CHAPTER II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

Many comprehensive reviews have appeared on various aspects of *E. histolytica*. Some of the latest in this context can be cited as reviews on the trophozoites of *E. histolytica*\(^30,31\) pathogenesis\(^32,33\) and immunological\(^34,35,36,37\) epidemiological\(^1,38\) and general aspects of the disease\(^39,40\). A concised review on some of the important aspects related to the present investigation upto May, 1992 has been presented.

Among the members of the genus *Entamoeba*, four species have been described from man: One occurring in the buccal cavity (*Entamoeba gingivalis*) and the other in the gut (*E. coli*, *E. hartmanni* and *E. histolytica*). Of these species, only *E. histolytica* is of any pathogenic significance. Apart from medical significance the *E. histolytica* attracted many scientists because of the unique characteristics of their metabolism and their lack of morphological differentiation as compared to eukaryotic cells.

*E. histolytica*, the causative organism of amoebiasis is a protozoan parasite. It exists in two forms: the trophozoite and cystic forms. The cystic form is a motile form of *E. histolytica* which is highly dynamic and pleomorphic ranging from 10 \(\mu\)m to 60 \(\mu\)m in diameter. The scanning electron microscopy of surface shows multiple pseudopodia and occasional filopodia\(^41,42\). In addition, a
prominent surface endocytic activity is reflected varying in number of phagocytic openings and pinocytic vesicles. Trophozoites dwell in the lumen of the large intestine, multiply and invade to produce typical amoebic lesions. The trophozoites subsequently get differentiate into a resistant form i.e. cyst. The cyst is protected by a relatively rigid cell wall which contains chitin. Cysts are passed in faeces and infection is reinitiated by tetra nucleated mature cyst in another human host through contamination of food and water. In countries where amoebiasis is an important health problem, a majority (approximately 90%) of individuals with colonic E. histolytica infections are carriers, while the remainder have invasive intestinal amoebiasis (W.H.O. 1981). The pathogenic effects and clinical features of amoebiasis depend upon dynamic interplay of a number of host and parasite factors. An understanding of both parasite and host factors and the conditions that modulate these factors would facilitate more accurate prediction of the outcome of the infection and also implementation of appropriate control measures.

E. histolytica has the ability to exert a lytic effect on tissues. The typical flask shaped lesions caused by the parasite are also a manifestation of lytic activity of amoebic trophozoites which destroy and digest tissues as they advance towards the healthy surroundings. Despite voluminous literature available on the mechanism of virulence of this parasite, relatively little is known
about the mechanism underlying the disease. Various hypothesis have been put forward to explain the disease process. These are being variation in virulence, production of toxic substances, contact dependent lysis, surface active lysosomal structure and ion influx changes in the target cell membrane etc.

**Variation in virulence**

The knowledge about the factors that determine the virulence of the *E. histolytica* and its conversion sometimes from a harmless commensal to an aggressive invader is rather limited. However, there are divergent opinions regarding the virulence of the parasite. The workers from U.S.A. and East European countries reported amoebic isolates from asymptomatic cyst carriers as invasive and virulent as those from the clinical patients\(^{10,45,46,47,48}\), while those from great Britain have reported such isolates as virulent or comparatively less virulent\(^{49,50,51}\). The other workers\(^ {11,52,53}\), presented evidences to indicate that all the isolates from *E. histolytica* either from asymptomatic cyst passers or from clinical patients were potentially capable of producing caecal infection in experimental animals. The basic criterion to determine virulence in animals employed by almost all workers has been the scoring system devised by Neal (1951).\(^ {54}\) However, it was pointed out that Neal scores failed to correlate with pathology of the caecum. The virulence indices which depend upon the
histopathological lesions, has been suggested to be a better index of virulence.\textsuperscript{55}

In general, the virulence of isolates of \textit{E. histolytica} is known to be lost after prolonged \textit{in vitro} maintenance.\textsuperscript{56,57,58} It has also been observed that inoculation of large number of amoebae growing in association with \textit{Crithidia} or in axenic state can infect and produce disease in liver of golden hamsters, brain of the newly born mice and caecum of guinea pigs.\textsuperscript{59,60,61,62} These investigations indicated the \textit{in vitro} maintenance lead only to alternation of virulence characteristics of the parasite. Certain manipulations have been shown to restore the virulence of attenuated strains in culture; such as incorporation of cholesterol in the culture medium\textsuperscript{62,63} intrahepatic inoculations of hamsters\textsuperscript{64}, inoculation in caecum of rat\textsuperscript{58}, reassociation with bacteria\textsuperscript{20,65}.

