CHAPTER II

ELECTROPHORETIC ANALYSIS OF SOLUBLE PROTEINS
INTRODUCTION

Parasite physiologists are faced with a significant problem of ever increasing number of new genera and species and their correct taxonomic position. The quest for such investigations by conventional taxonomists continues in spite of the sophisticated tools that are available today to ascertain their correct systematic position. The problem becomes more acute when morphologically closely related species, sub-species or strains are to be investigated. It becomes difficult to differentiate closely related species of the parasites purely on morphological criteria. This leads to the search for alternative methods which could provide more consistent and convincing results for the correct identification of the organisms through physiological, immunological and biochemical tools.

It has been widely accepted that the proteins are the first conceivable products of gene activity. Therefore, any variation in proteins would reflect the differences in the genetic constitution of an organism. The sequence of codons on the DNA strand specifies the sequence of amino acids in the protein coded. It has been suggested by Ferguson (1980) that the structural genes which code for specific proteins are governed by regulatory genes which are influenced by the environmental effect leading to the changes in morphology, physiology and behaviour. Though the phenotype of an individual organism may change due to various environmental interactions its genotype otherwise remains constant. However, the structure of specific proteins is a primary phenotype which remains constant throughout the individual's life and about one third of the proteins of many organisms exist in genetically determined polymorphic forms (Ferguson, 1980).

The intrinsic property of proteins being the charged molecules, has
provided an advantage that they can be resolved into different characteristic fractions according to their electrophoretic mobility. Sibley (1960) emphasized that electrophoresis could be used as a powerful analytical tool. Further, Wright (1974) pointed out that the electrophoresis technique could be used as a valid taxonomic tool, however, detailed studies are required on different groups of organisms.

The species specific protein profiles would not only help in the identification but also serve as an excellent source for specific diagnosis particularly in case of endoparasites. Among the helminths such studies have been carried out only on a few species. As evident from the studies in previous chapter, particularly in punched amphistomes, the seasonal biochemical changes are associated with the size and shape of gonads. Such morphometric changes can be influenced by the reproductive and metabolic state of the parasite. Therefore, morphological characters alone cannot be used as standard parameters for the characterization of closely related parasites. In the present study an attempt has been made to study the protein polymorphism as an aid to specific identification of a large number of closely related helminths infecting the gastrointestinal tract of the ruminants. The polyacrylamide gel electrophoresis of proteins in the presence of cationic detergent, sodium dodecyl sulphate would provide an opportunity to investigate the qualitative nature and molecular weight of the parasite protein fractions. Though the mobility of a protein band indicates almost nothing about its function, it provides a foundation and basic framework upon which interpretations of changes in the pattern of protein synthesis resulting from gene regulation, differentiation and many other dynamic processes can be explained (Dunn and Burghes, 1983 a, b). The qualitative differences can be ensured by the electrophoresis but complete characterization of polypeptides can only be possible after the application
of specific antibody binding and/or amino acid sequencing studies.

The variations in the ionic strength of buffers, pH and electric field etc., can alter protein mobilities during electrophoresis, therefore, the gradient slab gel electrophoresis was used so that the different parasite proteins are separated under the identical conditions.
LITERATURE REVIEW

The biochemical approach to the identification of organisms found its way two decades ago when Sibley (1960) emphasized the application of electrophoresis as an analytical tool for solving the taxonomic problems. Since then a number of studies using electrophoresis of proteins have been carried out on a wide variety of vertebrates and invertebrates.

According to Dessauer and Fox (1964), the electrophoresis showed greatest promise at the intra-specific and specific level in vertebrates. Among the invertebrates, insects (Sande and Karcher, 1960, Whittaker and West, 1962), sea cucumber (Manwell and Baker, 1963), snails (Wright and Ross, 1966), and plant parasitic nematodes (Evans, 1971) have been examined by electrophoresis. Polyacrylamide as a supporting medium for the electrophoretic separation of proteins has been widely used and several modifications in the technique have been proposed in recent years (see references in Hames and Rickwood, 1987).

