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Effect of Extraction Procedures on Disc Electrophoretic Patterns of *Schistosoma japonicum* Proteins*

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Introduction

Disc electrophoretic studies using polyacrylamide gel columns have been used in several areas of science since the technique was described by Ornstein and Davis (1962). However, the use of these techniques in studies of parasites has been rather limited.

Sodeman and Neuwissen (1966) separated the soluble proteins of an aqueous extract of *Plasmodium berghei* using disc electrophoresis. Sodeman (1967) investigated the components of a saline extract of *Schistosoma mansoni* using the same procedure and suggested that the excellent sensitivity of the technique could provide information equivalent to other refined biochemical separatory techniques. Recently Yoshimura (1969 a, b) and Yoshimura *et al.* (1969) have applied polyacrylamide gel electrophoresis to systematic studies of *Paragonimus westermani*, *P. ohirai*, *P. iloktsuenensis*, and *P. miyazaki*, reporting distinct protein patterns for each of these species. A comparison between pooled worm extracts of *S. mansoni* and *S. japonicum* has also indicated specific differences in electrophoretic profile (Yoshimura, 1968), but to date no studies have been reported on sex-specific proteins of the dioecious *Schistosoma*.

It is known that various chemical and physical conditions may denature a protein or influence its charge orchemical properties, thus changing its migrating pattern. The use of different extraction procedures and types of extracts (e.g., freshsaline extract, Sodeman, 1967; lyophilized extract, Yoshimura, 1968) makes comparison of the findings of different investigators difficult until such time as the effect of extraction procedures on disc electrophoretic patterns is known.

The purposes of this study were: (1) to determine if sex-related differences occur in the electrophoretic pattern of protein extracts of *Schistosoma japonicum*, and (2) to investigate the influence of extraction procedures on these patterns to determine a basis for comparison of different species and strains of parasites.

Materials and Methods

Source of Worms:

Adult *Schistosoma japonicum* (Yamanashi strain) were obtained from Swiss albino mice (406th Medical Laboratory inbred strain) by the Perf-o-suction technique of Radke *et al.* (1961) at 42 days post-infection. The worms were placed in 0.9% saline in a 37°C incubator for 4 hr to clear blood from the digestive system.

Preparation of Extracts:

After clearing, worms were grouped according to sex and homogenized in phosphate buffered saline (PBS), pH 6.9, at a ratio of 200 worms per ml PBS using a motor-driven

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tissue grinder. This homogenate was treated in several different ways. In one study it was immediately centrifuged at 2,680 g for 10 min. The protein content of the extract was then determined using the technique of Daughaday *et al.* (1952) as modified by Rutkowski *et al.* (1970). This extract was used immediately for disc electrophoretic runs and was designated as fresh extract.

In all other studies the original homogenate was sonicated for 4 min at a setting of 30 using a Biosonic ultramicro probe (Bronwill Scientific, Rochester, N. Y.). The resulting homogenate was subjected to the following treatments: (1) The sonicated homogenate was centrifuged and the protein content determined as above. This extract was used immediately for electrophoretic runs and designated fresh-sonicated extract. (2) The extract was centrifuged, protein content determined, and lyophilized. The lyophilized extract was later reconstituted to the original volume using distilled water, centrifuged, and the protein content redetermined. This was designated sonicated-lyophilized extract. (3) The sonicated extract was refrigerated at 4°C and used the following day. This was termed refrigerated extract. (4) The original sonicated homogenate was incubated for 1 hr at 37°C prior to centrifugation and the protein content determined. This extract was referred to as incubated extract.

Electrophoretic Procedure :

Electrophoresis was carried out using the polyacrylamide technique of Davis (1964) with the modifications employed by Davis and Lindsay (1967), Davis (1969), and Davis and Takada (1969). A constant current of 5 mA per tube was employed. For each type of extract studied, 10–15 runs were made, each run consisting of 4–9 tubes. Additional tubes containing normal human serum were included in each run as a control to check the quality of chemicals and electrophoretic separation. If the control tubes did not show excellent separation of the human serum after electrophoresis and staining, then all tubes in that run were discarded.

