher (1953) deal with the significant contributions to medical and biological problems. The theory and methods have been well summarized by Longsworth and MacInnes (1939), Longsworth (1942, 1952), Abramson et al. (1942), Alberty (1948) and Moore and Abramson (1950).

In immunological studies, if the assumption is correct that all antibodies are proteins, then antibody production ultimately resolves itself into a study of protein metabolism (Cannon, 1945). Electrophoresis affords an excellent tool for the study of the protein content of body fluids, which, in turn, reflects the physiological state of the animal (Reiner, 1952). It is the consensus of opinion among parasitologists that the basic mechanisms operative in the immune response against animal parasites are essentially the same as those functioning against other infectious agents

(Taliaferro, 1940a; Culbertson, 1951). Hence, electrophoresis should prove a valuable method in elucidating the many problems in parasitic immunology, as it has in other fields of immunological study. Luetscher (loc. cit.), for example, points to the significant contributions made by employing this method in establishing the relationship of antibody to the plasma proteins.

In spite of its great potential for the clarification of many questions in parasitic immunology (e.g., host-parasite relationships, host response to parasitic organisms, parasite physiology and biochemistry) the field of parasitology has sorely lagged in taking advantage of this technique. Explanations for this, noted by Stauber (1954) in his review of the applications of electrophoresis in parasitology, are the high cost of the apparatus and its inaccessibility in areas rich in clinical material in medical parasitology. The development of zone electrophoresis should stimulate efforts in this direction by virtue of its economy, simplicity and requirements for smaller amounts of sample (Tiselius and Flodin, 1953).

From Stauber's (loc. cit.) review it is readily evident that the majority of electrophoretic studies in parasitology have been concerned with protozoan infections. Furthermore, these studies were primarily directed towards clinical considerations. The paucity of electrophoresis applications to the helminths is particularly striking and no work is known to

have been reported on host serum protein changes induced by a larval cestode infestation. In the above review, aside from the quantitative and qualitative changes noted in the serum proteins of infected hosts, the "specific antibody has not yet been properly demonstrated and isolated in the increased gamma-globulin fraction for any animal parasitic infection... ." The closest approach to the latter has been made by Wright and Oliver-González (1943). They demonstrated the presence of antibodies against Trichinella spiralis adults and larvae in the increased and electrophoretically isolated gamma-globulin of immune rabbit serum by the in vitro precipitin test. However, following in vitro absorption of the immune serum, no significant change occurred in any of the electrophoretic components.

By an increased application of the electrophoretic method in the field of parasitology the resolution of such situations as encountered by Wright and Oliver-González (loc. cit.) and other questions may be markedly hastened.

LITERATURE

Immunological studies in parasitology during the first thirty years of the present century were dominated by a search for efficacious clinical diagnostic tests. Taliaferro's (1929) important monograph collated the early significant literature in the field of parasitic immunology up through this period. In the subsequent period, an increased and vigorous attack has been made on the mechanisms and processes of animal parasite immunity. The resultant of these efforts has been the accrual of a body of evidence establishing the humoral and cellular aspects of animal parasite immunity. Through the study of cestodes, "the larval infections with the taenoid tapeworms have furnished the clearest-cut evidence of antibody action of any of the metazoan parasites" (Taliaferro, 1940b). Dealing in general with the immunological results of this latter period are the résumés of Taliaferro (1934, 1940b, 1948), Culbertson (1938, 1941, 1951) and Chandler (1953). Summaries dealing with more specific segments of parasite immunity are those of Taliaferro (1940a) for helminth acquired immunity and Ackert (1942) for natural resistance in helminths. Peters (1936) and Larsh (1945, 1951) have reviewed the important phases of cestode immunity.

An immune response requires stimulation of the host tissues by foreign antigens. Parasites possessing a parenteral

stage meet this criterion. Intestinal lumen-dwelling forms elicit little, if any, immunological response by virtue of the failure of their antigens to reach the host tissues or to do so in an effective concentration. Most of the evidence demonstrating that lumen-dwelling forms initiate a negligible, or no, immune response comes from studies on adult tapeworms. Living adult Hymenolepsis nana introduced directly into the mouse intestine failed to protect against a superimposed infection with ova (Hearin, 1941). When the worms of an initial infection with H. diminuta were eliminated from the rat intestine by chemotherapeutic or direct surgical means, the results suggested that the resistance of young rats to a second infection was a manifestation of the "crowding" effect (Chandler, 1939). Oral and parenteral attempts to immunize other rats were negative (Chandler, 1940). Cats and kittens were not protected against a superinfection with cysts of Taenia taeniaeformis (Miller, 1931e, 1932d). The presence of the adult Multiceps glomeratus conferred no immunity against a second feeding of scolices to dogs (Clapham, 1940). Young chickens reinfected with Raillietina cesticillus had apparently little or no acquired immunity against the superimposed infection (Luttermoser, 1938).

Chandler (1948) feels that the bulk of the evidence supports the contention that lumen-dwelling tapeworms induce little, if any, immunity. In a discussion of Chandler's view, Stoll (1948) takes issue with the negative reports on

immunity in lumen-dwelling cestodes. The basis of his argument is the failure of workers to take cognizance of the fact that, in nature, there is a constant reinfection of hosts with infective forms. The eventual result of this repeated reinfection is a gradual quantitative build up of antigenic materials from the parasite. By being absorbed into the host system, the antigens ultimately induce an immune response. It is his opinion that more attention must be given to the adequate reinfection of hosts in testing for the presence of immunity.

There have been successful attempts to effect an immune response against adult cestodes residing in the gut lumen. Dogs that were heavily infected with Taenia pisiformis received intravenous injections of worm extracts. They appeared to have developed an immunity to the toxic reaction that follows such an injection in non-infected dogs (Essex et al, 1930). There is evidence that, in lambs under one year of age, immunity develops against Moniezia expansa reinfection in cases where the lamb has recovered from an infection and continues to graze in infected regions. A local immunity involving the intestinal mucosa is its suggested basis (Seddon, 1931). A report of the resistance of rats to superinfection with Hymenolepis diminuta has been made, though only three rats were used (Palais, 1934). Kittens were partially immunized against T. taeniaeformis and dogs against Dibothriocephalus latus by subcutaneous injections, and the feeding of homologous larval emulsions (Ohira, 1935). Attempts to

immunize adult and young dogs against the adult Echinococcus granulosus resulted in partial protection following immunization with hydatid cyst material (Turner et al., 1936).

It is apparent that the question of immunity against intestinal-dwelling cestodes is far from conclusively clarified. This may be extended to lumen-dwelling helminths in general, for Burlingame and Chandler (1941) present similar negative conclusions for an acanthocephalid intestinal worm, Moniliformis dubius, in rats. However, for the present, we may accept with reservations the view that worms living in the intestinal lumen elicit little, or no, immune response by the host.

Larval cestodes present another situation. Since they undergo a parenteral phase or reside in the host tissue, there has been conclusive evidence of a humoral response against them. Rabbits infected with Coenurus glomeratus exhibited positive intradermal tests against the cyst fluid antigen (Clapham, 1940). In four cases of human Cysticercus cellulosae, and in C. cellulosae infected pigs, precipitins were demonstrated in the serum of each (Trawiński and Rothfeld, 1935; Trawiński, 1936). Monkeys infected with Sparganum mansonoides had their sera tested against the specific antigen by the complement fixation test with positive results. Sera from normal humans and monkeys gave negative tests (Mueller and Chapman, 1937). When monkeys were immunized with adult S. mansonoides injections, evidence of protection against infection with the larvae was manifested by a failure

to develop elephantiasis and the prompt encapsulation of the spargana (Mueller, 1938). Hymemolepis nana is unique among cestodes in that it can undergo its adult intestinal lumen phase and parenteral larval, cysticercoid, stage in the same host. It passes its larval cycle within the villi of the small intestine. Resistance to superinfection has been reported in mice and rats (Shorb, 1933; Hunninen, 1935). However, it has been reported possible to superimpose an infection in both mice and rats, (Shorb, loc. cit.). A 75 percent reduction in the number of cysticercoids resulted following repeated injections of fresh adult worm material into mice (Larsh, 1944). The humoral basis of immunity against H. nana is evident from the positive results obtained by the passive transfer of immunity to mice with sera from infected animals and by in vitro serological tests with infected mouse serum (Hearin, 1941; Larsh, 1943). Oxen previously infected with ova of Taenia saginata exhibited a resistance to a second infection when compared to controls. A group of oxen allowed to graze on a pasture known to be infested with T. saginata eggs showed no evidence of a secondary infection applied by drenching though control animals showed normal infections. The proposition is made, on the basis of these results, that cattle be naturally immunized by allowing them to graze on known infested pastures (Penfold and Penfold, 1937). Similar demonstrations of larval cestode immunity have been made in sheep artificially immunized against hydatid cysts (Turner et al., 1937); and for Cysticercus pisiformis in artificially and passively protected rabbits (Miller and Kerr, 1932; Kerr, 1935).

C. fasciolaris, which has been subjected to intensive immunological studies in rats, is the larval stage of the cyclophyllidean cestode, T. taeniaeformis. The adult is the common cestode in the small intestine of cats, and also occurs in dogs, foxes and other closely related carnivores. Rats, mice, other rodents and also rabbits harbor the larval stage in their livers, though extra-hepatic sites have been recorded in rats (Bullock and Curtis, 1925) and rabbits (Mahon, 1954).

Wepfer in 1688 and Hartmann in 1695 were the first to report the finding of C. fasciolaris. The life cycle was experimentally established by Küchenmeister in 1851 by feeding cysts to cats. This work was confirmed and extended by Leuckart in 1854 by feeding eggs to white mice and the recovered larvae to cats (Sambon, 1924).

C. fasciolaris is morphologically characterized by the presence of several immature segments between the scolex and terminal bladder. The anatomy of the fully developed larvae has been studied by Rees (1951). Sambon (loc. cit.) suggested the term "strobilocercus" which is commonly used for this larval cestode.

The early development of C. fasciolaris in experimentally infected rats has been studied by Crusz (1948). Ten days after infection, the larva is an elongate bladder and no cells appear specifically differentiated. On the twenty-fifth day the scolex-anlage appears in most larvae, and by the thirty-sixth day the final phase of the bladder and invaginated scolex differentiation occurs.

Bullock and Curtis (1924, 1926) have described the host tissue reaction to the larva. Upon the ingestion of the eggs by the rat, the onchospheres are liberated in the small intestine (Bullock et al., 1934). They proceed to penetrate the intestinal mucosa, enter into the blood vessels of the gut wall, and become lodged in the capillaries of the liver via the portal circulation. The localization of the larvae in the liver is not necessitated by any particular requirements found therein by the parasite. It has been adequately shown to be the result of mechanical filtration by the liver (Bullock and Curtis, 1925). Within twenty-one hours following infection, a larva has been observed in the liver capillary. By the sixth day the larva are grossly visible on the liver surface.

Liver reaction to the parasite can be conveniently summarized into two main stages: first, the exudative inflammation and necrosis of liver tissue bordering the larvae that lasts about eight to ten days following infection with varying degrees of cirrhosis and fatty infiltration occurring in heavy infections; and secondly, the active proliferative stage that begins about the time the first stage ends, reaches its maximum phase between the fifteenth to twentieth day and then gradually subsides with the resultant formation of fibrous-walled cysts. The cells involved in the proliferative reaction appear to arise from liver vascular endothelium and connective tissue.

Leonard (1940) has studied the liver tissue response of rabbits non-immunized and passively immunized against C. pisiformis. An extension of the above work on C. fasciolaris to immunized rats would be extremely interesting.

C. fasciolaris is considered to be a benign parasite in rats (Miller and Dawley, 1928). The larvae do cause sarcomas arising from their cyst walls (Bullock and Curtis, 1926), with evidence of the causative agent being associated with the calcium carbonate corpuscles of the parasite (Dunning and Curtis, 1946). Adenomatous lesions of the rat stomach have been attributed to the larval infection (Blumberg and Gardner, 1940), and myeloid changes in the spleens of rats, mice and hamsters appear related to this organism (Hoeppli and Feng, 1933). Evidence presented in this thesis suggests liver dysfunction in heavily infected rats. However, the infection does not appear to be particularly detrimental to the host's existence under conditions in nature.

Significant contributions to parasite immunology have been rendered from studies utilizing C. fasciolaris. Conclusive demonstrations of acquired (Miller, 1931d), artificial (Miller, 1931c) and passive (Miller and Gardiner, 1932c) immunity against a metazoan parasite have emanated from these efforts.

Campbell (1938a) pointed to the suitability of C. crassicollis (= fasciolaris) to immunological studies on the basis of its life cycle. He stated, "....(a) the degree of infection

can be easily regulated, (b) an accurate measure of the intensity of infection can be obtained, due to the localization and size of the parasite, (c) any infections of C. crassiccllis other than the experimental one, can be detected by variation in the size of cysts, and (d) during the early stage of its migration the parasite is in direct contact with the host's blood and therefore with any humoral antibodies that might be present."

Miller (1931a, d) showed that rats infected with C. fasciolaris of from two to six months duration exhibited protection against a superinfection. Even a single large cyst in the liver of rats is protective against a second invasion. When the larvae were surgically removed from the liver cysts, the acquired immunity was still virtually complete sixty days following their removal (Miller and Massie, 1932).

Artificial immunization is also effective in rats, though less so than in acquired immunity (Miller, 1931d). Using fresh larval and powdered adult worm suspensions, Miller (1930, 1931c) showed that either, injected usually intraperitoneally, was equally effective in producing immunity. Infections in control rats develop normally, whereas complete or nearly complete inhibition was effected in the injected animals.

Miller (1931b, 1932e) immunized his animals with a series of six intraperitoneal injections given on alternate days with a rest period between the third and fourth injection. Immunity was found to be complete after the third injection

and still present 167 days after the last immunizing dose. However, initiating the injections following infection of the rats with eggs resulted in no protection. Living larvae and fragments thereof were protective when placed in the peritoneal cavity. Lipid extracted antigen was successfully employed, but negative results were given by powdered Taenia pisiformis, the common dog tapeworm.

Further evaluation of non-specific worm materials was made by Miller (1932b, 1935a). He found that though, as already noted, powdered T. pisiformis was ineffective, the introduction of living whole worms or portions thereof into the peritoneal cavity resulted in a high degree of immunity. The results on this and other related species of tapeworms suggests some degree of common antigenicity as a significant immune response was initiated by other related species.

There is also conclusive evidence of the humoral nature of the immune response encompassed in the passive immunity studies. Sera collected from infected and artificially immunized rats were shown to protect rats when injected intraperitoneally (Miller and Gardiner, 1932a, b, c). Some evidence is also presented of the slight protective property of normal serum (Miller and Gardiner, 1932a, c).

Miller (1932c, 1934) has shown that the effectiveness of immune serum has decreased when injected nine days after infection and was no longer protective when given on the tenth day. This is attributed to the effective walling off

of the larvae by the host at this time, making them inaccessible to the immune serum. It also corresponds to the early proliferative stage described by Bullock and Curtis (1924). Complete immunity persisted up to 36 days after injection of anti-serum in some rats (Miller and Gardiner, 1932c).

Immune serum in the amount of 1 ml. per 525 grams body weight afforded complete protection in rats. A direct quantitative relationship exists between the size of the infection and protectiveness of the serum. Serum from rats with eighteen or more cysts was most effective, and those with only dead or less than eighteen living cysts had a lesser value. Within four days following infection, serum from the donor rats exhibited some immune capacity, and serum from a rat containing 128 living cysts of ten days duration prevented the development of living cysts in the recipient of the anti-sera (Miller and Gardiner, 1934).

Passive transfer of immunity to the young of infected and artificially immune mother rats has been noted. It lasts in the young for about six weeks. Whether it is passed on to the young in the milk and/or transplacentally is not known (Miller 1932a, 1935b).

Important points brought out in the extensive work of Miller and his group briefly review above are as follows: 1) rats already infected are immune to a superimposed infection. The degree of immunity is proportional to the size of the infection and is detectable within four days after

infection. 2) Active immunization can be induced with intraperitoneal injections of adult and larval worm material. Immunity is evident after the third injection of an immunizing schedule of six injections given on alternate days and skipping one between the third and fourth. 3) Protection can be passively transferred if the anti-serum is given to the recipient animal within nine days after infection.

Campbell (1938a) has extended the work on C. fasciolaris, demonstrating an "early" and "late" immunity involving two antibodies. He confirmed the quantitative relationship between the size of infection and degree of serum protectiveness. In rats averaging 38 cysts, the serum showed some protective ability by the fifteenth day and was maximally effective by the twenty-eighth day after infection. Serum from very heavily infected rats collected on the seventh day was about 75 percent protective, and maximally protective by the fourteenth day.

Two antibody mechanisms were evident. One destroys the larvae prior to encystment ("early immunity") and is present within serum collected on the eleventh day after infection. The other destroys larvae after they have encysted ("late immunity") and is found in serum collected from animals on the twenty-eighth day.

Campbell (1938b) was able to absorb out the "early" immune factor from serum collected from rats eleven days after infection, and from artificially immunized rat and

rabbit serum. He obtained negative results with serum of rats obtained on the twenty-eighth day following infection and containing the "late immunity" antibody. The latter probably explains Miller and Gardiner's (1932c) negative absorption experiment.

The non-absorbable antibodies that are responsible for the "late immunity" in a natural infection are probably a response to metabolic products or some other antigen elaborated by and necessary to the growth of the larvae (Campbell 1938b, c). A cysticercocidal factor has been reported from infected rat serum, though a similar property is exerted by physiological saline, and normal rat and guinea pig serum. It is suggested that it may be the "late immunity" factor (Chen, 1950).

Campbell (1936, 1937, 1939a) has shown that different chemical fractions of the parasite are variable in their ability to produce artificial immunization. Polysaccharide fractions from the larvae produced non-protective antibodies in injected rabbits. Intraperitoneal injections in rats of whole worm material and chemical fractions such as "globulin", nucleoprotein and "albumins" from fresh worm material produced a strong resistance, whereas albumins from dried worm material were of little value. Different fractions also produced different degrees of "early" and "late" immunity. However, no definite conclusions relative to the latter are drawn.

Dual antibody production is far from exclusive to this parasite. Similar results have been reported for Cysticercus pisiformis in rabbits (Leonard and Leonard, 1941). Among other helminths, convincing evidence has been obtained that the protective antibodies against Nippostrongylus muris are those produced against its excretions and secretions, and not those against the somatic antigens (Thorson, 1954). Rabbits infected with Trichinella spiralis show evidence of producing antilarval and antiadult humoral factors (Oliver-González, 1941).

These demonstrations leave little doubt that the host produces multiple humoral factors against the complex of antigens present in animal parasites. Not only are somatic antigens available, but also parasite metabolic products to the host antibody synthesizing mechanism. The relative importance of these antigens to the resistance manifestations in animal parasite immunity is still an inadequately explored region (Chandler, 1953).

The purpose of the study herein reported was twofold. The first was to ascertain the effects of a C. fasciolaris infection in rats as reflected in the protein metabolism. Liver tissue involvement in this infection would be expected to influence the host's tissue metabolism. Secondly, an attempt was made to relate the rat's humoral response against the parasite to the serum proteins. That a dual antibody production against this infection occurs in rats

has been established (Campbell, 1938a). By analyses of sera from infected and artificially immunized rats, it was hoped to determine in which protein fraction(s) the antibodies are produced. Such information would enlighten our knowledge of the immune mechanisms against metazoan parasites. It was felt that electrophoresis afforded an excellent tool for these purposes.

MATERIALS AND METHODS

A. Animal Care and Management

Animals utilized in this study were cats and albino rats. Cats of unknown age were acquired from private sources in and around East Lansing, Michigan. Upon arrival in the laboratory, the cats were placed in wire-bottomed cages and immunized against distemper with feline distemper vaccine.* Immunization was accomplished by giving two 2 ml. injections of vaccine intraperitoneally about 7-10 days apart.

