DISCUSSION

1. Host specificity of the malaria parasite P. (Garnhamella) coturnixae

We are still far from understanding the nature and cause of a particular host for a particular plasmodium. In some instances the specificity is absolute and in others it is partial. Thus Rhesus monkey is totally resistant to P. vivax and P. falciparum (Jeffrey, 1961) and canary to P. Gallinaceum (Huff, 1957). In these instances the particular species of animal is absolutely resistant to all forms of the particular parasite. However there are other instances, where it has been shown that in a particular species of animal, only the tissue phase of the plasmodium could develop but no erythrocytic form was demonstrable (Bray, 1957). The reverse also happens in some cases where the erythrocytic phase can be observed but the tissue phase fails to develop (Vincke, 1954; Coradetti, 1955; Garnham and Lainson, 1957). There can be all ranges of specificity. Some parasites have a rather narrow range, e.g. P. Gallinaceum, a natural parasite of chick can infect the goose with readiness and duck with difficulty although both are from the same family (Brumpt, 1936). There are others where the range is wider e.g. P. lophurae which infects duck and chicken readily can even infect mammalian erythrocytes if the parasites are injected as parasitized erythrocytes into the immature chick embryo (McGhee, 1950).

Plasmodium (Garnhamella) coturnixae can infect Coturnix coromandelica and Coturnix coturnixae but when injected intramuscularly the parasite fails to infect chick, duck, canary, pigeon and other quails (Sarkar and Ray, 1969). The following experiments were carried out to study the extent and case of the specificity of the host to P. (Garnhamela) coturnixae.

A) Repeated inoculation

Repeated subcutaneous inoculation of heavily infected blood into chickens failed to infect them. Although intravenous inoculation was not carried out, in this instance, the dose that was used was rather massive. To compare, a single subcutaneous infection of a fifth of the dose was sufficient to bring about heavy parasitemia in a susceptible bird like the coturnix. A subcutaneous injection of parasitized erythrocytes produced parasitemia by gaining entrance into the lymphatics and thence into the blood of the injected animal; hence a subcutaneous injection is almost equivalent to an intravenous injection provided the injection does not cause a rupture of the capillaries. The chickens presumably have a normal lymphatic system and therefore, failure of such massive inoculation for such a prolonged time to produce parasitemia speaks of complete resistance of this species of bird to this species of plasmodium.

B) Use of immunosuppressive agents

If resistance of a certain species of bird to a certain species of bird malaria be due to naturally occurring immune bodies, it was noteworthy that the use of 11-oxycorticosteroids, which are lympholytic could not overcome the resistance. The corticosteroids, are effective immunosuppressive agents if given in the preinductive phase. We, however, do not know and cannot identify the antigens which give rise to naturally occurring antibodies and hence the use of corticosteroids may not be effective to suppress the production of antibodies because the phenomenon occurred much earlier before the introduction of either the corticosteroid or the malaria parasite. The corticosteroids are also lympholytic agents, but the actual recognition may be accomplished by a few cells only which always survive in the partial lympholysis by corticosteroids.

Incubation of infected coturnix erythrocytes with chick serum

The plasma, of resistant bird was unable to prevent the parasite to produce infection in the susceptible bird. Similar experiment was done by Trager and McGhee (1950) with hen plasma. Hens are naturally resistant to P. lophurae, when the plasma of of hen was injected in young chick or chick embryo the parasitemia was found to be relatively decreased even when the plasma was heated for 1/2 hr at 65°C. The authors suggested that the euglobulin factor is responsible for the resistance. In our experiments, however, the chick plasma had no effect on the degree of

parasitemia in the susceptible host, even though the Coturnix erythrocytes were agglutinated by the chicken serum.

2. Hemoglobin

General characteristic of Avian hemoglobin in electrophoretic field

Observing the tendency of avian hemoglobin to separate into two components on paper electrophoresis, efforts were made to separate them by acrylamide gel electrophoresis, a technique of relatively higher resolving power. This technique gave good separation of the kinds of hemoglobins. The chick hemoglobin and Coturnix hemoglobin were found to consist of two distinct bands, completely separated from each other (Fig. 6). In the avian group, the presence of two kinds of hemoglobin is a common phenomenon. Gatzer and Allison (1960) reviewed hemoglobins in the avian system. They reported that the following birds of different groups had two different kinds of hemoglobin.

