DISCUSSION

Amebic antigen preparations commonly employed in amebic serology were initially described by Kessel et al.\textsuperscript{100}. Subsequently, a large number of workers including Fife\textsuperscript{99} and Thompson et al.\textsuperscript{121} have advocated the use of standardized antigen preparations in various serologic tests. It was felt that the use of such a standardized antigen preparation can be successfully employed not only for making a critical assessment of the immunological competence of the host, but also for a comparative evaluation of the results of amebic serology as reported from various laboratories around the world. To date, innumerable studies have been carried out in an effort to describe the serological properties of \textit{E. histolytica} antigen preparations. But the identity of functional groups of antigens in these extracts, and their specificities in various serologic tests still remain somewhat speculative. Recently, a few studies including the present one have provided certain valuable information regarding the immunogenicity of amebic antigens.

The fractionation of axenic and monoxenic antigen preparations in this study has revealed the heterogeneity of ameba antigens. The results of this study are in conformity with those of Ali - Khan and Meerovitch\textsuperscript{86}.
The antigenic extracts used for column chromatographic separations were initially estimated to contain about 5 mg/ml protein. The axenic antigen preparations contained a sufficiently higher protein concentration. However, the protein concentration obtainable from axenic antigen preparations in this study was much less than the values reported earlier by Lunde and Diamond\textsuperscript{125}. It is quite possible that this difference in protein yield may apparently be due to a slight variation in the technic which was employed in extracting the antigen. The fraction of amebic antigens on Sephadex G-200 in the present investigations has revealed the presence of four major fractions having different antigenic specificities. The elution behaviour of these fractions suggests that the first two fractions ($F_1$ and $F_2$) were high molecular weight proteins. The two isolated components were found to be highly antigenic. Isolated antigen fraction $F_1$ in various serologic tests was found antigenically more potent than fraction $F_2$. Similar findings have also been reported by Krupp\textsuperscript{159} in one of her investigations. The above worker has also reported in this study that, the antibodies generated in experimental animals against this high molecular weight antigen fraction were of a protective nature. The guinea pigs immunized with this fraction were subsequently able to tolerate the challenging lethal doses of the causative agent. The tissues from such animals also did not show the presence of amebic lesions in
histopathological studies, whereas the tissues from all the control animals did.

The antigenicity of the isolated fractions from a Sephadex G-200 column has been discussed earlier by this author. On the basis of these results and in the light of similar work by other workers, it may be concluded that fraction F₁ is highly reactive and is capable of conferring specificity to the antigen-antibody reactions in which it participates as one of the reactants. The specificity of its serological behaviour has also been confirmed by Ali - Khan and Meervitch in their investigations. Although, Lushbaugh and Miller have attributed the antigenicity of amoeba due to the presence of glycocalyx as one of the components of the antigen complex.

Further, the characterisation of antigen on the basis of molecular weight estimations in this laboratory and elsewhere has also revealed that fraction F₁ is a high molecular weight protein and as such, it acts as a potent antigen in various antigen-antibody reactions. The approximate molecular weight of this fraction is estimated to be in the range of $600 \times 10^3$ to $900 \times 10^3$. Though, similar work cited earlier has shown that the molecular weight of fraction F₁ (NHH:200) on Sephadex G-150 was estimated in the range of $14 \times 10^3$ to $45 \times 10^3$. In these studies, the molecular weights of the components from third
fraction \((F_3)\) were not determined because of molecular heterogeneity.

