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Disc electrophoretic comparison between *Schistosoma japonicum* and *S. mansoni* adult worms

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Electrophoretic techniques have proved to be extremely reliable for characterizing and identifying proteins and numerous carbohydrates. A logical application of these techniques is to determine discrete differences in the protein substances found in animals. By determining these differences it is possible to establish biochemical taxonomic "keys" which better establish the relationships existing at the family and genus levels.

Sibley (1960) first described in detail the application of electrophoretic procedures to taxonomic studies on various organisms. He reported the results of an electrophoretic study of the egg-white proteins from 359 species of non-passerine birds.

Ornstein & Davis (1962) described a new electrophoretic technique using a polyacrylamide gel column. Since then, modifications of their procedure have been employed in various fields of science because of its extreme sensitivity, ease of handling, speed and reproducibility. Davis & Lindsay (1964, 1967) and Davis (1967) employed this procedure to aid in differentiating various taxa within the gastropod family Hydrobiidae. As described by Lillevik & Schloemer (1961), Davis *et al.*, (1964, 1967) named the electrophoretic pattern of each species of snail a "fingerprint" and suggested that "fingerprints" could help resolve some taxonomic problems occurring in the Gastropoda. Davis (1967) and Davis & Lindsay (1967) reported that electrophoretic data should be analyzed not only on the basis of patterns and number of separation bands but also on the Rf values of the bands. Recently, Sodeman & Meuwissen (1966) carried out the separation of soluble proteins in aqueous extracts of *Plasmodium berghei* using this electrophoretic procedure.

As far as the author knows, polyacrylamide electrophoresis has never been used to resolve some of taxonomic problems which occur in parasites. Stauber (1954) has reviewed the application of electrophoretic techniques to studies on parasitic diseases. He suggested that "application of paper-strip microelectrophoresis and the even newer immunoelectrophoresis in agar gel to this field will greatly expand our knowledge both of the parasites and of their pathological effects on the host". Quite recently, Sodeman (1967) applied disc electrophoresis as an analytical tool for the investigation of *S. mansoni* saline extract and he suggested that this technique could provide important information equivalent to other immunochemical methods.

The purpose of the present study is to investigate protein component differences between crude saline adult worm extracts of *S. japonicum* and *S. mansoni* by means of polyacrylamide electrophoresis and to determine if this procedure can be used to establish definitive species characteristics.

Materials and Methods

S. japonicum (Yamanashi strain) worms were obtained from infected rabbits and mice while *S. mansoni* (Puerto Rican strain) worms were harvested from mice by the

Perf-O-suction technique (Radke *et al.*, 1961). Worms were washed twice with physiological saline and once with distilled water before lyophilization.

Adult schistosome saline extracts were prepared by homogenizing 100 mg of the dry worm in 2 ml of 0.9% saline solution first with a motar driven tissue grinder and then with a microhomogenizer (50000 rpm/60 seconds) suspended in an ice water bath.

The homogenized *S. japonicum* worms were centrifuged twice at 2000 rpm for 5 minutes at 2°C while the *S. mansoni* worm homogenate was centrifuged twice at 1500 rpm for 5 minutes at 2°C. The supernatant obtained was used in these studies.

The protein content was determined by Biuret reaction. Protein standard (crystalline bovine albumin, College of American Pathologists, Chicago, Illinois) was utilized in preparation of the standard curve for protein determination. Chromometry was done by means of Shimadzu spectrophotometer. The protein contents in the saline extracts of *S. japonicum* and *S. mansoni* are indicated in Table 1.

Table 1 Protein contents (mg/ml) in whole worm extracts of adult *Schistosoma japonicum* and *S. mansoni*

Whole worm extracts	No. of tests	Mean	Standard deviation	Standard error of the mean
S. japonicum	9	37.81	10.00	3.33
S. mansoni	9	30.01	9.60	3.20

Electrophoresis was carried out by the method of Davis (1964) using a 7.5% polyacrylamide lower gel and 5.0% spacer gel with a tris-glycine buffer solution (pH 8.4–8.6). The electrophoretic apparatus used was according to the modification of Davis & Lindsay (1967). A constant current of 5 ma per gel column was maintained until the Bromphenol Blue tracking dye had migrated 32.5 mm.

