Chapter V
DISCUSSION
A. INCIDENCE OF ENTAMOEBA HISTOLYTICA

A perusal of the data on the epidemiological surveys carried out by earlier workers from India (Table I) and the results of two surveys carried out during the present study (Tables IV and V) indicates that:

(i) Amoebiasis still constitutes a serious health problem in India, inflicting a considerable percentage of populations in both urban and rural areas. A reliable diagnosis of asymptomatic and clinical cases would be most desirable for the control of the disease.

(ii) Most of the workers have placed great reliance on the examination of wet smear of stool samples for the diagnosis of amoebiasis. It is surprising that only a few workers have realized the limitations of this method when used alone for diagnosis of amoebiasis and have employed other complementary techniques for diagnosis such as concentration by zinc sulphate floatation or formal-ether sedimentation (Srivastava, 1960; Bell, 1957; Grewal, 1968; Mitra, 1970; Mathur and Kaur, 1974; Sharma et al., 1978a), culture methods (Chuttani et al., 1961; Prakash and Tandon, 1966; Rao et al., 1971b), and permanent stained smears (Chuttani et al., 1961; Sharma et al., 1978a). In the present study, detection of coproantibodies among cases of intestinal amoebiasis, using indirect haemagglutination test was evaluated for the first time in seroepidemiologic study of amoebiasis (Sharma et al., 1978a).
In spite of the introduction of effective and powerful chemotherapeutic agents like metronidazole, amoebiasis continues to prevail without any abatement in its incidence. Apparently adequate public health control measures have not been implemented to check the spread of infection. This calls for effective measures for diagnosis and radical treatment of the infected food-handlers, proper disposal of the night soil and prevention of contamination of the public water supply and eatables with the infected faecal material.

In the present investigations, 10.9 per cent of the hospital patients with gastrointestinal disorders were found to harbour *E. histolytica*. There was a slight variation in the incidence of *E. histolytica* as obtained in the two surveys (Tables IV and V) which might be a reflection of the seasonal variation in the incidence of amoebiasis. However, in both the surveys, there was a close similarity in the stool-positivity (12.4 and 9.5 per cent respectively for the first and second surveys) and the serologic positivity, as obtained with indirect haemagglutination test for coproantibodies (12.6 and 10.3 per cent respectively).

### B. STUDIES ON *E. HISTOLYTICA* ANTIGEN

The WHO Expert Committee on Amoebiasis (1969) observed that in the past, although it was "feasible to develop useful immunodiagnostic procedures for amoebiasis", complexity of the antigen preparations made from amoebae grown in *vitro* with culture-associates, had hampered the standardization of these tests. Obviously, the use of crude and insensitive amoebic antigens containing significant quantities of non-*E. histolytica* proteins, was the chief cause of the conflicting results in the
serological studies on amoebiasis (DeBlasi and Magaudda-Borzi, 1958). As a consequence, different workers reported different degrees of sensitivity and specificity of the various serodiagnostic tests. Thus in the gel-diffusion precipitin test, Atchley et al., (1963) observed that the sensitivity of the procedure depended upon the concentration of amoebic proteins in the antigen preparations, while Sen et al., (1961) and Maddison (1965) obtained non-specific precipitin bands (welchii" bands) against bacterial associates of E.histolytica used for the preparation of antigen. In the present study, it has been demonstrated that axenic axenic E.histolytica antigen gives sharp precipitin lines with sera from cases of invasive amoebiasis, while the precipitin bands resulting from use of the crude amoebic antigen were diffused and lacked the sharpness. In the indirect haemagglutination test, there appears to be very little agreement among the earlier workers regarding the threshold IHA titre which should be considered specific for diagnosis of amoebiasis. Thus Kessel et al., (1965) considered an IHA titre of 1 in 8, Milgram et al., (1966) of 1 in 256, Healy (1968) of 1 in 128, Krupp (1970) of 1 in 80 and Prakash et al., (1970) of 1 in 256 as specifically significant for the diagnosis of amoebiasis. Similarly, in indirect fluorescent antibody test, different workers reported different IPA titres as diagnostically significant (see Table II).

