CHANGES IN THE ELECTROPHORETIC PATTERN OF SERA OF DOGS SUFFERING FROM VARIOUS DISEASES.

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INTRODUCTION.

A considerable amount of work has been done by different investigators using the technique of electrophoresis on the sera of human beings suffering from various disease conditions. From a comprehensive review of these results, Marrack and Hoch (1949) have concluded that changes in the electrophoretic patterns cannot be regarded as characteristic of or specific for any particular disease, the main changes being a reduction of albumin, an increase in \( \gamma \)-globulin, or a moderate increase of \( \alpha \)-globulin. They regard these changes in the same light as determinations of erythrocyte sedimentation rate in that they are "a measure of the clinical state of the patient, rather than specific evidence of disease". Hence, the greater precision of electrophoretic examination of pathological sera would provide no real advantage over the usual chemical methods.

In the present work a study has been made of the sera of dogs suffering from three diseases commonly occurring in the vicinity of Onderstepoort (South Africa). The results of these investigations provide evidence leading to conclusions differing from those quoted above, and more in accordance with the views of other workers in this field (Zöllner et al., 1950, Reid et al., 1951).

The diseases studied are those due to infections with \textit{Babesia canis}, \textit{Rickettsia canis}, and the virus of canine distemper. Cases of the latter disease were representative of the different clinical forms in which it occurs, including the neurotropic form.

METHOD AND APPARATUS.

The electrophoresis apparatus used in these studies is the modified apparatus developed by Polson (1945). The registration of the moving boundaries was done by the scale method of Lamm (1928). All determinations were made in borate buffer at \( \text{pH} 8.6 \) in a water bath thermostatically controlled at \( 2^\circ \text{C} \). Observations were made only on the descending boundary.

MATERIAL.

The sera used were collected from animals presented for treatment from the vicinity of Onderstepoort. It was, therefore, difficult to obtain reliable information as to the duration of disease before material was obtained for examination. In a few cases dogs were experimentally infected.

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RESULTS.

These are, for the present purpose, presented graphically. Quantitative determinations for comparison were not made since in most cases it was not possible to obtain sera of these dogs before the infection had taken place. The deviations found in the patterns were sufficiently pronounced to make the lack of quantitative determination no disability. In a few instances the serum protein pattern was followed for a period during convalescence.

THE ELECTROPHORETIC PATTERN FOR NORMAL SERUM.

Like the sera of various animals and man, that of the dog can be shown to contain various fractions or components. Tiselius (1937) who pioneered electrophoretic analysis named these albumin, α-globulin, β-globulin and γ-globulin in order of their migration rate in a solution on the alkaline side of neutrality when an electric current is passed through. It has subsequently become generally known that prolongation of electrophoresis results in further resolution of these components. Those of the globulins were thus referred to as α1 and α2, and β1 and β2, while Hoch and Morris (1945) differentiated between subfractions of albumin named the A1 and A2. The latter were found to be present in approximately equal amounts and had migratory velocities which differed by only a small percentage.

For the purposes of comparison the electrophoretic diagrams of three normal dogs are given in fig. 1.

ELECTROPHORETIC PATTERNS IN CANINE BABESIOSIS.

Details of the symptomatology of the disease due to Babesia canis infection, with emphasis on atypical forms, have been given in a recent publication (Malherbe and Parkin, 1951). In this paper there is a discussion on the rôle of changes in the serum proteins in the pathogenesis of certain circulatory disturbances sometimes found in the disease.

The sera of nine infected dogs were studied, some of them serially during the course of convalescence. In fig. 2 are given the electrophoretic diagrams of three more or less acute cases. Internal parasites were eliminated as a complicating factor, so that changes in the sera may be regarded as being due to the infection.

Fig. 2 (a) represents the serum pattern of a dog with the peracute form of the disease. The parasitaemia had developed suddenly, and specific treatment (normally very efficient) did not prevent fatal termination within a matter of hours. The electrophoretic findings were: A1 and A2 decreased, α-globulin increased, β-globulin normal and γ-globulin slightly increased.

Fig. 2 (b). This dog had been treated some twelve hours previously for a "cerebral" form of the disease (see Malherbe and Parkin, 1951). The findings were A1 and A2 decreased, α-globulin increased, β- and γ-globulin normal.

Fig. 2 (c). In this case specific treatment was followed by recovery. Serum analysis before treatment showed: A1 and A2 decreased, α1-globulin markedly increased, β- and β2-globulin normal, and γ-globulin increased.

