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TEMPORAL RELATION BETWEEN A SERUM ELECTRO-ADSORBING FRACTION AND THE COURSE OF ACUTE *PLASMODIUM BERGHEI* MALARIA IN RATS*

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ABSTRACT: The temporal relations of a serum electroadsorbing factor (S. E. A. F.) that, quantitatively electroadsorbed from the serum of normal and *Plasmodium berghei*-infected rats, were determined by a modification of the immunoelectroadsorption (I. E. A.) technique. The S. E. A. F. electroadsorbed independently of the presence of a specific *P. berghei* antigen. It tended to increase slowly with time in normal rats. It was found to increase markedly and abruptly after the 5th day of *P. berghei* infection, and its pattern of increase appeared to accompany the typical pattern of acute *P. berghei* anemia and parasitemia. The S. E. A. F. remained elevated for varying periods after the resolution of infection. Its role in normal and pathophysiologic processes is presently unknown, as is its origin or identity. Its appearance interferes with the I. E. A. test, and reference is made to studies describing a resolution to this difficulty.

The serum alterations accompanying the course of acute malaria are complex,[1] and their relation to pathogenesis and specific pathophysiologic events remain poorly defined. Nevertheless, temporal correlations of these serum patterns to clinical and specific pathologic changes are important guideposts and, accordingly, have been the subject of intensive study. This is especially true with regard to anemia in malaria and its attendant parasitemia.[2,3] We here introduce yet another facet in this complex mosaic of temporally related patterns.

While evaluating the immunoelectroadsorption (I. E. A.) method of Mathot *et al.*[4] for serologic detection and measurement of specific *Plasmodium berghei* antibody in infected rats, we noted a *P. berghei* nonspecific electroadsorbing serum factor. This factor remained relatively close to pre-infection levels for several days after challenge with *P. berghei*. An abrupt increase then began that continued upward through the peak of the disease. The serum electroadsorbing factor (S. E. A. F.) tended to remain at elevated levels in surviving animals for varying periods after resolution of the disease. Because it electroadsorbed independently of the presence of specific malarial antigen, it obscured the possibility of measuring plasmodial antibody by the I. E. A. technique as carried out here.[4]

Elevated electroadsorbing materials in serum have been reported to occur in other systems,[5] but until presently, no systematic study as to their relation to various normal or pathologic states have appeared.

The present work concerns itself with the detection measurement and temporal relation of the rat S. E. A. F. in normal and *P. berghei*-infected animals.

METHODS

Immunoelectroadsorption (I. E. A.) and Serum Electroadsorption (S. E. A.) Techniques

I. E. A. was performed according to the procedure as originally described.[4] Modification of the procedure was made as noted below for S. E. A. F. determinations.

In the original I. E. A. procedure, a thin film of antigen was electroadsorbed onto the surface of a negatively charged metallized glass slide. The slide was then placed in the test serum, the polarity reversed so that the slide was positively charged, and a second film deposited. The thickness of the original antigen film and that of the serum-antigen films were determined to within one Angstrom (Å) unit by an especially adapted ellipsometer.[6] The thickness of the serum film was then calculated and taken to represent the level of antibody present in the original serum.

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To ascertain the presence of *P. berghei* nonspecific serum electroadsorbing substances in the test series under consideration, we made a comparison between the amount of material electroadsorbed from serum both in the presence and absence of specific *P. berghei* antigen on the slide. Serum samples for this comparison were diluted 1:10 in barbital buffer (pH 7.7; 0.03 M). I.E.A. (antigen on slide) and S. E. A. (blank slide) were carried out on a nickel-chromium (inconel) metallized glass slide positively charged for 30 seconds at a current of 300 microamperes (μamp).