Development of methods for the axenic cultivation of E.histolytica (Diamond, 1968), modified by Singh et al., (1973, 1974), provided the basis for preparation of standard, purified antigen of E.histolytica. A standard antigen prepared from axenic E.histolytica should contain a known amount of the total amoebic proteins (Healy, 1977, personal communication). In the present study, therefore, great importance was attached to the use of a standard axenic antigen of E.histolytica for diagnostic work.
Protein contents of a particular batch of amoebae would depend upon the growth-phase at which they are harvested. It could, thus, vary from 0.03 mg to 1.4 mg of protein per million amoebae. However, it is significant to note that quantitation of the protein contents of the antigen could be made and correlated with the number of trophozoites of E. histolytica.

It was also found feasible to lyophilize the axenic antigen in small aliquots and store it at -20°C without any alteration in its serologic reactivity.

In the present investigations in which purified and improved amoebic antigen prepared from axenic E. histolytica was employed, it was possible to obtain reproducible results with maximum specificity in the different serologic tests like IHA for coproantibodies and precipitin tests, IHA and IF tests for circulating antibodies.

C. STUDIES ON COPROANTIBODIES

Results of the present study (Tables VI, VII, VIII and IX) indicate that the concomitant presence of other intestinal parasites like Entamoeba coli, Iodamoeba butschlii, Giardia lamblia, Ascaris lumbricoides, hookworm, Enterobius vermicularis, etc., did not influence the outcome of IHA test for amoebic coproantibodies. Krupp (1970) and Vinayak et al., (1974) had demonstrated that the IHA titres for circulating antibodies against E. histolytica were not altered by the concomitant infections with other intestinal protozoa and helminths. That a similar situation exists for the coproantibody is corroborated by the observation that during the first survey (1976-77), only 8 out of 213 cases (3.7 per cent) and during the second survey (1978) only 6 out of 92 cases (6.5 per cent) of the non-amoebic parasitic disorders gave a false positive reaction. The possibility that these false positive reactions may be an indication of past infection with E. histolytica cannot be ruled out.
Findings of the present investigation are in agreement with those of Mahajan et al., (1972b) who employed crude antigen of *E. histolytica* prepared from bacterial cultures and demonstrated that an IHA titre of coproantibodies above 1 in 64 (i.e. 1 in 128 and above) should be considered diagnostic for intestinal amoebiasis. They also reported that IHA titres were consistently 1 in 64 or lower in cases with other intestinal parasites or in healthy cases. In the present study, a larger number of non-amoebic cases were studied to demonstrate the specificity of the test.

Results obtained in the present study are, however, at variance with those obtained by Shaalan and Baker (1970), who employed complement fixation test in order to detect coproantibodies. This variation can be explained in view of the biologic properties of the chief immunoglobulin class encountered in the intestinal lumen. Several workers have reported the preponderance of secretory immunoglobulin Ig A in the enteric contents in cases of gastrointestinal infections (Heremans, 1976; Tomasi 1970, 1976; Blecka, 1978). That immunoglobulin Ig A, whether serum or secretory, has a very poor ability for the fixation of complement, has also been demonstrated by several workers (Heremans, 1973; Ishizaka, et al., 1965; Ishizaka, et al., 1966; Tomasi, 1970). It was appreciated by Shaalan and Baker (1970) that poor positivity rate obtained by them might have been due to the poor reactivity of Ig A in complement fixation test and it was suggested that the utilization of other serologic techniques might increase the yield of positive results. Since immunoglobulin Ig A has been reported to be as reactive as IgG in the agglutination reactions (Eddie et al., 1971), detection of coproantibody by IHA test in the present study has been found to have better diagnostic value.
A comparative study of the behaviour of fresh, tanned sheep red cells and glutaraldehyde treated sheep red cells in the IHA test for detection of amoebic coproantibodies has been made for the first time in the present study and the results indicate that both methods had identical sensitivity and similar specificity. The chief advantage of the use of glutaraldehyde treated sheep cells was that they remained stable and unaltered in their sensitivity to adsorb amoebic antigen for long periods of time which facilitated their storage at 4°C. That also ensured availability of the cells from the same batch in the different series of test thus eliminating batch to batch variations. Krupp (1969b) had found it essential to store the glutaraldehyde-treated sheep erythrocytes at -70°C after these had been shell-frozen. In the present study, however, storage of the glutaraldehyde treated sheep cells at 4°C did not seem to influence their behaviour in the IHA. Similar observation was also reported by Ali-khan (1974). No clumping was observed in these preparations and patterns obtained with them were superior and more reproducible than those with fresh tanned cells. The two-step method of stabilizing sheep erythrocytes advocated by Farshy and Healy (1974) has not apparently been evaluated further. However, chief advantage of the method of Farshy and Healy (1974) was reported to be the feasibility of storing the treated sheep cells at 4°C. Results of the present study (Table XXIII) indicated that successful storage could also be achieved even with the glutaraldehyde-treated sheep erythrocytes.

