STUDIES ON CYTOPHILIC AND OPSONIN-ADHERING ANTIBODIES IN CATTLE, BUFFALO & RABBIT SERA

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This is to certify that the research work embodied in this thesis entitled "Studies on Cytophilic and Opsonin-Adhering Antibodies in Cattle, Buffalo and Rabbit Sera" is the original work carried out by Shri Ashok Kumar Sarmah, in partial fulfilment of the requirements for M.V.Sc. degree of Agra University, under my supervision and guidance during the academic session, 1975-76.

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C O N T E N T S

INTRODUCTION

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The sera of normal animals at times, with innumerable components of varied molecular complexity, display some sort of reactivity with a vast array of 'foreign' or immunologically unacceptable material. These serum components have been referred to as natural antibodies. The reactions attributed to natural antibodies have been reported to occur against a wide variety of micro-organisms, viz. bacteria, animal viruses, bacteriophages, protozoa, metazoan parasites etc. Existence of natural antibodies against heterologous and isologous erythrocytes have also been reported (Malkoff, 1900; Landsteiner, 1945).

These natural antibodies which might also be cytophilic or opsonic in nature are of immense importance in relation to the discriminative behaviour of the phagocytes. Evidence exists indicating that the phagocytosis of foreign or immunologically unacceptable particles by polymorpho-nuclear leukocytes depends mostly on the prior interaction of specific serum factors with the surface of the particles which are thereby rendered attractive to the phagocytic cells (Boyden, 1963; Boyden and Sorkin, 1964).

The term cytophilic antibody was first coined by Boyden and Sorkin (1960). Boyden, Sorkin and Sparck (1960) during the course of their investigation observed that certain

rabbit antisera to human serum albumin (HSA) when mixed with normal rabbit spleen cells, got fixed to these cells and conferred upon them the capacity to specifically adsorb HSA. Boyden (1964) subsequently defined cytophilic antibody as a globulin component of immune serum which becomes attached to certain cells in such a way that the cells are subsequently capable of specifically adsorbing antigen.

Berken and Benacerraf (1966) used the term 'cytophilic' to include those antibodies also which fixed to macrophages after combination with antigen and detected these antibodies by 'passive indirect technique'. Berken and Benacerraf (1966) considered cytophilin to be that property of opsonising antibody which provides the receptors to permit the binding of the antibody to the macrophage cell membrane in preparation for phagocytosis. Nelson and Mildenhall (1968) defined cytophilic antibodies as those present in the serum but capable of becoming attached to cells and conferring on these cells an affinity for, or reactivity with, antigen. They further suggested that this generic term should be qualified according to the type of cell to which the antibody is attached, e.g., macrophage, mast cell, spleen cell etc.

Amongst the various types of cells capable of binding

with cytophilic antibodies, macrophages and mast cells are worth to be noted. Boyden (1963, 1964) first reported the existence of cytophilic antibodies specific for macrophages which was later on confirmed by other workers (Jonas, Curner, Nelson and Coombs, 1965; Berken and Benacerraf, 1966). It is now believed that due to the attraction between a cytophilic site on the FC portion of the antibody molecule and a corresponding receptor site on the cell surface, cytophilic antibodies are capable of attaching to the macrophage molecules. Macrophages with specific cytophilic antibodies on their surfaces are capable of destroying the foreign mammalian target cells by a complement independent non-phagocytic contact mechanism. Besides, macrophages coated with cytophilic antibodies are also believed to play an important role in cellular immunity and delayed hypersensitivity (Nelson and Mildenhall, 1966, 1967; Lokaj, 1972; Ptak, 1973).

The binding of cytophilic antibodies to cells probably facilitates their migration across certain biological membranes, protects them against degradation and thus rendering them to be capable of arming leukocytes for aggression against any foreign agents.

Cytophic antibodies have been reported to occur in the immunoglobulin classes IgG, IgM and IgE.

Macrophage cytophilic antibodies have been reported to be produced by mice in response to a number of antigens, including sheep erythrocytes. The cytophilic antibodies produced during a secondary response to sheep erythrocytes are mostly found to be of IgG type while antibodies produced during the primary response are of IgM type (Tizard, 1969; Brown and Carpenter, 1971). Cytophilic antibodies in rabbits have been shown to be 7S IgG (Jerkin, 1963).

Opsonin-adhering antibodies are the serum proteins which promote phagocytosis by coating the particulates such as bacteria and effete mammalian target cells. The term opsonin was first employed in 1904 by Wright and Douglas who observed that fresh serum of normal animals promoted phagocytosis of various bacteria. Subsequently, other workers put forward their evidences in favour of the view that at least low levels of opsonin-adhering antibodies exist in normal serum for a large variety of bacteria as well as inert particles such as carbon, polysterene etc. (Jenkin and Rowley, 1961; Mouton, Bouthillier, Biozzi and Stiffel, 1963).

It is believed that the opsonin-adhering antibodies act by bringing about or promoting the attachment of particles to the surface of macrophages. These antibodies could be

cytophilic in nature by virtue of a change in the configuration of the H chains on the FC fragment after combining with the antigen and thus attach to a specific receptor site for cytophilic antibodies.

The phenomenon of phagocytosis by opsonin-adhering antibodies may, however, be resisted by certain pathogenic organisms such as pneumococci and β-hemolytic streptococci which are capable of synthesizing certain specialised biochemical components such as surface polysaccharides which enable them to evade the opsonic action of natural opsonins. Thus, in such circumstances, the host is forced to synthesize specific opsonin-adhering antibodies to abrogate the anti-phagotytic action of capsular polysaccharide.

Since there seems to have no information regarding the occurrence of natural cytophilic and opsonin-adhering antibodies against sheep red blood cells in domestic animals, it is proposed to carry out investigation on naturally occurring cytophilic and opsonin-adhering antibodies of heterophilic nature against sheep red blood cells in cattle and buffalo sera.

There are several reports regarding the production

of cytophilic antibodies in laboratory animals against various microbial agents e.g. Escherichia coli (Parish, 1966; Mittal, 1972), Salmonella paratyphi (Uhr, 1965), Sal. gallinarum, Sal. typhimurium, Sal. enteritidis (Padmanaban, 1976), Trypanosoma brucei (Tizard and Soltys, 1971). However, perusal of literature shows that there is no information available on the production of cytophilic and opsonin-adhering antibodies in large animals, e.g. cattle and buffaloes in response to vaccination. Attempts have, therefore, been made to demonstrate the production of these antibodies in ~~cattle and~~ buffaloes in response to vaccination with haemorrhagic septicaemia oil adjuvant vaccine and subsequent challenge infection with virulent Pasteurella multocida.

Studies have also been carried out to investigate the nature of artificially induced cytophilic and opsonin-adhering antibodies in rabbits against sheep red blood cells. Since the nature of the cellular receptors on the cell membranes, for the cytophilic and opsonin-adhering antibodies, is not clearly understood, attempts have been made for their characterization on the basis of the effects of treatment with various substances like, trypsin, papain and homologous normal serum. Sorkin (1964) also reported that rabbit spleen cells treated with proteolytic enzymes lost their capacity to

take up rabbit cytophilic antibodies to human serum albumin. It is expected that similar experiments with rabbit antibodies and heterologous macrophage system might also yield useful information on the nature of cellular receptors in the present investigation.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

There are ample evidences which show that serum of normal animals contains innumerable components with varied molecular complexity which are reactive with a vast array of different antigenic determinants, having almost similar physico-chemical and biological properties as those of antibodies that appear following immunisation. These serum components which display some sort of reactivity with foreign materials under varied conditions, have been referred to as natural antibodies.

Boyden (1956) defined the natural antibody in a broad sense to denote any of a family of molecules (probably always a protein and in mammals, probably always globulin) which are present in the body fluids of normal animals and which have the capacity to combine specially with antigens, but not with the immunologically acceptable molecules normally present in the body fluids.

It is believed that in vertebrate sera, natural antibodies are present as a consequence of previous antigenic stimulation with the test antigen or with foreign macromolecules that share determinant groups with the test antigen. There is much evidence that many antigenic determinant groups, particularly those occurring on polysaccharides are

shared by many micro-organisms as well as animal and plant tissues e.g. Forssman antigen (Forssman, 1911; Buchbinder, 1935; Boyd, 1936; Jenkin, 1963).

Natural antibodies have been reported to occur against a large varieties of bacteria (Skarnes and Watson, 1957; Shilo, 1959; Lovell, 1932, 1934; Gibson, 1930; Mackie and Finkelstein, 1930, 1931, 1932; Michael, Whity and Landy, 1962), animal viruses (Svehag and Mandel, 1964), fungi (Brody and Finch, 1960), Metazoan parasites (Sewell, 1963), heterologous serum proteins (Gruber and Cognet, 1956), mammalian tissue cells (Landy et al., 1960), starch particles (Nelson and Lebrun, 1956; Turk, 1959; Blum, 1964), heterologous and isologous erythrocytes (Malkoff, 1900; Landsteiner, 1945).

Gibson (1930) carried out a series of experiments for detection of natural antibodies against Shigella flexneri, Sh. dysenteriae, Proteus x19, Proteus morgani, Salmonella typhi, Sal. paratyphi A, Sal. paratyphi B, Pseudomonas aeruginosa, Klebsiella pneumoniae and several strains of Escherichia coli and Vibrio cholerae in the sera of ox, rabbit, guinea pig, horse, sheep, man, rat and cat. During his investigations, Gibson (1930) found that ox sera were most active, pig and horse sera were less so and human, cat,

rabbit, guinea pig and rat sera were progressively weaker.

Mackie and Pinkelstein (1930, 1931, 1932) tested normal serum from ox, sheep, horse, pig, rabbit, rat, cat, guinea pig, pigeon and man against a variety of bacteria including Vibrio cholerae, Salmonella species, Dysentary bacilli, Proteus bacilli, Brucella abortus, Br. melitensis, meningococci and so on. They found complement fixing and bactericidal antibodies in a large number of sera and showed that the bactericidal effect depended on the joint action of complement and a heat stable factor which could be removed by absorption.

Boyden (1936) reported that normal serum of certain species contains specific anti-sheep red cell antibody which is neither hemolytic nor hemagglutinating.

HETEROPHILE ANTIBODIES

The non-specific reactions attributed to heterophile antibodies have been reported by various workers from time to time and now it is well established that the reason for this apparent non-specificity is due to certain closely related cross-reacting antigens.

The existence of such cross-reacting antigens in

the tissue of different unrelated species of animals was reported earlier by Ehrlich and Morgenroth (1901). Later Frouin (1907) and Frouin and Lisbonne (1911) showed that serum of rabbits specifically immunized with the yolk of hen's eggs lysed the erythrocytes of various species of animals. Forssman (1911) discovered that when rabbits were injected with saline extracts of certain organs of the guinea pig or other animals, it led to the production of antibodies cross-reacting with antigens on the surface of sheep red blood cells.

Iseki (1952) reported the existence of Forssman antigens, related to the antigens of sheep red blood cells in various strains of Salmonella of group B. He found that the O somatic antigens of Salmonella paratyphi B, Sal. stanley and Sal. heidelberg produce in rabbits, antibodies which cross-react with the antigens of the sheep red blood cells. Buchbinder (1936) found a heterophile antigen in bacteria of hemorrhagic-septicaemia group and also in the erythrocytes of a wide variety of birds. He observed that when the bird erythrocytes are treated with rabbit antisera against these organisms, the erythrocytes were agglutinated or lysed. Finland and Curnen (1938, 1940) observed that horse antisera against type II pneumococci agglutinated human red cells of any blood group. But in contrast, the rabbit

anti-pneumococcus 14 sera failed to agglutinate cells of group A and AB and sheep red blood cells in low titres. MacDuffe and Kabat (1936) also observed certain substances having blood group A reactivity in the hog gastric mucosa, saliva and stomach linings of horses, the omasum of cows, housedust and Type XIV specific pneumococcus polysaccharide.

Springer (1936) reported that the closeness of the antigenic relationship between red blood cells and bacterial antigen is evident from the fact that the blood group agglutinins can inhibit the growth of these bacteria. These heterophile antibodies can act as bactericidin for them (Muschel and Osawa, 1960). Oliver and Gonzalez (1962) suggested that certain common parasitic bacteria and viruses of human-being contain antigens inducing sheep-cell agglutinins in man. Paul and Bunnel (1932) reported high titres of antibodies to sheep red blood cells in the serum of patients suffering from glandular fever.

Naturally occurring antibody to sheep cells in bovine sera has the specificity of a Forssman antibody. Ingram and Barnum (1965) observed that the level of naturally occurring Forssman antibody has a seasonal variation which is similar to that of conglutinin too, except that it is not significantly affected by calving or infectious diseases. Coombs (1954)

found that the Forssman antibody in the guinea pig serum failed to absorb guinea pig complement, which was thus an unsatisfactory complement for demonstrating antibody in pig. Wiggin (1953) reported that the procomplementary effect of pig serum was due to the naturally occurring Forssman antibodies in pig serum, which could be removed by absorption of the serum with washed sheep red blood cells.

The original idea that the Forssman antigen of mammalian tissue was lipoidial in nature (Forssman, 1930) was later regarded to be a macro-molecule consisting of lipocarbohydrate loosely bound to protein. Morgan and Partridge (1936, 1937, 1940, 1941) showed that O somatic antigen of Gram-negative bacteria, derived from Shigella dysenteriae, stimulated the production of sheep red blood cell hemolysins when injected into rabbits.

CYTOPHILIC ANTIBODIES

Boyden and Sorkin (1960, 1961) first detected the cytophilic binding property of immunoglobulins by incubating macrophages with antibodies to soluble protein antigens such as serum albumin and then reacting these treated cells with radioactively labelled antigen. Boyden (1964) found that in guinea pigs cytophilic antibodies were present 19 days after

primary immunization with sheep red blood cells incorporated in Freund's complete adjuvant. Jonas, Gurner, Nelson and Coombs (1965) reported that after three weeks of primary immunization of guinea pigs with sheep red blood cells incorporated in Freund's complete adjuvant by footpad route and one week after booster dose of sheep red blood cells in saline injected intradermally, had very high titers of cytophilic antibodies whereas those which were immunised with sheep red blood cells incorporated in Freund's incomplete adjuvant had very low titers or no cytophilic antibody at all.

Nelson and Hildenhall (1968) investigated the effect of route of injection of sheep red blood cells in Freund's complete adjuvant and of booster doses of sheep red blood cells in saline intradermally, on the production of cytophilic antibodies in guinea pigs. They found that sera obtained after intradermal, intra-peritoneal or footpad injection of sheep red blood cells contained moderate amounts of cytophilic antibodies but very little or no cytophilic antibody could be detected in the sera of guinea pigs obtained two weeks after subcutaneous injections. They further observed that cytophilic antibodies were present inconsistently 6, 7, 8 and 9 days after immunization, but were present consistently 14 days after immunization.

Gowland (1963, 1968) reported that in guinea pigs

cytophilic antibodies were generally absent 7 days and 3 month after immunization but consistently present 3 to 4 weeks after immunization when sheep red blood cells were used as antigen.

There is a paucity of information about the factors affecting the production of cytophilic antibodies to soluble antigens. Blazkovec (1966) immunised guinea pigs with complexes of Human serum albumin and rabbit anti-HSA antibodies incorporated in Freund's complete or incomplete adjuvant and injected into the footpads. He observed that the animals which were not skin tested showed no cytophilic antibodies 1, 2, 3 or 4 weeks after immunization. But he was able to observe cytophilic antibodies in the guinea pigs which were immunized with complexes in Freund's complete adjuvant after skin testing, titre reaching the peak 7 days after skin testing. Nelson and Hildenhall (1968) found that guinea pigs immunized intradermally with human serum albumin incorporated in Freund's complete adjuvant, invariably showed moderate titers of cytophilic antibodies after two weeks of immunization. Studies on guinea pig cytophilic antibodies directed against human serum albumin by Magino, Spar, Daniel and Blazkovec (1972, b) revealed that immune complexes prepared with homologous immune globulin were highly immunogenic than were complexes prepared with a heterologous source

of immune globulin.

Production of cytophilic antibodies in mice to sheep red blood cells has been reported by various workers. Nelson and Mildenhall (1967) carried out some investigations on the production of cytophilic antibodies in non-inbred Swiss mice following immunization with sheep red blood cells in saline, Freund's complete or incomplete adjuvant, injected subcutaneously or intraperitoneally. They found that in primarily immunized mice, cytophilic antibodies were consistently present when the animals had received sheep red blood cells incorporated in Freund's complete or incomplete adjuvant either through subcutaneous or intraperitoneal route, the titer reaching its maximum after one week of immunization but no cytophilic antibodies could be detected after 4 weeks of primary immunization alone. They also observed a secondary cytophilic antibody response in mice after injecting sheep red blood cells suspended in saline into the footpads. Such secondary cytophilic antibody response was reported to be more consistent when the primarily injected sheep red blood cells were incorporated in Freund's complete adjuvant, but inconsistent when primarily injected sheep red blood cells were incorporated in saline. Nelson and Mildenhall (1967) further observed that cytophilic antibodies present at 7 days (early antibodies) differed from those appearing after

secondary immunisation (hyper immune antibodies). The "early antibodies" gave strongly positive reactions even at low dilutions. They were very much sensitive to freezing and thawing and attached to a trypsin sensitive receptors, unlike the receptors for "hyperimmune antibodies" which were trypsin resistant.

Berken and Benacerraf (1966) showed the production of cytophilic antibodies in mice by using multiple injection of sheep red blood cells in saline.

Lukaj (1969) reported that the formation of cytophilic antibodies in mice could be induced by immunization with sheep red blood cells with and without Freund's complete adjuvant. Nelson ~~and Ray~~ (1969) studied the production of cytophilic antibodies by inbred strains of mice (A/J and C57Bl/6J) after immunizing them subcutaneously with sheep red blood cells in Freund's complete adjuvant. They observed that peritoneal macrophages freshly isolated from C57Bl/6J had a natural affinity for sheep erythrocytes and thus not suitable for the titration of cytophilic antibodies. Titration of A/J cytophilic antibodies produced especially after primary immunization, on A/J macrophages gave very weak or negative reactions, whereas the same sera gave strong reaction with Swiss macrophages. They concluded that in comparison to Swiss

mice, C57Bl/6J and A/J mice produced less detectable cytophilic antibodies. Tizard (1969, 1970, 1971) also reported that cytophilic antibodies could be produced in mice against sheep erythrocytes incorporated in saline after primary or secondary immunization through intraperitoneal route. Brown (1971) found that antigens (sheep erythrocytes) emulsified in Freund's complete adjuvant induced the formation of cytophilic antibodies in high titer. He was able to isolate the cytophilic antibody in purified form by permitting it to attach to macrophages *in vitro* and eluting it at 56°C. The production of mouse cytophilic antibodies to antigens other than sheep erythrocytes has been studied by some workers. Parish (1966) found cytophilic antibodies in the sera of mice immunised with bovine plasma albumin, bovine gamma globulin or *E. coli* somatic polysaccharide. Bowley et al. (1964) reported the existence of mouse cytophilic antibodies to *Salmonella typhimurium*. Their evidence was based on the ability of peritoneal cells from immune mice to protect normal mice against a challenge infection with the homologous organisms.

Hoy and Nelson (1967, 1969) studied the production of cytophilic antibodies in C57Bl/6J mice directed against the histocompatibility antigens (H2) of A/J mice, using the A/J tumour Sarcoma I as a particle which attaches to sensitized macrophages. They noted that these cytophilic antibodies are

produced after grafts of either A/J skin or the tumour Sarcoma I itself.

Mittal (1972) reported the production of cytophilic antibodies in mice and rabbits against a smooth and a rough strain of E. coli. He showed that cytophilic antibodies in mice are produced in response to the antigens, possibly the flagellar antigens, possessed only by the live smooth organisms, but no cytophilic antibodies were demonstrable in mouse anti-3662 serum when capsular or somatic antigens of E. coli 3662 strain coated on sheep erythrocytes were used as test antigens.

There is not much information available about the production of macrophage cytophilic antibodies in rabbits. Boyden and Sorkin (1960, 1961) found antibodies cytophilic for spleen cells in rabbits directed against Human serum albumin with the aid of Freund's complete adjuvant. They obtained maximum titers at 1 week after a booster injection of antigen in saline. Berken and Benacerraf (1966) found cytophilic antibodies in the sera of rabbits, immunized with eight intravenous injections of stroma of sheep erythrocytes for over a period of two weeks and bled 10 days after the last injection. Kossard and Nelson (1968) found high titers of cytophilic antibodies in the sera of rabbits which were

immunized with sheep erythrocytes or human serum albumin in Freund's complete adjuvant intradermally followed by an intraperitoneal injection of the test antigen in saline two weeks later and bled after a further week.

Lokaj (1969) demonstrated cytophilic antibodies in rabbit amboceptor (rabbits hyperimmunized by sheep erythrocytes) using macrophages from rabbits, mice and guinea pigs. He obtained highest cytophilic antibody titer with guinea pig macrophages. He further observed that cytophilic activity for macrophages of a given species was best absorbed by homologous cells. Hagimet al (1972) reported the existence of rabbit cytophilic antibodies directed against human serum albumin (HSA) complexed with homologous immune globulins. He further maintained that rabbits sensitised after incorporating the immune precipitates in Freund's complete adjuvant significantly produced high levels of cytophilic anti-human serum albumin antibody following skin testing. Tizard and Soltys (1971) detected cytophilic antibodies to Trypanosoma brucei 1 week after infection of rabbits with a mouse adapted substrain of the organism, employing macrophage monolayer method utilising intact trypanosomes as antigen.

