2. REVIEW OF LITERATURE

2.1. Historical aspects

Centuries ago, in nearly every civilizing country of the world, dysenteric cases had been recorded in the medical documents of those epochs. Some of these cases might well have been amoebic, but it is impossible to identify them as such, due to the then prevailing ignorance of their aetiological factor. Amoebic dysentery has doubtlessly been much involved through the ages when man seems to have wallowed more in his own excrement then he does at present. There is evidence, nonetheless, that the great Hippocrates was aware of distinction between diarrhoea and dysentery (Martinez Baez, 1975).

Lambl (1860) is frequently given credit for the first observation of amoebae in human stool, but he attached no significance to this finding. Lewis (1870) and Cunningham (1871) both working in India, and later Grassi (1879) and others in Italy, described amoebae in the stools of sick and healthy persons, but it is likely that these were *Entamoeba coli*. The first description of *Entamoeba histolytica* is almost certainly that of Lösch (1875). Lösch gave an excellent description of his observations on the organisms which he found in a patient suffering from chronic diarrhoea. He named it *Amoeba coli*.

Koch (1883) described amoebae from a case of dysentery and in the wall of a liver abscess. Kartulis (1885, 1887, 1904) also observed amoebae in the stools of dysenteric patients, in the pus from liver abscess and in some cases in the brain abscess as well. Kartulis was also able to produce dysentery in cats by rectal injection of faeces from cases of dysentery. Kruse and
Pasquale (1894) induced dysentery in cats by injecting liver pus containing amoebae, into the rectum. Meanwhile Councilman and Lafleur (1891) published their admirable work on intestinal and hepatic amoebiasis. They created the term "amoebic dysentery" and "amoebic abscess of the liver". In 1903, Schaudinn named the aetiological agent of amoebiasis as *Entamoeba histolytica*. Strong (1907) referred to the possibility of the existence of pathogenic and nonpathogenic strains of amoebae in human beings and later Walker and Sellards (1913) adduced proofs in support of this view. Dobell (1919), published his classic treatise, *The Amoebae Living in Man*, in which he gave a most comprehensive, lucid and precise summary of all valuable information on this subject.

2.2. *In vitro* cultivation of *E. histolytica*

(a) With bacterial associates:

Although much information was obtained from clinical and pathological study of human infections with *E. histolytica*, a new horizon of investigations was opened with the discovery of methods for its *in vitro* cultivation. The first reproducible technique of cultivation of *E. histolytica* was developed by Boeck and Drbohlav (1925). Earlier, Cutler (1918) claimed to have cultivated it on blood-bouillon and egg-infusions, but this work could not be confirmed by others, notably Dobell (1919) and Wenyon (1926). Boeck and Drbohlav (1925) used a diphasic medium consisting of either, Locke-Egg-Serum (LES) or Lock-Egg-Albumin (LEA), as solid base, overlaid with inactivated serum or solution of crystalline egg-albumin, without starch. Craig (1926) devised a monophasic medium consisting of inactivated serum in Locke's solution also without starch. Boeck and Drbohlav medium *(B&D medium)*
was further improved by several workers. Dobell and Laidlaw (1926) and practically all subsequent workers, irrespective of whether they modified the earlier culture media or developed new ones, added starch grains to the liquid phase to enable the amoebae to grow and multiply rapidly. The media containing glucose was readily fermented by bacteria, producing acid which made the media unsuitable for the growth of amoebae. Cleveland and Sanders (1930) devised a liver-infusion-agar medium for isolation and maintenance of *E. histolytica*. Jones (1946) prepared a simple modification of Pavlova's medium (1938) consisting of buffered saline-marmite-serum, with rice starch. This medium is easy to prepare and has been used extensively for screening of antiamoebic compounds. Nelson (1947) developed means of preparing media from alcoholic extracts of various tissues or hen's egg yolk. Shaffer and Frye (1948) and Shaffer et al., (1949) developed a clear thioglycollate culture medium preconditioned with a streptobacillus.

The development of *invitro* methods for the cultivation of *E. histolytica* ushered in an entirely new phase of investigation concerning intrinsic and extrinsic factors governing the life of this parasite. Further experiments concentrated on the control of associated bacteria, determination of essential ingredients, optimal pH and oxygen tension for survival and growth of *E. histolytica*.