The characterisation of ameba antigen in these studies was further carried out in polyacrylamide gel electrophoresis. Homogeneous isolates from a Sephadex G-200 column were found to exhibit charge heterogeneity in polyacrylamide gel electrophoresis. On the basis of their net molecular charge, the antigen fractions \(F_1\), \(F_2\) and \(F_3\) were resolved into more than 11 protein bands. The charge heterogeneity of these fractions was, therefore, confirmed on the basis of mobility patterns obtained in PAGE electrophoresis. But antigen fraction \(F_4\) showed only one slow moving band, confirming its high molecular weight. Other fractions seem to have several protein components as evident from different mobilities, thus indicating molecular weight and charge differences. Ali Khan and Meierovitch\(^\text{36,37}\) have shown some 21 bands from DKB strain cultured in association with crithidia. The difference in the resolution of these bands may perhaps also be due to the strain variations. But overall results on the antigenicity of these fractions in the present investigations, and by several other workers\(^\text{32,34,86,87,88,123,161}\) are in agreement that antigenic extract from \(E. \text{histolytica}\) consists of several different antigenic components. The antigenic make up of \(E. \text{histolytica}\), no doubt, also shows some strain
variations. But certain common antigenic moieties represented by identical arcs in agar-gel precipitation tests are suggestive of the antigenic similarity of the various strains of parasitic amebae. Similarly, the other isolated antigen fractions, specially the two high molecular weight fractions, act as potent antigens and maintain their identities in various tests. The disparity in the results of some of the above cited literature must also be due to the genetic variations amongst the different strains. Consequentially, the general variations are responsible for giving rise to slight differences in the constituent proteins of the antigenic determinants. After all, it is the genome of a species which is directly responsible for reflecting the phenotypic expression of a strain, including the nature and the number of constituent antigen proteins.

In the opinion of this author, the purification of the chromatographically isolated fractions along with an analysis of the chemical composition and the type of polypeptide chains should be required to provide further information about the nature and behaviour of functional ameba antigens.

Besides the characterisation of antigens, these investigations were also aimed to characterise the anti-
amebic circulating antibody. The elution profiles obtainable from immunized rabbit sera samples on DEAE-cellulose columns are similar to the chromatograms of Yap et al.162. The above workers have fractionated hyperimmune rabbit antiserum on DEAE-cellulose and Sephadex G-200 columns. Results of their study are in conformity with this work. They have also indicated that IgG was eluted in peak I on DEAE-cellulose columns. Whereas, antibody in this immunoglobulin was eluted as peak II in the Sephadex G-200 column eluant. The results of the present investigations further collaborate their findings that IgG is the most reactive anti-amebic immunoglobulin possessing the specific antibody activity. The manifestation of this antibody in the formation of specific antigen-antibody complex has also been demonstrated by means of immunodiffusion, passive hemagglutination and indirect fluorescent antibody tests. According to the above workers and several others163, IgG is perhaps the most or the only reactive immunoglobulin responsible for the manifestation of most of the antigen-antibody reactions.

The results of this study from salt fractionated immune and normal serum were also more or less similar. The heterogeneity of the immunoglobulins isolated on DEAE-cellulose columns was also checked on the basis of their molecular size in Sephadex G-200 columns. These
results on characterisation of antibody activity by column chromatographic studies are also in agreement with those of Maddison et al. These workers in a painstaking study on two human sera samples have demonstrated that IgG is the most reactive anti-amebic immunoglobulin. The results of the present studies, though based on a large number of sera samples, are quite similar to those reported from two cases only. In the present study, the sequential sera samples obtained from immunizing rabbits were also simultaneously assayed for the appearance of different types of antibodies in relation to the time of antigenic stimulation. Certain broad-based generalisations on the basis of the above study can be made as follows:

1. **Immunoglobulin G** is undoubtedly the most reactive component of antiamebic antibody in human, and rabbit sera as well.

2. **Specific IgG antibodies** are amply demonstrable both in recent and past infections. Ameba specific gamma globulin G is also mostly responsible for the formation of antigen-antibody complex in a large number of serologic tests.

3. In sequential sera samples, ameba-specific IgM antibodies are the ones which are predominantly detectable during
periods of initial antigenic stimulation. Immediately after first immunization dose, the IgM antibodies are found in higher titres in experimental animals.

The above findings are in agreement with those of Kane et al.\textsuperscript{129}. Though Ali-Khan and Meerovitch\textsuperscript{39} did not find a sequential release of IgM and IgG immunoglobulins in that order. They are of the opinion that antiamebic immunoglobulins appear rather simultaneously during primary immune response, as a result of active immunization. They further indicated in this study that hemagglutinin activity was present in both the IgG and IgM classes of antibodies. Subsequently, the booster injection considerably increased the IgM hemagglutinin activity. But the increase in IgM hemagglutinin was, somewhat, transitory. Its reactivity was found decreased by 12th week.