The production of cytophilic antibodies in chicken has been reported by Borsellino, Albano, Bellavia and Salerno

(1973). They observed that chicken cytophilic antibodies which were produced following intravenous injection of human serum albumin without adjuvant closely resembled mammalian cytophilic antibodies. McBride, Leckband and Schierman (1969) observed that chicken isoantibodies specific for erythrocyte isoantigens determined by the A and B blood group loci have cytophilic properties which could be demonstrated by the attachment of antibody coated erythrocytes to macrophages.

Cytophilic antibodies have also been demonstrated in Syrian hamster by Fortis and Coe (1975), using sheep erythrocyte rosetting technique.

The production of cytophilic antibodies in human beings against certain antigen and in diseased conditions has been reported by several workers. Buck and Kalhoff (1972) reported the existence of cytophilic antibodies of heterophile nature in psoriatic patients. By using cell rosetting technique, they found that 77% of the psoriatic patients had cytophilic antibodies of heterophilic nature. Tynan and Zwolinski (1972) demonstrated the macrophage cytophilic antibodies in the sera of patients suffering from pulmonary tuberculosis, by using passive direct and indirect method. Mitchell, Malcolm, Margalit, Gregg and McIntosh

(1972) demonstrated macrophage cytophilic antibodies in the serum of 25 patients with acute myelocytic or lymphocytic leukemia.

Physico-Chemical Properties of Cytophilic Antibody

The physico-chemical characteristics of cytophilic antibodies in different species have been studied by several workers. Jonas et al. (1965) and Berken and Benacerraf (1966) demonstrated by means of starch block electrophoresis that majority of the cytophilic antibodies in the sera of guinea pigs immunised with antigen in Freund's complete adjuvant belong to 7S gamma-globulin type. Nelson and Mildenhall (1968) and Gowland (1968a) also found the same results by using the DEAE-cellulose chromatography. By using sucrose density gradient ultra-centrifugation of guinea pig anti-sheep erythrocyte antiserum, Berken and Benacerraf (1966) reported that the cytophilic antibodies were 7S globulins. They have also reported that guinea pig cytophilic antibodies have some complement fixing capacity, but Nelson and Mildenhall (1968) did not find any correlation in the levels of cytophilic and complement fixing antibodies of guinea pig sera. Uhr (1965) reported that guinea pigs immunised with *B. naratynbi* S flagella without adjuvant had "primary 19S antibody" in the sera obtained after 5 days of

primary immunisation, primary "7S antibody" in the sera obtained after 14 days of primary immunisation and "Secondary 7S antibody" in the sera obtained after a secondary immunisation.

In mice also cytophilic antibodies are found among 7S gammag-globulins. However, cytophilic antibodies produced after primary immunization alone are not necessarily 7S gammag-globulin. Parish (1965) reported that cytophilic antibodies to bovine plasma albumin and bovine gamma-globulin were almost entirely present in the 7S gammag-globulin fraction but almost entirely absent from 7S gamma$_1$-globulin fraction of the whole gamma-globulin. Parish (1966) also reported that mouse cytophilic antibodies to somatic polysaccharides of *E. coli* were probably 19S (IgM) immunoglobulins. Turner *et al.* (1964) have reported that vaccination of mice with a living attenuated culture of *Sal. typhimurium* results in the production of two types of immunoglobulins. The antibody produced in the first phase is characterized by a 19S macro-globulin response which disappears after about one months, to leave the 7S type antibody which persists for at least six months.

Nelson *et al.* (1967) reported that in the sera of

Swiss mice which were immunized with sheep erythrocytes in saline, complete adjuvant or incomplete adjuvant, followed by a booster dose of sheep erythrocyte in saline, most of the cytophilic antibodies were found in the 7S gamma$_2$-globulin fraction of the whole serum. Hay and Nelson (1967) further reported that fractionation of C57Bl/6J anti-A/J antisera has revealed cytophilic antibodies in three fractions containing respectively 7S gamma$_2$-globulins, 19S globulins and albumin plus a fast α_1-globulin. Nelson (1970) also found a similar fast α_1-globulin in the sera of Swiss mice obtained after seven days of immunization with sheep erythrocytes in Freund's adjuvant. Lokaj (1968) reported that cytophilic activity was present mainly in the 7S fraction and also in the 19S fraction of the serum of mice twice revaccinated with sheep erythrocytes. Tizard (1969) showed that cytophilic antibodies elicited in mice after a single injection of sheep erythrocytes were macroglobulins and those formed during a secondary response were of IgG type.

Rowley, Turner and Jenkin (1964) reported the existence of mouse 19S cytophilic antibodies to S. typhimurium. Levenson, Braude and Chernokhvostva (1969) found that secondary cytophilic antibodies present in the serum of mice in the early period after immunization with Vi antigen of S. typhi, belong to the class of gamma M-globulin.

Very little information is available regarding the physico-chemical properties of rabbit cytophilic antibodies. Lokaj (1969) reported that cytophilic activity of rabbit anboceptor for guinea pig macrophages was found in 7S as well as in 19S type antibodies obtained by chromatography on Sephadex G-200 columns.

By Sephadex G-200 chromatography and immunoelectrophoresis Borsellino et al. (1973) have shown that chicken cytophilic antibodies directed against human serum albumin belong to 7S globulin type.

Tyran and Zwolinski (1972) demonstrated that cytophilic antibodies in the sera of patients suffering from pulmonary tuberculosis belong to IgG class. They further observed that almost all cytophilic activity was present in the IgGg immunoglobulin fraction and no cytophilic activity was found in IgM immunoglobulins.

The Nature of the Macrophage Membrane Receptors for Cytophilic Antibody

Howard and Benacerraf (1966) and Davey and Asherson (1967) investigated the nature of the macrophage receptors for cytophilic antibodies in guinea pigs. They found that those receptors were not susceptible to attack by proteolytic

enzymes (trypsin, chymotrypsin, papin, ficin and pronase) but were susceptible to Phospholipase A, lecithinase C, Naja naja venom and agents reacting with phospholipids and -SH group. The observations of Howard and Benacerraf (1966) indicated that free SH groups play an important part in the receptor's activity of guinea pig macrophages for cytophilic antibody, whereas those of Davey and Ashersen (1967) indicated that phospholipid found in the cell receptors play a major role. Kossard and Nelson (1968b) observed that receptors on the mouse and guinea pig macrophages for 7S gamma-globulin cytophilic antibodies were resistant to the treatment with various proteolytic enzymes, whereas those on mouse macrophages for α_1-globulin antibodies were susceptible to such treatment. They suggested that in case of mouse macrophages for α_1-globulin cytophilic antibodies, a peptide bond is involved either in the receptor site itself or the attachment of the receptors to other structures in the macrophage cell membrane. Both Nelson and Boyden (1967) and Fizard (1969) have found that the receptor for mouse 19S cytophilic antibody is trypsin-sensitive. Sorkin (1964) also found that trypsin treated rabbit macrophages failed to absorb spleen cell cytophilic antibodies.

Attachment of Cytophilic Antibodies to Different Cells

Boyden (1964) first confirmed the existence of antibodies specifically cytophilic for macrophages. However, Sorkin (1964) reported that rabbit cytophilic antibodies could bind to granulocytes and lymphocytes. Koller and Sorkin (1963) also found that the antibody which they examined was cytophilic for mast cells and liver cells. Jones et al. (1965) and Howard and Benacerraf (1966) found that mouse and guinea pig sera containing macrophage cytophilic antibodies do not sensitize neutrophils, eosinophils, lymphocytes or fibroblasts. Rabbit antibodies cytophilic for lymphocytes have also been described by Uhr (1963), Rose, Kite, Doebbler and Brown (1963) and Bussard (1964) as occurring in antisera to thyroglobulin, bovine serum albumin and bacteriophage. Uhr (1965) reported that complexes of flagellar antigens and guinea pig antibodies are capable of attaching to lymphocytes and plasma cells as well as to macrophages. Berken and Benacerraf (1966) have reported that mouse, guinea pig and rabbit alveolar macrophages adsorbed more cytophilic antibodies in comparison to their peritoneal macrophages. Bussard (1966) found that rabbit anti-sheep erythrocyte macrophage cytophilic antibodies can not only sensitize rabbit and guinea pig macrophages but also guinea pig small lymphocytes and small round cells, presumably lymphocytes in rabbit

spleen. Tizard (1969) reported that only cells seen to adsorb cytophilic antibody in the mouse were cells of the mononuclear phagocytic type i.e. peritoneal, alveolar, spleen and bonemarrow macrophages, blood monocytes, some Kupffer cells and phagocytic mononuclear cells obtained from the brains of embryo mice.

Factors Affecting the Attachment of Cytophilic Antibodies to Cell Receptors

Effect of Temperature

The effect of temperature upon the uptake of cytophilic antibodies has been reported by several workers. Rose and Brown (1962) reported that temperature has little effect on the uptake of rabbit cytophilic antibody. Jonas at al. (1965) reported by using guinea pig peritoneal macrophages that changes in temperature did not significantly affect the adsorption of guinea pig macrophage cytophilic antibody. They found that uptake of antigen was similar at 37°C, room temperature and 4°C. Berken and Benacerraf (1966) observed sensitization to be higher when the cells and serum were incubated at 37°C than at room temperature or 4°C. Koszard and Nelson (1968a) found that guinea pig peritoneal macrophages were slightly less readily sensitized at 37°C than at lower temperatures. Tizard (1970, 1971) found that both mouse IgG and IgM macrophage cytophilic antibodies were bound to cells much more strongly at 4°C than at 37°C.

It has also been reported that when macrophages carrying cytophilic antibodies are incubated in the absence of free serum or antigen at different temperatures, there is some loss of antibodies from cell surface and thus subsequent uptake of antigen is decreased (Uhr, 1966; Berken and Benacerraf, 1966; Kossard and Nelson, 1968a).

Inhibitory Effect of Non-Specific Cytophilic Immunoglobulins

The attachment of cytophilic antibodies to macrophages is markedly affected by normal serum, containing non-specific cytophilic antibodies which compete against the specific cytophilic antibodies under test for available cell receptors (Berken and Benacerraf, 1966; Inchley, Grey and Uhr, 1970; Jonas et al., 1965; Kossard and Nelson, 1968a; Lutaj, 1969).

Kossard and Nelson (1968a) found that this inhibitory effect is very much pronounced in the case of guinea pig and rabbit cytophilic antibodies and homologous normal serum and in the case of mouse hyperimmune serum containing 7S gamma-globulin, but not found consistently between α_1-globulin cytophilic antibodies to sheep erythrocytes and normal mouse serum. Berken and Benacerraff (1966) reported that uptake of cytophilic antibodies by guinea pig macrophages is more markedly inhibited by the serum containing cytophilic

antibodies to another unrelated antigen than by normal guinea pig serum. But Kessard and Nelson (1968a) could not confirm it. They observed that incubation of guinea pig macrophages with normal serum which had adsorbed cytophilic antibodies has a "desensitising" effect, macrophages so treated subsequently adsorb less antigen than those macrophages which were incubated at the same temperature for the same period of time in the absence of serum. They further noted that this effect was very pronounced at 37°C but slight at room temperature. Rizard (1969) reported that pooled inactivated serum from normal mice adsorbed three times at 4°C with a quarter of its volume of packed sheep erythrocytes, inhibited the adsorption of cytophilic antibodies. Lokaj (1970) investigated the ability of guinea pig sera and the eluates from passively sensitized guinea pig macrophages to inhibit the uptake of cytophilic antibodies by homologous macrophages. He observed that normal serum, serum from animals immunized with sheep erythrocytes in Freund's complete adjuvant and eluates from macrophages passively sensitized with these sera inhibit the uptake of guinea pig and chicken red cells cytophilic antibodies by homologous macrophages. The ability of the cytophilic antibodies to sensitize heterologous macrophages and the inhibitory effect of heterologous normal sera for cytophilic antibodies have been studied by

Berken and Benacerraf (1966) and Kossard and Nelson (1968a). They observed that guinea pig cytophilic antibodies are adsorbed to a less extent or not at all by guinea pig macrophages. They further observed that although guinea pig cytophilic antibodies do not sensitize mouse macrophages, normal guinea pig serum inhibits the uptake of mouse hyperimmunised antibodies by mouse macrophages.

Effects on Macrophages by Treatment with Various Agents

Treatment of macrophages with various agents may affect their ability to take up cytophilic antibody. Treatment of guinea pig macrophages with proteolytic enzymes like trypsin, papain, α-chymotrypsin or ficin increases their ability to take up cytophilic antibodies (Howard and Benacerraf, 1966; Davey and Asherson, 1967; Kossard and Nelson, 1968b). Kossard and Nelson (1968b) found that similar treatment of guinea pig macrophages passively sensitised in vitro with cytophilic antibody does not affect their ability to take up antigen. Kossard and Nelson (1968b) further observed that the treatment of mouse macrophages with trypsin or papain does not abolish and may slightly increase their ability to take up homologous cytophilic antibodies from hyperimmune sera. Similar treatment, however, reduces their capacity to take up cytophilic antibodies from "early" sera obtained after 7 days of

immunization with sheep erythrocytes in saline. They also noted that treatment of mouse macrophages with serum after passive sensitization in vitro apparently removes all the cytophilic antibody taken up from early sera, but has much less effect on the cytophilic antibodies taken up from hyperimmune sera. Tizard (1969) found that mouse macrophages treated with trypsin did not adsorb cytophilic antibody formed during a primary response in mice, however, an identical treatment did not usually alter their capacity to adsorb cytophilic antibody from a secondary response.

Effect of β-Mercaptoethanol Treatment

The effect of β-Mercaptoethanol treatment of whole serum has been reported by several workers (Berken and Benacerraf, 1966; Nelson and Mildenhall, 1967; Lokaj, 1968; Tizard, 1969; Brown & Carpenter, 1971; Borsellino et al., 1973).

Berken and Benacerraf (1966) and Nelson and Mildenhall (1967) reported that treatment of guinea pig whole serum alone with β-mercaptoethanol does not consistently affect the cytophilic activity, but treatment with β-mercaptoethanol followed by alkylation with iodoacetamide consistently diminishes the cytophilic activity. However, Berken and Benacerraf (1966) found that cytophilic antibody activity

of one sample was partly susceptible to 2-mercaptoethanol. Lokaj (1968) also observed that cytophilic antibody activity of mouse serum immunized with sheep erythrocytes was inhibited by 2-mercaptoethanol treatment. Tizard (1969) observed that treatment of mice serum obtained after primary immunization with sheep erythrocytes, with 2-mercaptoethanol, destroyed the cytophilic antibody activity. But he did not find any apparent effect of 2-mercaptoethanol upon the cytophilic antibody produced during a secondary response. Brown and Carpenter (1971) reported that mouse cytophilic antibodies produced in response to sheep erythrocytes emulsified in Freund's complete adjuvant were not affected by treatment with 2-mercaptoethanol. Borsellino et al. (1973) also found that cytophilic antibody activity of late immune sera in chicken against human serum albumin (HSA) was resistant to the action of 2-ME.

Effect of EDTA and Heat

Borken and Benacerraf (1966) carried out some experiments to determine whether cytophilic antibody require calcium or magnesium ions or complement to bind to macrophages. They observed that heating guinea pig anti-SRBC sera for 1 hour at 56°C does not alter the cytophilic properties or titre of the sera; furthermore, the passive sensitization of normal

macrophages is not interfered with when the antiserum is treated with 0.1 M NagEDTA.

Nelson (1970) carried out some experiments to determine whether reactions ascribable to cytophilic antibodies are due to complement or complement fixing antibodies or not. He found that after the incubation of SRBC in the saline dilution of mouse early sera which had been untreated, heated to 56°C for 20 minutes, diluted in 0.01 M EDTA or both heated and diluted in EDTA, the reactivity of cytophilic antibody was slightly weakened by heat, EDTA or a combination of the two, but was not completely abolished.

Cytophilic Antibody in Delayed Hypersensitivity

The possible role of macrophage cytophilic antibodies in the development of delayed type hypersensitivity has been studied by various workers (Cole and Favour, 1956; Ehrenkrang and Waksman, 1956; Raffel and Newel, 1958; Rauch and Favour, 1960; Tauji et al., 1964; Boyden, 1964; Nelson and Boyden, 1964; Kochan & Bendel et al., 1966; Gowland, 1966; Holtzer, 1967; Holtzer and Winkler, 1967; Mulliger, Blankovec and Sorkin, 1968; Nelson and Hildenhall, 1968; Dupuy, Percy and Good, 1969; Zembala and Asherson, 1970; Askenase, 1971; Lokaj, 1972; Kostiala, 1972; Ptak, 1973).

Cole and Favour (1955) and Rauch and Favour (1960) demonstrated passive transfer of delayed type hypersensitivity in guinea pigs by a fraction containing mostly an α-globulin. But Ehrenkranz and Waksman (1956) could not confirm the observations of Cole and Favour (1955).

Raffel and Nevel (1955), Boyden (1964) and Nelson and Boyden (1964) reported that guinea pigs immunized with protein antigens or sheep erythrocytes in Freund's complete adjuvant consistently develop delayed-type hypersensitivity as revealed by delayed skin reaction to the antigen, whereas guinea pigs immunized with antigen incorporated in Freund's incomplete adjuvant failed to elicit the reaction. Asherson and Loewi (1965) found that serum from guinea pigs with delayed type hypersensitivity could potentiate the effectiveness of lymphoid cells in the systemic passive transfer of delayed cutaneous reactivity. Gowland (1966) found that there is no relationship between the titer of cytophilic antibody and the intensity of delayed skin reactions in guinea pigs. Holtzer (1967), Holtzer and Winkler (1967) also did not find any correlation between the titers of cytophilic antibody and delayed hypersensitivity in guinea pigs. Nelson and Mildenhall (1968) carried out some investigations on the factors affecting the production of cytophilic antibodies by guinea pigs in response to immunization with

sheep erythrocytes incorporated in Freund's complete adjuvant, and its relationship with the development of delayed type hypersensitivity. But they did not find any close relationship between the intensity of delayed skin reactions and titers of cytophilic antibodies present in the sera of individual animals after two weeks of sensitisation. They further observed that guinea pigs injected once or twice with sheep erythrocytes or human serum albumin in saline before being injected with the same antigen in adjuvant had weaker delayed type of sensitivity and lower cytophilic antibody titers than the animals receiving only antigen in saline. Turk and Polak (1967) achieved local passive transfer of delayed cutaneous sensitivity in two out of six attempts with purified peritoneal macrophages from hyperimmune strain II guinea pigs. But they were not clear about the significance of the results since Arthus like reactivity was also transferred and purified macrophages were ineffective in strain XII guinea pigs. Halliger et al. (1968) found that in guinea pigs, local passive transfer of delayed skin reactivity to sheep erythrocytes has been effected by normal peritoneal cells with cytophilic antibodies in vitro. They passively sensitised normal guinea pig peritoneal cells with serum from guinea pigs immunised with sheep erythrocytes

in Freund's complete adjuvant. When these sensitized cells were injected, together with the antigen intradermally into normal recipients, a skin lesion developed at the site of injection which reached to maximum severity after 24 hours of injection. Injection of antigen together with serum from hypersensitive animals gave rise to an Arthus-type reaction with maximum severity within 4 to 12 hours. Dupuy et al. (1969) reported that a cytophilic factor, by passively transferred cells may play an important role in delayed type of hypersensitivity in guinea pigs. They were able to transfer delayed skin reactivity to normal guinea pigs by small quantities of fresh plasma from X-irradiated hypersensitive donors using tuberculin PPD as antigen. They found that plasma from non-irradiated PPD-sensitive donors did not transfe any skin reaction, but they observed that when 3×10^8 viable nucleated spleen cells from normal guinea pigs were incubated with 5 ml of plasma from irradiated sensitised donors and after washing were injected into normal guinea pigs, the recipients exhibited delayed skin reaction to tuberculin PPD when tested 6 days later.

Nelson and Mildenhall (1967) reported that a relationshi exists between the primary production of macrophage cytophilic antibodies and the development of delayed type hypersensitivity

in mice. They observed that cytophilic antibodies and delayed type hypersensitivity developed consistently when the antigen (sheep erythrocytes) was incorporated in Freund's complete adjuvant and injected intraperitoneally and when subcutaneous route was used, cytophilic antibodies and delayed type hypersensitivity developed after the antigen had been incorporated in saline.

The existence and role of cell bound cytophilic antibodies in delayed type hypersensitivity has also been reported by Zembala and Asherson (1970) in mice. They were able to transfer the contact sensitivity to oxazolone by means of serum from sensitive to normal mice. They also observed that contact sensitivity could be transferred by normal purified peritoneal macrophages passively sensitised with immune serum. Askenase (1971) found that in contact hypersensitivity to oxazolone in CBA mice, skin reactions which are delayed in time, can be transferred with macrophages from sensitized donors. He also noted that the transferred reactivity can be abolished by treatment of macrophages with trypsin or antimouse gamma-globulin.

