

Subtitle: Comparative Studies in the Pathology and Host Physiology of Malarias. Serum Protein Alterations in P. Coatneyi Malaria. A comparison of Cellulose Acetate and Polyacrilamide Disc Electrophoretic Patterns.

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Numerous observations have been made on the alterations of serum proteins during the course of malaria. The subject was reviewed by Stauber (1954) and the more recent knowledge summarized by Sadun et al (1966). In general, it has been found that gamma globulin increases, albumin decreases, and there is no consistent pattern of change in the alpha and beta globulins.

This present paper describes changes in serum proteins during P. coatneyi malaria of rhesus monkeys. In a previous paper in this series (Desowitz et al, 1967) it was shown that this parasite produced a wide spectrum of disease ranging from acute fatal to mild chronic. It was of interest, therefore, to determine whether the different types of infection would produce characteristic derangements in serum protein components.

Two methods of serum protein analysis have been employed in this study; conventional electrophoresis on cellulose acetate and disc electrophoresis. The latter is a relatively new technique in which separation is dependent not only on electrical charge but also the weight of the molecule. It will be seen that alterations are evident by disc electrophoresis that are not detectable by conventional zone electrophoresis.

#### METHODS

The course of infection and concomittant serum chemistry, haematologic, and histologic alterations for all infected rhesus monkeys were described previously (Desowitz et al, 1967). An aliquot of serum was obtained from all bleedings, usually at weekly intervals, for the estimation of total protein by the method of Weichselbaum (1946) and for thymol turbidity by the technique of Shank and Hoagland (1946). Microzone electrophoresis on cellulose acetate strips was performed and analyzed by the Spinco Analytrol apparatus and polyacrilamide disc electrophoresis by the Canalco model 12 (Canalco Co., Rockville, Md) and then scanned with the Densicord Model E densitometer (Photovolt Corp., New York).

#### RESULTS

##### Zone electrophoresis:

Serum proteins, as measured by cellulose acetate zone electrophoresis, for four representative infections are shown in fig. 1. Also illustrated in this figure are the course of parasitaemia and serum thymol turbidity. In all animals studied there was an increase in gamma globulin. In the Indian rhesus (KL series) per cent increase over the preinfection value ranged between 140% to 200% while in the Thai rhesus (MS and SP series) the increment was from 40% to 70%. In both groups there was no appreciable rise in gamma globulin during the first 20 days of infection, the period of the primary parasitaemic attack. In the Indian rhesus it increased with relative rapidity after this period while in the Thai rhesus, as will be seen from fig. 1, the incremental slope was much more gradual. The increase in thymol turbidity seemed to reflect the development of the hypergammaglobulinaemia. The rise in gamma globulin and thymol turbidity were, approximately, concurrent and proportional. There also appeared to be a relationship between gamma-globulin level and the course of the parasitaemia. In the Indian rhesus, which exhibited a rapid and marked rise of gamma-globulin, the primary attack was terminated about 30th to 40th day and the parasitaemia was scanty thereafter. In contrast, as noted previously, gamma-globulin increase in Thai rhesus was more gradual and lower. In these animals the primary parasitaemia usually persisted somewhat longer and the parasites were more numerous during the chronic phase.

In all animals, the beta globulin increased to varying degrees. Usually this rise was concurrent with that of the gamma-globulin. During the first 30 to 40 days of infection the alpha-globulin also rose. In some animals this was of a transient nature while in others the elevation persisted throughout the entire period of observation. Albumin decreased in all animals during the primary parasitaemia. There was a gradual recovery toward normal or greater than preinfection levels in the Thai rhesus while in the Indian rhesus the albumin level tended to remain depressed during chronicity.

#### Disc electrophoresis

Unlike conventional zone electrophoresis, disc electrophoresis is dependent upon the combined effect of molecular sieving in a polyacrilamide gel matrix and electrophoresis in a discontinuous buffer system. This resolves many more protein fractions than do the zone techniques. Eventually a new taxonomy of serum proteins may have to be devised to accommodate this new method. Several workers have attempted to correlate disc and electrophoretic fractions but there is still considerable confusion. Fig. 2 attempts to summarize the identification of disc serum electrophoretic components as proposed by a number of investigators. The regions of 19S and 7S globulins have been localized with reasonable certainty although both components show considerable heterogeneity. This is particularly true of the 7S globulin region. The identification of the IgM is still tentative and is based upon disc electrophoresis of a fraction isolated by column chromatography (Desowitz and Russell, unpublished results). The transferrin ( $\beta_1$ -globulin region) was, in some animals composed of two distinct components.