Major advancements in the methods to detect amoebic coproantibodies by serological means as revealed in the present studies, are enumerated below:
(i) Use of improved and purified amoebic antigen prepared from axenic _E. histolytica_ has been made for the first time in such studies which resulted in obtaining reproducible and reliable IHA results.

(ii) A highly specific serologic test has been developed for the detection of amoebic coproantibodies and it was demonstrated for the first time that presence of other intestinal pathogens did not influence the results of IHA for detection of coproantibodies in intestinal amoebiasis.

(iii) A large number of asymptomatic subjects who pass out cysts of _E. histolytica_ could be detected by means of this test. It is a significant observation in view of the very low titres of circulating antibody usually obtained in such cases.

It is evidently clear that this test could be potentially applied in the diagnosis of amoebic cases among hospital patients and also in the seroepidemiologic studies on amoebiasis.

**D. STUDIES ON SERUM ANTIBODIES**

The chief aim of present investigations was to develop reliable and standard serologic methods using improved axenic antigen of _E. histolytica_ which would help the clinician to diagnose amoebic cases among hospital patients who present with vague intra-abdominal symptoms, particularly:

(i) A large and tender liver with possible abscess formation (which could be amoebic or pyogenic), hepatoma or other liver disease: It is relatively easy to diagnose patients with frank amoebic liver abscess when the condition is present with all its typical signs and symptoms such as constant pain localized to the region of
right hypochondrium associated with tenderness in the lower right intercostal region. However, some of the patients do not present these features and they present complex problems for diagnosis. Serodiagnostic tests developed in this Institute during the course of present investigations, have shown their potential value in the differential diagnosis of amoebic liver abscess among these patients.

(ii) Tender hepatomegaly, which could be either due to infectious hepatitis or other clinical conditions like malaria and non-specific ulcerative colitis: It is well known that about 50 per cent cases of amoebic dysentery might also present with tender hepatomegaly. It would be desirable to identify amoebic cases among these patients in order to institute specific anti-amoebic therapy where indicated. In the present investigations, value of different serologic tests has been ascertained thoroughly towards this end.

(iii) Dysentery or diarrhoea: In the untreated amoebic patients, microscopic examination of the faeces is often helpful in the specific diagnosis. But when the patients have been partly or inadequately treated, diagnosis by identification of *E. histolytica* generally yields a negative result. Moreover, in certain conditions where a fulminating amoebic dysentery may have involved the entire colon, closely resembling condition encountered in the non-specific ulcerative colitis, specific diagnosis on clinical findings alone may be made extremely difficult. A serologic test would help the clinician to appreciate the extent of involvement of *E. histolytica* in such conditions.
In the present study which was mainly confined to the adults, the commonest age group to be affected by amoebiasis was found to be 50 to 45 years (Table XV). Sepulveda (1976a) commented that amoebiasis appeared to have a preference for men in the most productive period of their life i.e. the middle-age. It was also found that a larger number of males (28 cases) than females (15 cases) were prone to the amoebic infection. This is in agreement with the observations made by WHO (1969).

The commonest sign in all the patients of groups IIA, IIB and III was the pain in abdomen (91.35 per cent). Among the patients with amoebic liver abscess (Group III), this pain was confined to the right hypochondrium while among patients of hepato-intestinal amoebiasis (Group IIA) it was generalized in the region of abdomen. Next common symptoms were loss of appetite (76.54 per cent cases), history of loose motions or past history of loose motions (45.68 per cent). A comparison of the symptoms suggestive of amoebic liver abscess and those of tender hepatomegaly of obscure aetiology suggested that pain in the region of liver associated with heaviness in the right hypochondrium, hyper-pyrexia, difficulty in breathing and a sense of breathlessness, and sudden deterioration of a prolonged illness indicated diagnosis of amoebic liver abscess while symptoms like generalized pain in the abdominal region was indicative of the non-amoebic involvement of liver. History of dysentery was usually not present in these cases, although past history of dysentery could be obtained.