The cats were fed ground meal** mixed with about an equal amount of warm water, canned cat food***, and pasteurized, homogenized milk.

Rats used were of the Wistar or Sprague-Dawley strain and were purchased from commercial sources. Since females have been shown to be more resistant than males to an infection with Cysticercus fasciolaris (Campbell, 1939b; Campbell and Melcher, 1940), only males were utilized. Five to six weeks old rats were shipped with litter mates kept separate. Upon receipt, they were placed in wire-bottomed cages and kept in a room isolated from all other laboratory animals. This isolation was particularly important insofar as it reduced

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*Pitman-Moore Company, Indianapolis, Indiana.

**Ken-L-Meal, Quaker Oats Company, Chicago, Illinois

*** "Three Little Kittens"

the danger of accidental infection with eggs from the adult worm in the feces of infected cats.

Food and water were available ad libitum. All rats were fed a pellet ration*.

B. Bleeding, Infection and Immunization Procedures

For each species of animal, the serum protein electrophoretic pattern is relatively constant with respect to the quantity and number of components present (Deutsch and Goodloe, 1945; Moore, 1945). However, a number of factors such as age (Heim and Schechtman, 1954), sex (Moore, 1948), strain (Thompson et al., 1954), season of the year (Hill and Trevorrow, 1942), severe injury (Gjessing et al., 1947), and protein depletion induced by plasmapheresis and protein deficient diets (Chow et al., 1948) cause significant variations in the serum pattern of an animal. In order to obviate such factors and to take into consideration the relatively small total blood volume of rats (Burke, 1954), the following procedure was used to obtain an adequate amount of serum. All litter mates were segregated equally, when possible, into control and experimental groups. The evening prior to the day of bleeding, food was withdrawn from the cages of the animals to be bled to reduce serum lipid turbidity. The experimental animals and the corresponding litter mate controls

*Miller's Eaties, Battle Creek Dog Food Company, Battle Creek, Michigan.

were lightly anaesthetized by a 0.05-0.10 ml. intraperitoneal injection of Halatal.* This was supplemented with ether to accomplish complete anesthesia. The rats were immobilized in a supine position on a board by means of rubber bands attached to the board. Under aseptic conditions, each rat was maximally bled by intracardiac puncture and then discarded. The blood was placed in sterile test tubes, allowed to clot overnight at room temperature in a slanted position and the serum then collected. To insure an adequate amount of serum for testing, generally 2-3 rats were bled per experimental and control group. The serum collected from each rat was pooled, centrifuged for a minimum of 30 minutes at about 2,000 r.p.m. and the clear serum drawn off. Analysis of the serum was made immediately or it was stored in a 4° C. cold room until it could be analysed.

In order to have a source of Taenia taeniaeformis eggs, cats were infected with encysted cysticerci maintained in stock rats. The cyst was introduced on to the back of the tongue of the cat who was forced to swallow it whole by gently restraining the cat from chewing. Each cat received 3-4 cysts. Adults with gravid segments developed in about six weeks.

On the day rats were to be infected with eggs, an infected cat was sacrificed using ether or chloroform. The adult worms were recovered from the small intestine and the terminal

*Halatal, Jensen-Salsbery Laboratories, Inc., Kansas City, Mo.

gravid proglottids cut off. Eggs were recovered from the gravid proglottids by teasing them apart with dissecting needles in 0.85 percent NaCl. Quantification of the eggs was accomplished by using a hemacytometer. After adequate mixing of the eggs to insure a uniform suspension a sample was drawn off with a pipette drawn out to a fine point. The same pipette was used for all determinations. The chamber was charged and the number of eggs in the entire ruled area was counted. The average of ten such counts was taken and converted into number of eggs per ml. of egg suspension. Rats were infected intragastrically by injecting 1 ml. of the evenly dispersed egg suspension through a No. 8 French catheter that was passed orally into the stomach.

Cysticerci, whole and lyophilized, were used as antigen for artificial immunization. Whole cysticerci were obtained from infected stock rats and washed 3-4 times with sterile 0.85 percent NaCl. The excess saline was blotted off with filter paper and the larvae then stored at minus 30° C. until used. When needed for immunization, the cysticerci were ground into a paste with a mortar and pestle and made up into a 10 or 20 percent suspension in 0.85 percent NaCl. Cysticerci were lyophilized by placing them in 2 ml. ampoules. They were then quickly frozen at minus 70-75° C. in a 95 percent alcohol-dry ice mixture and then placed under vacuum (50-100 microns Hg.) for 48 hours at room temperature. At the end of this time, the necks of the ampoules were sealed with an oxygen

torch. Until used, the lyophilized material was stored at minus 30° C. For immunizing injections, the lyophilized larvae were ground into a fine powder and made up into 2.5 or 5.0 percent suspensions in 0.85 percent saline.

Rats were immunized according to the schedule given by Miller (1931c). Intraperitoneal injections of 1 ml. of worm suspension were given on alternate days for a total of six injections. A rest period was interposed between the third and fourth injection. Hence, if the first injection was made on the first day of a month, the schedule for the six injections would be as follows: 1-3-5-9-11-13. Injections given subsequent to the above standard series of injections will be noted under the experimental protocols of this section.

Immune (from infected or artificially immunized rats) and control sera for passive immunization were obtained by pooling the sera from 8-10 rats for each group. Since the sera were used no later than 3 days after they were collected, they were stored at 4° C. with no preservative added. Each recipient of immune or normal serum was injected with 1 ml. of serum intraperitoneally within 24 hours before or after being infected with Taenia taeniaeformis eggs. Since a limited amount of serum was available for passive transfer of immunity, the amount of serum injected was not based on body weight. Miller and Gardiner's (1934) and Campbell's (1938a, b) studies suggested that injections of serum not

based on body weight would serve the purpose of this investigation.

The number of larvae present in the livers of infected rats was determined by counting the total number of cysts visible on the surface of the entire liver. All animals were routinely autopsied and the livers examined for the presence of cysts from an accidental infection. Any animal found to be accidentally infected was discarded.

C. Total Protein Determinations

Serum total protein was determined by the biuret reaction. Weichselbaum's (1946) biuret reagent was used. A standard curve was constructed on semi-log graph paper by using a standard Bovine Albumin solution* whose nitrogen content was specified (about 10 mg. of protein nitrogen per ml.). Conversion into grams percent of protein was effected by multiplying the nitrogen content by the factor 6.25. The use and advantages of this standard solution were presented by Bernhard and Scher (1951).

Sera and standard solutions to be analysed for total protein were diluted 1:20 by adding 0.5 ml. of serum to 9.5 ml. of distilled water and mixing by gently inverting several times. To 4.0 ml. of diluted serum were added 4.0 ml. of biuret reagent and then well mixed by inversion. A blank

*Protein Standard Solution (Crystalline Bovine Albumin), Armour and Company, Chicago, Illinois.

was prepared by substituting 4.0 ml. of distilled water for the diluted samples. As a check on the standard curve, the known albumin solution was frequently run with the sera being determined. The solutions were allowed to stand one hour at room temperature for maximal color development and then read on a photelometer* at a wave length of 525 millimicrons**. From the percent transmission of each sample, the total serum protein could be obtained directly from the standard calibrated curve.

D. Electrophoresis

All sera were analysed by the moving boundary electrophoresis technique, using the Perkin-Elmer Model 38 Tiselius Electrophoresis Apparatus. Moore and White (1948) have described this instrument and further descriptive and procedural particulars are available in the Perkin-Elmer Instruction Manual (1951). The barbiturate (veronal and sodium veronal) buffer of pH 8.6, 0.1 ionic strength described by Longsworth (1942) was used throughout this study.

The particular electrophoresis procedure employed in this study was as follows:

Sera to be analysed were diluted with buffer 1:2, generally 2 ml. serum and 4 ml. buffer. The diluted serum

*Cenco-Sheard-Sanford Photelometer, Cat. No. 41000, Central Scientific Company, Chicago.

**Green Filter, Cat. No. 87309B.

was placed into a dialyzing membrane of seamless regenerated viscose process cellulose* and dialyzed by the mechanical stirring method of Reiner and Fenichel (1948) at room temperature. Dialysis was carried out for one hour against 400 ml. of buffer; and, was followed by further dialysis for four hours against 600 ml. of fresh buffer. The equilibrated system was then left overnight in a 4° C. cold room (15-18 hours).

The 2 ml. capacity cell was greased, assembled and also allowed to stand overnight at 4° C. Filling and complete assembling of the cell and buffer bottles were done in the 4° C. cold room. The complete assembly was then ready to be placed in the water-bath of the electrophoresis apparatus.

All analyses were made at 7.5 milliamperes with an average voltage of 112 volts for 7600 seconds. The potential gradient or electric field strength, in volts/cm., averaged 8.18 v/cm. and the bath temperature averaged 0.5° C.

Electrophoretic patterns were obtained by photographing the ascending and descending limbs of the cell by the modified scanning method of Longsworth (1939, 1946). To aid in establishing the base line for area measurements, a scanning photograph of each cell limb was made prior to shifting the initial boundary into view. By superimposing this photograph on the base of the pattern photographed after the boundaries have migrated, the base line of the latter photograph could

*Visking Corporation, Chicago, Illinois.

be more accurately established (Longsworth and MacInnes, 1940).

The electrophoretic patterns were photographed with Kodak M Plates and developed by standard photographic methods.

Component areas and mobilities were calculated from the descending limb patterns (Longsworth and MacInnes, 1940). A two-fold enlargement of the pattern was projected from a photographic enlarger and the outline traced on paper. Component areas were delineated by dropping an ordinate between the minimal point of adjacent areas to the base line (Tiselius and Kabat, 1939).

Each area was measured with a planimeter* in arbitrary units. The relative percentage of each component was obtained by dividing the area of each component by the total area, exclusive of the epsilon-boundary anomaly. Conversion into grams percent protein for each component was readily accomplished by relating the relative percentage of each component to the total protein of the serum sample.

Mobilities of each component were ascertained by measuring, in centimeters, the distance of an ordinate dividing the component area in half from the midpoint of the initial boundary (Longsworth and MacInnes, loc. cit.; Moore and Abramson, 1950). The latter boundary is photographed just prior to turning on the current. By fitting this distance into the equation given

*K and E No. 4236 Compensating polar planimeter, Keuffel and Esser Company, New York.

by Longsworth and MacInnes (loc. cit.) the mobility (μ) was obtained in x 10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$. The average component mobilities determined for the sera analysed in this study were as follows:

albumin	5.7
alpha 1 -globulin ...	4.8
alpha 2 -globulin ..	3.9
beta-globulin	2.6
gamma-globulin	1.6

For conductivity measurements, the conductance of the equilibrated buffer was determined in lieu of the dialyzed sample as suggested by Moore, et al. (1949).

E. Experimental Protocols

1. Artificially Infected Rats

Experiment I. Wistar strain rats, all of the same age, were grouped into experimental and control groups by segregating litter mates. Rats from the experimental reservoir were initially infected with about 1,200 Taenia taeniaeformis eggs at 8 weeks of age. Infected rats and their litter mate controls were bled at 3-day intervals following infection up to 15 days. Final bleedings were made 21, 28 and 35 days after initial infection.

To relate the protective capacity of the rats to the results obtained from the electrophoretic analysis of serum, animals initially infected when 8 weeks of age were reinfected with about 1,800 eggs on the day of the last bleeding. Their

litter mates were also infected to serve as a control on the viability of the eggs used in the second infection. All were autopsied 4 weeks later.

Experiment II. The results of experiment I suggested that a repeat be made in order to obtain a higher level of infection in the rats. Wistar strain rats were again separated, by litter mates, into control and experimental reservoirs. When 8 weeks old, the experimental rats were infected with about 7,000 eggs. Bleedings were made, of experimental rats and corresponding litter mate controls, at 4-day intervals up to 28 days. A final bleeding was done on the thirty-fifth day after infection.

To test again the protective capacity of the sera collected on the thirty-fifth day by the in vivo method, sera collected from infected and control rats were used for passive immunization of other rats. Rats, 13 weeks old, were separated into three groups so that litter mates were distributed as evenly as possible. One group received normal rat serum, the second infected rat serum and the third no serum. Each rat was injected intraperitoneally with 2 ml. of the respective sera within 24 hours after being infected with about 1,200 eggs. They were autopsied 5 weeks after infection with the eggs.

Experiment III. In order to determine in which fraction(s) of the serum proteins the "early" and "late" immune factors

are found, this experiment was undertaken. About 3,000 eggs were given to 8 week old Sprague-Dawley rats. Infected and control litter mates were bled at 4 day intervals up to the twelfth day. The sera collected from normal and infected rats on the twelfth day were absorbed, according to Campbell's (1938b) method, by adding enough fresh larval paste to make a final 2 percent suspension. Both pre- and post-absorbed sera were analysed electrophoretically.

To correlate any changes in the absorbed sera electrophoretic patterns with the protective capacity of the sera, the sera were tested in vivo by passive immunization. For this purpose, 5 groups of 10 weeks old rats were set up by distributing litter mates as equally as possible. They were grouped as follows:

1. received no serum
2. received normal rat serum
3. received normal rat serum that was absorbed
4. received infected rat serum
5. received infected rat serum that was absorbed

Each rat received 1 ml. of serum intraperitoneally and within 24 hours after receiving the serum they were all infected with about 3,000 eggs. All rats were necropsied 4 weeks after infection.

The procedure outlined for the 12 day bleeding was repeated on the thirty-fifth day following the initial infection. It was hoped to discern the "late" immune factor from the "early" immune factor by relating any changes obtained here to the results on the sera from the 12 day bleeding. The

only details that differed were in the use of lyophilized cysts for sera absorption and a new lot of eggs (dosage of 3,500). Also, the rats were 13 weeks and 4 days old when infected.

2. Artificially Immunized Rats

Experiment IV. Since "early immunity" is apparently a response against larval somatic antigens (Campbell, 1938b, c) it seemed reasonable to establish the protein fraction in which these antibodies are present by immunizing rats with worm material. Hence, Wistar rats received a standard series of 6 intraperitoneal injections of 1 ml. of a 2.5 percent saline (0.85 percent sodium chloride) suspension of lyophilized cysticerci. Injections were started when the rats were 7 weeks old. Bleedings of immunized and control litter mates were made at weekly intervals after the first injection for a total of 6 weeks. Two days before the third and fourth weekly bleedings, each rat received an extra injection of 1 ml. of a 5 percent suspension of lyophilized cysticerci.

To test the effectiveness of the immunization, on the day of the last bleeding injected and litter mate controls were each infected with about 2,000 eggs. They were necropsied 5 weeks later.

Experiment V. From the results of experiment IV, it was decided to use whole larvae, stored at minus 30° C., as

the antigen. A 10 percent saline (0.85 percent sodium chloride) suspension of ground larval paste was injected intraperitoneally (1 ml./injection) for the standard series of 6 injections. Injections were initiated when the Sprague-Dawley rats were 8 weeks old. A final injection of a 20 percent suspension was given 4 days prior to the last bleeding.

Immunized and control litter mates were bled at 4 day intervals following the first injection for a total of 28 days.

Sera collected on the twenty-eighth day were utilized for absorption studies. The procedure outlined in experiment III was followed. Lyophilized adults were used for absorbing the sera.

In the passive immunization study, rats 12 weeks and 4 days old were infected with about 1,500 eggs within 24 hours after receiving the serum (1 ml. intraperitoneally). They were autopsied 4 weeks after infection.

RESULTS

A. Artificially Infected Rats

Electrophoretic analysis of the sera from infected rats of experiment I revealed no significant changes in the total protein, A/G ratio (albumin/total globulin) or serum protein components. All of the electrophoretic data, in terms of both grams percent protein and relative percent composition, as well as the total proteins and A/G ratios presented in this thesis were analyzed statistically. The "t" value was determined for the mean of the differences between the experimental and control groups. Any "t" value occurring at or beyond the 5 percent level of probability was considered significant. A value at or exceeding the 1 percent level of probability was considered highly significant. The results of the electrophoretic analyses are presented in Table I. A look at the average values shows minor differences between the control and infected groups. No consistent trend was obtained for any of the serum protein components.

Eggs used to infect the animals had apparently a low percentage of viable onchospheres. A total average of 23.5 living and 3.9 dead cysts were found in the livers of the animals bled for serum analysis. The largest average number

of larvae were found in the rats bled on the fifteenth day. Table II shows the number of cysts found in the rats bled for serum analysis.

Though no changes were discernible in the serum, rats reinfected on the day of the last bleeding were almost completely immune to the second infection. It is of further interest that the average number of cysts from the initial infection was nearly a third less than that of the rats bled for serum analysis (Table III, Figures 13 and 14).

Hence, it is evident that the initial infection had rendered the animals resistant to a superimposed infection in spite of the failure of any changes to appear in the serum proteins.

The results of experiment I led to the natural desire to determine the results of a heavier infection in the rats. From the changes in the serum proteins of experiment II, it seems apparent that the results of experiment I are explainable on the basis of the small number of cysts resulting from the initial infection. Comparison of Tables II and V shows that the rats bled for serum analysis in experiment II had a total average number of cysts nearly seven times that of the animals of experiment I.

Results of the sera analyses of experiment II are presented in Table IV. No significant differences were obtained between the total protein and alpha 1 -globulin. The gamma-globulin of the infected group was significantly increased.

TABLE I

ELECTROPHORETIC ANALYSIS OF EXPERIMENT I

GROUP	Rat No.	Age (Days) Bled	Days Since Infected	Total Protein (Gm.%)	Electrophoresis (Gm.%)						
					Albumin	Total Globulin	Globulins				A/G
							Alpha 1-	Alpha 2-	Beta	Gamma	
Control	10,11,12	59	-	5.25	2.80	2.45	.79	.36	.94	.36	1.14
	13,14,15	62	-	5.58	2.91	2.67	.86	.41	.88	.52	1.09
	16,17,18	65	-	6.00	3.31	2.69	.88	.52	1.03	.26	1.23
	19,110,111	68	-	6.00	3.62	2.38	.77	.46	.86	.29	1.52
	112,113	71	-	6.48	3.70	2.78	.92	.52	.99	.35	1.33
	115,116,117	77	-	6.22	3.46	2.76	1.24	.32	.91	.29	1.25
	118,119,120	84	-	6.06	3.15	2.91	.82	.62	.85	.62	1.08
	121,122	91	-	5.65	3.03	2.62	.85	.47	.93	.37	1.16
			Average	5.91	3.25	2.66	.89	.46	.92	.38	1.23
Infected	125,126	59	3	5.14	2.81	2.33	.80	.34	.89	.30	1.21
	127,128,129	62	6	5.40	2.87	2.53	.93	.40	.88	.32	1.13
	130,131,132	65	9	5.40	2.94	2.46	.85	.41	.82	.38	1.20
	133,134,135	68	12	5.70	3.28	2.42	.73	.54	.85	.30	1.36
	136,137,138	71	15	6.57	3.70	2.87	.69	.60	.98	.60	1.29
	139,140,141	77	21	5.89	3.09	2.80	1.03	.34	.90	.53	1.11
	142,143	84	28	6.75	3.28	3.47	1.07	.62	1.12	.66	0.95
	145,146	91	35	6.09	3.08	3.01	1.01	.57	1.04	.39	1.02
			Average	5.87	3.13	2.74	.89	.48	.94	.44	1.16
	Level of Significance			None	None	None	None	None	None	None	None

TABLE II

LARVAE IN LIVERS OF RATS BLED FOR SERUM ANALYSIS OF EXPERIMENT I

Days Since Infected	Rat No.	Cysts			
		Total		Average	
		Living	Dead	Living	Dead
3	124	*			
	125	**			
	126				
6	127				
	128	**			
	129				
9	130	20	***		
	131	23		26.3	***
	132	36			
12	133	10			
	134	7	***	8.7	***
	135	9			
15	136	50	8		
	137	26	4	32.7	5.7
	138	22	5		
21	139	36	4		
	140	34	5	28.3	3.0
	141	15	0		
28	142	16	1		
	143	19	0	17.5	0.5
	144	*			
35	145	41	9		
	146	12	3	26.5	6.0
	147	*			
	Average	23.5	3.9		

* Discarded - accidental infection
** Not yet grossly visible
*** Not distinguishable from living

TABLE III

LARVAE IN LIVERS OF RATS REINFECTED TO DETERMINE PRESENCE OF IMMUNITY INDUCED FROM INITIAL INFECTION OF EXPERIMENT I

Rat No.	Cysts					
	Reinfected Group				Control Group	
	From initial infection		From second infection		For second infection	
	Living	Dead	Living	Dead	Living	Dead
1	*		4	7	124	13
2	20	0	2	1	120	5
3	16	0	0	7	177	3
4	12	0	1	0	72	10
5	*		2	4	62	11
6	**				143	15
7	9	0	0	1	173	21
8	4	0	1	2	92	3
9	11	0	1	0	119	10
10	4	0	0	1	78	24
Average	8.6	0	1.2	2.6	116.0	11.5

* No large cyst typical of initial infection seen.
** Discarded - accidental infection.

