

## Host Identification of Arthropod Bloodmeals by Agar Electrophoresis\*)

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With 4 Figures

### Resumen

Se investigó un método relativamente fácil y poco costoso de electroforesis rápida en gel, adecuado para la identificación de los huéspedes en las comidas de sangre de varios artrópodos.

Esto se hizo porque el conocimiento de la preferencia, que tienen dichos animales hematófagos por ciertos huéspedes, es de importancia primordial en el control de la mayoría de las enfermedades transmitidas por estos artrópodos.

Las moviidades relativas de las proteínas en los sueros y de la hemoglobina (Hb) fueron tomadas como criterios de identificación.

Se discuten las condiciones electroforéticas. Usualmente fueron obtenidos resultados óptimos usando agar "Difco Noble" 0.9%; buffer pH 8.4; un potencial iónico en los compartimientos de electrodos de 0.10 y para la preparación del agar de 0.05; condiciones eléctricas 25—60 V. por cm.; una corriente de aproximadamente 25 m.A; temperatura cerca de 4° C (suministrada por la evaporación de éter de petróleo de punto de ebullición de 40° C a 60° C) y un tiempo de electroforesis aproximadamente de 23 minutos. Se discute en detalle el método de la preparación de las comidas de sangre de los artrópodos y su aplicación a las láminas para estudio. Dos minutos después de comenzar el experimento electroforético fue aparente que la comida de sangre era adecuada para la prueba de identificación. Cuando fue visible una migración de más de una fracción de Hb hacia el ánodo, la digestión por el artrópodo estaba demasiado avanzada para poder identificar el huésped. Después de un cierto tiempo de digestión (para las comidas de sangre por los artrópodos en conejos después de un día, y para la mayoría de estas comidas en el hombre, después de 48 horas) la Hb no migró hacia el cátodo como una entidad compacta, sino que se separó en tres o más componentes con diferentes sensibilidades a la bencidina. Por lo menos tres de los componentes migraron

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hacia el ánodo, y por lo menos una fracción siempre se localizó en la posición de la albúmina en el ferograma.

Comidas de sangre de *Aedes togoi*, *Aedes aegypti*, *Culex fatigans*, *Anopheles* sp., *Rhodnius prolixus*, *Triatoma infestans*, *Triatoma dimidiata*, *Cimex lectularius*, *Pediculus humanus*, *Ornithodoros moubata*, *Ornithodoros savignyi* y *Ornithodoros rudis* tomadas del hombre, mono *Cebus*, y rhesus, perro, gato, conejo, curí, rata, ratón, gallina, paloma, culebra, sapo y rana fueron estudiadas y comparadas con el suero y sangre hemolizada de las especies huéspedes. El valor de esta técnica es comprobado por el hecho de que ferogramas similares fueron obtenidos de las mismas comidas de sangre de huéspedes tomadas por artrópodos muy diferentes taxonómicamente. La digestión de la sangre del vertebrado por diferentes artrópodos parece ser en general cuantitativamente similar, pero diferente en su velocidad. Fue posible identificar el huésped por los patrones de las comidas de sangre de los artrópodos, muchos de ellos por examen macroscópico y sin calcular la movilidad relativa.

Es muy dicente que la movilidad del suero-albúmina de una especie permanece inalterada durante todo el período de la digestión del artrópodo. Las características principales de los patrones de cinco especies huéspedes están resumidas en una clave y los cuadros originales de las láminas de ferogramas, así como los diagramas de las movilidades relativas de la mayoría de las especies huéspedes examinadas están dados en fotografías y tablas.

### Summary

A relatively easy and inexpensive method of rapid gel electrophoresis suitable for the host identification of arthropod bloodmeals was investigated. Electrophoretic conditions (preparation of agar and buffer, voltage, temperature, time, etc.) are discussed. The method of preparing and applying the arthropod bloodmeal to the slides is discussed in detail. Two minutes after the start of the electrophoretic run the suitability of the arthropod meal for testing was apparent. When there was visible migration of more than one Hb fraction towards the anode, digestion has proceeded too far for host identification.

Bloodmeals of *Aedes togoi*, *Aedes aegypti*, *Culex fatigans*, *Anopheles* sp., *Rhodnius prolixus*, *Triatoma infestans*, *Triatoma dimidiata*, *Cimex lectularius*, *Pediculus humanus*, *Ornithodoros moubata*, *Ornithodoros savignyi* and *Ornithodoros rudis* from man, *Cebus* and rhesus monkey, dog, cat, rabbit, guinea pig, hamster, rat, mouse, chicken, pigeon, snake, toad and frog were studied and compared with the serum and hemolyzed blood of the host species. It was possible to identify the host from the pattern of the arthropod bloodmeals. A number of details on arthropod digestion processes are given.

### Introduction

It is well known that in the epidemiology and control programs of all arthropod-borne diseases, the host preferences of hematophagous arthropods are of primary importance.

It is known that there are specific differences in the electrophoretic protein spectra of animals. A few workers have even compiled classification keys based on electrophoresis of serum (DESSAUER and FOX, 1956; SIBLEY, 1960). The individual variations in such spectra are usually smaller than the differences between various species (STURKIE

and NEWMAN, 1951; STEPHEN, 1956; RAMIREZ and DESSAUER, 1956; ZWEIG and CRENSHAW, 1957).

In the present studies, arthropod bloodmeals were subjected to electrophoretic examination with the object of identifying the host, not with the object of studying the digestive process as such. However, some results of digestion have been considered and are discussed. First, it was necessary to determine whether, after a reasonable digestion time in the arthropod, there was still sufficient material present in the bloodmeal to obtain a characteristic electrophoretic pattern. If a sufficiently constant specific pattern could be demonstrated, a key could be compiled (BANGHAM and BLUMBERG, 1956; ASHTON, 1956; GRATZER and ALLISON, 1960). In a few cases a study was made of the differences and similarities between digested blood, serum and hemolysed blood of the same species.

