The morphology and life history of parasitic protozoa affecting man are fairly well known at the present time as the result of contributions made during the last hundred years or so by pioneers of microscopy and parasitology. But the knowledge about the chemical composition of these parasites and of the various structures included in each is rather meagre. Comparatively little is known relating to the presence of enzymes and their localisation and of the various simple and conjugated proteins, carbohydrates, lipoids, inorganic constituents, pigments, vitamins etc., in the protozoan parasites of man.

The thesis is based on cytochemical investigation of the important species of parasitic protozoa affecting man or belonging to closely similar species and occurring in animals. The cytochemical patterns of the parasites which belong to the classes Mastigophora, Rhizopoda, Sporozoa and Ciliata, in different phases of their life cycle and in different environmental conditions such as the natural habitat and artificial culture medium, have been worked out and the results presented in 13 chapters (Chapters III -- XV). This has been followed by detailed
discussion about the probable physiological significance of the presence of various chemical groups and enzymes in the different organelles or parts of the protozoa (Chapters XVI — XIX). The thesis thus contains an account of the chemical constitution and probable functions of the various structures in a representative group of parasitic protozoa affecting man.

The Chapter I contains a brief account of the development of cytochemical methods since the days of Raspail and the objects of the present investigation. It also indicates the importance of application of histochemical and cytochemical techniques to the problems of mechanism of drug action, drug resistance, host-parasite relationship, and parasitic physiology.

The Chapter II contains an account of the various cytochemical techniques employed in the present investigation. It has been indicated that most of the methods are adaptations of histochemical methods for the study of tissue sections to the study of wet fixed smears. Suitable methods of fixation for each technique has also been mentioned in detail. Two new techniques, viz., ribonuclease digestion of living culture of L. donovani, and of wet preparations for pyronin and methyl green staining individually, have been described.
The question of validity of localisation and specificity of the reactions has been discussed briefly whenever necessary.

As already indicated the cytochemical pattern of the parasites studied has been described in the Chapters III—XV. In most instances the cytochemical features have been described for the first time or are based on the description published earlier. Available literature, if any, on the subject has been reviewed in each instance. The results obtained may be summarised as follows:

The mitochondria were demonstrated in all the protozoa studied for the presence of these structures and in most instances for the first time. These varied in size and shape according to the size of the parasite and were dot-, small or larger rod-shaped structures in the endoplasm. Oxidation-reduction phenomena could be demonstrated in all the parasites except Balantidium coli in which the colour of janus green B persisted for more than 6 hours.

The Golgi complex was demonstrated by supravital staining in all the protozoa studied as neutral red vacuoles which appeared as spheroids under the phase
contrast microscope.

The protoplasm of the protozoa including organs of locomotion when present, has been found to contain simple protein; the endoplasm to contain RNA and the nucleus both DNA and RNA, the distribution and relative proportion of the two nucleic acids varying in different species. It has been observed that in trophic forms of the parasites, those with small amounts of DNA in the nucleus show strong reaction for RNA in the cytoplasm, e.g., *P. berghei*, *E. histolytica*; and those with larger complement of DNA in the nucleus, e.g., *E. coli*, *L. donovani*, show relatively weaker reaction for RNA. The oysts however show strong reaction for ribonucleic acid.

Glycogen could be found in the endoplasm of both the trophic and cystic forms of the amoebas, the genital and intestinal flagellates, and in *E. coli*. It could not be demonstrated in the malaria parasite (except for the presence of polysaccharides in the wall of the oocyst), the leptomonads of *L. donovani*, and in *Trypanosoma equiperdum*. Leishmania form of *L. donovani* however contains polysaccharide. It could be concluded that this lack of glycogen in the forms present in the blood or in culture medium, was due to the ready availability of more readily metabolised monosaccharides in the environment for the protozoa in question.
Mucopolysaccharides could be demonstrated in several parasitic protozoa, e.g., *I. butschlii*, *E. nana*, *G. intestinalis*.

Lipoids were found to be present in the body wall of the parasites studied. Small granules or dots reacting for the presence of lipoids could be found in many of the parasites. These corresponded in distribution to the Golgi complex and the mitochondria.