A long interval of time may elapse between the date of original infection with *E. histolytica* and the apperance of clinical symptoms suggestive of the onset of hepatic amoebiasis. It was remarked by Stamm (1970) that hepatic amoebiasis might develop 20 or more years after the probable time of original infection. WHO (1969) offered two possible
explanations for such a long latent period: "One is that there is a true latent period, when amoebae lurk somewhere in the tissues without producing any clinical manifestations, eventually some stimulus causes them to multiply in an area of the liver and produce an abscess. The other explanation is that an original infection induces a state of hypersensitivity in the patient, and a later reinfection produces hypersensitivity reactions in the liver that lead to an abscess."

Results of the physical examination reveal that all patients of amoebic liver abscess present signs of tender hepatomegaly with 39.1 per cent of cases in whom liver was palpable more than 6 cms. below the costal margin, 21.8 per cent of cases in whom liver was palpable from 4 to 6 cms. below the costal margin, and 39.1 per cent of cases in whom liver was palpable from 2 to 4 cms. below costal margin (Table VIII).

Association of jaundice with amoebic liver abscess was once considered to be completely improbable, but it has now been realized that 23 to 31 per cent of the cases of amoebic liver abscess might also be suffering from jaundice at the same time (Joshi et al., 1972; Datta et al., 1973). In the present study also, five patients, all belonging to Group III, had jaundice, i.e. 21.7 per cent of the cases of amoebic liver abscess were found to have jaundice.
(a) Indirect haemagglutination test

Results of the present study indicate that indirect haemagglutination (IHA) test possesses a high degree of specificity for diagnosis of the patients with extra-intestinal amoebiasis. It is also evident that IHA test can be of definite advantage in differentiating amoebic liver abscess from the pyogenic abscess of the liver. A positive IHA reaction in 100 per cent of the 23 cases of amoebic liver abscess (Group III) compares favourably with the results reported by Kessel et al. (1965), Milgram et al. (1966), and Krupp (1970) who obtained 100, 96 and 87 per cent positive IHA test, respectively, among cases of liver abscess. The present study shows the high diagnostic value of IHA test for detecting liver abscess cases. Slightly lower positivity percentage reported by Prakash et al. (1969) could be attributable to the lower potency of the amoebic antigen used in that study. Of 16 patients of amoebic liver abscess, only 12 were positive with IHA and of these, 7 cases yielded extremely low IHA titres (1 in 4 to 1 in 64). Vinayak et al. (1974), however, reported 89.5 per cent of the 57 cases of amoebic liver abscess to be positive in the IHA test in which a titre of 1 in 16 was considered specific for amoebiasis. Relatively high percentage of false positive results (16.3 per cent) in the patients with "miscellaneous non-amoebic diseases" slightly devalues the positive results of IHA test reported by Vinayak et al. (1974).

The results of IHA test with sera of cases of uncomplicated acute amoebic dysentery also indicated that the test could be used for diagnosing a large number of such cases. Thus 77.7 per cent cases of Group I had IHA titres of 1 in 128 or above. Results of Kessel et al. (1965) and Maddison et al. (1965b) which yielded very high rates of positivity (97.5 per cent and 100 per cent,
respectively) probably reflected the characteristic fulminating type of amoebic dysentery which was known to be endemic in the populations studied by these workers. However, it may be pointed out that a very low IHA titre (1 in 8) was employed as the diagnostic titre in these studies which also contributed towards the higher yield of positive results. In other studies also where a lower IHA titre such as 1 in 16 (Vinayak et al., 1974), has been employed as the diagnostic titre, higher percentage of positive results has been reported. Results obtained in the present study, however, compare favourably with those reported by Milgram et al. (1966), Pasquel et al. (1968) and Krupp (1970). Lower percentages of positive IHA results were obtained by Prakash et al. (1969) and Kasliwal et al. (1970) probably because of lower potency of the amoebic antigen employed in these studies.

