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A CARPET BEETLE LARVA (COLEOPTERA : DERMESTIDAE) FROM THE DIGESTIVE TRACT OF A WOMAN

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

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INTRODUCTION

Studies on *Cysticercus fasciolaris* infection have made significant contributions to our knowledge of immunity against parasites. Conclusive demonstrations of acquired (Miller, 1931b), artificial (Miller, 1931a) and passive (Miller and Gardiner, 1932) immunity against a metazoan parasite emanated from these efforts. Miller and his group showed: (1) infected rats were immune to a superimposed infection, (2) intraperitoneal injections of worm material induced immunity and (3) protection could be passively transferred.

Campbell (1938a, b, c) demonstrated an "early" and "late" immunity involving at least two antibody mechanisms. The former destroys the larvae prior to encystment in the liver, is present in the serum collected on the 11th day after infection and is absorbable with homologous worm material. Late immunity is evidenced following encystment, is present in serum collected 28 days after infection and is non-absorbable. The "early immunity" is probably exerted against the larval somatic antigens and the "late immunity" may involve a response against metabolic products and/or essential metabolites of the parasite.

It is likely that the basic mechanisms operative in the immune response against the animal parasites are essentially the same as those functioning against other infectious agents (Culbertson, 1951). Electrophoresis proved a valuable tool in immunological studies of other fields and has not been adequately applied to the problems of immunity to animal parasites. In Stauber's (1954) review of the applications of electrophoresis to parasitology, he has noted the failure to demonstrate conclusively in which serum protein fraction(s) antibodies against animal parasites are found. Such information would contribute much to our understanding of the relation between the basic immune response against animal parasites and other infectious agents.

The purpose of the study herein reported was twofold. First, it was desired to ascertain the effects of a *C. fasciolaris* infection in rats as reflected in the protein metabolism. As this parasite usually localizes in the liver with a resultant liver tissue involvement (Bullock and Curtis, 1924, 1926), any alterations in liver function would likely be manifested in the rat's serum protein metabolism. Secondly, an attempt was made to relate the rat's humoral response against the parasite to the

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serum proteins. By analyses of sera from infected and artificially immunized rats, it was hoped to determine in which protein fraction(s) the antibodies appeared.

MATERIALS AND METHODS

General Procedures:

Cats were used for maintaining a source of *Taenia taeniaeformis* eggs. All cats, upon arrival in the laboratory, were immunized against feline distemper with feline distemper vaccine (Pitman-Moore Company, Indianapolis, Indiana).

Rats used in an experiment were of the Wistar or Sprague-Dawley strains. Males were employed throughout because of the greater resistance of females to an infection with *Cysticercus fasciolaris* (Campbell and Melcher, 1940). The rats were kept in a room completely isolated from all other laboratory animals. Food (Miller's Eaties, Battle Creek Dog Food Company, Battle Creek, Michigan) and water were available *ad libitum.*

Several factors such as age (Heim and Schechtman, 1954), sex (Moore, 1948) and plasmapheresis (Chow *et al.,* 1948) induce significant variations in the serum electrophoretic pattern of an animal. These were obviated as much as possible when serum was acquired for electrophoretic analysis by having all littermates segregated as equally as possible into control and experimental groups. Food was withdrawn the evening prior to the day of bleeding from the animals to be bled.

Blood was obtained by maximally bleeding each rat, under sodium pentobarbital anesthesia supplemented by ether, by intracardiac puncture under aseptic conditions and then discarding the rat. After clotting at room temperature overnight, the serum was collected and clarified by centrifugation for 30 minutes at about 2,000 rpm. The clarified serum was analyzed immediately or, when necessary, stored at 4°C. for not longer than 48 hours before analysis. To insure an adequate amount of serum for testing, sera collected from 2–3 rats per experimental and control group were pooled.

Eggs used for infecting rats were obtained by sacrificing an infected cat on the day rats were to be infected. The gravid segments of the recovered worms were teased apart and the eggs recovered. Quantification of the eggs was accomplished by using a hemacytometer. Rats were infected intragastrically by introducing one ml. of the evenly dispersed egg suspension (in 0.85% sodium chloride) through a No. 8 French catheter that was orally passed into the stomach.