Fraction IgA of the immune serum does not appear to have any specific role as an antiamebic immunoglobulin. Its level also appeared, more or less, constant during the entire course of immunization. These findings are in agreement with those of Abioye et al.\textsuperscript{96} and Ali-Khan and Meerovitch\textsuperscript{39}. The above workers were also not able to find any significant relationship between serum levels of IgA and amebic antigen stimulation. Ali-Khan and Meerovitch\textsuperscript{39} were also not able to determine the presence of IgA conclusively in their test sera samples.
The sequence in which antibodies appear during the course of immunization in experimental animals may not parallel the immunological events in naturally acquired cases of human amebiasis. But continued antigenic stimulation and the subsequent appearance of antibodies during the course of secondary immune responses in experimental animals is perhaps a situation, which is not very different from naturally acquired human infections. The sequence of immunological response from such experimental animals appears to correlate well with the events that occur in cases of invasive amebiasis with liver involvement. In both these cases, there is a considerable antibody production. Such antibodies mostly belong to IgG and IgM classes of immunoglobulins. Most of the hemagglutinating antibody activity appears in IgG (7S) class. But antibodies belonging to IgM (19S) class are also readily detectable by means of complement fixing activity of the immune sera samples. The IgM antibodies are not completely lacking in hemagglutinin type of reactions though.

The detection of antiamebic antibody activity from the isolated rabbit serum fractions also clearly indicates that the IgA fraction of the immune serum does not seem to possess the circulating antibodies against E. histolytica antigen. Some of the IgA activity, if any, must have been eluted along with IgG fractions on DEAE-cellulose and
Sephadex G-200 columns. The level of IgA immunoglobulin during the entire course of immunization appeared to be more or less constant. In order to determine the individual role of the various immunoglobulins in the serologic reactions of amebiasis, a large number of techniques like inactivation of IgM with mercaptoethanol, etc., have so far been variously employed. But the available information on this particular aspect is still somewhat lacking in many ways. Consequently, more work is needed to unravel the exact role of each type of antibody in different antigen-antibody reactions. On the basis of the evidence derived from chromatographic studies on sequentially obtained rabbit sera samples it may be concluded, that an immunologically experienced animal is capable of mounting a specific antiamebic humoral immune response. Although, the antibody production in secondary response sera is of a much greater magnitude. Some of these antibodies perhaps persist for much longer periods of time, also. The follow up work for the detection of antibodies from experimental animals was, however, not done in this study. Though similar studies in human subjects have been done earlier. In the present studies, the investigations on antigen-antibody reactions in human subjects were mainly carried out to assess the usefulness of the commonly employed serodiagnostic tests. Since most of the seroepidemiological survey work is also based on the results of these tests,
therefore, an attempt was made to include a large number of sera in these investigations. In general, the immunology of amebiasis has variously been studied by making use of the complement fixation, immobilization, fluorescent antibody, precipitin, indirect hemagglutination and skin tests. In fact, most of these tests have been used either to determine the potential diagnostic value of these techniques, or for studying the strain variations amongst the pathogenic amebae.

The results on the detection of antiamebic antibodies in this study are almost in perfect agreement with a large number of earlier investigations of a similar nature. The results of the present study based on indirect hemagglutination, complement fixation and bentonite slide flocculation tests on human sera samples clearly indicate that ameba specific antibodies can be reliably detected by these methods. A large percentage (60 per cent) of cases showed the IHA titre in the range of 512 and above, whereas about 10 per cent cases showed a titre value around 1024. Krupp and Powell have carried out extensive studies on antibody response to invasive amebiasis in Durban, South Africa. They have found that immunodiffusion and indirect hemagglutination tests are almost similar in their ability to detect antibodies. Around 95 per cent positive results have been obtained by them and various other workers as well. Some discrepancies
which may be apparent in large scale IHA tests are generally found in cases where only low titres (1:243 or less) are obtainable. But a definite correlation between the severity of infection or on the extent of tissue invasion and the level of antibody titre cannot be reliably established on the basis of IHA tests. Although Krupp\(^{164}\) is of the opinion, that antigenic stimulation as a result of more severe tissue invasion results in a better antibody response. She further claims that antibody response, in cases of amebic colitis, is an indication of the degree of tissue invasion. This fact, however, could not have been established from the results of the present investigations. In spite of good immune response, as reflected by a fairly high level of IHA titre, amebic antibodies were not found to protect against the reinfection. Because the frequency of reinfection in a large number of human subjects was not observed to decrease in any way. Such hemagglutinin, type of antibodies were usually found to fade away in about six months time. In a few follow up cases, the IHA titre was found to be significantly low after about six months following successful treatment.