Extracts were diluted with spacer gel to give sample gels containing the appropriate amounts of protein. All male extracts, female fresh-sonicated, and female sonicated-lyophilized extracts resulted in good separation of bands using 200 μg of protein per tube. Fresh, refrigerated, and incubated female extracts were increased to 300 μg per tube in order to produce relatively sharp bands. For quantitative studies, gels containing no protein and designated as blank gels were prepared in the same manner as gels containing extrect; however, the sample gel consisted of spacer gel diluted with PBS at a ratio of 3 : 1 or 4 : 1. Electrophoresis, staining, and destaining of blank gels were accomplished using the same procedure as for gels containing extract. All gels were stained in 1 % aniline blue black for 1 hr and destained electrically in 10 % acetic acid. Gels were stored in 8 % acetic acid in glass tubes.

For each extract, 8–10 gels, each from a different run, were selected for densitometric analysis. Densitometric tracings were made using a Densicord Densitometer, Model 542 (Photovolt Corporation, N. Y.) using a quasi-logarithmic setting of D-1. An integrating recorder attached to the densitometer marked off density units beneath the tracing.

Analysis of Data :

The separated components of each extract were analyzed in terms of overall densitometric profile, Rf values, and quantitative comparisons of major peaks. The calculation of the relative distribution of protein in the sample, spacer, and lower gels with each extraction procedure was done by first determining the total number of densitometric units under each portion of the densitometric curve. This number of units was corrected by subtracting the number of standard units which occurred in the length of each portion of the trace. These were calculated from a units per length ratio which was obtained from a tracing which correlated the integrating recorder with chart speed without moving the gel column. The number of density units found in the blank gels was determined and corrected in a similar manner. The blank units were then sub-

tracted from the corrected density units in the corresponding layers of the experimental gels and the resulting units used to calculate the percent distribution of protein in each of the three layers.

Rf values were calculated as described by Davis and Lindsay (1967). When fractions were too faint to locate their position on the densitometric tracing, Rf values were determined from measurement of the original gel column. The total experimental error in determining any single Rf value was calculated as 0.016, thus agreeing with the error reported by Davis and Lindsay (1967), where 0.014 was attributed to variation in measurement. Accordingly, fractions in gels which differed by Rf of 0.016 or less were not considered significantly different.

Quantitative comparisons of the protein in major bands were made for all male extracts and for fresh-sonicated and sonicated-lyophilized female extracts. Major bands (or major peaks) were defined as those prominent peaks which were easily identified in both gels and densitometric tracings and which occurred in all gels of a particular extract. A comparison of the heights of major peaks gives an indication of the relative amounts of proteins in the band since a protein sample of 220 μg was used in each gel column, and because the height of a peak on a densitometric tracing is dependent on the density of the band. The heights of the peaks were measured in percent of 1 O.D. (optical density), where 1 O.D. equalled no transmittal of light through the gel. Since 300 μg were used in each sample with fresh, refrigerated, and incubated female extracts, the relative heights of the densitometric peaks were not directly comparable with the corresponding bands from the above extracts; therefore, quantitative band analyses were not made.

In summarizing results for tabular presentation, data from all gel columns of a particular extract were averaged. Student's "T" tests (Snedecor, 1956) were used for statistical analysis. The use of the term "significant" in this paper has a statistical connotation ($p=0.05$). When the effects of different extraction procedures on protein separation were compared using statistical analysis, the results from fresh-sonicated extract were always used as a basis of comparison since, as described below, this extract gave the best separation.

Results

Effect of Extraction Procedures on Soluble Protein:

The influence of extraction procedures on total soluble protein is shown in Table 1. Both sonication and incubation increased the

Table 1 Effect of various procedures on the mean soluble protein content of extract from adult *Schistosoma japonicum* (% increase or decrease)

Extraction procedure (treatment)	Effect of treatment on soluble protein content (%)*	
	Male extract ($\bar{X}\pm$S.D.)**	Female extract ($\bar{X}\pm$S.D.)**
Sonication	+19.0± 4.0	+17.0±7.4
Lyophilization	−38.8±13.3	−38.6±9.8
Refrigeration	−28.8±11.7	−33.4±2.7
Incubation	+17.0±10.1	+ 7.1±1.9

* Each average is based on 12–16 protein determinations. The soluble protein content of sonicated extract was compared with the values for fresh extract. The protein contents of lyophilized, refrigerated, and incubated extracts were compared with the protein values for sonicated extract. The average amount of protein in fresh male extract was 901±220 mg/ml; in fresh female extract, 940±197 mg/ml.

** $\bar{X}$=Mean; S.D.=Standard deviation.

levels of soluble protein when compared with amounts obtained prior to treatment. Lyophilization and refrigeration of extracts decreased soluble protein compared with the amount of protein in the original sonicated extract.

