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IMMUNO-ELECTROPHORETIC COMPARISON OF PLASMODIAL ANTIGENS[1]

by

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Until quite recently, antigenic differentiation of plasmodia has been based only on cross-immunity reactions, in the appropriate host the assumption being that such reactions must reflect the molecular structure of the parasites. Some confusion exists in defining strains and species on this basis, since strain differences in one group, for example, of mammalian plasmodia, may be greater than species differences in another group, for example of the avian plasmodia. The analysis of the antigenic composition of the plasmodia by some means other than cross-immunity might help to clarify this problem and might also contribute to our understanding of the phylogenetic interrelations among avian, mammalian and other plasmodia.

We have been working on techniques for such a comparative study with the aid of immuno-electrophoresis, and have to date collected observations on two rodent plasmodia, *Plasmodium berghei* and *Plasmodium vinckei*, (Spira & Zuckerman, 1962: Zuckerman, 1964); and on the fowl parasite, *P. gallinaceum* (Spira & Zuckerman, 1964). Our working plan is theoretically simple, and is briefly as follows:

(1) To obtain plasmodial antigen as free as possible of host cell contamination.

(2) With the aid of antiserum against such an unfractionated preparation to establish its antigenic pattern by immuno-electrophoresis.

(3) To compare antigenic patterns within a strain or species (i.e., sporozoites as compared with erythrocytic stages) as well as between strains and species; to determine in each case which antigens are shared by the two groups studied and which are peculiar to one or another of the groups.

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(4) To fractionate the total plasmodial preparation with a view to defining the chemical and immunological characteristics of each separate antigen.

For some of these items we already have partial solutions, others still remain to be studied.

We first worked with P. vinckei in mice. We treated highly infected mouse blood with saponin to lyse the red cells, then ground the still viable plasmodia in a Hughes press. This preparation contained 5 to 10 mg protein/ml. This product injected into the rabbit together with Freund's adjuvant, allowed an antiserum to be obtained. Electrophoresis of the plasmodia ground in barbiturate agar at pH 8.6 and development with potent antiserum, gave six to eight precipitation arcs (Fig. 1).

Appropriate controls with ground mouse erythrocytes and anti-mouse-erythrocyte rabbit serum, which reacted strongly with one another, showed that the plasmodial product was free of red cell components detectable by this technique.

A similar study of P. berghei in mice also yielded about six precipitation arcs (Fig. 2). This has since been confirmed by Diggs (1964), who demonstrated five plasmodial antigens; and by Banki & Bucci (1964), who obtained 10 precipitation arcs. Most of the precipitating antigens were shared by the two rodent plasmodia but each had also one or two unshared antigens, as was to be expected of two plasmodia which do not cross-immunize (Fig. 3).

In the study with P. gallinaceum we faced certain additional technical problems:

(1) The concentration of saponin adequate for dissolving the stromata of mouse red cells (1:10 000) was insufficient in the case of fowl red cells; we had to double its concentration (1:5000) in order to obtain lysis.

(2) Disposing of the nuclei of avian red cells created another problem. We first washed the saponin-treated residues, consisting of red cell nuclei with parasites adhering to them, in a phosphate buffer solution containing Mg^{++} and glucose at pH 7.7 (Sherman & Hull, 1960). The suspension was then incubated for half an hour at 37°C in the presence of 630 000 units of DNA-ase in a total volume of 10 ml of the above buffer, with occasional gentle stirring.

Following this treatment the red cell nuclei had dissolved, leaving a viable sediment of parasites. Judging from their staining properties, most of the parasites at this stage were still normal. Trituration of the plasmodia and subsequent conduct of the experiments were as in the rodent malaria studies. The gallinaceum preparation contained 10 to 15 mg protein/ml.

Immuno-electrophoresis of the P. gallinaceum preparation developed with homologous rabbit antiserum yielded 12 precipitation arcs (Fig. 4).

Due to imperfections in our earlier technique of eliminating red cell components, our first anti-gallinaceum antiserum also contained an anti-red-cell antibody. The contaminating red cell antigen had an electrophoretic mobility quite distinct from that of any of the plasmodial antigens. Later plasmodial products were more satisfactory, and did not cross-react with anti-red-cell antibody.

How do the P. gallinaceum antigens relate to those of the two rodent plasmodia?

(1) They all have approximately the same range of electrophoretic mobilities, and move to positions on both sides of the point of origin.

(2) A larger number of precipitinogens have been demonstrated in P. gallinaceum than in the two rodent plasmodia. Whether this difference is real or related to the technique employed is not clear. The total doses of plasmodial preparation used in immunizing rabbits were 25 to 35 mg protein for the rodent parasites and 40 to 50 mg protein for P. gallinaceum.

(3) Cross-reactions among the three species yielded interesting information. In all cases, reactions in which a plasmodial preparation was developed against its homologous antiserum revealed a larger number of antigens than did any cross-reaction, as was to be expected.

The two rodent plasmodia, as already pointed out, shared most of their antigens, while only one (in P. berghei) or two (in P. vinckei) were species specific. Anyone who has worked with these two species of parasite will agree that they are biologically distinct, differing in such fundamental charaters as morbidity and mortality rates in various hosts; and in their preferences for given host cell types (Zuckerman, 1957).