(b) **Attempts of eliminating bacterial flora from *E. histolytica* cultures.**

Attempts to cultivate *E. histolytica* without the association of any bacterial flora have been made since long. Rees et al. (1941) first attempted to culture
parasitic amoebae without bacterial associates. Rees et al. (1950) obtained amoebic cysts free from bacteria by microisolation, and Faust et al. (1948) did so by use of antibiotics. However, the purified cysts or amoebae were reassociated with a single known bacterium (Rees and Reardon, 1945; Rees et al., 1953) suitable for supporting abundant growth. Shaffer and Frye (1948) and Shaffer et al. (1949) cultivated amoebae with a streptobacillus without any demonstrable bacterial multiplication. Phillips (1950, 1951) and Pan (1960) described procedures for the monoxenic cultivation of E. histolytica with Trypanosoma cruzi and other trypanosomatids. These workers usually substituted a trypanosome, in place of the streptobacillus in the "pre-conditioned" medium used by Shaffer and his colleagues (1948, 1949). This was done by gradually eliminating the bacteria from the amoeba-bacteria culture and simultaneously adding a pure culture of trypanosomes, usually T. cruzi.

From the foregoing review it is evident that the results with various methods employed by different workers for obtaining cultures of E. histolytica with a single bacterium or with trypanosomatids, were unpredictable and unsatisfactory from the point of view of rapid isolation of different strains of the parasite for experimental investigations. Diamond (1961) for the first time grew E. histolytica axenically (strain NIH:200) in a diphasic medium. Later, Diamond (1968a, 1968b) ultimately succeeded in cultivating E. histolytica and E. histolytica-like amoebae monoxenically (with trypanosomatids) and axenically in a monophasic liquid TP-S-1 medium. However, Diamond's (1968b) medium was slightly modified by Dutta and Yadava (1972) in which cysteine HCl concentration was increased from 0.1% to 0.2% and ascorbic acid was omitted from the medium.
Inactivated horse serum was replaced by the inactivated adult buffalo serum. Recently, Diamond et al. (1978) have developed a new medium for axenic cultivation of *E. histolytica*. In the new medium, designated as TYI-S-33, Panmede an essential ingredient of TP-S-1 medium was substituted by yeast extract and the medium was supplemented with iron ammonium citrate, vitamin B<sub>12</sub>, thioctic acid and Tween 80. This medium is believed to give much better growth of amoebae than TP-S-1 medium.

(c) Effect of steroids on growth of *E. histolytica*

Intensive studies have been made to determine the optimal ingredients for the cultivation of *E. histolytica*. Cholesterol has frequently been mentioned as a requirement or stimulant for the growth and multiplication of *E. histolytica* in culture (Snyder and Meloney, 1943; Hansen and Anderson, 1948; Sharma, 1959). Griffin and McCarten (1949) developed more luxuriant amoebic cultures by incorporating a very small amount of oleic acid (20 mg./ml.) in the culture medium in addition to cholesterol, although they found that 60 mg./ml. concentration of the former was toxic and prevented survival of the amoebae. Cedillos et al. (1961) however, demonstrated inhibitory effect of cholesterol, dihydrocholesterol and other steroids on the cultures of *E. histolytica*.

(d) pH and *E. histolytica*

Boeck and Drbohlav (1925) reported that *E. histolytica* grew best in cultures having an initial pH of 7.2 to 7.8. After 24 hours the pH was 6.0 or lower because of the fermentation activity of bacteria. Balamuth and Thompson (1955) reported growth of *E. histolytica* at pH 5.4, 6.0 and 8.5. Studies on axenic
cultures of \(E.\) histolytica have shown that the best growth is obtained when the pH of the medium is between 6.0 and 6.5 (Eaton, 1977; Elsdon-Dew, 1978).