For the S. E. A. F evaluation of a given serially studied normal control or infected animal, either an inconel slide with the serum diluted 1:10 as above or a chromium-coated slide with the serum diluted 1:20 was used. A comparison of the S. E. A. F. between different animals was made by comparing the ratio of the Å thickness of S. E. A. F. electroadsorbed on any given day of the study for a given animal divided by the Å thickness of the S. E. A. F. electroadsorbed from that animal serum on day zero of the study. The ratio thus obtained was multiplied by 100,

$$\frac{\text{Å thickness Day N}}{\text{Å thickness Day 0}} \times 100 = \text{S. E. A. F. ratio}$$

P. berghei *Antigen*

This was prepared according to the method of D'Antonio *et al.*[5] The major complement-fixing fraction was tested as the antigen coating.

Serum Collection and Hematologic Determinations

Serum specimens were collected daily from tail blood of rats into microhematocrit tubes. The blood was allowed to coagulate and the serum separated from the clot in a microhematocrit centrifuge.

Total red blood cells per cmm were determined on tail blood with a Coulter counter. Thin blood films were made at the same time for determination of parasitized and nonparasitized cells.

Animals and Infection

Sprague-Dawley and Carworth Farm rats were divided into two groups according to weight. The lighter group ranged from 50 to 160 g and the heavier group from 161 to 240 g.

Animals from each group were infected on day zero by the intraperitoneal injection of *P. berghei*-infected rat blood. The NK65 strain of *P. berghei* was used for the temporal pattern and specificity studies and the NYU-2 strain for study of change in electroadsorption with variation in current.

Noninfected animals from each of the above groups acted as normal controls.

As a check on the influence of the electroadsorption procedure itself on the S. E. A. F. determinations, serum samples collected serially from a group of normal and *Haemonchus contortus*-infected lambs, and handled in the same manner as the rat serum, were also tested.

TABLE 1

Comparison of electroadsorption ratios (× 100) from rat serum during course of P. berghei *infection with and without specific plasmodial antigen on slide*

Day of infection	Specific antigen on slide	Blank slide
0	100	100
1	96	104
2	100	104
3	98	100
4	119	122
5	116	111
6	174	132
7	205	186
8	299	233
9	279	416
10	254	300

Current and the Degree of Serum Electroadsorption

The influence of current variation on the S. E. A. F. of normal serum and that infected with *P. berghei* (10 days, NYU-2) was studied by diluting each serum 1:20 (veronal buffer, pH 7.7; 0.03 M). A freshly diluted sample was initially adsorbed by momentary immersion of a clean metallized glass slide. This always resulted in a physical adsorption of 14.5 ± 0.5 Å. The preadsorbed serum samples were then electroadsorbed for 60 seconds on a positively charged slide at current intensities varying from 20 to 100 μamp. The electroadsorption in Å versus current was then plotted, and the curves obtained for the normal and *P. berghei* serum were compared.

RESULTS

Serum Electroadsorption and Specificity

The results of the malarial-antigen specificity study are indicated in Table 1. The ratio of

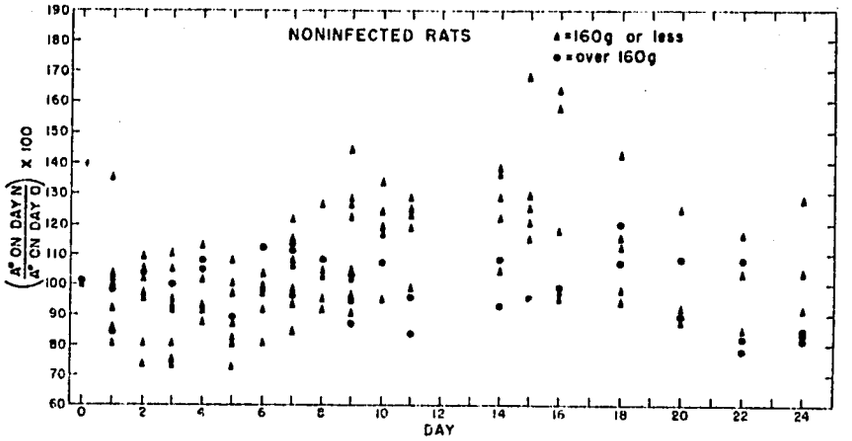

FIGURE 1. Scatter graph of daily serum electroadsorption ratios (× 100) for normal rats weighing under 160 grams (▲) and over 160 g (●).

thickness of material electroadsorbed from the rat serum remained relatively steady from the start of infection on day zero to about day five. From day five on, the ratio progressively increased. The pattern of increase measured by the slide coated with the major complement-fixing component of *P. berghei* antigen was similar to that measured when a blank slide containing no antigen was used.