Total globulin, alpha 2 -globulin and beta-globulin showed highly significant increases in the infected animals. Highly significant decreases were obtained in the albumin and A/G ratio of the infected rats.

Figures 1 to 10 are the serum electrophoretic patterns showing the changes occurring at various days following infection. Of particular interest are the marked changes occurring on the eighth, twelfth and sixteenth days after infection. On the fourth day, the only noticeable difference between the infected and control groups was the increased alpha 2 -globulin (Table IV, Figures 1 and 2). The alpha 2 -globulin of the infected rats persisted at a fairly constant level above the control animals throughout the entire experiment (Table IV). Sera on the eighth day showed a sharp increase in the beta-globulin and total globulin, with a marked decrease in the albumin and A/G ratio (Figures 3, 4, 11 and 12). By the twelfth day the beta-globulin and total globulin dropped sharply to a lower level while the albumin and A/G ratio rose markedly. The albumin was at about a control level on the twelfth day, had essentially the same value as the controls on the sixteenth day and maintained itself below control values throughout the remainder of the experiment (Figures 5 to 8, 11 and 12). Referring again to Figures 3 to 8 and 12, there was a sharp rise in gamma-globulin that occurred on the twelfth day with a marked drop to a lower level by the sixteenth day.

From Figures 7 to 12 and Table IV it can be seen that the differences between the serum proteins of the infected and control groups from the sixteenth day on are fairly constant and maintained themselves in the sera obtained on the thirty-fifth day following infection. Also, it appeared that protein metabolism was most drastically affected for approximately two weeks; between the fourth and sixteenth days following infection. Table V shows that there were marked quantitative differences in the average number of larvae in the rats bled for serum analysis on the various days, but these differences per se appeared to have no effect beyond the sixteenth day. However, before definite conclusions can be made on this point further studies are indicated.

That a protective humoral factor was present in the serum of the infected rats obtained on the thirty-fifth day was clearly evident from the complete protection afforded rats passively immunized with this serum. Though rats receiving serum from non-infected rats had fewer living cysts than the rats receiving no serum, the total number (living plus dead) of cysts in both groups were about the same (Table VI, Figures 15 to 17). The slight protective capacity of normal rat serum was observed in the other passive immunization studies conducted herein and Miller and Gardiner (1932a, c) have also reported this. Also, it should be noted that the rats utilized throughout this study appeared to show an age resistance to infection with the tapeworm eggs. In

TABLE IV

ELECTROPHORETIC ANALYSIS OF EXPERIMENT II

GROUP	Rat No.	Age (Days) Bled	Days Since Infected	Total Protein (Gm.%)	Electrophoresis (Gm.%)						
					Albumin	Total Globulin	Globulins				A/G
							Alpha 1-	Alpha 2-	Beta	Gamma	
Control	22	60	-	5.89	3.19	2.70	.84	.52	.78	.56	1.18
	24	64	-	5.47	2.78	2.69	.87	.51	.88	.43	1.03
	26, 27	68	-	5.43	3.02	2.41	.81	.41	.76	.43	1.25
	28,29	72	-	5.22	2.91	2.31	.84	.45	.75	.27	1.26
	211	76	-	5.75	3.27	2.48	.94	.45	.77	.32	1.32
	212,213	80	-	6.19	3.62	2.57	.71	.54	.92	.40	1.41
	214,215	84	-	6.41	3.47	2.94	1.19	.51	.94	.30	1.18
	216-223	91	-	6.66	3.58	3.08	1.17	.59	.91	.41	1.16
			Average	5.88	3.23	2.65	.92	.50	.84	.39	1.22
Infected	224,225,226	60	4	6.05	3.08	2.97	.82	.72	.88	.55	1.04
	227	64	8	5.73	2.11	3.62	.92	.74	1.45	.51	0.58
	229,230	68	12	6.03	2.88	3.15	.81	.44	.90	1.00	0.91
	231,232	72	16	5.45	2.93	2.52	.60	.52	.90	.50	1.16
	233,234	76	20	5.75	2.88	2.87	.65	.64	1.09	.49	1.00
	235,236	80	24	6.21	3.30	2.91	.77	.63	1.02	.49	1.13
	237,238	84	28	6.19	3.03	3.16	.87	.64	1.14	.51	0.96
	239-247	91	35	6.79	3.16	3.63	.93	.75	1.34	.61	0.87
			Average	6.02	2.92	3.10	.80	.64	1.09	.58	.96
			Level of Significance	None	Exceeds 1%	Exceeds 1%	None	Exceeds 1%	Exceeds 1%	Exceeds 5%	Exceeds 1%

TABLE V

LARVAE IN LIVERS OF RATS BLED FOR SERUM ANALYSIS OF EXPERIMENT II

Days Since Infected	Rat No.	Cysts			
		Total		Average	
		Living	Dead	Living	Dead
4	224				
	225	*			
	226				
8	227	160	***	160	***
	228	**			
12	229	219	11	215.5	10
	230	212	9		
16	231	98	7	104	6
	232	110	5		
20	233	235	14	221	8.5
	234	207	3		
24	235	312	33	211	38.5
	236	110	44		
28	237	132	2	139	5
	238	146	8		
35	239	56	12		
	240	19	2		
	241	60	10		
	242	128	18		
	243	127	7	130.3	10.7
	244	117	1		
	245	209	20		
	246	182	16		
	247	275	10		
	248	**			
	Average	155.7	12.2		

* Not yet grossly visible
** Discarded - accidental infection
*** Not distinguishable from living

PLATE I

Serum electrophoretic patterns of rats bled on fourth day of experiment II.

Fig. 1. Control rats

Fig. 2. Infected rats

PLATE I

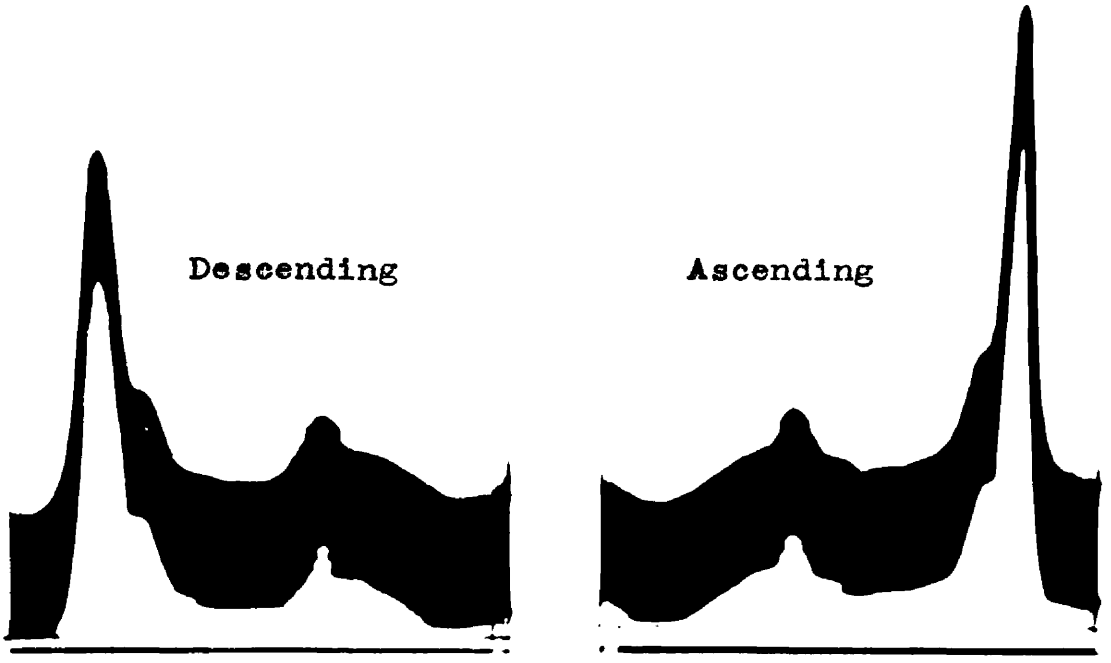

Figure 1

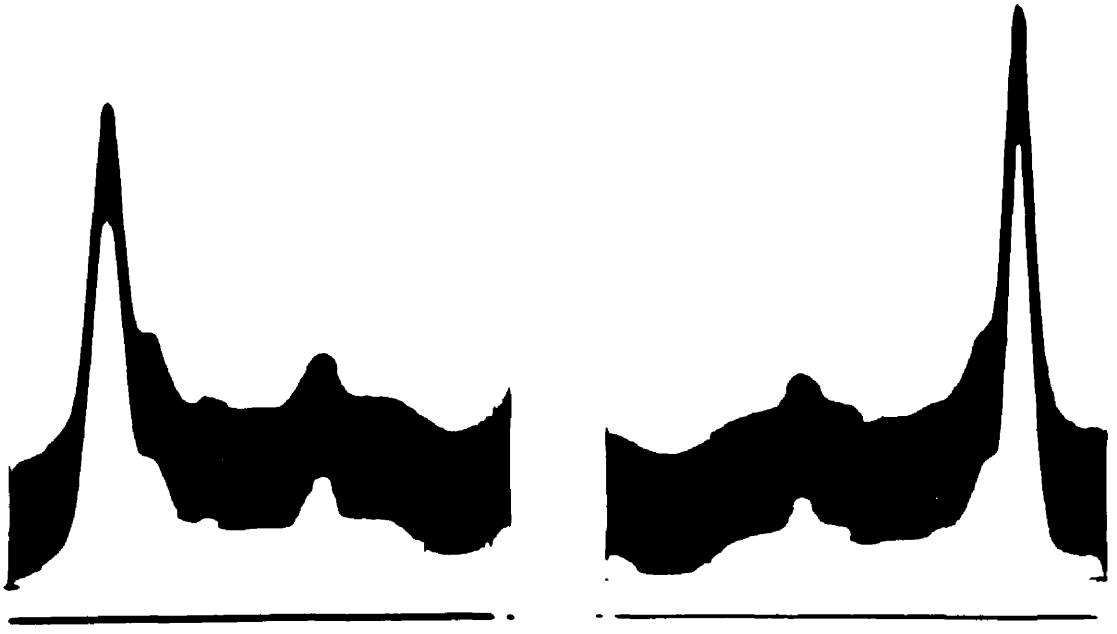

Figure 2

PLATE II

Serum electrophoretic patterns of rats bled on eighth day of experiment II.

Fig. 3. Control rats

Fig. 4. Infected rats

PLATE II

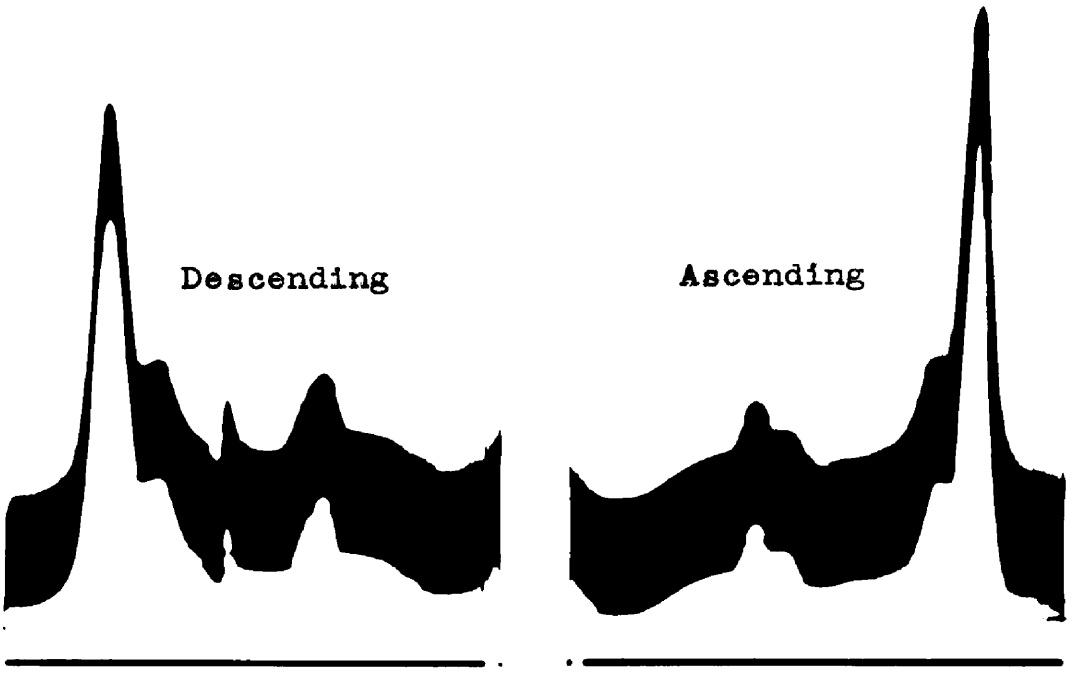

Figure 3

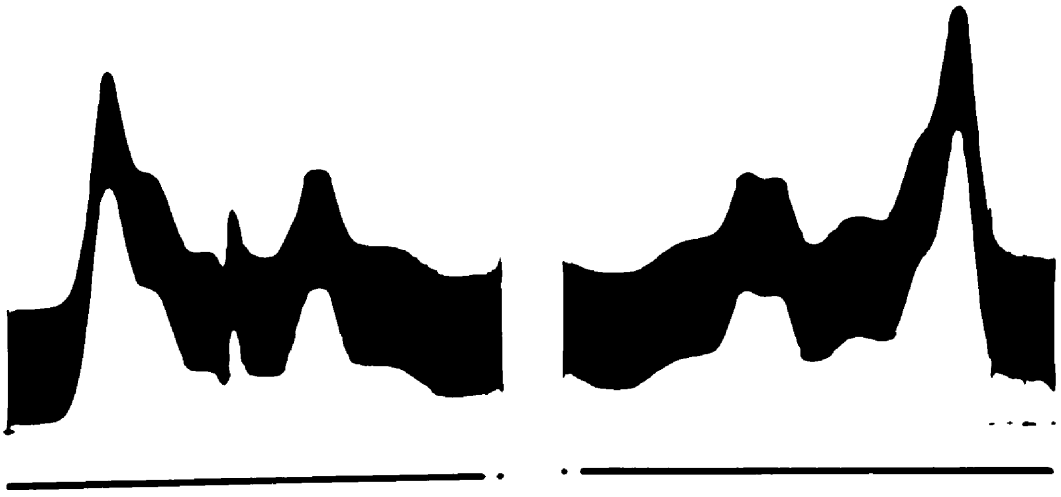

Figure 4

PLATE III

Serum electrophoretic patterns of rats bled on twelfth day of experiment II.

Fig. 5. Control rats

Fig. 6. Infected rats

PLATE III

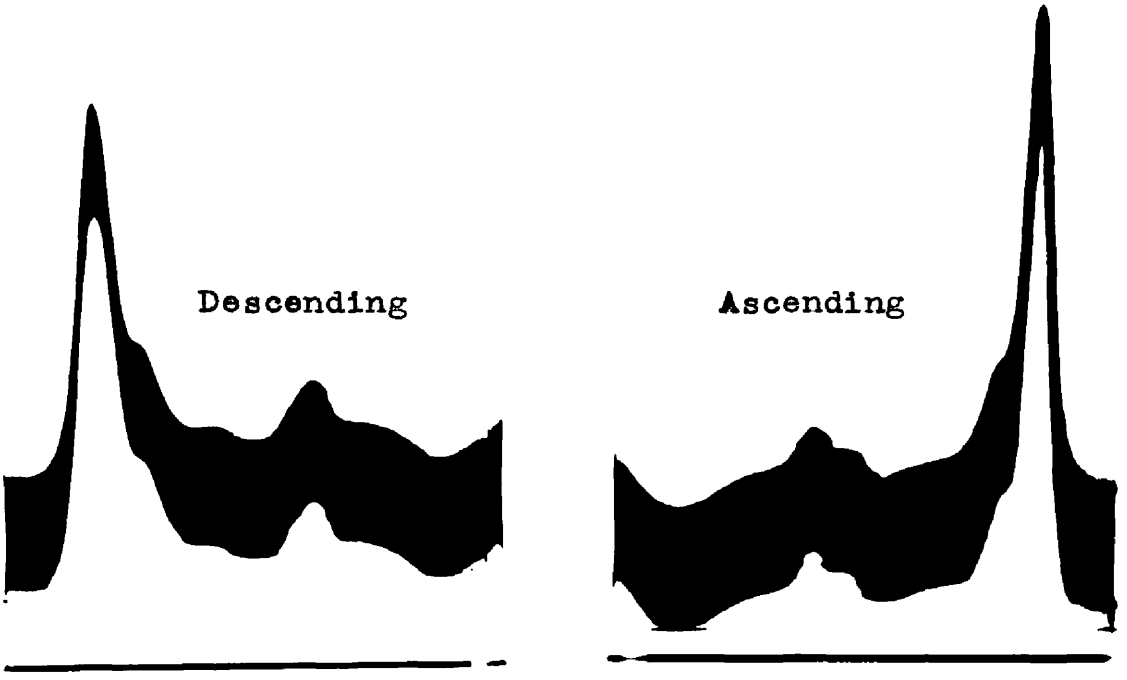

Figure 5

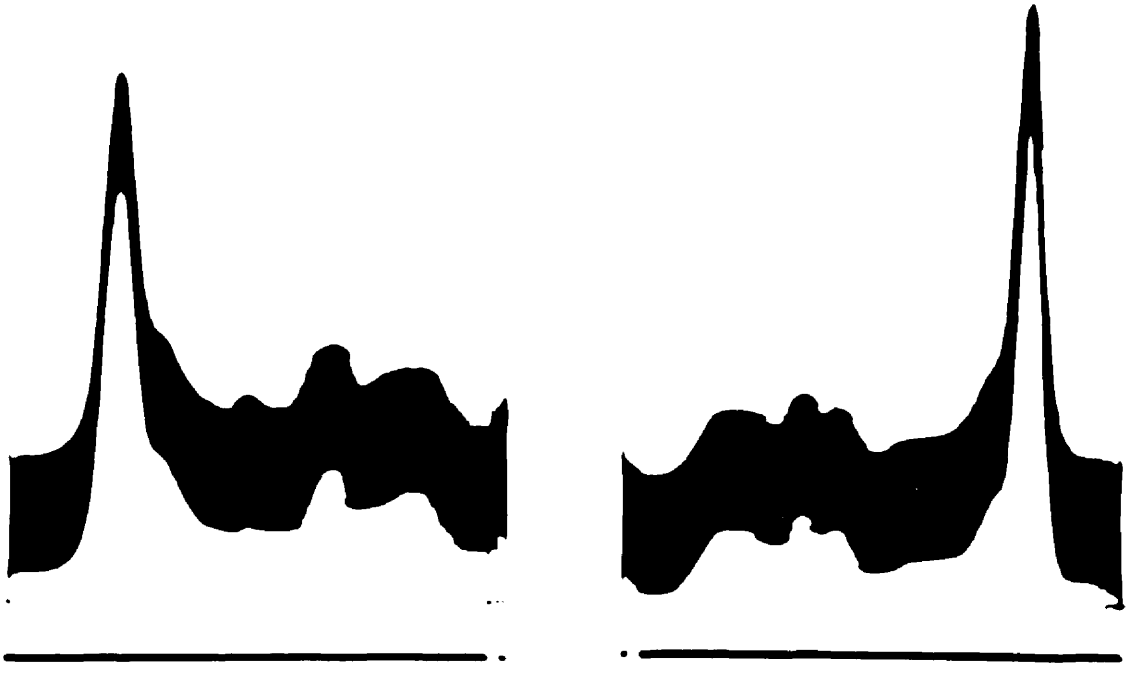

Figure 6

PLATE IV

Serum electrophoretic patterns of rats bled on sixteenth day of experiment II.