Whole cysticerci were used as antigen for artificial immunization. They were stored at minus 30° C., ground into a paste with a mortar and pestle and made into a 10 or 20 percent suspension in saline (0.85% sodium chloride) for use in the intraperitoneal injections of the rats being immunized. For the sera absorption studies fresh larvae and lyophilized larvae and adults were used as the homologous antigen. Campbell's (1938b) method was employed for serum absorption, adding enough antigen to the serum to make a final 2% suspension.

Immune (from infected or artificially immunized rats) and control sera for passive immunization studies were obtained by pooling the sera from 8–10 rats per group. Each rat received intraperitoneally 1 or 2 ml. of serum, depending upon amount of serum available, within 24 hours prior to or following their being infected with *Taenia taeniaeformis* eggs.

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 necropsied and any found harboring cysts from an accidental infection were discarded.

Total Protein Determinations:

Serum total protein was determined by the biuret reaction, using Weichselbaum's (1946) biuret reagent. A standard curve was calibrated using a crystalline bovine albumin solution (Armour and Co., Chicago) with a specified nitrogen content (about 10 mg. of protein nitrogen per ml.) as the standard protein solution. The standard solution was converted into grams percent of protein by multiplying the nitrogen content by the factor 6.25. Readings were made on a photelometer at 525 millimicrons.

Electrophoresis:

All sera were analyzed by the moving boundary electrophoresis technique using the Perkin-Elmer Model 38 Tiselius Electrophoresis Apparatus. Barbiturate (veronal and sodium veronal) buffer of pH 8.6 and 0.1 ionic strength was used throughout this study.

Sera were diluted with buffer 1 : 2, generally 2 ml. serum and 4 ml. buffer. Dialysis was performed by the mechanical stirring method of Reiner and Fenichel (1948) at room temperature with the diluted serum enclosed in a membrane of seamless regenerated viscose process cellulose (Visking Corp., Chicago). Dialysis of the diluted sera was effected against 400 ml. of buffer for 1 hour followed by 600 ml. of fresh buffer for 4 hours.

The 2 ml. capacity cell was used and the following average particulars employed: 7.5 milliamperes at 112 volts for 7600 seconds; potential gradient of 8.18 v/cm; bath temperature of 0.5° C.

Ascending and descending limbs were photographed by the scanning method (Longsworth, 1946). All calculations were made from two-fold enlargement tracings of the descending limb patterns. The base line was established with the aid of a scanning photograph of the cell limb taken prior to shifting the initial boundary into view (Longsworth and MacInnes, 1940). Area measurements were made by delineating each area according to the method of Tiselius and Kabat (1939); then, the total area (exclusive of the epsilon-boundary) and component areas were determined with a planimeter. By appropriate calculations, each serum protein component was obtained in terms of relative percent composition and grams percent protein. Mobilities were obtained by using the equation given by Longsworth and MacInnes (1940).

Experimental Protocols:

Experiment I. Eight-week-old Wistar strain rats were each infected with 7,000 eggs. Bleedings of infected and corresponding littermate controls were made at 4-day intervals thereafter up to 28 days. A final bleeding was made on the 35th day.

To test by the *in vivo* method the protective capacity of the sera collected on the 35th day, 13-week-old rats were separated into 3 groups with littermates distributed as equally as possible. Within 24 hours after being infected with 1,200 eggs, each rat of one group received intraperitoneally 2 ml. of normal rat serum,

the second group 2 ml. of infected rat serum, and the third group no serum. They were all autopsied 5 weeks after infection.

Experiment II. This experiment was essentially a repeat of Experiment I and was undertaken in an effort to establish in which serum protein fraction(s) the "early" and "late" immune factors are present. Eight-week-old Sprague-Dawley rats were each infected with 3,000 eggs. Sera were collected from normal and infected rats on the 4th, 8th and 12th day after infection. The 12th day sera were absorbed with fresh larval paste and reanalyzed electrophoretically.

To correlate any changes in the electrophoretic patterns with the protective capacity of the sera, the latter were tested by passive immunization. Distributing littermates as equally as possible, 5 groups of 10-week-old rats were set up as follows: 1. received no serum; 2. received normal rat serum; 3. received normal-absorbed rat serum; 4. received infected rat serum; 5. received infected-absorbed rat serum. Each rat received one ml. of serum intraperitoneally and was then infected within 24 hours with 3,000 eggs. Necropsy was performed 4 weeks after infection.