The serological identification of arthropod bloodmeals is an intricate and rather costly process, requiring extensive organization, equipment and a trained staff, therefore it can be carried out only in a few highly specialized centers. As an electrophoretic method, micro agar gel electrophoresis was chosen, because it is possible to analyse very small quantities of protein with this technique (MILLS and SMITH, 1951; GIRI, 1956).

During the course of the experiments it was found that the sensitivity of the method permitted measurements of certain aspect of arthropod digestion, in particular protein breakdown processes. For instance, it was possible to detect differences in digestive activities of *Aedes togoi* when the bloodmeals were obtained from various host species.

## Techniques

### A. Methods of obtaining the arthropod bloodmeals

The most frequently used samples were bloodmeals from men as source. Other bloodmeals were from rhesus and *Cebus* monkeys, rabbit, hamster, guinea pig, cat, dog, sheep, goat, mouse, rat, chicken, pigeon, snake, toad and frog.

During feeding, the hosts were usually anaesthetized with an intraperitoneal injection of nembutal. Small cages with batches of about 30 mosquitoes (*Aedes togoi*, *A. aegypti*, *Anopheles* sp., *Culex fatigans*) were placed on the animals. In order to insure feeding, the mosquitoes were starved 24 to 48 hours in humid conditions. It was found that those mosquitoes which fed at all did so within about 10 minutes after application to the host. In a series of individual observations on mosquitoes it was found that the duration of feeding was from 20 to 50 seconds. *Ornithodoros* sp., *Rhodnius prolixus*, *Triatoma* spp. and *Pediculus humanus* were fed singly.

## B. Preparation of the arthropod bloodmeal samples

The extracted bloodmeal had to be prepared carefully in order to avoid gaping of the slit during the run, and agar depressions which result in trailing of the sample (fig. 3 E 2).

Removal of the intestinal content from the arthropod was done under a dissecting microscope. The gut contents were ground with 0.004 ml. homologous buffer in a microhomogenizer which was made by grinding a glass rod (4 mm. diameter) into the bottom of a thick walled short test tube (diameter of 5.5 mm.) with carborundum powder. The sample was taken up in a capillary of 1.8 mm. or less diameter, sealed at both ends and centrifuged for at least 30 minutes at about 600 g. In handling such small quantities of material, considerable caution was necessary to avoid loss of material or increase of ionic strength by evaporation. The narrow diameter of the capillary sometimes caused blockage during centrifugation. There was always a plug of fatty material above the supernatant and it was important to avoid contamination of the clear sample.

When trailing occurred it was overcome by centrifuging the sample again for a longer period. The sample was often filtered in order to reduce the time needed for preparation. Filtration had to be carried out in a capillary tube by packing the capillary with a small plug of cotton wool before centrifuging. Unless analysed immediately, the samples were stored in sealed capillaries at about 4° C. Disappearance during storage of the labile beta 2 globulin has been described (RABAËY, 1959), and was noticed as soon as 24 hours after storage.

## C. Preparation of buffer and agar

The best results were obtained with stock barbital buffer, pH 8.4, ionic strength 0.10 (R / sodium barbital 17.0 gram., HCl 1 N 23.5 ml., aqua destil. ad. 1.000 ml.). This stock solution was used for the electrode compartment.

For preparing the agar, the stock buffer was diluted 1 : 1 with distilled water pH 7.0—7.2 to prepare a buffer of 0.05 ionic strength and poured in a 500 ml. Erlenmeyer flask. This was placed in a water bath at 100° C. A long necked flask was placed upside down in the opening of the Erlenmeyer to reduce water evaporation. After 10 minutes, the desired quantity of agar was added (0.9 gm. Difco Special Noble Agar in 100.0 ml. buffer; RABAËY, 1959; WIEME, 1959 b). Heating was continued, and the flask shaken from time to time until the solution was absolutely clear with no undissolved agar particles. After exactly 20 minutes the Erlenmeyer was removed from the water bath, the neck cleaned and the flask cooled under running water. When the solution was 50°—55° C the contents were poured into the electrophoretic tank compartments and into Petri dishes. The flask was replaced in

the water bath. The Petri dishes were left untouched while gelification proceeded, producing a smooth horizontal surface.

After the layer in the Petri dishes had set (about 10 minutes at 18°C), microscope slides were placed on it and the remaining agar solution, measured in a warm cylinder, poured over them. Accurate calculation insured the exact amount of agar solution required to produce the desired thickness of agar film (0.6—0.8 mm.; 0.8—1.1 mm.; 1.0—1.4 mm.) on the slides. After gelification of this second layer (about 10 minutes at 18°C) the container was covered and placed in the cold room (4°C) overnight.

Small variations in the concentration of the agar did not cause differences in the results (RABAEY, 1959). Except in one case, concentrations of agar lower than 0.9% were not used because of the difficulty in handling this soft material. The age of the agar did not produce marked differences in results. Both slides and blocks have been used up to two weeks after preparation. Drying out of the agar was prevented by keeping the slides in the Petri dishes sealed with vaseline (paraffinum molle album).