S. E. A. F. Levels

Figure 1 graphically depicts the scatter of S. E. A. F. ratios for normal animals from day to day in the two weight groups. The daily scatter drifted slowly upward for the lighter animals. The scatter for the heavier animals tended to remain in the starting range and was somewhat more uniformly distributed.

The infected group of lighter weight (Fig. 2) maintained a fairly uniform close distribution of the S. E. A. F. ratios until day five. An abrupt steep upward trend began immediately following day five and continued upward in a fairly uniform manner, reaching a cluster of maximum values on day nine. As the infection subsided in the surviving animals, a leveling off and then a downward trend set in. Some values began to approach preinfection ranges by days 22 and 24.

A similar, though less steep and less compact distribution of S.E.A.F. ratios began immediately after day five for the heavier animals (Fig. 3). Maximum values here, too, were attained on the 9th day with a leveling off and a tendency to

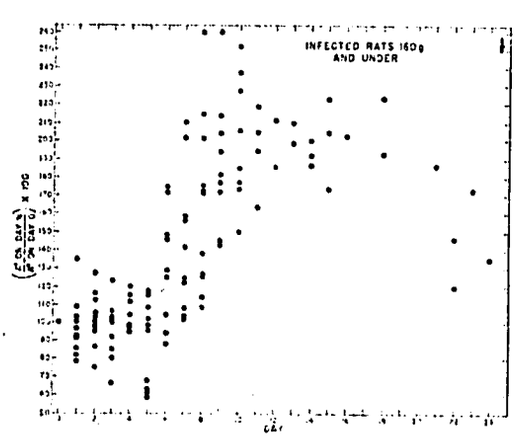

FIGURE 2. Scatter graph of daily serum electroadsorption ratios (× 100) for *P. berghei*-infected rats weighing under 160 g.

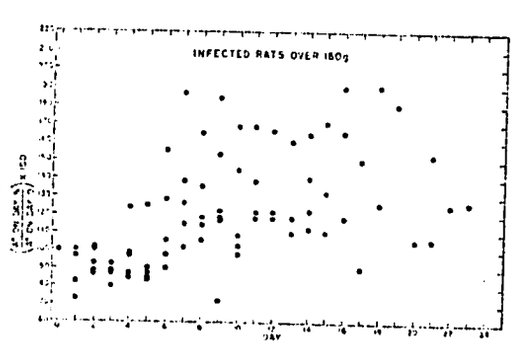

FIGURE 3. Scatter graph of daily serum electroadsorption ratios (× 100) for *P. berghei*-infected rats weighing over 160 g.

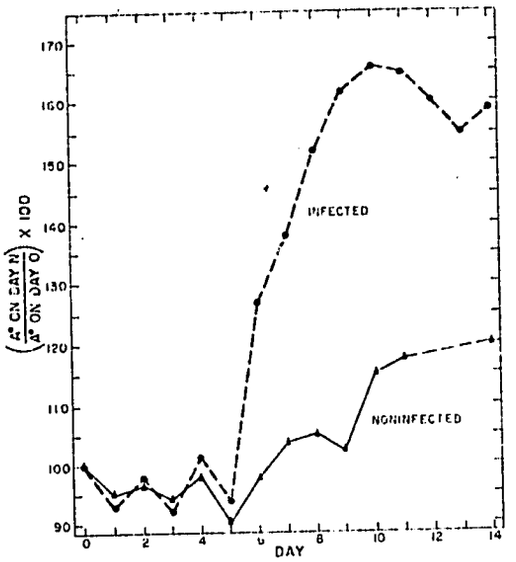

FIGURE 4. Average values of electroadsorption ratios for infected and noninfected rats.