The procedure outlined for the 12 day bleeding was repeated on the 35th day. Details differed only in the use of lyophilized larvae for sera absorption, a new lot of eggs (dosage of 3,500) and rats 13 weeks and 4 days old for passive immunization.

Experiment III. In order to circumvent the changes in the serum electrophoretic patterns of infected rats induced by liver damage, this study was undertaken. Sprague-Dawley rats were artificially immunized by a series of 6 intraperitoneal injections of a 10% suspension of ground larval paste. The injections (1 ml./injection) were initiated when the rats were 8 weeks old and were given on alternate days with a rest period between the 3rd and 4th injections. A final injection of a 20% suspension was given 4 days prior to the last bleeding.

Immunized and control rats were bled at 4 day intervals following the first injection up to the 28th day. Sera collected on the 28th day were utilized for absorption studies. The procedure outlined in Experiment II was followed. Lyophilized adults were used for absorbing the sera.

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

RESULTS

Experiments I and II:

Results of the sera analyses of Experiment I are presented in Table I. All of the electrophoretic data, as expressed in terms of both grams percent protein and relative percent composition, the total proteins and A/G (Albumin: Total Globulin) ratios obtained in this study were analyzed statistically. The "t" value was determined for the mean of the differences between the control and experimental groups. "t" values at or beyond the 5% and the 1% levels of probability were considered significant and highly significant, respectively. An examination of Table I shows that the differences occurring in the total protein and alpha 1-globulin were not significant. The gamma-globulin of the infected group was significantly increased. Total globulin, alpha 2-globulin and beta-globulin of the

TABLE I. *Electrophoretic analysis, Experiment I*

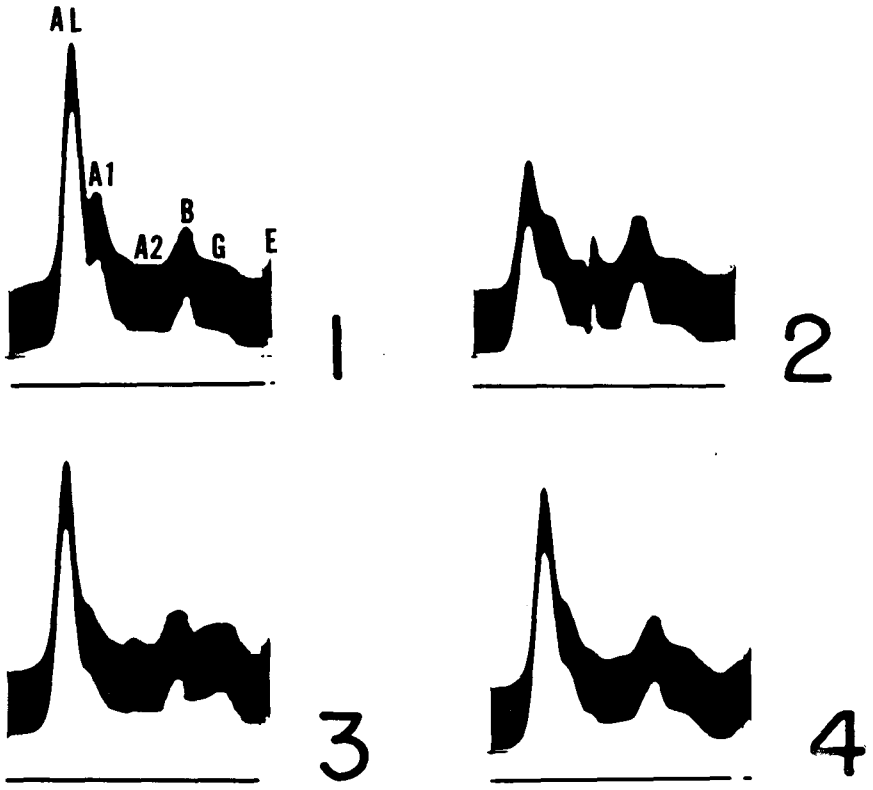
No. rats used	Age (Days) bled	Days Since Infected	Total Protein (Gm.%)	Electrophoresis (Gm. %)						A/G Ratio
				Albumin	Total Globulin	Globulins				
						Alpha 1-	Alpha 2-	Beta-	Gamma-	
Control Group:										
1	60	–	5.89	3.19	2.70	.84	.52	.78	.56	1.18
1	64	–	5.47	2.78	2.69	.87	.51	.88	.43	1.03
2	68	–	5.43	3.02	2.41	.81	.41	.76	.43	1.25
2	72	–	5.22	2.91	2.31	.84	.45	.75	.27	1.26
1	76	–	5.75	3.27	2.48	.94	.45	.77	.32	1.32
2	80	–	6.19	3.62	2.57	.71	.54	.92	.40	1.41
2	84	–	6.41	3.47	2.94	1.19	.51	.94	.30	1.18
6	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 Group:										
3	60	4	6.05	3.08	2.97	.82	.72	.88	.55	1.04
1	64	8	5.73	2.11	3.62	.92	.74	1.45	.51	.58
2	68	12	6.03	2.88	3.15	.81	.44	.90	1.00	.91
2	72	16	5.45	2.93	2.52	.60	.52	.90	.50	1.16
2	76	20	5.75	2.88	2.87	.65	.64	1.09	.49	1.00
2	80	24	6.21	3.30	2.91	.77	.63	1.02	.49	1.13
2	84	28	6.19	3.03	3.16	.87	.64	1.14	.51	.96
9	91	35	6.79	3.16	3.63	.93	.75	1.34	.61	.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 IV—*Electrophoretic analysis, Experiment III*