#### D. Filling the tank (fig. 1)

The tank was filled to a point just above the inner edge of the median crossbars with the previously prepared agar solution. A cover

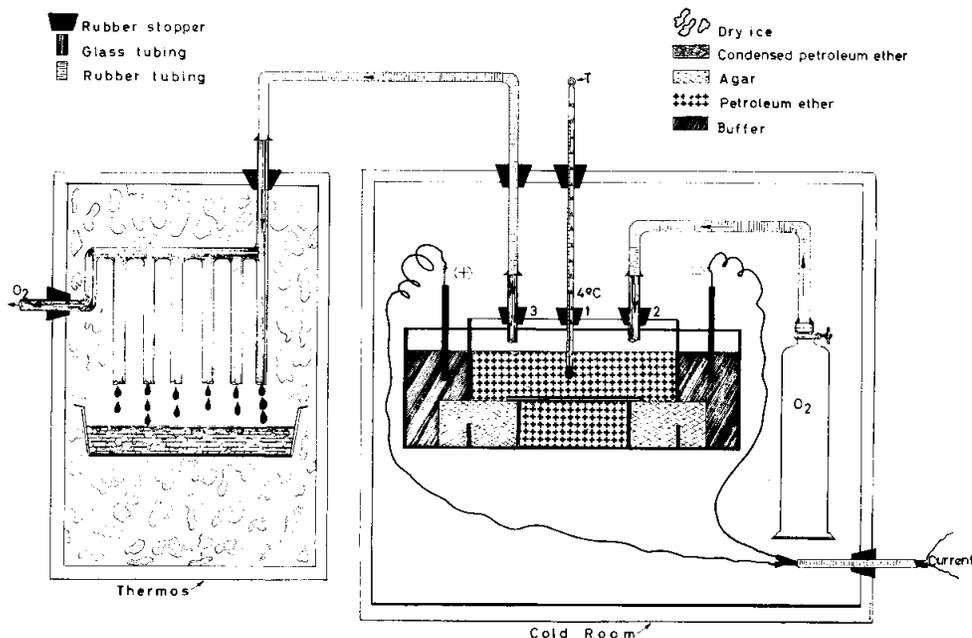


Fig. 1. Electrophoretic tank in partial section. Top of the tank and cooling system not shown (see fig. 2). All measurements in mm. Legend: A = large agar blocks, B = buffer compartments, C = agar connection slit, P = petroleum ether compartment, S = slide with agar film below, in contact with large agar blocks, (+) and (-) = electrodes; stippled areas represent agar.

was placed on the central part of the tank after the gel had set and the whole tank left overnight in a cold room (ca. 4° C). Next day the agar from the central compartment and the lateral half of the outer buffer compartments was cut out with a sharp knife. The lateral part of the outer compartments were filled with stock barbital buffer (pH 8.4, ionic strength 0.10) and platinum electrodes inserted. The central compartment was filled with petroleum ether (b. p. 40°—60° C) and a cover placed on the tank.

#### E. Application of the Sample

This operation requires considerable practice. As with all other manipulations involving these slides it had to be carried out under a dissecting microscope. To prevent contraction of the agar from the edges of the slide it was cut out of the Petri dishes immediately before use. The slit was located at the center of the slide and the length of the slit (3 to 10 mm.) adapted to the quantity of the sample available. Optimal results were obtained with slits 4—5 mm. long.

The slit was cut in the agar with a piece of razor blade (0.08 mm. thick) fixed to the short side of a microscope slide. The slit was then dried with a piece of filter paper, slightly narrower than the length of the slit and ca. 15 mm. long. After drying, the slit was inspected and any loose pieces of agar which could cause trailing (fig. 3 E 2) were removed with a fine needle. Slits prepared in this way allowed application of approximately 0.004 ml. of sample without spreading over the surface of the agar layer.

Because mobilities of sample fractions are calculated as relative distances, bovine albumin and depolymerized dextran (Macrodex, Poviet; formula under "Fixation of the slide") were used as standards. These could be applied either separately as minute drops (0.002 ml.) on each side of the slit, or as a single drop of the mixed solutions (25 volume parts Macrodex, 6 volume parts bovine albumin 30%, 69 volume parts homologous buffer) at the center of the slide.

Immediately after application of standards the sample was evenly applied to the slit with a finely drawn capillary pipet (diameter about 0.08 mm.). Special care is necessary to avoid air bubbles. The preceding operations were performed under standard conditions and thus approximately identical volumes could be applied each time (ca. 0.003—0.004 ml. filled a 5 mm. long slit). The slide was then placed face downwards on the agar blocks in the petroleum ether compartment of the apparatus (fig. 1), and the run started. The whole process of preparing the slide and application of the sample was completed as rapidly as possible to avoid drying of the agar.

## F. Electrical conditions, temperature and starting of electrophoresis

From the results of a number of experiments best results were obtained with a voltage of 25—60 volt per cm., giving a current of 18—24 m. A. with the system maintained at a constant temperature (temperatures between 1—7° C were used).

A good quality power pack was used to convert A. C. to D. C. (alternating current gives rise to the Joule effect resulting in distortion of the electric field and heat production). Regular observation of the power pack and A. V. O. meter were necessary to maintain constant voltage and current during the run. Variations in current were a sensitive indication of undesirable temperature changes in the agar film on the slide or in the surrounding petroleum ether (RABAEY, 1959; VAN SANDE, 1961; WIEME, 1959, 1959 a). An increase of 10% in the current could be allowed without distortion of the band pattern but when intensity increased beyond that point, the lid of the tank was removed partly or entirely, and an air current passed over the petroleum ether surface (ZAK, 1958).

The point was to obtain the highest possible field strength and to prevent disturbing temperature gradients in the agar film. Such gradients cause distortion of the final protein pattern. In later experiments a better method for regulating temperature was devised. The electrophoretic tank was covered with a plastic top in which 3 holes were bored. A thermometer was placed in one, another was connected with a cylinder of compressed oxygen and the third was the exit for escaping gas. A constant temperature was maintained in the electrophoretic tank by evaporation of the petroleum ether with oxygen. The escaping oxygen containing petroleum ether vapor was conducted from the tank via the third hole by way of a glass spiral into a thermos flask containing dry ice. The petroleum ether then condensed in the thermos flask and could be reused. To reduce the problem of temperature control, all work was done in a cold room (4° C) (see Fig. 2).

Duration of the electrophoretic run was between 15 and 30 minutes. Best results were obtained with a time of approximately 23 minutes. The polarity of the electrodes was reversed after each run, so pH shifts in the agar blocks were kept at a minimum and the tank could be used a number of times (about 50 runs).

To avoid contamination of the large agar blocks with sample material which had run off the slides during a previous run, thin agar underlayers were placed on the blocks. These were moved after each run to avoid back migration of proteins when the polarity of the electrodes was reversed.