(e) **Effect of redox-potential on \(E.\) histolytica**

Another important aspect of the growth requirements of \(E.\) histolytica is anaerobiosis (Jahn, 1934; Snyder and Meleney, 1943; Chang, 1946; Balamuth and Howard, 1946). It was discovered that preconditioning of the medium with suitable bacterium created strongly negative oxidation reduction (O-R) potential which was favourable for the profuse growth and multiplication of amoebae. Balamuth and Brent (1954) and Balamuth (1963) found that amoebic growth was inhibited by 0.1% oxygen and the amoebae were killed by 2.0% oxygen. Different chemical substances were used to maintain reduced potential. Shaffer et al. (1948) and Griffin and McCarten (1950) employed thioglycollate, while Hansen (1950) used cysteine.

Inspite of the fact that strongly negative O-R potential is necessary for rapid growth of \(E.\) histolytica, Wittner (1968) and Montalvo et al. (1971) claimed that amoebae growing axenically consumed oxygen. Studies on the influence of O-R potential on axenic amoebae have been conducted by Singh et al. (1973, 1974) and Dutta and Yadava (1976). Strongly negative O-R potential was found to be very important for initiating cultures from small number of amoebae (Dutta, 1976).

2.3. **Cryopreservation of \(E.\) histolytica**

Fulton and Smith (1953) first reported the successful cryopreservation of \(E.\) histolytica growing with bacterial associates. Diamond (1964) developed a successful technique of preserving axenic \(E.\) invadens in liquid nitrogen. Successful cryopreservation of
axenic *E. histolytica* strains, KK-9 and 200:NIH, was accomplished by Gordon *et al.* (1969). They could recover viable trophozoites from frozen samples kept at -196°C in liquid nitrogen for up to 5 months. Neal *et al.* (1974) in contrast pointed out distinct differences between results of deep freezing axenic and monoxenic *E. histolytica*. Axenically grown amoebae, strain 200:NIH, suffered more damage and showed poor survival. Recently, Raether and Uphoff (1976) reported a viable recovery of monoxenic and axenic *E. histolytica* after the storage period of 5.1 and 1.9 years respectively in liquid nitrogen.

2.4. Clone culture of *E. histolytica*

Methods of obtaining clones of *E. histolytica* by using a complex micromanipulator have been described (Rees, 1942; Baernstein *et al.* 1957; Rees *et al.* 1960). Isolated trophozoites were usually cultured either in association with *Trypanosoma cruzi* (Phillips, 1950; Phillips and Rees, 1950) or with bacteria in KSre medium (Das, 1972; Rees *et al.* 1960). Recently, Farri (1977, 1978) also described techniques for isolation of clones of *E. histolytica* from bacterial cultures. Gillin and Diamond (1978a, 1978b) developed a new technique of growing axenic *E. histolytica* colonies in agar from clonal amoebae.

2.5. Studies on the virulence of *E. histolytica* strains

Many studies conducted to test the pathogenicity of *E. histolytica* have shown that different strains of this organism have different pathogenic potentials, at least under the environmental situations in which they have been studied.
Faust (1961) gave a critical account of the work done by various workers on the pathogenicity of different strains of *E. histolytica*. The strains from acute cases of amoebic dysentery generally produced intensive ulceration in the experimental animals, while those from carrier cases differed markedly in virulence (Rogava, 1956; Hunminen and Boone, 1957; Neal, 1957). Singh *et al.* (1958) and Schensovich and Soloviev (1963) found that strains from acute cases were highly virulent to rats. The strains from carriers either produced intense ulceration, minimal ulceration or no ulceration. It is rather important in studies on virulence of strains in experimental animals that the trophozoites inoculated intracaecally should be able to survive and colonize the host. Unless this happens the results are apt to be misleading and of little value. Singh *et al.* (1963) and Das and Singh (1965) developed methods for getting 100 percent infection of *E. histolytica* in rats and guinea pigs. However, such infections are commonly restricted to the intestinal tract and are of very limited use for observations on extra-intestinal amoebiasis.

The development of extra-intestinal amoebiasis, especially in the hamster liver has also been attempted by many workers. Three main methods have been developed for the production of amoebic liver abscess. These include the intrahepatic injection of *E. histolytica* trophozoites (Reinertson and Thompson, 1951), intraperitoneal injection of amoebae in the region of umbilicus and liver region (Jarumilinta and Kaeegraith, 1962) and gelatine sponge method (Jarumilinta, 1966).
All these methods involved laparotomy or damage to the liver, involving risk of secondary infection. Dutta (1970) reported the production of hepatic amoebiasis in hamsters by intraperitoneal inoculation of trophozoites of *E. histolytica*, without laparotomy.