The results of immunodiffusion (ID) tests were found to be more or less similar in their ability to detect the antibodies. About 90 per cent and above human sera samples were detected as positive in these tests. Generally, this
test was not found as sensitive as IHA test. On the basis of this test, of course, it could be concluded that 
*E. histolytica* comprises of a multiple antigen system. The antibody production, in fact, takes place against all or a large number of constituent amebic antigens. The persistence of antibodies over longer periods of time can also be reliably detected by the ID tests. Although the results obtained from both of these tests cannot be reliably interpreted for establishing the clinical status of the patient. Such positive tests may at times be an indication only of a past infection. A previous episode often leaves a fairly high titre of amebic antibodies which may be detectable over a long period of time. Antigenic stimulation as a result of an 'occult' infection may also give rise to antibody production. Although, the antibody titres in such cases is generally very low. Some workers have called the detection of such low levels of antibody from an endemic area as the 'background noise'. This phenomenon often complicates the interpretation of results obtained from seroepidemiological surveys of the endemic areas. Complement fixation test is good only up to a limited extent. In the experience gained during the course of these investigations, it may be concluded that complement fixing antibodies are more readily detectable in cases of recent infection. One plausible interpretation of this could be that immunoglobulin M antibodies are more predominantly detected during the course
of initial, or continued antigenic stimulation. Since complement fixation test is more dependent on IgM, therefore, an increased titre of such antibodies may be an indication of an amebic infection of a more recent origin. Of course, this sort of finding is well documented in the case of polio virus. The kinetics of gamma M (19S) and gamma G (7S) antibodies in serum has been extensively studied by Svehag and Mandel, Uhr and Finkelstein, and Smith and Eitzman. According to these workers, gamma M antibody depends on the continued presence of antigen. It has also been reported by the above workers that 19S antibody generally appears early and is subsequently replaced by 7S antibody. The results from bentonite slide flocculation tests indicate that this is a quick and fairly reliable test for initial screening purposes. But this procedure at its best is a quantitative test and, therefore, cannot be used for an accurate evaluation of antibody levels. The results of immunofluorescence tests are also in agreement with the results of a large number of other studies. Ambrose-Thomas has recently described 412 positive results out of 412 cases tested by means of immunofluorescence technic. The results of the present study also indicated that this is a very accurate test procedure for detecting deep seated acute infections of liver amebiasis. Therefore, amebiasis of hepatic location can be reliably diagnosed on the basis of fairly high serum titres obtainable from immunofluorescence tests.
Since some very basic questions in the case of ameba antigen stimulation still remain somewhat unanswered, no satisfactory explanations seem to be forthcoming on some of the following aspects:

1. Is there an optimal interval between primary and secondary stimulation?

2. Does the length of this interval depend on the primary antigen dose?

3. Does a direct proportionality exist between the secondary antigen dose and the magnitude of secondary antibody response elicited?

Speaking of parasitic immunology, several factors should, in fact, be taken into account before assessing the immune responses in amebic infections. The ability of the host, of course, to recognise the antigen is the most important factor to be considered for understanding the host parasite interaction. Other factors which influence the antibody production are: the duration of antigen stimulation, or the state of the disease, the degree of tissue invasion and the number of amebae entering the blood stream. Individual variations in the production of antibodies are mainly dependent on the 'immunocompetence' or
'immunological responsiveness' of the host. The persistence of a high antibody titre depends on the retention of antigen in the tissues. Garvey and Campbell\textsuperscript{173} and Saha et al.\textsuperscript{174} claim that some antigens are retained by liver and spleen for a considerable period of time. These antigens persist usually in combination with ribonucleic acid. Such RNA-antigen complexes are responsible for continued antibody production. Swart and Warren\textsuperscript{175} tried to study different types of antigen-antibody reactions by obtaining rabbit serum directed against physiologically derived (PD) and somatic \textit{E. histolytica} antigens. They have reported in this study that \textit{E. histolytica} under \textit{in vitro} conditions does not secrete or excrete any antigenic material into the medium.