No. rats used	Age (Days) bled	Days since first injection	No. of injections received	Total Protein (Gm. %)	Electrophoresis (Gm. %)						A/G Ratio
					Albumin	Total Globulin	Globulins				
							Alpha 1-	Alpha 2-	Beta-	Gamma-	
Control Group:											
3	60	-	-	5.90	3.15	2.75	.97	.66	.75	.37	1.15
3	64	-	-	6.06	3.05	3.01	.88	.53	1.19	.41	1.01
3	68	-	-	6.29	3.26	3.03	.99	.67	1.04	.33	1.08
3	72	-	-	5.50	2.84	2.66	1.10	.46	.79	.31	1.07
2	76	-	-	5.77	3.00	2.77	1.06	.46	.89	.36	1.08
2	80	-	-	6.48	3.35	3.13	1.14	.53	.97	.49	1.07
9	84	-	-	6.13	3.14	2.99	1.08	.57	.91	.43	1.05
			Average	6.02	3.11	2.91	1.03	.55	.93	.39	1.07
Immunized Group:											
3	60	4	2	6.21	3.38	2.83	1.00	.62	.91	.30	1.19
3	64	8	3	6.06	2.94	3.12	1.02	.64	1.08	.38	.94
3	68	12	5	6.19	3.02	3.17	1.11	.64	.94	.48	.95
3	72	16	6	5.65	2.77	2.88	1.02	.56	.87	.43	.96
2	76	20	6	6.10	3.16	2.94	1.12	.49	.99	.34	1.07
2	80	24	6	6.34	3.25	3.09	1.14	.50	.95	.50	1.05
10	84	28	7	5.98	2.78	3.20	1.08	.54	.97	.61	.87
			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

infected animals showed significant increases; the albumin and A/G ratio had decreased significantly.

Of particular interest are the marked changes that occurred on the 8th, 12th and 16th days after infection. On the 4th day, the only noticeable change was the increased alpha 2-globulin of the infected group; alpha 2-globulin persisted at a fairly constant level above the control group throughout the experimental period (Table I). A sharp increase in the beta- and total globulins and marked decrease in the albumin and A/G ratio appeared in the infected group on the 8th day (Figures 2 and 5). By the 12th day the beta- and total globulins dropped sharply to



FIGS. 1 to 4. Sera electrophoretic patterns (descending limb) of Experiment I. 1, normal; 2, 8th day after infection; 3, 12th day after infection; 4, 35th day after infection (AL, albumin; A1, alpha 1-globulin; A2, alpha 2-globulin; B, beta-globulin; G, gamma-globulin; E, epsilon-boundary.

levels still above those of the controls while the albumin and A/G ratio rose markedly to values still below those of the uninfected rats. The albumin was near the control value on the 12th day, had essentially the same value as the controls on the 16th day and thereafter maintained itself below the control albumin level (Figure 5). A sharp rise in the gamma-globulin of the infected rats was seen on the 12th day and abruptly returned to a lower level above that of the controls on the 16th day (Figures 3 and 5).