Among the trematode parasites, Schistosoma mansoni and S. japonicum have been used for their protein polymorphism using disc electrophoresis (Sodeman, 1967; Yoshimura, 1968). It was found that the saline extract of schistosome species produce characteristic species specific electrophoretic patterns which may serve as useful tools in the identification of these parasites (Yoshimura, 1968). Using the disc electrophoresis, Ruff et al. (1973) have also identified different strains of S. japonicum on the basis of their geographical distribution. Various species of the genus Paragonimus have been identified on the basis of their characteristic protein patterns, particularly, P. westermani, P. ohirai and P. miyazaki. The morphological features of the adult worms
of these species are difficult to differentiate because the taxonomic characteris-
tics are not well established (Yoshimura, 1969 a, b). The most striking species
specific differences in the polypeptide profile as well as glycoprotein profile
have been found in schistosomes (Aronstein & Strand, 1983).

Among cestodes, the Diphyllobothrium group has been used to study
their protein profile by isoelectric focussing which allows protein separation
on the basis of their isoelectric points. Bylund and Djupsund (1977) have
analysed the soluble proteins of this cestode to delimit various species. Similarly
Kumartilake and Thompson (1979) obtained consistent variation in the protein
profile of different strains of E. granulosus as well as generic differences
in Taenia crassiceps, and Mesocestoides corti. Bursey et al. (1980) have
also applied polyacrylamide gel electrophoresis for the separation of different
species of Taenia.

The electrophoretic studies carried out by Evans (1971) suggest that
protein and enzyme patterns of nematode tissue extracts, have the potential
to be used as stable taxonomic characters. Similar conclusions have been
drawn by Dickson et al., (1970) while using Meloidogynae species. More recently,
using the isoelectric focussing, the soluble protein profile of seven strains
of Trichinella spiralis isolates have been found to exhibit specific variations
in their electrophoretic mobility and isoelectric points (Fukumoto et al., 1987).

However, helminths in general, and amphistomes in particular, which
infect a wide array of ruminants in the Indian sub-continent, have remained
neglected as far as the biochemical approach to identification is concerned.
Although paramphistomes have received considerable attention of taxonomists
and a large portion of the literature is confined to morphological descriptions.
As a result, much confusion has arisen from incoisistencies in the use of
morphological and histological criteria to separate various species which simply look alike. Despite the comprehensive systematic studies (Fischoeder, 1901, 1903; Stiles and Goldberger, 1910; Maplestone 1923; Stunkard, 1929; Fukui, 1929; Nasmark, 1937; Yamaguti, 1958, 1971; Mukherjee and Chauhan, 1965; Gupta and Nakhasi, 1977; Sey, 1979; Eduardo, 1980) no single satisfactory scheme for classifying amphistomes has evolved.

In a preliminary study, Alam and Nizami (1988) identified generic differences in the protein profile of the amphistomes *G. explanatum*, *G. crumenifer* and *P. epiclitum*, and stressed that the biochemical taxonomy can be used for the identification of amphistomes. Therefore, in the present study a large number of helminths inhabiting same or different hosts and habitats as well as closely related genera, species and strains have been used and gradient polyacrylamide SDS-slab gel electrophoresis technique has been applied in order to find out the qualitative similarities and dissimilarities between the parasite proteins.
MATERIALS AND METHODS

To investigate the protein polymorphism in helminths, a number of species inhabiting same or different host and habitat were used. Among the trematodes, *G. explanatum* and *F. gigantica* from the liver and *G. crumenifer*, *F. elongatus*, *O. scoliocoelium*, *P. epielitum* (pink and yellow varieties), *C. calicophorum*, *C. cauliorchis* were collected from buffalo rumen, whereas *G. crumenifer* and *S. globipunctata* were collected from the rumen and intestine of goat respectively.