The decline in virulence followed by axenisation of amoebae occurs gradually over many weeks and months of maintenance of parasite \textit{in vitro}. It is reasonable to assume that changes in the metabolism during axenization or failure of amoebae to undergo encytment in axenic state could be influencing the pathogenic capacity of the amoebic isolates. However, a large number of trophozoites of isolate HK-9, cultured axenically over a very long period of time produced lesions upon intrahepatic inoculation of hamsters.\textsuperscript{59} As in case of polyxenic amoebic isolates the
capacity of axenic amoebae to produce abscess vary. It has been documented that IP-106 of *E. histolytica* in axenic state inoculated into hamsters intrahepatically and intraperitoneally produced abscess in the liver. Similarly NIH : 200 or cholesterol incorporated NIH : 200 V has also been shown to produce hepatic abscess in hamsters. No correlation however exists between the virulence and the length of time the amoebic isolate were in axenic state. Besides cholesterol which is known to enhance virulence of parasite, administration of ferric ammonium citrate or ferrous gluconate intraperitoneally has been reported to enhance severity and incidence of infection by axenic isolates.

Presence of various viruses have been reported in axenic isolates of *E. histolytica* e.g. the cytoplasmic icosahedral or beeded viruses. Although certain moderate changes in virulence could be demonstrated due to association of viruses with amoebae, no recognizable pattern in virulence change could be suggested owing to limited data available. Clones of amoebae from one isolate would differ in their virulence and it has been suggested that virulence is also subjected to unpredictable variations. Recently, five clones of axenic *E. histolytica* HM1: grown as discrete colonies in semi solid agar medium were adapted in liquid medium and labelled as HM1-C-121, HM1-C-131, HM1-C-143, HM1-C-144 and HM1-C-145. These workers observed that the clone-C-121 was more...
cytotoxic to the cultured baby hamster kidney (BHK) cells while all the other clones were significantly (p <0.001) less cytotoxic as compared to the cloned HM1-C-121 and uncloned E. histolytica (HM1). These investigators felt that axenic E. histolytica culture (HM1) consists of several populations (clones) and cytotoxic potentials would depend upon the population which predominantly multiplies and outgrows other populations in the culture system.

The degree of virulence of cultured E. histolytica varies according to the strain and culture conditions. Certain cell surface properties such as adhesion to epithelial cells, susceptibility to agglutination by con-A, destruction of tissue cultured cell monolayers, phagocytosis, production of soluble cyto-pathogenic substances appear to characterize pathogenic strains.

Attempts have been made to distinguish pathogenic and non-pathogenic isolates by the presence of enzymes in the amoebic extracts. Hyaluronidase activity has been demonstrated in numerous strains of E. histolytica but the enzyme activity was not correlated with pathogenicity. Other enzymes of both pathogenic and non-pathogenic strains include trypsin, pepsin, gelatinase and hydrolytic enzymes of casein, fibrin and haemoglobin but did not show any variations.
It was Sargeaunt and his colleagues, who reported first that invasive amoebic isolates could be differentiated by their electrophoretic isoenzyme patterns on starch gel electrophoresis. The amoebae based on this technique have been classified into 23 zymodemes i.e. population of parasites that possesses like forms of specific enzyme. Basically 4 isoenzymes i.e. L-malate: NADP oxidoreductase (ME), glucose phosphate isomerase (GPI), phosphoglucomutase (PGM) and hexokinase (HK, a misnomer, used for glucokinase) have been used to identify these zymodemes. Of the 23 zymodemes so far identified, nine have been isolated from patients with the clinical evidence of tissue invasiveness. These zymodemes are II, VI, VII, XI, XII, XIV, XIX, XX and XXI. The electrophoretic mobilities of these enzyme systems are reported as arbitrary positions on the starch gel electrophoresis by Sargeaunt and his colleagues. According to these investigators, ME of E. histolytica produces, characteristically a single band always in the same position where as HK always shows double bands in either of two positions. PGM and GPI vary with each zymodeme, a few showing a single band, but most have double bands, with the exception of triple banded pattern in GPI of zymodeme XIX. The bands of PGM and GPI is also constant in any one of 4 positions labelled as alpha, beta, gamma and delta. However in addition recently HK has been shown with three bands in the clones of HMI axenic E.
The virulence judged by the clinical status of the patient correlates with $B$ position of PGM and two fast moving bands of HK on starch gel electrophoresis of either polyxenic or axenic isolates of *E. histolytica*. The fastest moving bands in the polyxenic isolates of amoebae are of bacterial origin. These can be easily distinguished without any difficulty from amoebae associated bands.