Boonpucknavig et al.\textsuperscript{176} reporting on the antigen-antibody system in amebiasis are of the view that homogenate containing functional antigens are mostly particulate. The antigens are 'confined to particles of microsomal density'. The antigen-antibody interaction is usually dependent on the antigenicity of the antigen molecule, the duration of antigenic stimulation and, the manner of antigen incorporation into reticuloendothelial cells - particularly the antigen-antibody interaction at the cellular level. Studies on antigen-antibody system in these investigations confirm the molecular heterogeneity of the functional antigens. The type and differences obtainable in the shape of precipitin
curves are indicative of a heterogeneous population of different molecular species participating in antigen-antibody reactions, as evidenced by precipitin titration curves.

In these investigations narrow equivalence zones were obtained in all such antigen-antibody systems in which the molecular weight of antigen protein was in the range of $40 \times 10^3$ - $160 \times 10^3$. Broader zones of equivalence were suggestive of the fact that the antibodies in the mixture were available against many antigenic determinants - confirming the multiple antigen system of a *E. histolytica* cell. The chemical nature of the various antigen fractions and their immunological properties have already been discussed by this author elsewhere. More recently, McLaughlin and Meerovitch\(^\text{177}\) and Meerovitch\(^\text{178}\) have elucidated the antigenic properties of cell fractions of *E. invadens* and *E. histolytica* by studying the subcellular distribution of antigenic sites. Antigens derived from cytoplasmic constituents both membranous (enzymic) and free antigens were characterised. The antigens were successively isolated as plasma membrane, light vesicle, heavy vesicle, lysosomal and cytosol fractions respectively. It was shown in this study that light vesicle and cytosol fractions were found to be more antigenic than other fractions. All the membranous fractions also exhibited a unique acid phosphohydrolase activity. Soluble and lysosomal fractions
contained a high acid protease content. The rabbit and human antisera against *E. histolytica* was further shown to inhibit cathepsin D activity towards hemoglobin. In their conclusions, the above workers have shown that plasma membrane of *E. histolytica* is hardly antigenic. Therefore, the antibodies are mostly produced against internal enzymatic antigens, following the lysis of a large number of invading amebae in the tissue lesions. They further claim that such anti-enzyme antibodies may perhaps also function as enzyme inhibitors - exerting a certain degree of protection.

Present investigations were further carried out to study certain parameters of cell mediated immunity and to establish the protective role, if any, of the ameba specific antibodies. Such studies were considered essential for making an overall assessment of the elicited immune responses, particularly with reference to the existence of protective immunity. After all certain basic requirements like the destruction of the invading amebae and the neutralisation of their pathogenicity have to be experimentally ascertained before one could assume the existence of such an immunity. The protective role of antibodies was studied by immunizing the experimental animals (rabbit) with ameba antigen in combination with HCG and tetanus toxoid. The antigen was combined with the nonspecific vaccines in the hope of increasing the potency of ameba antigens, as also for enhancing
the immunocompetence of antibody producing cells. The animals were subsequently challenged and the degree of protection was assessed by histopathological studies. To a certain extent, these results are in agreement with those of Krupp and others\textsuperscript{159,179} in which they have demonstrated the protection of experimental animals following the immunization with a high molecular weight antigen fraction. The results obtained from unprotected animals were similar to the observations made by a number of other workers\textsuperscript{42,180,181} on the appearance of typical pathology of invasive amebiasis. The results obtained from protected animals are also in conformity with those of Sepulveda et al.\textsuperscript{182} who have described the induction of antiamebic immunity in hamsters. In an anatomicopathological study, these workers have demonstrated the production of immunity in their experimental animals. The animals were inoculated with axenic amebae mixed with Freund's complete adjuvant. The injections were given through subcutaneous and intraperitoneal routes. The animals were sacrificed ten days later, following a challenging dose consisting of 125 million amebae. Their results suggest that typical amebic lesions, following the above procedure, were found only in 29.1 per cent cases. Whereas, about 1.3 per cent of the control group showed the production of amebic lesions. These results were found to be statistically significant ($P \leq 0.001$). Results of similar histopathological studies have also been reported by other Mexican workers\textsuperscript{183,184,185}. All these workers have
reported the production of immunity in hamsters following the vaccination with axenic and monoxenic cultures. These workers have reported a high grade immunity after vaccination with monoxenic cultures. The lesions produced by axenically grown amebae were mainly of the inflammatory and granulomatous type. On the other hand, the monoxenic amebae were reported to give rise to typical necrotic lesions. Similarly, they have reported that intrahepatic inoculations of amebae in vaccinated animals could not produce as many lesions as were obtained from unprotected controls.