All the worms were processed separately for the extraction of proteins. The worms were washed with saline premaintained at 37±2 °C, and were homogenized in 0.1 M phosphate buffer, pH 7.4, containing 0.25 M sucrose, in a glass-teflon tissue homogenizer with a motor driven pestle. The homogenate was centrifuged at 1000 × g for 10 min to remove debris and unbroken cells. The clear homogenate was treated with ammonium sulphate for extraction and partial purification of total proteins. Following gradual addition of ammonium sulphate (65% w/v) to the homogenate, constant stirring was done and any change in the pH was checked so that pH remained between 7 and 7.4. At 65% ammonium sulphate concentration, majority of the proteins are precipitated. The precipitated proteins were centrifuged at 6000 × g and the pellet was suspended in 0.1 M Tris-HCl, pH 7.4, washed twice in the same buffer. Finally the pellet was air dried and subsequently the dried proteins were solubilized in sample buffer containing 2.0% sodium dodecyl sulphate (SDS), 5% β-mercaptoethanol, 10% aqueous bromophenol blue in 0.12 M Tris-HCl, pH 6.75 (Laemmli, 1970) and stored in liquid nitrogen for subsequent use.

Protein concentrations of the samples were determined by the method of Bradford (1976) as modified by Spector (1978).
The electrophoresis of proteins in presence of SDS was essentially carried out as described by Laemmli (1970) with some minor modifications. The separating slab gels were prepared by a linear gradient of 7-15% polyacrylamide which was allowed to polymerize at room temperature for about 45 min. Following polymerization it was overlaid with a stacking gel consisting of 4% polyacrylamide. Since the stacking gel shrinks on storage, it was always prepared fresh just before the start of electrophoresis.

**Preparation of solutions:**

**A. Acrylamide**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide</td>
<td>30 gm</td>
</tr>
<tr>
<td>Bis-Acrylamide</td>
<td>0.8 gm</td>
</tr>
</tbody>
</table>

Make up the final volume to 100 ml with double distilled water (DDW).

**B. Stacking gel buffer**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>5.98 gm</td>
</tr>
<tr>
<td>Temed</td>
<td>0.46 ml</td>
</tr>
<tr>
<td>SDS</td>
<td>0.80 gm</td>
</tr>
</tbody>
</table>

Dilute to 80 ml with DDW and adjust pH 6.7 with IN HCl.

**C. Separating gel buffer**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>36.00 gm</td>
</tr>
<tr>
<td>Temed</td>
<td>0.23 ml</td>
</tr>
<tr>
<td>SDS</td>
<td>0.80 gm</td>
</tr>
</tbody>
</table>

Dilute to 80 ml DDW and adjust to pH 8.3 with IN HCl.

**D. Running buffer**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>9.09 gm</td>
</tr>
<tr>
<td>Glycine</td>
<td>43.20 gm</td>
</tr>
<tr>
<td>SDS</td>
<td>3.00 gm</td>
</tr>
</tbody>
</table>
Adjust to pH 8.3 with IN HCl and used 1:2 dilution with DDW.

E. Ammonium per sulphate

Always prepared fresh at 100 mg/ml double distilled water.

The separating gradient 7 to 15% gel and 4% stacking gel were prepared by taking the appropriate volumes of acrylamide, buffer and distilled water as follows:

<table>
<thead>
<tr>
<th>Solution</th>
<th>For 7% gel</th>
<th>For 15% gel</th>
<th>For 4% gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acrylam: (A)</td>
<td>4.67 ml</td>
<td>10.00 ml</td>
<td>2.00 ml</td>
</tr>
<tr>
<td>2. Buffer</td>
<td>3.10 ml (C)</td>
<td>3.10 ml (C)</td>
<td>2.00 ml (B)</td>
</tr>
<tr>
<td>3. DDW</td>
<td>12.23 ml</td>
<td>6.90 ml</td>
<td>10.80 ml</td>
</tr>
<tr>
<td>4. APS (E)</td>
<td>20 µl</td>
<td>20 µl</td>
<td>200 µl</td>
</tr>
</tbody>
</table>

Always freshly prepared ammonium sulphate was added in separating gel solutions and mixed thoroughly before pouring into the chambers of gradient gel maker. The gel solution was poured into the glass plates mould using 1.5 mm thick spacers so that 1.5 mm thick slab gel of linear gradient of acrylamide (7%-15%) is formed. Once the solution is poured, it was carefully overlaid with few drops of distilled water and left for polymerization at room temperature for 45 min. After the polymerization, distilled water was aspirated and then 4% stacking gel was prepared.