The various pathogenic zymodemes appear to have different geographical distributions e.g. zymodeme II, XI, XX and XII are restricted to south Africa, whereas zymodeme XIV appears to be restricted to India and Indian subcontinent and The Great Britain. The non pathogenic zymodeme I, and III also appear to be restricted to India and Indian subcontinent. However, pathogenic zymodemes II with XIV and Zymodeme I and XVI as non-pathogenic zymodemes have been demonstrated from Bangladesh on cellulose acetate electrophoresis. Recently pathogenic zymodemes II and VI and non pathogenic zymodemes I, IV from clones of *E. histolytica* have been demonstrated in India (Hyderabad) also.

Though certain zymodemes have been associated with the diseased clinical status of the host, these might still be present in the asymptomatic carriers. Pathogenic zymodemes have been isolated in 17 out of 303 South African subjects confirmed to be asymptomatic carriers.

These authors used polyacrylamide gel
electrophrosis techniques to differentiate pathogenic and non-pathogenic amoebic isolates. The first author\textsuperscript{17} used horizontal polyacrylamide gel electrophoresis, while the other worker\textsuperscript{86} employed vertical polyacrylamide gel electrophoresis. Both the authors used the Rf values to differentiate the pathogenic and non-pathogenic amoebae. The method of measuring Rf value of the band of lysates of amoebae was different from than the usually employed ones. They measured Rf values with reference to mobilities of axenic band(s) nearest to the application point and they had taken this as unity.

There are contradictory reports regarding the stability of zymodemes of \textit{E. histolytica} isolates. The finding of non-pathogenic zymodeme in conjunction with a pathogenic one in any single host has never been reported.\textsuperscript{22} Moreover, the author\textsuperscript{22} further stressed that alteration of isoenzyme pattern i.e. shifts from non-pathogenic to pathogenic or vice versa was never demonstrated in longitudinal culture studies which were conducted in the presence of viable bacteria in Robinson’s medium. Moreover short term interaction for one hour of axenically grown amoebae with bacteria which markedly enhanced their virulence did not cause any change in pathogenic zymodeme.\textsuperscript{90} These authors suggested that zymodemes are stable and reproducible. Similar reports were made by other authors.\textsuperscript{15}
Recently, the effects of axenisation on the zymodeme status of *E. histolytica* isolates has been demonstrated. The authors studied the stability of the characteristic isoenzyme patterns of axenic cultures of pathogenic and non-pathogenic of *E. histolytica* and after axenisation. The initial isolations were made in either Robinson’s or Diamond’s TYS-GM medium. Cultures were then transferred into Diamond’s TYIS-33 medium containing various combinations of antibiotics to limit and eventually eradicate concomitant bacteria. Cultures were supplemented with *trypanosomes* or *crithidia* until monoxenic culture with either of these flagellates had been established. At the stage where prolific monoxenic growths had been established, the amoebae were gradually weaned off the concomitant organisms into axenic culture. Successful axenic culture was only achieved with pathogenic zymodemes while only monoxenic growth was obtained with non-pathogenic zymodemes. Conversions from pathogenic to non-pathogenic zymodemes and vice versa were not observed in any case. Similar behavior has been demonstrated in a case of non-pathogenic clone MAV-I which was changed to monoxenization. The clone was tested in vitro and in vivo. This clone MAV-I had *F. symbiosum* in the culture medium and belonged to zymodeme I. For comparative studies clone A of MSM1:1MSS belonging to zymodeme II was grown in the presence of *F. symbiosum* for several generations before the experiments, were conducted. Both the clones were
grown under similar conditions separately. Starch gel electrophoresis for isoenzymes was employed. No change in their original isoenzyme pattern was observed for both the clones.

In contrast, it has been reported that isoenzymes are not stable.\textsuperscript{19,20} The authors suggested that bacterial associates appeared to have influence on the electrophoretic mobilities of isoenzymes and virulence of the parasite. They took an amoebic isolate CDC:07:84:4 belonging to non-pathogenic zymodeme I. They converted this isolate into pathogenic zymodeme II by suppressing the growth of associated flora with antibiotics while X-ray irradiated bacterial flora used as a temporary supplement for growth in Diamond's axenic medium. Following the transfer to this culture medium without viable bacteria a gradual increase in virulence of trphozoites was also observed. The zymodeme became pathogenic 37-40 days after the transfer. They offered two explanation for the observed change in zymodeme and virulence:

1. A zymodeme is not a stable inherent property of amoebae.
2. The original isolate consists of two zymodeme populations.