In contrast to the above studies, the experimental protocols in these investigations were somewhat differently designed. In these investigations, an attempt was made to invoke the immune responses by stimulating the immune mechanisms of the host with a potent nonspecific antigen. Such nonspecific stimulation of the host's immune mechanisms for enhancing the immune responses has earlier been tried in cases of other diseases. Such stimulation of nonspecific immunity by bacterial lipopolysaccharides has been reported by Howard et al. In the present studies ECG stimulation, as compared to tetanus toxoid, was found more effective for invoking the over all immune responses. The animals protected by immunization with ameba antigen along with ECG were found to generate protective antibodies. Apparently, such animals showed about one hundred per cent protection against seemingly
lethal doses of the causative agent. The immunity obtainable from ameba antigen in combination with tetanus toxoid, or ameba antigen alone, was not able to afford a high degree of protection. Only antigen - BCG combination appeared to give rise to a high grade immunity. A large scale cellular infiltration with marked leukocyte migration was evident in the tissue sections from the protected animals only. Such areas showing leukocytic infiltration further indicated the involvement of a reaction of the inflammatory nature. On the other hand, typical amebic lesions were developed in unprotected animals - displaying a complete lack of immunity.

On the basis of the comparative results obtained from immunisation and histopathological studies, it may be concluded that antiamebic antibodies are capable of affording protection to the host. The protective antibodies are produced as a result of a combined stimulation both for humoral and cellular immune mechanisms. Such an enhanced immune response can be successfully obtained by immunizing the animals with ameba antigen along with a potent vaccine for simultaneously stimulating the nonspecific immune mechanisms of the host as well. The development of acquired immunity, and a certain degree of resistance to reinfection in dogs has earlier been reported by Swartsvelder and Avant. They have also shown that preinoculation blood transfusions from animals refractory to infection were able to provide certain degree of protection.
to the recipient animals. This passive transfer of immunity was obviously due to the presence of humoral antibodies.

The results of present investigations on \textit{in vitro} inhibition of amebic growth in the presence of hyperimmune serum and antiamebic immunoglobulin G seems to confirm the above findings on passive immunity. The results of \textit{in vitro} studies are also in agreement with similar findings reported from Mexico\textsuperscript{187,188}. These workers have shown the cytopathogenic effect of human immune serum on ameba trophozoites. The addition of complement and Ca and Mg ions to hyperimmune serum appeared to enhance its cytopathogenic effect. These workers further claimed that antiamebic antibody has a complement activating ability. The disintegration of parasite, according to them, is caused due to a progressive membrane destruction.