In Passeriformes : Zonotrichia leucophrys gambelii, Melospiza lincolnin, M. melodia, Spizella passerina arizonae and then other birds.

In Piciformes : Coloptes auratus borealis, C. cafer collaris.

Anseriformes : Anas platyrhynchas.

In Galliformes : Domestic fowl (Gallus domesticus), Meleagris gallopavo, Phasianus colchins, Numida meleagris, Gallus gallus, Aptenodytes forsteri.

The birds in our experiments, namely the Coturnix and chick are under the group galliformes and had two different kinds of hemoglobin. That the chickens have two different kinds of hemoglobins were also reported by other workers (Huisman et al., 1958; Gatzer and Allison, 1960; Cabannes and Serain, 1955; Saha et al., 1957).

Fowl hemoglobin was sometimes found to consist of three different kinds of molecule (Rodnan and Ebough, 1956, 1957; Dutta et al., 1958, D. Amelio and Salvo, 1959). The separation in these instances was carried out on starch gel. In pigeons, paper electrophoresis and agar gel electrophoresis of hemoglobin showed a broad zone whereas ion exchange chromatography resolved two components (Saha, 1959). Two hemoglobins were demonstrated in the duck by Cabannes and Serain (1955). In great horned owl only one band of hemoglobin was demonstrated (Abercromibie et al., 1969).

Hemoglobin during infection

Blood for hemoglobin was collected from the Coturnix at the height of infection and was studied quantitatively and qualitatively for the hemoglobin; results were compared with blood from non-infected birds. It was found that the same kinds of hemoglobin were present during the infection, although the hemoglobin contents were lower in the infected blood than in the

non-infected blood. The Coturnix, therefore, when infected, produced the same two kinds of hemoglobin molecules as the uninfected Coturnix. Sherman and Hull (1960a) also found that the character of the hemoglobins of the chick did not change after infection with P. lophurae. It is, therefore, apparent that hemoglobin does not alter malaria infection.

Quantitative analysis of the kinds of hemoglobin

Quantitative analysis of the different kinds of hemoglobin was done by eluting the bands of hemoglobin from the gel and then reading the optical densities at 540 m/u. The results showed that the anodically fast moving component of both Coturnix and chick hemoglobin was a quarter of the total hemoglobin (Fig. 7).

Study of hemoglobin by possible chain separation

Hemoglobin becomes broken down by 8M urea and 1% mercaptoethanol into the constituent chains. The chains showed separate mobilities on electrophoresis (Abercromibie et al., 1969).

Upon urea treatment of the hemolyzate and subsequent separation of the hemoglobins on polyacrylamide gels, the band pattern was found to be different in Coturnix from the chick. The fast moving band of both the chick and the Coturnix was more or

less similar. The second band of Coturnix, however, had a mobility which was different from the second band of the chick (Fig. 8). The slowest moving band possibly representing alpha chains showed similar mobility in the Coturnix and chick. The results, therefore, show that there was a dissimilarity in a component of hemoglobin molecule of the Coturnix from that of the chick.

The importance of hemoglobin in malaria parasites' nutrition and host specificity has been already discussed in the literature review. Hemoglobin is the chief nutritive source of the malaria parasite (Moulder, 1962; Sherman et al., 1968; Rudzinska and Trager, 1957, 1959; Rudzinska et al., 1960; Flectcher and Maegraith, 1962; Ristic and Kreier, 1964). The uptake of nutrients from the host cells by intracellular parasites generally occurs across the cell membrane of the parasite (Rudzinska et al., 1965).

All type of hemoglobin is not efficiently utilized by the malaria parasite e.g., sickle cell hemoglobin and fetal hemoglobin. Again the same hemoglobin can not be utilised by all species of malaria parasite. Different strains of mice, not possessing the same type of hemoglobin showed different susceptibility to *P. berghei* (Greenberg and Kendrick, 1959). The fact that the human malaria parasite may be susceptible or non-susceptible to other animals may in part be due to their different hemoglobin structure (Manwell, 1963). Even in human population the

susceptibility to P. falciparum differ due to variations of hemoglobin (Bowman et al., 1971).