Effect of Extraction Procedures on Protein Distribution:

Gels representing fresh-sonicated and refrigerated extracts, and a blank gel with no

protein are shown in Fig. 1. Sharp bands with good separation were obtained with fresh-sonicated and sonicated-lyophilized extracts. Fresh, refrigerated, and incubated extracts gave fainter bands ; refrigerated gels shown in Fig. 1, D and E, represent the latter situation. The distribution of protein in the sample, spacer, and lower gel layers, when different extracts underwent electrophoresis, is shown in Table 2. Essentially, no significant difference was found in the amount of protein in the three layers. An average of 12 %–17 % of the protein remained in the sample gel layer, little or no protein was found in the spacer gel, while most of the protein migrated into the lower gel during electrophoresis. However, with male incubated and female refrigerated extracts, a small but significant decrease was found in the percent of protein in the sample gel coupled with a small but significant increase in the percent of protein in the lower gel, compared with the distribution found in fresh-sonicated extracts. Although the heavy stain in the sample and spacer gels suggested that large amounts of protein were trapped in these layers, this was not the situation when comparisons were made with densitometric tracings of blank gels. With schistosome extract, the staining density was no greater in spacer gel layers and only slightly greater in the sample gel layers (Fig. 2, B and C) when compared with the density of the same areas of the gel in the blank gels (Fig. 2, A and A′) in which no protein was added.

Effect of Extraction Procedures on Protein Band Migration :

Although the various extraction procedures used did not greatly affect the total amount of protein in the lower gel, as shown above, considerable influence was noted on the migration and intensity of the individual protein bands. The Rf values and frequency of occurrence are given in Table 3 for male extracts and Table 4 for female extracts. Representative densitometric profiles of each extract are shown in Figs. 3–7.

Fresh-sonicated extract gave the best separation of bands among the different extraction procedures for male worms. Using this extract, a total of 30 distinct bands were identified of which 24 were found in all of the gels examined (Table 3). Bands 8, 11, 14, 17, 18, 21, and 27 were major fractions (i.e., those showing prominent peaks). The peaks at bands 17 and 18 were especially prominent in gels (Fig. 2B) and densitometric

Table 2 Protein distribution in sample, spacer, and lower gel layers using various extracts

Sex	Extract	Distribution of protein in gel (%) ($\bar{X}$±S.D.)*		
		Sample	Spacer	Lower
Male	Fresh	14.1±3.7	5.4±4.2	80.5±6.4
	Fresh-sonicated	17.2±2.8	0.5±0.7	84.5±1.8
	Sonicated-lyophilized	16.7±4.3	2.6±2.6	80.6±4.9
	Refrigerated	15.6±3.1	1.1±1.9	83.1±3.8
	Incubated	12.0±1.8**	0.8±1.4	87.2±2.0**
Female	Fresh	13.5±1.0	0±0	86.5±1.0
	Fresh-sonicated	16.2±3.7	0±0	83.5±3.7
	Sonicated-lyophilized	13.5±1.7	3.2±4.2	82.4±7.5
	Refrigerated	12.0±1.9**	0±0	87.9±1.6**
	Incubated	13.2±2.0	0±0	86.8±2.0

* $\bar{X}$=Mean ; S.D.=Standard deviation.

** Significant difference from fresh-sonicated extract (p=0.05).

Table 3 Mean Rf value and frequency of occurrence of protein fractions separated from various extracts of adult male *Schistosoma japonicum*