Fig. 7. Control rats

Fig. 8. Infected rats

PLATE IV

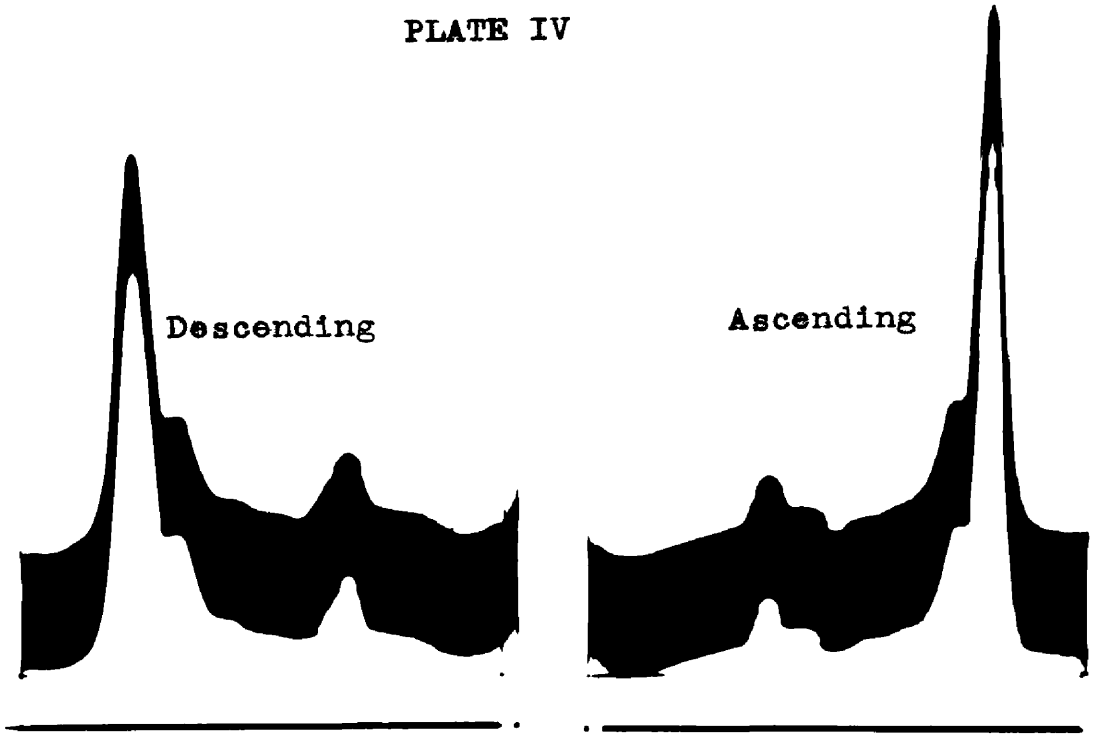

Figure 7

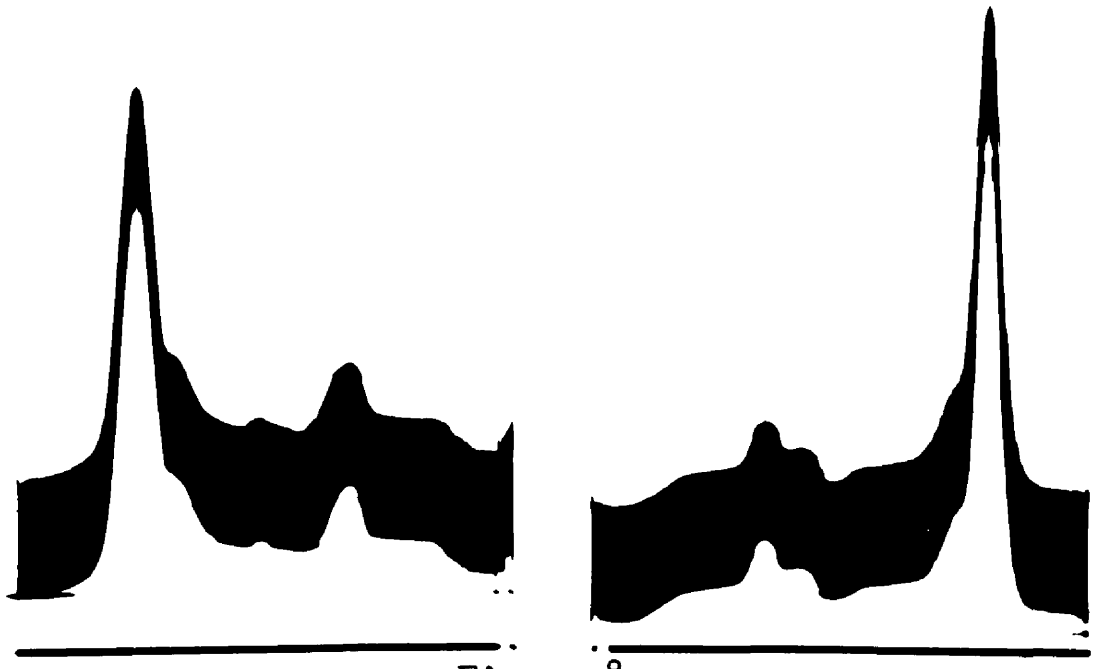

Figure 8

PLATE V

Serum electrophoretic patterns of rats bled on thirty-fifth day of experiment II.

Fig. 9. Control rats

Fig. 10. Infected rats

PLATE V

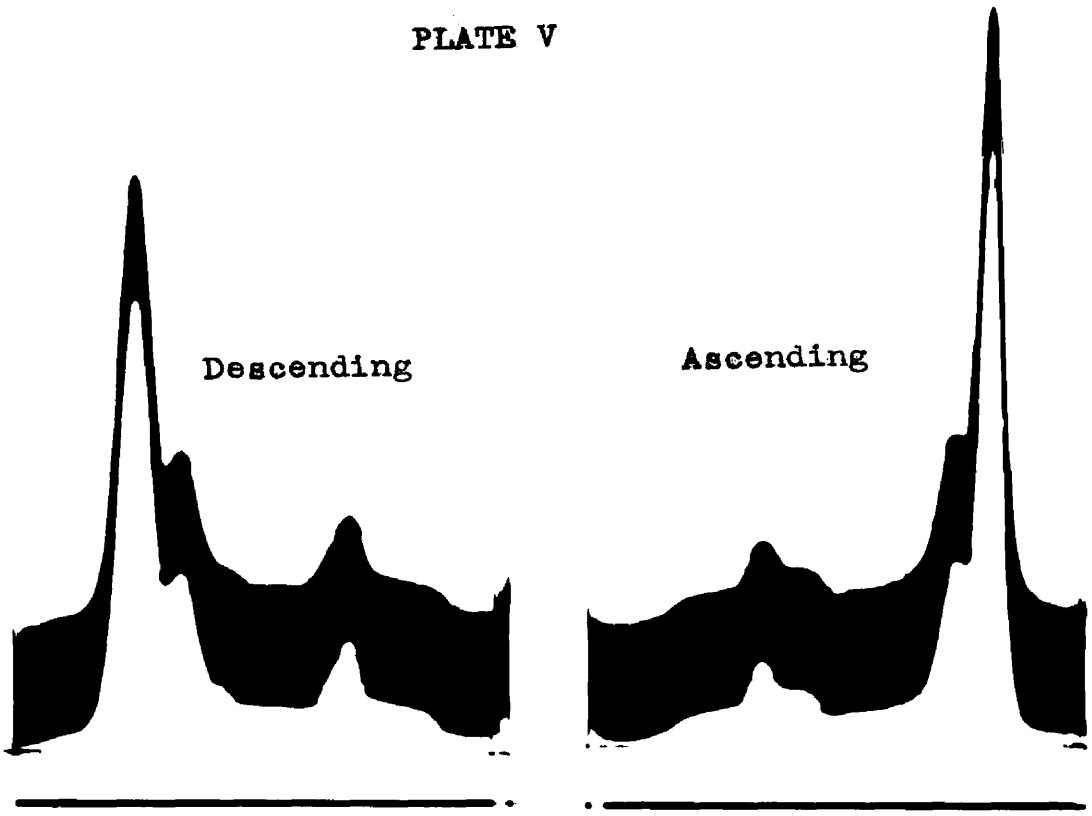

Figure 9

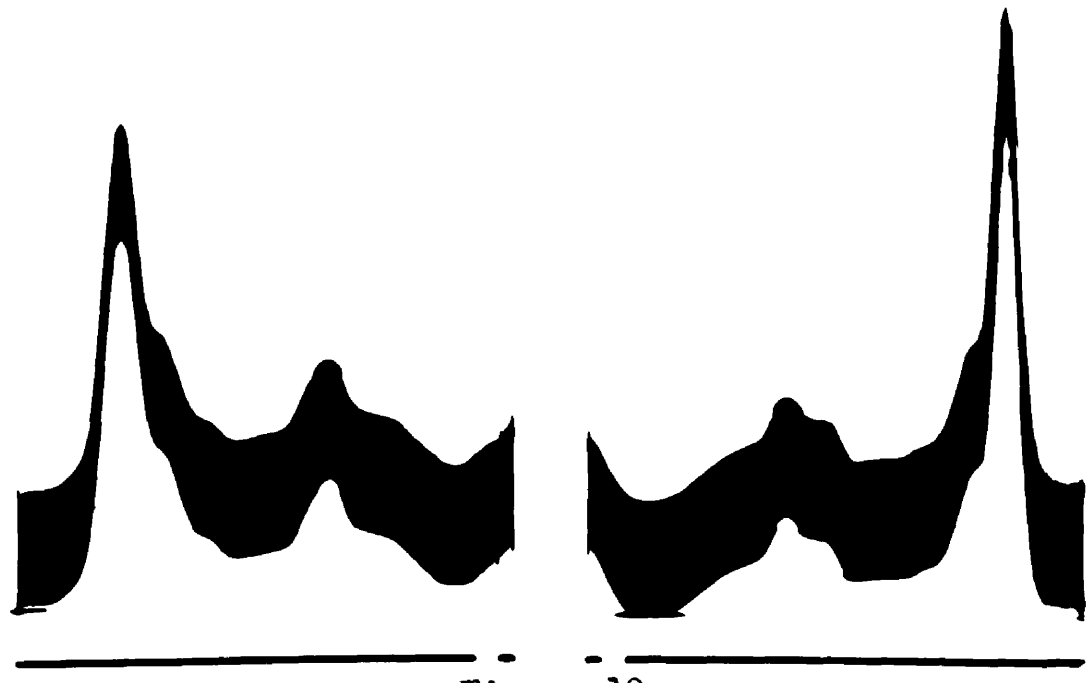

Figure 10

Fig. 11

Relation of Albumin, Total Globulin and A/G Ratio to Infection of Experiment II

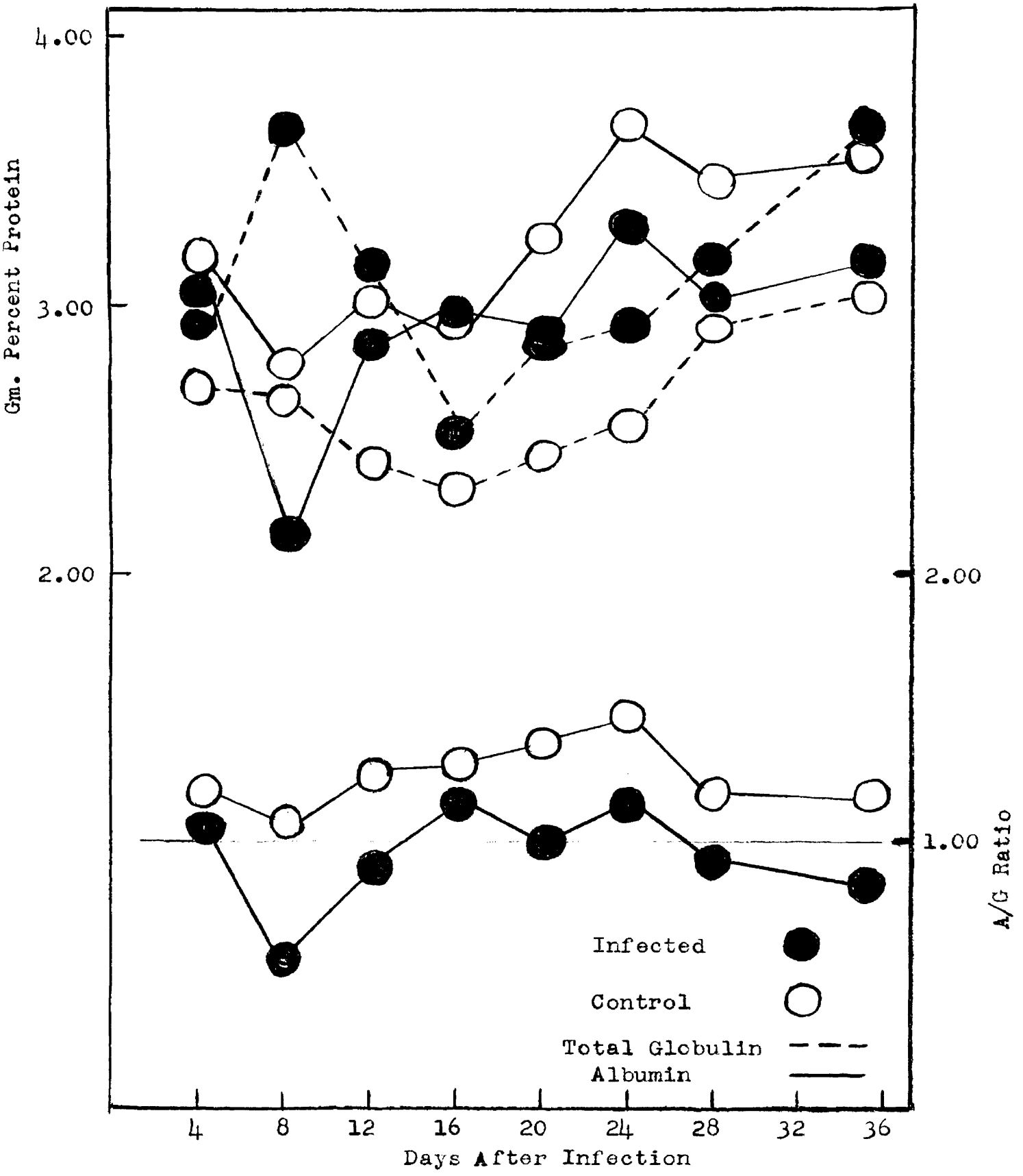

Fig. 12

Relation of Beta- and Gamma-Globulins to Infection of Experiment II

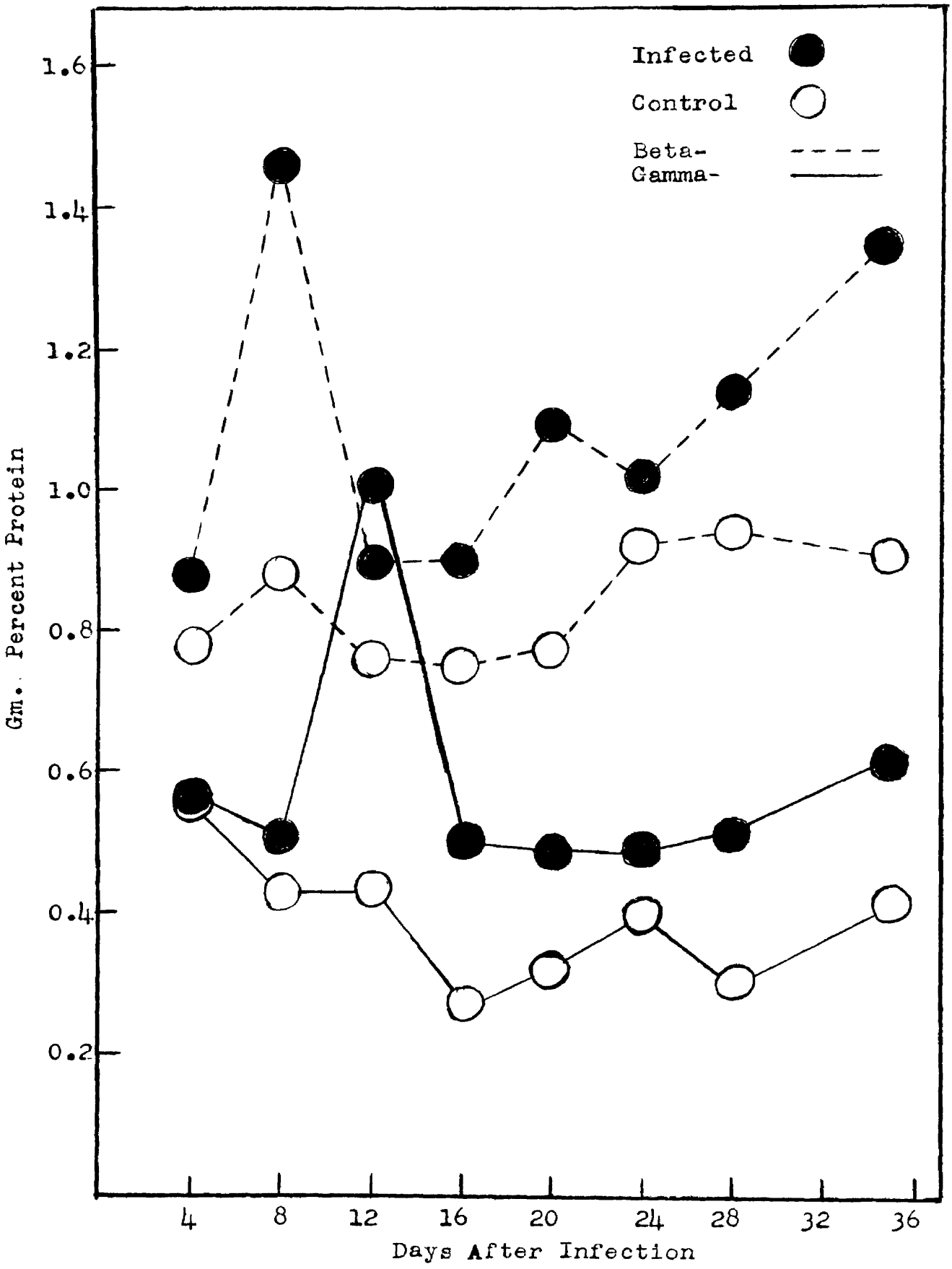

TABLE VI

LARVAE IN LIVERS OF RATS PASSIVELY IMMUNIZED WITH SERUM FROM RATS BLED ON THIRTY-FIFTH DAY OF EXPERIMENT II

Rat No.	Injected with Serum from rats:				No Serum	
	Infected		Non-infected			
	Cysts		Cysts		Cysts	
	Living	Dead	Living	Dead	Living	Dead
1	0	0	8	74	23	23
2	0	0	15	35	2	16
3	0	0	7	50	*	
4	0	0	1	29	58	13
5	0	0	6	4	22	31
6	0	0	1	20	18	28
7	0	0	*		1	30
Average	0	0	6.3	35.3	20.7	23.5

* Discarded - accidental infection

PLATE VI

Livers from rats used in reinfection study of experiment I.