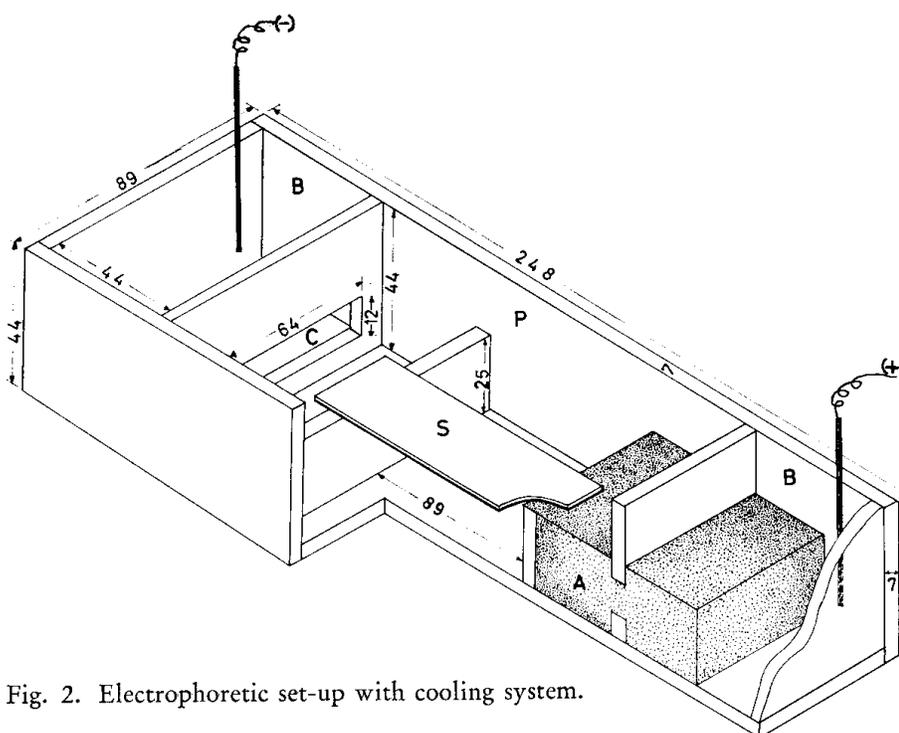


Fig. 2. Electrophoretic set-up with cooling system.

#### G. Fixation of the slide

The slides were fixed immediately after the run in the following solution:

R. / ethanol	70 vol.	Fixation takes
acetic acid	5 vol.	about 30 minutes
distilled water to	100 vol.	(WIEME, 1959 b).

Fixation has a fourfold effect:

1. Precipitation of the proteins which become visible within 5 minutes.
2. The Macrodex (Holland) becomes visible after ca. 30 minutes.

R. / Macrodex Poviet (Holland):

Depolymerized dextran	6 gram
Sodium chloride	0.9 gram
Distilled water to	100 ml.

(GRABAR and WILLIAMS, 1963; RABAEY, 1959; VAN SANDE, 1959, 1961; URIEL and GRABAR, 1956, 1956 a; WIEME, 1959 b).

3. Some of the buffer salts of the agar are removed.
4. Some of the water is removed from the agar.

#### H. Drying of the slide

After fixation the slides were dried for several hours in an oven at approximately 37° C. It was found that drying was more satisfactory when the slide was placed face downwards on a sheet of filter paper if precautions were taken to avoid trapping air bubbles between the agar

and the filter paper. This treatment removed more buffer salts and ensured even drying. Removal of the slide from the filter paper presented no difficulties if the temperature of the oven was not allowed to rise above 37° C. However, when the filter paper could not be removed without serious damage to the agar film the slide was placed in distilled water until the filter paper could be removed.

Later it was found more satisfactory to remove the filter paper when the agar was only partly dry (ca. 10 minutes). It is imperative that the agar gel dry into a thin, entirely crackless, transparent film before staining is undertaken.

## I. Staining methods

### (a) Serum proteins

The best results were achieved with amido black 10 B.

R. / Amino black 10 B	0.50 gram
Mercuri Chloride	5.00 gram
Acetic Acid	50.00 ml.
Distilled water to	100.00 ml.

Optimal staining took about 15 minutes and there was no advantage to longer staining.

### (b) Hemoglobin

In order to locate the presence of Hb components a number of slides or parts of slides were treated with benzidine before fixation or drying.

R. / benzidine dihydrochloride	1.0 gram
Glacial acid	20.0 ml.
distilled water	30.0 ml.
ethanol 90%	50.0 ml.

Stored in dark bottle at 4° C. Immediately before use 0.5 ml. of fresh 0.6% H<sub>2</sub>O<sub>2</sub> solution was added to 1.0 ml. of the stock benzidine solution (KRUPP et al., 1960).

## J. Differentiation

The stained slides were rinsed in several baths of a solution of 5% acetic acid in distilled water.

The differentiation was very rapid within 3—5 minutes. However, when the wet agar film was stained without previous drying, differentiation took about 12 hours (GRASSMAN and HANNING, 1952). After differentiation the slide was redried.

## K. Storage of the pherograms

The dry slides were stored without further treatment or were mounted with clear colourless Depex under long cover slips. No better results were obtained by clearing in toluol before mounting.

When long cover slips were used for the electrophoretic run, these were always mounted on normal microscope slides to avoid breakage (RABAËY, 1959). Unmounted pherograms, now 7 years old, are unchanged.

#### L. Examination of the pherograms

The distance between bovine albumin and Macrodex was taken as unity (1.00). Relative mobilities (Mr.) of the protein fractions in the samples were measured against this standard. Relative mobilities were measured by enlarging the slide approximately 10 times by means of a slide projector. The distance between the bovine albumin and Macrodex spots on the screen was a constant, but arbitrarily chosen, distance. By enlarging the slide, measurement of relative distance of migration of the protein bands was not only far more accurate, but much faster.