Each run included a sample of normal human serum as a control to check on the condition of the gels. In order to determine the optimal dilution for the sample gel, the saline extracts were diluted with the spacer gel at ratios of 1 : 3, 1 : 4, 1 : 5, 1 : 7 and 1 : 10. One tenth ml of the mixture was applied to the polymerization which was made above the spacer gel in each gel column. The resulting separation pattern indicated that the 1 : 3–1 : 7 dilutions were optimum and produced distinct separations. The mean protein amounts placed in the 1 : 3, 1 : 5 and 1 : 7 dilutions were 945 μg, 630 μg and 473 μg for *S. japonicum* while those for *S. mansoni* were 750 μg, 500 μg and 375 μg respectively. The protein amounts in the sample gels are high in both species as compared with the amount (about 200 μg of serum protein) recommended by Davis (1964).

From 10–21 runs, each containing 7–9 gel columns were made for respective worm saline extracts. The electrophoresed gel columns were fixed and stained in Amido Schwarz 10 B. After an overnight staining the gels were destained in acetic acid.

Ten to 15 gel columns with protein migrations of 34.5 to 35.5 mm were used to analyze each sample. Results were analyzed in terms of the number of fractions separated, the Rf value of each fraction and the densitometric patterns obtained from tracings (Densicord, Photovolt Corp., New York). Rf values were calculated by measuring the fraction position with a ruler divided into 0.5 mm units.

Densitometric tracings of the dense fractions were distinct, however, the very faint fractions could not always be clearly distinguished. In the very faint fractions, the position was determined by multiplying the Rf value by the length of the chart.

Standard deviations of prominent fractions were calculated and fractions with the greatest deviation for each species were noted. Mean Rf values were used for comparing data according to the methods of Davis & Lindsay (1967), who stated that: "... when average Rf values differed by 0.018

they indicated a significant statistical difference (using standard t test). Where Rf values were only 0.017 mm apart, it was necessary to determine whether or not there was a significant difference." Accordingly, when the Rf difference of a particular fraction was 0.017 or over between two species, it was statistically analyzed by the "t" test.

Results

S. japonicum.

A total of 24 distinct protein fractions were resolved from *S. japonicum* obtained from mice (mouse-SJ), of which 22 were highly reproducible, i.e., found in 100% of the gels (Table 2).

There were two types of variation in the electrophoretic pattern between fractions 16 and 17. Type I - fractions 16 and 17 were located relatively close to each other; this type of pattern was found in approximately 70% of the gels examined. Type II - fraction 17 was shifted slightly towards the front band and usually resulted in the occasional appearance of fraction 16a. This was detected in about 30% of gels. The variations of the electrophoretic patterns due

Table 2 Mean Rf values of the protein fractions separated from adult *Schistosoma japonicum* worms obtained from mice and rabbits

Protein fraction number	*S. japonicum* from mice			*S. japonicum* from rabbits			Significant difference (t test)
	Mean Rf values	Standard deviation	Occurrence frequency of fraction (% run)	Mean Rf values	Standard deviation	Occurrence frequency of fraction (% run)	
1	0.026		100.0	0.025		100.0	
1a	0.046†		100.0	0.046		33.3	
2	—		—	0.062		93.3	
3	0.079		100.0	0.082		100.0	
4	*0.124	0.009	100.0	*0.127	0.010	100.0	
5	0.159		100.0	0.163		100.0	
6	0.190		100.0	0.200		100.0	
7	0.227		100.0	0.235		100.0	
8	*0.289	0.010	100.0	*0.291	0.013	100.0	
9	0.335		100.0	0.336		100.0	
10	0.368		100.0	0.372		100.0	
10a	—		—	0.413		43.8	
11	*0.439	0.012	100.0	*0.438	0.015	100.0	
12	*0.461	0.013	100.0	*0.464	0.014	100.0	
13	0.490		100.0	0.495		100.0	
14	0.524		100.0	0.525		100.0	
15	0.557		100.0	0.561		100.0	
16	0.598		100.0	0.605		100.0	
16a	0.620		30.0	0.635		66.6	
17	*0.632	0.021	100.0	*0.660	0.019	100.0	Yes, $p < 1.0\%$
18	0.674		100.0	0.687		100.0	
19	*0.759	0.014	100.0	*0.763	0.014	100.0	
20	0.848		100.0	0.836		93.3	
21	0.888		100.0	0.886		93.3	
21a	0.957		40.0	0.961		33.3	
22	1.000		100.0	1.000		100.0	

* Prominent protein fraction

† See text

to the migration of the fraction 17 are presented in A-F of Figs. 1-3. The deviation of fraction 17 was the greatest among all the fractions identified from mouse-SJ, the standard deviation being 0.021.

