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Electrophoresis in infectious diseases

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Electrophoresis is simply the migration of charged colloid particles in an electric field toward the oppositly charged electrode. Proteins posses an electrical charge as a result of their component amino acids, which exist in varying degrees of polarity depending on the pH of the solution in which the protein is dispersed. These amino acids posses both an amino (or positive) group, and a carboxyl (or negative) group. Which charge is dominant depends on the degree of depression of the opposite charge or group. Thus, in alkaline solutions, the positive amino ionization is depressed, leaving the solution more negative and with the amino acid tending to migrate toward the positive electrode, while in acid solutions, the situation is reversed. Moreover, the degree of acidity of alkalinity will tend to result in corresponding degrees of charge, and since different proteins have different isoelectric points, they will also tend to have different degrees of charge at the same pH, and therefore show different tendencies toward migration in an electrically charged field. These tendencies are apparent to a degree in the rate of migration of the proteins toward the charged poles, and taking advantage of this varying rate, differently charged proteins should be separable to some degree when exposed

for a prolonged period to an electric field. This, in general, is the principle in the separation of the different electrophoretic components. The rate of migration of the colloid particles also depends on other poorly understood factors, however, so that proteins of similar function do not necessarily migrate at a similar rate of speed. However, since antibodies are generally considered to be protein in nature, it was thought that they might migrate in a sufficiently distinctive manner that they would perhaps be separable from the rest of the blood, or add distinctive enough characteristics to the usual blood picture to be of some diagnostic value.

The Normal Electrophoretic Picture

The normal electrophoretic picture is highly variable, rather marked individual differences existing between persons who are clinically considered to be normals. Moreover, a rather limited number of values have been published for so-called normals, many of these using different techniques or buffer solutions, so that as a result, only rather gross differences in the values of any one sera can be considered of significance, lesser differences tending to overlap with the present "normals". (1)

All methods of electrophoresis resolve normal human

sera into a faster moving albumen component, followed in order by alpha, beta, and gamma globulin components. Varying the composition and pH of the buffer solution will tend to further subdivide or alter the pattern in many cases. In his early writings, Tiselius (2) stated that the beta and gamma fractions might possibly be subject to further subdivision, as the diffuseness of their boundaries indicated that they were more heterogeneous than the alpha fraction. However, the components into which the Tiselius apparatus divides the plasma proteins do not represent homogeneous molecular species, either with respect to size, charge distribution, solubility, or physiological function, so that even though the fractions are further broken down, the seraration of specific immunological components in the present type of apparatus is not likely (3).

The Function of the Globulins

Despite these objections, the function of the components so far distinguished has been determined to some extent, indicating the possibility that the physical properties which make the groups serarable by the apparatus also determine, to some extent, the physiological activity of the groups components. Nevertheless, the proteins of interrelated function do not always posses

comparable chemical and physical properties, and therefore are not always in the same electrophoretically separable fractions (3).

At present it has been determined (4)that the sera of a febrile person shows an increase in the alpha globulin component, as manifested by an alpha/albumin ratio of approximately twice normal and a decreased albumin/globulin ratio. An increase in the alpha component also takes place with any considerable inflamation or tissue destruction, regardless of cause(5).

The first studies of immune sera (utilizing re-crystallized egg albumen as the antigen) indicated that an increase in gamma globulin was present (2), an observation which has been amply substantiated by work with many other antigens. (However, in the case of many virus caused diseases, an increase in the gamma fraction was not noticeable (6), and in the case of sensitivity to rag weed pollen, no perceptiable change was observable in the gamma fraction, although the antibody could be demonstrated in this fraction by passive transfer experiments (7).) Further evidence that immune bodies are concentrated in the gamma fraction is offered by the discovery that fetal sera is relativly rich in gamma globulin, the absolute concentration being above that for

the maternal sample. This discovery is compatable with the high degree of immunity in the new born infant (8). However, studies on children with a congenital defect in protein formation which results in an almost complete lack of gamma globulin (1 and 9) indicate that antibodies are apparently concentrated elsewhere too. These children show no clinical susceptibility to infection, and were particularly free from the usual colds and sore throats. Moreover, the Dick and Shick tests also indicated that antibodies were present. The Mantoux was negative, however, despite a calcific focus in the chest, and injection of Diptheria toxoid, Typhoid vaccine, and Pertussis vaccine did not result in a positive Widal, the presence of Pertussis antibodies, or any rise in serum proteins. Therefore, it is quite possible that humoral antibody is not the only basis for the individuals resistance to infection, and that cellular immunity may play a definite role in producing antibodies rapidly (9).