The difference in the hemoglobin molecule in the present study may be the cause of host specificity. This slight variation must be in the globin molecule, so that the structure of the globin molecule is oriented in such a fashion that this particular parasite cannot engulf this hemoglobin. The cell surface of the parasite can recognise this change and the pinocytic uptake of the hemoglobin is impaired. The non-susceptible bird, chick in the present study is susceptible to another species of parasite P. gallinaceum. It is likely that digestive enzyme system of all the malaria parasites are more or less same. Therefore, it is hardly possible that the hemoglobin digested by one species of parasite can not be digested by the other species of parasite.

3. Serum sodium and potassium

Evans et al. (1956) described the gene affecting the cation composition of red cell. They stated that in most breeds of sheep some animals are found with high sodium and low potassium in erythrocytes. Different hemo-parasites have different choice for cation concentration. Babesia, a blood cell parasite, very similar to the malaria parasite were found in animals whose erythrocytes have relatively high concentrations of sodium. Plasmodium prefer high potassium concentration. McGhee's (1950) experiment

with chick embryo was a significant one. A particular malaria parasite P. lophurae selected erythrocytes of 3 species out of 16 in mammalian red cells. The potassium content of these erythrocytes were high, although the erythrocytes were derived from three separate order of mammals, Rodentia, Lagomorpha and Artiodactyla. Therefore, the specificity of a host was assumed to be regulated by a high potassium concentration of the erythrocytes. A particular malaria parasite might have a particular choice for a particular concentration of potassium and sodium. Sherman and Tanigoshi (1971) showed in peking ducklings that sodium and potassium concentration of the parasite was similar with that of the erythrocytes. Therefore, it was presumed that there was a relationship between the particular parasite's cation composition with that of the particular erythrocytes. In our experiments sodium and potassium concentrations of both the susceptible and the non-susceptible birds were similar; it appears, therefore, that the cation concentration of the erythrocytes did not confer any specificity for the Plasmodium (garnhamelia) coturnixae.

4. The role of host gamma globulin in the development of the Malaria parasite

When methods were developed for fractionation of serum proteins by electrophoresis a number of workers studied the electrophoretic pattern of host serum after infection by hemoparasites. Infection of the Coturnix by P. (garnhamella) coturnixae led to an

increase of gamma globulin (Fig. 9), such increase lasts throughout the period of infection. This result is in agreement with previous reports of electrophoretic investigations of the host serum during malarial infection, (Dole and Emersion, 1945; Schinazi, 1957; Sherman and Hall, 1960b). An attack of malaria like many other acute infections, is associated with hypoalbuminaemia and a rise in globulin concentration (reviewed by Gutman, 1948; Stauber, 1954). This increase of gamma globulin may or may not be associated with the development of so called protective immunoglobulins against the parasite. Sengers (1971) showed that gamma globulin increased and albumin decreased in swiss mice after intraperitoneal inoculation of P. berghei. The rise of gamma globulin level may have some relation to the production of opsonin. Leddy and Vaughan (1964) reported that high molecular weight gamma globulins sensitized trypsin treated erythrocytes to phagocytosis by cells of the reticuloendothelial system. Schroeder and Ristic (1968) also showed the presence of serum factors associated with erythrophagocytosis in Calves with Anaplasmosis. The normalization of the gamma globulin fraction was coincidental with the removal of parasites, suggesting that gamma globulin might contain an agglutinin or opsonin which was bound to the parasite and/or the parasite erythrocyte complex might be removed from the blood stream with some amount of fecility (Sherman and Hall, 1960b).

The beta globulin showed significant rise in chicks infected with P. lophurae (Sherman and Hall, 1960b). In our studies, however, no such rise could be demonstrated.

Normal chickens have high gamma globulin concentration whereas normal Coturnix have a low gamma globulin. The low gamma globulin level in Coturnix may or may not have some role to play in the susceptibility of the bird to the particular plasmodium. The birds which are resistant to this malaria parasite P. (Garnhamella) coturnixae have a comparatively higher serum gamma globulin content. The role of gamma globulin in the immunity of malaria parasite was studied by a number of authors (Taylor et al., 1949; Box and Gingrich, 1958). Antibodies were shown to be associated with gamma globulin (Cohen and McGregor, 1963). They were present in the 7S gamma globulin (Cohen et al., 1961; Cohen and McGregor, 1963; Edozien, 1964; Curtain et al., 1964) and 19S gamma globulin (Abele et al., 1965). The acquisition of immunity was associated with increase of gamma globulin level. Thus inhabitants of endemic malarious regions had high gamma globulin levels (Holmes et al., 1951). Gamma globulin synthesis was considerably greater in adult Gambians exposed to malarial infection than in protected subjects, i.e. receiving antimalarial drug (Cohen and McGregor, 1963). Even after several years of residence in the United Kingdom, West Africans continue to synthesize gamma globulin at almost twice the rate observed in healthy Europeans; this might be due to environmental influences of early life, or to genetically determined differences in the rate of gamma globulin synthesis. In the present study, the higher gamma globulin concentration in the resistant birds, like the chick may be due to exposure to similar parasites in early life or, as a result of concomitant presence of other