Furthermore they do not cross-immunize (Rodhain, 1954). Despite their biological disparity, the structural differences so far observed between them are limited to one or at the most two specific antigens, upon which our interest is focused. We have no information as yet on the possible occurrence in plasmodia of other antigens which may induce the production of precipitins in rabbits.

In cross-reactions between rodent plasmodial preparations and *P. gallinaceum* we found less antigenic congruence than between the two rodent plasmodia; as, indeed, was to be expected in view of their phylogenies. Triturated *P. vinckei* exposed to anti-*gallinaceum* rabbit serum yielded three arcs identical with the homologous *gallinaceum*-anti-*gallinaceum* arcs but much fainter (Fig. 4).

Thus only three out of the 12 *gallinaceum* antigens were shared by *P. vinckei*. In the reciprocal test of triturated *P. gallinaceum* antigen against anti-*vinckei* serum we obtained only one arc, so faint as to be revealed only after staining with amido black. The same *P. gallinaceum* preparation gave no cross-reaction with potent anti-*berghei* serum.

Thus, whereas observed antigenic congruence is extensive between the two rodent plasmodia studied; it is either limited or lacking between the fowel- *versus* the rodent-parasites.

Our experiments obviously represent only a beginning. We feel, however, that it is highly desirable to pursue comparative studies on antigenic relationship of plasmodia since this research may bring to light the phylogenetic relationships now insufficiently defined. It may also help in correlating immunological relationships with molecular composition among plasmodial strains and species.

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RESUME

Jusqu'à une date récente, pour établir une différenciation antigénique entre les divers plasmodiums, on s'est fondé uniquement sur les réactions croisées d'immunité chez un hôte approprié. Mais les différences entre souches peuvent, à l'intérieur d'un même groupe - par exemple les plasmodiums hébergés par les mammifères - être plus grandes que les différences entre espèces dans un autre groupe, tel que les plasmodiums aviaires. C'est pourquoi l'analyse de la composition antigénique des plasmodiums par d'autres moyens que l'immunité croisée pourrait aider à éclaircir la situation et à mieux connaître les relations phylogénétiques existant entre les diverses espèces de plasmodiums, dont celles indiquées plus haut.

On a mis au point des techniques permettant d'étudier la composition antigénique de certains plasmodiums en recourant à l'immuno-électrophorèse. Des observations ont été faites par ces méthodes sur Plasmodium berghei, P. vinckei et P. gallinaceum.

L'analyse immuno-électrophorétique d'un extrait de P. vinckei exposé à un antisérum homologue de lapin a donné 6 à 8 arcs de précipitation; dans les mêmes conditions P. berghei a donné environ 6 arcs.

La plupart des antigènes précipitants existaient chez les deux plasmodiums hébergés par les rongeurs, mais chacun d'eux possédait également un ou deux antigènes qui n'existaient pas chez l'autre, comme on pouvait d'ailleurs s'y attendre avec deux plasmodiums ne donnant pas d'immunité croisée.

L'analyse immuno-électrophorétique d'un extrait de P. gallinaceum exposé à un antisérum homologue de lapin a donné 12 arcs de précipitation.

Dans les réactions croisées entre les plasmodiums de rongeurs et P. gallinaceum, on a observé une concordance antigénique moindre qu'entre les deux plasmodiums hébergés par les rongeurs. Des extraits de P. vinckei exposés à un sérum de lapin anti-gallinaceum ont produit 3 arcs identiques aux arcs homologues gallinaceum-anti-gallinaceum, quoique beaucoup plus atténués. Ainsi, 3 seulement des 12 antigènes de gallinaceum existaient également chez P. vinckei. Dans l'épreuve inverse de l'antigène de P. gallinaceum mis en présence du sérum anti-vinckei, on n'a obtenu qu'un seul arc très atténué. La même préparation de P. gallinaceum n'a donné aucune réaction croisée avec un sérum actif anti-bergh

Des études comparatives se poursuivent sur les relations antigéniques existant entre les divers plasmodiums, afin de préciser les rapports phylogénétiques encore imparfaitement connus.

