SUMMARY
The concept of pathogenic amoebae has undergone radical change since 1958, when pathogenesis of environmental isolates of amoebae was discovered and it was established that *E. histolytica* was not the only pathogen. This is now well known that two genera namely *Acanthamoeba* and *Naegleria* amongst the free-living amoebae are potentially pathogenic to man and animals. These pathogenic amoebae are plentiful, ubiquitous in nature and world-wide in distribution.

These three amoebae differ in their pathogenicity. *E. histolytica* causes the endogenous amoebiasis, focus of infection being in colon and other organs like liver, lung, pericardeum brain, skin etc., are also affected through hematogenous spread of trophozoites. *Naegleria* and *Acanthamoeba* are reported to have main site of infection in brain. *Naegleria* generally causing fatal primary amoebic meningoencephalitis (PAME) and *Acanthamoeba* producing chronic granulomatous amoebic encephalitis (GAE).

Infections caused by *E. histolytica* can be cured by the powerful amoebicides available today, but no effective drug has yet been found to cure PAME or GAE. Moreover, the immune responses of cases suffering from PAME or GAE have not been evaluated. No serious attempt has been made to study host parasite relationship or the immunological profile of experimentally infected animals. In view of the scanty information available in the immunology of
pathogenic amoebae, it was considered of interest to study the dynamics of immune responses against amoebiasis caused by three pathogenic amoebae which belong to different genera namely Entamoeba histolytica, Naegleria aerobia (=N. fowleri) and Acanthamoeba culbertsoni.

Pathological studies on free-living amoebae

Pathogenicity of Naerobia (ATCC-73 strain) and A. culbertsoni A-1 strain) was tested using mice of different weights, i.e. 10-15 gm., 15-20 gm. and 20-25 g. It was observed that after intranasal instillation/intracerebral inoculation, the mice up to 20 gms weight show 100% mortality whereas in 20-25 g mice only 50-60% mortality was observed. Pathogenicity of free-living amoebae was also tested in guinea-pigs (wt. 250 g. av.) through intranasal route which showed 50% mortality for both N. aerobia and A. culbertsoni infections.

Axenic cultivation of pathogenic Amoebae:

a. Axenic cultivation of E. histolytica

Axenic cultivation of three pathogenic amoebae was performed. Different commonly used media were compared for growth of axenic E. histolytica. These media were Diamond's monophasic TPS-1 medium (1968), modified TPS-1 medium defined by Dutta and Yadava (1972), Diamonds new medium TYIS-33 and Hi-Medium (Diamond base) which is commercially available. It was inferred from this study that modified TPS-1 medium is best suitable medium for axenic cultivation of E. histolytica, as 38-40 fold growth
of amoebae was achieved in this medium from small inocula. Suitability of other media were in the following order.

Diamond's TPS-1 medium gave 16-66 fold growth
Diamond's TYIS-33 medium gave 20 fold growth
Himedia - gave 3.48 fold growth.

In the present study it was observed that Hi-media supported poor growth but when the Hi-media was replaced by modified TPS-1 medium in culture tubes, the same cultures showed 30-fold growth when harvested after 5 days of replacement. This is again a proof for best suitability of modified TPS-1 medium for axenic cultivation of E.histolytica.

Axenic cultivation of Naegleria aerobia:

Naegleria aerobia was cultured axenically in Nelson's medium (1970). An attempt was made to replace the foetal calf serum by bovine serum in Nelson's medium. It has been concluded from the present work that foetal calf serum can easily be replaced by bovine serum, as both the sera support the growth of N.aerobia.

Axenic cultivation of Acanthamoeba culbertsoni:

A.culbertsoni was cultured axenically in Balamuth's medium (1975). Bulk cultivation in flasks and roux bottles was done for preparation of antigen while culture was maintained in tubes.
Antigen preparation of pathogenic amoebae

Antigen of three pathogenic amoebae was made separately by pooling the amoebae showing exponential growth in cultures. Polled amoebae were washed thrice with chilled physiological saline. Sonicated amoebae were centrifuged and supernatants were collected for soluble antigens. These antigens were also lyophilized and stored at -70°C. The protein contents of three amoebae were estimated by the technique of Lowry et al. (1951). It was found that E. histolytica antigen has protein in range of 0.7 to 1.0 mg/10^6 amoebae, N. aerobia in range of 0.09 to 0.13 mg/10^6 amoebae and A. culbertsoni in range of 0.14 to 0.236 mg/10^6 amoebae. Protein contents of the amoebae depended on growth conditions of amoebae.

Optimal concentrations of three amoebic antigens for application of indirect haemagglutination test, gel diffusion precipitin test, skin test and migration inhibition test were derived.

Induction of specific immune response in guinea-pigs against three pathogenic amoebae

a) Six different doses of three antigen proteins emulsified with 0.2 ml Freund’s complete adjuvant were given to six batches of guinea-pigs via foot pads for standardization of optimum dose to provoke cellular and humoral immune response in experimental animals. Doses were 10 μg, 50 μg, 100 μg, 200 μg, 500 and
2000 µg of amoebic proteins in case of *E. histolytica*. For *N. aerobia* and *A. culbertsoni* also the above doses were used except that 2000 µg dose was replaced by 1000 µg. Two groups of guinea-pigs were used as controls. One of these was injected with 0.2 ml F.C.A. while the other remained as such.

b) 200 µg of *E. histolytica* antigen and 50 µg of *N. aerobia* and *A. culbertsoni* antigens were found to be optimum doses for inducing adequate delayed hypersensitivity in guinea-pigs. Humoral components of immune reaction were also quite expressable with 200 µg and 50 µg sensitizing antigen dose, as indicated by indirect haemagglutination and gel diffusion precipitin test.