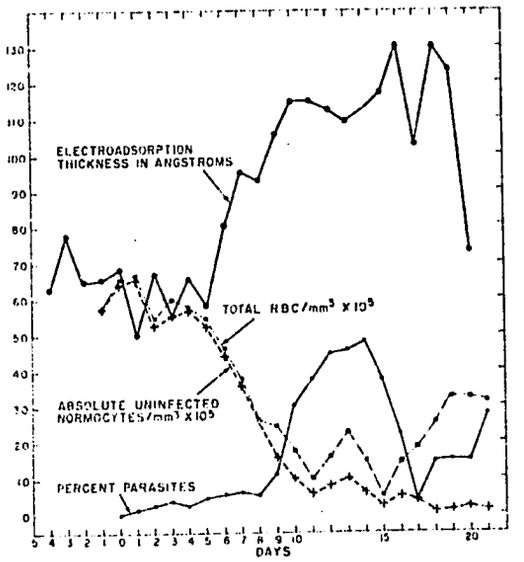

FIGURE 5. Hematologic and electroadsorption patterns during the course of *P. berghei* infection in the rat.

approach preinfection ranges by the 22nd to 24th days.

The over-all influence of these trends is depicted as composite values for the normal and infected group in Figure 4. As in the scatter graphs, the average S. E. A. F. ratios in the infected animals abruptly climbed upward after day five. The noninfected animals also showed a slight upward movement after day five and reflect the tendency for upward drift noted in the lighter group.

Table 2 depicts the S. E. A. F. ratios for the normal and *H. contortus*-infected lambs. The S. E. A. F. ratios of these animals tended to remain at or slightly below their initial levels.

TABLE 2

Average serum electroadsorption ratios (× 100) with mean deviation of serum from normal and H. contortus-*infected lambs*

Week	Average S. E. A. F. ratio (× 100) ± M.D.*
0	100.0 ± 0.0
2	98.5 ± 3.5
3	97.9 ± 5.5
4	96.0 ± 6.3
5	93.8 ± 6.0
6	91.5 ± 1.5
7	90.8 ± 2.8
8	89.4 ± 2.4

* Mean deviation.

Hematologic and Serum Electroadsorption Patterns during P. berghei *Infection*

Figure 5 graphically expresses typical peripheral-blood changes[2] in relation to the absolute Å thickness of electroadsorbed material.

As indicated by Figure 5, the red-blood cells per cmm remained at an elevated level until the 5th day of infection; a dramatic daily decline then ensued. This daily decrease in the erythrocyte count was primarily due to the loss of uninfected normocytes, which declined from an initial level of five million per cubic millimeter just before the onset of anemia to less than one million per cubic millimeter at the time of crisis.

Before the onset of the loss of uninfected normocytes, the S. E. A. F. values tended to remain within a relatively narrow range. On day five of the infection, the S. E. A. F. increased dramatically and continued to increase daily thereafter. The highest levels were obtained during the peak period of infection.

The parasitemia accompanied, but did not run strictly parallel to, the above events. The percentage of infected cells present rose slowly from the onset of infection to about the 9th day, when

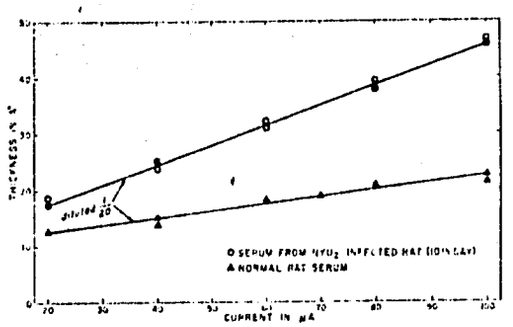

FIGURE 6. Electroadsorption of serum from normal and *P. berghei*-infected rats with various currents.

a sharp and progressive rise in parasitemia began with a peak occurring on day 14.