From Table I and Figure 5 it can be seen that the differences between the significantly altered serum proteins of the control and infected rats from the 16th day on were relatively constant and are still apparent in the serum pattern of the

35th day (Figure 4). It appeared that the protein metabolism was most drastically affected for approximately two weeks (between the 4th and 16th days) after infection.

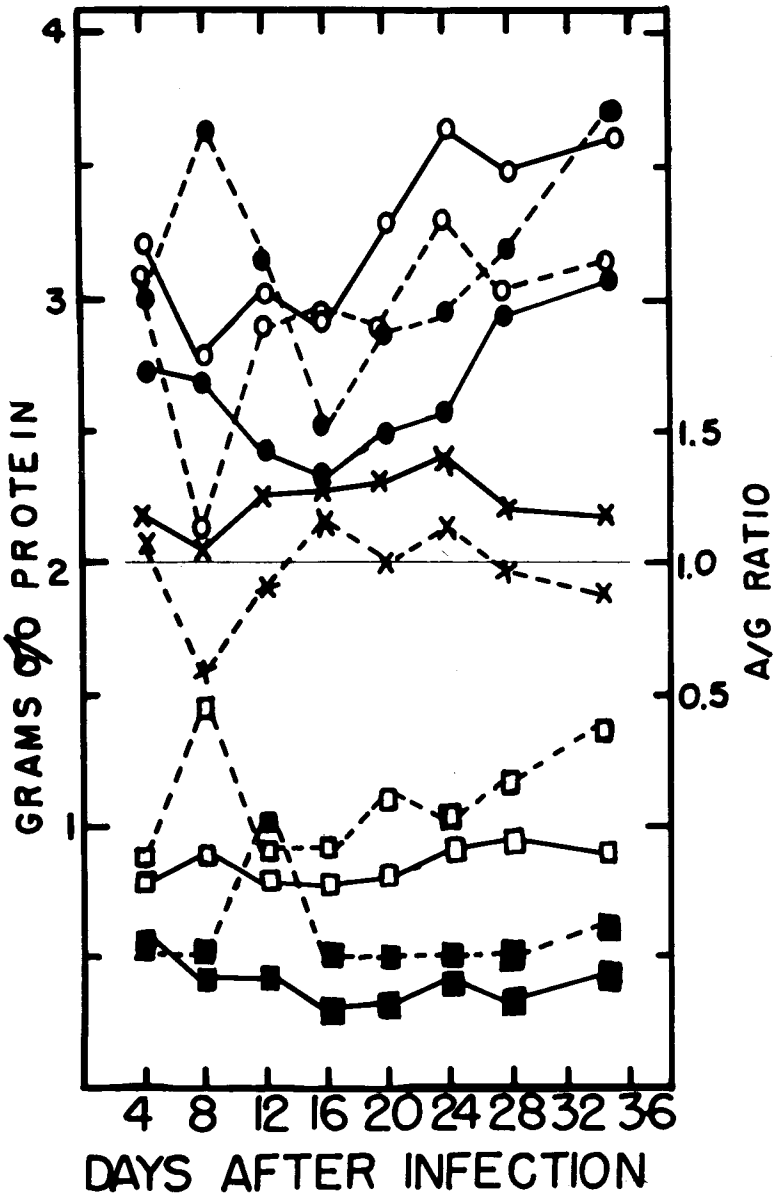


FIG. 5. Relationship of total globulin, albumin, A/G ratio, beta- and gamma-globulins to time of infection. Total globulin, ●; albumin, O; A/G ratio, X; beta-globulin, □; gamma-globulin, ■; control, ———; infected, - - - -.