In fig. 3 three stages of the infection in one dog are presented. Fig. 3 (a) shows the pattern during the acute infection, before treatment: A1 decreased, A2 only slightly decreased, α1-globulin increased, α2 only slightly increased, β-globulin and γ-globulin normal. Fig. 3 (b) shows the pattern five days after treatment,
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i.e. during the recovery phase. Here the only notable deviation from the normal pattern was an increase of $\gamma$-globulin. Five days later the diagram shown in fig. 3 (c) was obtained, and the $\gamma$-globulin still found to be elevated.

An interesting series is pictured in fig. 4. It consists of five sera from one dog, taken on presentation and then serially during a prolonged convalescence. The diagrams cover a period of about three weeks. Recovery from this disease in its uncomplicated form is usually rapid after specific treatment. In the present instance, clinical icterus due to severe hepatic damage was much in evidence when the animal was presented for treatment. Instead of resuming feeding on the day after treatment, the dog showed no interest in food till the fifth day afterwards. The icterus remained intense for a week and only started disappearing during the second week after treatment. The $A_1$ component showed a pronounced drop while $A_2$ remained high; $\alpha$-globulin was not markedly changed, while $\beta$-globulin was much increased. A high $\gamma$-globulin was very much in evidence. On about the fourteenth day there was, judging by haematological findings, an "abortive" relapse in which no parasites could be demonstrated in the peripheral blood. This appears to be reflected in fig. 4 (d) where the improvement in $A_1$ seemed to be interrupted by a slight fall, coupled with an elevation of the $\gamma$-globulin peak.

**Electrophoretic Patterns in Rickettsiosis.**

This disease as it occurs in South Africa has been described by Neitz and Thomas (1938), and Malherbe (1947). At first thought to be highly fatal, it was later found to be a comparatively mild disease provided it was uncomplicated by other infections, e.g. babesiosis, ankylostomiasis, or organic disease such as nephritis.

Sera from five different dogs infected with *Rickettsia canis* (uncomplicated) were examined electrophoretically. The diagrams obtained are presented in fig. 5.

The changes found in these cases were associated with an absolute increase in the globulin moiety. At the same time, there was a sharp falling away of the faster migrating portion of albumin, $A_1$, while the $A_2$ was hardly affected. $\alpha$-globulin was somewhat increased but $\alpha_2$ to a considerably greater extent. $\beta_1$-globulin was greatly increased and $\beta_2$ to a lesser extent. The elevation of the $\gamma$-globulin peak was moderate.

**Electrophoretic Patterns in Distemper.**

This disease, as has been indicated, was studied in its different clinical manifestations, the "classical" respiratory and gastroenteric form (fig. 6a), "nervous" distemper with encephalitis (fig. 6b), and the so-called "hard pad disease" (figs. 6c, d). These show that not only were there no obvious characteristic differences between the various clinical forms, but none of them gave evidence of any particular deviation from the normal serum pattern.

These sera were in all cases collected when the disease was in an advanced stage of development, some being in extremis. The diagrams can, therefore, be taken as representing the maximum change possible in this disease. In a few cases a moderate lowering of the serum albumin was noted, but this was regarded as a reflection of the emaciated condition of the animal.
DISCUSSION.

The limited number of dogs infected with the three diseases and of which the sera were examined by means of electrophoresis, do not permit of any categorical opinions as to exactly which changes or combinations of changes comprise the pattern for the particular disease. The results obtained, however, do provide an indication of fairly characteristic differences which might, in certain cases, be of value where the simpler methods of blood smear examination and determination of the haematological picture fail to provide the required differentiation.

There exists in the case of distemper no certain method of diagnosis and reliance has to be placed on a subjective appraisal of the clinical signs, often with consideration of the epizootiology. Demonstration of inclusion bodies in the conjunctival epithelium has in our hands not proved to be of material assistance. The essentially unchanged electrophoretic serum pattern is then of limited value except in differentiating between doubtful distemper and rickettsiosis or babesiosis. The last-mentioned two disease can, as a rule, be diagnosed with certainty from properly prepared blood smears (Malherbe, 1948). Cases of proved babesiosis with consistently negative smears do, however, occur (Malherbe and Parkin, 1951) and every case of rickettsiosis goes through phases when the Rickettsias cannot be demonstrated in the monocytes of the peripheral blood.

It is in these cases that electrophoresis may prove to be of material assistance. Further study of many more cases would, however, be required to reach any final evaluation of the specificity of this method.