The absolute mobilities of the fractions, as shown on the slide pictures (fig. 3) depend upon the experimental conditions-voltage, time, m. A. and temperature. Photographs of the original slides run under different conditions of voltage, m. A., time and temperature are shown in fig. 3. Comparison with fig. 4, showing the results obtained by calculating the relative mobilities against the bovine albumin and Macrodex standards, indicates how comparable data can be obtained despite variations in experimental conditions.

This study was not concerned with optical density of the protein bands because no photometric scanner could be adapted to use on the slides.

### Results and observations

#### A. The serum proteins

In normal human serum the alpha-1-globulin component was faint and close to the albumin, the alpha-2- and beta-globulins appeared as compact bands, and the gamma globulins usually as one diffuse band (fig. 3 F). Sometimes there was a faint beta-2 band between the beta-1 and gamma globulins. Owing to electro-osmosis the gamma and beta-globulins migrated toward the cathode. The relative mobility of the albumin fraction was different in nearly all species examined. The rat and guinea pig albumin migration was very slow (fig. 3 I, J, K, L).

Human and monkey albumin migration was slightly slower than the bovine albumin standard (fig. 3 E, F). Rabbit albumin (fig. 3 G) migration was much faster than the bovine albumin standard, fowl and snake albumin migration was slightly faster (fig. 3 M, N) and the mouse and toad albumin (fig. 3 H) migrated with about the same speed as the bovine albumin. The pherograms of the various animal species were all different, and thus distinguishable from each other (fig. 3, 4). Similar

results were found by various authors (DEUTSCH and GOODLOE, 1945; ASHTON, 1958; HARRIS et al., 1958; RIVA, 1957; VAN ROYEN, 1951; WUNDERLEY, 1959).

#### B. Observations on hemoglobin (Hb) and remarks on arthropod digestion

A single free Hb band was usually observed in the pherograms of *Aedes togoi* bloodmeals within about 24 hours, and in *Rhodnius prolixus* and *Ornithodoros moubata* for as long as 14 days (fig. 3 A—E, H—J, N). This dark, benzidine positive band was situated in the position of the beta-globulin. In the arthropod meal pherogram of the guinea pig, however, the position was always characteristic and in contact with the slit (fig. 3 L).

In arthropods fed on monkeys, and once in those fed on a human, this band was split into two closely adjacent bands. At the cathode side of the slit, and in contact with it, was a heavily stained benzidine positive band at the hapto-gobulin position. This band was present in the arthropod bloodmeals and in hemolyzed blood of men and monkeys. The binding of Hb to serum hapto-globulin (fig. 3 A-D, F, H-J) has been described by several workers (ALLISON 1961; JAYLE et al., 1952; VAN ROYEN, 1951; WIEME, 1959 b). After a longer period of digestion the free hemoglobin begins to move to the cathode. In the animals fed on guinea pigs, however, the free Hb band keeps the characteristic position very near the slit (fig. 3 L 2).

When the *Aedes togoi* meals were digested for more than 24 hours the three Hb fractions soon after the start of the electrophoresis, moved toward the anode. In bloodmeals from humans this happened after a longer period of digestion (about 2 days). In the pherograms of arthropod bloodmeals on most hosts, three very narrow bands could be found about 4 mm. from the anode side after a run of 2 minutes. After 5 minutes the bands were rather diffuse and broad, and spread over a length of about 4 cm. towards the anode. The middle band is always the narrowest and darkest. After about 12 minutes, depending on the electrical conditions, the band nearest the anode moves off the slide (fig. 3 K, L). The middle Hb band is always found at the position of the albumin of the species, even after a run of 35 minutes. This suggests that this Hb fraction is in some way associated with the albumin. The band nearest the slit takes an extremely diffuse position between the slit and the albumin.

The splitting off of these Hb bands can clearly be seen with the naked eye during and after the electrophoretic run. All three are benzidine positive. However, the fraction nearest the anode, which migrated off the slide in about 12 minutes, is very weakly benzidine positive.