The potential usefulness of such a specific and sensitive test as IHA becomes apparent when one considers the difficulties encountered in the differential diagnosis of amoebic and non-specific ulcerative colitis. Juniper and Minshew (1968) found this test to be extremely helpful in diagnosis of cases of acute amoebic colitis, especially when the identification of E. histolytica in the stool was hindered by the ingestion of interfering substances. Healy and Kraft (1972) who tested sera from 511 subjects with inflammatory bowel diseases (ulcerative colitis or Crohn's disease) by amoebic IHA, found that only 1 per cent of these were positive as against 2 per cent positive in the control group. They emphasized the positive application of this test in differentiating the fulminating kind of amoebic dysentery with possible involvement of the entire colon which closely simulated nonspecific ulcerative colitis.
The usefulness of this test is further enhanced by the fact that its outcome is not influenced by the presence of other non-amoebic pathogens, protozoal, helminthic or bacterial, in the gut (Krupp, 1970; Vinayak et al., 1974; Sharma et al., 1978a).

Various workers have reported relatively high percentage of positive results in the cases of so-called "amoebic hepatitis", although amoebic etiology has only rarely been demonstrated among cases labelled with this entity (Doxiades et al., 1961; Reddy et al., 1969). Results obtained by Vinayak et al. (1974), Kotcher et al. (1970) and Prakash et al. (1969) who reported 57.6, 85 and 54 per cent of the cases of "amoebic hepatitis" respectively, to be positive in the IHA test might be due to the lower diagnostic titres employed as already indicated. Moreover, apparently no attempt has been made so far to assess the specificity of IHA test in cases of tender hepatomegaly of obscure etiology in which involvement of _E. histolytica_ is ruled out. In the present study, using a titre of 1 in 128 as diagnostic, 22.4 per cent of the 49 cases of tender hepatomegaly (Group II) were found to be positive. The significance of this test is revealed when its behaviour is studied in the subgroups IIA and IIB which respectively included patients with and without amoebic dysentery. Thus, 72.5 per cent of the cases of tender hepatomegaly associated with overt amoebic dysentery (IIA) were found to be positive in the IHA test, while only 3 of 38 cases of Group IIB gave a positive result.

The specificity of the test is further strengthened by the results obtained in the non-amoebic hospital patients and healthy subjects. Of 100 hospital patients (Group IV) who did not present any laboratory or clinical evidence of amoebiasis, only 3 gave a positive IHA result; similarly of 44 healthy subjects (Group V) only 1 subject was found to be positive in the IHA test (Table AIII).
In order to make the test more meaningful and its titres more reproducible, a comparative study of IHA test, using fresh sheep erythrocytes and stable (glutaraldehyde-treated) sheep erythrocytes was undertaken. As reported by Krupp (1969b) and also observed during the early phase of the present studies, using fresh, tanned sheep red cells, replicate titres for the same sera were seldom obtained with different batches of red cells. It has been reported by Poulak and Lauf (1969) that red blood cells of different batches showed variations in the physico-chemical and serological properties of their membranes. Even the same batch of fresh red blood cells was shown to undergo changes in the physiological and metabolic properties during storage (Marks and Johnson, 1958). Ping et al. (1967) investigated the feasibility of employing preserved red cells in the haemagglutination assays and found that the treatment of red cells with glutaraldehyde "appeared to fulfil the desired requirements."

Results presented in Table XXIII indicate that the stable (glutaraldehyde-treated) sheep erythrocytes could be stored at 4°C and used in the IHA test for several months. Titres obtained with these cells were reproducible within the limits of two dilutions when same sera were tested at different intervals of time. However, similar sensitivity and identical specificity was obtained when a comparison was made between the IHA results obtained with fresh sheep erythrocytes and the stable erythrocytes. These results are in agreement with those of Krupp (1969b) and Ali-Khan (1974).

In another modification of IHA, use of buffered chromic chloride was reported to be valuable in saving time for the preparation of sensitized red blood cells (Poston, 1974). However, in the present study, this procedure was found to markedly reduce the sensitivity of IHA test as the maximum IHA titre obtained did not exceed 1 in 8 while
the corresponding titre obtained with tanned red cells was 1 in 64000. Recently, Ortiz-Ortiz et al. (1978), however, reported that the procedure of Poston (1974) could be usefully employed in the amoebic IHA but their results remain to be confirmed.