Before carrying out electrophoresis all the protein samples in Laemmli's sample buffer were boiled for 5 min in water bath, pre-maintained at 100°C. The gels were pre-run at 5 mA for 30 min after which the power was disconnected. The tank buffer was changed prior to the application of samples containing 80-100 µgm protein of each parasite on the stacking gel. The gel slab thickness was 1.5 mm, with 130 mm total gel length consisting of
10 mm stacking gel and 120 mm gradient separating gel. The electrophoresis was initially carried out at 5 mA per slab until the tracking dye entered the separating gel, and then the current was increased to 15 mA for 8-10 hours in a refrigerator to minimize the heating effect. The standard molecular weight markers were simultaneously run in one lane of the same gel slab. The low molecular weight standards, purchased from Sigma Chemical Co. (U.S.A.), contained the following highly purified proteins: lactalbumin (14.4 Kd), trypsin inhibitor (20.1 Kd), carbonic anhydrase (29 Kd), ovalbumin (43 Kd), bovine albumin (monomer) (66 Kd). The high molecular weight proteins included hexokinase (96 Kd) and \( \beta \)-globulin (150 Kd).

After electrophoresis, the side spacers were removed and the gel casting glass plates were pried apart to obtain the gel. The gels were fixed in methanol: acetic acid: water mixed in the ratio of 45:10:45, for 1 h at room temperature. The gels were then stained for 2 h in 0.25 percent coomassie brilliant blue R-250 (Sigma) prepared in the fixative. The overstained gels were initially destained in the same fixative without dye and finally destained in methanol: acetic acid: double distilled water (5:7:88) until the background was clear and protein bands became distinct. The gels were photographed under trans-illumination with a Canon AE-1 camera using ORWO, 125 ASA, black and white film.

**Densitometry:**

For the quantitation of protein bands, the individual lanes in the gel representing different parasite proteins were precisely cut with a fine razor blade and each lane was scanned on a Systronics densitometer (model 301) using green filter.
UPGMA system of classification:

The data were subjected to UPGMA system of classification as described by Ferguson (1980) and finally the dendrograms were constructed according to the method of Sneath and Sokal (1962).
RESULTS

In order to find out the molecular heterogeneity in various species of helminths, their soluble proteins were subjected to SDS-gradient PAGE and the results reveal that the individual parasite proteins were separated into various fractions according to their electrophoretic mobilities as well as molecular weight. The meristic variations in the polypeptide profile of different parasites are shown in Fig. 2.1.

It was found that the apparent molecular weight of different polypeptides of helminths ranged from 10 Kd to 200 Kd, but the majority of bands were observed between 29 Kd and 150 Kd (Fig. 2.1) when compared with the standard molecular weight markers. The molecular weight of parasite proteins was determined with the help of standard calibration graph (Fig. 2.2) showing the relative mobility of standard molecular weight markers. The high molecular weight protein bands above 200 Kd were inconspicuous and only a few bands were observed in *G. crumenifer* and *F. elongatus* while in other trematodes such molecular weight proteins were not detected.