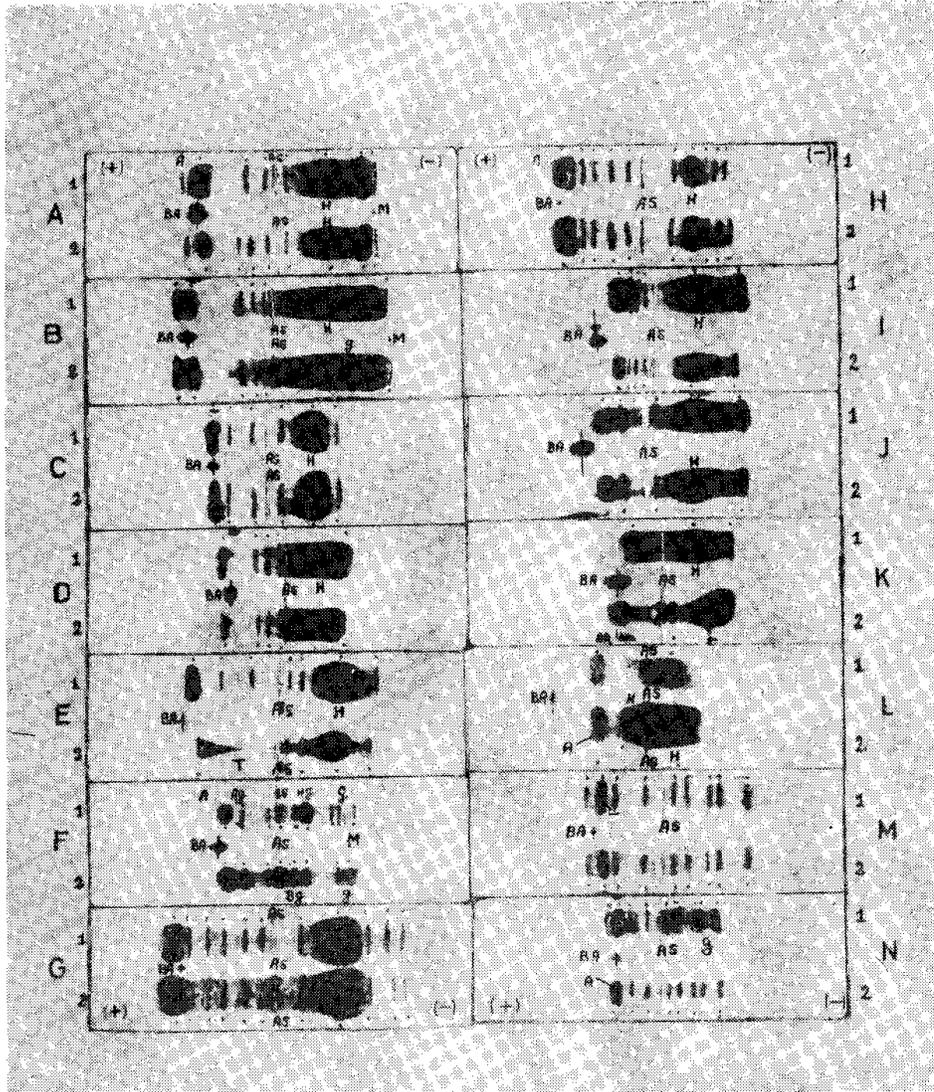


Fig. 3. Photographs of original slides.

Explanation:

- A. 1, 2. *Ornithodoros moubata* bloodmeals (goat) 12 days after ingestion, electrophoretic run 22 min., 40 V.
- B. 1, 2. *Rhodnius prolixus* bloodmeals (goat) 2 days after ingestion, electrophoretic run 26 min., 60 V.
- C. 1, 2. *Rhodnius prolixus* bloodmeals (sheep) 5 days after ingestion, electrophoretic run 20 min., 35 V.
- D. 1, 2. *Rhodnius prolixus* bloodmeals (dog) 13 days after ingestion, electrophoretic run 18 min., 35 V.
- E. 1. *Rhodnius prolixus* bloodmeals (human) 3 weeks after ingestion, electrophoretic run 28 min., 70 V.
- E. 2. Previous sample showing trailing due to a small agar particle left in the application slit. Electrophoretic conditions as in E. 1.

- F. 1. Human serum slightly hemolyzed, showing beta-globulins towards the anode. Electrophoretic run 18 min., 30 V.
- F. 2. Previous sample, kept at 4° C for 48 hours before application. Electrophoretic conditions as in F. 1.
- G. 1, 2. *Ornithodoros moubata* bloodmeal (rabbit) 11 days after ingestion, electrophoretic run 23 min., 60 V.
- H. 1, 2. *Aedes togoi* bloodmeal (mouse) 20 hours after ingestion, electrophoretic run 26 min., 55 V. (Unchanged hemoglobin still present)
- I. 1. *Rhodnius prolixus* bloodmeal (rat) 12 days after ingestion, electrophoretic run 18 min., 35 V.
- I. 2. *Aedes togoi* bloodmeals (rat) 22 hours after ingestion- electrophoretic run as in I. 1.
- J. 1. *Rhodnius prolixus* bloodmeal (rat) 16 days after ingestion, electrophoretic run 23 min., 40 V.
- J. 2. *Ornithodoros moubata* bloodmeal (rat) 3 weeks after ingestion, electrophoretic conditions as in J. 1.
- K. 1. *Aedes togoi* bloodmeal (rat) 28 hours after ingestion, electrophoretic run 16 min., 25 V.
- K. 2. *Aedes togoi* bloodmeal (rabbit) 31 hours after ingestion, electrophoretic run as in K. 1. Slide K clearly shows the different position of albumins and hemoglobins in rat and rabbit, and the distinct band in the beta-globulin position typical of the rabbit (compare with G. 1 and 2).
- L. 1. *Rhodnius prolixus* bloodmeal (guinea pig) 4 weeks after ingestion, electrophoretic run 23 min., 60 V.
- L. 2. *Aedes togoi* bloodmeal (guinea pig) 38 hours after ingestion, electrophoretic conditions as in L. 1. Slide L shows the very short migration of guinea pig albumin and the typical position of the hemoglobin.
- M. 1. *Rhodnius prolixus* bloodmeal (chicken) 10 days after ingestion, electrophoretic run 19 min., 45 V.
- M. 2. Previous conditions but kept 4 days at 4° C before application. Electrophoretic conditions as in M. 1.
- N. 1. Serum of chicken slightly hemolyzed, electrophoretic run 23 min., 32 V.
- N. 2. Serum of chicken not hemolyzed, electrophoretic run as in N. 1.

Legend: A = albumin of host, AR = artefact, AS = application slit, BA = bovine albumin, BS = beta-globulin position, G = gamma-globulin position, H = hemoglobin, HG = hapto-globulin position, M = dextran (Macrodex), T = trailing, (+) and (-) = electrodes.

The rate of digestion of the bloodmeal can thus be determined during the first 2 minutes of electrophoresis. If the splitting of the narrow Hb bands is observed, the feed is usually not suitable for identification, because only the Hb and albumin bands can be demonstrated on the stained slide. In *Aedes togoi* fed on humans and monkeys this phenomenon appeared after 40—46 hours. In *Aedes* fed on other animals it appeared after about 24 hours, and in *Rhodnius prolixus*, *Triatoma* sp. and *Ornithodoros* sp. it was observed only after respectively 9—14, 14—18 and 22—38 days of digestion.

Certainly many factors in addition to the length of time elapsing since the arthropod was fed are responsible for the actual degree of

digestion. These include: laboratory maintenance vs field conditions, activity of the individual arthropod, temperature and other environmental factors, individual variations within the species both of the arthropod and its host. In the laboratory, anesthesia of the host was necessary. A variety of anesthetics were tried, and Nembutal had the least apparent effect upon the arthropods. Possible effects of host anesthesia on arthropod digestion are unknown.