In mouse-SJ, fraction la was always isolated; conversely, fraction 2 was never observed. In addition, fraction 18 may occasionally resolve into 2 fuzzy bands.

Six of the total fractions separated in densitometric tracings appeared sharp and dense. The six prominent peaks were B, D, G, H, I and K as indicated in A-F of Figs. 2 and 3.

Occasionally a very faint fraction with Rf 0.402-0.410 was observed between fractions 10 and 11; this fraction seemed to correspond to fraction 10a identified from the worms of rabbits (see Table 2). Another faint fraction, whose Rf was 0.799-0.816, was located between fraction 19 and 20.

S. japonicum from rabbits (rabbit-SJ) gave a total of 26 distinct separations of protein fractions, of which 22 were highly reproducible, i.e., found in 93.3 to 100% of the gels. Rf values for the fractions are listed

Table 3 Comparison of the protein fractions separated from adult *Schistosoma japonicum* and *S. mansoni* worms obtained from mice

Protein fraction number	*S. japonicum*		*S. mansoni*		
	Mean Rf values	Occurrence frequency of fraction (% run)	Mean Rf values	Standard deviation	Occurrence frequency of fraction (% run)
1	0.026	100.0	0.014		100.0
1a	0.046	100.0	0.044		100.0
3	0.079	100.0	0.066		60.0
3A	—	—	*0.103	0.006	100.0
4	*0.124	100.0	—		—
5	0.159	100.0	0.161		20.0
6	0.190	100.0	0.183		60.0
6A	—	—	*0.210	0.006	100.0
7	0.227	100.0	—		—
8	*0.289	100.0	*0.290	0.009	100.0
9	0.335	100.0	*0.322	0.007	100.0
10	0.368	100.0	*0.359	0.005	100.0
10A	—	—	0.394	0.010	90.0
11	*0.439	100.0	*0.431	0.006	100.0
12	*0.461	100.0	*0.452	0.007	100.0
13	0.490	100.0	0.478		100.0
14	0.524	100.0	0.529		100.0
15	0.557	100.0	0.555		100.0
16	0.598	100.0	—		—
16a	0.620	30.0	0.618		100.0
17	*0.632	100.0	—		—
18	0.674	100.0	*0.667	0.008	100.0
18A	—	—	0.712		100.0
19	*0.759	100.0	0.760		100.0
19A	—	—	0.826		100.0
20	0.848	100.0	—		—
21	0.888	100.0	0.900		100.0
21a	0.957	40.0	0.958		100.0
22	1.000	100.0	1.000		100.0

* Prominent protein fraction

in Table 2.

Variations in electrophoretic pattern, as described in mouse-SJ were also observed. In rabbit-SJ, however, the pattern Type I was isolated from about 45% of gels studied whereas the Type II was observed from 55% of gels. Pattern variations which corresponded to those of mouse-SJ, were presented in A'-F' of Figs. 1-3 respectively. The position of the fraction 17 with Rf 0.660, however, was shifted more to the front than that in mouse-SJ. The standard deviation for this fraction was 0.019 which was the greatest among the fractions recovered from rabbit-SJ.

As described in mouse-SJ, the corresponding six dense fractions showed prominent peaks in densitometric tracings (Figs. 2 & 3., A'-F'). Fraction 10 showed a very prominent peak in the densitometric tracing as seen in D'-F' of Fig. 3. Rarely was a faint fraction resolved at the position of Rf 0.801.

S. mansoni.

A total of 24 protein fractions were resolved from *S. mansoni* (SM) as listed in Table 3. Of these fractions, 21 were highly reproducible, i.e., identified from 90 to 100% of the gels. The electrophoretic pattern of SM was relatively uniform when compared with that of SJ. The region near fraction 6 was usually blurred as shown in A of Figs. 4 & 5. Fraction 5, which had a prominent peak in the densitometric tracings, was sometimes resolved instead of fraction 6 as seen in B of Figs. 4 & 5. Fractions 1, la and 3 in B of Fig. 5 showed sharp peaks but no qualitative difference was observed between A and B.

Eight of the 24 fractions demonstrated dense and prominent peaks in the densitometric tracings; these 8 fractions corresponded to the peaks A, C, D, E, F, G, H and J as seen in Fig. 5.

In SM, the densities of fractions 14, 15, 17, 19, 19A and 21 were usually not high enough to determine their Rf values by direct measurements with a ruler, especially fractions 19A and 21.