It is thought that the immune bodies are concentrated in the gamma fraction in many cases due to an asymetric charge distribution on the gamma globulins, resulting in a strong interaction with other proteins and electrolytes (3). (On albumen the charges are nearly symetrical.) There is some quantitative evidence that such interaction does take place in the case of immunization. Gamma

globulin of both normal and immune horse sera is similar in mobility, but different in antigenic behavior, and their molecular weights are correspondingly different; that of normal gamma globulin being about 150,000, while that of the immune gamma globulin is about 910,000, or approximatly six times the normal weight, indicating that agregation has taken place.

The Electrophoretic Pattern in Disease Leues

No adequate normal pattern has been established for serologically positive syphylitic sera. J. A. Cooper (11) states that shortly after infection takes place, a decreased albumin/globulin ratio, with an increase in all three globulins is evident electrophoretically, associated with a normal total serum protein. This situation is said to persist throughout the course of the disease unless therapy takes place. On serological examination of the electrophoretically isolated fractions, the reagen for both the Wassermann and Kahn tests was present in the gamma fraction (11, 12). However, in false positive sera, one or more of the globulin components was said to be within normal limits, as contrasted to truly syphilitic positive sera, in which the quantity of all three globulins was elevated. He also states in another

article (13) that when utilizing fresh sera, the beta anomaly, which is always present in the descending limb of the apparatus, was absent in thirteen syphilitic patients in the primary, secondary, and tertiary stages, and believes that possibly this anomaly may be present in otherwise false positive patients.

These optimistic viewpoints were not shared by G. R. Cooper, however (14), who utilized a series of twenty eight syphilitic patients, thirteen normal individuals, and thirty two false positive patients to show that there was no difference in the beta anomaly between normal and syphylitic patients, and that false positive and normal seras so overlapped with each other that individual cases could not be differentiated electrophoretically (although averages of the groups showed slight variations). Moreover, he could make no correlation statistically between the quantity of gamma globulin and the serologic activity. Other workers (15, 16) have shown that the Wassermann antibody migrated in th area between the beta and gamma fractions, principally on the gamma side, in both false positive cases and in syphilitic patients, but no differentiation can be made between the electrophoretic patterns produced.

Tuberculosis

Siebert and Nelson (17, 18), in working with tuberculous rabbits, discovered a number of characteristic changes in the electrophoretic pattern. The albumin was found to be lower than normal and to decrease progressivly throughout the course of the disease, while alpha globulin increased and a new X component appeared early in the disease. The X component and alpha globulin findings were similar after sensitization to tuberculin protein. The alpha increase is not associated with immunity, and may be evidence of sensitization to tuberculin protein. However, it may also be evidence of a protein sensitization in many diseases. Also of interest is the fact that the X component, which migrates slightly faster than albumin, is of the same mobility as that of a component of Purified Tuberculin Protein. Late in the course of the disease - just before death - the beta component increases. Moreover, the beta globulin was found to be lower in concentration in the normal sera of the more resistant strains of rabbits, but the gamma globulin concentration was of little significance in this respect.

In humans with advanced tuberculosis, a decrease in albumin (which becomes more pronounced with the progression of the disease) and an increase in alpha and gamma

occured, and it is therefore theorized that gamma may accompany an increased resistance to the disease. The X component also appeared, although it was not present in any of the normal controls except one who had been sensitized by contact with the disease. The X component moreover, was seen to increase with a declining course, and to disappear with improvement. The increase was particularly apparent in cases of extreme severity, while the gamma globulin was likened to the increase in beta globulin in the rabbits.