Qualitative changes in any serum protein component as manifested by the appearance of new components or alterations in their mobilities were not observed in any of the studies herein reported. However, qualitative changes as evidenced by the presence of a protective humoral factor did occur. Rats passively immun-

ized with the sera of infected rats, collected on the 35th day, were completely protected against infection.

Experiment II was an effort to demonstrate in which serum protein fractions(s) the antibody against the larvae appeared. Though comparison of Experiments I and II was limited by the fewer analyses made in the latter, the results obtained in Experiment II were in general similar to those of Experiment I. The data of Experiment II were not as quantitatively significant as those of Experiment I. Total protein and alpha 1-globulin were again not significantly altered. Total and beta-globulins showed significant increases and the A/G ratio a significant decrease in the infected animals. Whereas albumin, alpha 2- and gamma-globulins were significantly altered in Experiment I, the differences noted in Experiment II were not statistically adequate. The average results of the electrophoretic analyses of Experiment II are given in Table II.

TABLE II. *Average values for electrophoretic analysis, Experiment II.*

Group	Total Protein (Gm.%)	Electrophoresis (Gm. %)						
		Albumin	Total Globulin	Globulins				A/G Ratio
				Alpha 1-	Alpha 2-	Beta-	Gamma-	
Control	5.64	2.91	2.73	1.01	.54	.81	.37	1.08
Infected	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%

It is of interest to note here that the abrupt changes observed on the 8th and 12th days of Experiment I were also apparent in Experiment II, but to a lesser degree. The explanation appears to lie in the fewer cysts present in the rats bled on these days. In Experiment I rats bled on the 8th day had an average of 160 cysts and those on the 12th day 225 cysts. Rats bled on the corresponding days of Experiment II averaged 66 and 124 cysts, respectively. Furthermore, in a preliminary experiment extending over a period of 35 days the total average number of cysts present was 27. No significant changes were obtained in any of the protein components, total protein or A/G ratio. However, qualitative changes were assumed to have occurred as rats re-infected on the 35th day after the initial infection were almost completely protected against the second infection. Hence, it is evident that the quantitative changes obtained in Experiments I and II are for the most part, if not entirely, a reflection of the extent of liver involvement.

Campbell (1938b) demonstrated that the "early" immune factor is absorbable from serum collected on the 11th day following infection. Sera collected from infected and non-infected rats on the 12th 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.

That the sera collected on the 12th day contained protective humoral bodies was evident from the results of the passive immunization study. Also, some absorption of antibodies from the sera was accomplished though it appeared to be somewhat inadequate and possibly accounts for the failure to induce any change in the electrophoretic pattern (Table III).

Negative results were also obtained in the absorption studies conducted on the 35th day sera. Antibodies were also present in the 35th day serum of the infected

rats as both pre- and post-absorbed sera afforded complete protection to passively protected rats (Table III). Failure to obtain evidence of any absorption of anti-

TABLE III—*Average number of larvae in livers of rats passively immunized with serum collected from rats on the 12th and 35th days, Experiment II*

Day serum collected	Rat serum received				
	None	Normal	Normal-absorbed	Immunized	Immunized-absorbed
	Cysts	Cysts	Cysts	Cysts	Cysts
	Living - Dead	Living - Dead	Living - Dead	Living - Dead	Living - Dead
12th*	251.7 - 46.7	171.0 - 20.0	144.7 - 33.0	0 - 11.0	0 - 45.7
35th**	12.0 - 99.0	6.8 - 31.3	0.75 - 43.0	0 - 0	0 - 0

*Each group contained 3 rats
**Each group contained 4 rats

body in the rats receiving the post-absorbed serum is in agreement with the non-absorbability characteristic of the "late" immune factor with worm material (Campbell 1938b, c).

Though the "late" immune factor was not absorbable by the method employed, it was felt that it might be possible to relate changes hoped for in the absorbed 12- and 35-day sera. Thereby, evidence may have presented itself enabling the establishment of the serum protein component(s) in which the "early" immune antibody is produced with a possible extension to the "late" immune factor.

Experiment III:

Antibody induced with larval worm material is absorbable (Campbell, 1938b), and it seemed that this afforded an excellent approach to determining the serum protein component in which the "early" immune factor is produced; and also enabled the elimination of changes superimposed by liver involvement in infected rats.