Fig. 13. Liver of rat (No. 1) that received eggs of initial and second infection.

Fig. 14. Liver of rat (No. 1) that received eggs only of second infection.

Livers of rats used in passive immunization study of experiment II.

Fig. 15. Liver of rat (No. 1) that received serum from infected rats.

Fig. 16. Liver of rat (No. 7) that received serum from non-infected rats.

Fig. 17. Liver of rat (No. 5) that received no serum.

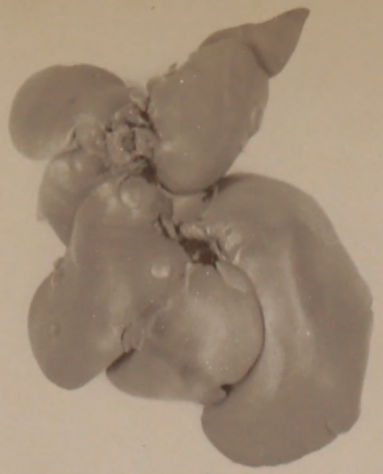

Fig. 13

Fig. 14

Fig. 15

Fig. 16

Fig. 17

PLATE VI

the passive immunization study just noted, the rats receiving normal or no serum had cysts that were smaller than those obtained in rats infected at an earlier age. Older rats used for infection showed smaller numbers of cysts than young rats. A decrease in susceptibility to this infection with an increase in age of rats has been reported by Curtis *et al.* (1933) and Greenfield (1942). However, Miller and Massie (1932) questioned the existence of age immunity in rats against this parasite.

In an effort to demonstrate in which protein fraction(s) the antibody against the larvae are produced, experiment III was undertaken. Though comparison of experiments II and III was limited by the fewer analyses made in the latter experiment, the same general results were evident in experiment III as were obtained in experiment II. The results of experiment III were not as significant as those of experiment II. Total protein and alpha 1 -globulin were not significantly changed. The total globulin and beta-globulin were significantly increased in the infected animals and the A/G ratio was reduced significantly. However, the albumin, alpha 2- and gamma-globulins showed no significant differences whereas in experiment II they did. The electrophoretic results are given in Table VII and Figures 18 to 25. An explanation would appear to lie in the smaller number of cysts present in rats bled on the eighth and twelfth days following infection (Table VIII).

The average number of cysts present were about half that in the rats bled on the corresponding days of experiment II (Tables V and VIII).

Campbell (1938b) demonstrated that the "early" immune factor is absorbable from the serum collected on the eleventh day following infection. Sera collected from infected and non-infected rats on the twelfth day after infection were analyzed electrophoretically before and after absorption with fresh larval paste. No significant alterations between the pre- and post-absorbed patterns were obtained. In Table VII, only the A/G ratios are given for the post-absorbed sera and serve to show the lack of any significant change between the patterns.

That the sera collected on the twelfth day contained protective humoral bodies was evident from the results obtained in the passive immunization study. Table IX and Figures 26 to 30 show that the serum from the infected rats was almost completely protective. Furthermore, some absorption of the antibodies was accomplished. However, it would appear that the absorption was inadequate and possibly accounts for the failure to induce any change in the electrophoretic pattern.

Sera collected on the thirty-fifth day also failed to show any difference between the pre- and post-absorbed serum patterns (Table VII). The pre-absorbed serum from the infected rats, when used for passive immunization, was also

found to contain antibodies as it afforded complete protection to the recipient rats. There was no evidence of any absorption of antibody in rats that received the absorbed-immune serum. They were also completely protected (Table IX, Figures 31 to 35). The non-absorbability of the "late" immune factor that has been shown to be present in 35-day serum would agree with Campbell's (1938b, c) results.

On the basis of these absorption experiments, no conjecture is possible with respect to the protein component(s) in which the "early" and "late" immune factors are produced. Though the "late" immune antibody is not absorbable by the method employed, it was felt that it might be possible to relate the changes that were hoped for in the absorbed 12- and 35-day sera. Thereby, definite information might have been procured on the protein-component site of the "early" immune antibody with some evidence presenting itself for the localization of the "late" immune factor.

B. Artificially Immunized Rats

Using larval worm material, it has been shown that rats could be artificially immunized (Miller, 1930, 1931c). Also, immunity thus induced was absorbable (Campbell, 1938b). Therefore, the next logical approach to the problem of ascertaining in which serum protein fraction the "early" immune factor is produced would be to follow the serum changes in such immunized rats.

TABLE VII

ELECTROPHORETIC ANALYSIS OF EXPERIMENT III

GROUP	Rat No.	Age (Days) Bled	Days Since Infected	Total Protein (Gm.%)	Electrophoresis (Gm.%)						
					Albumin	Total Globulin	Globulins				A/G
							Alpha 1-	Alpha 2-	Beta	Gamma	
Control	30, 31	60	-	5.37	2.96	2.41	.81	.56	.68	.36	1.23
	33	64	-	5.67	2.92	2.75	.96	.55	.86	.38	1.06
	34-311	68	-	5.99	3.00	2.99	1.25	.49	.89	.36	1.00* .92**
	313-321	91	-	5.52	2.77	2.75	1.01	.56	.81	.37	1.01* .91**
			Average	5.64	2.91	2.73	1.01	.54	.81	.37	1.08***
Infected	322,323	60	4	5.7	3.03	2.67	.83	.57	.79	.48	1.13
	324,325	64	8	5.37	2.33	3.04	.93	.69	1.14	.28	.77
	326-334	68	12	5.89	2.74	3.15	1.02	.49	1.02	.62	.87* .86**
	335-344	91	35	5.67	2.53	3.14	.95	.60	1.12	.47	.81* .75**
			Average	5.66	2.66	3.00	.93	.59	1.02	.46	.90***
	Level of Significance			None	None	Exceeds 5%	None	None	5%	None	Exceeds 5%

* - Before absorption
** - After absorption
*** - A/G ratios after absorption not used to calculate average

TABLE VIII

LARVAE IN LIVERS OF RATS BLED FOR SERUM ANALYSIS OF EXPERIMENT III

Days Since Infected	Rat No.	Cysts			
		Total		Average	
		Living	Dead	Living	Dead
4	322	*			
	323				
8	324	70	**	66	**
	325	62			
12	326	160	10		
	327	111	4		
	328	119	2		
	329	104	2	119.3	5.0
	330	198	10		
	331	95	4		
	332	98	6		
	333	89	2		
	334	100	5		
35	335	220	3		
	336	154	4		
	337	133	12		
	338	91	2		
	339	87	7	110.3	5.6
	340	58	5		
	341	60	2		
	342	108	10		
	343	107	3		
	344	85	8		
Average		110.0	5.3		

* Not yet grossly visible
** Not distinguishable from living

PLATE VII

Serum electrophoretic patterns of rats bled on fourth day of experiment III.

Fig. 18. Control rats

Fig. 19. Infected rats

PLATE VII

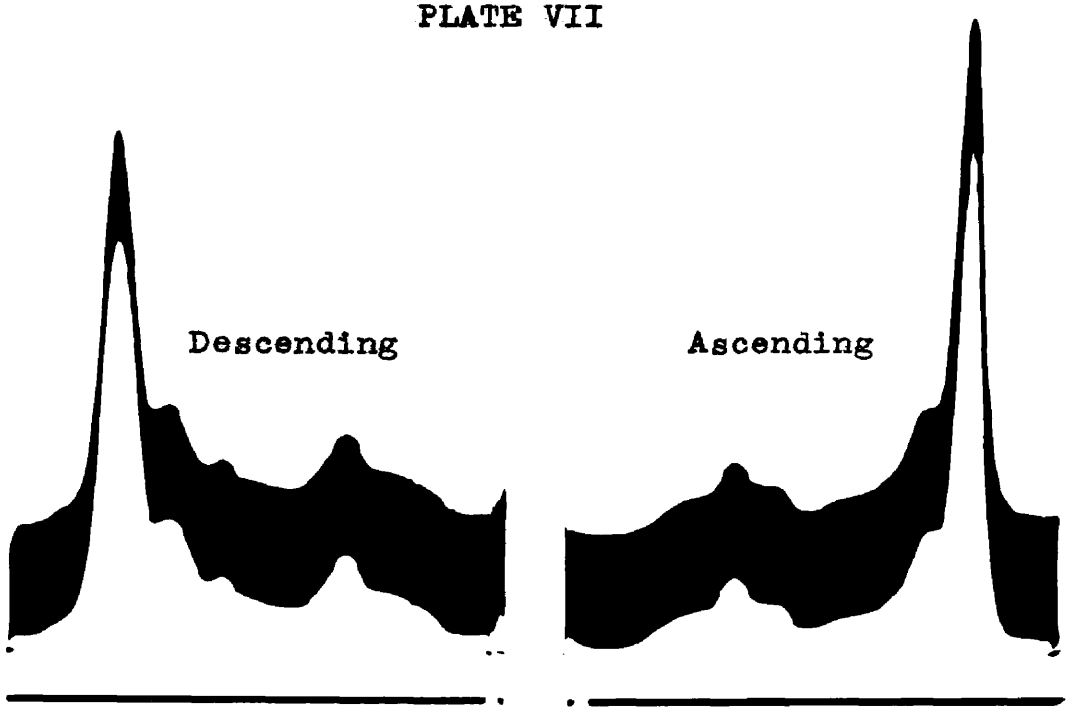

Figure 18

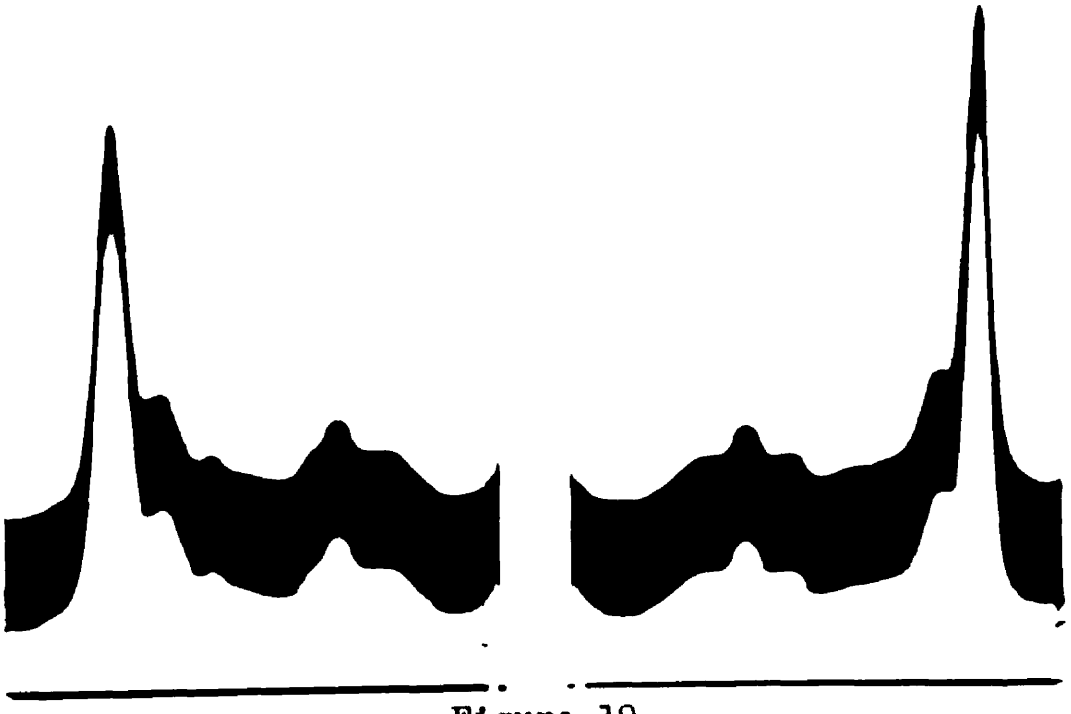

Figure 19

PLATE VIII

Serum electrophoretic patterns of rats bled on eighth day of experiment III.

Fig. 20. Control rats

Fig. 21. Infected rats

PLATE VIII

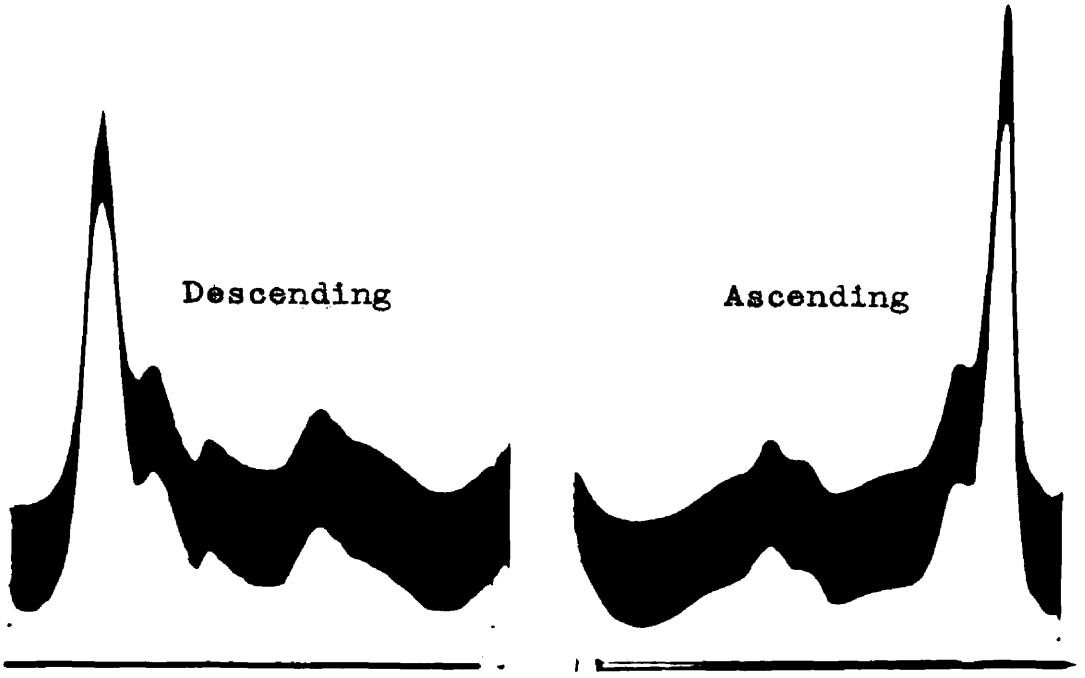

Figure 20

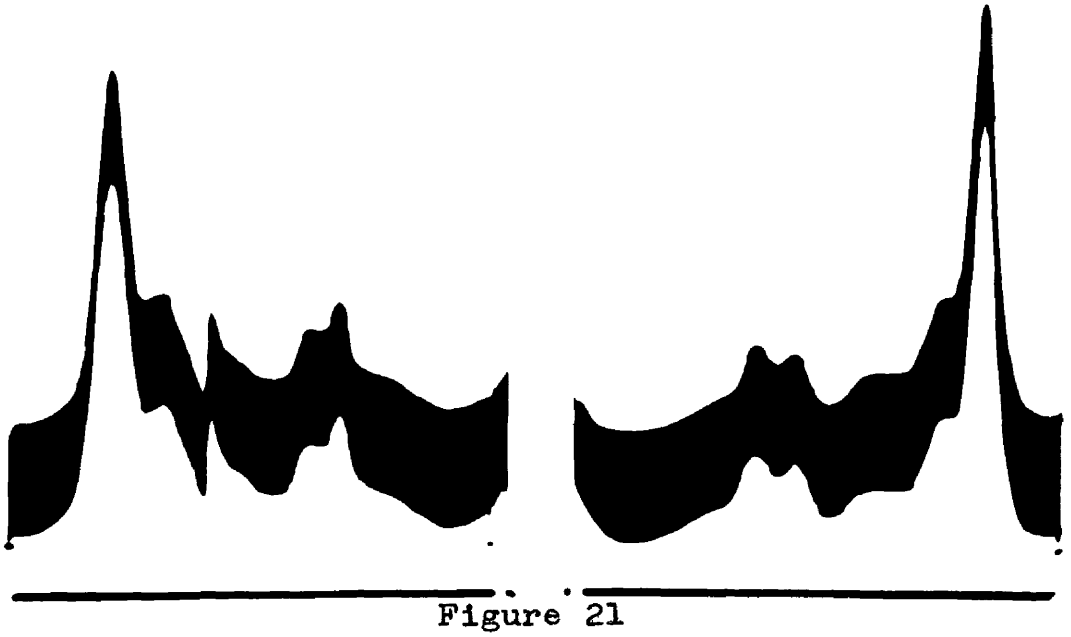

Figure 21

PLATE IX

Serum electrophoretic patterns of rats bled on twelfth day of experiment III.