FIG. 1

TRACING OF IMMUNOELECTROPHORETIC PATTERN OF *P. VINCKEI* EXTRACT EXPOSED TO HOMOLOGOUS RABBIT ANTISERUM

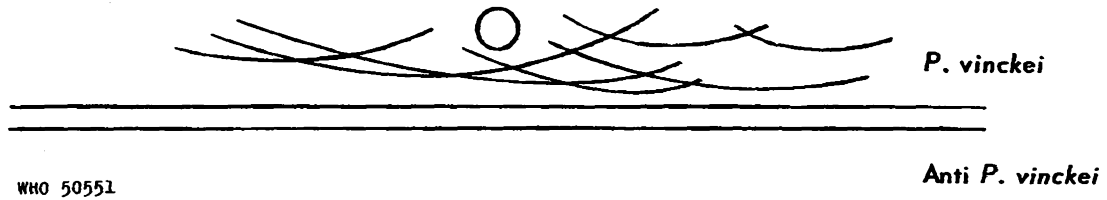


FIG. 2

TRACING OF IMMUNOELECTROPHORETIC PATTERN OF *P. BERGHEI* EXTRACT EXPOSED TO HOMOLOGOUS RABBIT ANTISERUM

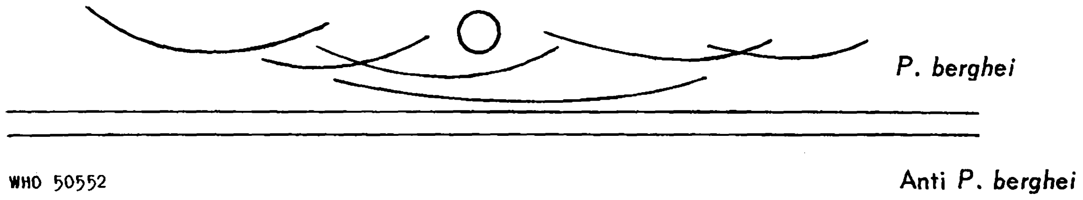


FIG. 3

TRACING OF IMMUNOELECTROPHORETIC PATTERNS OF *P. VINCKEI* AND *P. BERGHEI* EXTRACTS EXPOSED TO HOMOLOGOUS AND HETEROLOGOUS RABBIT ANTISERUM. NOTE SPECIFIC ANTIGENS MARKED BY ARROWS

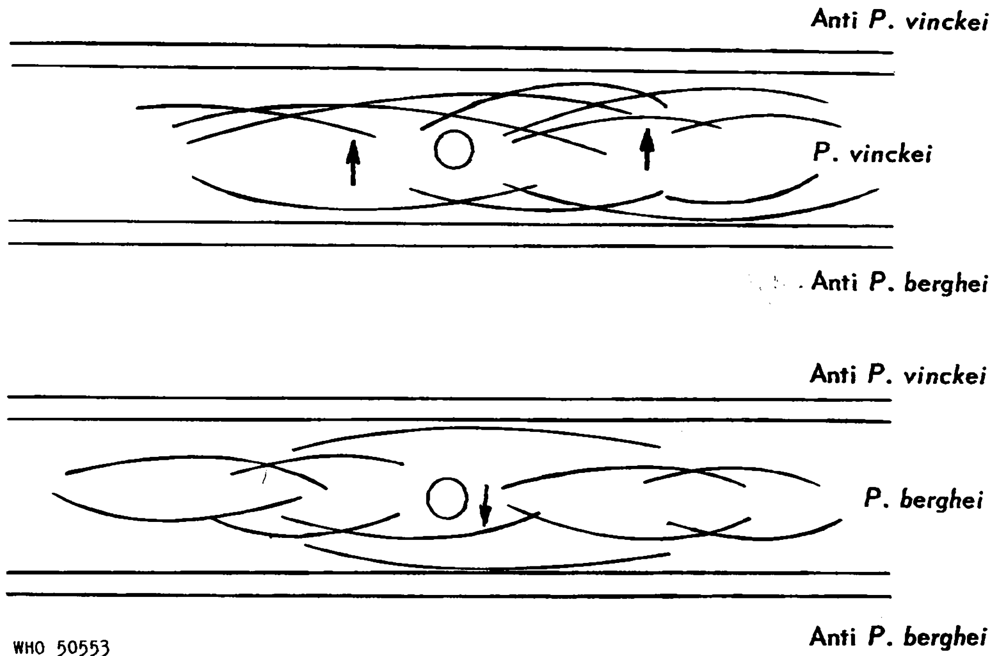
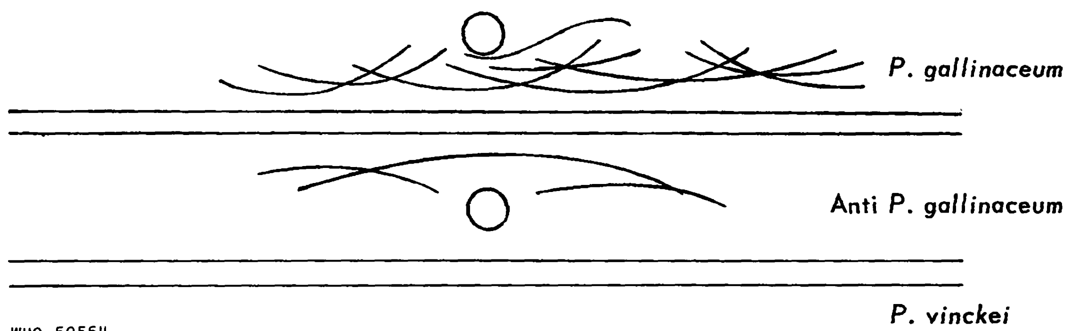


FIG. 4

TRACING OF IMMUNOELECTROPHORETIC PATTERNS OF *P. GALLINACEUM* AND *P. VINCKEI* EXTRACTS EXPOSED TO ANTI *GALLINACEUM* RABBIT ANTISERUM



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