

Separation of *Plasmodium berghei*-parasitized rat erythrocytes by means of carrier-free electrophoresis*

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A carrier-free electrophoresis apparatus was used to isolate rat erythrocytes parasitized with Plasmodium berghei. The region of high electrophoretic mobility yielded noninfected erythrocytes, whereas that of low electrophoretic mobility yielded erythrocytes infected with viable parasites. Over 98% purity of parasitized erythrocytes was obtained when blood at an advanced stage of parasitaemia was used. Merozoite-rich fractions were also observed. This continuous one-step separation method should provide large numbers of parasitized erythrocytes for use in immunological and biochemical studies of malaria.

Malaria is still one of the most widespread diseases in the world, affecting some 200 million people (1). The development of a safe and effective vaccine has been advocated by the World Health Organization and the framework of this research development was established in 1976.^a The preparation of potential vaccine material, using blood-stage parasites, is one of the main aspects of this research programme and one barrier to this project has recently been removed by the successful continuous cultivation of *Plasmodium falciparum* (2, 3). The next step will be the purification and collection of large amounts of purified parasites of a specific stage that can be used to induce protective immunity in the host.

A carrier-free electrophoresis apparatus has been described by Hannig (4). The apparatus has proved highly successful in separating homologous cell populations from heterologous cell groups (5) and also for the isolation and purification of subcellular

organelles (6). The major advantages of the procedure are rapid separation of the cells under conditions producing the minimum of artefacts and a scale of separation allowing quantitative studies of each fraction. Approximately 10^8 – 10^9 cells may be effectively separated in an hour and electrophoresis can be continued for a day without changing the electrophoretic pattern. In addition, nearly 100% recovery can be achieved on completion of the separation procedure. Until now, the apparatus had not been tested for its ability to isolate infected cells; the present paper shows that erythrocytes infected with *Plasmodium berghei* (NK65) can be isolated from noninfected erythrocytes by this apparatus.

MATERIALS, METHODS, AND RESULTS

Wistar rats weighing about 50 g were each inoculated with 10^7 parasitized erythrocytes. On the sixth day following inoculation, when parasitaemia was 40–60%, the rats were exsanguinated by cardiac puncture. Since even a little coagulation can considerably alter the fractionation pattern, the blood was rapidly taken into a heparinized syringe and care was taken to prevent the entry of air. Two millilitres of infected blood was diluted with 10 ml of separation buffer and centrifuged at 655 g for 10 min. After removal of the supernatant, the erythrocytes were washed and resuspended in the same buffer to give a 1% cell suspension (10^7 – 10^8 erythrocytes/ml). Nine millilitres of this erythrocyte suspension was applied to a carrier-free electro-

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^a WHO unpublished document TDR/1M/76.3.

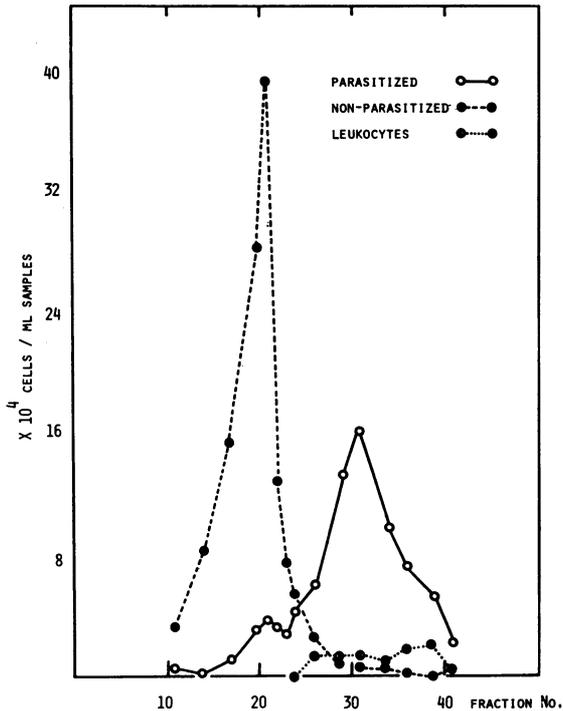


Fig. 1. Electrophoretic distribution profiles of parasitized and nonparasitized erythrocytes and leukocytes from *Plasmodium berghei*-infected rats.

phoretic separator.^a In the present experiment, 3 mmol/litre triethanolamine acetate buffer, pH 7.4, containing 1 mmol/litre of EDTA and 0.25 mol/litre of sucrose was used as the separation buffer.