In the same year the second possibility was excluded.\textsuperscript{20} They repeated the experiment with a clone culture of an authentic non-pathogenic strain (SAW 1734 R
The results obtained with clone culture were similar to those obtained in the previous experiment and confirmed that an amoebae possessing a non pathogenic zymodeme could change to pathogenic upon change in growth conditions. Similar results were demonstrated by other workers. They took a non cloned and a cloned isolate of non-pathogenic (zymodeme I) of E. histolytica. These were maintained in Diamond’s TPS-I medium in the presence of Crithidia for 52 days. Irradiated E. coli of a single addition rendered the culture axenic. The analysis of zymodeme pattern established that both isolates had transformed to zymodeme II. The reestablishment of cultures in the presence of microflora from the original cultures resulted in a return of zymodeme I. The other possible way of changing zymodemes of amoebic isolates was suggested as a result of genetic exchange. Later on the author with his colleagues corroborated the above findings by demonstrating biological genetic exchange both in vivo and in vitro experiments. In vivo studies they mixed clone cultures of zymodemes II and XIV. The mixture was injected to rats and they got XX zymodeme (after 3 days) in passage in culture. Similarly, they mixed clones of zymodemes II and XIX, XIV and XIX, separately and injected to rats and they found new zymodemes from the passage of rats (after 3 days). In vitro, they demonstrated that by mixing in culture zymodemes of E. histolytica clones showing a different pattern from the 2 parents could be obtained. Two new
zymodemes have thus been produced, which belong to the third
generation of a family of *E. histolytica* zymodemes
apparently obtained by genetic exchange. For example, they
mixed the clones of zymodeme of II and XIV and got new
electrophoretic mobilities of zymodeme II (after 48 hours),
zymodeme XX (after two weeks). The long standing controversy
on inter conversion of non pathogenic and pathogenic or vice
versa has been recently resolved by conducting the
experiments under strict experimental conditions taking
considerable precautions for variables proposed to be
involved in triggering of conversion. In non of the
experiments change in isoenzyme pattern was observed. 92
Hence they believed that isoenzymes are stable and that all
available evidence, other than the reported coeversion,
points to pathogenic and non pathogenic *E. histolytica* as
being distinct entities. These observations were also
corroborated by using DNA probes for differentiating
pathogenic and non pathogenic *E. histolytica*.

In recent years DNA probes93,94 and monoclonal
antibodies95 also have been used to distinguish between
pathogenic and non pathogenic amoebic isolates. Ribosomal
RNA analysis has been done recently and compared SSU-RNA
genes of pathogenic and non pathogenic amoebic isolates.96
They proved by this method that pathogenic and non
pathogenic forms of *E. histolytica* are genetically distinct.
Antigenic Characterization of *E. histolytica*

There are two sources of antigens i.e. cultured amoebic trophozoites and the amoebic cysts. The homogenate or crude extract of amoebic trophozoites has basic pattern of 14 antigens by immunoelectrophoresis. By two dimensional immunoelectrophoresis 30 precipitable antigens were demonstrated. The sonicated extract of trophozoites has been reported to possess more than 59 antigenic polypeptide by SDS-PAGE. However, by molecular exclusion chromatography, 3-5 peaks have been identified. The haemagglutination, precipitation and complement fixation activities reside in high molecular fraction. The ribonucleic acid (RNA) protein and lysosomal fractions have also been obtained from amoebic trophozoites. The existence of surface associated antigens of amoebic trophozoites have been indicated by treatment of amoebic trophozoites with immune sera, surface labelling of fluorescent labelling immune serum, abrogation of surface labelling after absorption of immune serum with trophozoites and the antibody mediated lysis of trophozoites by complement. By labelling the surface membrane of intact amoebae with $^{125}$I, 12 polypeptides on autoradiography after SDS-PAGE of the cell homogenate have been identified. The molecular weights of nine major surface associated antigens ranged from more than 150,000 to 9,000 daltons. The 12 major polypeptides with molecular
weights have been obtained from axenic \textit{E.histolytica} HK-9 with molecular weight ranging from 12-200 K daltons. These proteins were found to be glycoprotein in nature and tightly bound to cell surface. These surface associated antigens are undoubted important imported in modulating the immune responses and disease processes. Recently these cell associated molecules have been found for target cell lysis. These molecules provide some lead to differentiating amoebae into pathogenic and non pathogenic. Enhanced expression of 29 Kda cell associated amoebic protein has been observed in pathogenic amoebic isolates.\textsuperscript{25} Similarly there exists epitopic differences on 17D K Da cell associated amoebic protein of pathogenic and non pathogenic amoebic isolates.\textsuperscript{26} However a 96 Kda adheive protein has only been found on pathogenic population of amoebae.\textsuperscript{27} Several other surface associated amoebic protein with molecular mass of 210, 160, 190, 70, 50 and 24 Kda has been suggested to be adhesive molecules of amoebic trophozoites which mediated attachement of amoebae to erythrocytes.\textsuperscript{28} A 112 Kda adhesive molecule identified by use of monoclonal antibodies.\textsuperscript{110} It has been reported that antigens in electron dense granules (EDG) from \textit{E.histolytica} to contain protein of 95, 68, 28 Kda. The EDG has been found to be differentially expressed by amoebic isolates for, symptomatic cases and asymptomatic carriers.\textsuperscript{111}