Cytophilic Antibodies and Resistance to Infection

One of the major forms of cell mediated immune response

is the state of acquired "cellular immunity". Mackaness and Blanden (1967) reported that this acquired cellular immunity is manifested by an increase in the phagocytic and bactericidal abilities of macrophages and usually arises as the result of infection by an organism capable of intracellular multiplication such as Mycobacterium tuberculosis or Listeria monocytogenes. Mackaness (1971) observed that infection with such an organism stimulates the appearance of a new population of small lymphocytes which migrate to the site of infection, where after interaction with the antigen, they influence the surrounding macrophages so that their power of combating the invading organism is increased. These stimulated macrophages exhibit enhanced phagocytosis and destruction of any foreign agents they come in contact with.

OPSONIN-ADHERING ANTIBODIES

Wright and Douglas (1903, 1904) described opsonins as thermolabile substances present in normal serum which act non-specifically on a variety of bacteria to make them liable to phagocytosis. Muir and Martin (1906a) reported that opsonins resemble complement and could be removed from the fresh serum by antigen-antibody complexes. Ecker, Weisberger and Pillemer (1942) [illegible] Ecker, Pillemer and Kuhn (1942) measured the opsonic action of large numbers of sera from various mammalian species on Staphylococci, both

alone and with antibody. However, they could not establish any constant associationship between the opsonic activity and the four components of complement. But Maaløe (1947) concluded that the normal thermolabile factors responsible for bactericidal and opsonic effects in serum were identical and that all the four components of complement were necessary for both effects. While working with rabbit granulocytes, Hirsh and Strauss (1964) also found that some bacterial strains required no rabbit serum factors for phagocytosis, some opsonized by heat-labile opsonins, or by antibody and some encapsulated strains opsonizable only by antibody. They also found that the heat-labile opsonins were constant in amount in the samples of sera tested, inactivated by hydrazine and ammonia, not present in the serum gamma-globulins and non-specific in action. But since neither Ca^{+} nor Mg^{++} were necessary for opsonization, they concluded that heat-labile opsonins were neither antibody nor the complete complement system.

Physico-Chemical Characteristics of Opsonin Adhering Antibodies

Inspite of their paramount importance in the host defence mechanism, the opsonin adhering antibodies have not yet been substantially charaterised. Allen, Seba and Holmar

(1972) carried out some investigations for the isolation, identification and characterization of the opsonins. They found that opsonic antibody could be precipitated quantitatively by 35% ammonium sulphate which migrated exclusively as an α_2-globulin in free flow electrophoresis. They also observed that the purified factor promoted phagocytosis of gelatinised lipid emulsion in vitro and caused aggregation of both the lipid emulsion and colloidal gold. Allen et al. (1973) also reported that the isolated factors could be stored in a lyophilised form at -16°C with the retention of its phagocytosis stimulatory activity for about 90 days.

Parish (1965) carried out an investigation to differentiate between cytophilic antibody and opsonin antibody by a macrophage phagocytic system. He observed that opsonin antibodies which are detectable by passive direct technique, are not cytophilic prior to combination with the antigen. By agar gel electrophoresis, Parish (1965) separated the opsonizing activity from the cytophilic activity of mouse anti-bovine plasma albumin and anti-bovine gamma globulin.

Tizard (1969a, b) also carried out some experiments to differentiate antigen-adherence due to cytophilic and opsonin antibody in mice. He observed that in the serum of mice taken after 6 days of primary immunization with sheep

erythrocytes, opsonic antibodies were mainly present in the second main peak eluted from Sephadex G-200 and on electrophoresis it was found widely distributed throughout the gamma region. But serum taken 6 days after a second or subsequent immunization revealed that the opsonin-antibodies were present in the fractions containing immuno-globulin G on gel filtration, ion-exchange chromatography and preparative electrophoresis. He also suggested that the opsonins could be heterogenous and be found in macroblobulin containing fractions.

NATURE OF COMPLETE AND INCOMPLETE ANTIBODIES

Ravin and Ehlberg (1970) studied the complete and incomplete antibodies in rabbits immunized with 90% suspension of SRBC. They determined the titre of complete antibodies by active hemagglutination test and the titre of incomplete antibodies by the indirect Coomb's test using ass anti-rabbit globulin serum. They found that both the complete and incomplete antibodies belong to IgM class as no change in the titres was noticed after 2-ME treatment. Klykova and Prokopenko (1975) also studied complete and incomplete anti-bodies in rabbits immunized with different doses of SRBC. They found that decrease in the titres of complete antibodies takes place more rapidly than that of incomplete. They also

observed that titres of complete antibodies were higher in the reaction with trypsinized SRBC than in the reaction with native SRBC, while the titres of incomplete antibodies were almost identical in the reaction with native and trypsinized SRBC. They further reported that at all periods of the investigation, the titres of incomplete antibodies to native SRBC were higher than the titres of complete antibodies.

MATERIALS AND METHODS

MATERIALS AND METHODS

DEMONSTRATION OF NATURALLY OCCURRING CYTOPHILIC, OPSONIN-ADHERING AND HETEROPHILE ANTIBODIES IN CATTLE AND BUFFALO SERA

Sera Samples of Cattle and Buffaloes for Cytophilic, Opsonin-Adhering and Heterophile Antibodies

Blood samples were collected from apparently healthy cattle and buffaloes of various age groups, maintained at IVRI Dairy Farm. About 10 ml of blood was collected from each animal through jugular vein puncture under strict sterile condition in sterilized test tubes and allowed to clot at room temperature. The blood clots were broken with the help of a sterile pasteur pipette and after centrifugation the sera samples were separated and stored at -20°C until used.

The test sera samples were heat-inactivated at 56°C for 30 minutes in a water bath prior to use.

Preparation of Physiological Solution

Normal Saline Solution (NSS)

0.85% saline solution was prepared by dissolving 8.5 gm of sodium chloride in 1000 ml of distilled water.

The saline solution was then sterilized by autoclaving at 15 pound pressure for 15 minutes.

Phosphate-Buffer Saline (PBS)

Phosphate buffer saline (PBS) 7.2 was prepared by dissolving the following ingredients in 500 ml of distilled water :

NaCl	...	36.0 gm
Na_2HPO_4 (anhydrous)	...	7.4 gm
KH_2PO_4 (anhydrous)	...	2.15 gm

The solution was then autoclaved at 10 pound pressure for 15 minutes.

Hank's Solution

Hank's solution was prepared by dissolving the following ingredients in 995 ml of distilled water :

NaCl	...	8.0 gm
KCl	...	0.4 gm
Na_2HPO_4	...	0.06 gm
KH_2PO_4	...	0.06 gm
Dextrose	...	2.00 gm
Phenol red (0.4%)	.	5.00 ml
$NaHCO_3$	...	0.36 gm

The solution was autoclaved at 5 pound pressure for 10 minutes.

Sheep Red Blood Cell Preparations

Alsevers' Solution

The modified Alsevers' solution (Mushel and Love, 1955) was prepared by dissolving the following ingredients in 100 ml of distilled water :

Glucose	...	2.05 gm
Sodium chloride	...	0.42 gm
Sodium citrate	...	0.80 gm
Citric acid	...	0.055 gm

The solution was autoclaved at 10 pound pressure for 15 minutes and stored at 4°C until used.

Sheep Red Blood Cells (SRBC)

A healthy sheep was bled through jugular vein puncture at weekly interval and the blood was mixed with an equal volume of Alsevers' solution. The sheep blood was centrifuged at 1,500 r.p.m. in a clinical centrifuge machine for 10 minutes, the supernatant along with the buffy-coat being removed. The SRBC were then washed thrice in saline and centrifuged at the same speed for 10 minutes to prepare the

packed SRBC. Finally a 1% suspension of the SRBC was prepared from the packed cells in normal saline.

Collection of Peritoneal-Macrophages for Cytophilic and Opsonin-Adhering Antibody Assay

Peritoneal-macrophages from the unstimulated peritoneal cavities of normal, healthy, adult mice, rats and buffaloes were collected for the assay of cytophilic and opsonin-adhering antibodies.

Normal, adult mice and rats of either sex weighing about 16-20 gms and 80-100 gms respectively were killed under chloroform anaesthesia. The abdominal skin was moistened with absolute alcohol and the skin was reflected. 5 ml of Hank's solution was introduced into the peritoneal cavity and after gentle agitation, the washings were withdrawn with a sterile pasteur pipette and placed in a sterile plastic tube. Washings from the peritoneal cavities of at least two mice or rats were pooled. These washings were the source of peritoneal-macrophages.

Buffalo peritoneal-macrophages were collected from apparently healthy adult buffaloes of either sex slaughtered at the Municipal Slaughter House, Bareilly. Through a sterile pasteur pipette, about 5 ml of peritoneal-fluid was collected by opening the peritoneal-cavity and placed

in a sterile plastic tube containing an equal amount of Hank's solution.

Method to Study the Cytophilic and Opsonin-Adhering Antibodies

The "rosette" test originally used by Boyden (1964) was employed in the present investigations with slight modification to study the cytophilic and opsonin-adhering antibodies.

Test for Cytophilic Antibody

0.2 ml of peritoneal fluid containing macrophages obtained from the unstimulated peritoneal-cavities of normal mice, rats and buffaloes were cultured in the chamber of acid-free micro-cavity slide at room temperature for 45 minutes. The cell-monolayer so obtained was then washed with Phosphate buffer saline pH 7.2 (PBS) and 0.2 ml of neat test serum was added to it. After incubating at room temperature for further 30 minutes, the monolayer was washed three times with PBS and finally 0.2 ml of 1% SRBC suspension was added to the monolayer. Half an hour later, the monolayer was thoroughly washed with PBS to remove the unattached SRBC from the monolayers. Finally the monolayer was stained with Leishman's stain.

Controls were prepared by adding 0.2 ml of 1% SRBC suspension alone to the monolayer.

Scoring of Results

The degree of attachment of SRBC to the peritoneal-macrophages was expressed in terms of percentage of rosettes formed. At least 200 macrophages were examined and each macrophage having more than two SRBC on its periphery was regarded as positive for rosette. Macrophage having two or less than two SRBC on its periphery was recorded as negative.

Test for Onsonin-Adhering Antibody

Peritoneal-macrophage monolayers of mice, rats and buffaloes were obtained by culturing 0.2 ml of peritoneal washings at room temperature for 45 minutes. The monolayer was then thoroughly washed with PBS and 0.2 ml of heat test serum together with 0.2 ml of 1% SRBC suspension was added to the monolayer and incubated at room temperature for 30 minutes. The monolayer was then thoroughly washed with PBS to remove the unattached SRBC from the monolayer. Finally the monolayer was stained with Leishman's stain. Controls were prepared by adding 0.2 ml of SRBC suspension alone to the monolayer.

Scoring of Results

The degree of attachment of SRBC to the peritoneal-macrophages was expressed in terms of percentage of rosettes formed. At least two hundred macrophages were examined and each macrophage having more than two SRBC on its periphery was recorded as positive for rosette. Macrophage having two or less than two SRBC on its periphery was recorded as negative for rosette.

ADSORPTION STUDIES OF CYTOPHILIC AND OPSONIN-ADHERING ANTIBODIES IN CATTLE AND BUFFALO SERA

Preparation of Cell Suspensions

To carry out the adsorption studies of cytophilic and opsonin-adhering antibodies in normal cattle and buffalo sera, peritoneal, lymphnode, liver and spleen cells of normal mice were used.

Peritoneal-Cell Suspension

Five apparently healthy normal adult mice were killed under chloroform anaesthesia, the abdominal skin was moistened with absolute alcohol and reflected. The peritoneal cavity was opened and thoroughly washed with about 5 ml of Hank's solution. The peritoneal washing was then collected with a sterilized pasteur pipette and placed in a sterile plastic tube.

The peritoneal washings from five mice were pooled and centrifuged at 1500 r.p.m. for 10 minutes in a graduated centrifuge tube. After centrifugation the supernatant was discarded and finally a 10% suspension of the peritoneal cells was made in 0.5 ml amount in Hank's solution.

Lymph-Node Cell Suspension

Mesenteric lymph-nodes from healthy, normal mice were cut into fragments with a scissor and with a sterile forceps the fragments were teased apart through a sterilized rubber sieve. Hank's solution was added to the lymph-node cells so obtained which were then centrifuged at 1500 r.p.m. for 10 minutes. After centrifugation the supernatant was discarded and finally a 10% suspension of lymph-node cells was made in 0.5 ml amount in Hank's solution.

Liver Cell Suspension

Liver from normal mice were cut into fragments with a sterilized scissor and teased apart through a sterilized rubber sieve. The liver cells so obtained were immediately immersed in Hank's solution and centrifuged at 1500 r.p.m. for 10 minutes. After centrifugation, the supernatant was discarded and finally a 10% suspension of the liver cells was made in 0.5 ml amount in Hank's solution.

Spleen Cell Suspension

Spleens from normal mice were cut into fragments with a sterilized scissor and teased apart through a sterilized rubber sieve. The cells were then immediately immersed in Hank's solution and then centrifuged at 1500 r.p.m. for 10 minutes. After centrifugation the supernatant was discarded and finally a 10% spleen cell suspension was made in 0.5 ml amount in Hank's solution.

Sheep Red Blood Cell Suspension

A 10% SRBC suspension was prepared in saline from the packed SRBC.

Absorption of Test Sera with Cell Suspensions

0.5 ml amount of various cell suspensions were centrifuged. The supernatant was discarded and to the sediment, 0.5 ml of the pooled test serum each from cattle and buffaloes was added. The serum-cell mixtures were then incubated at 37°C for 30 minutes, followed by an overnight incubation at 4°C for adsorption and then centrifuged at 1500 r.p.m. for 15 minutes. The supernatants were collected and used in the test system to study the effect of adsorption.

2-Mercaptoethanol Treatment of Serum

5 ml of 0.2 M 2-mercaptoethanol (Merck) solution was prepared in PBS. A 0.5 ml of 0.2 M 2-mercaptoethanol solution was added to 0.5 ml of the pooled test serum and incubated in a water bath at 37°C for 1 hour. The serum mercaptoethanol mixtures were then dialysed against normal saline for 24 hours at 4°C. During the dialysis period the saline was changed thrice. The 2-mercaptoethanol treated sera samples were then tested for cytophilic and opsonin-adhering antibodies.

TEST FOR HETEROPHILE ANTIBODIES

Sera samples of cattle and buffaloes of various age groups were tested for heterophile antibodies against SRBC. The sera samples were heat-inactivated at 56°C for 30 minutes before being employed in the test.

In the present investigation two methods were used to study the heterophile antibodies against SRBC. The methods were :

1. Direct conglutination test
2. Direct agglutination test

1. Direct Conglutination Test

Production of Immunoconglutinin in Rabbits

To study the heterophile antibodies against SRBC by direct conglutination test, immunoconglutinin was first raised in rabbits.

10 mg of sterile Kaolin powder was added to 1 ml of fresh horse serum. The serum-Kaolin mixture was then kept for 10 minutes at 37 C in a water bath, with frequent shaking of the suspension. By light centrifugation the kaolin was deposited, washed twice in saline and finally resuspended to the original volume of serum. The kaolin suspension in saline was then kept at 4°C for 1 hour and the supernatant was used as inoculum.

Two rabbits received five injections of 1 ml each of the suspension intravenously at an interval of two days. Eight days after the last injection, the rabbits were bled. The sera samples were separated and heat-inactivated at 56°C for 30 minutes, and then absorbed with 10% SRBC twice at 37°C for 30 minutes in a water bath to remove the heterophile antibodies, if any. Titration of an individual serum was carried out for the presence of immunoconglutinin by using aloxinated SRBC. Finally the sera samples were pooled and stored at -20°C until used.

Titration Procedure for Heterophile Antibodies by Direct Conglutination Test

Two-fold dilutions of the test sera were made in normal saline beginning with neat to 1/640. To each dilution of the serum, a 0.2 ml of fresh horse complement in a dilution of 1/10 was added. A 0.2 ml of a 1% SRBC suspension was added to each tube. Finally, a 0.2 ml of rabbit immunoconglutinin in a dilution of 1/500 (containing 4 minimal conglutinating doses) was added to each dilution.

Three control tubes were kept as shown in the protocol.

All the tubes including the controls were incubated at 37°C for 30 minutes in a water bath. The tubes were then centrifuged at 1500 r.p.m. for 1 minute and the degree of conglutination for heterophile antibodies was read by the resuspension technique of Coombs, Coombs and Ingram (1961). The results were recorded as follows :

4+ = strong clumping
3+ = diffuse clumping but all particles were clumped
2+ = weak clumping with some unclumped particles
1+ = trace of clumping
0 = no clumping

Protocol for the titration of heterophile antibodies by Direct Conglutination Test

Reagents	Tube No.									Controls		
	1	2	3	4	5	6	7	8	9	10	11	12
Normal saline	-	0.8	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Test serum	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2 (1/5 dil)	-	0.2 (1/5 dil)
Dilutions of serum	Neat	(1/5)	(1/10)	(1/20)	(1/40)	(1/80)	(1/160)	(1/320)	(1/640)			
Horse complement (1/10)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-	0.2	0.2
1% SRBC suspension	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Rabbit IK (1/200 dil)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-

Discard 0.2 ml from tube No. 2.
Finally the tubes were shaken properly for thorough mixing and then incubated at 37°C for 30 minutes.

The tube showing a minimum reaction of 2+ was regarded as positive for heterophile antibody.

2. Direct Agglutination Test

Double-fold dilutions of the test sera were made in normal saline starting from neat to 640. Finally to each dilution, a 0.2 ml of 1% SRBC suspension was added. A control tube containing saline and SRBC suspension was also kept as shown in the protocol.

The tubes were incubated at 37°C for 30 minutes in a water bath and then centrifuged at 1500 r.p.m. for 1 minute and the degree of agglutination for heterophile antibodies was read. The results were recorded as follows :

4+ = strong clumping
3+ = diffuse clumping but all particles were clumped
2+ = weak clumping with some unclumped particles
1+ = trace of clumping
0 = no clumping

The tube showing a minimum reaction of 2+ was recorded as positive for heterophile antibody.

Protocol for the titration of heterophile antibodies by Direct Agglutination Test

Reagents	Tube No.									
	1	2	3	4	5	6	7	8	9	Control 10
Normal saline	-	0.8	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Test serum	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-
Dilutions of serum	Neat	(1/5)	(1/10)	(1/20)	(1/40)	(1/80)	(1/160)	(1/320)	(1/640)	-
1% SRBC suspension	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Discard 0.6 ml from the tube No. 2. The tubes were shaken for proper mixing and then incubated at 37°C for 30 minutes.

DEMONSTRATION OF CYTOPHILIC AND OPSONIN-ADHERING ANTIBODIES IN BUFFALO-CALVES IN RESPONSE TO VACCINATION AND CHALLENGE INFECTION WITH PASTEURELLA MULTOCIDA

Six healthy, 1 to 1½ year old male buffalo-calves of local breed were selected for the experiment. The buffalo-calves were vaccinated each with 3 ml dose of haemorrhagic septicaemia (HS) oil adjuvant vaccine intramuscularly. On the 21st day of post-vaccination, the vaccinated calves were challenged with 1 ml of 10^{-1} dilution of freshly passaged 18 hour-old broth culture of Pasteurella multocida (P_{52}) subcutaneously.

These animals were bled at 0 day and at 7th, 14th and 21st day after vaccination and also after 24 hours and 48 hours of challenge infection. As such, sera samples from altogether 6 bleedings were collected and stored at -20°C to be tested for the presence of cytophilic and opsonin-adhering antibodies against Pasteurella multocida.

Cultivation of Pasteurella multocida (P_{52}) in Broth

For the cultivation of Pasteurella multocida (P_{52}) organisms, nutrient broth was prepared as follows :

NaCl	...	3 gm
Na_2HPO_4	...	2 gm
Peptone	...	10 gm
Lab. lemco	...	10 gm
Dist. water	...	1000 ml

The pH was adjusted to 7.6. The medium was then autoclaved at 15 pound pressure for 15 minutes.

The tubes containing the broth were then inoculated with one loop ful culture of the Pasteurella multocida (P_{52}) organisms and then kept at 37°C for 18 hours. The 18 hour-old broth culture of the P_{52} strain was then sedimented by centrifugation at 2000 r.p.m. for 20 minutes in a clinical centrifuge and finally resuspended to 1% suspension in PBS. The prepared suspension was used as test antigen for the titration of cytophilic and opsonin-adhering antibodies in the test sera.

Test for Cytophilic and Opsonin-Adhering Antibodies in Buffalo Sera Against Past. multocida Antigen

The mouse peritoneal-macrophage monolayer was prepared and 1% suspension of live Past. multocida in PBS was used as test antigen for assaying the test sera both for cytophilic and opsonin-adhering antibodies by rosette technique as described earlier.

STUDIES ON RABBIT CYTOPHILIC AND OPSONIN-ARMISRING ANTIBODIES DIRECTED AGAINST SHEEP RED BLOOD CELLS

Six adult rabbits of either sex weighing 2-2½ kg were used in this experiment. These animals were divided into three groups and housed in separate cages.

Antigens : A 5% suspension of SRBC was used in the test to immunize the rabbits. The sheep blood was centrifuged and washed thrice in normal saline. Finally sheep red blood cells were made upto 5% concentration in normal saline.

Immunization Procedures in Rabbits

Group I : Two rabbits No. 7960 and 1344 were immunized each with 0.3 ml of 5% SRBC suspension in saline subcutaneously.

Group II : Two rabbits No. 1375 and 1350 were immunized with 0.6 ml of an emulsion of an equal volume of 5% SRBC in Freund's incomplete adjuvant subcutaneously.