infection as reported by Fairly (1945), Cox (1968) and Cox and Turner (1970). These chickens were taken from the government poultry firm as seven days old bird and were maintained in our laboratory. It is hardly possible that the chicks had any previous experience of this parasite. It is probable, therefore, that a gamma globulin level (either high or low) is a genetically determined factor.

5. Occurrence of naturally occurring antibodies in the resistant birds

The high gamma globulin level may, presumably contain some naturally occurring antibodies against the parasite. The following investigations were carried out in order to establish whether there are any naturally occurring antibodies in the serum of the resistant bird.

Agglutination test

In 1951, Stauber et al. carried out studies of *in vitro* agglutinations of erythrocyte free avian plasmodia and demonstrated group specific and species specific agglutinogens on the plasmodia. Our experiments also show that such agglutinogens can be demonstrated in the *Plasmodium (Garnhamella) coturnixae* too.

They showed that the specific plasmodium could be agglutinated in the presence of serum from infected birds. So, as a consequence of the infection the serum must have developed

agglutinins against the plasmodium. We prepared strong saline extract of the erythrocyte free plasmodium (Garnhamella) coturnixae with the idea that such an extract would contain membrane specific antigens. When tanned sheep red cells were coated with this extract and then incubated in the presence of infected serum, agglutination of the coated erythrocytes could be detected at high dilutions. So, the infected serum must contain some agglutinins to the parasite. In vivo, however, infected coturnix erythrocytes do not agglutinate. This is because the parasite is intracellular and hence is not exposed to the agglutinins present in the infected serum. In other wards, the plasmodia are not free.

The results also show that when such coated sheep erythrocytes are incubated with normal sera of naturally resistant birds like chick and titir, there was agglutination of the sheep erythrocytes. In contrast when such coated sheep erythrocytes were incubated with normal coturnix serum no agglutination could be detected. It appears possible, therefore, that those birds which are resistant to the parasite contains some natural agglutinins against this particular species of parasite. So, when in their natural habitat they get inoculated with the sporozoites they are probably agglutinated by such agglutinins and the agglutinated mass is disposed off as a mass of foreign bodies.

6. The role of erythrocyte surface membrane in determining the host specificity of malaria parasite

The membrane of a cell is a world by itself. It has properties which are distinct from other properties of the cell (Ponder,

1961). It allows certain molecules to pass through and bars the entry of others. It has on itself certain characteristic sites which binds only certain specific molecules and thus produces configurational changes on the molecules with consequent alterations in properties (Passow, 1964). It has complementory sites for attachment of molecules like the viruses with concomitant activation of certain enzymes which finally results in injection by the viral nucleic acid (Gottschalk, 1957). An obvious analogy, can therefore, be drawn whether the specificity of a malarial parasite for a particular kind of erythrocytes could reside in some properties of the cell membrane of the susceptible host. Can it be that the erythrocytes of the susceptible hosts has on their cell membrane, sites which are complementary to the cell membrane of the malaria parasite? Under that circumstance these sites can allow certain bonds like hydrogen bonds, or Van der Waals forces to form between the membrane of the erythrocytes and the membrane of the plasmodium. Such complementary sites may be lacking in the case of the erythrocytes of the resistant host and the particular plasmodium.

Attempts were, therefore, made to study the binding of the parasite to the membrane of the erythrocyte of the susceptible host. But they were, on the whole, unsuccessful. People even tried to modify the surface membrane of the erythrocyte by treatment with various agents like trypsin, neuraminidase etc., with very little success. We also carried out some experiments on this line, again, with limited success.