Since the SDS-PAGE technique was rigorously standardised, the resolution of proteins was quite distinct. Even a cursory glance at the electropherograms of polypeptides and their densitometric scans (Fig. 2.3, 2.4 and 2.5) reveal that in each parasite specific polypeptides were present. The total number and parasite specific protein bands have been summarised in Table 2.1. A total of twenty seven and twenty one fractions of proteins were obtained in the liver trematodes *G. explanatum* and *F. gigantica* respectively. In *G. explanatum*, 40 Kd polypeptide and in *F. gigantica*, 16 and 18 Kd polypeptides were the characteristic proteins. However, a maximum of thirty protein bands with a specific 165 Kd polypeptide were recognised in *G. crumenifer* of buffalo.
FIG. 2.1: Gradient SDS-polyacrylamide gel electrophoresis of proteins of various helminth parasites. S: Standard Molecular Weight (Kd) marker proteins 14–150 Kd. Different lanes representing the protein profile of: *Paramphistomum epiclitum* (yellow: PY; and pink: PP varieties), *Fasciola gigantica* (Pg), *Gastrothylax crumenifer* (Gxb), *G. crumenifer* of goat origin (Gxg), *Fischoederius elongatus* (Fis), *Gigantocotyle explanatum* (Gg), *Orthocoelium scoliocoelium* (Os), *Calicophoron calicophorum* (Ca), *C. cauliorchis* (Cb) and *Stilesia globipunctata* (Sg). Arrow indicates the position of the 27 Kd polypeptide in rumen amphistomes.
FIG. 2.2: The calibration curve of the standard molecular weight markers. a: γ-globulin, b: hexokinase, c: bovine albumin, d: ovalbumin, e: carbonic anhydrase, f: trypsin inhibitor, g: lactalbumin.
Table 2.1: Total number of protein bands and molecular weights of species specific polypeptides of some helminths.

<table>
<thead>
<tr>
<th>Parasites</th>
<th>Host-Habitat</th>
<th>Total Number of protein bands</th>
<th>Parasite specific polypeptide (Mₚ - Kd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. explanatum</td>
<td>Buffalo-liver</td>
<td>27</td>
<td>40</td>
</tr>
<tr>
<td>F. gigantica</td>
<td>Buffalo-liver</td>
<td>21</td>
<td>16, 18</td>
</tr>
<tr>
<td>G. crumenifer</td>
<td>Buffalo-rumen</td>
<td>30</td>
<td>165</td>
</tr>
<tr>
<td>G. crumenifer</td>
<td>Goat-rumen</td>
<td>24</td>
<td>85, 96</td>
</tr>
<tr>
<td>F. elongatus</td>
<td>Buffalo-rumen</td>
<td>22</td>
<td>80</td>
</tr>
<tr>
<td>P. epiclitum (Pink)</td>
<td>Buffalo-rumen</td>
<td>22</td>
<td>60</td>
</tr>
<tr>
<td>P. epiclum (Yellow)</td>
<td>Buffalo-rumen</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>O. scoliocoeleum</td>
<td>Buffalo-rumen</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>C. calicophorum</td>
<td>Buffalo-rumen</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>C. cauliorechis</td>
<td>Buffalo-rumen</td>
<td>23</td>
<td>36</td>
</tr>
<tr>
<td>S. globipunctata</td>
<td>Goat-intestine</td>
<td>18</td>
<td>&gt; 200</td>
</tr>
</tbody>
</table>
FIG. 2.3: Electropherograms with corresponding densitometric scans of SDS-polyacrylamide gels stained with coomassie brilliant blue R-250 for general proteins of, a: Gigantocotyle explanatum, b: Fasciola gigantica, c: Gastrothylax crumenifer, d: G. crumenifer (goat). Arrows indicate the direction of protein migration. Numbers on the scans represent the bands.
FIG. 2.4: Electropherograms with corresponding densitometric scans of SDS-polyacrylamide gels stained with coomassie brilliant blue R-250 for general proteins of, a: *Fischoederius elongatus*, b: *Paramphistomum epiclitum* (Pink variety), c: *P. epiclitum* (Yellow variety), d: *Orthocoelium scoliocoelium*. Numbers on the scans represent the bands.
FIG. 2.5: Electropherograms with corresponding densitometric scans of SDS-polyacrylamide gels stained with coomassie brilliant blue R-250 for general proteins of, a: Calicophoron calicophorum, b: C. caulorchis, c: Stilesia globipunctata. Numbers on the scans represent the bands.
origin. Interestingly, differences were also observed in the polypeptide profile of *G. crumenifer* collected from goat in which two distinct protein bands, 85 Kd and 96 Kd, were detected which were absent in *G. crumenifer* of buffalo origin. Another pouched amphistome *F. elongatus* was found to possess a total of twenty two distinct protein bands, of which 80 Kd protein was specific for this trematode. The two varieties of *P. epiclitum* used in this study also exhibit variation in the relative mobilities of their proteins, total number of bands and their molecular weights. The yellow variety of *P. epiclitum* was found to contain 25 protein fractions with a characteristic 24 Kd protein while in pink variety only 22 fractions were separated and a 60 Kd band was found to be specific for this variety. In *C. calicophorum* and *C. cauliorchis* 18 and 23 protein bands were found with 29 Kd and 36 Kd specific proteins, respectively. Further a protein of 27 Kd was found to be more distinct and common in all the rumen trematodes (Fig. 2.1). On comparing the protein profile of trematodes with that of cestode *S. globipunctata* it was found that the total number of protein bands were less in cestode but the characteristic identification bands were in high molecular weight range (> 200 Kd).