(b) Virulence of axenic *E. histolytica*:

The earlier investigations conducted on germ free and monocontaminated guinea pigs suggested that *E. histolytica* is unable to produce lesions in the intestine of these animals in the absence of bacteria (Phillips and Bartgis, 1954; Phillips *et al.*, 1955, 1958, 1972). It was supposed that the bacteria contribute in establishing amoebic infection in the lumen by providing a suitable environment, until the amoebae invade the tissue. There is also evidence that bacteria may have a direct effect on severity and pathogenesis of the several manifestations of the disease (Phillips and Gorstein, 1966). It was believed by some workers earlier that bacteria might supply a factor responsible for virulence of amoebae (Wittner and Rosenbaum, 1970).

In the recent decade several workers have made an attempt to study the pathogenesis of purely axenic cultures of *E. histolytica*. Virulent strains of *E. histolytica* continuously cultivated in axenic conditions lose their invasiveness for laboratory rodents. The loss of virulence is accompanied with concurrent loss of encystment potential (Phillips, 1973). However, there is good evidence that amoeba cultivated for months in axenic condition or in association with *Crithidia* without the development of cysts, did not lose virulence (Diamond *et al.*, 1974a; Bos, 1973).
Diamond et al. (1973) have reviewed the literature on studies of experimental animal infections induced with axenically cultivated *E. histolytica*. They reported that three amoebic strains, cultivated in axenic culture for two to seven years, were able to produce lesions in the hamster liver. Limited trials with jirds showed their susceptibility to hepatic infection. Diamond et al. (1974a) showed a wide variation in virulence of different strains of axenic amoebae. They did not found any apparent correlation between virulence and length of time the amoebae were in axenic culture. Diamond et al. (1974b), showed the clawed jird, *Meriones unguiculatus* to be susceptible to hepatic infection with axenic *E. histolytica*.

Mattern and Keister (1977a, 1977b) developed multiple amoebic lesions by intracerebral inoculation of axenic cultures of *E. histolytica*, in newborn Swiss mice. The degree of pathogenicity was different for different strains. These workers also found newborn hamsters to be highly susceptible to infection by intrahepatic inoculation of axenically cultured *E. histolytica*. Ghadirian and Meirovitch (1976) showed that the I2-106 strain of *E. histolytica* had not lost its virulence after eleven years of axenic cultivation. They could demonstrate the pathogenic potential of axenic amoebae inoculated intrahepatically, intraperitoneally, and intra-caecally in the golden hamsters.

2.6. Restoration of virulence of *E. histolytica*

The question whether a virulent strain of *E. histolytica* retains its virulence in a bacteria free culture remains of topical interest (Diamond et al. 1973). Diminished pathogenicity of *E. histolytica* is observed to a lesser degree in trophozoites maintained
in monoxenic cultures with trypanosomatids (Luttermoser and Phillips, 1952; Phillips and Bartgis, 1954; Bos and Hage, 1975), and in cultures with mixed bacterial flora (Vincent and Neal, 1960). Although the factors responsible for loss of virulence remain obscure, certain manipulations have been shown to restore virulence of attenuated strains.

The possibility of restoring the virulence of bacteria free amoebae by reassociation with intact bacteria is known (Phillips and Bartgis, 1954; Wittner and Rosenbaum, 1970; Bos, 1973), although with some axenized strains this method failed (Diamond et al., 1973). The axenic strain, NIH:200, also remained avirulent in experiments conducted by Phillips (1973). It has been therefore of interest to determine whether organisms associated with *E. histolytica* in vitro, such as *Crithidia* sp. (A-C cultures) affect the virulence of axenic cultures. Michel and Westphal (1970) observed enhanced pathogenesis of monoxenic cultures in the liver of golden hamsters. Raether (1975) also confirmed that (A-C cultures) were more virulent as compared to axenic ones.