A 112 Kda adhesive has been found absent in non pathogenic amoebic isolate in zymodeme I, while it was present in pathogenic zymodeme II.\textsuperscript{91} The authors further
demonstrated polypeptide analysis of amoebic isolate belonging to pathogenic zymodeme I and amoebic isolate belonging to zymodeme II. They found no polypeptide variations between these two amoebic isolates on SDS PAGE. Similarly four axenic amoebic isolates of \textit{E.histolytica}, which varied in virulence character determined by development of liver abscesses in hamsters were subjected to SDS-PAGE showed most of the antigens were common.\textsuperscript{112}

The cyst antigen of \textit{E.histolytica} has been analysed partially because of non-availability of pure cyst in culture. However the cyst antigen of \textit{E.invadens} cross reactivity with \textit{E.histolytica} antibodies has been demonstrated.\textsuperscript{113}

\textbf{Action of anti-amoebic compounds on \textit{E.histolytica} Isolates}

In spite of the development of powerful amoebicidal drugs during the last 25 years which are claimed to kill the trophic stage of \textit{E.histolytica} at all sites in the body, it has not been possible to eradicate amoebic infections completely from the treated cases. The W.H.O. (1969)\textsuperscript{114} Expert Group has expressed the following opinion regarding the use of luminal amoebicides for treatment of asymptomatic cases of intestinal amoebiasis: "of the many such drugs that are available, none is completely reliable in eradicating amoebae".

In the ideal situation, the development of new amoebicides or more accurately anti-amoebic compounds which
are compounds with activity against *E. histolytica* should initially proceed with study of parasite-specific metabolic pathways and their inhibition, followed by whole parasite *in vitro* studies, experimental *in vivo* and finally clinical trial. There are many antiamoebic compounds being used in clinical practice. Some of the compounds studied in detailed for amoebicidal activity *in vitro* are listed below:

I. **Nitromidazoles**  
   (a) Metronidazole,  
   (b) Panidazole,  
   (c) Tinidazole,  
   (d) Orindazole,  
   (e) Nimorazole.

II. **Antibacterial compounds**  
   (a) Oxytetracycline,  
   (b) Gentamycin,  
   (c) Chlortetracycline,  
   (d) Tetracycline,  
   (e) Paromomycin,  
   (f) Furazolidone.

III. **Miscellaneous compounds**  
   (a) Carbarsone,  
   (b) Chloroquine sulphate,  
   (c) Iodohydroxyquinoline,  
   (d) Sulphonic acid,  
   (e) Iodochlorohydroxyquinoline,  
   (f) Chlorobetamide,  
   (g) Clefamide,  
   (h) Phanquone,  
   (i) Diloxanide,  
   (j) Niridazole,  
   (k) Dehydroemetine,  
   (l) Emetine.

There are several reports on the clinical use of combinations of antiamoebic drugs and antibiotics for the treatment of amoebiasis. Some of the combinations have synergistic action on *E. histolytica*. Combination of diiodohydroxyquinoline and chloroquine sulphate had been studied on axenic cultures. The author reported that in lower concentration of diiodohydroxyquinoline (2.0 µg/ml)
and chloroquine sulphate (50 µg/ml) showed additive action on amoebae. Combined use of these drugs at higher concentration (diiodohydroxy quinoline 4.0 - 8.0 µg/ml and chloroquine sulphate 100-200 µg/ml were also tested but no additive or synergistic action was observed.

Several other combinations of antiamoebic drugs have been recently tested for their additive or synergistic action on *E. histolytica*. The experiments were carried out with a combination of diloxanide furoate and various dilutions of metronidazole and the results suggested some additive action on *E. histolytica* isolates. Similarly a combination of furazolidone and enterovioform showed an additive effect on axenic amoebae. Sensitivity of *E. histolytica* to furazolidone and iodochlrohydroxyquin, separately and combined has been demonstrated. This combination also showed an additive effect on the symptomatic amoebic cases and asymptomatic carriers.