Occasionally, a very faint fraction was located at the position of Rf 0.246.

Discussion

Quite recently, Sodeman (1967) reported that a minimum of 22 distinct protein discs were identified from saline extracts of *S. mansoni*. In this study, a total of 24 protein fractions, of which 21-23 were highly reproducible, were resolved from *S. mansoni*.

A comparsion of *S. japonicum* and *S. mansoni* indicated that each schistosome species had a very characteristic electrophoretic pattern. *S. japonicum* was characterized by 6 prominent protein fractions. Conversely, *S. mansoni* was characterized by 8 prominent protein peaks and by the fact that the gel region between fraction 16 and the front band was devoid of high density protein fractions except fraction 18.

When only the electrophoretic patterns were considered, the peaks B, I and K were characteristic for. *S. japonicum* while peaks A, C, E, F and J were characteristic for *S. mansoni* (Figs. 2, 3 & 5).

However, the two species have relatively similar patterns in the midgel region (fractions 8-13). Three characteristic "common" protein components for the two species (peaks D, G and H) were found in this area. These three components may be genus specific. The most characteristic difference in their electrophoretic patterns is present in the gel region between fraction 16 and the front band. *S. japonicum* demonstrates 2 very characteristic protein fractions (peaks I and K). The densities of the components found in this area are high as compared with those of *S. mansoni*. *S. mansoni* indicates only one relatively high density fraction (peak J) in this area.

When the Rf values of individual fractions resolved from *S. japonicum* are compared with those from *S. mansoni*, it was noted that five fractions in the former, and five in the latter were considered to be species specific as seen in Table 3. It

can also be surmised that fraction 19A is more specific for *S. mansoni* because the fraction with Rf 0.799–0.816, supposedly corresponding to 19A, was rarely identified from *S. japonicum*. The above described differences for the respective schistosome parasites may be a useful tool in the identification of the parasites at the level of body components.

In order to determine whether the difference in *S. japonicum* worms recovered from two different hosts is significant or not, their electrophoretic patterns and protein fraction separations were compared. The electrophoretic patterns of *S. japonicum* obtained from two different hosts, indicated very similar separations. However, peaks G and H were generally more sharp and dense in *S. japonicum* from mice. The major difference between *S. japonicum* from two hosts is in fractions la and 2. Fraction 2 was not identified from the worms recovered from mice while fraction la was always isolated. As pointed out by Sodeman & Meuwissen (1966) and Davis & Lindsay (1967), the gel region near the origin is usually packed with many large and slow migrating components so that it is very difficult to interpret these fractions. In addition, such fraction resolutions are frequently influenced by an inevitable technical error, i.e., the minute protein concentration difference of the sample gel.

The most significant difference was recognized in the migrational characteristic of the fraction 17, i.e., its Rf value; the difference between Rf 0.632 (mouse-SJ) and 0.660 (rabbit-SJ) was highly significant ($p<1.0\%$; Table 2). However, comparing this fraction in individual gels, there could be observed a lot of matched gels, with very similar electrophoretic patterns as well as very close Rf values (Table 4, Figs. 1 & 2). Moreover, the density and staining quality of fraction 17 in the respective gels were identical. Thus, these two fractions with statistically significant differences in mean Rf values may actually be the same fraction. This phenomenon resembles the results obtained by Davis & Lindsay (1967), who stated that the differences in 4 populations of *Pomatiopsis lapidaria* were demonstrated by the migrational difference in identifiable components and of new or different fractions. Disadvantages of disc electrophoresis which accompany the advantages of multicomponent separation were already discussed by Davis & Lindsay (1967). Similar disadvantages were also noted in the present studies. In addition, when whole worm extracts are used there is a chance that the host protein components

Table 4 Comparisons of fraction 17 from six electrophoretically matched pairs of *Schistosoma japonicum* adult worms obtained from mice and rabbits

Protein fraction	*S. japonicum* from mice	*S. japonicum* from rabbits	Significant difference
*17	0.613	0.629	
	0.621	0.625	
	0.625	0.629	
	0.668	0.676	
	0.647	0.671	
	0.672	0.671	
Mean Rf	0.641	0.650	No
Mean Rf values for total series†	0.632	0.660	Yes, $p<1.0\%$

* Prominent protein fraction

† See Table 2

may be involved in the separated protein fractions. However, two suppositions can be made; 1) it is unlikely that the extract used for electrophoresis would contain large amounts of host protein components because of significant dilutions of the extracts, 2) when comparing the fractions identified from *S. japonicum* with those from *S. mansoni* obtained from the same host (mouse), the dilution factor should result in "relatively weak fractions" common to both species. These problems require further study.