The virulence enhancing effect of serial liver passage is also well known, but most experiments are usually not carried out under strictly sterile conditions. Recently Lushbaugh et al. (1978) could develop a significant increase in virulence of the axenically grown amoebae by serial liver passage. The invasiveness of attenuated strains of *E. histolytica* cultivated in bacterial cultures has also been revived by liver passage in hamsters (Neal, 1958; Neal and Vincent, 1956; Vincent and Neal, 1960; DeCarneri, 1958) or by caecal or colic passage (Healy and Gleason, 1966; Chang, 1945; Thompson et al., 1954).
The enhancing effect of cholesterol on the virulence of amoebae is also well documented. Singh (1959) found that strains of *E. histolytica* non-invasive to rat became invasive when the bacterial cultures of amoebae were fed with fine particles of cholesterol. Sharma (1959) also observed that cholesterol made a non-invasive strain virulent to rat. Biagi F. et al. (1962) demonstrated that the production of amoebic liver abscesses occurs with great frequency in hypercholesterolemic guinea pigs. Neal and Stewart (1960), on the contrary, failed to increase the virulence of non-invasive strains by feeding the amoebae with cholesterol. Das and Singh (1965) further studied the effect of cholesterol on the virulence of two attenuated strains of *E. histolytica* and three strains from carrier cases. All the strains became highly virulent to rats when the amoebae were fed in culture with cholesterol. A detailed study carried out by Singh et al. (1971), on the effect of cholesterol, rat caecal and hamster liver passages on virulence of three non-invasive strains, showed a marked increase in their virulence.

Bos and Van de Griend (1977) described a method of restoring virulence and toxicity of axenic *E. histolytica* by feeding the amoebae with cholesterol in TP-S-1 medium. By applying this method, Keervitch and Ghadirian (1978a, 1978b) could restore the lost pathogenicity of two strains of *E. histolytica* grown axenically for 5-6 years, by supplementing the culture medium with cholesterol through a number of transfers. Certain sterols such as progesterone, testosterone and hydrocortisone are also known to favour the production of liver abscess by amoeba (Biagi-F. et al., 1963). The exact metabolic or physiologic mechanism of this
phenomenon is not known, but it is believed that the effect is exerted upon the host or upon the host parasite relationship, rather than directly upon the parasite.

2.7. Mechanism of pathogenicity of cholesterol fed cultures:

The mechanism by which cholesterol triggers the virulence of an avirulent amoeba still remains uncertain. It is an established fact that cholesterol influences the permeability of various cell membranes (Kruyff, et al., 1972), but Bos and Van de Grievd (1978) pointed out that cholesterol does not seem to cause a change in permeability of the amoebic cell membrane. Meerovitch and Ghadirian (1978b) assumed that particulate cholesterol is instrumental in altering the pathogenicity of amoebae, especially Entamoeba trophozoites as these are obligate phagotrophs. They further assumed that cholesterol or its metabolic by-products, upon ingestion and digestion by the amoebae, alter their metabolic processes in such a way that more or stronger proteolytic enzymes are produced and released through the surface active lysosomal mechanisms (Eaton et al., 1970). The following aspects may be considered to play some role in pathogenesis of amoebiasis.

(a) Cytochemical studies on acid phosphatase activity of E. histolytica

Extensive investigations have been made on the lysosomal hydrolases in E. histolytica and other anaerobic amoebae. Acid phosphatase which is the marker enzyme for lysosomes, was demonstrated in the cytoplasm of E. histolytica (Carrera, 1950; Carrera and Changus, 1948; Sharma et al., 1970). Later, ultrastructural cytochemical studies on E. histolytica by Eaton et al. (1969, 1970) and Trevino-Garcia Manzo et al. (1971) showed high acid phosphatase activity in surface
active lysosomes, cytoplasmic or interior lysosomes, food vacuoles and also in the intranuclear bodies. Kairalla et al. (1975) isolated the phagosomes of axenic *E. histolytica* and demonstrated the acid phosphatase activity, on the inner surface of phagosome membranes, on the outer surface of the material surrounding the individual beads and on the outer surface of double unit membrane laminae within the phagosomes. Acid phosphatase activity of *E. histolytica* during interaction with *Toxoplasma gondii* has been investigated by Fastag de Shor et al. (1975) and they showed the presence of this enzyme in the pilopodia of *E. histolytica*, on the edges of vacuoles and also in the diffuse form in cytoplasm. Intense activity was seen in vacuoles containing the toxoplasma.