In the recent years, with the zymodeme classification of amoebic isolates the problem of treatment of non pathogenic *E. histolytica* took a new sphere. Some authors of British origin proposed that only those found to be infected with pathogenic zymodemes should be treated while those of U.S.A. recommended all the individuals who harbour *E. histolytica* whether pathogenic or non-pathogenic should be treated. While others stressed upon new drugs for treatment of amoebiasis as there were failures of treatment with metronidazole and other
conventional drugs both in liver and intestinal cases.

Recently, the effect of metronidazole and emetine separately has been studied on a few amoebic isolates belonging to pathogenic or non-pathogenic zymodemes. The investigators observed similar concentrations of both the anti-amoebic compounds required for amoebic isolates of pathogenic or non-pathogenic zymodemes.

Metronidazole, its derivatives and furazolidone have a selective antimicrobial action on a large variety of prokaryotic and eukaryotic pathogens. Metronidazole and this class of compounds are relatively non-toxic with exception of certain neurotoxic effects in a few heavily treated patients and all the side effects of these compounds are transient and reversible on the withdrawal of the drug. Long standing clinical trials ruling out the risk of carcinogenic & mutagenic side effects made it a drug of choice for therapeutic uses and also recognised as being a valuable prophylactic agent against anaerobic infection: for instance, after bowel and genital tract surgery had no serious side effect. Since it is selected for the present study, it is thought appropriate to summarize the mode of action of metronidazole and have a better understanding of the basis of amoebicidal and facilitation of invasive action.
Mode of Action

The mode of microbicidal/amoebicidal action of a drug depends upon (a) entry of drug into target cell (b) its reductive activation (c) the toxic effect of the reduced product or products (d) the release of inactive end products. For instance Fig. (A) represents the scheme of action of metronidazole on an anerobic microorganisms. All the experimental data up to date suggests cytotoxicity is the result of the reduction of nitro group of the drug after the entry into target cell to form a toxic derivative(s), which is short lived and converted into biologically inactive derivative(s). Common characteristic of highly susceptible organisms is their anaerobic nature and having a redox system that is of great significance in metabolism of these pathogens and play no/only minor role in other organisms.

Reductive activation

The nitro group of metronidazole is easily reduced by intact cells and cell free extracts of susceptible anaerobic organisms. The electron necessary for the reduction are of metabolic origin and suppression of metabolism results in diminished activity of metronidazole. The direct donors of electrons are assumed to be a ferredoxin - like electron transport proteins of low redox potential (doxins) and the reduction itself is assumed to be a non-enzymatic chemical reaction of the reduced doxin with the nitro compound. The reduction of metronidazole occurs most likely in one electron step (Fig. B) assumed to lead first to the
formation of a free radical anion, which can be reoxidized by oxygen to the original compound with the production of superoxide, a toxic derivative of oxygen. The end products of the reduction lack cytotoxic activity and represent mostly fragments of the molecules. Of these acetamide and 2-hydroxyethyl oxamic acid were detected for metronidazole. Similarly the end products of furazolidone (Fig. B1) as 3-(4-cyano-2-oxobutylideneamino) -2-oxazolidone and the other 2,3-dihydroxy -3-cyanomethyl -2-hydroxy -5-nitro 1 α , -2-di- (2-oxo-oxazolidin-3yl) iminomethylfuro [2,3-b] furan. have been identified in biological systems. The compounds with lower electron affinity (e.g. the 4-nitro analogue of metronidazole) have no biologic activity since none of the electron donors present in living cells can reduce them. Nitroimidazoles with higher electron affinity are active on anaerobic organisms since they can be reduced easily by the same doxins that are responsible for the reduction of metronidazole. At the same time, they seem to be activated effectively by additional intracellular electron donors present in both anaerobic and other organisms as in the case of E.histolytica isolates.

Mechanism of Cytotoxicity

The mechanisms involved in the cytotoxic action of metronidazole are not understood in any detail. The toxicity is not only due to final products of reduction but to some unstable intermediate products possibly less reduced than
the final products of reduction. Susceptible protozoan cells exposed to metronidazole show morphologic evidence of damage to various components. Higher concentrations of the drug lead to a general disintegration of cells. These data suggest that toxic intermediate attack multiple sites in the target cells.