When normal coturnix erythrocytes were incubated with P. (Garnhamella) coturnixae we could get attachment of the parasite in 1% to 2% of the cells (Figs. 12 and b) whereas when chick erythrocytes were similarly treated there were no attachment at all. The stages in the life cycle of the malaria parasite are such that the parasites undergo progressive developmental changes. The morphological changes associated with the development of the schizont from the merozoit are visible indication of tremendous alterations which are taking place; they may also involve alterations in the surface membrane and if the penetration of the parasite into the erythrocyte involve a specific binding between the complementary sites, it is quite probable that further development from the stage of merozoite would result in loss of the binding sites. Therefore, these later stages of the parasite may not be infective. What we started with, was a collection of free parasites obtained from infected erythrocytes. Among these only a few percent of the parasite would be merozoite and hence infective. Therefore, even though we got only 1% to 2% of binding in the incubated susceptible erythrocytes the number is not insignificant in consideration of the smaller quantity of the infective merozoites.

It has been recently shown that the cell membrane of certain types of lymphocytes have multiple binding sites for membranes of heterologous erythrocytes(Lay et al., 1971). As a result, when such lymphocytes are incubated with those heterologous erythrocytes, quite a number of the red cells become attached to the cell membrane of the lymphocytes to form a rosette - like pattern.

We coated sheep erythrocytes with extracts of the plasmodia and then incubated them with the erythrocytes of the coturnix and the chickens. It was our purpose to see if such coated sheep cells would attach the erythrocytes of the susceptible host and not those of the resistant. No rosette formation was observed. The sheep erythrocytes are smaller in diameter than either the coturnix or the chick red cell. After one of the larger erythrocyte becomes attached to the coated sheep erythrocyte, further attachment would be rendered progressively difficult because of lack of space. So, rosette formation is hardly likely to occur under such circumstances.

The fact, that in about a quarter of the instances the coturnix erythrocyte showed contact with the coated sheep cell and in less than a tenth instance only the chicken erythrocyte made contact with the coated cell only shows that the cells of the susceptible host could possibly bind the plasmodium more effectively than the cells of the resistant host.

7. Non-susceptibility of chick embryo

The resistance of a non-susceptible host to infection by a malaria parasite might be due to the presence in the resistant host of naturally occurring antibodies which, by association with the parasites render them incapable of invading the erythrocytes. If, therefore, the resistance be really due to an immunologic phenomenon, the question appears pertinent whether a resistant species can acquire infection if the parasites are introduced early

in embryonic life before the immunologic apparatus had developed. In the chick, the immunologic mechanism develops around the 13th day of embryonic life. The failure of the chick to take up P. (Garnhamella) coturnixae, even when it was injected on the 11th day of embryonic life, therefore, implies either :

a) that the amount of heterologous infected erythrocytes and other blood cells failed to induce tolerance to them.

or b) that free merozoites were not significantly present in the injected blood.

or c) that the susceptibility of a host for a particular parasite does not depend on an immunological mechanism.

The chick embryo on or about the 11th day weighs about 10 gm; even if we allow 10% of the body weight as blood, it has only about 1 ml. of blood. Since 0.1 ml. of infected Coturnix blood was injected, the amount of injected blood was 10% of its own blood volume; such an amount ordinarily should be enough to produce tolerance in the 11th day old embryo. Since we did not test the chick's tolerance by grafting Coturnix tissues, we cannot say definitely that a tolerance had been produced in the injected chicken. But since a considerable amount of blood was injected it would be improbable that a state of tolerance was not established in the treated chickens.

Ordinarily, when a susceptible species like the Coturnix is injected intravenously with infected Coturnix blood the

parasite appears in about 4-5 days' time in the peripheral blood. Such infection must have resulted from at least 4-6 generations of the merozoite to schizont cycle. Since the life span of an avian erythrocyte is of the order of more than 3 weeks, it is hardly likely that the parasites could be released from the infected erythrocytes to infect the injected host. It is more probable that free merozoites must be present in the injected blood. In a smear preparation of the infected blood, we also come across occasionally merozoites lying free of any erythrocytes in the stained specimen. It can, therefore, be presumed that the free merozoites could not infect the chick embryonic erythrocytes, or probably even the chicken erythrocytes after hatching.

