

DETECTION OF MALARIAL ANTIBODIES BY COUNTER CURRENT IMMUNOELECTROPHORESIS

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SYNOPSIS

This is the first published report of the use of counter current-immunoelectrophoresis for the detection of malarial antibodies. The method has worked satisfactorily in rats infected with *Plasmodium berghei* and the antibodies could be detected on the fifth day of infection. Investigations are now in progress to find out if the technique could be used in human malaria.

Amongst the various serological tests used for the detection of malarial antibodies the two most commonly employed are the fluorescent antibody test (FAT) and the passive haemagglutination test (HAT). Both these tests have provided valuable information on various aspects of malarial immunity (Voller, 1971). However, both FAT and HAT have certain limitations. In the case of FAT accurate titration is difficult as the assessment of the degree of fluorescence is highly subjective. In addition, fluorescein conjugated sera although commercially available are expensive. HAT is more sensitive than FAT and accurate titration is possible, but the technical problems associated with the preparation of red cells and their sensitization are usually too complex and time consuming for a laboratory involved with routine diagnosis. Recently, an agar electrophoretic method generally known as counter-current immunoelectrophoresis has been employed for the detection of antigens and antibodies in serum (Mason *et al*, 1972; Hellwege *et al*, 1972; Banffer, 1972). The advantages associated with this technique are that it is a one step procedure, is fast and simple to perform. The instrumentation required is inexpensive and the technique is about 10 times more sensitive than the ordinary double diffusion method of Ouchterlony. The essence of the procedure is that an anodally moving antigen becomes complexed with a cathodally moving antibody due to the process of electro-endosmosis. The positive reaction is re-

vealed by the appearance of a visible precipitate. To the best of our knowledge counter-current immunoelectrophoresis has not been used previously for the detection of malarial antibodies. In this paper we are reporting on its use in *Plasmodium berghei* infection in rats.

MATERIAL AND METHODS

1. *Antigen preparation*—Heparinized *P. berghei* infected mouse blood showing a parasitaemia of about 40-50% was passed through a 5 cm. column of Whatman powder (standard grade) in physiological saline to remove leukocytes. The red cells were then packed and separated by centrifugation for 10 minutes at 250 × G and lysed with isotonic ammonium chloride solution in tris-buffer of pH 7.2 (Boyle, 1968). We have found that this method of lysis preserves the morphology and viability of the parasites, while the red cells are completely lysed. The usefulness of ammonium chloride for this purpose has also been reported by other workers (Martin *et al*, 1971). After lysis the released parasites were washed three times in physiological saline by centrifugation. The final sediment was diluted 1:5 in physiological saline and sonicated. The sonicated material was then centrifuged at 10,000 × G for 30 minutes and the supernatant used as the antigen. Aliquots of antigen were stored at -20°C.

2. *Antiserum preparation*—Six adult rats each weighing approximately 300 gm. were injected intraperitoneally with 0.2 ml. of blood containing approximately 100×10^6 *P. berghei* parasites. Blood samples were obtained daily for 12 days by transection of the tail and the serum collected.

3. *Counter-current immunoelectrophoresis of the test*—Ionagar (Oxoid No. 2) was dissolved in a 1:2 dilution of barbitone buffer (pH 8.6, ionic strength 0.083) in distilled water to give a final concentration of 1.5%. The agar was then poured into plastic plates to a depth of 1 mm. and wells were cut in pairs, about 6 mm. apart from centre

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to centre and 3 mm. in diameter. 8 microliters volume of antigen or antiserum was introduced into the appropriate well and electrophoresis was

carried out in a Hyland electrophoresis unit (California, U.S.A.) with constant output current of 50 milliamperes at 120 volts for 90 minutes.

RESULTS AND DISCUSSION

Positive reaction first appeared in some rats as early as 5 days after the date of injection. The reaction was in the form of one or two bands between the antigen and antibody wells (Fig. 1). Control serum consisting of normal rat serum produced no band against the malarial antigen. Positive control using immune rabbit serum gave a distinct band. Rats tested up to six months after exposure to *P. berghei* remained positive. In these experiments undiluted antibody was used, therefore, we do not know how the test compares in terms of sensitivity to FAT or HAT. We are now investigating the problem and the possible use of this method in the detection of antibodies to human malaria.

REFERENCES

1. Banffer, J.R.J.: "Demonstration of Precipitins against a Treponemal Antigen by Counter-Immuno-electrophoresis." *Lancet*, i, 996, 1972.
2. Boyle, W.: "An Extension of the ^{51}Cr -Release Assay for the Estimation of Mouse Cytotoxins." *Transplantation*, 6, 761, 1968.
3. Hellwege, H.H., Fisher, K. and Blaker, F.: "Diagnostic Value of Candida Precipitins." *Lancet*, ii, 386, 1972.
4. Martin, W.J., Finerty, J. and Rosenthal, A.: "Isolation of *P. Berghei* (Malaria) Parasites by Ammonium Chloride Lysis of Infected Erythrocytes." *Nature*, 233, 260, 1971.
5. Mason, E.C., Shaw, A.E., Harding, M.J. and Witney, K.J.: "High Voltage Immuno-electro-osmophoresis in Australia Antigen Screening of Blood Donors." *Med. J. Austr.*, 1, 1020, 1972.
6. Voller, A.: "The Detection and Measurement of Malarial Antibodies." *Trans. Roy. Soc. Trop. Med. Hyg.*, 65, 111, 1971.

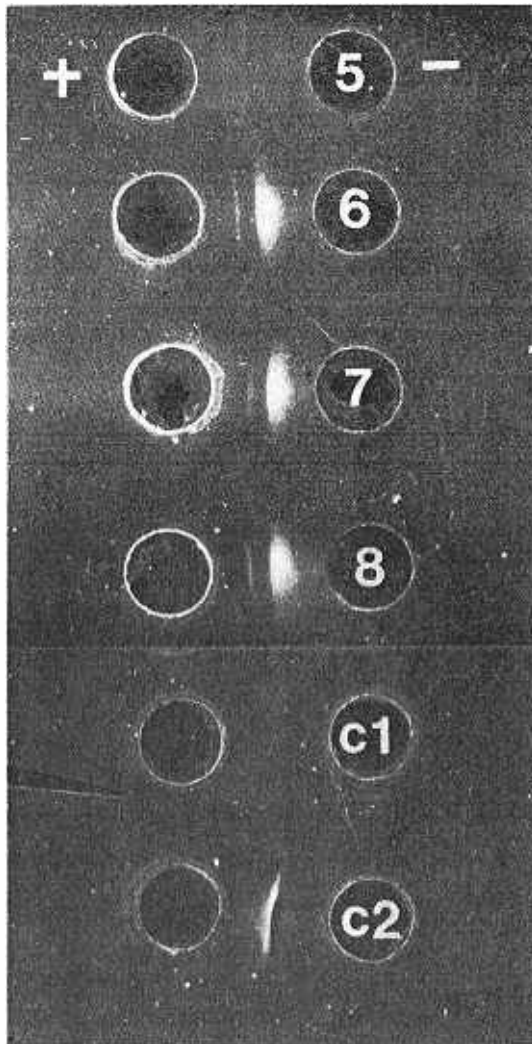


Fig. 1. Shows the appearance of a light band on the 5th day of infection. Subsequently, 2 bands appeared which were seen clearly throughout the test period.

C1 = Normal rat serum against malarial antigen.
C2 = Immune rabbit serum against malarial antigen.