An interaction with DNA of the reactive metabolite or metabolites of nitroimidazoles is the most widely held explanation of their toxic action on general cells and amoebic cells.\textsuperscript{37} This explanation is supported also by the fact that non-susceptible cells often shown mutations, if exposed to nitroimidazoles. No such interaction was observed with RNA or proteins. In vitro, exposure of trophozoites to metronidazole at a concentration of 50 $\mu$g/ml for short periods (upto 1 hr) had no significant effect on the virulence of amoebae. The presence of metronidazole, however markedly inhibited the stimulation of virulence that occurs after association with bacteria under anaerobic conditions.\textsuperscript{37} Since the uptake of metronidazole molecules by trophozoites is known to be a function of their rate of reduction by ferredoxin, the increased in vitro uptake in the presence of bacteria, was a clear indication of an acceleration in the cell’s electron transport system or an increase in the reducing power of amoebic cell.\textsuperscript{37}

**Resistance to metronidazole**

Lowered susceptibility to metronidazole is not observed frequently in microbial species that usually are highly susceptible to this drug. For further details refer
Another important observation is that strains of microbes like *T. vaginalis* show normal susceptibility in *in vitro* assays performed under anaerobic conditions, but in certain aerobic *in vitro* assays their susceptibility is significantly reduced to that of non-resistant strains.\(^\text{122}\) The results suggest that the inhibitory activity of aerobiasis on metronidazole activation is increased in these strains, possible due to changes in the activating systems, more so in the redox state.\(^\text{122}\)

Successful anaerobic cells must protect themselves from a number of highly reactive species of molecules and radicals generated directly or indirectly during the reduction of oxygen. Products toxic to anaerobic organisms are known to build up rapidly in culture media exposed to air and may actually arise from agents added to the medium to lower the oxidation-reduction potential.\(^\text{37}\) These reactive products may slowly damage cell components such as sulphydryl and metalloprotein groups, cause a rise in the oxidation-reduction potential of the cell and inhibit the electron transport thus consuming the reducing power of the cells. Experiments were carried out under microphilic conditions showed that virulence of amoebic trophozoites, which is known to have no catalase activity or the electron transport chain (cytochromes) increased considerably in the presence of metronidazole. Further, hydrogen peroxide has been shown to be very toxic to amoebae.\(^\text{37}\) *In vitro*, trophozoites of *E. histolytica* exposed to fluxes of oxygen,
hydrogen peroxide and hydroxyl ions (OH) generated enzymically by glucose oxidase and xanthine oxidase reactions respectively demonstrated that the E. histolytica trophozoites were resistant to oxygen, but killed by hydrogen peroxide alone. Hydroxyl group (OH) and oxygen were not required for effective amoebicidal activity. The addition of a peroxidase and halide enhanced amoebic trophozoite killing by hydrogen peroxide because of the very low levels of superoxide dismutase and glutathione peroxidase with the absence of catalase, which are involved in maintaining the reduction potential of E. histolytica amoebic cells.

The clinical enigma on the physiopathology and chemotherapy of E. histolytica could be dealt with an understanding of the metabolism of E. histolytica particularly in finer details as amoebic isolates differ in the virulence potential. The present status reviewed by Reeves and others brought forth some of the unique metabolism of E. histolytica. For instance, the statement of WHO (1981) is quoted: "understanding of nuclear division is inadequate at the microscopic level and completely lacking at molecular level. The role of virus-like cellular inclusions is not clear in amoebac axenically grown, much less so in vitro. Knowledge of the metabolism of carbohydrates, is reasonably complete, but the areas of protein, lipid and nucleic acid metabolism are virtually untouched".
E. histolytica is an anaerobic organism but it can not only thrive/live in the presence of oxygen when available but has a high affinity for oxygen. A study of literature reveal that E. histolytica grown axenically have a complete, but circumscribed, metabolic capability and that, when living in contact with other cells, they have a faculty for expanding their metabolic competence. Though carbohydrate metabolism and membrane components is explored in detail, the other aspects such as nucleic acids, lipids, proteins etc are still in a rudimentary state.