(b) Biochemical studies on lysosomal enzymes of *E. histolytica*:

Proteolytic enzymes have been extensively investigated as a possible mechanism for invasion of the host cells. Jarumilinta and Maegraith (1969), Biagi and Beltran (1969) and Neal (1971) concluded that it was not possible to distinguish clearly between invasive and non-invasive strains of *E. histolytica* on the basis of patterns of their enzymes. *E. histolytica* has cytotoxic properties as evidenced by its ability to kill leucocytes (Jarumilinta and Kradolfer, 1964; Artigas et al., 1966). Eaton et al. (1970), Chevez et al. (1972), and Knight et al. (1975) reported that toxic effect is contact dependent between the amoebae and leucocytes. Chevez et al. (1975) working on the mechanism of pathogenesis of *E. histolytica* in the hamster liver reported that intense cytolytic activity and varacious phagocytosis are responsible for the pathogenic activity of *E. histolytica*. Recent studies by Lushbaugh et al. (1978a)
and Bos (1979) demonstrated cytopathic effect (CPE) of cell free extracts of amoebae on the cultured monolayer cells. Said-fernandez and Revilla (1978) also reported that total extract from *E. histolytica* trophozoites exerted a dose dependent toxic and lytic effect on RBCs.

(c) **Ultrastructural studies on *E. histolytica***

Eaton *et al.* (1969) described trigger organelle on the surface of the amoebae, and suggested their positive role in cytopathogenic activity of amoebae. Although this mechanism has also been described by Miller *et al.* (1972) and Trevino-Garcia Manzo *et al.* (1974), its presence could not be confirmed by Magaudda *et al.* (1970), El-Hashimi and Pittman (1970), Griffin (1972) and Knight *et al.* (1975). According to Eaton *et al.* (1970) the trigger organelle of *E. histolytica* can exert cytolytic effect on cells in the absence of bacteria, the effect being contact dependent. The toxic products were not liberated into the medium. Pittman *et al.* (1973, 1975), recognised clear vesicles resembling to surface-active lysosomes, in electron micrographs of amoebae. Deas and Miller (1977) observed the presence of blebs like structures in *E. histolytica*. They also observed that surface lysosomes and triggers were plasmalemmal extensions of membrane bound cytotoxic hydrolases.

(d) **Con A induced agglutination of *E. histolytica***

Martinez- Palomo *et al.* (1973) first reported the method of selective agglutination of pathogenic *E. histolytica* strains by con A. Trissl *et al.* (1975) made quantitative study of con A induced agglutination of strains of *E. histolytica*, *E. invadens*, *E. moshkovskii* and *E. histolytica*-like Laredo strain. Das (1977)
carried out a selective agglutination study of pathogenic and non-pathogenic strains of *E. histolytica*. Effect of cholesterol feeding on the selective agglutination of axenic amoebae by con A was further described by Bos and Van de Griend (1977, 1978). The above studies showed that low concentration of con A agglutinate only the virulent strains of amoebae.

2.8. Chemotherapeutic studies on *E. histolytica*:

Even before the *E. histolytica* was discovered, attempts were made to cure the "bloody fluxes" with ipecac bark (during the 17th century). In the succeeding years, the antiamoebic alkaloid, the emetine, was isolated from the roots of *Cephaelis* or *Psychotria ipecacuanha*, a plant native in Brazil, and cultivated in India. Rogers (1912) published an important work on the treatment of amoebiasis under the title of "The salts of emetine". However, in order to discover new amoebicides, in vitro screening against *E. histolytica* was considered an important prerequisite.