Group III : Two rabbits No. 1378 and 1362 were immunized with 0.6 ml of an emulsion of an equal volume of 5% SRBC in Freund's complete adjuvant (containing Mycobacteriumphlei) subcutaneously.

The rabbits in all the 3 groups were then boosted

on the 21st day of primary immunisation with 0.6 ml of 5% SRBC in saline by subcutaneous route.

Bleeding and Storage of Sera

All the rabbits were bled at 0 day, 7th day, 21st day and on 28th day after primary immunization, through cardiac puncture. The blood was allowed to clot at room temperature. After clot retraction, the serum was separated by centrifugation and finally pooled as per the groups and stored at -20°C until use.

Cells

For the titration of cytophilic and opsonin-adhering antibodies, peritoneal-macrophages from the unstimulated peritoneal cavities of normal mice and guinea pigs of either sex weighing 16-20 gms and 250-300 gms respectively were obtained as described previously and used in the test for the monolayer culture.

Enzyme Treatment of Macrophages

In the present study Trypsin (1:250, Difco) and Papain (British Drug House) were used.

Trypsin (1:250 Difco) was dissolved in PBS at a concentration of 1 mg/ml just before use. Papain (British

Drug House) was added to PBS at a concentration of 1 mg/ml and left for 24 hours at 4°C. The solution was filtered and to it, cysteine hydrochloride (Diamalt, A.G.) was added to a final concentration of 1 mg/ml.

The culture chambers containing monolayers of mouse peritoneal-macrophages were washed with PBS after a period of 45 minutes incubation. The culture chambers were then filled with the solutions of Trypsin and Papain in PBS. Control chambers were filled with PBS only. After incubation at room temperature for 30 minutes, the chambers were again thoroughly washed in PBS.

The monolayers of macrophages so treated were then tested by the usual procedures to study the effect of these enzymes upon the subsequent uptake of SRBC by the macrophages.

Test for Inhibition of Sensitisation by Normal Serum

Serum from an apparently healthy normal rabbit was diluted to 20% and 50% in PBS. The monolayers of mouse peritoneal macrophages were then treated each with the 20%, 50% and undiluted normal rabbit serum, and incubated at room temperature for 30 minutes. The treated monolayers were then thoroughly washed with PBS and finally tested for the inhibitory effect of normal serum on the uptake of rabbit cytophilic and opsonin-adhering antibodies by mouse peritoneal macrophages in the usual way.

Test for Effect of Temperature on Sensitization

The monolayers of mouse peritoneal macrophages obtained by incubating at room temperature for 45 minutes were sensitized at 4°C, 37°C and at room temperature (23°C) with 0.2 ml of each of the serial dilutions of antisera for 30 minutes. For cytophilic antibody assay, 0.2 ml of 1% SRBC suspension was then added to the sensitized macrophages, which were then washed with PBS. For opsonin-adhering antibody assay, 0.2 ml of 1% SRBC suspension was added to the monolayers along with the test sera.

Test for the Effect of Heat and Ethylene-Diaminetetraacetate (EDTA) on Antisera

To study the effect of heat and EDTA on the cytophilic and opsonin-adhering antibody activities of rabbit anti-sheep red blood cell sera, the sera samples used in the tests were : untreated, heated at 56°C for 30 minutes, diluted in 0.01 M EDTA or both heated and diluted in 0.01 M EDTA.

The monolayers of mouse peritoneal-macrophages were then sensitized with serial dilutions of each of the untreated or treated sera as mentioned above and tested for cytophilic and opsonin-adhering antibodies by using 0.2 ml of 1% SRBC suspension in the usual way.

2-Mercaptoethanol Treatment of Antisera

The 2-mercaptoethanol (Merck) treatment of rabbit anti-sheep red blood cell sera was done in the similar manner as described earlier.

TEST FOR COMPLETE AND INCOMPLETE ANTIBODIES IN RABBIT ANTI-SHEEP RED BLOOD CELL SERA

Isolation of Globulins from Normal Rabbit Sera for the Production of Anti-Globulin Serum

Two apparently healthy adult male rabbits were bled through cardio puncture. The blood was allowed to clot at room temperature. After clot retraction, the serum was separated by centrifugation.

For isolation of rabbit globulins, 20 ml of normal rabbit serum was mixed with an equal volume of saturated solution of ammonium sulphate. The serum-ammonium sulphate mixture was then gently shaken and kept for overnight incubation at 4°C. It was then centrifuged at 2000 r.p.m. for 20 minutes. The precipitate containing globulin and ammonium sulphate was collected and dissolved in 10 ml of distilled water and dialysed against normal saline for two days. Globulins were then reconstituted to the original volume of serum in NSS. Finally the globulin solution was mixed with an equal volume of oil adjuvant so as to get an emulsion.

Production of Anti-Rabbit Globulins in Goats

Two adult apparently healthy Black Bengal goats, one he goat and one she goat, were inoculated with 2 ml of rabbit globulins in adjuvant by intramuscular route. The same goats were boosted with 2 ml rabbit globulins in adjuvant after 1 week of primary immunization by intramuscular route. On the 21st day of primary immunization, the two goats received a further boosting dose of 2 ml rabbit globulins without adjuvant intravenously.

Both the goats were bled through jugular vein puncture one week after the last injection. The blood was allowed to clot at room temperature and after retraction of the clot, the serum was separated by centrifugation. The serum was pooled and stored at -20°C until used.

Procedure for the Titration of Anti-Globulin Serum

A 0.5% SRBC suspension was prepared and sensitised with an equal volume of 1/200 heat-inactivated rabbit anti-sheep red blood cell serum. A 0.5% SRBC suspension was also sensitised with an equal volume of 1/200 normal heat-inactivated rabbit serum. The mixtures were then incubated at 37°C for 30 minutes, centrifuged at 1500 r.p.m. for 10 minutes, washed thrice in saline and resuspended to 0.5%.

Protocol for the titration of antiglobulin sera

Reagents	Tube No. 1	2	3	4	5	6	7	8	9	10	Control 11	12
N.S.S.	0.9	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-	0.2
Antiglobulin sera	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2 (1/100 dil)	-
Dilution of antiglobulin sera	(1/100)	(1/200)	(1/400)	(1/800)	(1/1600)	(1/3200)	(1/6400)	(1/12800)	(1/25600)	(1/51200)		
0.5 suspension of SRBC sensitized with (1/200) rabbit haemolysin	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-	0.2
Or 0.5% suspension of SRBC sensitized with (1/200) normal rabbit sera	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-

Amounts indicated are in ml. Final volume in each tube was 0.6 ml.

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Two rows of tubes were set up. Serial two-fold dilutions of heat-inactivated goat anti-rabbit globulin serum (absorbed with 10% SRBC) were prepared in 0.2 ml aliquots in both the rows starting from 1/100 upto 1/6400. Controls were also prepared with sensitized SRBC in saline alone and also a control of the first dilution (1/100) of the anti-globulin serum plus normal SRBC.

To each dilution of the anti-globulin sera, a 0.2 ml of 0.5% SRBC sensitized with rabbit anti-sheep red blood cell serum was added in the first row. In the second row, to each dilution, a 0.2 ml of 0.5% SRBC sensitized with normal rabbit serum was added.

The tubes were then gently shaken and incubated at 37°C for 1 hour. After incubation the tubes were lightly centrifuged and degree of agglutination was recorded by macroscopic examination of the cell suspension.

The results were recorded as follows :

4+ = strong clumping
3+ = diffuse clumping, not all particles are clumped
2+ = weak clumping with some unclumped particles
1+ = traces of clumping
0 = no clumping

The tubes showing a minimum of 2+ reaction was taken as positive.

Trypsinization of SRBC

The method originally employed by Morton and Pickles (1961) for trypsinization of RBC was used in the present experiment with slight modification for trypsinization of SRBC.

SRBC were packed and one part of the packed SRBC was mixed with four volumes of a solution containing 1 gm of a commercial trypsin preparation in 5 ml of 0.05 N HCl, diluted with 4.5 parts of 0.1 M PBS, pH 7.7. The mixture was incubated at 37°C for 30 minutes. The red blood cells were then centrifuged and resuspended to 0.8% suspension.

Preparation of Native SRBC Suspension

A 0.8% suspension of SRBC was prepared in normal saline from the packed, washed SRBC as described earlier.

2-Mercaptoethanol Treatment of Serum

The test sera samples were treated with 0.2 M 2-mercaptoethanol as described earlier.

Test Proper for Complete and Incomplete Antibodies

The titration for complete antibodies was carried out

by the direct haemagglutination test while titres of incomplete antibodies were determined by direct anti-globulin haemagglutination test.

Direct-Haemagglutination Test for Complete Antibodies

Four rows of tubes were set up. In the first tube of the first two rows, 0.8 ml of normal saline was taken and to the subsequent tubes, 0.2 ml of saline was added. 0.2 ml of heat-inactivated test serum was added to each of the first tubes in the first two rows and after thorough mixing, 0.2 ml was withdrawn from the first tube and transferred to the second. After mixing properly, 0.2 ml was withdrawn from the second tube and transferred to the third tube and the same procedure was followed upto the last tube and thus a two-fold dilution was made starting from 1/5 to 1/16,3840. In the similar way, in the third and fourth row a two-fold dilution of the 2-ME treated serum was obtained starting from 1/5 to 1/16,3840. Finally, 0.4 ml each was discarded from the first tube of each row.

Then 0.2 ml of 0.5% suspension of native SRBC was added to each of the dilutions of the first and third row, and 0.2 ml of 0.5% suspension of trypsinised SRBC to each of the dilutions of second and fourth row.

Protocol for the titration of complete and incomplete antibodies

Reagents	Tube No.												Control	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
N.S.S.	0.8	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Test serum (heat-inactivated or 2 ME treated)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-	-
Dilutions of serum	1/5	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	-	-
0.5/suspension of SRBC (Native or trypsinized)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Incubate at room temperature for 3 hours and record the titre for complete antibodies. Amounts indicated are in ml. Final volume in each tube was 0.6 ml. Discard 0.6 ml from Tube No. 1. Control tube No. 13 & 14 - No antibody.
To the tubes negative for complete antibodies, add 0.2 ml of 1:100 dilution of goat anti-rabbit globulin serum. Incubate at room temperature for 3 hours and record the titre for incomplete antibodies.

Controls were prepared by adding 0.2 ml of 0.5% suspension of native and trypsinized SRBC in saline.

The tubes were gently shaken and after three hours of incubation at room temperature, the test was read.

Scoring of Results

The test was read as follows :

4+ = strong clumping
3+ = diffuse granular clumping, not all particles are clumped
2+ = weak clumping with some unclumped particles
1+ = traces of clumping
0 = no clumping

Tube showing a minimum of 2+ reaction was taken as positive for complete antibody.

Direct Anti-Globulin Haemagglutination Test for Incomplete Antibodies

Direct anti-globulin test was carried out for incomplete antibodies by washing the tubes that were negative for complete antibodies thrice in normal saline. After thorough washing, 0.2 ml of 1/100 goat anti-rabbit globulin serum was added to each of the dilutions of test sera.

The tubes were then shaken gently and incubated at room temperature for 3 hours and then results were recorded.

Scoring of Results

The test was read as follows :

4+ = strong clumping
3+ = diffuse granular clumping, but all particles are clumped
2+ = weak clumping with some unclumped particles
1+ = traces of clumping
0 = no clumping

Tube showing a minimum of 2+ reaction was taken as positive for incomplete antibody.

RESULTS

RESULTS

NATURALLY OCCURRING CYTOPHILLIC AND OPSONIN-ADHERING ANTIBODIES IN CATTLE AND BUFFALO SERA

A total of 24 cattle and 24 buffalo sera samples were tested for naturally occurring cytophilic and opsonin-adhering antibodies against SRBC. The results of the present investigation are shown in Tables 1 and 2.

Using mouse, rat and buffalo peritoneal macrophages in the test, it was found that the degree of sensitization (expressed in terms of percentage of rosettes formed) by both cytophilic and opsonin-adhering antibodies being highest with rat peritoneal-macrophages, followed by mouse and buffalo peritoneal-macrophages respectively.

Cytophilic Antibody

Cattle Sera

Calf : The mean percentage of rosettes formed due to cytophilic antibody being 71.60 ± 1.43 with rat peritoneal-macrophages, and with mouse peritoneal-macrophages, it was found to be 68.75 ± 2.16 and with buffalo peritoneal macrophages, it was 58.43 ± 0.86 as shown in Table 1.

Adult : The mean percentage of rosettes formed due to cytophilic antibody was 70.3 ± 1.24 with rat peritoneal

Table 1

Cytophilic and opsonin-adhering antibodies in cattle sera

Species	Sample No.	Peritoneal macrophages	Cytophilic antibody (per cent rosettes formed)	Opsonin-adhering antibody (per cent rosettes formed)
Cattle	12(N)	Rat	81.4	80.0
		Mouse	75.7	83.1
		Buffalo	68.2	80.4
	B339	Rat	66.8	74.06
		Mouse	77.4	75.0
		Buffalo	65.1	61.6
	J236	Rat	68.8	72.5
		Mouse	82.5	72.1
		Buffalo	57.1	62.1
	B342	Rat	73.9	83.2
		Mouse	69.9	70.4
		Buffalo	58.1	61.7
	B349	Rat	75.6	80.2
		Mouse	70.2	72.1
		Buffalo	64.3	65.8
(Calf)	B229	Rat	77.6	74.0
		Mouse	69.6	73.01
		Buffalo	62.3	60.8
	F_g66	Rat	73.9	80.1
		Mouse	61.06	75.1
		Buffalo	60.09	66.04
	F_g166	Rat	72.3	70.2
		Mouse	62.06	73.5
		Buffalo	56.8	65.9
	F_g271	Rat	67.1	77.5
		Mouse	69.1	78.2
		Buffalo	57.8	66.1

Table 1 (contd)

Species	Sample No.	Peritoneal macrophages	Cytophilic antibody (per cent rosettes formed)	Opsonin-adhering antibody (per cent rosettes formed)
	$F_2$272	Rat	66.6	81.4
		Mouse	64.2	74.3
		Buffalo	53.3	64.1
	J305	Rat	66.1	80.6
		Mouse	72.8	78.3
		Buffalo	57.01	69.2
	$F_2$165	Rat	69.2	81.4
		Mouse	67.5	83.1
		Buffalo	61.1	71.7
Mean value		Rat	71.60±1.43	77.92±1.20
		Mouse	68.78±2.16	78.98±1.17
		Buffalo	58.43±0.88	64.53±1.04
Cattle	001	Rat	76.0	86.6
		Mouse	71.1	72.4
		Buffalo	57.2	58.4
	002	Rat	74.5	79.2
		Mouse	66.1	78.3
		Buffalo	51.3	57.2
	003	Rat	67.5	77.5
		Mouse	67.2	86.4
		Buffalo	58.5	60.4
	004	Rat	68.4	75.6
		Mouse	73.4	76.0
		Buffalo	60.6	67.9
	005	Rat	69.1	80.00
		Mouse	72.3	73.90
		Buffalo	65.1	58.50

Table 1 (contd)

Species	Sample No.	Peritoneal macrophages	Cytophilic antibody (per cent rosettes formed)	Opsonin-adhering antibody (per cent rosettes formed)
(Adult)	006	Rat	66.8	68.8
		Mouse	68.2	69.1
		Buffalo	57.1	61.8
	007	Rat	64.2	75.7
		Mouse	67.7	64.1
		Buffalo	49.3	55.3
	008	Rat	72.5	79.6
		Mouse	70.0	69.5
		Buffalo	55.3	57.6
	009	Rat	72.5	81.3
		Mouse	65.8	73.00
		Buffalo	54.8	56.9
	010	Rat	77.6	72.1
		Mouse	61.5	65.5
		Buffalo	55.3	60.4
	0110	Rat	66.1	71.1
		Mouse	70.0	74.2
		Buffalo	59.5	65.7
	0111	Rat	68.4	83.9
		Mouse	60.0	60.03
		Buffalo	57.5	59.2
Mean value		Rat	70.3±1.24	77.6±1.51
		Mouse	67.7±1.17	71.70±1.99
		Buffalo	55.9±0.93	59.7±1.08

Table 2

Cytophilic and opsonin-adhering antibodies in buffalo sera

Species	Sample No.	Peritoneal macrophages	Cytophilic antibody (per cent rosettes formed)	Opsonin-adhering antibody (per cent rosettes formed)
Buffalo	023	Rat	70.1	76.9
		Mouse	71.2	71.4
		Buffalo	60.7	72.1
	024	Rat	75.7	81.3
		Mouse	68.5	72.8
		Buffalo	65.2	74.5
	025	Rat	63.9	83.9
		Mouse	64.4	68.6
		Buffalo	67.1	76.3
	026	Rat	66.1	80.5
		Mouse	64.1	71.08
		Buffalo	67.3	67.5
(Calf)	027	Rat	65.3	77.5
		Mouse	65.3	72.9
		Buffalo	62.5	69.2
	028	Rat	68.1	79.2
		Mouse	61.1	74.5
		Buffalo	61.4	66.3
	030	Rat	73.05	72.4
		Mouse	67.4	70.6
		Buffalo	60.08	65.9
	031	Rat	64.3	82.6
		Mouse	64.4	57.08
		Buffalo	64.3	70.7

Table 2 (contd)

Species	Sample No.	Peritoneal macrophages	Cytophilic antibody (per cent rosettes formed)	Opsonin-adhering antibody (per cent rosettes formed)
	033	Rat	61.1	75.5
		Mouse	57.9	64.1
		Buffalo	56.5	57.4
	034	Rat	59.6	60.3
		Mouse	58.3	73.5
		Buffalo	54.01	49.3
	038	Rat	61.6	71.9
		Mouse	58.7	74.2
		Buffalo	58.6	55.3
	039	Rat	59.3	68.8
		Mouse	64.4	61.3
		Buffalo	50.6	59.8
Mean value		Rat	65.76±1.50	77.58±1.30
		Mouse	63.72±1.17	71.00±1.71
		Buffalo	59.88±1.36	66.25±2.41
Buffalo	013	Rat	70.2	86.1
		Mouse	44.7	73.4
		Buffalo	54.6	63.09
	014	Rat	69.8	73.6
		Mouse	49.6	74.04
		Buffalo	56.9	59.08
	015	Rat	69.3	73.7
		Mouse	51.2	69.7
		Buffalo	54.6	68.2
	016	Rat	68.9	73.1
		Mouse	49.72	72.1
		Buffalo	43.3	50.02

Table 2 (contd)

Species	Sample No.	Peritoneal macrophages	Cytophilic antibody (per cent rosettes formed)	Opsonin-adhering antibody (per cent rosettes formed)
(Adult)	017	Rat	70.6	73.5
		Mouse	49.5	70.3
		Buffalo	54.7	48.1
	018	Rat	72.8	71.1
		Mouse	50.06	72.5
		Buffalo	33.4	45.8
	019	Rat	71.3	79.1
		Mouse	46.2	71.5
		Buffalo	56.6	63.3
	020	Rat	72.6	77.6
		Mouse	52.8	72.1
		Buffalo	50.08	58.6
	021	Rat	65.9	65.2
		Mouse	57.2	54.6
		Buffalo	57.1	58.1
	022	Rat	70.6	60.04
		Mouse	50.5	54.4
		Buffalo	53.7	50.5
	032	Rat	66.3	66.2
		Mouse	59.1	42.2
		Buffalo	65.3	71.1
	040	Rat	61.08	64.9
		Mouse	66.6	72.1
		Buffalo	52.4	56.6
Mean value		Rat	69.11±0.95	72.01±2.06
		Mouse	52.26±1.74	66.67±2.96
		Buffalo	52.22±2.25	57.95±2.05

macrophages, 67.7 ± 1.17 with mouse peritoneal macrophages and with buffalo peritoneal cells, it was found to be 65.9 ± 0.93 (Table 1).

Buffalo Sera

Calf : The mean percentage of rosettes formed due to cytophilic antibody of buffalo calf sera was 65.76 ± 1.50 with rat peritoneal-macrophages, 63.72 ± 1.17 with mouse peritoneal-macrophages and 59.88 ± 1.38 with buffalo peritoneal-macrophages.

Adult : The mean percentage of rosettes formed due to cytophilic antibody of buffalo adult sera with rat peritoneal-macrophages was 69.11 ± 0.95 with mouse peritoneal-macrophages, 52.36 ± 1.74 and 52.22 ± 2.25 with buffalo peritoneal-macrophages (Table 2).

Opsonin-Adhering Antibody

Cattle Sera

Calf : The mean percentage of rosettes formed due to the opsonin-adhering antibodies was 77.92 ± 1.20 with rat peritoneal-macrophages, 75.93 ± 1.17 with mouse peritoneal-macrophages and 64.53 ± 1.04 with buffalo peritoneal-macrophages as shown in Table 1.

Adult : The mean percentage of rosettes formed with rat peritoneal-macrophages was 77.6 ± 1.51 with mouse peritoneal-macrophages 71.79 ± 1.99 and 69.7 ± 1.08 with buffalo peritoneal-macrophages as shown in Table 1.

Buffalo Sera

Calf : The mean percentage of rosettes formed with rat peritoneal-macrophages was 77.53 ± 1.30, with mouse peritoneal-macrophages 71.00 ± 1.71 and 65.25 ± 2.41 with buffalo peritoneal-macrophages (Table 2).