In general, it could be summarized as follows:

The unique nature of carbohydrate metabolism is characterized by having unusual enzyme characteristics, with hardly any metabolic regulations except the thermodynamic explanations of enzyme regulations i.e. the rate of reversible reaction shows as equilibrium is approached. Some of the salient features of metabolic pathway for utilization of glucose as presented by Reeves is depicted in the figures Ca., Cb. The steps are shown, schematically in this figure. The enzymes catalysing steps 3, 7, 10, 11 and 14 through to 19 are not known to occur widely in other eukaryotic organisms. A brief description of the unusual steps in this glycolytic pathway is as follows:-
FIG. D(a): PATHWAYS FOR PYRUVATE FORMATION FROM PEP

10: PYRUVATE PHOSPHATE DIKINASE (2.7.9.1)
11: PEP - CARBOXYPHOSPHOTRANSFERASE (4.1.1.38)
12: MALATE DEHYDROGENASE (1.1.1.37)
13: MALATE DEHYDROGENASE (DECARBOXYLATING) (1.1.1.40)

REGARDING NUMBERS IN DIFFERENT STEPS REFER FIG. C a
FIG. D (b): PATHWAYS FOR PYRUVATE DEGRADATION

14: PYRUVATE SYNTHETASE  (1.2.7.1)
15: ACETYL-COA SYNTHETASE (ADP FORMING)  (6.2.1.13)
16: ACETALDEHYDE DEHYDROGENASE (ACYLATING)  (1.2.1.10)
17: NADP-LINKED ALCOHOL DEHYDROGENASE  (1.1.1.1)
18: NON-ENZYMIC HYDROLYSIS
19: NADPH-DEPENDENT ALCOHOL DEHYDROGENASE  (1.1.1.2)
a: SERINE DEHYDRATASE

REGARDING NUMBERS IN DIFFERENT STEPS REFER FIG. C a.
is being deprived of mitochondria, i.e. having no citric acid cycle or electron transport system, the energy (ATP) for metabolic activity and reducing units (NADPH) for reductive synthesis has to be derived from glycolysis. Thus it could be visualized that the modified (unusual) steps in the glycolytic pathway are mostly met by pyrophosphate (PPi) and the reduced units are produced by alternative pathway of pyruvate metabolism (Figs.Da and Db) as there is no oxidative pathway (Hexosemonophosphate pathway – HMP) is operative. Glucose is degraded to ethanol and carbon dioxide in anaerobic conditions (Fig. Cb) and exposed to oxygen, it gives acetate, ethanol, and carbon dioxide (Fig. Ca).

The glycogen Cycle

The enzymes which catalase glycogen formation and glycogenolysis in *E. histolytica* appear to be those normally found in eukaryotic organisms. Phosphoglucomutase and glucose-1-phosphate uridylyltransferase are cytoplasmic enzymes, but glycogen phosphorylase occurs loosely bound to particulate glycogen which is slowly liberated in homogenates by the action of amylase.

Since amoebae utilize PPI, glycogen cycling in this organism proceed from Glucose -1- phosphate to glycogen and back to glucose -1- phosphate without the expenditure of a highly energy phosphate bond. The overall effect of one cycle is to convert one molecule of orthophosphate and nucleoside triphosphate into a nucleoside diphosphate and
PPi. Glycogen cycling may be the principal source of the elevated intra cellular PPi concentrations found in the organism.

**Electron Transport**

Under anaerobic conditions the electron transport is the route by which electrons liberated at the pyruvate oxidation step (Fig. Db) are transferred to NAD. Under aerobic conditions, by the route by which electrons from reduced flavins transfer to oxygen without the production of peroxide.

The amoebic glycolytic system affords two sites where transhydrogenation occurs between the NAD and NADP to maintain a suitable reduced and oxidized nucleotide balance. One of these sites is at the level of ethanol and acetalaldehyde oxidation and reduction. There are two alcohol dehydrogenases, one linked to NAD the other to NADP. These two enzymes are located in the cytoplasmic compartment and they will function as a system to maintain a thermodynamically balanced concentration of the four enzyme loop between phosphoenol pyruvate and pyruvate. These enzymes are all physiologically reversible. One complete clockwise cycle, pyruvate to pyruvate, around this loop effects the net change:

\[ \text{NAD} + \text{NADPH} + \text{PPi} + \text{AMP} \rightarrow \text{NADH} + \text{NADP} + \text{ATP} \]

The another alternative pathway for the dissimilation of glucose via gluconate Entner - Doudoroff pathway is still
an enigma. The presence of the enzyme glucose-6-phosphate dehydrogenase, the first enzyme in HMP pathway responsible for the production of 6-phosphogluconate is controversial. However, glucose could be fermented via gluconate to pyruvate and glyceraldehyde and to ethanol. The enzymes catalase these steps have been detected in *E.histolytica* isolates.

Thus the foregoing review reveals that functional, biochemical and molecular comparative studies of non-pathogenic and pathogenic *E.histolytica* isolates, as well as detection of specific molecules in pathogenic amoebic isolates absent or altered in comparison to that of non-pathogenic amoebic isolates are of immense interest in the understanding of molecular basis of pathogenicity of this parasite because of its unique morphology and biochemical setup.