In conclusion, the present data suggested that disc electrophoresis could be employed as an aid for the identification of parasites. The procedure may be of great advantage in differentiating closely related parasite species which have very similar morphological characteristics or which resemble each other from various biological aspects. The high reproducibility of electrophoretic patterns for each sample makes it possible to compare the results obtained from relatively small amounts of material. This procedure would of course also be useful for large parasites such as *Ascaris lumbricoides*, *Fasciola hepatica* and others.

Summary

To determine the species characteristics of *Schistosoma japonicum* and *S. mansoni* adult worms, the differences in the protein fraction separations as well as their electrophoretic patterns were qualitatively established by disc electrophoresis.

Saline extracts of adult *S. japonicum* worms yielded a minimum of 26 protein fraction separations of which 22–23 were highly reproducible. *S. japonicum* adult worms recovered from mice and rabbits demonstrated identical electrophoretic patterns with at least 6 very prominent fractions. Slight pattern variations were observed in the migration of fraction 17 (characteristic protein fraction for *S. japonicum*). The position of the fraction 17 in *S. japonicum* from rabbits was shifted towards the front when compared with that from worms obtained from mice.

From saline extracts of adult *S. mansoni*, a minimum of 24 protein fractions were identified, of which 21–23 were very reproducible. In the electrophoretic pattern, there were 8 very prominent protein fractions.

A comparison of *S. japonicum* and *S. mansoni* recovered from the same host (mouse) was made. It was found that both *S. japonicum* and *S. mansoni* had characteristic electrophoretic patterns; the former was characterized by the described prominent protein peaks in the densitometric tracing and the fact that the gel region between mid-gel and the front band was composed of high density protein fractions; the latter was characterized by the fact that gel region mentioned above was devoid of high density components and by several prominent protein peaks in its densitometric tracing.

When comparing the individual separations of protein fractions identified from the two schistosome parasites, 5 separations from *S. japonicum* and 5 from *S. mansoni* were found to be distinctly specific for the respective schistosome species.

Of the prominent protein fractions identified from two species, there were 3 very prominent characteristic fractions common to both schistosomes.

The specific protein fractions as well as the characteristic pattern established for each species may be useful tools in the identification of these parasites.

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日本住血吸虫ならびにマンソン住血吸虫成虫のディスク電気泳動像の比較

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Schistosoma japonicum および *S. mansoni* 成虫の種類差を検討するため，これらのディスク電気泳動像ならびに電気泳動によって分離された個々の蛋白分画の比較を行なった．電気泳動実験には凍結乾燥虫体の生理食塩水抽出液を用いた．

S. japonicum からは26本の蛋白分画が分離され，これらの内22～23本は再現性の高いものであった．本種の泳動像には少なくとも6本の著明な蛋白分画が認められ，これらの分画が本種の泳動像を特徴づけている．マウスならびに家兎から得た *S. japonicum* の電気泳動像には有意な差異を認めなかった．

S. mansoni からは24本の蛋白分画が分離され，それらの内21～23本は再現性の高いものであった．その泳動像には少なくとも8本の著明な蛋白分画が常に認められ，これらが本種の特徴的泳動像を形作っている．

マウスから得た *S. japonicum* ならびに *S. mansoni* を比較したところ，各々が特徴的な電気泳動像を示すことが解った．前者の泳動像では，ゲルの中央部と front band との間に認められる蛋白分画の density が高いのに対して，後者のそれではこの部位に認められる蛋白分画の density の低いことが特徴である．

2種の住血吸虫から分離された個々の蛋白分画を比較したところ，*S. japonicum*の 5本，また *S. mansoni* の5本は各々に特異的な分画であろうと考えられた．また2種から分離された11本の著明蛋白分画中3本は2種に共通の特徴的なものであった．