Band no.	Extraction procedure*				
	F	FS	SL	R	I
1	.008 (38) **	—	—	.009 (50)	.008 (63)
2	.017 (100)	.014 (100)	.013 (100)	.017 (100)	.018 (63)
3	.031 (75)	.034 (100)	.037 (100)	.031 (100)	.031 (75)
4	.047 (88)	—	—	.042 (38)	.044 (75)
5	.067 (88)	.070 (100)	.067 (100)	.066 (100)	.064 (100)
6	.088 (75)	.089 (38)	.088 (75)	.088 (100)	.091 (100)
7	.113 (100)	.106 (100)	.111 (100)	.106 (100)	.113 (63)
7A	.124 (13)	—	—	—	—
8	.146 (100) †	.139 (100) †	.142 (100)	.137 (100) †	.139 (100) †
9	.168 (38)	.161 (25)	—	.162 (100)	.168 (88)
10	.182 (63)	.177 (100)	.177 (100) †	.182 (100)	.182 (63)
11	.219 (100) †	.226 (100) †	.215 (100)	.223 (100)	.221 (88)
12	.258 (88)	.252 (100)	.252 (100)	.248 (100)	.247 (63)
13	—	—	—	.294 (38)	.287 (75)
14	.312 (100) †	.317 (100) †	.318 (100) †	.312 (100)	.324 (75)
14A	—	—	—	.349 (100)	—
15	.354 (100)	.356 (100)	.367 (38)		.366 (100)
16	.398 (100)	.391 (100)	.402 (100)	.400 (100)	.395 (50)
16A	.416 (50)	.414 (50)	—	—	.427 (38)
17	.455 (100) †	.450 (100) †	.448 (100) †	.440 (100) †	.449 (100)
18	.480 (100) †	.476 (100) †	.474 (100) †	.469 (100) †	.479 (100)
18A	—	.496 (13)	.504 (100)	.495 (63)	.499 (25)
19	.514 (100)	.513 (100)	.526 (100)	.517 (100)	.520 (63)
19A	—	—	—	—	—
20	.568 (50)	.568 (100)	.562 (100)	.554 (88)	.552 (88)
20A	—	—	.600 (13)	—	.593 (88)
21	.606 (100) †	.610 (100) †	.608 (100) †	.602 (100)	—
22	.636 (100)	.636 (100)	.635 (100)	.629 (100)	.636 (50)
23	.671 (100)	.674 (100)	.668 (100)	.670 (100)	.666 (75)
24	.704 (63)	.691 (13)	—	.692 (100)	.699 (75)
25	—	—	—	—	—
26	.741 (38)	.731 (100)	.732 (100)	.742 (75)	.729 (75)
27	.758 (100) †	.759 (100) †	.757 (100) †	.757 (63)	.752 (88)
28	.798 (63)	.792 (100)	.782 (100)	.789 (100)	.796 (88)
28A	—	—	—	—	.840 (63)
29	.874 (100)	.872 (100)	.867 (100)	.872 (100)	.874 (100)
29A	—	.948 (13)	—	—	—
30	.974 (100)	.978 (100)	.972 (100)	.978 (100)	.975 (100)
31	1.000 (100)	1.000 (100)	1.000 (100)	1.000 (100)	1.000 (100)
Total no. of bands	31	30	27	31	33
No. of 100% bands	17	24	24	24	8
No. of 100% bands common to FS	17	—	23	21	7

* F=fresh ; FS=fresh-sonicated ; SL=sonicated-lyophilized ; R=refrigerated ; I=incubated

** Mean Rf value ; the percentage of experiments in which the fraction was resolved is shown within parentheses

† Designates major bands

tracings (Fig. 3, A and A′).

With fresh extract a total of 31 bands were resolved. Only 17 of these bands were found in all gels; however, they did correlate to bands found in fresh-sonicated extract (Table 3). In general, the bands resolved from fresh extract were not as sharp as those obtained with fresh-sonicated extract. In particular, bands 17 and 18, although still visible, were noticeably less prominent (Fig. 4, A and A′).

Using sonicated-lyophilized extract, several changes in the densitometric profile were observed compared with fresh-sonicated extract. Twenty-four bands were found in all of the gels examined; however, band 15 which had a 100 % occurrence frequency in fresh-sonicated extract was found in only 38 % of the gels using sonicated-lyophilized extract, and band 18A which was resolved in only 13 % of the fresh-sonicated gels was observed in 100 % of the sonicated-lyophilized gels (Table 3). The major changes in profile involved prominent bands. Bands 8 and 11 were no longer major peaks but band 10 became readily visible (Fig. 4, B and B′).

With refrigerated extract, 31 bands were resolved of which 24 were found in all gels examined. Only 21 of the latter 24 bands were found in all gels of the fresh-sonicated extract. Bands 6, 9, and 24 which had been resolved in 38 %, 25 %, and 13 % of the gels, respectively, using fresh-sonicated extract were found in all gels with refrigerated extract (Table 3). In the region of bands 14A and 15 a single band was resolved in all the gels of refrigerated extract which were examined. This band showed a large variation in Rf value ranging from Rf values comparable to band 14A up to values comparable with band 15. Statistical annlysis of the Rf value showed no significant differences compared with the Rf values obtained for bands 14A or 15 using gels of other extracts. The overall sharpness of bands resolved from refrigerated extract was greatly reduced compared with gels containing fresh-sonicated male extract. Bands 8, 17, and 18 were still major bands; however, bands 11, 14, 21, and 27 were no longer prominent (Fig. 5, A and A′). In soms gels band 21 gave a higher peak than band 22 and in other gels the reverse was true.