Scarlet Fever vs Rheumatic Fever

Luetscher (19) states that in rheumatic fever, the plasma showed an increase in gamma, the appearence of P_2 and B_1 components, an increase in fibrinogen, and a decrease in albumin. This was partially confirmed by Rutstein, Clarke, and Taran (20), who determined that there was an increased gamma/albumin and gamma/total serum protein ratios in the acute stages of rheumatic fever, which continued at lower levels during inactivity.

Dole, Watson, Rothard, Braun, and Winfield (21), in a study of scarlet fever patients who developed rheumatic fever, discovered that there was a decrease in albumin and a decreased albumin/globulin ratio in scarlet fever, with a slow rise to normal during convalescence, which rise is delayed by the development of rheumatic

fever. There was also an increase in $alpha_1$ to two or three times its normal value during scarlet fever, which declined to normal even in the face of a developing rheumatic fever, while $alpha_2$, which was elevated also in scarlet fever, remained increased during rheumatic fever. An increase in gamma also appeared during scarlet fever, but did not decrease with absorbtion by living streptococci of the type causing the disease, and was not correlated with rheumatic fever as such. There were no changes which distinguished between rheumatic fever and scarlet fever.

Rheumatic Fever vs Rheumatoid Arthritis

According to Dole, Rothard, and Winfield (22), the patterns in eash are identical, being similar to that inferred above (21); a decreased albumin and albumin/globulin ratio, becoming normal with inactivity; an elevated alpha to as much as twice normal early, returning to normal with convalescence; a slightly decreased beta throughout the course; and a gamma globulin concentration of over 35% of normal.

Virus Diseases

Most antiviral sera do not show unusual electrophoretic patterns. However, by serological tests on electrophoretically seperated fractions, it was determined by Bourdillon and Lennette (23), and Wyckoff and Rhian

(6), that the antibodies in influenzal horse serum and in the tissues of human influenzal patients were associated with fractions of a mobility close to that of gamma globulin. In the horse serum the titers ran parallel with the concentration of gamma globulin, but in man there was very little compliment fixing antigen in the blood and the determinations had to be run on lung tissue extracts.

Using antiserum to Western Equine Encephalitis, Morgan (24) determined that although the antibody could be demonstrated in the alpha, beta, and gamma globulin fractions, there was no change from the normal in these fractions electrophoretically, and that even with heating for a half hour at 56°C, there was no change in their appearance. Koprowski, Moore, and Richmond (25), determined that the antibody in Japanese B encephalitis was associated entirely with the gamma globulin fraction, while in the case of Venezuelan and Western equine encephalitis, the antibody was associated with the beta and gamma fractions (or perhaps between the beta and gamma fractions). This is in contrast with Morgan's report in regard to the antibody content of the alpha globulin fraction. However, in no case was there any alteration in the electrophoretic pattern from that of the normal.

Pneumonia

Tiselius and Kabat (26), in determining the electrophoretic pattern of type I antipneumococcic rabbit antisera, discerned no new protein components, but did note an increase of the gamma component which was markedly reduced by specific precipitation without effecting the other components. It was thus determined electrophoretically that 19.6% of the total serum protein was antibody -a figure that compared well with a chemical analysis for antibody nitrogen showing the total serum protein to be 18.6% antibody. Utilizing type I antipneumococcic horse serum, a new component was discovered between the beta and gamma globulins, which disappeared when the antibody was precipitated. This latter finding has not been generally confirmed by others (27, 28), however, the antibody usually being found in the gamma globulin. Blix (29) demonstrated an elevation of the alpha and beta components in the sera of humans in the acute phase of pneumonia (the increase was probably due to the increased temperature of the patient). van der Scheer, Bohnel, Clarke, and Wyckoff (30) also demonstrated the antibody in the gamma component in antipneumococcic rabbit sera (although two rabbits showed it in the T peak, and one other in the gamma' peak). Moreover, they demonstrated that purer and larger amounts of antibodies

were produced by prolonged hyperimmunization, and could be demonstrated electrophoretically by the size of the gamma peak produced. Van der Scheer and Wyckoff (31) also demonstrated that the gamma component decreased when immunization ceased.