ディスク電気泳動像に見られたこれらの特徴によって *S. japonicum* および *S. mansoni* 成虫を容易に区別することが出来る．

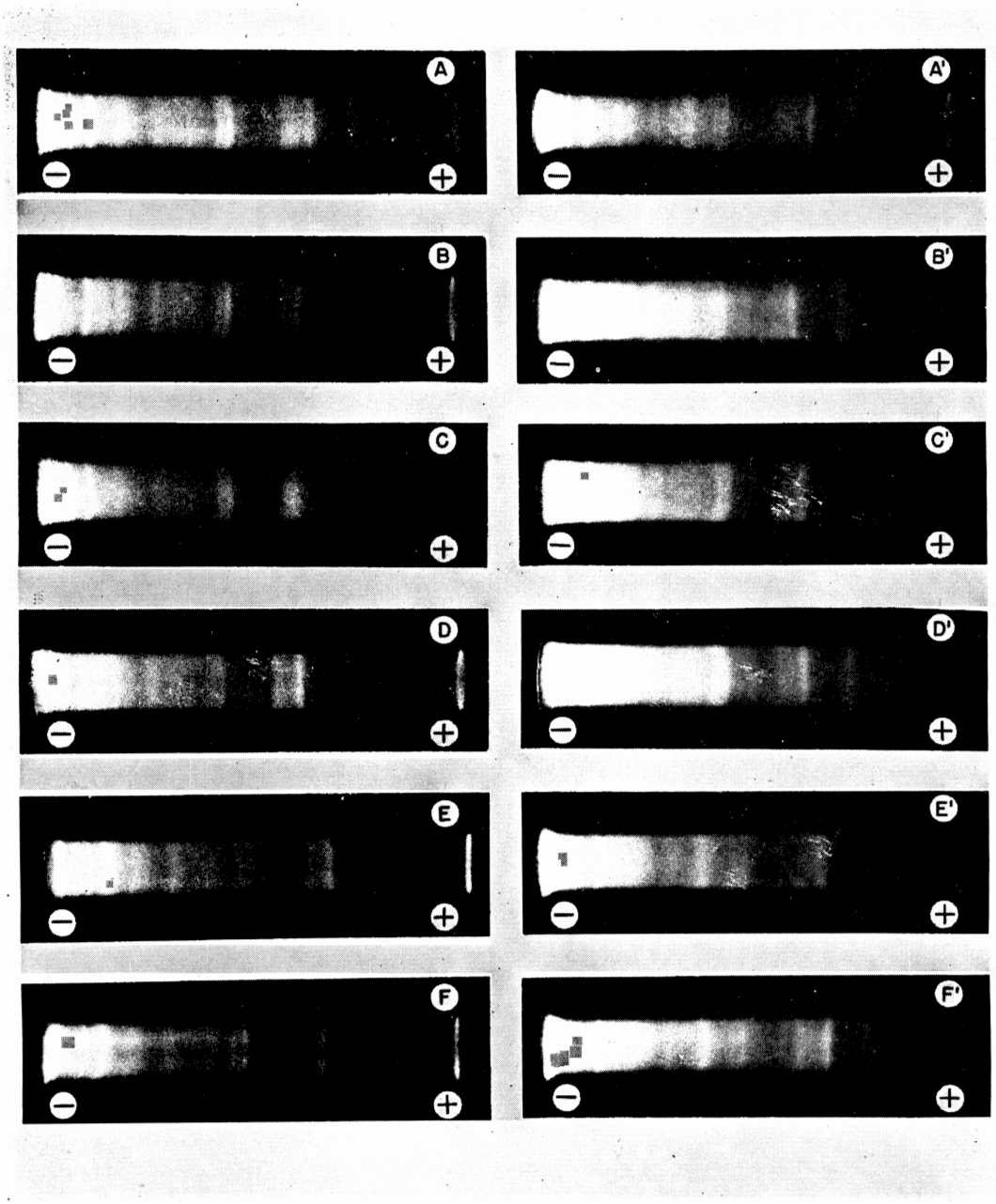
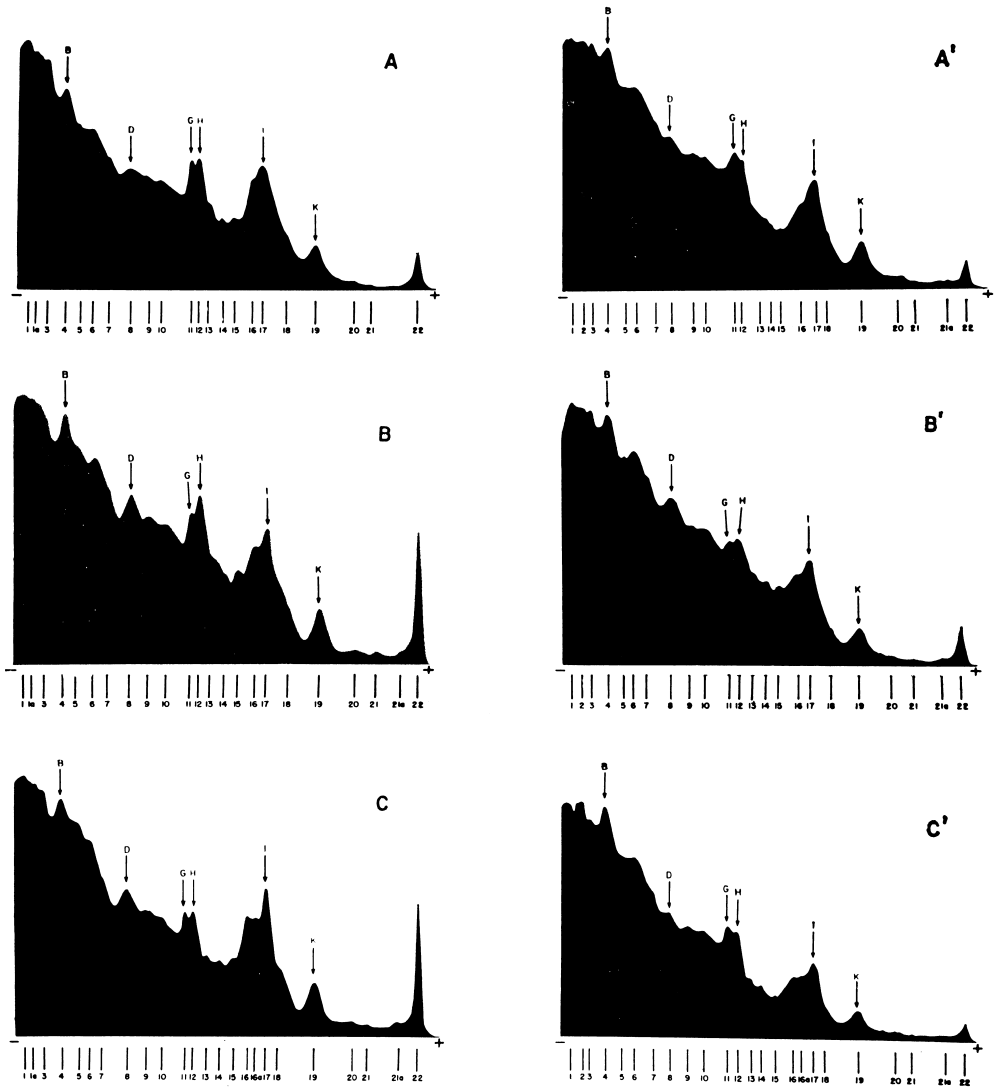