(a) In vitro assessment of amoebicidal activity of drugs:

Soon after the successful cultivation of *E. histolytica*, attempts were made to initiate in vitro drug testing. Dobell and Laidlaw (1926) demonstrated high antiamoebic activity of emetine. It soon became apparent, that different investigators obtained different results, because of the variable distribution of drugs in the diphasic media used for drug testing. In 1928, Laidlaw *et al.*, introduced a buffered, all fluid, serum saline medium for in vitro drug testing. Dobell in 1947, further modified the above method by employing *Escherichia coli* in monobacterial culture, and included an indicator of reducing activity in the test medium. Hansen (1949) compared amoebicidal activity of aureomycin...
in cotton stoppered tubes (aerobic) and petroleum sealed tubes (anaerobic). The end point recorded in anaerobic tubes was much higher than the aerobically incubated tubes. Similar results were recorded by Bradin and Hansen (1950) and Nakamura and Anderson (1951). Thompson et al. (1950) conducted antiamoebic tests using anaerobic jars. Balamuth (1952) reported that since bacteria play an important role in adjusting the O-R potentials in amoeba-bacteria cultures, any antibacterial agent by yielding the more positive redox potential, would be indirectly detrimental and possibly lethal to amoebae.

In order to maintain low redox potential necessary for growth of amoebae during drug testing, L-cysteine hydrochloride and thioglycollate have been used in Shaffer-Fry medium (Shaffer et al. 1948). However, a wide variety of live as well as resting (penicillin treated) bacteria decompose cysteine and produce ammonia and hydrogen sulphide, which may be injurious to amoebae. Brackett and Bliznick (1947) pointed out that in order to differentiate between the amoebastatic and amoebicidal effects of a drug one should ascertain whether a "fold increase" occurs between the number of amoebae in the inoculum and the number produced on incubation. To answer this question, Phillips (1951) devised an effective method by using micro-isolation and microculture techniques for testing antiamoebic activity of drugs and antibiotics. Hrenoff and Nakamura (1951) used amoeba trypanosomes cultures to study the effect of fumagillin. Before the axenic culture of E. histolytica became available, appraisal of amoebicidal action of broad spectrum antimicrobial agents was complicated by the difficulty of distinguishing direct versus indirect effect of these agents on E. histolytica. Balamuth (1955) and Balamuth and Thompson (1955)
described in detail the criteria used by different workers, using cultures of *E. histolytica* maintained with bacterial associates or *T. cruzi*, to distinguish between direct Vs. indirect action of test compounds upon *E. histolytica* trophozoites.

Developments in the axenic culture of *E. histolytica* provided opportunity to study more precisely direct action of antiamoebic agents *in vitro*. Diamond and Bartgis (1971) first studied the antiamoebic action of emetine, metronidazole, and paromomycin sulphate in axenic culture of *E. histolytica*. Dutta and Yadava (1972) carried out a detailed study of known antiamoebic drugs. Yadava and Dutta (1973a) further studied the combined action of antiamoebic drugs and antibiotics against axenic *E. histolytica*. In 1973b, these workers compared the amoebicidal action of antibiotics on *E. histolytica* grown axenically and with bacterial or trypanosome associates. Dutta and Yadava (1976) investigated the influence of pH on amoebicidal activity of emetine and dehydroemetine against axenic *E. histolytica*.

The work published on *in vitro* amoebicidal activity of drugs in monophasic media is regarded merely as comparative. Apart from differences in susceptibilities of different strains of *E. histolytica* to drugs, their activity might vary according to the associates of amoeba in culture, the constitution of culture medium, the pH and redox potential of the medium before and after incubation, the temperature and length of incubation, etc. For instance Laidlaw et al. (1928), using emetine obtained end point at 0.2 μg/ml, whereas St. John (1933) reported that emetine at 100 μg/ml may or may not kill *E. histolytica* in 24 hours. Bradner and Rawson (1951) gave end-points at 30 μg./ml. in *E. histolytica- T. cruzi* cultures and 60 μg./ml. in
E. histolytica-mixed bacteria cultures. Similarly the activity of other systemic and luminal amoebicides was reported to vary considerably in different types of cultures.