Adult : The mean percentage of rosettes formed due to the opsonin-adhering antibodies of adult buffalo sera was 72.01 ± 2.06 with rat peritoneal-macrophages, 66.57 ± 2.93 with mouse peritoneal-macrophages and 57.95 ± 2.05 with buffalo peritoneal-macrophages (Table 2).

Cytophilic and Opsonin-Adhering Antibody in the Adsorbed Sera

The activity of cytophilic and opsonin-adhering antibodies was profoundly reduced when pooled cattle and buffalo sera samples were adsorbed with BMBC, peritoneal cells, spleen cells, lymph-node cells and liver cells. The results are shown in Table 3.

Table 3

Effect of adsorption of pooled cattle and buffalo sera with various cells

Species	Per cent rosettes formed due to cytophilic antibody						Per cent rosettes formed due to opsonin-adhering antibody					
	Before adsorption	After adsorption with SRBC	Peritoneal cells	Spleen cells	Lymph node cells	Liver cells	Before adsorption	After adsorption with SRBC	Peritoneal cells	Spleen cells	Lymph node cells	Liver cells
Cattle :												
Calf	60.2	7.88	8.75	11.06	10.2	11.3	66.3	8.4	9.7	11.6	11.4	11.7
Adult	54.9	6.8	7.5	9.1	10.0	11.3	57.7	7.4	8.7	10.3	11.1	11.01
Buffalo :												
Calf	60.5	7.92	9.6	10.5	11.5	13.2	75.1	8.4	10.2	10.9	10.3	13.3
Adult	54.9	6.04	8.9	8.97	9.12	10.2	62.7	6.3	8.13	9.2	9.5	10.1

Amongst the cells used for adsorption studies, SRBC had highest adsorption effect on both cytophilic and opsonin-adhering antibodies of cattle and buffalo sera, while adsorption on to liver cells by both cytophilic and opsonin-adhering antibodies was found to be poorest.

2-Mercaptoethanol Sensitivity

2-ME treatment has significantly reduced the activity of both cytophilic and opsonin-adhering antibodies of cattle and buffalo sera. The results are shown in Table 4.

Testing Sera Samples for Heterophile Antibodies

The cattle and buffalo sera that were tested for cytophilic and opsonin-adhering antibodies were also tested for agglutinins against SRBC. The distribution of agglutinins in the cattle and buffalo sera is charted in Table 5.

Among the two tests, that were employed for the titration of agglutinins, direct conglutination test was found to be more sensitive.

Heterophile Antibodies in Cattle Sera

No agglutinins could be detected in both calf and adult cattle sera by direct agglutination test, while using direct conglutination test, it was found that titre of agglutinins ranged from < 5 to 5 in calf sera with a mean of 1.33.

Table 4

Effect of 2-ME treatment upon cytophilic and opsonin-adhering antibodies of pooled cattle and buffalo sera

Species	Per cent rosettes formed due to cytophilic antibody		Per cent rosettes formed due to opsonin-adhering antibody	
	Before treatment with 2-ME	After treatment with 2-ME	Before treatment with 2-ME	After treatment with 2-ME
Cattle :				
Calf	60.2	9.09	66.3	11.6
Adult	51.3	6.27	57.7	11.3
Buffalo :				
Calf	59.5	9.42	75.1	9.58
Adult	54.9	10.6	62.7	10.66

Table 5

Levels* of heterophile antibody in buffalo sera against SRBC

Species	Sample No.	Heterophile antibody titres against SRBC	
		Direct congluti-nation test	Direct agglutina-tion test
Buffalo :			
Calf	023	20	40
	024	40	40
	025	40	80
	026	40	20
	027	80	80
	028	80	40
	030	80	40
	031	40	40
	033	160	80
	034	320	160
	038	320	160
	039	80	80
Mean value		108.33	71.66
Adult	013	160	80
	014	80	80
	015	80	80
	016	80	80
	017	40	20
	018	40	20
	019	80	40
	020	80	40
	021	320	160
	022	20	40
	032	80	40
	040	40	20
Mean value		91.6	58.3

Table 5 (contd)

Levels* of heterophile antibody in cattle sera against SRBC

Species	Sample No.	Heterophile antibody titres against SRBC	
		Direct conglutination test	Direct agglutination test
Cattle :			
	12(N)	0	0
	B339	0	0
	J286	0	0
	B342	0	0
	B349	0	0
Calf	B329	0	0
	$F_2$566	0	0
	$F_2$156	5	0
	$F_2$271	5	0
	$F_2$272	0	0
	J305	5	0
	$F_2$155	1	0
Mean value		1.33	0
	001	0	0
	002	0	0
	003	0	0
	004	0	0
	005	0	0
	006	0	0
Adult	007	0	0
	008	0	0
	009	0	0
	010	0	0
	0110	0	0
	0111	0	0
Mean value		0	0

* Reciprocal of the highest dilutions of the serum giving 50% conglutination.

Heterophile Antibodies in Buffalo Sera

Heterophile antibodies have been detected in both the sera of both young and adult buffaloes.

In buffalo calf sera the titres ranged from 20 to 320 as detected by direct conglutination test with a mean of 103.33. By direct agglutination test, the titres were found in between 20 to 160 with a mean of 71.66.

In adult buffalo sera the titres ranged from 20 to 320 as detected by direct conglutination test with a mean of 91.6, while by direct agglutination test the titre was found in between 20 to 160 with a mean of 58.3.

PRODUCTION OF CYTOPHILIC AND OPSONIN-ADHERING ANTIBODIES IN BUFFALO CALVES IN RESPONSE TO VACCINATION AND SUBSEQUENT CHALLENGE INFECTION WITH PASTEURELLA MULTOCIDA

Cytophilic and opsonin-adhering antibodies were also detected in the sera of buffalo calves vaccinated with H.S. oil adjuvant vaccine and then subsequently challenged with Pasteurella multocida (P_{52}) organisms. It was observed that the titres of both cytophilic and opsonin-adhering antibodies against Pasteurella multocida (P_{52}) increased gradually after the vaccination and reached the maximum following challenge infection. The results are shown in Table 6 and Fig. 1.

Table 6

Titres* of cytophilic and opsonin-adhering antibodies in the sera of buffalo-calves vaccinated with H.S. oil adjuvant vaccine and then challenged with Pasteurella multocida (P_{52}) organisms

Buffalo calf No.	Cytophilic antibody titre on the day						Opsonin-adhering antibody titre on the day					
	Pre-vaccination	Post vaccination 7	14	21	Post challenge 1	2	Pre-vaccination	Post vaccination 7	14	21	Post challenge 1	2
9	0	40	80	160	320	320	0	40	80	160	320	320
12	0	40	80	160	160	320	0	40	80	160	320	320
143	0	40	40	80	160	320	0	80	80	160	320	320
14	20	40	160	160	320	320	20	80	160	160	320	320
2	0	80	80	160	320	320	0	80	160	160	320	320
3	40	80	80	160	320	320	40	80	80	160	320	320
Mean value	10.00	53.33	86.66	146.66	266.66	320.00	10.00	66.66	106.66	160.00	320.00	320.00

*Reciprocal of the highest dilution of the serum showing rosette formation in 50% of the cells counted

Cytophilic Antibodies

The rosettes of Pnat. multocida (Pgp) due to cytophilic antibodies on mouse peritoneal macrophages sensitized with 21 day post-vaccination serum and 2 day post-challenge serum of buffalo calf No. 143 are shown in Plates I & II respectively.

Pre-Vaccination Sera

Cytophilic antibodies were detected in the pre-vaccination sera of only two buffalo calves No. 14 and 3, out of six, the titres being 20 and 40 respectively. In other experimental calves, it was not detectable. The mean value of cytophilic antibody in the prevaccination sera was 10.00.

Post-Vaccination Sera

Cytophilic antibodies were detected invariably in all the post-vaccination sera sample tested.

The titres of cytophilic antibodies in the 7th day post-vaccination sera ranged from 40 to 80. In the sera of buffalo calves No. 2 & 3, the titre was found to be as high as 80, while in other experimental calves (No. 9, 12, 143 and 14) the titre was 40. The mean titre being 53.33.

On 14th day post-vaccination sera of the buffalo calves,

the titres were found in between 40 and 160. In the buffalo calves No. 143 and 14 the titres were found to be 40 and 160 respectively, while in other calves (No. 9, 12, 8 and 3) the titre was found to be 80. The mean titre on the 14th day post-vaccination sera was 86.66.

The titres of cytophilic antibodies in the 21st day post-vaccination sera ranged from 80 to 160. The titre was found to be 80 in the buffalo calf No. 143, while in other calves the titre was found to be 160. The mean titre being 146.66.

Post-Challenge Sera

The titres of cytophilic antibodies in 1 day post-challenged sera ranged from 160 to 320. The titre was found to be 160 in the buffalo calf No. 12 and 143, while in others it was found to be 320. The mean titre of cytophilic antibod on 1st day post-challenge sera was 263.66.

The titre of cytophilic antibodies in 2nd day post-challenge sera was 320 in all the buffalo calves. The titre on the 2nd day post-challenge sera being 320.00.

Opsonin-Adhering Antibodies

Rosettes of Past. multocida (P_{52}) due to opsonin-adhering antibodies on mouse peritoneal macrophages sensitized

with 21 day post-vaccination and 2 day post-challenge sera of buffalo calf No. 143 are shown in Plates II & IV respectively

Pre-Vaccination Sera

Opsonin-adhering antibodies were detected in the pre-vaccination serum of two buffalo calves No. 14 and 3, the titres being 20 and 40 respectively. However, in the pre-vaccination sera of other experimental calves opsonin-adhering antibodies were not detected. The mean titre of opsonin-adhering antibody in the pre-vaccination sera was 10.

Post-Vaccination Sera

The titres of opsonin-adhering antibodies in the 7th day post-vaccination sera ranged from 40 to 80. In the serum of the buffalo calves No. 9 and 12, the titre was 40 and in other buffalo calves, the titre was 80. The mean titre being 66.66.

In the 14th day post-vaccination sera, the titres of opsonin-adhering antibodies ranged from 80 to 160. In the serum of buffalo calf No. 14 and 2, the titre was found to be 160, while in other calves the titre was 80. The mean titre on the 14th day post-vaccination sera was 106.66.

The titre of opsonin-adhering antibodies in the 21 day

post-vaccination sera was 160 in all the buffalo calves with a mean of 160.00.

Post-Challenge Sera

The titre of opsonin-adhering antibodies in the sera of all the buffalo calves after one day of challenge infection was 320 with a mean of 320.

The titre of opsonin-adhering antibodies was found to be 320 in all the buffalo calves after two days of challenge infection with a mean of 320.00.

RABBIT CYTOPHILIC AND OPSONIN-ADHERING ANTIBODIES DIRECTED AGAINST SHEEP RED BLOOD CELLS

The titrations of cytophilic and opsonin-adhering antibodies of the rabbit anti-SRBC sera were carried out with mouse and guinea pig peritoneal macrophages. The rabbit antisera sensitized both guinea pig and mouse peritoneal macrophages, the apparent uptake of antigen being greater by guinea pig peritoneal-macrophages than that by mouse-peritoneal macrophages with hyperimmune sera. The sensitization of guinea pig and mouse peritoneal-macrophages by antisera obtained on the 7th day and 21st day of primary immunization was same in both the Freund's incomplete as well as saline group while with guinea pig peritoneal macrophages the sensitization was

found to be greater by the sera of Freund's complete adjuvant group obtained on 7 and 21 day of primary immunization. The results are shown in Tables 7 & 8 and Fig. 2.

Primary Response

Cytophilic Antibodies

Cytophilic antibodies were detectable to moderately high titres in the pools of sera obtained on the 7 and 21 days of primary immunization with SRBC in saline by subcutaneous route. The titres being 40 and 80 respectively on the 7 and 21 day with both mouse and guinea pig peritoneal macrophages.

The cytophilic antibodies were detectable to relatively low titres in the pools of sera obtained on the 7 and 21 days of primary immunization with SRBC in Freund's incomplete adjuvant subcutaneously. The titres being 10 and 20 on the 7 and 21 day respectively with both mouse and guinea pig peritoneal macrophages. However, the cytophilic antibodies were detectable to very low to moderate titres in the pools of sera obtained on the 7 and 21 days of primary immunization with SRBC in Freund's complete adjuvant subcutaneously. The titres being 10 and 20 on the 7 and 21 day respectively with mouse peritoneal macrophages and 20 and 80 with guinea pig peritoneal-macrophag

Table 7

Sensitization of mouse and guinea pig macrophages by rabbit cytophilic and opsonin-adhering antibodies against sheep red blood cells

Group No.	Dilution of serum	Uptake of sheep red blood cells due to cytophilic antibody by macrophages of Mouse 0 Day	Mouse 7 Day	Mouse 21 Day	Mouse 28 Day	Guinea pig 0 Day	Guinea pig 7 Day	Guinea pig 21 Day	Guinea pig 28 Day	Uptake of sheep red blood cells due to opsonin-adhering antibody by macrophages of Mouse 0 Day	Mouse 7 Day	Mouse 21 Day	Mouse 28 Day	Guinea pig 0 Day	Guinea pig 7 Day	Guinea pig 21 Day	Guinea pig 28 Day
I (Saline Group)	Heat	-	++++	++++	++++	-	++++	++++	++++	-	++++	++++	++++	-	++++	++++	++++
	10	-	+++	+++	+++	-	+++	++++	++++	-	+++	++++	++++	-	+++	++++	++++
	20	-	++	++	+++	-	++	+++	+++	-	+++	++++	++++	-	+++	++++	++++
	40	-	++	++	+++	-	++	++	+++	-	++	+++	++	-	++	++	+++
	80	-	+	++	++	-	+	++	+++	-	+	++	+++	-	+	++	+++
	160	-	-	+	++	-	-	+	+++	-	-	+	++	-	+	+	++
	320	-	-	-	-	-	-	-	++	-	-	-	+	-	-	-	+
	640	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II (F.incomplete adjuvant group)	Heat	-	++	+++	++++	-	+++	++++	++++	-	+++	++++	++++	-	+++	++++	++++
	10	-	++	++	+++	-	++	+++	++++	-	++	+++	++++	-	++	+++	++++
	20	-	-	++	++	-	+	++	++++	-	+	++	+++	-	+	++	+++
	40	-	-	-	++	-	-	+	+++	-	-	-	++	-	-	-	++
	80	-	-	-	+	-	-	-	++	-	-	-	+	-	-	-	++
	160	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-	++
	320	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
	640	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III (F.complete adjuvant group)	Heat	-	+++	+++	++++	-	+++	++++	++++	-	+++	++++	++++	-	++	++++	++++
	10	-	++	++	++++	-	++	++++	++++	-	++	+++	++++	-	++	++++	++++
	20	-	+	++	+++	-	++	+++	++++	-	+	++	++++	-	++	++++	++++
	40	-	-	+	+++	-	+	++	+++	-	-	+	+++	-	-	++	+++
	80	-	-	-	+++	-	-	++	+++	-	-	-	+++	-	-	++	+++
	160	-	-	-	+++	-	-	+	+++	-	-	-	+++	-	-	+	++
	320	-	-	-	++	-	-	-	++	-	-	-	++	-	-	-	++
	640	-	-	-	+	-	-	-	++	-	-	-	+	-	-	-	++

Table 8

Titres* of rabbit cytophilic and opsonin-adhering antibodies on mouse and guinea pig peritoneal macrophages

Group No.	Serum on the day	Cytophilic antibody titre on peritoneal-macrophages of Mouse	Cytophilic antibody titre on peritoneal-macrophages of Guinea pig	Opsonin-adhering antibody on peritoneal-macrophages of Mouse	Opsonin-adhering antibody on peritoneal-macrophages of Guinea pig
I (Saline Group)	0	-	-	-	-
	7	40	40	40	40
	21	80	80	80	80
	28	160	320	160	320
II (F. incomplete adjuvant group)	0	-	-	-	-
	7	10	10	10	10
	21	20	20	20	20
	28	40	160	40	320
III (F. complete adjuvant group)	0	-	-	-	-
	7	10	20	10	20
	21	20	80	20	80
	28	320	640	320	640

* Reciprocal of highest dilution of the serum showing rosette formation in 50% of the cells counted.

Opsonin-Adhering Antibodies

The primary production of opsonin-adhering antibodi followed the same pattern like that of cytophilic antibodi

The apparent titres being 40 and 80 respectively on the 7 and 21 day of primary immunization with SRBC in sali on both mouse and guinea pig peritoneal-macrophages.

The titres in the Freund's incomplete adjuvant grou being 10 and 20 respectively on the 7 and 21 day of primar immunization with both mouse and guinea pig peritoneal-macrophages.

The titres of opsonin-adhering antibodies on mouse peritoneal-macrophages were found to be 10 and 20 on the 7 and 21 day of primary immunization with SRBC in Freund's complete adjuvant and 20 and 80 on guinea pig peritoneal-macrophages.

Secondary Response

Cytophilic Antibodies

The term 'secondary response' is used to denote the antibody titres of the hyperimmune sera obtained on the 7th day after being boosted.

Rosettes of SRBC due to cytophilic antibodies on

mouse as well as guinea pig peritoneal macrophages sensiti with rabbit anti-SRBC hyperimmune sera of Freund's complet adjuvant group are shown in Plates V and VII respectively.

Among rabbits immunized by the subcutaneous route, cytophilic antibodies appeared in high titres in the hyper immune sera of those which had originally received SRBC in Freund's complete adjuvant. The titres being 320 on mouse peritoneal-macrophages and 640 on guinea pig peritoneal-macrophages.

The rabbits which had earlier received SRBC in sali also showed moderately high titres of cytophilic antibodie the titres being 160 on mouse peritoneal-macrophages and 320 on guinea pig peritoneal-macrophages.

Lowest cytophilic antibody titres were found in the hyperimmune sera of rabbits which had earlier received SR in Freund's incomplete adjuvant. The titre being 40 on mo peritoneal-macrophages and 160 on guinea pig peritoneal-macrophages.

Opsonin-Adhering Antibodies

Rosettes of SRBC due to opsonin-adhering antibodie on mouse and guinea pig peritoneal-macrophages sensitized

with rabbit anti-SRBC hyperimmune sera of Freund's complete adjuvant group are shown in Plates VI and VIII respectively

Among the groups of rabbits immunized, highest titres of the opsonin-adhering antibodies were detected in those, which had earlier received SRBC in Freund's complete adjuvant. The titres being 320 and 640 on mouse and guinea pig peritoneal-macrophages respectively.

Opsonin-adhering antibodies were also detected to moderately high titres in SRBC in saline. The titres being 160 and 320 on mouse and guinea pig peritoneal-macrophages respectively.

But the opsonin-adhering antibodies showed a peculiar pattern of titres in the hyperimmune sera of those which were earlier immunized with SRBC in Freund's incomplete adjuvant. The titres being 40 on mouse peritoneal-macrophages and 320 on guinea pig peritoneal-macrophages.

Enzyme Treatment

Experiments were designed to study the effects of treatment of mouse peritoneal-macrophages with trypsin and papain, on the subsequent uptake of cytophilic and opsonin adhering antibodies.

The results are shown in Tables 9, 10, 11, 12, 13 and 14. They are expressed as the apparent titres of cyto phillic and opsonin-adhering antibodies in 'early' and 'hyp immune' sera titrated on normal macrophages (PBS treated) and on macrophages treated with trypsin and papain.

Trypsin Treatment

Early Sera

Macrophages treated with trypsin did not show any inc.ease in sensitization either by cytophilic or opsonin-adhering antibodies formed during a primary response in rabbits (group II) immunized with SRBC in Freund's incompl adjuvant. However, identical treatments with early serum from rabbits (group I & group III) immunized with SRBC in saline or in Freund's complete adjuvant, showed increased sensitization due to the cytophilic and opsonin-adhering antibodies as shown in Tables 9 and 11.

Hyperimmune Sera

Macrophages treated with trypsin showed an overall increase in sensitization due to the cytophilic and opson adhering antibodies of all the three groups of hyperimmun sera. The highest sensitization was noticed when trypsin macrophages were exposed to hyperimmune serum of rabbits immunized earlier with SRBC in Freund's complete adjuvant group as shown in Tables 10 and 11.