After 100 min of operation, each tube contained 8.1 ml of a suspension of erythrocytes with different mobilities. Aliquots of 1 ml were taken from each tube for erythrocyte counting; the remainder was then centrifuged and the resulting sediments re-suspended in the same buffer to give a volume of about 0.5 ml in each tube. The viability of the parasites in the separated infected erythrocytes of fraction tubes 26, 31, 36, and 41 was tested by injecting 0.15 ml of the suspension from each tube into two mice. All mice injected with erythrocytes from these fraction tubes died of parasitaemia.

Each specimen was spread on glass slides, fixed with methanol, and stained with Giemsa solution to determine the percentage contamination with parasitized erythrocytes and leukocytes. Electro-

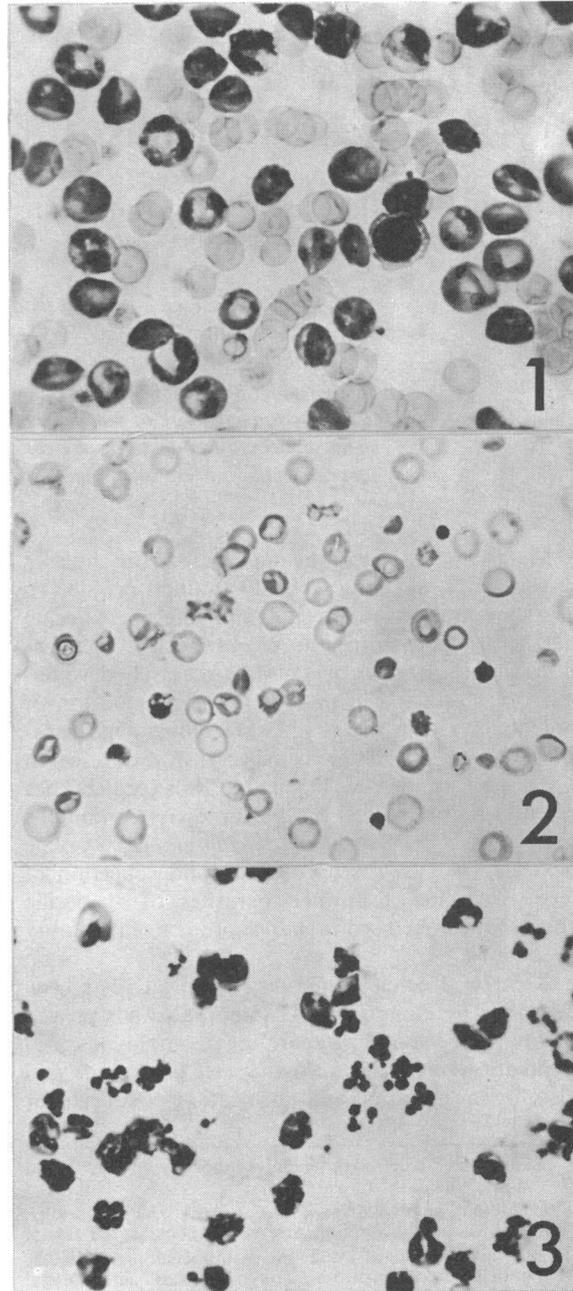


Fig. 2. Photomicrographs of original and separated erythrocytes. (1) Original blood sample from a rat infected with *Plasmodium berghei* (NK65); parasitaemia 41.7%. (2) Noninfected erythrocytes separated by electrophoresis (tube 20 in Fig. 1); purity 97.4%. (3) Infected erythrocytes from tube 28 in Fig. 1—note merozoite; purity 98.5%.