Table IV presents the results of the electrophoretic analyses of this experiment. No statistically significant changes were obtained.

Electrophoretic patterns of the pre- and post-absorbed sera collected on the 28th day showed no significant alterations in any of the protein components. Immune sera showed protective capacity (Table V.) The qualitative changes might

TABLE V—*Average number of larvae in livers of rats passively immunized with serum collected from rats on 28th day of Experiment III*

Rat serum received				
None* Cysts	Normal Cysts	Normal - absorbed Cysts	Immunized Cysts	Immunized - absorbed Cysts
Living - Dead	Living - Dead	Living - Dead	Living - Dead	Living - Dead
19.0 - 58.5	22.5 - 49.3	19.3 - 34.0	2.0 - 17.0	11.3 - 35

*Each group contained 4 rats

be due to antibodies produced in response to immunization. Also, absorption had been moderately successful in spite of the failure of the pre- and post-absorbed sera patterns to indicate any significant changes. This may also be a reflection of inadequate absorption.

DISCUSSION

The serum protein changes noted in the infected rats cannot be regarded as specific. If anything, the significant quantitative differences noted are attributable essentially to the liver tissue involvement resulting from the infection. Hypo-

albuminemia and hyperglobulinemia are characteristic of many conditions including parasitic infectons (Stauber, 1954) and liver involvement (Gutman, 1948). Significant decreases in the A/G ratios reflected the rise in total globulins and decreased albumin though the total protein was unaffected. Failure of the total protein to indicate quantitative alterations in serum protein components is not unusual in liver conditions (Gutman, *loc. cit.*).

Among the globulins, gamma-globulin is most frequently increased in liver involvement with changes in other globulins varying with different liver conditions (Gutman, 1948). The significant increments in the alpha 2-, beta- and gamma-globulins noted in the heavily infected animals are essentially what would be expected in a condition of liver involvement. Also, the severity of the condition (i.e., number of larvae in the liver) influences the degree of serum protein changes (Gray and Barron, 1943).

Evidence indicates that the liver is the primary site of albumin synthesis (Madden and Whipple, 1940; Peters and Anfinsen, 1950) and that albumin production is impaired with a resultant hypoalbuminemia when liver parenchyma is damaged (Martin, 1949). Other studies suggest that alpha 1-globulin is formed largely in the liver (Roberts and White, 1949) and that globulins in general are mainly produced in extrahepatic sites (Abrams and Cohen, 1949).

That hyperglobulinemia is a physiological compensation for the decreased albumin in order to maintain the blood's colloidal osmotic pressure is highly questionable (Gutman, 1948). Several factors have been incriminated as responsible for the globulin disturbances in liver diseases. Gamma-globulin changes have been attributed to liver damage (Lamirande, 1952) and liver mesenchymal cell activity (Popper *et al.*, 1951; Franklin *et al.*, 1951). Alterations in the alpha-globulins are considered to reflect liver damage (Popper *et al., loc. cit.*), proliferative activity (Lamirande, 1952) and possibly a non-specific response to tissue destruction in the case of alpha 2-globulin (Seibert *et al.*, 1947). Liver proliferative activity may be concerned in beta-globulin responses (Lamirande, 1952).

From the above, the serum protein changes noted in the infected rats appear correlated with the liver tissue reaction to the infection described by Bullock and Curtis (1924). For approximatly 8–10 days following infection, liver cell degeneration predominates. The marked decrease in albumin found on the 8th day is indicative of impaired liver function as a result of the liver damage. On the 12th and 16th days albumin approached or was at the control level and coincided with the time liver cells were rapidly recovering. As albumin persisted below normal values thereafter, it is felt that the traumatic effects occasioned by the growth in size of the larvae and their cyst wall on the liver cells were moderately adverse to albumin synthesis. Though not statistically significant, the lowered alpha 1-globulin in the infected rats may also be related to liver damage.