Disc electrophoresis of serial serum samples from infected monkeys evidenced certain progressive alterations not detectable by the conventional zone technique. The most notable of these changes was the consistent increase in macroglobulins in all monkeys studied. The major macroglobulin elevation was in the fraction tentatively identified as IgM. The increase in IgM occurred approximately between the 25th and 40th day although one animal, MS2, the elevation was not detected until the 77th day. The gradual increase of IgG, occurred concurrently with that of the IgM. The transferrin component also increased and this probably reflected the rise in  $\beta$ -globulin noted in the zone electrophoretograms. Alterations in post albumins (probably  $\approx 1$  components) varied from animal to animal as it did in the zone technique. Two typical examples of disc electrophoretograms of serial serum samples are shown in fig. 3.

## DISCUSSION

The increase in gamma-globulin during *P. coatneyi* conforms to the general picture of hypergammaglobulinaemia described for many other malaria infections. Disc electrophoresis revealed increased concentrations of IgM and IgG although as would be expected the major moiety of the immunoglobulins was of the latter type. There seemed to be a discernible causal relationship between gamma globulin production and the course of parasitaemia. Kuvin et al (1962), Abele et al (1965), Lunn et al (1966) and Tobie et al (1966) have made similar observations on the influence of immunoglobulin level on the parasitaemia. Although the notion may be heretical in this era of sophisticated serology, we suggest that the direct measurement of the immunoglobulins may be as good as or a better indicator of functional immunity than many of the available serologic techniques. A number of studies such as that by Lunn et al (1966) have revealed a discrepancy between gamma globulin and antibody levels and that the immunoglobulin concentration seemed to represent a more rational causal relationship to the immunologic factors affecting the dynamic balance of the host-parasite system. An additional deficiency in present day serology applied to malaria is that the techniques in common use do not seem to measure, directly, protective antibody (Targett and Voller, 1965; Mahoney et al, 1966).

While the role of the 7S immunoglobulins in malaria has been well defined by McGregor and his colleagues (Cohen et al, 1961; Cohen and McGregor, 1963; McGregor et al, 1963) less is known of the macroglobulins participation in the immune response. It has recently been demonstrated that there was a marked increase of IgM during the primary attack and relapses of volunteers infected with *P. vivax* and *P. cynomolgi* (Tobie, 1965; Abele et al, 1965; Tobie et al, 1966). Elevated IgM has also been found in the sera of people living under conditions of hyperendemic malaria (Turner and Voller, 1966). This present study

employing disc electrophoresis has also shown this increase in immunoglobulin. We have also found, in confirmation to Tobie et al: (1966) observation, that during the primary attack both IgM and IgG show a coincidental elevation. As Tobie et al have pointed out, this finding is contradictory to the accepted concept of sequential immunoglobulin synthesis. The functional role of IgM in malaria is still unknown. While it does not seem to possess protective properties (Cohen et al, 1961) the sephadex gel filtration IgM fraction was serologically positive with the fluorescent antibody test. It should be pointed out, however, that we have found by disc electrophoresis, contamination of IgG in similarly isolated fractions of IgM and this may be at least partly responsible for the serologic reaction. There is also the interesting suggestion by Houba and Allison (1966) that some of the IgM in people living in hyperendemic areas may be of a type related to rheumatoid-factor-like macroglobulin. Obviously, better serologic techniques which are truly indicative of the various functional immune mechanisms will have to be developed before the roles of the different immunoglobulins can be determined.

Despite the often repeated observation of lowered serum albumin during the clinically active stages of malaria little is known as to the underlying causative mechanism. Maeraith (1948) has hypothesized that the fall in albumin concentration is due to a functional hepatic derangement in albumin synthesis. However, in the absence of any data on total albumin pool, rates of synthesis and turnover it is not possible to explain the hypoalbuminaemia. It should be noted that in our animals there was no significant loss of albumin in the urine (Desowitz et al, 1967).