Table 2

Effect of trypsin treatment of mouse peritoneal-macrophages before exposure to rabbit anti-sheep red blood cell serum (Early) on the uptake of sheep red blood cells

Group No.	Dilution of serum	Uptake of SRBC due to cytophilic antibody by macrophages treated with		Uptake of SRBC due to opsonin-adhering antibody by macrophages treated with	
		PBS	Trypsin	PBS	Trypsin
I (Saline Group)	Neat	++++	++++	++++	++++
	10	+++	++++	+++	++++
	20	++	++++	++	++++
	40	+	+++	++	+++
	80	+	++	+	++
	160	-	++	-	+++
	320	-	+	-	++
	640	-	-	-	++
II (F. incomplete adjuvant group)	Neat	++	++	++	++
	10	+	+	+	+
	20	-	-	-	-
	40	-	-	-	-
	80	-	-	-	-
	160	-	-	-	-
	320	-	-	-	-
	640	-	-	-	-
III (F. complete adjuvant group)	Neat	++	++++	++	++++
	10	++	++++	++	++++
	20	+	+++	+	+++
	40	-	++	-	++
	80	-	-	-	-
	160	-	-	-	-
	320	-	-	-	-
	640	-	-	-	-

Table 10

Effect of trypsin treatment of mouse peritoneal-macrophages before exposure to rabbit anti-sheep red blood cell serum (Hyperimmune) on the uptake of sheep red blood cells

Group No.	Dilution of serum	Uptake of SRBC due to cytophilic antibody by macrophages treated with		Uptake of SRBC due to opsonin-adhering antibody by macrophages treated with	
		PBS	Trypsin	PBS	Trypsin
I (Saline Group)	Heat	++++	++++	++++	++++
	10	+++	++++	++++	++++
	20	+++	++++	+++	++++
	40	+++	+++	+++	++++
	80	++	++++	+++	++++
	160	++	+++	++	+++
	320	-	++	-	+
	640	-	-	-	-
II (F. incomplete adjuvant group)	Heat	++++	++++	++++	++++
	10	+++	++++	++++	++++
	20	+++	++++	+++	++++
	40	++	++++	++	+++
	80	+	++	+	++
	160	-	-	-	-
	320	-	-	-	-
	640	-	-	-	-
III (F. complete adjuvant group)	Heat	++++	++++	++++	++++
	10	++++	++++	++++	++++
	20	+++	++++	++++	++++
	40	+++	+++	+++	++++
	80	+++	++++	+++	++++
	160	++	+++	+++	++++
	320	++	+++	++	+++
	640	-	+	++	+++

Table 11

Effect of trypsin treatment on mouse peritoneal macrophages before exposure to cytophilic and opsonin-adhering antibodies on the sequence uptake of sheep red blood cells

Group No.	Serum	Titre* on macrophages exposed to PBS or trypsin			
		Cytophilic antibody		Opsonin-adhering antibody	
		PBS	Trypsin	PBS	Trypsin
I (Saline Group)	Early	20	160	40	160
	Hyperimmune	160	320	160	320
II (F. incomplete adjuvant group)	Early	1	1	1	1
	Hyperimmune	40	80	40	80
III (F. complete adjuvant group)	Early	10	20	10	20
	Hyperimmune	320	640	640	640

*Reciprocal of the highest dilution of the serum showing rosette formation in 50% of the cells counted.

Table 12

Effect of Papain treatment of mouse peritoneal macrophages before exposure to rabbit anti-sheep red blood cell serum (Early) on the uptake of sheep red blood cells

Group No.	Dilution of serum	Uptake of SRBC due to cytophilic antibody by macrophages treated with		Uptake of SRBC due to opsonin-adhering antibody by macrophages treated with	
		PBS	Papain	PBS	Papain
I (Saline Group)	Neat	++++	++++	++++	++++
	10	+++	+++	+++	+++
	20	++	++	++	++
	40	+	+	++	++
	80	+	+	+	+
	160	-	-	-	-
	320	-	-	-	-
	640	-	-	-	-
II (F. incomplete adjuvant group)	Neat	++	++	++	++
	10	+	+	+	+
	20	-	-	-	-
	40	-	-	-	-
	80	-	-	-	-
	160	-	-	-	-
	320	-	-	-	-
	640	-	-	-	-
III (F. complete adjuvant group)	Neat	++	++	++	++
	10	++	++	++	++
	20	+	+	+	+
	40	-	-	-	-
	80	-	-	-	-
	160	-	-	-	-
	320	-	-	-	-
	640	-	-	-	-

Table 13

Effect of papain treatment of mouse peritoneal macrophage before exposure to rabbit anti-sheep red blood cell serum (Hyperimmune) on the uptake of sheep red blood cells

Group No.	Dilution of serum	Uptake of SRBC due to cytophilic antibody by macrophages treated with		Uptake of SRBC due to opsonin-adhering antibody by macrophages treated with	
		PBS	Papain	PBS	Papain
I (Saline Group)	Neat	++++	++++	++++	++++
	10	+++	++++	++++	++++
	20	+++	++++	+++	++++
	40	+++	+++	+++	+++
	80	++	+++	+++	+++
	160	++	++	++	+++
	320	-	++	-	++
	640	-	-	-	-
II (F. incomplete adjuvant group)	Neat	++++	++++	++++	++++
	10	+++	++++	++++	++++
	20	+++	+++	+++	++++
	40	++	+++	++	+++
	80	+	++	+	++
	160	-	-	-	-
	320	-	-	-	-
	640	-	-	-	-
III (F. complete adjuvant group)	Neat	++++	++++	++++	++++
	10	++++	++++	++++	++++
	20	+++	+++	++++	++++
	40	+++	+++	+++	++++
	80	+++	+++	+++	+++
	160	++	++	+++	+++
	320	++	++	++	+++
	640	-	++	++	++

Table 14

Effect of papain treatment on mouse peritoneal macrophage before exposure to cytophilic and opsonin-adhering antibodies on the subsequent uptake of sheep red blood cells

Group No.	Serum	Titre* on macrophages exposed to PBS or papain			
		Cytophilic antibody		Opsonin-adhering antibody	
		PBS	Papain	PBS	Papain
I (Saline Group)	Early	20	20	20	20
	Hyperimmune	160	320	160	320
II (F. incomplete adjuvant group)	Early	1	1	1	1
	Hyperimmune	40	80	40	80
III (F. complete adjuvant group)	Early	10	10	10	10
	Hyperimmune	320	640	640	640

*Reciprocal of the highest dilution of the serum showing rosette formation in 50% of the cells counted.

Papain Treatment

Early Sera

Macrophages treated with papain did not show any increase in sensitisation either by cytophilic or opsonin-adhering antibodies formed during a primary response in all the three pools of early sera. The degree of sensitisation on papain treated macrophages being same as on control (PBS treated) macrophages as shown in Tables 12 and 14.

Hyperimmune Sera

Identical treatments with hyperimmune serum from all the three pools, showed an increase in sensitization due to both cytophilic and opsonin-adhering antibodies, when compar to PBS treated control macrophages as shown in Tables 13 and 14.

It was also observed that trypsin and papain treatmen on mouse peritoneal-macrophages caused an increase in the size of the macrophages, as reflected by spreading of the macrophages to 2 to 3 times in diameter, on the glass surfac of the culture chamber, when compared to untreated macrophag

Inhibition of Adherence Reactions by Normal Homologous Serum

The results are shown in Table 15.

Table 15

Inhibition by homologous normal serum of the uptake of rabbit cytophilic and opsonin-adhering antibodies (Hyperimmune sera) by mouse peritoneal macrophages

Group No.	Dilution of serum	Attachment of sheep red blood cells due to cytophilic antibody on mouse peritoneal macrophages treated with				Attachment of sheep red blood cells due to opsonin-adhering antibody on mouse peritoneal macrophages treated with			
		None	2% normal rabbit serum	5% normal rabbit serum	Undiluted normal rabbit serum	None	2% normal rabbit serum	5% normal rabbit serum	Undiluted normal rabbit serum
I (Saline Group)	Neat	++++	+++	++	++	++++	++++	++++	++++
	10	+++	++	+	-	++++	++++	+++	++
	20	+++	++	-	-	+++	+++	++	++
	40	+++	+	-	-	+++	++	++	++
	80	++	-	-	-	++	+	++	+
	160	++	-	-	-	-	-	++	-
	320	-	-	-	-				
II (F. incomplete adjuvant group)	Neat	++++	+++	++	-	++++	++++	++++	++++
	10	+++	++	+	-	++++	++++	++++	++
	20	++	++	-	-	+++	+++	+++	+
	40	++	+	-	-	++	++	++	-
	80	+	-	-	-	-	-	-	-
	160	-	-	-	-	-	-	-	-
	320	-	-	-	-				
III (F. complete adjuvant group)	Neat	++++	+++	+++	++	++++	++++	++++	+++
	10	++++	++	++	-	++++	++++	++++	++
	20	+++	++	++	-	+++	++++	+++	++
	40	+++	++	+	-	+++	+++	+++	++
	80	+++	-	-	-	+++	+	++	+
	160	+++	-	-	-	++	-	-	-
	320	++	-	-	-	++	-	-	-

Inhibition of Cytophilic Antibody Uptake

It was observed that normal rabbit serum at a concentration of 20% had very little inhibitory effect on the uptake of hyperimmune cytophilic antibodies of all the three pools of sera.

The inhibitory effect of normal rabbit serum at a concentration of 50% was more pronounced, on the uptake of cytophilic antibodies of hyperimmune sera of animals earlier immunized with SRBC in saline and Freund's incomplete adjuvant. But 50% normal rabbit serum had relatively less inhibitory effect on the uptake of hyperimmune cytophilic antibodies from animals immunized with SRBC in Freund's complete adjuvant.

However, normal undiluted rabbit serum was found to have a marked inhibitory effect on the uptake of cytophilic antibodies of all the three pools of hyperimmune sera.

Inhibition of Opsonin-adhering Antibody Uptake

Normal rabbit serum at a concentration of 20 and 50% had no apparent inhibitory effect on the uptake of opsonin-adhering antibodies of all the three pools of sera. Likewise, normal undiluted rabbit serum also had no pronounced inhibitory effect on the uptake of opsonin-adhering antibodies of all the three pools of sera.

Effect of Temperature

The experiments were carried out at 4°, 23° (room temperature) and 37°C. The results are shown in Table 16.

It was observed that the degree of sensitisation due to the cytophilic and opsonin-adhering antibodies, was almost same in all the three pools of hyperimmune sera when the tests were carried out at 4°C, 23°C and at 37°C. No increase or decrease in sensitisation either due to cytophilic or opsonin-adhering antibodies could be detected at different temperatures.

2-Mercaptoethanol Sensitivity

The results are shown in Tables 17, 18 and 19.

Effect Upon Cytophilic Antibodies

Treatment of early sera with 2-mercaptoethanol reduced the cytophilic antibody activity in Freund's complete and incomplete adjuvant group. However, ME treatment of sera in saline group had no apparent effect. But 2-mercaptoethanol treatment had no apparent effect upon the cytophilic antibody formed during a secondary response in all the three pools of hyperimmune sera.

Table 16

Effect of temperature on the uptake of rabbit cytophilic and opsonin-adhering antibodies (Hyperimmune) by mouse peritoneal macrophages

Group No.	Dilution of serum	Attachment of SRBC due to cytophilic antibody to mouse peritoneal macrophage sensitized at temperature (Centigrade)			Attachment of SRBC due to opsonin-adhering antibody to mouse peritoneal macrophages sensitized at temperature (Centigrade)		
		37°	23°	4°	37°	23°	4°
I (Saline Group)	Neat	++++	++++	++++	++++	++++	++++
	10	+++	+++	+++	++++	++++	++++
	20	+++	+++	+++	++++	++++	++++
	40	+++	+++	+++	+++	+++	+++
	80	++	++	+++	+++	+++	+++
	160	++	++	++	++	++	++
	320	-	-	-	+	+	+
	640	-	-	-	-	-	-
II (F. incomplete adjuvant group)	Neat	++++	++++	++++	++++	++++	++++
	10	+++	+++	+++	++++	++++	++++
	20	++	++	++	+++	+++	+++
	40	++	++	++	++	++	++
	80	+	+	+	+	+	+
	160	-	-	-	-	-	-
	320	-	-	-	-	-	-
	640	-	-	-	-	-	-
III (F. complete adjuvant group)	Neat	++++	++++	++++	++++	++++	++++
	10	++++	++++	++++	++++	++++	++++
	20	+++	+++	+++	++++	++++	++++
	40	+++	+++	+++	+++	+++	+++
	80	+++	+++	+++	+++	+++	+++
	160	+++	+++	+++	+++	+++	+++
	320	++	++	++	++	++	++
	640	+	+	+	+	+	+

Table 17

Effect of 2-mercaptoethanol treatment on cytophilic and opsonin-adhering antibodies of rabbit anti-sheep red blood cell serum (Early)

Group No.	Dilution of serum	Attachment of sheep red blood cells due to cytophilic antibody after treated with		Attachment of sheep red blood cells due to opsonin-adhering antibody after treatment with	
		None	ME	None	ME
I (Saline Group)	1/5	++++	++++	++++	++++
	1/10	+++	+++	+++	+++
	1/20	++	++	++	++
	1/40	+	+	++	++
II (F. incomplete adjuvant group)	1/5	++	+	++	++
	1/10	+	-	+	+
	1/20	-	-	-	-
	1/40	-	-	-	-
III (F. complete adjuvant group)	1/5	+++	++	+++	+++
	1/10	++	+	++	++
	1/20	-	-	-	-
	1/40	-	-	-	-

Table 18

Effect of 2-mercaptoethanol treatment on cytophilic and opsonin-adhering antibodies of rabbit anti-sheep red blood cell serum (Hyperimmune)

Group No.	Dilution of serum	Attachment of sheep red blood cells due to cytophilic antibody after treatment of serum with		Attachment of sheep red blood cells due to opsonin-adhering antibody after treatment of serum with	
		None	ME	None	ME
I (Saline Group)	1/5	++++	++++	++++	++++
	1/10	+++	+++	++++	++++
	1/20	+++	+++	++++	+++
	1/40	+++	+++	+++	+++
	1/80	++	++	++	++
	1/160	++	++	+	+
	1/320	-	-	-	-
	1/640	-	-		
II (F. incomplete adjuvant group)	1/5	+++	+++	+++	+++
	1/10	+++	+++	+++	+++
	1/20	+++	+++	+++	+++
	1/40	+++	+++	+++	+++
	1/80	++	++	++	++
	1/160	++	++	-	-
	1/320	-	-	-	-
	1/640	-	-		
III (F. complete adjuvant group)	1/5	++++	++++	++++	++++
	1/10	+++	+++	++++	++++
	1/20	+++	+++	++++	++++
	1/40	+++	+++	++++	++++
	1/80	+++	+++	+++	+++
	1/160	++	++	++	++
	1/320	++	++	+	++
	1/640	-	-		

Table 19

Effect of 2-mercaptoethanol treatment on cytophilic and opsonin-adhering antibodies of rabbit anti-sheep red blood cell serum

Group No.	Serum	Cytophilic antibody titre* after treatment of serum with None	Cytophilic antibody titre* after treatment of serum with ME	Opsonin-adhering antibody titre after treatment of serum with None	Opsonin-adhering antibody titre after treatment of serum with ME
I (Saline Group)	Early	20	10	40	40
	Hyperimmune	160	160	160	160
II (F. incomplete adjuvant group)	Early	5	0	5	5
	Hyperimmune	160	160	160	160
III (F. complete adjuvant group)	Early	10	5	10	10
	Hyperimmune	320	320	640	640

*Reciprocal of the highest dilution of the serum showing rosette formation in 50% of the cells counted.

Effect Upon Opsonin-Adhering Antibodies

It was observed that 2-mercaptoethanol treatment of early and hyperimmune sera had no apparent effect upon the opsonin-adhering antibodies.

Heat and EDTA Treatment

The results of the heat and EDTA treatment of early and hyperimmune sera are shown in Tables 20 and 21.

Effect Upon Early Sera

It was observed that the cytophilic and opsonin-adhering antibody activity was slightly weakened in all the three pools of sera, when the early sera was diluted in EDTA or heated and diluted in EDTA. But no such effect was seen in the early sera which was fresh (unheated) or heated at 56°C for 30 minutes.

Effect Upon Hyperimmune Sera

None of the treatment was found to have any effect upon the cytophilic and opsonin-adhering antibodies of all the three pools of hyperimmune sera tested.

However, an intense phagocytosis of SRBC by opsonin-adhering antibodies was seen in fresh (unheated) hyperimmune

Table 20

Effect of EDTA and heat on rabbit anti-sheep red blood cell serum
(Early)

Group No.	Treatment of serum	Cytophilic antibody activity at dilution 1/5	1/10	1/20	1/40	Opsonin-adhering antibody activity at dilution 1/5	1/10	1/20	1/40
I (Saline Group)	Nothing	++++	+++	-	++	++++	+++	++	++
	0.01 M EDTA	+++	++	+	+	+++	++	+	+
	56°C, 30 min	++++	+++	++	++	++++	+++	++	++
	56°C, 30 min + 0.01 M EDTA	+++	++	+	+	+++	++	+	+
II (F. incomplete adjuvant group)	Nothing	++	++	-	-	+++	++	+	-
	0.01 M EDTA	+	+	-	-	++	-	-	-
	56°C, 30 min	++	++	-	-	+++	++	+	-
	56°C, 30 min + 0.01 M EDTA	+	+	-	-	++	-	-	-
III (F. complete adjuvant group)	Nothing	+++	++	+	-	+++	++	+	-
	0.01 M EDTA	++	+	-	-	++	+	-	-
	56°C, 30 min	+++	++	+	-	+++	++	+	-
	56°C, 30 min + 0.01 M EDTA	++	+	-	-	++	+	-	-

Table 21

Effect of EDTA and heat on rabbit anti-sheep red blood cell serum (Hyperimmune)

Group No.	Treatment of serum	Cytophilic antibody activity at dilution 1/5	1/10	1/20	1/40	1/80	1/160	1/320	1/640	Opsonin-adhering antibody activity at dilution 1/5	1/10	1/20	1/40	1/80	1/160	1/320	1/640
I (Saline Group)	Nothing	+++	+++	+++	++	++	++	-	-	++++	++++	+++	+++	+++	++	-	-
	0.01 N EDTA	+++	+++	+++	++	++	++	-	-	++++	++++	+++	+++	+++	++	-	-
	56°C,30 min	+++	+++	+++	++	++	++	-	-	++++	++++	+++	+++	+++	++	-	-
	56°C,30 min +0.01M EDTA	+++	+++	+++	++	++	++	-	-	++++	++++	+++	+++	+++	++	-	-
II (F. incomplete adjuvant group)	Nothing	+++	+++	++	++	-	-	-	-	++++	++++	+++	+++	+++	++	-	-
	0.01 N EDTA	+++	+++	++	++	-	-	-	-	++++	++++	+++	+++	+++	++	-	-
	56°C,30 min	+++	+++	++	++	-	-	-	-	++++	++++	+++	+++	+++	++	-	-
	56°C,30 min +0.01M EDTA	+++	+++	++	++	-	-	-	-	++++	++++	+++	+++	+++	++	-	-
III (F. complete adjuvant group)	Nothing	++++	+++	+++	+++	+++	++	++	-	++++	++++	++++	++++	+++	+++	++	++
	0.01 N EDTA	++++	+++	+++	+++	+++	++	++	-	++++	++++	++++	++++	+++	+++	++	++
	56°C,30 min	++++	+++	+++	+++	+++	++	++	-	++++	++++	++++	++++	+++	+++	++	++
	56°C,30 min +0.01M EDTA	+++	+++	+++	+++	+++	++	++	-	++++	++++	++++	++++	+++	+++	++	++

sera of all the three pools upto a dilution of 1/40. Phagocytosis of SRBC due to cytophilic and opsonin-adhering antibodies by mouse peritoneal macrophages sensitised with rabbit anti-SRBC hyperimmune sera of Freund's incomplete adjuvant group is shown in Plates IX and X respectively.

Detection of Complete and Incomplete Antibodies

Titre of Goat Anti-Rabbit Globulin Sera

A very high titre of antiglobulin was detected in the goat anti-rabbit globulin sera. The titre was 25,600.

Complete and Incomplete Antibodies

Complete and incomplete antibodies have been detected in the sera of rabbits immunized with SRBC in saline, Freund's complete adjuvant and in Freund's incomplete adjuvant. The results are summarised in Table 22.

Complete Antibodies

The titres of complete antibodies were higher in the reaction with trypsinised SRBC than with native SRBC at all the period of the experiments.

Complete Antibodies in 7 Day Old Serum

Both untreated and ME-treated 7 day-old serum of all the groups had low titres of complete antibodies when tested

Table 22

Titres* of complete and incomplete antibodies in rabbit anti-sheep red blood cell serum against sheep red blood cells

Group No.	Antiserum on the day	Antigen	Complete antibody titre in the antiserum treated with		Incomplete antibody titre in the antiserum treated with	
			NH1	NE	NH1	NE
I (Saline Group)	7	Native SRBC	20	20	80	80
		Trypsinized SRBC	80	80	Nd	Nd
	21	Native SRBC	40	20	320	80
		Trypsinized SRBC	160	40	Nd	Nd
	28 (Hyperimmune)	Native SRBC	40	20	320	160
		Trypsinized SRBC	160	160	Nd	Nd
II (F. incomplete adjuvant group)	7	Native SRBC	10	5	40	40
		Trypsinized SRBC	40	10	Nd	Nd
	21	Native SRBC	20	20	160	80
		Trypsinized SRBC	80	80	Nd	Nd
	28 (Hyperimmune)	Native SRBC	20	10	320	160
		Trypsinized SRBC	160	80	Nd	Nd
III (F. complete adjuvant group)	7	Native SRBC	20	20	320	320
		Trypsinized SRBC	40	40	Nd	Nd
	21	Native SRBC	80	80	1280	1280
		Trypsinized SRBC	160	160	Nd	Nd
	28 (Hyperimmune)	Native SRBC	320	320	10240	2560
		Trypsinized SRBC	1280	1280	Nd	Nd

Nd = Not done

*Reciprocal of the highest dilution of the serum giving 50% agglutination.

with native and trypsinized SRBC. In group I, the titres were 20 and 80 with native and trypsinized SRBC respectively in both untreated and ME-treated serum.

The titres in group II were very low in both untreated and ME-treated serum. The titres in the untreated serum being 10 and 40 respectively with native and trypsinized SRBC, while in ME-treated serum, the titres being 5 and 10 with native and trypsinized SRBC respectively. In group III, the titres of complete antibodies in both untreated and ME-treated serum were 20 and 40 with native and trypsinized SRBC respectively.