The results of present investigations obtained from skin hypersensitivity studies further illustrate the presence of a cell mediated immune response. The appearance of such an immune hypersensitivity reaction has also been demonstrated by a host of early workers\textsuperscript{189,190,191,192}. They have shown a correlation of amebic infection with a demonstrable hypersensitivity of the delayed type. The passive transfer of skin-sensitizing antibodies through the immune serum has also been demonstrated by one of the above workers\textsuperscript{192}. Similarly, several other workers\textsuperscript{90,134,135,136} have also
reported the results of skin sensitivity tests which are in complete agreement with the present findings. The test procedure used for skin sensitivity experiments and the obtained results in the present investigations are, in fact, consistently similar to the several above cited studies. An immune hypersensitivity, ranging from immediate to delayed type of reaction has been variously reported earlier. Certain apparent disparities in the observed results may only be due to the differences in the employed antigen preparation. These seemingly minor differences do not appear contradictory to the basic findings of these investigations. Basically, two different types of reactions have so far been reported. The immediate type of wheal and flare reaction, which may be only due to the interaction of antigen with skin sensitizing antibodies in the sensitized subjects. Such reactions are usually the manifestations of the release of mediators from the mast cells. Whereas, the induction of delayed or cellular hypersensitivity reactions in experimental animals are characterized with the presence of mononuclear cells or with basophilic leukocytes, as in Jones. Note hypersensitivity reactions. More recently, the evidences in favour of a delayed type of hypersensitivity reaction, instead of immediate type, have been further provided by Kretschmer et al. and Landa et al. All the above cited studies including the present one are in support of the view, that an immune hypersensitivity reaction of the
delayed type is obtainable from experimental animals and as well as from human subjects. The presence of cellular immunity in amebiasis has been further demonstrated by inhibition of peripheral leukocyte migration tests\textsuperscript{199,200}. The liberation of migration inhibitor factor (MIF) for peritoneal cell migration in hamsters has also been demonstrated by the above workers. The absence of histologic lesions in the liver of animals with MIF suggests the mediation of a cellular immune mechanism, which may perhaps be participating in the dynamic processes related to the overall defense mechanisms of the host. The participation and presence of cellular immunity in amebic infections has also been demonstrated by experiments on blast transformation of lymphocytes. The in vitro experiments by Savanat et al.\textsuperscript{143} have confirmed that such blastogenic transformations induced by ameba antigen were of a specific nature and, the immune response varied from patient to patient.

It could be deduced from the above discussion on cellular and humoral responses that parasitic amebae induce a wide spectrum of antibodies by challenging both the humoral and cellular immune mechanisms of the host. Although, a strict demarcation cannot always be made between the humoral and the cellular components of immunity. The host, in fact, uses both humoral and cellular immune mechanisms for defence against the invading amebae. The T lymphocytes which are
normally required for the induction of cell mediated immunity also play a role in the recognition of B cells for humoral immune responses. The presence of an innate, protective cell mediated immunity (CMI) in invasive amebiasis can be indirectly inferred by the fact that treated cases of amebic liver abscess never develop a second infection. Likewise, all those people who are exposed to parasitic amebae do not develop clinical amebiasis, suggesting a certain degree of resistance with individual variations, of course. Sato\textsuperscript{201} has shown a fairly high degree of protection in all those animals who were thoroughly immunized. Thereby confirming the assumption of a large number of investigators that only prolonged antigenic stimulation, due to deeper tissue invasion by amebae, can result in the appearance of protective immunity.

In spite of a large number of recent advances in the research methodology and immunological techniques being presently employed in amebiasis immunology, there are still certain areas which merit further investigations\textsuperscript{202}. For example, the role of IgE antibodies in amebiasis need to be thoroughly investigated. The detection of IgE levels in amebiasis can be carried out by Mancini-type immunodiffusion using radiolabelled antisera. Certain more sensitive methods like radioimmunosorbent and radioallergosorbent tests can also be successfully employed for the estimation of specific IgE antibodies. Similarly, the role of IgA antibodies, if any,
in the intestinal mucosa of amebic patients should be critically evaluated. Tomasi has recently described the presence of such secretory immunoglobulins in other diseases.

At the moment, there is no denying of the fact that certain aspects of amebiasis immunology will perhaps remain beyond easy comprehension till such time that we have some more definite answers to the following questions:

1. What happens to the complement levels in clinical amebiasis?

2. What is the immunologic capacity of an amebiasis patient? Is he immunosuppressed?

3. Are antigen-antibody complexes detectable in the serum of a patient? Are blocking antibodies present in the serum?

4. Are the obtained cellular immune responses of a protective nature? Can we separate the Arthus type reaction from a true delayed hypersensitivity?