The third possibility that the non-susceptibility is not due to an immunologic phenomenon is a hard thing to swallow. All the indirect evidence indicates that the penetration by the parasite of the host erythrocyte is a surface phenomenon. There must be some receptors on the erythrocyte membrane which can form more or less stable bonds with the surface membrane of the Plasmodium in the susceptible species; in the resistant species such a structure may be absent in the stroma. Such bond formation will lead to configurational changes in both the host cell as well as the parasite; these three dimensional changes must be at the back of the penetration of the parasite. However, unless we can have more elegant methods of preparation of the membranes of the parasites and erythrocytes, the problem will be difficult to handle.

8. Conclusion

For the purpose of the present thesis we will leave aside the inoculation of the sporozoites and the development of the the tissue phase. We will only consider the continued infection of the erythrocytes by the merozoites instead.

The specificity of a particular plasmodium to a particular kind of erythrocytes probably depends on a number of factors.

a) At first, the parasites must attach themselves to the cell membrane of the erythrocyte. Considering the analogy of viruses for cells, where the specificity lies in the outer coat of the viruses, it may be presumed that the cell membrane of the plasmodium must play a very important role in determining the specificity for a particular kind of erythrocyte. The attachment of the plasmodium garnhamella to the erythrocytes of the susceptible species in contradistinction to those of the resistant, points in the same direction. The fact that we could get attachment in only a few percentage of the erythrocytes need not worry us too much. The preparation of free parasites that we could obtain with Trager technique still leaves much to be desired. It is possible that a layer of the host erythrocyte still envelops the cell membrane of the parasite; *in vivo*, probably because of continuous buffeting within the capillary endothelial tubes, such a membrane may be rubbed off and thus the merazoite cell membrane is freed and the parasite can attach themselves easily *in vivo* to the erythrocytes of the susceptible host.

b) After attachment to the cell membrane the parasite has to obtain entry into the intracellular space of the erythrocyte. Presumably, this is achieved by the activation of a protease or a lipase by the alteration and strain on the surface membrane consequent on the attachment of the parasite to the red cell. So, this process is co-incidental to the primary specific attachment of the parasite.

c) After it gains entry into the cell the parasite has to obtain its nourishment from the erythrocytes and also by diffusion from the plasma. If the erythrocyte cannot provide the right kind of nutrition for the parasite, naturally the life of the parasite will come to an end. Such nutrition involves not only the proximate constituents but also the minerals and vitamins. The factor of nutrition has been over emphasized in the studies of host-specificity. The parasite is a complete cell, equipped with nucleus, endoplasmic reticulum and mitochondria. In contrast to the viruses, it does not have to depend on the host cell for furnish it with the necessary apparatus for synthesizing either the DNA or the RNA or the protein synthesizing machinery. Presumably it can live on its own provided it has the raw materials it needs for its growth and reproduction. However, the fact that it is an intracellular parasite and it has not yet been possible to culture it <u>in vitro</u>, points to the provision of either a key compound or induction of some key enzymes by the host cell. The key compound has not yet been identified. It is certainly not any of the usually known nutrients. Hemoglobin is not the key compound. It is not possible that the host globin can be incorporated as such

into the proteins of the parasite, the parasite proteins have to be synthesized from amino acids. If hemoglobin is to be utilized, it must be broken down to the constituent amino acids, a proteolytic enzyme which has an absolute specificity for a particular polypeptide chain of a particular hemoglobin is almost impossible. It is, of course, possible that the right proportion of the amino acids or of the other unidentified nutrients may regulate the synthesis of an essential protein or co-enzyme necessary for the continued growth and survival.

d) The merozoites, upon introduction into the circulation of the host are suspended in an environment which may or may not be conducive to the attachment of the parasite to the host. If, for example the plasma contains agglutinins to some components of the parasite, the parasites would be agglutinated and such agglutinated mass would be dealt with by the phagocytes of the host very avidly. We found that the serum of the resistant bird possessed agglutinins against the parasite whereas none was obtained in the serum of the susceptible bird. While this fact may be significant in some cases, it cannot be the sole factor determining specificity, for such agglutinins also developed during the course of the infection.

Considering all these, we conclude that the specificity of a particular parasite is determined primarily by the cell membrane of the parasite in complementarity to the cell membrane of the host. The nutritive factors of the host cell and plasma

might also play some part in determining the specificity. The character of the immunoglobulins and other immuno-protective factors of the plasma might also determine the specificity in some cases.