c) 10 µg sensitizing dose instead of being too low was able to induce a delayed hypersensitive state which could be observed by *in vivo* testing with skin test. Even humoral components i.e. precipitins and agglutinin antibodies were quite expressable.

d) (2000 µg and 1000 µg) High sensitizing doses of the antigens for all the three amoebae gave poor induction of specific immune response as revealed by skin test, IHA test and GDP test. In skin test only pinpoint erythema without induration could be observed with these doses. In gel diffusion test also precipitins either failed to appear or appeared with very low intensity in sera of guinea pigs sensitized with these doses. This indicates that antigen, if used in higher
doses than the optimum amount, produced immune paralysis of host immune system.

Cellular immune reaction against pathogenic amoebae—

(E. histolytica, N. aerobia, A. culbertsoni)

Two parameters namely— skin test and migration inhibition test were applied for observing the cellular immune reaction of sensitized guinea pigs. Both these tests were positive on 4-8 day, post-sensitization and remained positive throughout the experiment of 60-90 days (60 days for free-living amoebae and 90 days for E. histolytica.) It was inferred from the present study that migration inhibition is a good in vitro correlate of skin test which is performed in vivo.

Humoral immune reaction against pathogenic amoebae (E. histolytica, N. aerobia, A. culbertsoni).

Indirect haemagglutination and gel-diffusion precipitin tests were employed to evaluate the humoral immune status of sensitized guinea pigs. It was inferred from this study that humoral response appears later in the immune system and becomes less expressable as compared to early institution of CMI which is persistent.

Observations of these tests indicated that agglutinins appear prior to the appearance of precipitins which are demonstrable on 15th day post-sensitization. Both of these antibodies persisted throughout the experiment but became lower in their intensities. It can also be con-
cluded from this study that a single injection of very low
dose (200 μg for *E. histolytica* and 50 μg for *N. aerobia*
and *A. culbertsoni*) was capable of producing an immune
response which persisted up to 90 days of experiment.

**Cross reactivity among the three pathogenic amoebae:**

In the present study, it was observed that there
was no cross reactivity among the three pathogenic amoebae
studied namely, *E. histolytica*, *N. aerobia*, and *A. culbertsoni*,
as judged by four tests, i.e., Intradermal test for skin
reaction, macrophage migration inhibition test, Indirect
haemagglutination test and gel-diffusion precipitin test.

**Immune status of guinea pigs infected with live N. aerobia
and A. culbertsoni.**

Although a number of serological tests (viz. Indirect
haemagglutination, precipitin, indirect fluorescent anti-
body, and enzyme linked immunosorbent assay etc.) are
available for the specific diagnosis of *E. histolytica*
infections, virtually no reliable test has been developed
for the early diagnosis of infections due to *Naegleria
aerobia* and *Acanthamoeba culbertsoni*. In the present
work, two parameters of the immune system namely intra-
dermal test for skin reaction (an *in vivo* correlate of
cellular immunity) and indirect haemagglutination (IHA)
test (for humoral immunity) were evaluated in the
experimentally infected animals. It was inferred that
in infected animals CMI does not play any explicit role
in immunity against *N. aerobia* and *A. culbertsoni*, as a very faint skin reaction was observed on day four post infection in infected guinea-pigs. Even this faint reaction could not be elicited from day 6 onwards. In infected mice also a less remarkable footpad reaction (analogous to skin test) was observed. These observations strongly suggest rapid deterioration of specific cellular immune response in the infected animals leading to fatal outcome.

The IHA test became positive a little later (day 8) but remained so through day 30 post-infection with the titers elevated to 1 in 512. Both these tests can be valuable in diagnosis of these infections. Skin test has the advantage of providing an early diagnosis which might be crucial for control of PAME, a rapidly fatal infection.

Specific cellular immune response as shown by persistent, strongly positive skin test was, however, induced in the guinea pigs vaccinated with killed *Naegleria aerobia* and *Acanthamoeba culbertsoni* emulsified in Freund's complete adjuvant.

**Fractionation of Axenic E. histolytica antigen**

Axenic *E. histolytica* antigen was fractionated on Sephadex G-200 column. Three fractions FI, FII and FIII with protein concentrations of 26.58%, 13.42% and 33.8% respectively were achieved. These fractions were characterized by indirect haemagglutination test and...
skin test. It was inferred in the present investigation that fraction I had the highest immunological reactivity in both the tests as compared to other two fractions. Fraction III had the minimal immunological reactivity as very low titers (1.2 and 1.32) of positive sera were obtained when tested with fraction III protein. In skin test also only pinpoint reaction could be obtained on challenge of sensitized guinea pigs with fraction III.

Incidence of *E. histolytica* was evaluated by means of microscopic examination of fresh stool samples. In the recent survey carried out in 1982, it was observed that incidence of *E. histolytica* was 4.5%.

**Sero logical studies on amoebic cases and random population:**

In this study indirect haemagglutination test, radial immuno-diffusion test (for evaluating the change in concentrations of different immunoglobulins) and microzone electrophorosis (for detecting the albumin globulin ratio of sera) were performed. It was inferred that cases with amoebic liver abscess show the highest GMRT, (geometrical mean reciprocal titer) by IHA test. IgG levels of sera from amoebic liver abscess cases were elevated whereas IgM and IgA levels were less effected. Albumin globulin ratio of sera from amoebic liver abscess cases were decreased as compared to those of normals.