(b) Gel-diffusion precipitin test

100 per cent of the patients with proved amoebic liver abscess were found to be positive for amoebic precipitins. These results are in fair agreement with those reported by several other workers like Nakamura (1961), Powell et al., (1965) and Maddison et al., (1965b). Vinayak et al., (1974a) and Atakpa et al. (1978) obtained a positive gel diffusion precipitin result in only 90.5 per cent and 89 per cent of the cases of proved amoebic liver abscess. They attributed these lower percentages to the possible inability of certain individual patients to mount a positive immune response. However, it was emphasized by Atakpa et al. (1978) that this test was particularly useful in distinguishing cases of amoebic liver abscess from those of hepatoma and other liver diseases. That Stamm et al., (1976) had observed a positive GDP result was invariably indicative of extant invasive amoebiasis of liver despite consistent failure to find *A. histolytica* in the stool. Mohapatra et al. (1978) reported only 26 out of 35 (74.3 per cent) patients of amoebic liver abscess to be positive with GDP test.

In the diagnosis of patients with amoebic dysentery (Group I), GDP test yielded only 22.2 per cent positive results. This does not reflect unfavourably upon the usefulness of this test since it is known that precipitins become demonstrable only during the later stages of the disease (Taylor and Truelove, 1961). This is also borne out by the observations made by Vinayak et al. (1975) and
Mohapatra et al. (1978) who found that GDP test became positive only after haemagglutinins had attained a high titre. The observation in the present study that only 22.2 per cent of the patients of amoebic dysentery had IHA titres above 1 in 256 appear to indicate that considerable tissue invasion by *A. histolytica* in this group had not taken, which explains the results obtained by GDP test. It was further observed that one patient who had a very high titre (1 in 128,000) also gave a strong precipitin reaction in which three distinct precipitin bands were obtained. Very high percentages of positive results have been reported by Maddison et al. (1965b), Powell et al. (1966), Auernheimer et al. (1966), who, respectively, found 91 per cent, 92 per cent and 85 per cent of the cases of amoebic dysentery to be positive in GDP test. Auernheimer et al. (1966) and Powell et al. (1966), however, pointed out emphatically that the amoebic dysentery in these patients was a severe disease with considerable tissue invasion. It was further corroborated by the observation that more than 50 per cent of the apparently asymptomatic subjects also gave a positive precipitin reaction (Auernheimer et al., 1966).

Results obtained for Group II could also be understood in the light of observations made above. The fact that none of the control subjects (which included 100 hospital patients with miscellaneous ailments and 44 healthy subjects) gave a positive result with GDP test, points towards the high degree of specificity that this test possesses. Powell et al. (1966) had suggested that this test could be used for differential diagnosis of post-dysenteric acute amoebic colitis and chronic, non-specific, ulcerative colitis. Results of present investigations also point towards the potential usefulness of GDP test in this regard.
(c) Immunoelectrophoresis (IEP) test:

Only selected sera were subjected to IEP test. The value of this test lies not as much in the specific diagnosis as in delineating variations in the antigenic response of individual hosts to *E. histolytica*. Krupp and Powell (1971) suggested that severity of the disease could be correlated with certain definite IEP patterns. Results of the present study, though preliminary in this respect, also indicate that a distinction could be made between patients with amoebic liver abscess and those with severe amoebic dysentery on the basis of IEP results. Patients with amoebic liver abscess showed development of more precipitin bands, majority of which seemed to possess a slow electrophoretic mobility (Figs. 13 & 14). Patients with amoebic dysentery, on the other hand, generally gave 1-3 precipitin bands. Krupp (1976) has discussed the value of this test in studying variations in the antigenic response against *E. histolytica* among different population groups. She commented that even when the basic pattern of IEP was strikingly similar within each population, individuals differed in their IEP patterns against *E. histolytica*. Capron et al. (1972) demonstrated that IEP was a valuable test in detection of amoebic patients by virtue of the presence of a particular band (analogous to band 4 of Krupp and Powell, 1971) in their IEP patterns.

(d) Counterimmunoelectrophoresis (CIEP) test

Counterimmunoelectrophoresis test appears to possess same specificity and sensitivity as the gel-diffusion precipitin test. However, its chief advantage lay in its speed and simplicity. The test was found to be 100 per cent positive in cases of amoebic liver abscess which is in agreement with the results reported by Krupp (1974a), and Mahajan et al., (1975a). But with the sera from patients of amoebic dysentery, only 22.2 per cent of the cases in
the present series yielded positive results as against 70 per cent positive results obtained by Krupp (1974a). Nevertheless, differences in the character of disease studied in the two investigations seem to account for the difference in the results of present investigation and that of Krupp (1974a). That this test possesses a high degree of specificity is apparent from the fact that no positive reaction was obtained with the sera from control subjects.