There is considerable evidence to show that disease of certain organs may produce particular changes of the electrophoretic pattern, but on this question there is as yet no unanimity. While Marrack and Hoch (1949) state that "the change of serum proteins characteristic of disease of the liver parenchyma is an increase of the \( \gamma \)-globulin", and also mention the reduction of albumin, sometimes to very low levels, Zollner, Eymer and Scheid (1950) stress the increase of \( \beta \)-globulin, as the most regular finding in liver damage. The latter authors also regard the drop of albumin as evidence of acute damage to the liver cells, where physiologically albumin is formed.

There is similarly much speculation as to the actual significance of \( \gamma \)-globulin. Tiselius and Kabat (1939) have shown that antibodies are quantitatively contained in the \( \gamma \)-globulins. \( \gamma \)-globulins, however, do not consist only of antibody, and Zollner et al. (1950) give the opinion that in acute infectious diseases "non-specific" \( \gamma \)-globulin apparently occurs together with immune bodies.

The babesiosis cases portrayed in fig. 2 show normal \( \beta \)-globulins and unaltered or slightly increased \( \gamma \)-globulin fractions. Clinically considered, these cases were too acute to have suffered any extensive damage to the liver so that the drop in albumin is the only indication of impaired functioning of this organ. The slight increase of \( \gamma \)-globulin in fig. 2 (a) and greater increase in fig. 2 (c) are construed as representing the degree of antibody production at the time of examination.

The case shown in the series of fig. 4 gave evidence of very severe toxic hepatitis when admitted. There was severe icterus of the mucous membranes and unpigmented skin and the direct van den Bergh reaction was strongly positive. Both the \( \beta \)- and \( \gamma \)-globulins were much increased. The changes due to liver damage are well demonstrated by a comparison between the fig. 4 diagrams and those of fig. 2. The "abortive" relapse appears to have been the cause of an exacerbation of antibody production (fig. 4d).
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A further point of interest in this series (fig. 4) is the seeming reluctance of the more slowly migrating albumin, $A_2$, to disappear, as compared with the fate of this fraction in the acute cases shown in fig. 2. This is considered as being due possibly to the large amount of bilirubin present in the serum, as this pigment is bound to the $A_2$ component, which is thereby prevented from acting in the normal way or being mobilized, as in the acute cases.

The case shown in fig. 3 can be regarded as intermediate between fig. 2 and fig. 4 in that the $A_2$ was also retained at a high level; but since recovery after treatment was rapid, all components returned to normal except that there was evidence of developments of immune bodies in the $\gamma$-globulin. This case does not seem to lend support for a contention that increased $\gamma$-globulin is a major manifestation of liver damage.

Icterus is hardly ever seen in rickettsiosis and the liver is not damaged to the same extent as in babesiosis. Our relatively limited experience of rickettsiosis tends to show that the kidneys are severely affected. The reason, therefore, for the persistence of $A_2$ is not clear. The disappearance of the faster migrating $A_1$ may possibly be attributed to a drain through the kidneys. This point requires further clarification, as does the reason for the general absolute increase in concentration of the globulin moiety.

Concerning distemper, the almost total lack of electrophoretic characterization in the various clinical forms must be regarded as evidence that the pathogenicity for organs is limited to those that have but little influence on the serum composition of the blood.

**SUMMARY.**

The sera of dogs suffering from three diseases commonly encountered in South Africa, viz. babesiosis, rickettsiosis and distemper, have been examined electrophoretically and compared with normal sera.

Diagrams of the electrophoretic patterns are given with a description of changes found in them. These changes are discussed in the light of the present knowledge of the diseases as they affect the body organs.

The potential value of electrophoresis in the differential diagnosis of these diseases is discussed, and it is concluded that the available information indicates a degree of specificity which could prove useful for this purpose.

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**REFERENCES.**


ELECTROPHORETIC PATTERN OF SERA OF DOGS.


Fig. 1.—Descending electrophoretic patterns of sera from three normal dogs.
Fig. 2.—Descending electrophoretic patterns of sera from three acute cases of babesiosis.
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Fig. 3.—Descending electrophoretic patterns of sera from one dog with babesiosis, taken at 5-day intervals.
Fig. 4.—Descending electrophoretic patterns of sera from one dog with babesiosis over a period of three weeks of delayed convalescence.
FIG. 5.—Descending electrophoretic patterns of sera from five dogs with rickettsiosis.
Fig. 6.—Descending electrophoretic patterns of sera from dogs with distemper, (a) "classical" distemper (serum slightly haemolyzed); (b) "nervous" distemper with encephalitis; (c) and (d) "hard pad disease".
ELECTROPHORETIC PATTERN OF SERA OF DOGS.

b.

c.

d.

24.