Tetanus

Wyckoff and van der Scheer demonstrated a new T component in antitetanal sera (32), of the same mobility as the antipneumococcic component of Tiselius and Kabat. In addition they demonstrated that the size of the T component increased as the amount of the antitoxin in the serum increased, but not in such a fashon that its area could be taken as a direct measure of the antitoxic activity. It was also noted that the area of the gamma component also increased - but not proportionally - as hyperimmunization proceeded.

Miscellaneous Diseases

A large number of different hyperimmune antisera were studied by van der Scheer and Wyckoff (33), and van der Scheer, Wyckoff, and Clarke (34). These studies demonstrated in general, that no hyperimmune sera so far studied has shown an increase in beta; that some antigens produce a T component (in horses); that a few result in an increase in the globulin component - gamma principally - (an increase not shown to be related to an increase in

antibody content); and that all the antitoxins examined showed a T component, while the anti bacterial protein and anticarbohydrate sera showed an increase in the gamma component. Thus Clostridium welchi, sordelli, oedematiens, tetani, and Corynebacterium diptheriae antisera all produced large T components; scarlet fever, botulinus, staphyloccus, histolyticus, Shiga and Flexner paradysenteriae, Swine Erysipelas, and vibron septic antitoxin sera resulted in both T and gamma components; and antipneumococcus, meningococcus, and hemorrhagic septicemia sera showed an elevated gamma fraction. They also established that in the horse, when prolonged hyperimmunization was carried out with pneumococcic and tetanus antigen, there was no change in the antibody mobility.

In a study by Dole, Emerson, and Braun (35), on the sera of Relapsing fever patients, the changes noted were thought to be those due to the host reaction to an acute episode, and not due to the presence of parasites in the body.

According to Stats, Perlman, Bullowa, and Goodkind (36), cold hemaglutinin appears to have a mobility similar to that of gamma globulin.

Typhoid antigen was studied by Linton, De Spain, Smith, and Krejci (37), using the growth products of four

strains of typhoid organisms; H, O, Ty2, and Ty58, the latter two containing the Vi antigen. The products of each strain were found to contain alpha, beta, and gamma globulin components, and in each case, each component gave some protection to mice against virulent typhoid organisms Ty36. Serological tests on the electrophoretically seperated fractions showed that in the H and O strains, serological reactivity was restricted to the gamma component. In the Ty2 and Ty58 strains, the beta component was found to be serologically active as well as the gamma component, and in type Ty2, the Vi antigen seemed to be in the beta component.

Fisher and Davis (38) studied the serum proteins of both active and inactive cases of sarcoid, and found that in the active cases there was a hyper gamma globulinemia and a decreased albumen, while in inactive cases there was a near normal, or only slightly increased gamma globulin concentration, and the albumin was higher than in active cases. They thought that such analysis might therefore be of some value in following the course of such diseases.

Allergy

In a study of human allergic serum by Newell, Sterling, Foxman, Burden, and Krejci (7), no difference could be found between the allergic and normal serum, although

in passive transfer experiments, the antibody could be demonstrated only in the gamma fraction.

Possible Clinical Applications of the Electrophoretic Apparatus

Although in its present state of refinement, the electrophoretic apparatus is of little value diagnostically, further experimentation to establish normals and increase the delicacy of the separations may increase its usefullness in this sphere.

The apparatus may be of value in the evaluation of various treatments and in following the course of diseases in which definite changes are produced in the sera. At the present time it would seem that tuberculosis could be followed in this manner.

It may be of value in demonstrating the relationship between some diseases - such as scarlet fever and rheumatic fever - or in differentiating between confusing diseases or tests - syphilitic versus false positive sera, for example.

However, a great deal of basic work on normals and on improving separation techniques will have to be done before the apparatus will be of much value clinically. Even then, the technical complexity of the apparatus and procedure in its present form will limit its use.

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