Fig. 1 Variation in the electrophoretic patterns of *Schistosoma japonicum* adult worms obtained from mice and rabbits

A -F : Electrophoretic patterns of *S. japonicum* obtained from mice.

A′-F′: Electrophoretic patterns of *S. japonicum* obtained from rabbits. These patterns closely match A F.

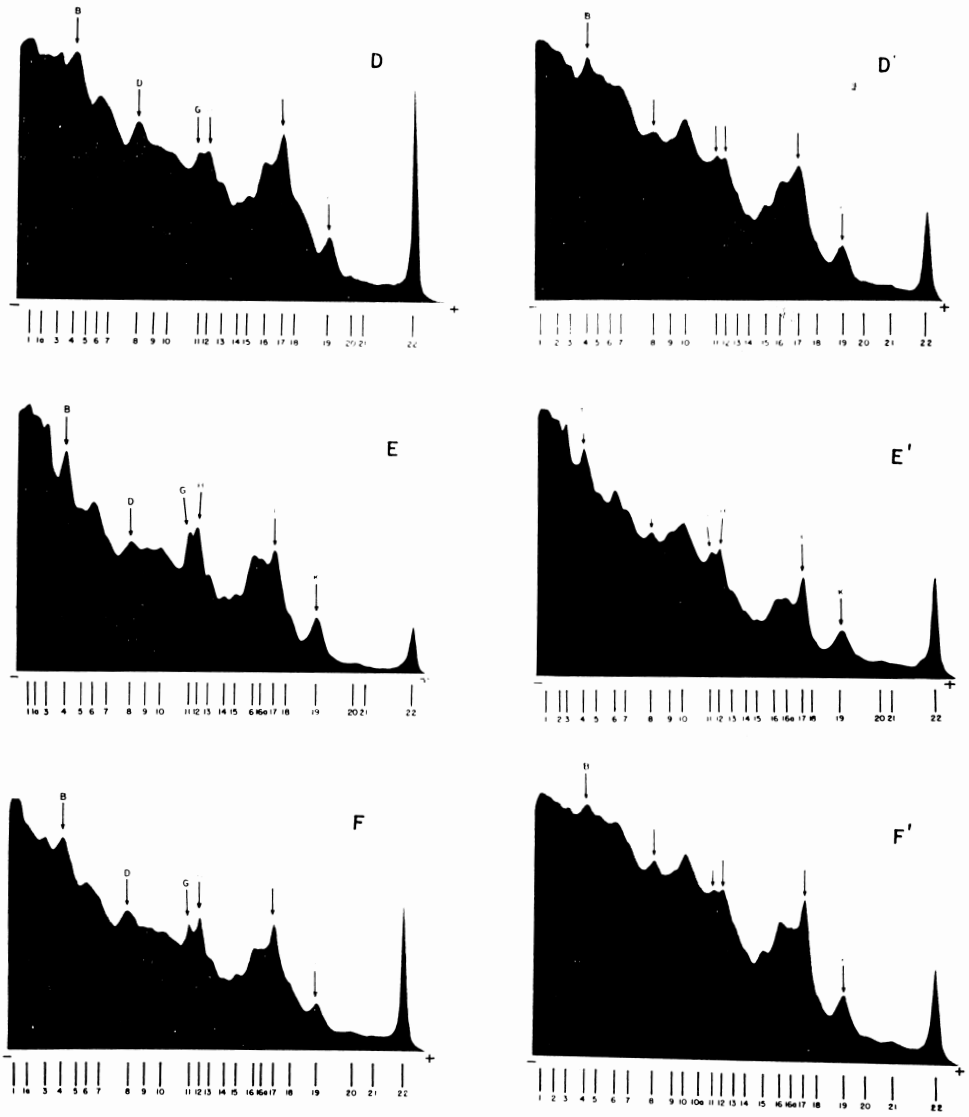


Figs. 2 & 3 Densitometric tracings of electrophoretic patterns from *Schistosoma japonicum* adult worms obtained from mice and rabbits

A F : *S. japonicum* obtained from mice

A′ F′: *S. japonicum* obtained from rabbits

These tracings correspond to the patterns shown in Fig. 1. The fractions are numbered below the densitometric tracings and the most characteristic fractions for the species are lettered.



Figs. 2 & 3 cont.

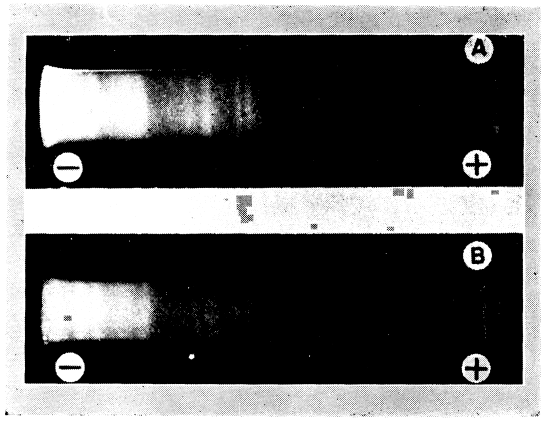
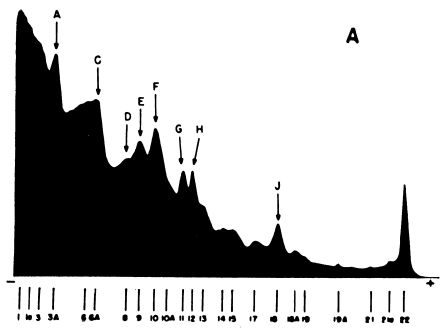


Fig. 4 Electrophoretic patterns of *Schistosoma mansoni* adult worms obtained from mice



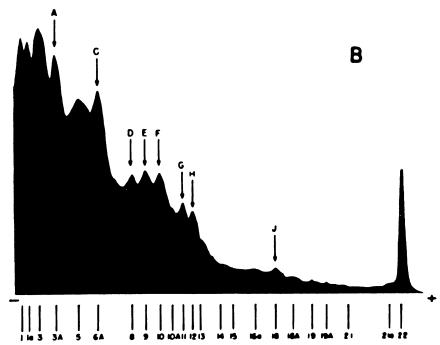


Fig. 5 Densitometric tracings of electrophoretic patterns from *Schistosoma mansoni* adult worms obtained from mice
A–B: Corresponded to A–B shown in Fig. 4