The data obtained from the relative mobilities of protein bands and their densitometric scans were subjected to the UPGMA system of classification using similarity coefficient for different protein profile for the construction of dendrograms. It can be seen from Fig. 2.6 that the over all amphistome group share about 28% similarity. However least similarity, about 12 to 14%, was found when the whole amphistome group was compared with the liver fluke, *F. gigantica*. It is evident from the dendrogram that the two varieties of *P. epiclitum* from buffalo and *G. crumenifer* from two different hosts (buffalo and goat) maintain independent identity with varying degree of similarities. However, the closely related species inhabiting the same
FIG. 2.6: (A) Similarity matrix and (B) Dendrogram constructed from the results obtained in gradient SDS-polyacrylamide gel electrophoresis of: 1: Gigan
cotyle explanatum, 2: Fasciola gigantica, 3: Gastrothylax crumenifer, 4: G. crumenifer (goat), 5: Fischoe
dorius elongatus, 6: Paramphistomum epiclitum (Yellow
variety), 7: P. epiclitum (Pink variety), 8: Orthocoe
lium scolicoelium, 9: Calicophoron calicophorum, 10: C. cauliarchis.
habitat exhibit greater degree of similarity as in case of rumen amphistomes. Whereas *G. explanatum*, infecting buffalo liver was found to share 36% similarity with the rest of the amphistomes of rumen.
DISCUSSION

The results of the present study clearly reveal pronounced variations in the protein profile of different parasites under study. Analysis of dendrograms not only show an overall similarity among the different trematodes, but at the same time it also reflects their differences.

The liver trematodes *F. gigantica* and *G. explanatum* which represent two different families, share only 28% similarity on the basis of their protein profile or in other words it can also be said that these parasites show 72% dissimilarity which reflect their taxonomic distance. However, the dissimilarity narrows down to 43% in the closely related genera, *G. crumenifer* and *F. elongatus* which exhibit 57% similarity. This shows that habitat may not be the only factor which influences the polypeptide profile of these endoparasites but there must be some genetic factors which regulate the similarities and diversities in the closely related taxonomic groups.

The pronounced generic differences in the protein profile of amphistomes have also been reported in a previous study (Alam and Nizami, 1988), where 18, 14 and 12 protein bands were recognized for *G. explanatum*, *G. crumenifer* and *P. epiclitum*, respectively, whereas, in the present study 27 and 30 protein bands have been observed in *G. explanatum* and *G. crumenifer*, respectively. Comparing these two studies the differences could be due to the use of different electrophoretic methods. Since the gradient SDS-polyacrylamide slab gel electrophoresis is more sensitive than the ordinary electrophoresis, more protein bands were resolved in the present study.