Fig. 22. Control rats

Fig. 23. Infected rats

PLATE IX

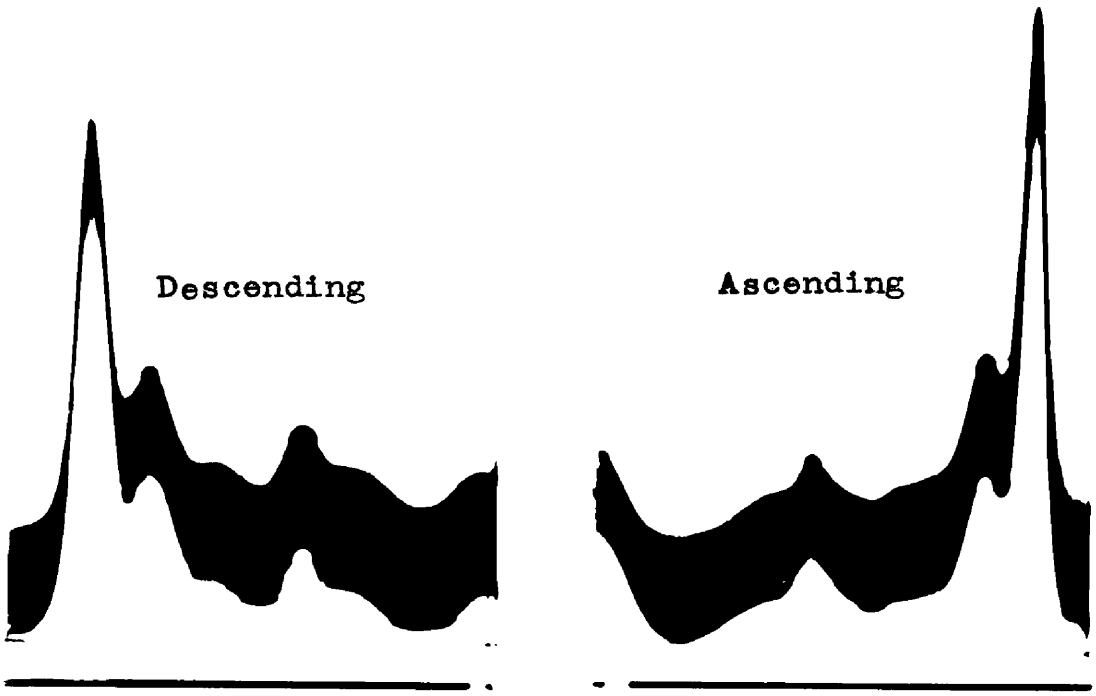

Figure 22

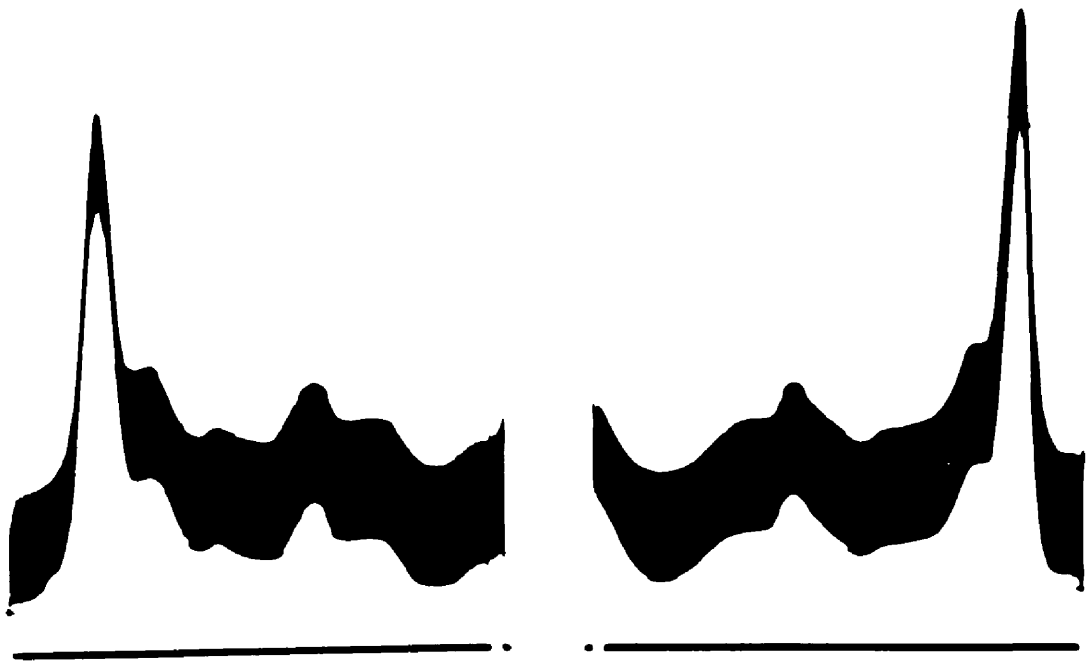

Figure 23

PLATE X

Serum electrophoretic patterns of rats bled on thirty-fifth day of experiment III.

Fig. 24. Control rats

Fig. 25. Infected rats

PLATE X

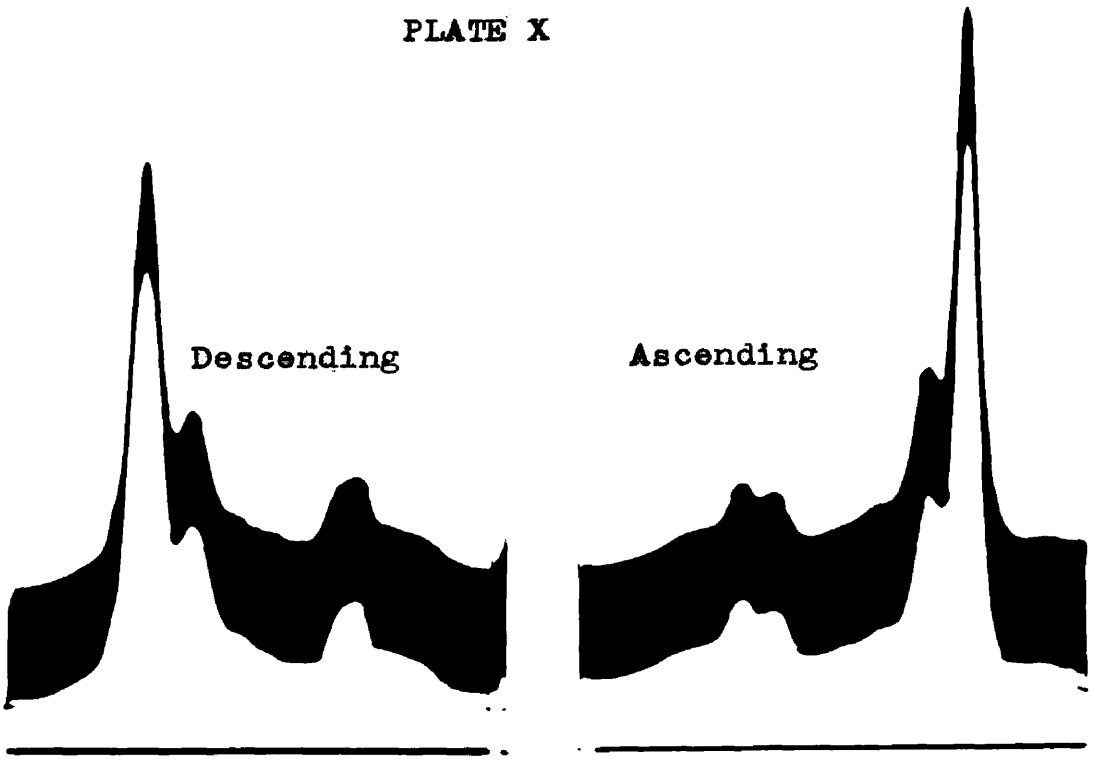

Figure 24

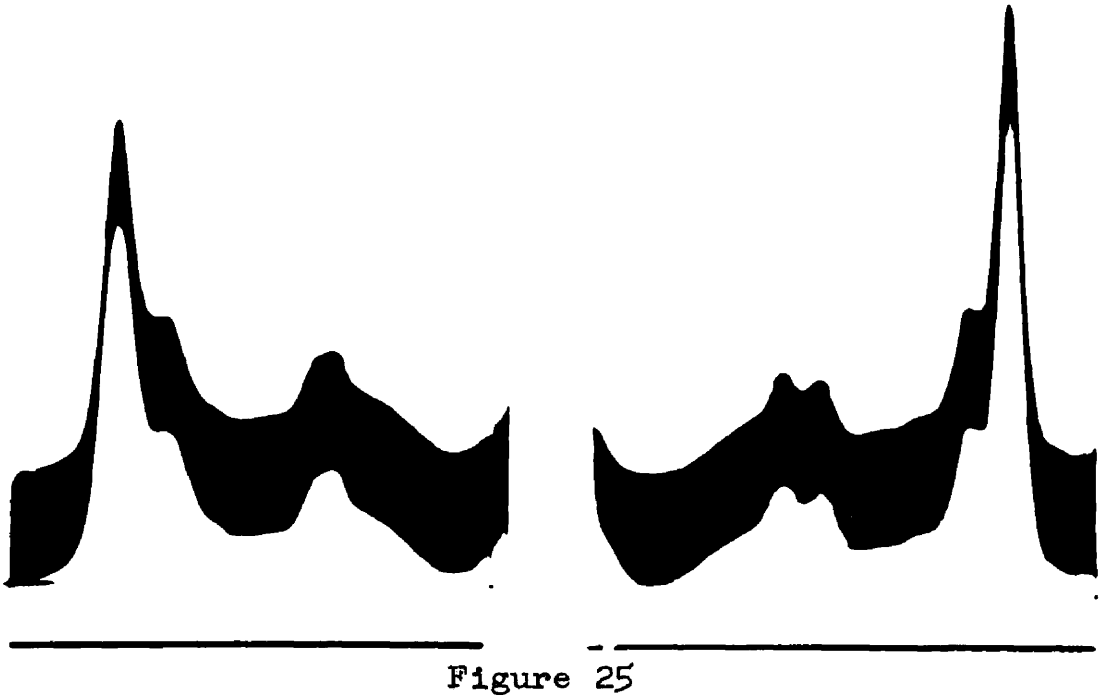

Figure 25

TABLE IX

LARVAE IN LIVERS OF RATS PASSIVELY IMMUNIZED WITH SERUM FROM RATS BLED ON TWELFTH AND THIRTY-FIFTH DAYS OF EXPERIMENT III

RAT NO.	Injected with Serum from Rats								No Serum	
	Infected		Infected and Absorbed		Non-infected		Non-infected and Absorbed			
	Cysts		Cysts		Cysts		Cysts		Cysts	
	Living	Dead	Living	Dead	Living	Dead	Living	Dead	Living	Dead
GROUP RECEIVING TWELFTH-DAY SERA:										
1	0	13	0	5	147	26	244	26	390	27
2	0	0	0	82	189	22	31	45	213	54
3	0	20	0	50	177	12	159	28	152	59
Average	0	11.0	0	45.7	171	20	144.7	33	251.7	46.7
GROUP RECEIVING THIRTY-FIFTH DAY SERA:										
1	0	0	0	0	15	36	1	32	42	224
2	0	0	0	0	0	23	0	17	1	50
3	0	0	0	0	12	56	2	93	5	105
4	0	0	0	0	0	10	0	30	0	17
Average	0	0	0	0	6.8	31.3	0.75	43	12	99

PLATE XI

Livers from rats passively immunized with sera from rats bled on twelfth day of experiment III.

Fig. 26. Liver of rat (No. 2) that received non-absorbed serum from infected rats.

Fig. 27. Liver of rat (No. 2) that received absorbed serum from infected rats.

Fig. 28. Liver of rat (No. 2) that received non-absorbed serum from non-infected rats.

Fig. 29. Liver of rat (No. 3) that received absorbed serum from non-infected rats.

Fig. 30. Liver of rat (No. 1) that received no serum.

Figure 26

Figure 27

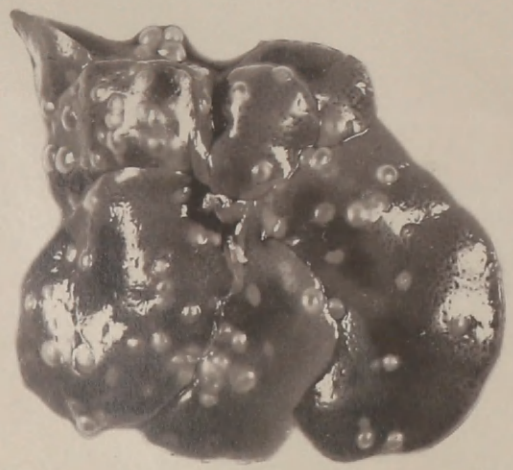

Figure 28

Figure 29

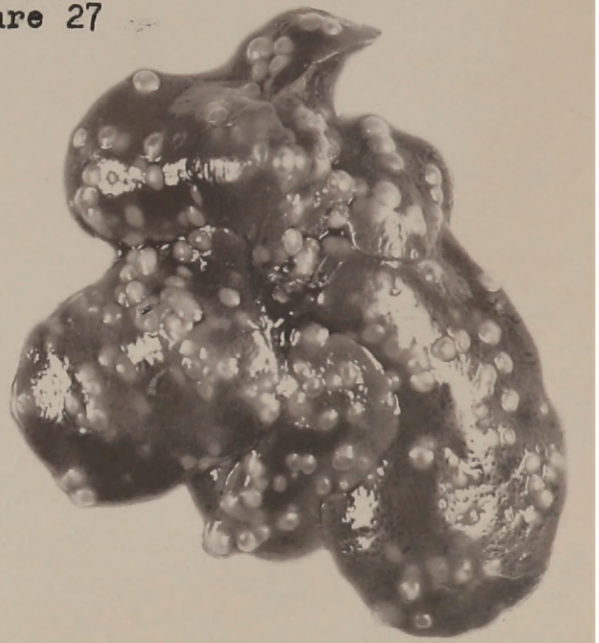

Figure 30

PLATE XI

PLATE XII

Livers from rats passively immunized with sera from rats bled on thirty-fifth day of experiment III.

Fig. 31. Liver of rat (No. 1) that received non-absorbed serum from infected rats.

Fig. 32. Liver of rat (No. 1) that received absorbed serum from infected rats.

Fig. 33. Liver of rat (No. 3) that received non-absorbed serum from non-infected rats.

Fig. 34. Liver of rat (No. 3) that received absorbed serum from non-infected rats.

Fig. 35. Liver of rat (No. 1) that received no serum.

Figure 31

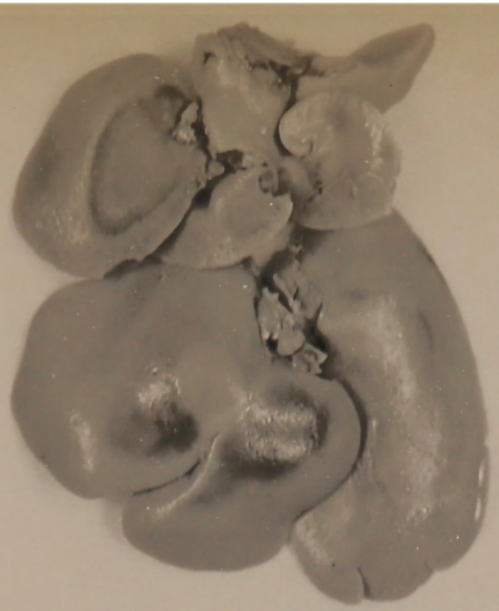

Figure 32

Figure 33

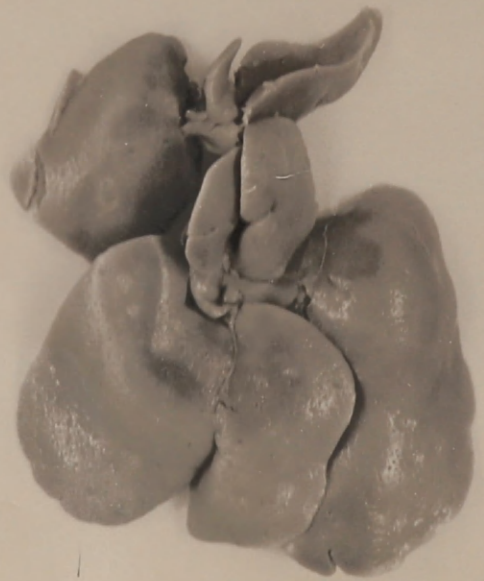

Figure 34

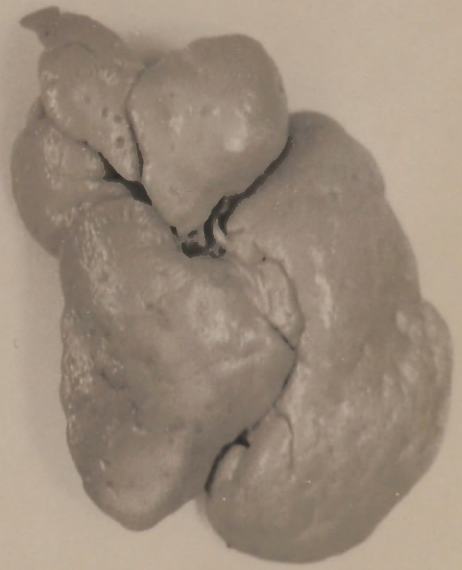

Figure 35

PLATE XII

Experiment IV was the first such attempt. No significant variations were obtained in the electrophoretic analyses carried out in this experiment. Table X presents the results of the electrophoretic determinations.

Although serum protein changes were not noted, the immunization was successful. Immunized rats were highly protected against an infection with Taenia taeniaeformis eggs as indicated in Table XI and Figures 36 to 39.

In experiment V, again no significant differences in the electrophoretic components were obtained (Table XII). The A/G ratio showed a tendency to be slightly reversed for the 8-, 12-, and 16-day sera. A fairly definite reversal appeared again in the 28-day serum which was obtained four days following the final injection of a 20 percent worm suspension. Also of interest was the fairly abrupt increase in the gamma-globulin of the 28-day serum. Figures 40 to 45 are serum electrophoretic patterns showing these moderate differences. Figures 40 and 41 are the patterns of 4-day serum showing virtually no differences exist between the immunized and control animals. The slight reversal in the A/G ratio of 16- and 28-day sera are indicated in Figures 42 to 45 and the slight increase in gamma-globulin is shown in Figures 44 and 45.

Electrophoretic patterns of the 28-day sera taken before and after absorption showed no significant variation in any of the serum components. This is indicated in the A/G ratios

given in Table XII for the 28-day sera. Table XIII and Figures 46-50 demonstrate that the immune serum exerted some protective capacity and that the immunization had induced the formation of protective antibodies. Also, absorption had been moderately successful though no changes were obtained between the pre- and post-absorbed sera patterns. This may also be a reflection of inadequate absorption.

TABLE X

ELECTROPHORETIC ANALYSIS OF EXPERIMENT IV

Rat No.	Age (Days) Bled	Days Since First Injection	Number of Injections Received	Total Protein (Gm%)	Electrophoresis (Gm.%)						
					Albumin	Total Globulin	Globulins				A/G
							Alpha 1-	Alpha 2-	Beta	Gamma	
CONTROL GROUP:											
41,42,43	56	-	-	6.02	3.34	2.68	.85	.58	.97	.28	1.25
44,45,46	63	-	-	6.35	3.21	3.14	.81	.78	1.03	.52	1.02
47,48,49	70	-	-	5.90	3.30	2.60	.86	.57	.84	.33	1.27
410,411, 412	77	-	-	5.72	3.11	2.61	.84	.56	.83	.38	1.19
414	84	-	-	5.72	3.24	2.48	.81	.51	.74	.42	1.31
415,416	91	-	-	5.65	3.03	2.62	.94	.49	.80	.39	1.16
			Average	5.89	3.21	2.69	.85	.58	.87	.39	1.20
IMMUNIZED GROUP:											
418,419	56	7	3	6.57	3.80	2.77	1.01	.57	.96	.23	1.36
421	63	14	6	5.80	2.85	2.95	.78	.87	.80	.50	0.97
423,424, 425	70	21	7	6.25	3.27	2.98	.85	.61	1.09	.43	1.10
426,427	77	28	8	6.05	2.92	3.13	.85	.66	1.18	.44	0.93
428,429	84	35	8	6.36	3.40	2.96	.86	.64	.92	.54	1.15
430,431	91	42	8	5.89	3.09	2.80	.88	.58	.97	.37	1.10
			Average	6.15	3.22	2.93	.87	.66	.99	.42	1.10
Level of Significance				None	None	None	None	None	None	None	None

TABLE XI

LARVAE IN LIVERS OF ARTIFICIALLY IMMUNIZED RATS TO TEST FOR PRESENCE OF IMMUNITY IN EXPERIMENT IV

Immunized Cysts		RAT NO.	Control Cysts	
Living	Dead		Living	Dead
0	0	1	322	127
68	77	2	246	72
1	68	3	202	76
*		4	180	60
8	46	5	114	39
1	17	6	22	24
25	90	7	35	20
92	82	8	*	
Average 27.9	54.3		160.1	59.7

*Discarded - accidental infection

PLATE XIII

Livers from rats infected after artificial immunization of experiment IV.

Fig. 36. Liver of immunized rat (No. 7).

Fig. 37. Liver of immunized rat (No. 1).

Fig. 38. Liver of control rat (No. 5).

Fig. 39. Liver of control rat (No. 3).