Major changes in protein separation occurred with incubated male extract compared with the bands obtained with male fresh-sonicated extract. Although 33 bands were resolved from incubated male extract, only eight of these were found in all gels. Bands 1, 4, 13, 20A, and 28A were found in some gels but were never present with fresh-sonicated extract (Table 3). A considerable variation was found in the electrophoretic profile. In 50 % of the electrophoretic runs a relatively typical male profile was obtained such as shown in Fig. 5B′. In the other runs a different profile was found (Fig. 5B). The type of pattern was consistent within any electrophoretic run using a single extract, but was also found with several different incubated extracts using different groups of chemicals to make the gels. In some cases, using the same chemicals, one extract would give a profile such as Fig. 5B while a second extract would give a profile such as Fig. 5B′. The difference in pattern was not due to "bad" chemicals or electrophoretic runs since the human serum control gels had good separation, but rather was due to a difference in the extracts. Only band 8 was prominent in all gels using incubated male extract. The major peaks which normally occurred in the area of band 21 using other extracts occurred in the position of bands 20A–22 in the type of profile shown in Fig. 5B′ and in the position of band 23, 24, or 26 in the second type of profile (Fig. 5B).

As with male extracts, the best resolution of female protein bands was obtained with fresh-sonicated extract. Using this extract a total of 31 bands was resolved of which 28 were 100 % reproducible (Table 4). On the densitometric tracings, only bands 7 and 11 were consistently prominent (Fig. 4, B and B′). A major peak occurred in the area of band 22 or 23; however, the location was not consistent.

With fresh female extract four more bands were found than with fresh-sonicated extract

Table 4 Mean Rf value and frequency of occurrence of protein fractions separated from various extracts of adult female *Schistosoma japonicum*

Band no.	Extraction procedure*				
	F	FS	SL	R	I
1	.009 (100) **	.008 (100)	.008 (100)	.007 (100)	.009 (100)
2	.019 (75)	.018 (100)	.016 (100)	.018 (88)	.022 (75)
3	.032 (50)	.035 (37.5)	.030 (67)	.031 (63)	.027 (38)
4	.048 (88)	.048 (100)	.048 (100)	.043 (63)	.053 (63)
5	.065 (75)	.065 (100)	.066 (100)	.062 (88)	.063 (50)
6	.082 (63)	.081 (100)	.080 (100)	.086 (100) †	.081 (88)
7	.106 (100) †	.102 (100) †	.097 (100) †	.100 (50)	.107 (88)
7A	.129 (50)	.120 (50)	.117 (78)	.129 (50)	.130 (25)
8	.150 (50)	.142 (100)	.145 (100)	.144 (75)	.139 (63)
9	.163 (63)	.164 (100)	.161 (33)	.168 (38)	.162 (50)
10	.181 (63)	.181 (100)	.179 (100)	.189 (75)	.189 (88)
11	.218 (100)	.228 (100) †	.222 (78)	.226 (63)	.221 (50)
12	.248 (75)	.256 (100)	.256 (100)	.245 (50)	.255 (75)
13	.297 (100)	.295 (100)	.300 (33)	.285 (100)	.299 (75)
14	—	—	—	—	—
14A	.337 (50)	.335 (38)	.337 (33)	.338 (50)	.329 (50)
15	.360 (88)	.370 (100)	.366 (100)	.360 (75)	.369 (100)
16	.394 (38)	.398 (100)	.394 (56)	—	—
16A	.424 (50)	—	.413 (44)	.418 (38)	.412 (25)
17	.452 (63)	.456 (100)	.451 (67)	.443 (63)	.455 (38)
18	.477 (50)	.482 (100)	.483 (100)	.483 (75)	.478 (38)
18A	.496 (50)	—	—	.495 (25)	.501 (50)
19	.520 (63)	.516 (100)	.517 (56)	—	—
19A	—	—	—	.540 (75)	.546 (25)
20	.564 (88)	.556 (100)	.560 (100)	.557 (38)	.566 (75)
20A	—	—	—	.593 (38)	—
21	.605 (63)	.601 (100)	.600 (100)	.604 (88)	.605 (38)
22	.640 (63)	.640 (100)	.641 (56)	.642 (100)	.636 (88)
23	.671 (63)	.663 (100)	.662 (100)	.668 (75)	.666 (50)
24	.691 (63)	.688 (100)	.692 (100)	.689 (50)	.697 (50)
25	.715 (50)	.705 (100)	.716 (22)	.717 (75)	.712 (50)
26	.743 (63)	.734 (100)	.733 (33)	.741 (38)	.739 (75)
27	.768 (50)	.767 (100)	.764 (44)	.764 (50)	.766 (75)
28	.797 (38)	—	—	—	.793 (63)
28A	.842 (13)	—	—	.841 (13)	.836 (38)
29	.875 (100)	.862 (100)	.865 (100)	.877 (100)	.869 (100)
29A	—	—	—	—	—
30	.970 (100)	.965 (100)	.975 (100)	.973 (100)	.976 (100)
31	1.000 (100)	1.000 (100)	1.000 (100)	1.000 (100)	1.000 (100)
Total no. of bands	35	31	32	33	34
No. of 100% bands	7	28	18	7	5
No. of 100% bands common to FS	7	—	18	7	5