### C. The patterns of bloodmeals

The patterns of arthropod bloodmeals of men and 15 other species showed a number of lines which had different positions on the pherogram after calculation of the relative mobilities. One factor allowing host identification was that the mobility of the serum albumin remains unaltered during the whole of the arthropod digestion and relative mobility is identical with that of the albumin in unhemolyzed serum.

The most marked difference between human and monkey arthropod bloodmeals was that in human feeds the band nearest the albumin was more heavily stained, while in monkey feeds that nearest the slit was more heavily stained. In the monkey feeds a pre-albumin band was visible. This fraction was not present in human feeds. In normal human serum this fraction always moves off the slide (WIEME, 1959 b).

In chicken the position of three bands rather close together at the anode side of the slit was characteristic (fig. 3 M). No less than 10 bands were present in the chicken feed of *Rhodnius prolixus* after 10 days of digestion (fig. 3 M). The guinea pig feed were easy to distinguish by the very short migration of the albumin, the position of the free hemoglobin in the hapto-globulin and beta-globulin position at the cathode side, and a small fraction at the anode of the slit (fig. 3 L). In the rabbit feed the bands at the cathode side of the slit were typical (fig. 3 G).

Other differences in pherograms between dog, sheep, goat, rat, mice and other species can be evaluated from the figures (3, 4).

## Discussion

The most important criteria for differentiation of the pherograms of the species examined are:

### A. Serum proteins

1. Relative mobility of the albumin fraction.
2. Different position and shape of the bands visible at the anode side of the application slit in the alpha-globulin and pre-albumin positions.

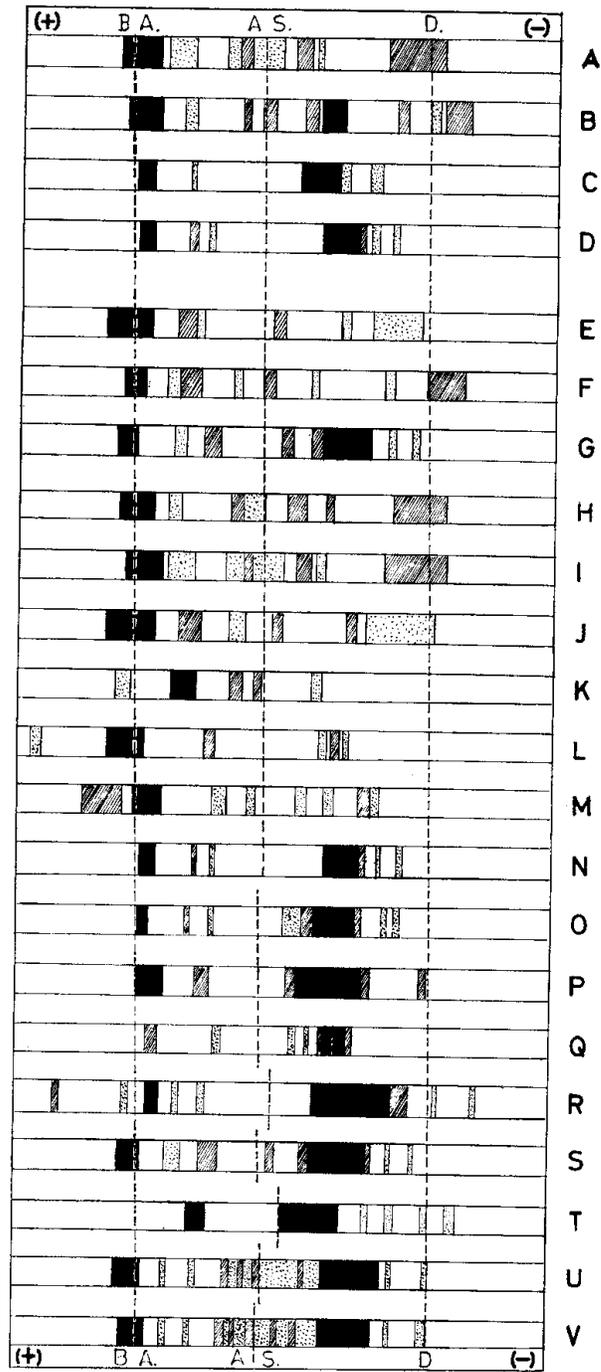


Fig. 4. Diagrams of relative mobilities.

- A: Human serum.
- B: Human serum slightly hemolyzed.
- C: Bloodmeal of *Aedes togoi* (human) 2 hours after ingestion.

- D: Bloodmeal of *Aedes togoi* (human) 48 hours after ingestion.
- E: rabbit serum.
- F: rabbit serum slightly hemolyzed.
- G: bloodmeal of *Aedes togoi* (rabbit) 24 hours after ingestion.
- H: human serum.
- I: Ibid.
- J: rabbit serum.
- K: Guinea pig serum.
- L: mouse serum.
- M: chicken serum.
- N: bloodmeal of *Aedes togoi* (human) 48 hours after ingestion.
- O: bloodmeal of *Rhodnius prolixus* (human) 14 days after ingestion.
- P: bloodmeal of *Aedes togoi* (rhesus monkey) 5 hours after ingestion.
- Q: bloodmeal of *Rhodnius prolixus* (rhesus monkey) 9 days after ingestion.
- S: bloodmeal of *Aedes togoi* (rabbit) 24 hours after ingestion.
- T: bloodmeal of *Rhodnius prolixus* (chicken) 10 days after ingestion.
- V: bloodmeal of *Ornithodoros moubata* (chicken) 10 days after ingestion.

Legend: AS = application slit, BA = bovine albumin, D = dextran (Macrodex), (+) and (-) = electrodes.