A proliferative stage involving liver mesenchymal cells starts about 8–10 days after infection and succeeds the degenerative phase. By the 12th day the proliferative stage is well underway and liver parenchyma cells also show active division. The marked increment in beta-globulin noted on the 8th day corresponded to the beginning of the proliferative stage. Continuance of an elevated beta-globulin throughout the period studied probably reflects proliferative activity in the liver. Maintenance of alpha 2-globulin increases in the infected rats would appear to be

due to proliferative activity and also in part a nonspecific response to liver tissue damage as on the 4th day (during degenerative phase) it was the only globulin showing a definite elevation. Gamma-globulin manifested an abrupt rise on the 12th day at which time proliferative activity is well underway. As mesenchymal cell activity is then prominent, the gamma-globulin increase may be associated with enhanced mesenchymal tissue activity and/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 20th day after infection, but never entirely ceases. The abrupt changes in the serum proteins were encountered during that period following infection (4–16th days) when liver tissue involvement was most pronounced. Protein metabolism appeared to have been markedly altered within this time. Following this period, concentration differences between infected and control rats were relatively stable. As liver tissue activity began subsiding at this time, it would seem that the protein metabolism had readjusted to an atypical and fairly steady state of activity.

The highest concentrations of beta- and gamma-globulins occurred about the time that the absorbable "early" immune factor is present (Campbell, 1938b). However, as a consequence of the negative results obtained in the absorption studies it is only possible to conjecture as to which protein component contained the humoral factor. The gamma-globulin might be favored, but it is to be borne in mind that antibody production is not restricted to any one protein component (Enders, 1944). Hence, in spite of the quantitative and qualitative changes noted it is only feasible at this time to infer therefrom that the alterations were primarily non-specific results engendered in the liver involvement. Other electrophoretic studies of parasitic infections have failed to delineate the specific and non-specific changes in the protein components (Wright and Oliver-Gonzalez, 1943; Leland, Lindquist and Lillevik, 1955).

Though the artificial immunization work eliminated the superimposition of liver damage effects, no evidence presented itself as to the protein component containing the "early" immune factor. There were no significant quantitative differences giving portent as to the localization of the qualitative changes exemplified in the passive immunization study. The absorption studies were also fruitless.

Several factors may enter into the failures encountered. The absorption methods used were empirical (Campbell, 1938b) and serve to emphasize the need in parasitic immunology of more exact quantitative techniques for antigen-antibody studies. Inability of the electrophoretic apparatus to detect the antibody produced and absorbed as well as inadequate absorption are to be considered. Immunizing materials and procedures used in addition to the bleeding schedule may enter into the situation.

Further immunization studies employing cyst-fluid material for determining the serum protein component site of the "late" immune factor, isolated protein fractions and the immunoelectrophoretic techniques (Williams and Grabar, 1955) could prove fruitful.

Electrophoretic studies of serum proteins serve as indicators of the physiological state of the animal. Investigations of this type would not only contribute to our understanding of antibody production in parasitic infections but also to the problem of the host's physiological response to parasitism. This, in turn, should facilitate elucidation of the overall host-parasite relationship complex.

SUMMARY

Sera from rats experimentally infected with *Cysticercus fasciolaris* were analyzed electrophoretically for various days following infection. A similar procedure was carried out on sera from rats artificially immunized with larval worm material. This is believed to be the first application of electrophoresis to the study of a cestode infection.

New components or alterations in the component mobilities were not obtained. However, qualitative changes in the serum proteins of infected and artificially immunized rats were evident from the positive results in passive immunization and reinfection studies.

In the infected rats the quantitative changes in serum protein components are related to the degree of infection. Rats having less than 66 cysts exhibited no significant quantitative changes. Significant quantitative increases in heavily infected rats occurred in the total, alpha 2, beta- and gamma globulins. Albumin and the A/G ratio were significantly decreased. Rats containing fewer larvae than the heavily infected rats showed significant differences only in the total globulin, beta-globulin and A/G ratio.

The apparent correlation of the serum protein changes with alterations in serum protein metabolism resulting from the liver tissue reaction against the infection is discussed. It is concluded that the serum protein changes were, for the most part, a non-specific response manifesting liver involvement.

Animals were artificially immunized in order to circumvent the changes superimposed by the liver response in the hope of determining the protein component containing the "early" immune factor. Significant quantitative differences were not obtained though immunity was produced.

Results of the efforts to ascertain the immunologically active protein component in infected and artificially immunized rats by electrophoretic analysis of serum before and after absorption with worm material were negative.

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