PLATE XIII

Figure 36

Figure 37

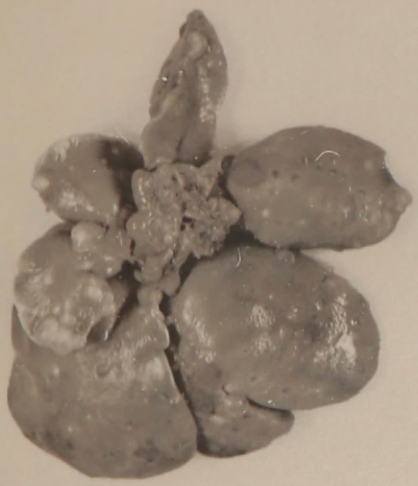

Figure 38

Figure 39

TABLE XII

ELECTROPHORETIC ANALYSIS OF EXPERIMENT V

Rat No.	Age (Days) Bled	Days Since First Injection	Number of Injections Received	Total Protein (Gm%)	Electrophoresis (Gm.%)						
					Albumin	Total Globulin	Globulins				A/G
							Alpha 1-	Alpha 2-	Beta	Gamma	
CONTROL GROUP:											
50,51,52	60	-	-	5.9	3.15	2.75	.97	.66	.75	.37	1.15
53,54,55	64	-	-	6.06	3.05	3.01	.88	.53	1.19	.41	1.01
56,57,58	68	-	-	6.29	3.26	3.03	.99	.67	1.04	.33	1.08
59,510,511	72	-	-	5.5	2.84	2.66	1.10	.46	.79	.31	1.07
512,513	76	-	-	5.77	3.00	2.77	1.06	.46	.89	.36	1.08
514,515	80	-	-	6.48	3.35	3.13	1.14	.53	.97	.49	1.07
516-524	84	-	-	6.13	3.14	2.99	1.08	.57	.91	.43	1.05* .92**
			Average	6.02	3.11	2.91	1.03	.55	.93	.39	1.07***
IMMUNIZED GROUP:											
525,526,527	60	4	2	6.21	3.38	2.83	1.00	.62	.91	.30	1.19
528,529,530	64	8	3	6.06	2.94	3.12	1.02	.64	1.08	.38	.94
531,532,533	68	12	5	6.19	3.02	3.17	1.11	.64	.94	.48	.95
534,535,536	72	16	6	5.65	2.77	2.88	1.02	.56	.87	.43	.96
537,538	76	20	6	6.10	3.16	2.94	1.12	.49	.99	.34	1.07
539,540	80	24	6	6.34	3.25	3.09	1.14	.50	.95	.50	1.05
541-550	84	28	7	5.98	2.78	3.20	1.08	.54	.97	.61	.87* .70**
			Average	6.08	3.04	3.03	1.07	.57	.96	.43	1.00***
			Level of Significance	None	None	None	None	None	None	None	None

* Before absorption

** After absorption

*** A/G ratio after absorption not used to calculate average

PLATE XIV

Serum electrophoretic patterns of rats bled on fourth day of experiment V.

Fig. 40. Control rats.

Fig. 41. Immunized rats.

PLATE XIV

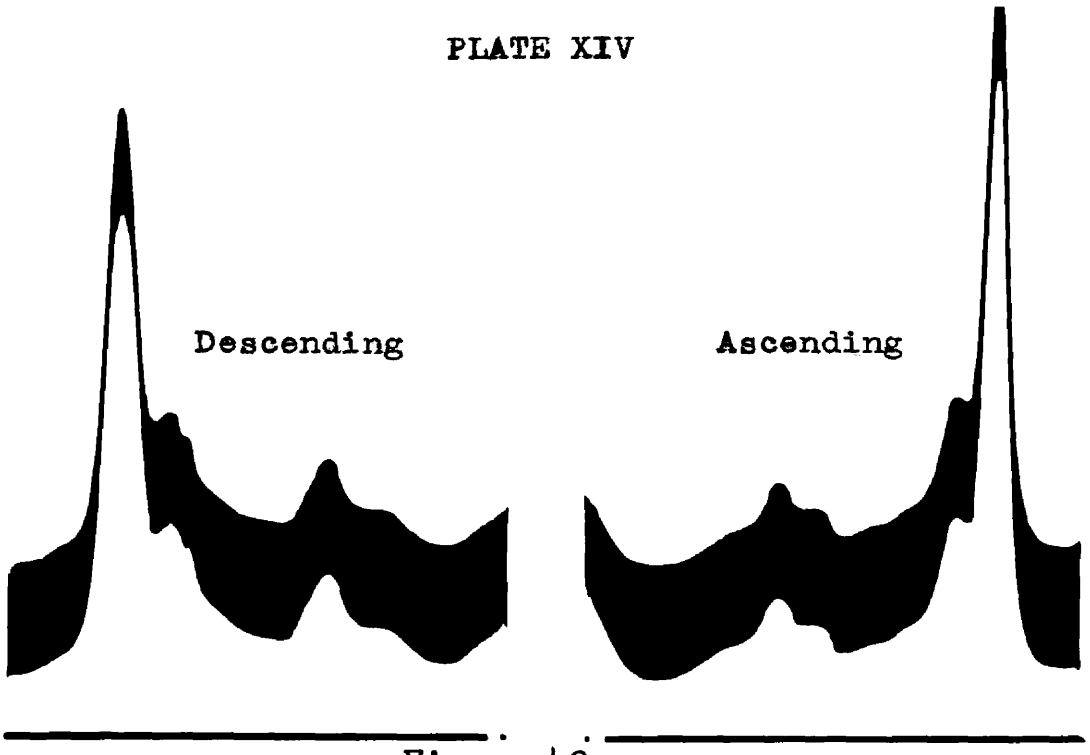

Figure 40

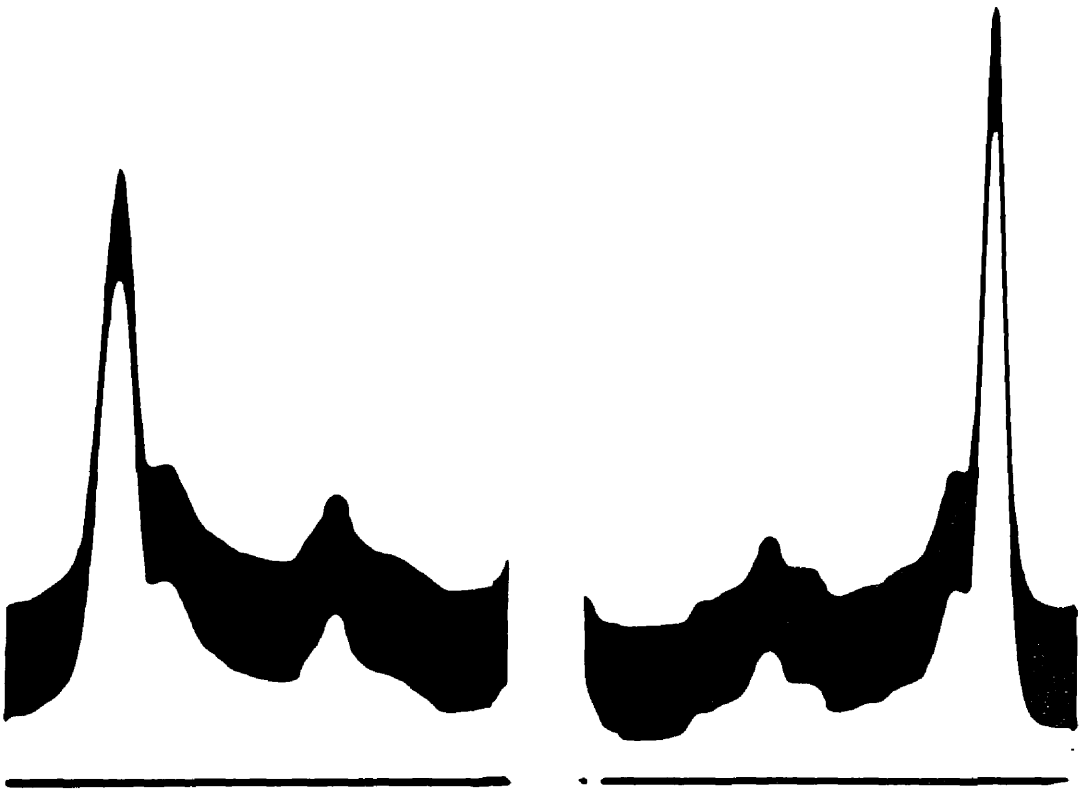

Figure 41

PLATE XV

Serum electrophoretic patterns of rats bled on sixteenth day of experiment V.

Fig. 42. Control rats.

Fig. 43. Immunized rats.

PLATE XV

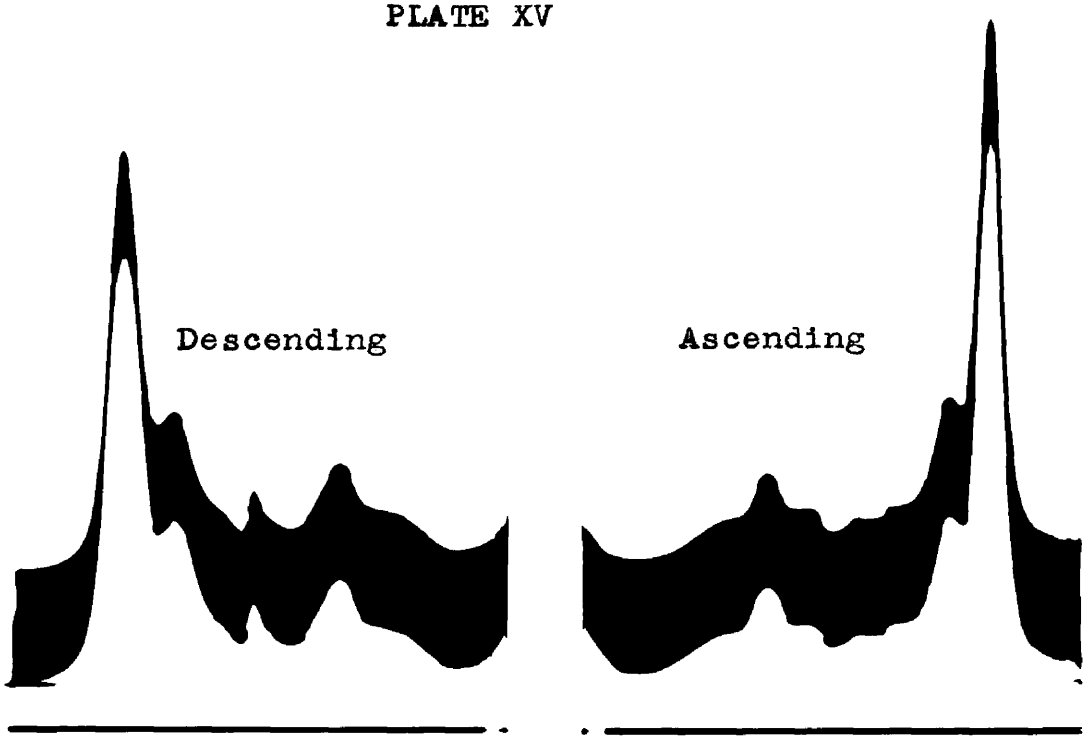

Figure 42

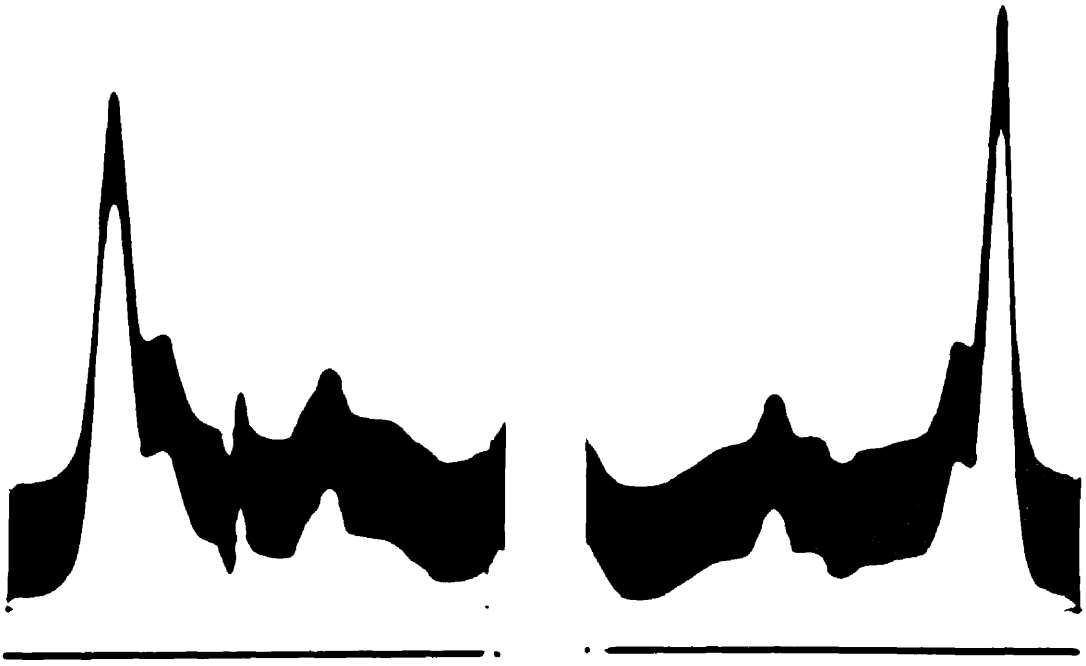

Figure 43

PLATE XVI

Serum electrophoretic patterns of rats bled on twenty-eighth day of experiment V.

Fig. 44. Control rats.

Fig. 45. Immunized rats.

PLATE XVI

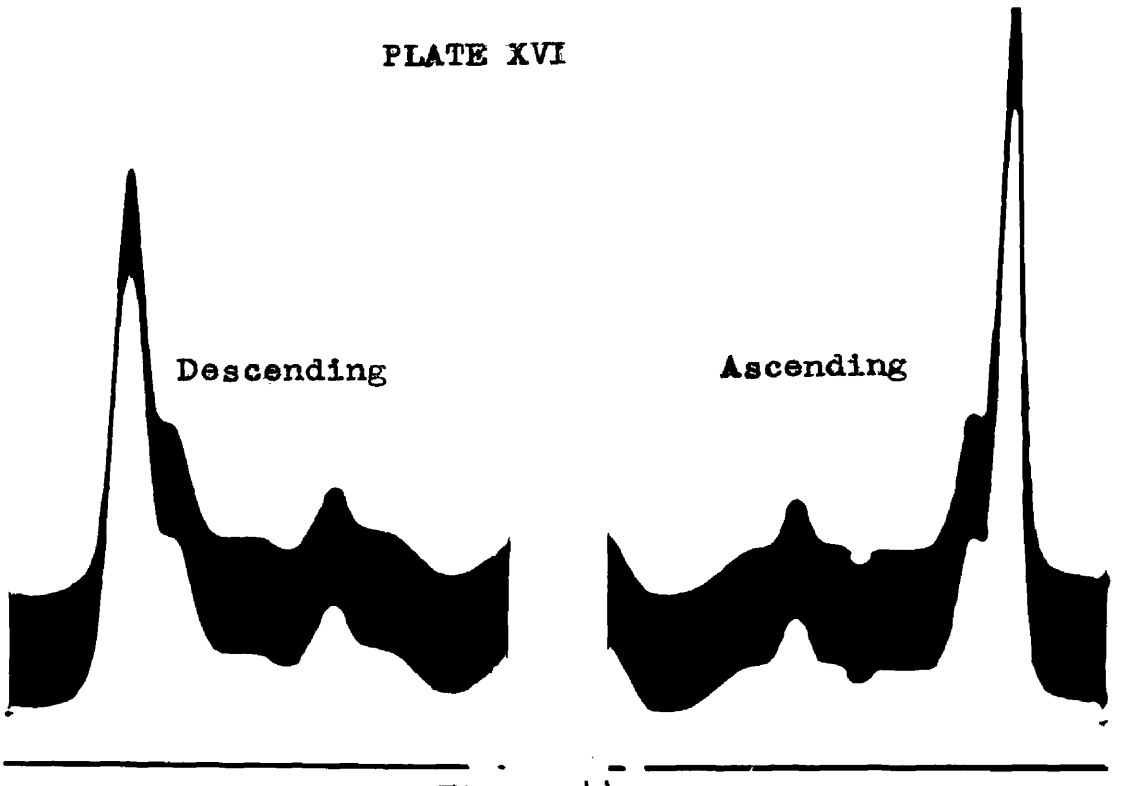

Figure 44

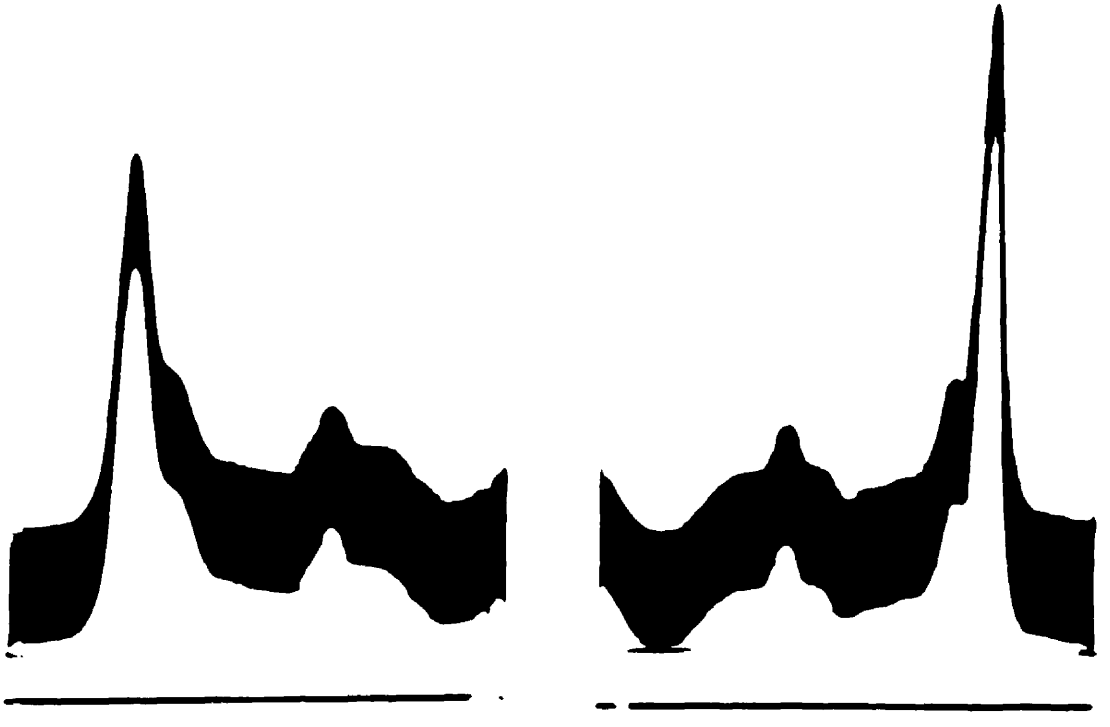

Figure 45

TABLE XIII

LARVAE IN LIVERS OF RATS PASSIVELY IMMUNIZED WITH SERUM FROM RATS BLED ON TWENTY-EIGHTH DAY OF EXPERIMENT V

RAT NO.	Injected with serum from rats								No Serum	
	Immunized and absorbed		Immunized		Non-immunized and absorbed		Non-immunized			
	Cysts		Cysts		Cysts		Cysts		Cysts	
	Living	Dead	Living	Dead	Living	Dead	Living	Dead	Living	Dead
1	33	39	6	23	49	57	6	25	6	34
2	2	29	1	28	11	48	9	60	10	37
3	10	52	0	2	15	17	30	43	40	75
4	0	20	1	15	2	14	45	69	20	88
Average	11.3	35.0	2.0	17.0	19.3	34.0	22.5	49.3	19.0	58.5

PLATE XVII

Livers from rats passively immunized with sera from rats bled on twenty-eighth day of experiment V.

Fig. 46. Liver of rat (No. 3) that received absorbed serum from immunized rats.

Fig. 47. Liver of rat (No. 4) that received non-absorbed serum from immunized rats.

Fig. 48. Liver of rat (No. 1) that received absorbed serum from control rats.