The diagram shows bands of three intensities. The darkest one towards the anode always corresponds with the host albumin, the darkest towards the cathode always corresponds with the free hemoglobin. Mobilities were measured relative to the center of the bands shown in the diagrams. Exceptions were the hemoglobin bands and the diffuse bands in the gamma-globulin position. The maximum density of these bands was sometimes displaced from the center of the band.

3. Difference in position and shape of the bands at the cathode side of the application slit in the beta-globulin position, if not covered by the free hemoglobin.
  4. Difference in position and shape of the bands at the cathode side of the application slit in the gamma globulin area.
  5. Presence or absence, shape and position of the postalbumin.
  6. Optical density differences between bands.
- B. Hemoglobin complex
1. Position of the free hemoglobin and eventual splitting of this band (attention should be given to the possibility of artefacts in this regard).
  2. Presence or absence of a heavy band in the hapto-globulin position at the cathode side of the application slit.
  3. Position of other benzidine positive bands.

It is well known that electrophoretic patterns of sera show individual differences within a species. The variation in intensity of certain globulin bands is often due to factors such as: species, strain, age, sex, thirst, pregnancy, nutritional state, hormone balance and diseases, etc. (ABRAMSON, 1936; STURKIE and NEWMAN, 1951; ASHTON, 1957; MAI-NARDI, 1957; JACOB and TAPPEN, 1956; VAN SANDE, 1959, 1961).

In general, the quantity of albumins are lowered in all pathological conditions. The alpha and beta-lipoproteins (carriers of ca. 75 % of the plasma lipids) are increased in many diseases. The gamma globulins (carriers of most antibodies) are increased in most infectious diseases (VAN ROYEN, 1951; WUHRMANN and WUNDERLEY, 1952).

Many accounts have been published of the electrophoresis of various animal hemoglobins, most of them using paper but others using starch gel (JAYLE et al., 1952; ITANO, 1953; BANGHAM and BLUMBERG, 1956; BANGHAM and LEHMANN, 1956; DESSAUER and FOX, 1956; FINE et al., 1956; JACOB and TAPPEN, 1956; GRATZER and ALLISON, 1960; ALLISON, 1961).

Multiple hemoglobins have been recorded from a number of animals. There have been several reports that the hemoglobin of lizards and snakes move more slowly, and that of amphibians more rapidly, than that of humans (STURKIE and NEWMAN, 1951; DESSAUER and FOX, 1956; STEPHEN, 1956). However, it is not known whether certain species differ consistently in the migration of the breakdown products of Hb due to arthropod digestion, and whether this difference can be used for host identification. Certainly, the pattern of the partly digested and undigested guinea pig Hb was different in position on the slide from that of other species investigated (WIGGLEWORTH, 1953).

It is not yet clear whether the non-appearance of certain bands in the various runs of arthropod meals is due to digestion by insect enzymes, or whether the concentration of these proteins was too low to be shown by the staining method used. Between the moment of killing the insect and the end of the electrophoretic run a period of at least 1 1/2 hours elapsed, due to the preparation of the feed. RABAEY (1959) showed that certain globulin bands in serum disappeared within one to two minutes when 0.05 % trypsin was added. If such enzymes are present in the arthropod digestive system, it would not be surprising if loss of bands occurred. According to RABAEY (1959) quantities of less than 0.1 gamma of protein can not be demonstrated by agar electrophoresis. Using the method described it was possible to detect bands when only 0.05 gamma of protein was available.

After certain digestion time (for rabbit blood one day, for human blood about 46 hours) the Hb does not migrate towards the cathode as a compact entity, but is split into 3 or more components with different sensitivities to benzidine. At least three of the components migrate toward the anode, and at least one fraction could always be located at the albumin position on the pherogram.

The value of this technique is shown by the fact that similar pherograms were obtained from bloodmeals of the same host from taxonomically very different arthropods.

Digestion by the different arthropods of vertebrate blood seems to

be quantitatively similar, but different in speed. At the present time the reasons for the marked differences between chicken and pigeon pherograms are not clear.

### Conclusion

It was possible to identify the following host animals by the relative mobilities of serum proteins and hemoglobins in the pherograms of their respective arthropod bloodmeals: man, monkey, dog, cat, rabbit, rat, mouse, guinea pig, hamster, chicken, pigeon, snake, toad, and frog. When fed on the same species the protein and hemoglobin bands were in the same characteristic positions for *Aedes togoi* (after 24 hours or less), *Rhodnius prolixus* (up to 9—11 days) and for *Ornithodoros moubata* (up to at least 2 weeks).

It is remarkable that the mobility of the serum albumin of a species is unaltered during the whole period of arthropod digestion.

When digestion has proceeded to the point at which the splitting of Hb occurred, only albumin and Hb bands were shown by the protein stain, and therefore, the meal was no longer identifiable (except in case of rat and guinea pig).

Within the limits of the number of species used in the experiments, the protein pattern of the bloodmeals was characteristic. The main characteristics of the patterns of five species are summarized in a simple key as example.

- |     |  |               |
|-----|--|---------------|
| 1a. | Mr of albumin 1.000 or more . . . . .  | 2             |
| 2a. | Two fractions on the anode and one the cathode side of application slit . . . . .  | rabbit        |
| 2b. | Three fractions on the anode side and one or two on the cathode side of the application slit . . . . .                                   | chicken       |
| 1b. | Mr of albumin less than 1.000 . . . . .  | 3             |
| 3a. | Mr of albumin 0.95—0.99 . . . . .  | 4             |
| 4a. | A pre-albumin fraction usually visible. Either a single band at Mr 0.75 or a darker band at Mr 0.75 and a pale band at Mr 0.85 . . . . . | rhesus monkey |
| 4b. | No pre-albumin present. Either a single band at Mr 0.85 or a darker band at Mr 0.85 and a paler band at Mr. 0.75 . . . . .               | human         |
| 3b. | Mr of albumin less than 0.90 . . . . .   | guinea pig    |

The results suggest that arthropod bloodmeals from a great variety of animals might be identified from their protein and Hb patterns by use of the agar gel electrophoretic method.

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