^a Elphor VAP-5 model, Bender & Hobein, Munich, Federal Republic of Germany.

phoretic distribution profiles of infected blood thus obtained are shown in Fig. 1. Clear distinction of the peaks of parasitized and nonparasitized erythrocytes was obtained. Photomicrographs of the original blood smear and of noninfected and infected erythrocytes after separation are shown in Fig. 2. From the electrophoretic behaviour of the two populations of erythrocytes, it was concluded that the infected erythrocytes showed apparently lower electrophoretic mobility than the noninfected ones. Although the reason for this phenomenon is unknown at the present time, the lower electrophoretic mobility of the former seems to be connected with invagination of the erythrocyte surface membrane during invasion by *Plasmodium*. It is known that various agents cause invagination of the erythrocyte surface membrane and such erythrocytes show lower electrophoretic mobility (7). The level of leukocyte contamination measured in fraction tubes 26–41 was 0.8–3.8% (Fig. 1). Platelets were not detected in great numbers in any of the specimens examined.

DISCUSSION

The technique described will open a new approach to the one-step separation of erythrocytes parasitized with plasmodia. By continuous operation of the apparatus, large amounts of parasitized erythrocytes can be made available for antigen studies. One major advantage of the present method is the

separation of merozoites for investigations into protective immunity induced by blood-stage parasites (8); mature schizonts and merozoites were abundant in fractions 28–30. However, quantitative differential counting of the isolated parasites was difficult owing to aggregation of parasites freed from disrupted infected erythrocytes, which are often very fragile at this level of parasitaemia.

Leukocytes were not satisfactorily removed from the infected blood by the one-step procedure, as shown in Fig. 1. In subsequent experiments, this disadvantage might be overcome by leaving blood in a test tube containing 2 volumes of a separation medium, such as plasma-gel or 3% gelatin solution in isotonic buffer, for 30 min so that the leukocytes float up into the supernatant. A higher level of purity could be achieved by repeating the procedure. Removal of leukocytes on a cellulose column (9) results in a considerable loss of erythrocytes, some of which are adsorbed to the cellulose powder.

The first supernatant obtained immediately after the withdrawal of infected blood contained considerable numbers of merozoites mixed with leukocytes and platelets. Merozoites could be obtained from this source by means of carrier-free electrophoresis.

The technique described is expected to provide large numbers of merozoites for future vaccine projects and will also provide a new means of studying membrane changes in parasitized cells.

RÉSUMÉ

SÉPARATION DES ÉRYTHROCYTES DE RAT INFECTÉS PAR *PLASMODIUM BERGHEI* AU MOYEN DE L'ÉLECTROPHORÈSE LIBRE

L'emploi pour cette expérience d'un appareil à électrophorèse libre a permis d'isoler des érythrocytes de rat infectés par *Plasmodium berghei*. Du sang présentant un taux de parasitémie de 40–60% a été prélevé sur des rats Wistar et immédiatement ajouté à la solution tampon utilisée pour l'expérience, soit 3 mmol/l d'acétate de triéthanolamine de pH 7,4 contenant 1 mmol/l d'EDTA et 0,25 mol/l de sucrose. Les érythrocytes obtenus par centrifugation ont ensuite été lavés puis réintroduits dans le même tampon et la suspension obtenue a été placée dans l'appareil. Les érythrocytes non infectés ont montré une plus grande affinité pour la zone de forte mobilité électrophorétique, alors que les érythrocytes infectés se rassemblaient dans la zone de plus faible mobilité. Bien que les profils de distribution des deux

groupes de cellules manifestent un certain chevauchement (figure 1), le contenu de certains tubes présente une uniformité correspondant à un taux d'érythrocytes infectés de 98%. On a constaté dans quelques tubes se situant dans cette zone de faible mobilité la présence de mérozoïtes. D'autre part, certaines fractions de ladite zone contenaient des leucocytes à raison de 0,8 à 3,8%. Ceux-ci pourraient sans doute être éliminés en introduisant le sang prélevé dans un milieu de séparation approprié avant de procéder à l'électrophorèse. Le mécanisme à l'origine de la différence de mobilité entre les érythrocytes infectés et non infectés n'est pas encore connu de manière précise, mais la méthode décrite permettra de disposer de grandes quantités d'érythrocytes parasités pour les études immunologiques et biochimiques.

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