Complete Antibodies in 21 Day Old Serum

The titres of complete antibodies in 21 day-old serum of group I and group III were found to be moderately higher as compared to group II. In group I, the titres were 40 and 160 with native and trypsinized SRBC respectively in the untreated serum, while in ME-treated serum the titres being 20 and 40 with native and trypsinized SRBC respectively.

In group II, the titres were 20 and 80 with native and trypsinized SRBC respectively in both untreated and ME-treated serum.

The titres of complete antibodies in group III were 80 and 160 with native and trypsinized SRBC respectively in both untreated and ME-treated serum.

Complete Antibodies in 28 Day Old (Hyperimmune) Serum

The titres of complete antibodies in hyperimmune serum were found to be highest in group III.

In group I, the titres were 40 and 160 with native and trypsinized SRBC respectively in untreated serum, while in ME-treated serum the titres being 20 and 160 with native and trypsinized SRBC respectively.

In group II, the titres were found to be 20 and 160 with native and trypsinized SRBC respectively in the untreated serum, while in the ME-treated serum, the titres were 10 and 80 respectively with native and trypsinized SRBC respectively. In group III, the titres of complete antibodies in both untreated and ME-treated serum, were 320 and 1280 with native and trypsinized SRBC respectively.

Incomplete Antibodies

Incomplete antibodies in both untreated and ME-treated serum were detected using native SRBC as the test antigen.

At all periods of the experiment the titres of incomplete antibodies in both untreated and ME-treated serum to native SRBC were higher than the titres of complete antibodies.

Incomplete Antibodies in 7 Day Old Serum

In group I, the titres of incomplete antibodies in both untreated and ME-treated serum to native SRBC were 80.

In group II, the titres in both untreated and ME-treated serum to native SRBC were 40.

In group III, the titres to native SRBC in both untreated and ME-treated serum were 320.

Incomplete Antibodies in 21 Day Old Serum

In group I, the titres of incomplete antibodies to native SRBC in untreated and ME-treated serum were 320 and 80 respectively.

In group II, the titres to native SRBC in untreated and ME-treated serum, were 160 and 80 respectively.

In group III, high titres were obtained in both untreated and ME-treated serum. The titres being 1280 in both the sera.

Incomplete Antibodies in Hyperimmune Serum

Highest titres of incomplete antibodies were found in the hyperimmune serum of group III.

In group I, the titres to native SRBC were 320 and 160

in the untreated and ME-treated serum respectively.

In group II, the titres were 320 and 160 in the untreated and ME serum respectively.

In group III, the titres to native SRBC were 10,240 in the untreated serum and 2,560 in the ME-treated serum.

FIG. 1. MEAN TITRES OF CYTOPHILIC & OPSONIN-ADHERING ANTIBODIES IN THE SERA OF BUFFALO CALVES IN RESPONSE TO VACCINATION & CHALLENGE INFECTION WITH P. MULTOCIDA

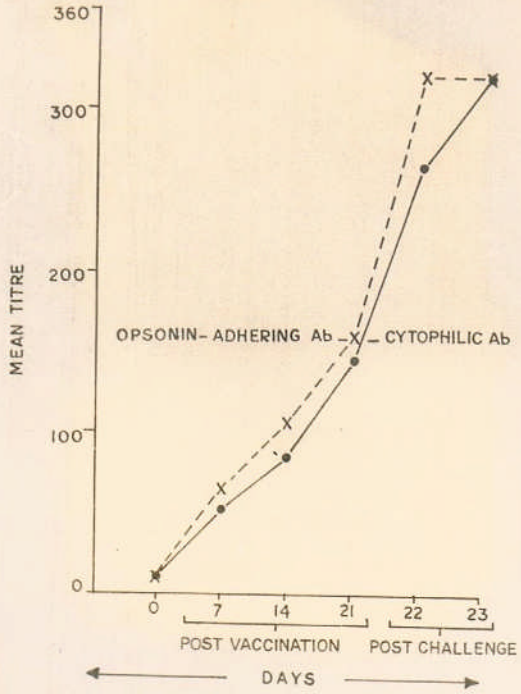


FIG. 2. LEVELS OF RABBIT ANTI-SRBC CYTOPHILIC AND OPSONIN-ADHERING ANTIBODIES TITRATED ON MOUSE AND GUINEA PIG PERITONEAL MACROPHAGES

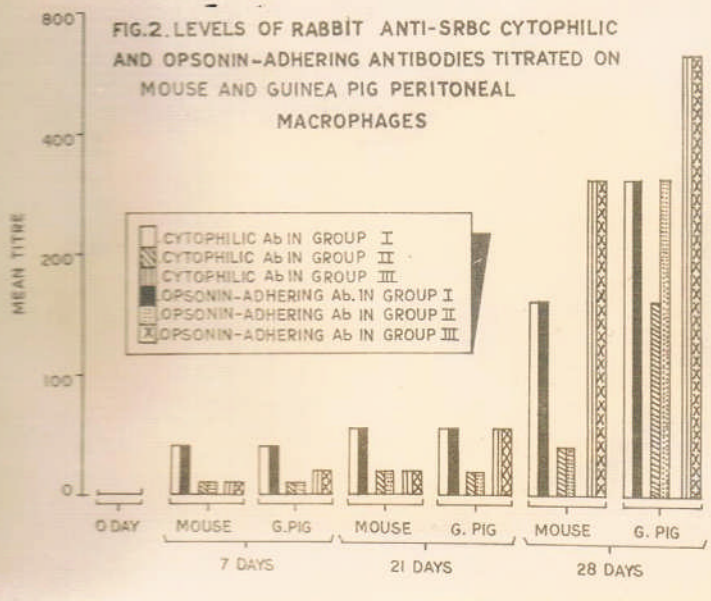
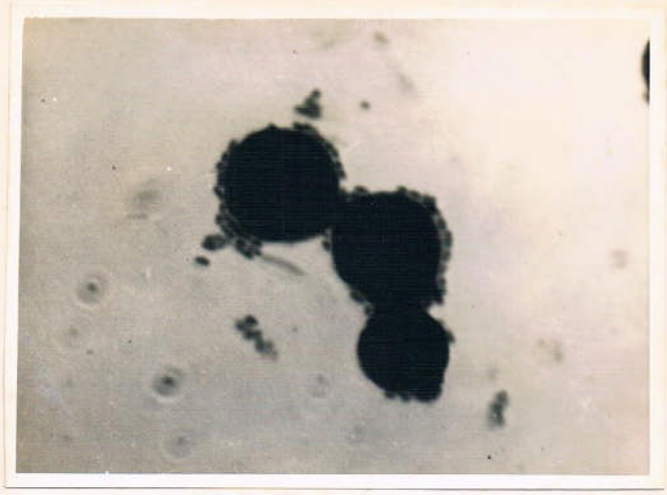


Fig. 1 - Mean titres of cytophilic and opsonin-adhering antibodies in the sera of buffalo calves in response to vaccination and challenge infection with *P. multocida*.

Fig. 2 - Levels of anti-SRBC cytophilic and opsonin-adhering antibodies titrated on mouse and guinea pig peritoneal macrophages.



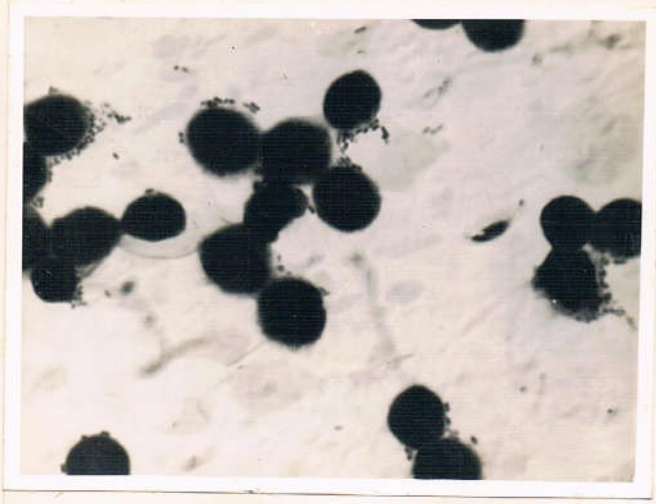
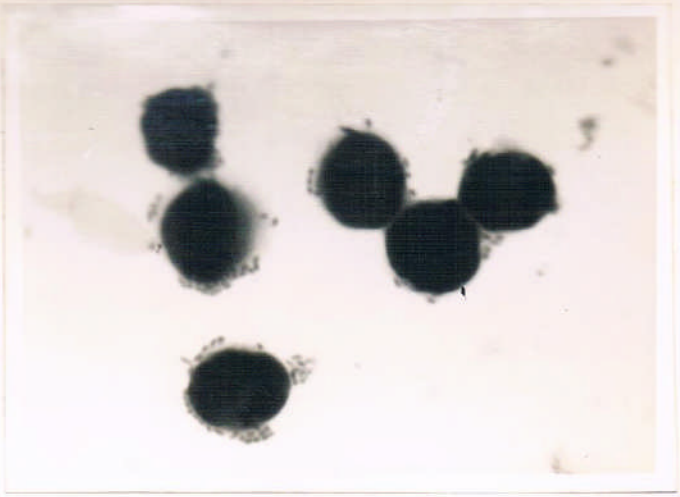


Plate I - Rosettes of Past. multocida (P_{52}) due to cytophilic antibodies on mouse peritoneal macrophages sensitized with 21 day post-vaccination serum of buffalo calf No. 143. X 1800

Plate II - Rosettes of Past. multocida (P_{52}) due to opsonin-adhering antibodies on mouse peritoneal macrophages sensitized with 21 day post-vaccination serum of buffalo calf No. 143. X 1250



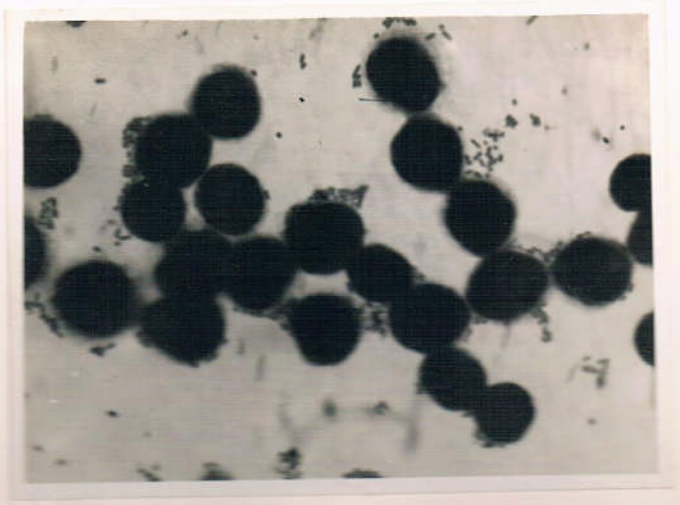
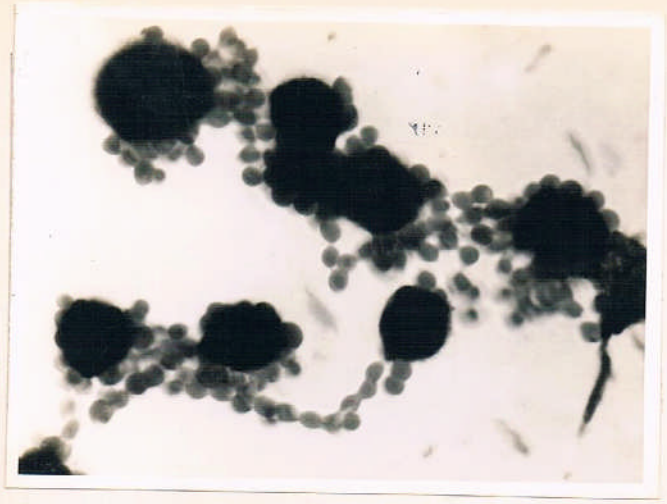


Plate III - Rosettes of Past. multocida (P_{52}) due to cytophilic antibodies on mouse peritoneal macrophages sensitized with 8 day post-challenge serum of buffalo calf No. 143. X 1650

Plate IV - Rosettes of Pas. multocida (P_{52}) due to opsonin-adhering antibodies on mouse peritoneal-macrophages sensitized with 8 day post-challenge serum of buffalo calf No. 143. X 1650

Plate V - Rosettes of sheep red blood cells due to cytophilic antibodies on mouse peritoneal-macrophages, sensitized with rabbit anti-SRBC hyperimmune sera of Freund's complete adjuvant group. X 1250

Plate VI - Rosettes of sheep red blood cells due to opsonin-adhering antibodies on mouse peritoneal macrophages, sensitized with rabbit anti-SRBC hyperimmune sera of Freund's complete adjuvant group. X 1250



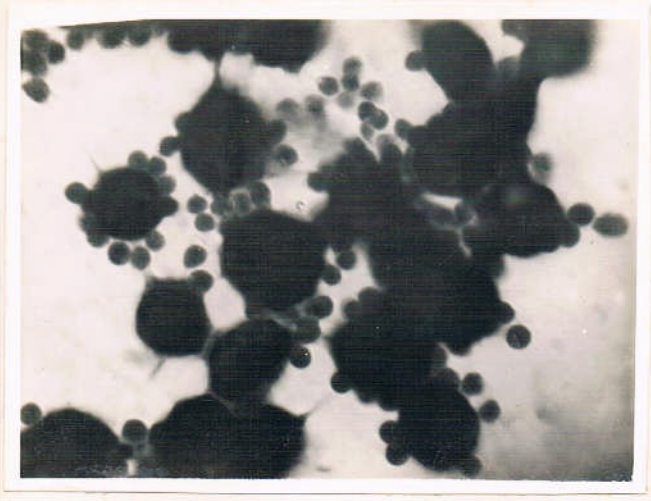
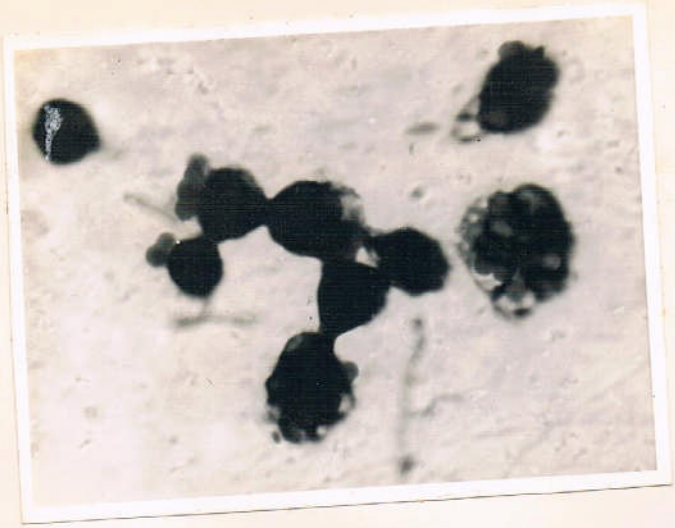


Plate VII - Rosettes of sheep red blood cells due to cytophilic antibodies on guinea pig peritoneal macrophages sensitised with rabbit anti-SRBC hyperimmune sera of Freund's complete adjuvant group. X 1250

Plate VIII - Rosettes of sheep red blood cells due to opsonin-adhering antibodies on guinea pig peritoneal-macrophages sensitised with rabbit anti-SRBC hyperimmune sera of Freund's complete adjuvant group. X 1250



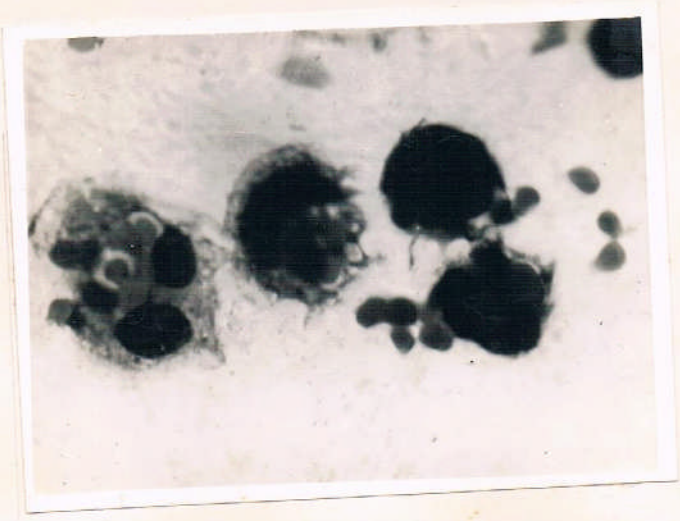
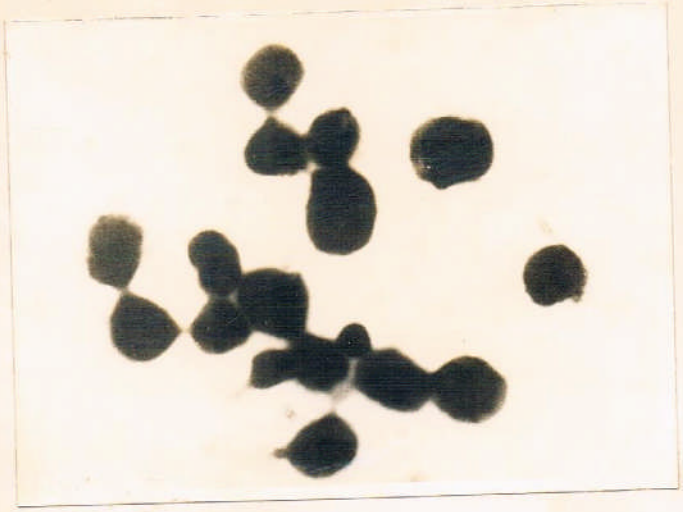


Plate IX - Phagocytosis of sheep red blood cells due to cytophilic antibodies by mouse peritoneal-macrophages sensitized with rabbit anti-SRBC hyperimmune sera of Freund's incomplete adjuvant group. X 1250

Plate X - Phagocytosis of sheep red blood cells due to opsonin-adhering antibodies by mouse peritoneal-macrophages sensitized with rabbit anti-SRBC hyperimmune sera of Freund's incomplete adjuvant group. X 1250



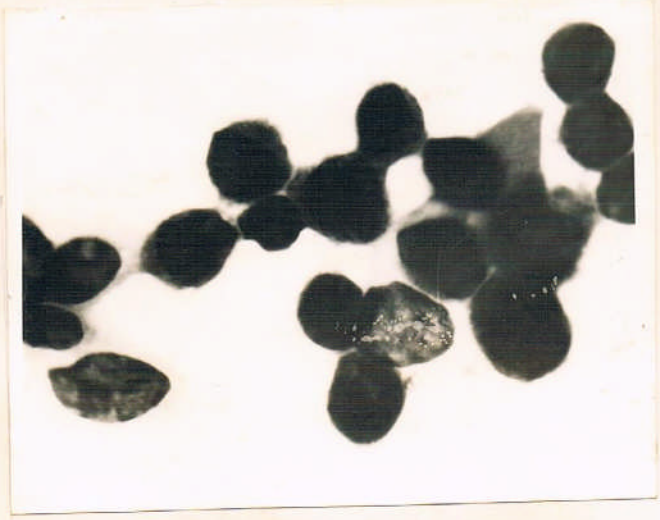


Plate XI - Negative control : No rosette formation on mouse peritoneal-macrophages. X 1250

Plate XII - Negative Control : No rosette formation on guinea pig peritoneal-macrophages. X 1250

DISCUSSION

DISCUSSION

DEMONSTRATION OF NATURALLY OCCURRING CYTOPHILIC, OPSONIN-ADHERING AND HETEROPHILE ANTIBODIES IN CATTLE AND BUFFALO SERA

The controversial topic with regard to the origin of natural antibodies against a vast array of different antigens has been discussed from time to time by several workers. Inspite of the tremendous amount of work that has been carried out in recent years to elucidate the nature and origin of natural antibodies, uncertainties and difficulties still prevail in the interpretation of the results.

The presence of naturally occurring cytophilic and opsonin-adhering antibodies might pose a question as to how these antibodies occur in the serum of cattle and buffaloes which were not deliberately immunized with SRBC. This could be well explained with the view that in the vertebrate sera all the natural antibodies occur as a consequence of previous antigenic stimulation with the test antigen or with other foreign macromolecules that share determinant groups with the test antigen. Therefore, it becomes apparent that these natural cytophilic and opsonin-adhering antibodies are not necessarily due to SRBC but due to certain other foreign macro-molecules that share determinant groups with SRBC and thus provide stimuli for the

production of these antibodies in cattle and buffaloes. A similar view was expressed by other workers with regard to the occurrence of natural antibodies in the serum of normal animals against various agents (Springer, Horton and Forbes, 1959; Cohen, Newton, Cherry and Updyke, 1963).

Sera of most animals contain naturally occurring antibodies such as the isoagglutinins in man or agglutinins for human group B red cells found in the serum of normal pigs or agglutinins for human group A red cells found in the serum of normal rabbits. In the same category, the traces of antibodies against the somatic antigens of Gram-negative bacteria such as Salmonella sp. or Escherichia coli could be classed. Springer et al. (1959) found anti-B activity in the sera of chickens raised under germ-free condition and they considered that this activity could be accounted for by the antigenic stimulation resulting from killed Gram-negative bacteria in the diet. Cohen et al. (1963) also found antibodies in the sera of mice raised under germ-free conditions reactive with coagulase positive Staphylococci. They concluded that the antibodies were not probably due to the presence of dead Staphylococci but might be due to other antigen in the food which cross-reacted with the components of Staph. aureus.