(b) Studies on the incorporation of radio-active precursors:

Emetine and structurally related compounds which are amoebicides are known to have inhibitory effect on the protein synthesis. Studies conducted by Entner and Grollman (1973) using $^{14}C$-leucine as amino acid precursor, reported emetine and cycloheximide as the potent protein-synthesis inhibitors for axenic E.histolytica. Other amoebicides studied were comparatively less inhibitory. Similar studies by Segura and Lopez-Revilla (1975) taking $^{3}H$-leucine as the precursor revealed inhibitory effect of emetine on the protein synthesis.

(c) In vivo testing of drugs against hepatic amoebiasis

(i) Studies on experimental animals

Although in vitro tests are used in primary screening of potential amoebicides, in vivo tests are necessary for final assessment of new drugs. Thompson and Reinertson (1951) used hamsters for testing action of drugs against amoebic hepatitis. Amoebae were injected directly into the liver, and drugs were assayed four days after infection by their ability to suppress the development of hepatic lesions and to eradicate amoebae from the liver. Emetine and chloroquine - the two drugs well-documented for treating extraintestinal amoebiasis in man - were active in this test. Williams (1959), recorded the extension of survival time as the criteria for in vivo activity of the drugs. Although some attempts have been made to utilize rabbits, guinea pigs, jirds and mice for the production of amoebic hepatitis,
the hamsters have been found most susceptible to amoebic infection. The disease in the hamsters spreads very fast, leading to necrosis of the liver tissue and ultimately resulting in the death of animals within 5-7 days.

Dale and Dobell (1917) first attempted to demonstrate the efficacy of preparations of emetine and several other drugs against experimental amoebiasis in kittens, without success. Clampit (1948) studied the effect of carbarsone, diodoquin and vioform in cats, with partial success. Thompson and Lilligren (1949) chose the dog for their experimental studies against amoebiasis. Anderson et al., (1947, 1949), studied the effect of certain thioderivatives of carbarsone oxide and antibiotics against E. histolytica in macaques. These workers found fumagillin as very effective amoebicide in macaques (1952). Recently, Burden et al. (1979) demonstrated the potent activity of bisamidines of 2,6-diaminoanthraquinone against caecal and hepatic E. histolytica infections in rats and hamsters, respectively.

(ii) Studies in clinical cases of amoebiasis

Although ipecacuanha had long been used for the treatment (Docker, 1858), the first landmark in clinical therapy was the introduction of emetine hydrochloride by Rogers (1912). However, despite its efficacy as a tissue amoebicide the drug frequently failed to eradicate amoebae from the bowel lumen, and, hence recurrence of symptoms was common. In an attempt to achieve great activity within the bowel lumen, oral emetine preparation, emetine bismuth iodide (EBI), was introduced. It yielded high cure rates in intestinal amoebiasis (Kanson-Bahr, 1941; Woodruff, 1959). Further development of numerous luminal amoebicides, chiefly arsenical and quinoline derivatives was ushered shortly after
World War I. In recent years, diloxanide preparations have also become popular.

Conan (1948, 1949) found chloroquine to be effective in amoebic liver abscess. Though chloroquine has achieved wide usage as a less toxic alternative to emetine, it is less active (Harinasuta, 1951; Wilmot et al., 1958). Nevertheless, it is still used as a supplementary medication. Dehydroemetine, a synthetic preparation, was introduced by Brossi et al. (1959) as an advance over emetine because of its more rapid excretion and a more favourable liver-heart concentration ratio. Metronidazole and related drugs have been extensively used for treatment of all forms of amoebiasis.

(iii) Combined use of antiamoebic drugs

The combined use of various luminal and systemic amoebicides has been shown to be adequate in eradicating the luminal and tissue dwelling trophozoites of *E. histolytica*. Powell et al. (1973) demonstrated high efficacy of a combination of metronidazole with diloxanide furoate in curing amoebic liver abscess as well in eliminating intestinal *E. histolytica*. The combined regimen of dehydroemetine and metronidazole was claimed to be more effective for the treatment of amoebic liver abscess (Cervantes et al., 1970). Scragg and Powell (1968) also recommended the combined usage of emetine and dehydroemetine with chloroquine for the treatment of liver abscesses. Tsai (1973) reported improved activity of a combination of emetine in the chloroquine as well as emetine + chloroquine and metronidazole, as compared to emetine alone.