(e) Indirect fluorescent antibody (IFA) test:

IFA test has been applied by several workers for the serodiagnosis of clinical cases of amoebiasis (Maddison et al., 1968; Jeanes, 1969; Agarwal et al., 1971; Ambroise-Thomas and Kien Truong, 1972; Ray et al., 1974; Boonpucknavig and Nairn, 1967; Cox and Nairn, 1969; Mithal et al., 1978). Results of some of these studies are briefly summarized in Table II. As is evident, there is a good deal of variation with regard to the positivity and diagnostic titres employed by different workers. These variations can be attributed to a number of factors such as differences in the quality of the fluorescent-conjugate with varying molar F/P ratios (Shu et al., 1975), in optimal dilutions of the FITC-conjugate employed and in the procedures employed for the preparation of antigen.

Results of the present studies are in agreement with those of the earlier workers as far as the value of IFA test for diagnosis of amoebic liver abscess cases is concerned. Nearly 100 per cent of these cases are positive with this test. Among the cases of acute intestinal amoebiasis, we found this test to be positive in 77.7 per cent of the cases, which is in agreement with the results reported by Jeanes (1969), Ambroise-Thomas and Kien Truong (1972) and Ray and coworkers (1974). Overall seropositivity among the 49 cases of tender hepatomegaly
in our study has been found to be 20.4 per cent. On clinical grounds, these cases could be grouped into two categories, namely, those showing tender hepatomegaly in association with overt amoebic dysentery (11 cases) and those without amoebiasis (38 cases). This study suggests that the cases of tender hepatomegaly with a positive amoebic IPA test should be specifically treated while those without amoebiasis and giving a negative IPA test constitute a distinct group which needs thorough clinical investigation before the antiamoebic therapy is instituted. It is felt that, in this group, hepatomegaly might be of the non-amoebic etiology.

The results of IPA test obtained with 100 non-amoebic hospital patients and 44 healthy subjects who gave very low positivity further enhances the value of this test for diagnosis of amoebiasis. The very low positivity (4.4 to 6 per cent) in these groups might be due to past exposure of the cases to amoebiasis.

E. EXPERIMENTAL STUDIES
I. Antiamoebic activity of the specific antisera

Information regarding importance of circulating antiamoebic antibodies for serodiagnostic purposes and epidemiologic surveys is well documented (Juniper et al., 1972; Stamm et al., 1976; and Connell and Elsdon-Jew, 1973). By and large, the humoral antibodies do not impart any protection against reinfection with E.histolytica in cases of intestinal amoebiasis (Krupp, 1970). However, Sepulveda (1976b) and Krupp (1975) have reported that hepatic amoebiasis cases, once cured, do not relapse, indicating development of protective immunity in these cases. The antiamoebic antibody of the immune human serum has been shown to play a protective role by exerting a cytolytic effect on the trophozoites of E.histolytica.
This antibody is localized chiefly in the IgG fraction of the immune serum. The evidence available so far thus strongly suggests that at least some component of the antibodies does exert lethal action on *E. histolytica* (Sharma et al., 1978b)

II. *Experimental induction of delayed dermal hypersensitivity*

Several workers have employed intradermal test for the diagnosis of amoebiasis (Scalas, 1923; Leal, 1953; Maddison et al., 1968; Savanat et al., 1973b; Meerovitch and Scott, 1973). In several earlier studies conflicting results were obtained with regard to the specificity of this test, evidently because of the poor quality of antigen employed. Lunde et al. (1969) prepared an antigen from the axenic *E. histolytica* and ascertained its skin-test potency in guinea-pigs. As a prelude to develop specific skin-test using standard amoebic antigen prepared from local strains of *E. histolytica*, studies were also carried out during the course of present investigations. Results obtained indicate that each of the 3 axenic strains of *E. histolytica* could be potentially used for diagnosis of amoebiasis by skin-test.

It was further observed that *E. histolytica* antigen with Freund's complete adjuvant could induce a state of delayed hypersensitivity in the experimental model. This may help explain some features of the pathology of amoebiasis such as development of liver abscess and immunology of amoebiasis such as development of cell mediated immune response in a recovering patient (WHO, 1969).