The species specific polypeptides with characteristic electrophoretic mobility were also recognized in case of *C. calicophorum* and *C. cauliorchis.*
The dendrogram reveals 74% similarity indicating their taxonomic proximity. However, some distinct intra-species differences in the protein profile were also noticed, particularly in *P. epiclitum*. The pink and yellow varieties of *P. epiclitum*, though morphologically similar but differentiated on the basis of their body colour, which are often found as concurrent infection, reveal about 82% similarities. Such differences and similarities within the same species indicate the possibility of existence of strains in *P. epiclitum*, which may either be of different geographical region or else the definitive host might have harboured the infection from different snail species. Various factors like the inter-state migration of buffaloes, lack of any quarantine measures and unrestricted grazing may contribute in the development of strains as pointed out by Firasat and Nizami (1989). However, further studies are required to ensure these assumptions.

Similarly Osikovski, *et al.* (1978) separated three species of the genus *Paramphistomum*, *P. microbothrium*, *P. cervi* and *P. microbothrioides* on the basis of their protein profile and suggested that protein electrophoresis can serve as a most powerful tool for biochemical taxonomy. Further Yoshimura *et al.* (1970) and Klimenko and Velichko (1972) also used protein profile to correctly identify the morphologically similar parasite species and also to synonymize several controversial species with weak taxonomic descriptions.

Most interesting intra-specific variation was noticed in the case of pouched amphistome, *G. crumenifer* collected from the rumen of two different hosts (buffalo and goat). Besides variation in the *M*<sub>p</sub> of their characteristic polypeptides, differences were also found in the total number and mobility of the protein bands, while showing only 66% similarity as revealed from the dendrograms. The results of the present study as well as the differences in the isozyme profile of malate and lactate dehydrogenase of *G. crumenifer*
isolated from buffalo and goat (Dhandayuthapani et al., 1983) indicate that such variations may be due to the fact that \textit{G. crumenifer} collected from buffalo and goat represent two different strains, however, the host physiology may also exert its influence on the parasite metabolism leading to their biochemical adaptations and variations.

Though the total number of protein bands in \textit{S. globipunctata} were less than the trematodes under study, the polypeptides of the higher molecular weight were more pronounced. It is possible that the absence of alimentary canal and the process of strobilization in cestodes may lead to the differences in their protein polymorphism as compared to trematodes.

It may be possible that the asexual reproduction and enormous fecundity, and niche segregation of helminths might have led to the variation in protein profiles which ultimately reflect variation in their genetic constitution. Kumartilake and Thompson (1979) have also suggested that the genetic constitution of parasites is reflected in their characteristic protein profiles. Further, according to Ferguson (1980) the genetic composition of a population will change gradually over the generations due to mutation, over production of offsprings and natural selection and only those genes are selected which are best adapted to the conditions of the macro- or micro-habitats.

The species or strain specific polypeptides not only represent the biochemical variation among the different parasites, they may also contribute to the possible existence of antigenic variations because Simpson (1986) pointed out that the heterogeneity in the polypeptides may be of considerable importance for the diagnostic and protective measures.

It has been pointed out that the biochemical unity must receive as much attention as biochemical diversity because the undue importance of
any one will give a distorted picture. In the present study, the presence of a polypeptide of 27 Kd, found to be common in all the rumen amphistomes which could be of great importance in the immunodiagnostic and prophylactic measures. The extensive cross reactivity among the helminths has also been reported by Hanna and Hillyer (1984), and Hanna et al. (1984) which may be a consequence of a common polypeptide which might be acting as common antigenic component in these parasites.

Thus it can be concluded that the parasite proteins are not only quantitatively different as revealed in the first chapter, but they also show some qualitative differences. On the basis of earlier reports (Yoshimura, 1968, 1969; Ruff et al., 1973; Bylund and Djupsund, 1977; Osikovski et al., 1978; Kumartilake and Thompson, 1979; and Fukumoto et al., 1987) as well as the results of the present investigation, it can be suggested that protein polymorphism can be used as a valid parameter for generic, specific and intra-specific characterization of helminth parasites. In addition to genetic factors, the biochemical and physiological similarities and differences among the parasites might exist as a result of host specificity and niche segregation which provide an opportunity to understand the physiological adaptations and biochemical evolution.