'Heterophile' is the name given to several groups of

antigens which occur in cells or fluid of apparently unrelated animals and which are so closely related immunologically, presumably by virtue of similar or identical haptenic groups, that they cross-react extensively with antibodies against any one member of the particular heterophile group. Their nature is not known, although attempts at purification indicate that they are perhaps mucopolysaccharides associated with lipid. Only those animal species which do not possess a heterophile antigen in their tissue would contain heterophile antibodies, apparently occurring naturally but more probably due to stimulation by micro-organisms which possess the antigen.

In the present investigation sera of apparently healthy cattle and buffaloes were tested for heterophile antibodies to SRBC by direct conglutination and direct agglutination test. The results of direct agglutination test show that the antibodies against SRBC in cattle sera are of non-agglutinating type. No heterophile antibodies could be detected in the adult cattle sera either by direct conglutination or direct agglutination test (Table 5). However, in calves it was detectable but to a very low level (< 5 to 10) by direct conglutination test. On the contrary, buffalo sera - both adult and calves - contained naturally occurring heterophile antibodies demonstrable by both direct agglutination test as well as by direct conglutination test (Table 5).

Ingram and Barnum (1965) also found a relatively high titre of heterophile antibodies in the calves as compared to cows. They also reported that the level of naturally occurring heterophile antibody has a seasonal variation like that of conglutinin, but not significantly affected by calving or infectious disease.

The present findings with respect to the level of heterophile antibodies in cattle sera could be explained in the light of the view expressed by Ingram and Barnum (1965) and Osterhoff (1962). Osterhoff (1962) found highest titre of naturally occurring anti-J and anti-Y_2 in cattle in the month of January and February and lowest titre in August and September, which he considered to be governed by environmental temperature. In the present investigation the cattle sera samples were collected during the month of August, September and early part of October. Thus it seems that the absence of heterophile antibodies in the adult cattle and lower level of these antibodies in the calves may possibly be due to the effect of season. In the buffalo sera also higher titres of heterophile antibodies were found in the calves as compared to the adults. Maurya, Mittal and Jaiswal (1976) reported the occurrence of natural heterophile antibodies in buffalo calves as well as in the adults. They found that only 3% of the buffalo calves had no detectable heterophile antibody titres

whereas about 34% adult buffaloes were found negative for heterophile antibodies.

It was observed that among the macrophages used in the test for the detection of naturally occurring cytophilic and opsonin-adhering antibodies, highest sensitization occurred with rat peritoneal-macrophages and lowest with buffalo peritoneal-macrophages (Tables 1 and 2). No definite conclusion could be drawn at the moment, as far as the disparity in the sensitization of macrophages by naturally occurring cytophilic and opsonin-adhering antibodies is concerned. However, it is assumed that the relatively higher sensitization of rat peritoneal-macrophages by these antibodies may be due in large part to accidental correspondence of determinant groups between the antibody molecules and macrophages rather than the systemic relationship between the species involved.

Boyden (1960, 1961) during his experiments on the adsorption of rabbit anti-HSA cytophilic antibodies by normal rabbit and guinea pig spleen cells found that adsorption of antiserum under appropriate conditions results in a greater loss of cytophilic antibody. Results of similar experiments carried out during the present investigation on adsorption of normal cattle and buffalo sera with normal peritoneal, spleen, lymphnode and liver cells of mouse and also with SRBC

showed a tremendous reduction in the activities of both cytophilic and opsonin-adhering antibodies (Table 3).

It was also observed that reduction in the activity of both cytophilic and opsonin-adhering antibodies were highest due to absorption with GMBC while comparatively lower reduction was seen on adsorption with liver cells (Table 3). This difference in the ability of the cells to adsorb naturally occurring cytophilic and opsonin-adhering antibodies could be accounted for a definite heterogeneity on the part of the cytophilic and opsonin-adhering antibodies.

Reduction studies with 2-ME showed that both cytophilic and opsonin-adhering antibodies were sensitive to 2-ME (Table 4). The results of the present investigations show that probably the cytophilic and opsonin-adhering antibodies of normal cattle and buffalo serum belong to the IgM class.

The presence of these naturally occurring heterophile antibodies in cattle and buffalo sera could be detected by direct conglutination test of Coombs et al. (1961). These antibodies are either very weak agglutinins or non-agglutinating type as in the case of cattle hence fail to be detected by direct agglutination test. However, these antibodies are able to fix complement and thus are demonstrable by either direct conglutination test or complement-fixation test.

The results of the present investigation give clear evidence to the fact that these naturally occurring sub-agglutinating but complement-fixing antibodies in cattle and buffalo sera are heterophilic in nature because they are removed from the serum on adsorption with sheep red blood cells. Since they are also removed on adsorption with various kinds of cells, lymphocytes, macrophages, spleen cells, liver cells, and also form a strong rosette when sensitised macrophage monolayer and sheep red blood cells are allowed to react together (Tables 1 & 2), it becomes evident that these heterophilic antibodies are also cytophilic in nature, i.e. they have affinity for certain receptors that are present on the cell membranes.

Detection of heterophile antibodies in cattle and buffalo sera by rosette technique does not seem to be reported earlier.

DEMONSTRATION OF CYTOPHILIC AND OPSONIN-ADHERING ANTIBODIES IN BUFFALO CALVES IN RESPONSE TO VACCINATION AND CHALLENGE INFECTION WITH PASTEURELLA MULTOCIDA

A great deal has been reported about the production of cytophilic and opsonin-adhering antibodies to various antigens in different laboratory animals. It is known that various micro-organisms adhere to the lymphoid cells of animals immunized with homologous organisms. Mouse cytophilic

antibodies to somatic antigens of E. coli have been reported by Parish (1966) and were presumed by him to be 19S. Uhr (1966) studied the cytophilic antibodies for macrophages in guinea pigs injected with Sal. paratyphi flagellae with and without adjuvant. He used the whole live bacteria as the antigen for detecting cytophilic antibodies. Mittal (1972) studied the production of cytophilic antibodies in rabbits and mice against a smooth and a rough strain of E. coli. He found that rabbit spleen cells, lymphnode cells as well as peritoneal-macrophages were effective in adsorbing the homologous cytophilic antibodies.

However, a review of literature shows that no such studies seem to have been carried out in large animals. Therefore, in the present investigation attempts were made to study the cytophilic and opsonin-adhering antibodies produced in buffalo calves in response to vaccination and subsequent challenge infection with Past. multocida.

Results of the present investigation show that cytophilic and opsonin-adhering antibodies are produced in buffalo-calves against Past. multocida. The titres of both cytophilic and opsonin-adhering antibodies show a gradual increase throughout the period of post-vaccination, reaching the maximum after challenge infection (Table 6).

The gradual increase in the titres of cytophilic and opsonin-adhering antibodies during the post-vaccination period might be due to the fact that the antigen (killed Past. multocida organisms) incorporated in oil adjuvant provides a persistent stimulation to the antibody forming cells of the host which subsequently produce the antibodies.

A subsequent challenge infection with the live Pasteurella organisms provides a further stronger stimulus and thus eliciting an intense and accelerated memory response in which the antibody titre reaches the maximum.

Higher level of opsonin adhering antibodies was found as compared to that of cytophilic antibodies during the entire period of vaccination.

Two calves showed the presence of natural cytophilic and opsonin-adhering antibodies against whole live P_{SG} strain of Past. multocida even before vaccination. It is difficult to say at the moment as to the type of antigen of Past. multocida involved in the production of cytophilic and opsonin-adhering antibodies during vaccination. Since the whole live Past. multocida organisms were used it is probable that these antibodies might have produced in response to the capsular antigen.

Further studies are warranted to ascertain the role

of various capsular and somatic antigenic components of the Past. multocida in the production of these types of antibodies and the possible role of these antibodies in the host defence mechanism against pasteurellosis should be explored.

STUDIES ON RABBIT CYTOPHILIC AND OPSONIN-ADHERING ANTIBODIES DIRECTED AGAINST SHEEP RED BLOOD CELLS

The production of cytophilic antibodies in rabbits to SRBC or HSA has been reported by several workers (Boyden and Sorkin, 1960, 1961; Berken and Benacerraf, 1966; Kossard and Nelson, 1968; Lokaj, 1969; Maginn, 1972; Blackovec et al., 1972). These investigators detected the presence of cytophilic antibody by the capacity of the antisera to confer upon normal cells the capability of specifically adsorbing antigen.

The present investigations carried out with rabbits immunized with SRBC in Freund's complete adjuvant, Freund's incomplete adjuvant or in saline showed cytophilic and opsonin-adhering antibodies in the sera when titrated on mouse and guinea pig peritoneal-macrophages.

The first portion of this study deals with the binding of rabbit cytophilic and opsonin-adhering antibodies to peritoneal-macrophages of heterologous origin.

Among the mouse and guinea pig peritoneal-macrophages

that were used in the titration of cytophilic and opsonin-adhering antibodies, the degree of sensitisation was found to be relatively higher with guinea pig peritoneal-macrophages than the mouse counterpart (Tables 7 and 8). This finding is in full corroboration with those of Berken and Benacerraf (1966) and Rossard and Nelson (1968) who found that sensitisation of guinea pig peritoneal macrophages was greater than that of rabbit by rabbit anti-SRBC or anti-HSA cytophilic antibody and less so with mouse peritoneal-macrophages. No ready explanation is available at the moment, as far as the sensitization of macrophages with rabbit anti-SRBC cytophilic and opsonin-adhering antibodies is concerned. But it is presumed that there is a species variation in the macrophage receptors for cytophilic and opsonin-adhering antibodies and that the macrophages might possess a variety of receptors.

Cytophilic and opsonin-adhering antibodies were detectable to a very low titre in the early sera of rabbits immunised with Freund's complete or incomplete adjuvant. Similar observations were also made by other investigators. Boyden (1964) found cytophilic antibodies after 19 days of primary immunization of guinea pig with SRBC in Freund's complete adjuvant by foot-pad route. Berken and Benacerraf (1966) also could not detect cytophilic antibody in guinea pigs until 8 days of primary immunization with SRBC in Freund's complete adjuvant.

Berken and Benacerraf (1966) found cytophilic antibodies in the sera of rabbits which had been immunized with 8 intravenous injections of SRBC stroma over a 2 week period and bled 10 days after the last injection. Kossard and Nelson (1968) found high titres of cytophilic antibodies in sera of rabbit which had been immunized by the intradermal injection of SRBC in Freund's complete adjuvant followed by an intraperitoneal injection of the antigen in saline two weeks later and bled after a further week.

The most likely explanation for this delayed appearance and low level of cytophilic and opsonin-adhering antibodies in the early sera of rabbits immunized with Freund's complete and incomplete adjuvant is that after the administration of the antigen with adjuvant, the antigen is retained in a depot at the site of inoculation, from where it is being slowly absorbed. This slow process of absorption of antigen from the site of inoculation subsequently results in the appearance of delayed or low level of cytophilic and opsonin-adhering antibodies. This explanation could be best supported with the findings that cytophilic and opsonin-adhering antibodies appeared in a moderately high titre in the early sera of rabbits immunized with SRBC in saline since the antigen incorporated in saline was quickly absorbed from the site of inoculation thus providing quick stimuli for antibody formation.

In the hyperimmune sera, highest titres of cytophilic and opsonin-adhering antibodies were found in the rabbits which were primarily immunized with SRBC in Freund's complete adjuvant, while lowest titres were detected in rabbits immunized with SRBC in Freund's incomplete adjuvant. Kossard and Nelson (1968) also found a high titre of cytophilic antibody in the hyperimmune sera of rabbits which were primarily immunized with SRBC or HSA in Freund's complete adjuvant.

The better immunological response in the rabbits immunized with SRBC in Freund's complete adjuvant, appears to be due in large part to the effect of Mycobacterium phlei present in the adjuvant. The mycobacterial adjuvant causes an increase in the number of immuno-competent precursor cells normally found in the lymphoid tissue.

The poor immunological response with regard to cytophilic and opsonin-adhering antibody production in rabbits immunized with SRBC in Freund's incomplete adjuvant might be due to the reason that the Freund's incomplete adjuvant lacks the essential mycobacterial component which enhances the antibody formation.

Diverse opinions exist with regard to the susceptibility of macrophage receptor sites to the treatment of various

proteolytic enzymes. Treatment of macrophages with various agents may affect their ability to take up cytophilic antibodies. Most of such treatments were carried out with a view to study the nature of the receptor sites on macrophages.

Results of the present investigations show that after the treatment of mouse peritoneal-macrophages with trypsin or papain, the subsequent uptake of cytophilic and opsonin-adhering antibodies of hyperimmune sera is increased profoundly (Tables 10, 11, 13 and 14). However, an identical treatment does not sufficiently cause an increase in the uptake of early cytophilic and opsonin-adhering antibodies. These findings are in agreement with those of Kossard and Nelson (1968), Howard and Bonaccerraf (1968), Maginn et al. (1972). However, the present findings are in partial disagreement with those of Tizard (1969) who reported that mouse peritoneal-macrophages treated with trypsin neither adsorb cytophilic antibody formed during a primary response, nor does an identical treatment usually alter their capacity to adsorb either cytophilic antibody from a secondary response or opsonized erythrocytes.

The most likely explanation with regard to the strikingly different effect of trypsin and papain treatment on the subsequent uptake of cytophilic and opsonin-adhering antibodies of early and hyperimmune sera is that there is a qualitative difference

in the cytophilic and opsonin-adhering antibodies of early and hyperimmune sera and that they attach to two different types of receptors present on the macrophages surface.

However, macrophages treated with either trypsin or papain show a marked increase in sensitization as reflected by an increase in the uptake of SRBC, when exposed to cytophilic and opsonin-adhering antibodies of hyperimmune sera. This shows that the macrophage receptors for hyperimmune cytophilic and opsonin-adhering antibodies are resistant to trypsin and papain.

The increase in sensitization of macrophages with hyperimmune cytophilic and opsonin-adhering antibodies by pre-treatment with the proteolytic enzymes is due to the fact that during the enzymatic treatment certain substances which mask some of the receptors, are removed from the surface of the macrophages. Since enzyme treated macrophages would have more receptor sites exposed, they could take more antibody molecule and so more antigen.

Jonas *et al.* (1965), Berken and Benacerraf (1966), Mossard and Nelson (1968), Tizard (1971) and Maginn *et al.* (1972) shown that the presence of normal serum inhibits the fixation of cytophilic antibody by cells. The present findings show that the presence of normal serum from rabbits depending

upon its concentration consistently interfers in the uptake of hyperimmune rabbit cytophilic antibodies by mouse peritoneal macrophages (Table 15). Maximum interference was evidenced with undiluted normal rabbit serum while less with 20% and 50% normal rabbit serum. However, the uptake of opsonin-adhering antibody was not found to be significantly inhibited even with undiluted normal rabbit serum.

The inhibitory effect of normal serum upon the uptake of specific cytophilic antibodies may be due to a competitive inhibition afforded by a pool of naturally occurring cytophilic antibodies of normal serum. But it seems difficult to interpret the absence of inhibitory effect of normal serum upon the uptake of specific opsonin-adhering antibody by mouse peritoneal-macrophages. It is presumed that probably the receptor sites on mouse peritoneal-macrophages are of two different types - one for the specific and the other for the naturally occurring opsonin-adhering antibodies. Therefore, the naturally occurring opsonin-adhering antibody cannot block the receptors of the specific opsonin-adhering antibodies, and thus when normal serum treated macrophages are exposed to hyperimmune serum, no inhibition on the uptake of specific opsonin-adhering antibody is seen.

As yet there is some unresolved confusion about the

effect of temperature upon the uptake of cytophilic and opsonin-adhering antibodies by macrophages. Rose and Brown (1962) found very little effect of temperature upon the uptake of rabbit cytophilic antibody. Jonas et al. (1965) using guinea pig peritoneal-macrophages found that the degree of sensitization was similar at 4°C, room temperature and 37°C. Barker and Benacerraf (1966) using guinea pig lung macrophages found sensitization to be greater at 37°C than at room temperature or 4°C. Kossard and Nelson (1968a) found that guinea pig peritoneal-macrophages are less readily sensitized at 37°C than at lower temperature. Tizard (1970, 1971) reported that both IgM and IgG mouse cytophilic antibodies bound strongly at 4°C at 37°C. Maginn et al. (1972) reported that the fixation of guinea pig and rabbit cytophilic antibodies to homologous spleen and peritoneal exudate cells was same either at 0°C or at 37°C.

A comparative study of the effect of different temperature upon the uptake of rabbit cytophilic and opsonin-adhering antibodies by mouse peritoneal-macrophages has shown that the uptake of these antibodies is similar at 4°, 23° or 37°C (Table 16).

The results of the present investigations are in corroboration with those of Rose and Brown (1962); and Jonas et al. (1965) who also did not find any significant effect of

temperature upon the uptake of cytophilic antibodies. However the present findings are in disagreement with those of Berken and Benacerraf (1966), Kossard and Nelson (1968), Tizard (1970, 1971), Maginn et al. (1972). These differences in the observations by various investigators might be due to differences in the immunization procedures used to induce the production of antibodies and also certain variations in techniques used for the titration of cytophilic and opsonin-adhering antibodies.

Different opinions have been expressed by different workers on the effect of 2-ME upon the cytophilic and opsonin-adhering antibodies. Kossard and Nelson (1968) holds the view that cytophilic antibody activity is not reduced by treatment of serum with 2-ME alone, but treatment of serum with 2-ME followed by iodoacetamide markedly diminishes the cytophilic antibody activity. Berken and Benacerraf (1966) also found that treatment of gamma-globulin fraction of guinea pig anti-sheep red blood cell serum results in the reduction of cytophilic antibody activity while still greater loss of activity was seen when 2-ME treatment was followed by alkylation with iodoacetamide. Results of the present investigation on the reduction studies with 2-ME have shown that the treatment of 2-ME reduces the cytophilic antibody activity of the early sera while those that appear during a

secondary response are not affected by 2-ME treatment (Tables 17, 18 and 19). However, the opsonin-adhering antibodies have been found to be unaffected by 2-ME treatment at any stage of antibody formation. Similar observations were also made by Tizard (1969) who also observed reduced cytophilic antibody activity in 2-ME treated mouse early sera, but no effect of 2-ME upon the cytophilic antibody formed during a secondary response. He also did not find any effect of 2-ME upon the opsonin-adhering antibody either formed during a primary or secondary response.

The results of the present investigation show that cytophilic antibodies that appear during a primary response are mainly IgM which are sensitive to mercaptoethanol, while the cytophilic antibodies that appear during a secondary response belong to IgG class which are resistant to mercaptoethanol. Maginn et al. (1972) also opined that reduction in the cytophilic antibody activity is associated with IgM fraction, while bulk of the IgG cytophilic antibody is insensitive to 2-ME.

The results of the experiments which were carried out to determine whether complement or complement-fixing antibodies were causing reactions falsely attributed to cytophilic and opsonin-adhering antibodies, conclusively showed that the reactions ascribable to cytophilic and opsonin-adhering

antibodies were not due to the complement or complement-fixing antibodies, and they act independently of complement. A slight reduction in the cytophilic and opsonin-adhering antibody activity was noticed in the early sera which were diluted in 0.01 M EDTA or heated at 56°C for 30 minutes and then diluted in 0.01 M EDTA (Tables 20 and 21). This finding was in corroboration with that of Nelson (1970) who also found that when mouse anti-SRBC early sera were diluted in 0.01 M EDTA or heated at 56°C for 30 minutes and then diluted in 0.01 M EDTA, a slight reduction in the cytophilic antibody activity occurred.

STUDIES ON RABBIT COMPLETE AND INCOMPLETE ANTIBODIES DIRECTED AGAINST SHEEP RED BLOOD CELLS

A parallel determination of the complete and incomplete antibodies in rabbits shows that the titres of both complete and incomplete antibodies gradually increase. The titre of both complete and incomplete antibodies appeared to be higher in the serum of rabbits immunized with SRBC in Freund's complete adjuvant. In all the three groups of rabbits, the titres of complete antibodies are higher in the reaction with trypsinized SRBC than in the reaction with native SRBC, in both 2-ME treated or untreated serum (Table 22).

These findings are in absolute agreement with those

of Klykova and Prokopenko (1975) who also found high titres of complete antibodies in the reaction with trypsinized SRBC than with native SRBC. Treatment of SRBC with trypsin probably alters the configuration of the red cells and expose additional antigenic determinants which react with the antibodies formed against the analogous determinants of red cells exposed by the action of trypsin. It was also observed that at all the period of the experiment the titres of incomplete antibodies to native SRBC were higher than the titres of complete antibodies. Klykova and Prokopenko (1975) also reported high titres of incomplete antibodies to native SRBC in comparison to the titre of complete antibodies.

These findings thus show that a marked difference in the dynamics of the titres of complete and incomplete antibodies exists in the sera of immunized rabbits. This difference in titres could indicate that the synthesis of complete and incomplete antibodies might occur independently as it is presumed that the complete and incomplete antibodies are formed against different antigenic determinants of the red cells (Klykova and Prokopenko, 1975).

The observed increase in the titre of complete antibodies with respect to trypsinized red cells could be explained in the light of the above mentioned assumption.