Fig. 49. Liver of rat (No. 3) that received non-absorbed serum from control rats.

Fig. 50. Liver of rat (No. 4) that received no serum.

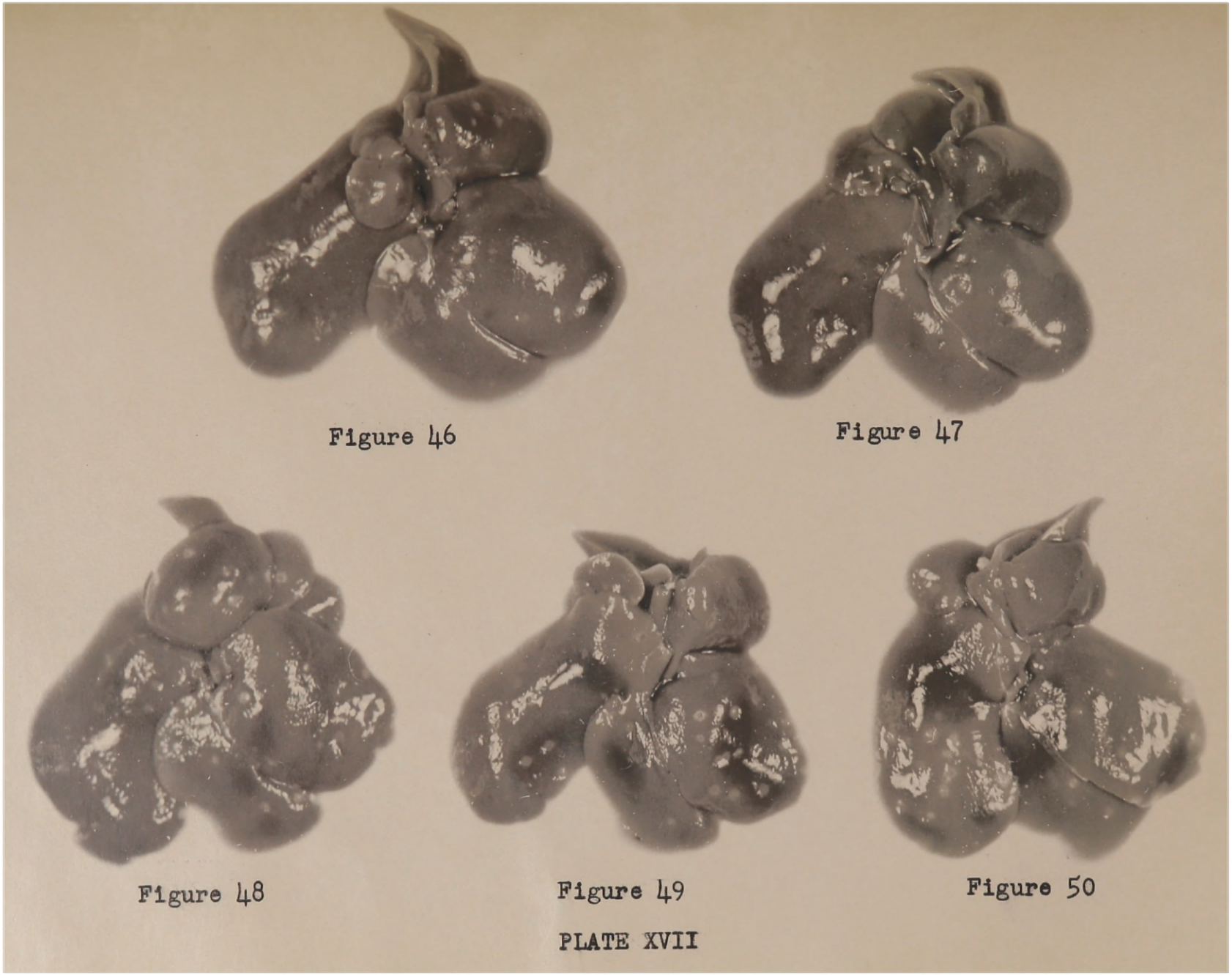

Figure 46

Figure 47

Figure 48

Figure 49

Figure 50

PLATE XVII

DISCUSSION

A. Artificially Infected Rats

From the results obtained with the rats infected with Cysticercus fasciolaris, one cannot ascribe any specificity to the serum protein changes noted. If anything, the differences obtained between the normal and infected groups can be attributed to the liver involvement resulting from the presence of the larvae. Hypoalbuminemia and hyperglobulinemia are characteristic changes for many diseases including liver involvement (Gutman, 1948). Total protein is frequently unaffected in liver conditions and is a poor indicator of the serum protein changes that are occurring (Gutman, loc. cit.). The results obtained in this study bear this out. A decrease in the A/G ratio reflects the rise in total globulins and decrease in albumin (Gray and Barron, 1943). Significant decreases in the A/G ratios of two experiments were so induced by highly significant decreases in albumin and highly significant total globulin increases.

Among the globulins, the gamma-globulin is most frequently increased in liver involvement with changes in the other globulins varying with different liver conditions (Gutman, 1948). The significant increase in gamma-globulin and highly

significant increases in the beta- and alpha 2 -globulins found in the heavily infected animals agree with what would be expected from a condition involving the liver. Also, the apparent relation of the number of larvae in the liver to the degree of serum protein changes conforms with the expectation that the severity of the condition influences the degree of abnormality (Gray and Barron, 1943).

The liver is considered to be the primary site of albumin synthesis, with globulins derived mainly from extra-hepatic locations (Madden and Whipple, 1940; Kass, 1945; Abrams and Cohen, 1949). Evidence indicates that the liver is the site of albumin production (Tumen and Bockus, 1937; Peters and Anfinsen, 1950) and liver parenchyma damage results in an impairment of albumin synthesis which is reflected in the decreased serum albumin (Martin, 1946, 1949; Franklin et al., 1951). Studies on rats have provided results suggesting that in addition to albumin, the liver is also largely responsible for alpha 1 -globulin formation. Partial hepatectomy resulted in decreases in the albumin and alpha 1 -globulin (Roberts and White, 1949).

Explanation of the hyperglobulinemia as being a compensatory response to the decreased albumin for maintenance of the osmotic pressure of the blood is highly questionable (Gutman, 1948; Franklin et al., 1951). Evidence suggests that liver damage in rats is associated with the decreased albumin and increased gamma-globulin while the alpha- and

beta-globulin increases reflect proliferative activity (Lamirande, 1952). Among the important factors listed by Popper *et al.* (1951) as responsible for the serum protein changes in liver diseases are a) liver cell damage, and b) the mesenchymal reaction. Changes in the albumin and alpha-globulin are attributed to the former and gamma-globulin differences are considered to reflect the latter. The increased gamma-globulin may be a response of the liver mesenchymal cells to breakdown products released during liver damage (Popper *et al.*, *loc. cit.*; Franklin *et al.*, 1951). Alpha 2 -globulin increase is possibly a non-specific response to tissue destruction (Shedlovsky and Scudder, 1942; Seibert *et al.*, 1947).

From the above, the serum protein changes noted in the heavily infected rats appears correlated with the liver tissue reaction to the infection described by Bullock and Curtis (1924). For approximately 8-10 days following infection, liver cell degeneration is the most pronounced change. The highly significant decrease in albumin found on the eighth day would indicate impaired liver function. On the twelfth and sixteenth days albumin was near or at the level of the control rats and coincided with the time liver cells appeared to be rapidly recovering. Persistence of the albumin, thereafter, below the normal level probably reflected the effects of growth in size of the larvae and their cyst wall. Though not significant, possibly the lowered alpha 1 -globulin

in the infected animals may also be related to the liver damage.

The degenerative phase is then followed by a proliferative stage involving mesenchymal liver cells and starts at about 8-10 days after infection. By the twelfth day the proliferative stage is well underway and liver cells also show active division. The marked rise found in the beta-globulin on the eighth day corresponded to the beginning of the proliferative stage. Maintenance of an elevated beta-globulin as well as the alpha 2 -globulin throughout the period studied probably is the result of the proliferative activity in the liver. Alpha 2 -globulin increase may also be in part a nonspecific response to liver cell damage since on the fourth day (during degenerative phase) it was the only globulin showing a definite increase. Gamma-globulin showed its most pronounced increase on the twelfth day at which time proliferative activity is well underway. As mesenchymal cell activity is prominent at this time, the increased gamma-globulin possibly is produced by those cells. It may merely manifest the enhanced activity of the mesenchymal tissue or be a response on their part to the products of liver cell degeneration that accumulated during the first week.

Proliferative activity begins to subside on about the twentieth day after infection, but never entirely ceases. The abrupt changes in serum protein components occurred between the fourth and sixteenth days after infection. This

coincides in general with that period following infection during which liver tissue involvement is most pronounced with the resultant marked disturbance in protein metabolism. After the sixteenth day, the concentration differences in the serum protein components between infected and control animals was relatively stable. As this occurred at approximately the time liver tissue activity began to subside, it may be that the protein metabolism had adjusted to a steady level of activity.

Since the marked increase in gamma-globulin on the twelfth day coincides with the time of appearance of the "early" immune factor that is absorbable (Campbell, 1938b), one might be predisposed to place the antibody in this fraction. However, the antibody could by a similar argument be attributed to the beta-globulin as it showed a pronounced increment on the eighth day. Antibody production is not confined to any one protein component (Enders, 1944). The sera from the rats collected on the twelfth day did have antibodies. Also, antibodies were present in sera of infected rats on the thirty-fifth day. Yet, absorption with larval worm material failed to reveal any changes in the sera-protein patterns. Even the lightly infected animals of the first experiment, where no changes occurred in any of serum proteins, had antibodies present on the thirty-fifth day after infection. The results showed that besides the quantitative changes in the serum proteins, qualitative changes also

occurred. However, the data do not permit ascribing any of the serum protein component changes to any specific response. All that could be reasonably inferred is that the changes are attributable to non-specific results engendered in the liver involvement. Other studies dealing with animal parasite infections have also resulted in changes not specifically attributable to the parasite, but rather to the result of a non-specific response (Dole et al., 1945; Berschn and Lurie, 1953; Leland, 1953; Olberg, 1955).

B. Artificially Immunized Rats

It had been hoped that by using worm material for immunizing rats, the protein component containing the antibody to the factor considered responsible for the "early immunity" would be found. The results obtained showed that antibody production was induced. Yet, even use of the massive injection of a 10 percent suspension of larval material failed to induce any significant increments in the protein components.

Though quantitative changes did not occur, qualitative changes were effected. Still, absorption of the anti-serum had no effect on the serum patterns.

All considered, several facts may explain these results. Rats, to begin with, are not good antibody producers. Also, the absorption methods used were empirical (Campbell, 1938b).

This serves to emphasize the need in parasitic immunology of more exact quantitative methods for the study of antigen-antibody reactions. The results further lead to the conjecture that antibody production is quantitatively small in the rat and beyond the resolving capacity of the electrophoretic apparatus used. Hence, antibody produced by infection and artificial immunization was insufficient in amount to be discerned by the apparatus. Similarly, evidence that some absorption of antibody occurred was obtained but in an amount too small to be detected by the method used.

In the artificially infected rats, there was present an added difficulty. The protein changes obtained are typical of that for a number of conditions wherein liver involvement is found. Thereby, these changes superimposed upon the antibody response would serve to obscure the latter.

The methods used in this study far from exhaust all the approaches to the problem of specifically locating the protein component in which the antibodies against this parasite are produced. Further studies employing different types of worm preparations, different immunizing procedures, and cyst fluid material are some possible avenues leading to the clarification of the problem. Application of the electrophoresis-convection technique might be most fruitful (Cann and Kirkwood, 1949). Use of cyst fluid material for immunization studies may throw direct light on the "late" immune factor.

Perhaps the most drastic need is that of quantitative methods for studying antigen-antibody reactions in animal parasite immunology. Precise quantitative methods have been developed for measuring antigens and antibodies (Kabat, 1943; Kabat and Mayer, 1948). These immunochemical techniques have been successfully applied to studies in bacterial immunology as well as protein and polysaccharide chemistry. Such methods allow for the accurate quantitative determination of interacting antigens and antibodies. The relation of chemical structure to immunological reactivity, determination of proportions of antibody to antigen yielding the maximal antibody precipitation and calculation of the amount of antibody in an antiserum are some of the things emanating from these methods. Such information in conjunction with data obtainable by electrophoresis would certainly advance the field of parasite immunology.

SUMMARY AND CONCLUSIONS

Sera from rats experimentally infected with the larval cestode, Cysticercus fasciolaris, were analyzed electrophoretically for various days following infection. Also, rats were immunized artificially with larval worm material and their sera analyzed by electrophoresis at different intervals following the initial injection. This is believed to be the first application of electrophoresis to a cestode infection.

The following results were obtained from the studies on the experimentally infected rats:

1. In lightly infected rats having about 38 cysts as their highest average total number (living plus dead), no significant quantitative differences were observed between infected and non-infected rats in any of the serum protein components. A qualitative change was evidenced by the ability of the lightly infected rats to resist a second infection.

2. Heavily infected rats having a total number of cysts averaging 66 or more showed significant quantitative changes among the serum protein components. Qualitative changes in the serum proteins were also evident among this group as use of their sera for passive immunization rendered the recipient rats resistant to an infection. These general

results obtained in the lightly and heavily infected animals lead to the expected and obvious conclusion that quantitative changes in serum protein metabolism are related to the degree of infection.

3. Specific changes in serum protein components noted in the heavily infected rats were significant increases in the total, alpha 2-, beta-, and gamma-globulins. Significant decreases in the albumin and A/G ratio were obtained. The total protein and alpha 1 -globulin showed no significant differences.

4. As related to time following infection, the following were noted in the heavily infected rats:

a. Four days after infection, the only apparent difference in serum protein components between infected and control rats was an increased alpha 2 -globulin in the former.

b. On the eighth day, the albumin showed a sharp decrease and beta-globulin a marked increase.

c. Twelve days after infection, the albumin had risen, beta-globulin dropped sharply, gamma-globulin showed a marked increase and dropped to a lower level on the sixteenth day.

d. From the sixteenth day after infection to the last day of the experiment (thirty-fifth), the quantitative differences between the control and infected rats maintained themselves at a fairly constant level.

5. In an effort to determine in which serum protein fraction(s) the antibodies against the parasite were produced, sera obtained 12 and 35 days after infection were absorbed with larval worm material. Electrophoretic patterns of the sera made before and after absorption revealed no significant differences. Evidence of some absorption of antibodies from the 12-day sera was demonstrated in passive immunization studies.

The following conclusions were drawn from the results secured on the infected rats:

1. None of the serum protein changes can be specifically attributed to the parasite.

2. The serum protein changes are typical of those resulting from liver tissue involvement, induced in this case by the parasite infection.

3. It appears that the protein changes were correlated with the liver tissue response to the infection.

4. For a period of about two weeks, between the fourth and sixteenth days after infection, serum protein metabolism appeared to be most drastically altered due to the liver involvement.

5. From the sixteenth day following infection throughout the remainder of the experimental period (to the thirty-fifth day after infection) the protein metabolism seemed to have adjusted to a stable level of activity.

6. Inadequate absorption and/or the limitations of the electrophoresis resolving capacity may explain the failure to note changes in the absorbed sera patterns.

From the studies on the animals artificially immunized with larval worm material, the following resulted:

1. Significant changes in the serum proteins were not obtained.

2. Antibody production was effected as evidenced by the in vivo studies. Hence, though quantitative changes were not noted, qualitative changes had occurred in the serum proteins.

3. Antisera absorbed with homologous antigen revealed no reduction in any serum protein component when pre- and post-absorbed sera electrophoretic patterns were analyzed.

4. Some absorption was effected as the in vivo studies showed.

It was concluded on the basis of the results obtained with the artificially immunized rats that:

1. The antibody response produced may have been quantitatively insufficient to be detected by the electrophoresis apparatus.

2. Similarly, absorption was in an amount too small to be detected by the method employed.

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AN ELECTROPHORETIC STUDY OF SERA FROM RATS ARTIFICIALLY INFECTED WITH AND IMMUNIZED AGAINST THE LARVAL CESTODE, *CYSTICERCUS FASCIOLARIS*

By

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AN ABSTRACT

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This study was undertaken to: (1) ascertain the effects of a larval cestode, Cysticercus fasciolaris, infection on rats as manifested by serum protein metabolism, and (2) to attempt to relate the humoral factors produced against this parasite to the serum proteins.

Serum protein analyses were made by the moving boundary electrophoresis method. Rats were experimentally infected with the larval cestode and their sera analyzed electrophoretically on various days following infection. A similar procedure was followed for rats artificially immunized against the parasite by a series of intraperitoneal injections of larval worm material.

The following results were obtained from the studies on the experimentally infected rats:

1. In lightly infected rats having about 38 cysts as their highest average total number (living plus dead), no significant quantitative differences were obtained between infected and non-infected rats in any of the serum protein components. A qualitative change was evidenced by the ability of the lightly infected rats to resist a second infection.

2. Heavily infected rats having a total number of cysts averaging 66 or more showed significant quantitative changes among the serum protein components. Qualitative changes in the serum proteins were also evident among this

group as use of their sera for passive immunization rendered the recipient rats resistant to an infection. These general results obtained in the lightly and heavily infected animals leads to the expected and obvious conclusion that quantitative changes in serum protein metabolism are related to the degree of infection.

3. Specific changes in serum protein components obtained in the heavily infected rats were significant increases in the total, alpha 2-, beta-, and gamma-globulins. Significant decreases for the albumin and A/G ratio were obtained. The total protein and alpha 1 -globulin showed no significant differences.

4. As related to time following infection, the following were noted in the heavily infected rats:

a) Four days after infection, the only apparent difference in serum protein components between infected and control rats was an increased alpha 2 -globulin in the former.

b) On the eighth day, the albumin showed a sharp decrease and beta-globulin a marked increase.

c) Twelve days after infection, the albumin had risen, beta-globulin dropped sharply, gamma-globulin showed a marked increase and dropped to a lower level on the sixteenth day.

d) From the sixteenth day after infection to the last day of the experiment (thirty-fifth), the quantitative

differences between the control and infected rats maintained themselves at a fairly constant level.

5. In an effort to determine in which serum protein fraction(s) the antibodies against the parasite were produced, sera obtained 12- and 35- days after infection were absorbed with larval worm material. Electrophoretic patterns of the sera made before and after absorption revealed no significant differences. Evidence of some absorption of antibodies from the 12-day sera was obtained in passive immunization studies.

The following conclusions were drawn from the results secured on the infected rats:

1. None of the serum protein changes can be specifically attributed to the parasite.

2. The serum protein changes are typical of that resulting from liver tissue involvement, induced in this case by the parasite infection.

3. It appears that the protein changes were correlated with the liver tissue response to the infection.

4. For a period of about two weeks, between the fourth and sixteenth days after infection, serum protein metabolism appeared to be most drastically altered due to the liver involvement.

5. From the sixteenth day following infection throughout the remainder of the experimental period (to

thirty-fifth day after infection) the protein metabolism seemed to have adjusted to a stable level of activity.

From the studies on the animals artificially immunized with larval worm material, the following resulted:

1. Significant changes in the serum proteins were not obtained.

2. Antibody production was effected as evidenced by the in vivo studies. Hence, though quantitative changes were not noted, qualitative changes had occurred in the serum proteins.

3. Antisera absorbed with homologous antigen revealed no reduction in any serum protein component when pre- and post-absorbed sera electrophoretic patterns were analyzed.

4. Some absorption was effected as the in vivo studies showed.

It was concluded on the basis of the results obtained with the artificially immunized rats that:

1. The antibody response produced may have been quantitatively insufficient to be detected by the electrophoresis apparatus.

2. Similarly, absorption was in an amount too small to be detected by the method employed.