#### SUMMARY

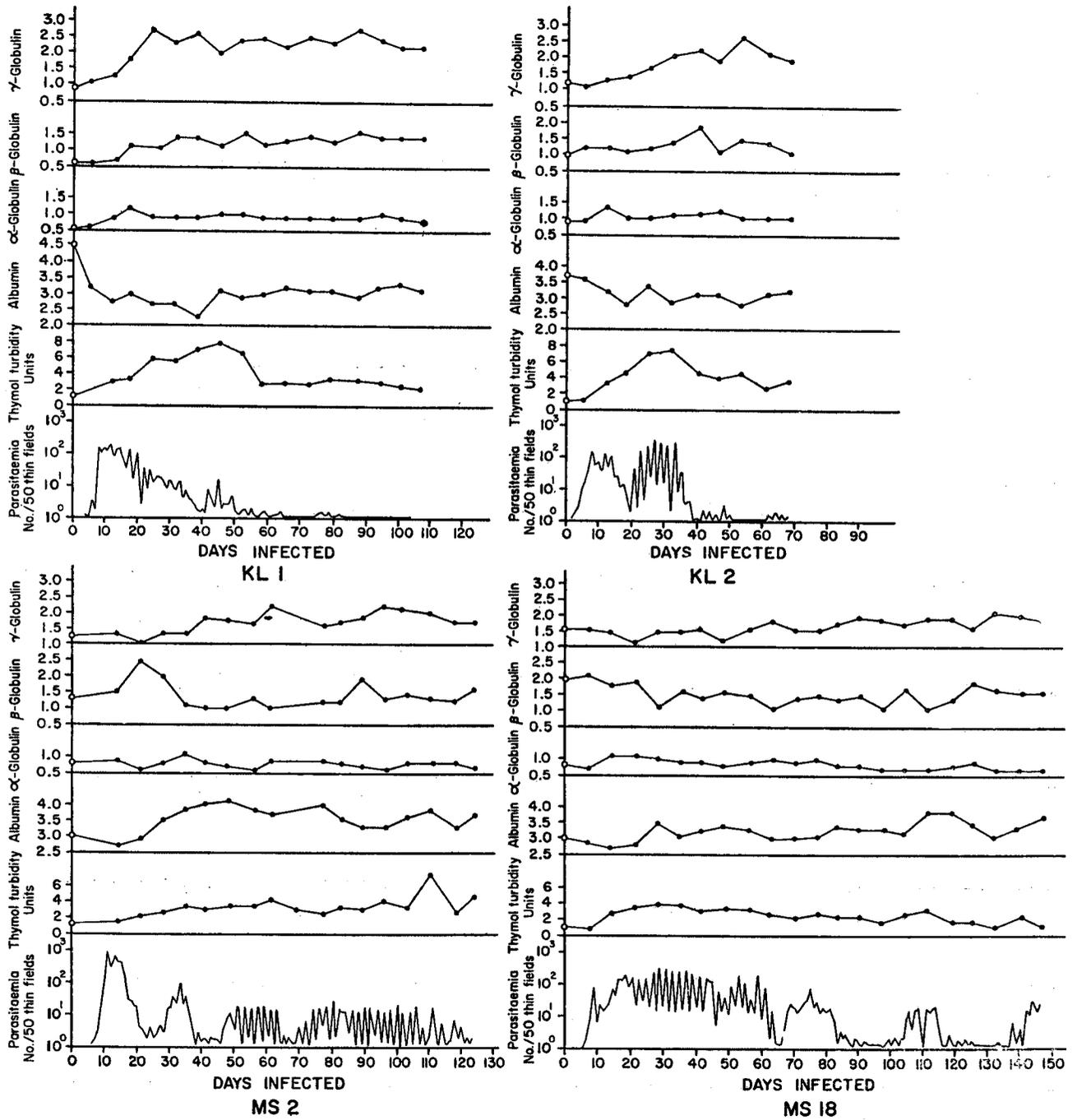
Alterations in serum proteins during the course of P. coatneyi malaria in rhesus monkeys have been measured by the cellulose acetate zone and polyacrilamide disc electrophoretic methods. Zone electrophoresis evidenced elevations of gamma, beta and alpha globulins during the course of infection. Albumin decreased during the primary infection. Disc electrophoresis indicated a concomitant increase of IgG and IgM after the 25th day of infection.