Resolution of the infection began on about day 14.

Electroadsorption Variation with Current

Figure 6 illustrates the electroadsorption thicknesses obtained from a normal and an NYU-2-infected rat serum. The amount of electroadsorption from the normal and infected serum samples increased in direct proportion to the current intensity from 20 to 100 μamp. The slope of increase for the infected serum was greater than that for the normal serum.

DISCUSSION

There is an electroadsorbing factor in the serum of rats both with and without malaria. It is presently unknown whether they are identical. The rat S. E. A. F., as evidenced in Table 1, electroadsorbs independently of the presence of specific plasmodial antigen. Artifact as a direct cause of the S. E. A. F. results appears ruled out by the difference noted in the slope of the electroadsorption curve obtained between normal and NYU-2 *P. berghei*-infected rat serum with current variation, the consistent pattern of change of the S. E. A. F. during the malarial infection, and the failure to obtain similar S. E. A. F. changes and variations in the serially collected serum samples of the normal and *H. contortus*-infected lambs.

The S. E. A. F. patterns were similar in the two strains of rats studied and appear independent of the *P. berghei* strain used.

Although the scatter of the S. E. A. F. ratios tends to distribute upward in the normal lighter animals and to a lesser degree in the heavier animals, it is impossible to state whether it is identical with the steeply increasing material noted during the course of *P. berghei* infection. The normal animals' upward trend, especially apparent in the lighter group, seriously suggests some physiological relation to the known rapid growth of younger rats or other stresses induced by the necessary handling and serial sample collections, or both. It is possible that the stresses induced by disease (*P. berghei* malaria in this case) may accentuate S. E. A. F. production. The question still remains whether the S. E. A. F. is participatory or dependent on changes occurring in the animal, or both.

The S. E. A. F. temporal pattern of marked increase during infection is highly suggestive. This increase is especially striking with respect to the acute malarial anemia (Fig. 5), an anemia characterized by a marked disparity between daily erythrocyte destruction and parasitemia.[2] The mechanism for this devastating, unremitting erythrocyte destruction is far from clear, and many attempts have been made to relate it to various serum components of both host and parasite origin.[2,8] The present data do not enable us to determine the pathophysiologic role, if any, of the S. E. A. F. in this process.

Electroadsorption of solubilized parasites did not give any significant thickness; thus the S. E. A. F. appears to be of host and not direct parasite origin, though such a result does not strictly rule out the role of the reported explasmodial serum antigens.[9,10] These have been related to anemia in various animal malarias[9,11] Other serum factors such as certain macroglobulins, cryoproteins, fibrinogen, and its related products[12-18] have also been reported to be involved in various types of anemia and malaria.

Hemoglobin as a serum contaminant has been ruled out as responsible for the S. E. A. F., since it does not electroadsorb in significant amounts even at high concentration.[10] The rapidly appearing, somewhat labile alpha (acute-phase) globulin of rats[20] presents a profile reminiscent of the S. E. A. fraction reported here and deserves close scrutiny. Other components to be considered include such things as 2 haptoglobin-hemoglobin and other complexes, an increase in an as yet undefined specific antibody or antibodies, as well as any combination of the myriad serum alterations associated with acute pathologic states.

Lastly, increases and variations of the S. E. A. F. in human serum has been the subject of an intensive study that suggest their identity with the serum gamma globulins.[5,16]

The S. E. A. F. as reported here deserves close scrutiny as to its biological significance in both normal and pathologic' conditions. In a more practical sense, refinement of its method of measurement as well as its characterization might offer a rapid sensitive test for detecting, differentiating, and evaluating certain physiologic and pathophysiologic states.

Studies carried out because of the interfering effects of the S. E. A. F. with the I. E. A. test have resulted in a modified technique whereby these effects may be overcome.[21,22]

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