The enzyme alkaline phosphatase was found to be present in the nuclei of protozoa. In the nuclei the sites of distribution generally corresponded to those of DNA (showing more intense reaction) and RNA (relatively weaker reaction). Chromosomes when visualised showed strong reaction and the spindle distinct but weaker reaction. The enzyme was present in the body surface, the flagella and the cilia, and their kinetoplast or blepharoplast or basal granule, in the undulating membrane, and occasionally it could be demonstrated in the mitochondria. The endoplasm showed the presence of enzyme but the reaction varied in different species and in different stages of life cycle or development. The ectoplasm usually showed weak reaction for alkaline phosphatase. Concentration of alkaline phosphatase could be seen around the food vacuoles in the amoebas, trichomonads and *E. coli*. The walls of certain cytoplasmic
structures, viz., the cytopyge and the contractile vacuoles, showed the presence of this enzyme.

The chromosome number of *L. donovani* was found to be six and it was the same in *T. equiperdum*. The trichomonads also appeared to have six chromosomes.

Variation of pattern of distribution of DNA-containing elements in the nucleus was observed, the karyosome in particular showing variation in shape and size. Apart of the changes recognisable as due to cell division, the variation in the shape and size of the karyosome and in the chromosome pattern in the nucleus was concluded to be indicating activity of these structures and the karyosome was apparently a very active component of the nucleus. As already mentioned, the nuclear distribution of alkaline phosphatase, particularly of areas of strong reaction, showed a close similarity to that of DNA.

The chemical composition and evolution of the chromatoid bars or bodies in entamoebic cysts could be determined. These were found to contain varying proportions of DNA and RNA in addition to simple protein. Alkaline and acid phosphatase were present in these structures. With the development of the cysts, the chromatoid bodies were found to get attenuated in size and ultimately they were found to be arranged in a line
in the inner wall of the cysts and showing reaction for acid phosphatase. These appear to be active elements concerned in turnover of DNA and RNA and ultimately its remnants go to strengthen the cyst wall; these could hardly be regarded as inert store of protein.

The Chapters XVI—XIX deal with the probable functions of the various cytochemical findings in the parasitic protozoa.

The activity of intermitotic chromosomes, particularly of the karyosome and their close association with alkaline phosphatase, has been regarded as evidence of protein synthesis in the nuclei; the contraction and relaxation of the karyosome and the chromatin elements elsewhere in nucleus mediated through alkaline phosphatase, producing globular protein molecules from polypeptide chains. The other possible site of protein synthesis is the cytoplasm where the ultramicroscopic particles, viz., the microsomes, containing RNA and alkaline phosphatase among others, are regarded as autoduplicating and the process is probably under the control of the nucleus.

Localisation of alkaline phosphatase along the cell wall of parasitic protozoa indicates that absorption of food materials or excretion of metabolic
end products through the body wall is mediated through enzyme action. It is possible that osmotic phenomena which according to the Goldacre hypothesis is due to unfolding and folding of protein molecules at the cell surface depend on the enzyme to great extent. The energy required for the folding and unfolding process is probably provided by the action of alkaline phosphatase on certain phosphoric acid esters present in the cell as postulated by Danielli.

There is some similarity of distribution of glycogen and alkaline phosphatase in the parasitic protozoa. This association is likely to be of importance in storage and metabolism of the polysaccharide, the phosphorylation and dephosphorylation being important reactions necessary for both processes.

Mucopolysaccharides probably have protective function in the parasites in which they have been demonstrated. Hyaluronic acid type of polysaccharides have been found in the nuclei of protozoa; it is probable that by virtue of their hydrophilic action, they serve to keep the environments of the chromatinic elements in a fluid state for nuclear division and other physiological activities.
The mitochondria are regarded as containing proteolytic enzymes and they have often been found arranged around food vacuoles. They may thus possess some digestive enzymes in parasitic protozoa. Cytochemical study of the mitochondria of parasitic protozoa have revealed that these structures are of great importance in metabolic process in the parasites. There is evidence that both anaerobic degradation of carbohydrate by the Embden-Meyerhoff sequence and the aerobic processes in Krebs' tricarboxylic cycle take place in the mitochondria of parasitic protozoa.

In view of their adsorbing neutral red, the Golgi complex of the parasitic protozoa have been concluded to possess the power of isolating potentially noxious substances in the cytoplasm.

Alkaline phosphatase has been found to be associated with the organelles of locomotion of certain parasitic protozoa, viz., the basal granules and the cilia, the blepharoplasts and the flagella, the kinetoplast and the flagellum. Strong reaction for alkaline phosphatase has been noted in the 'tail' of certain amoebae opposite to pseudopodial extrusion. It appears probable that the enzyme provides energy for the mechanical work involved in locomotion or movement by its action on phosphoric acid esters present in the structures.