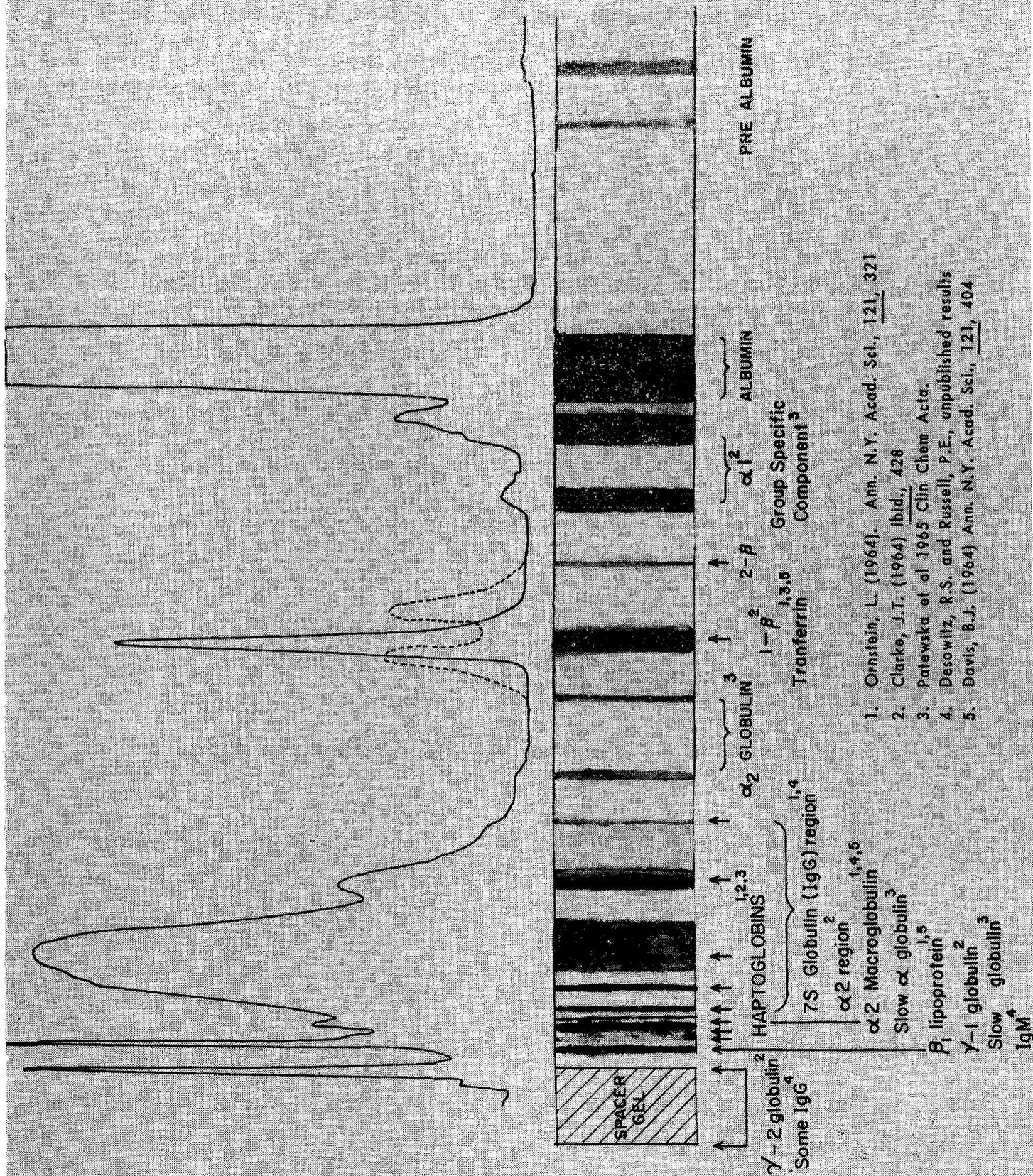
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Fig. 1: Serum proteins and parasitaemia during the course of four representative *P. coatneyi* infections

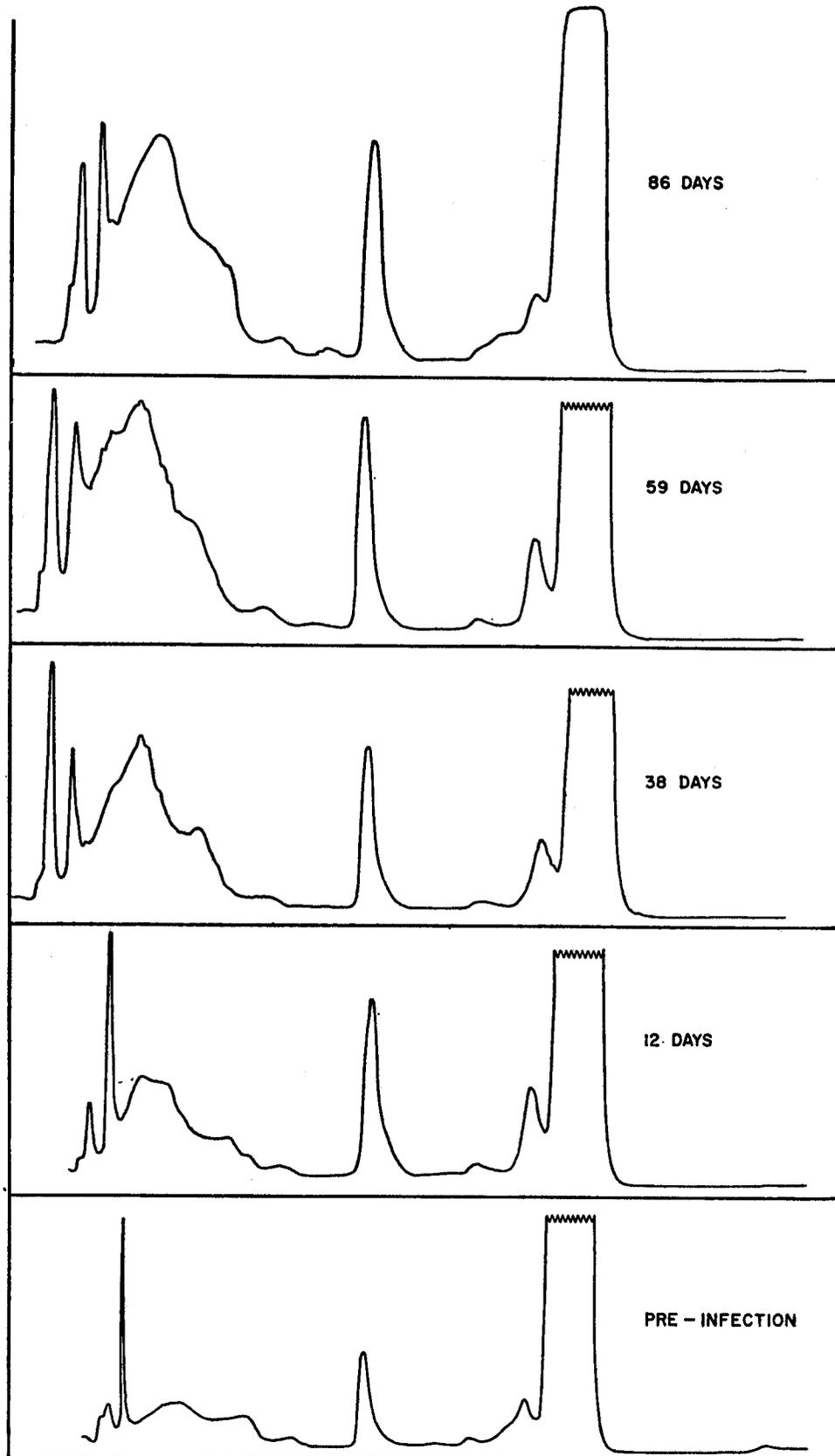




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Fig. 2. Identification of disc electrophoresis components according to various authors.

Fig. 3. Disc electrophoretic changes during the course of *P. coatneyi* infections



KL 1