* F=fresh ; FS=fresh-sonicated ; SL=sonicated-lyophilized ; R=refrigerated ; I=incubated

** Mean Rf value ; the percentage of experiments in which the fraction was resolved is shown within parentheses

† Designates major bands

(Table 4). However, these bands (16A, 18A, 28, and 28A) were relatively rare in occurrence and were found only in 50 %, 50 %, 38 %, and 13 % of the gels, respectively. Only seven of the bands resolved were 100 % reproducible using fresh extract. Densitometric tracings of the gels showed a decrease in the sharpness of the bands with band 7 being the only 100 % reproducible major peak (Fig. 6, A and A′). The major peak in the latter portion of the tracing again varied in position between band 22 and 23.

Lyophilization of the sonicated extract produced greater changes in the electrophoretic separation with female extract than it did with male extract. Table 4 shows that the number of 100 % reproducible bands was reduced from 28 (fresh-sonicated female extract) to 18 (sonicated-lyophilized female extract). Band 7 was the only reproducible major peak consistently identified (Fig. 6, B and B′). An additional major band occurred in the region of bands 20 and 21 but the location varied between gels.

Refrigeration of female extract greatly reduced the number of reproducible bands (7) compared with fresh-sonicated extract (28) as shown in Table 4. A shift in the major peak was observed from band 7 (Fig. 3, B and B′) to band 6 (Fig. 7, A and A′). In the latter portion of the gel a prominent peak was sometimes found in the position of band 20. In all gels an additional prominent peak was found; however, the location varied from band 22 to band 24.

Incubation of female extract resulted in the resolution of 34 bands; however, only five of these were 100 % reproducible (Table 4). As with male extracts, incubation seemed to produce two different profiles. The "typical" female profile, as shown in Fig. 7B, was found in approximately 60 % of the electrophoretic runs examined. A prominent peak occurred at the position of bands 7 and 11 in all gels of this type and a third prominent peak was found in the area of bands 22 and 23. With the second type of profile, as shown in Fig. 7B, bands 7 and 11 were not major bands and the prominent peak in the latter area of the gel was found in the position of band 25 or 26.

Effect of Extraction Procedures on the Relative Protein Content of Major Bands:

The height of each major band (measured in percent of 1 O.D.) was used as an indication of the relative amounts of protein for all extracts containing 220 μg of protein per gel column as shown in Table 5. Since fresh-

Table 5 Quantitative comparison of major electrophoretic bands using various extracts based on the height (%) of each band on the densitometric tracing

Band no.	Extract*						
	Male					Female	
	F	FS	SL	R	I	FS	SL
7	39.3±4.4	43.4±5.4	46.9± 7.2	42.3±8.5	26.8±13.5**	36.1±8.4	43.6±5.8
8	55.3±5.8	48.8±5.6	51.8± 7.2	48.8±8.6	41.0±14.1	25.8±7.2	25.6±5.9
11	33.1±4.6	32.6±6.2	40.4± 5.3	29.5±7.3	31.4±11.2	20.4±5.1	17.3±4.1
14	26.2±6.6	22.2±4.8	20.3± 5.5	19.9±5.7	25.5± 9.1	—†	—†
17	15.8±3.2**	25.8±2.8	39.6± 8.3**	22.1±5.2	20.8± 5.2	9.3±2.8	8.0±3.0
18	14.9±3.2**	27.6±4.8	45.4±10.3**	21.1±4.8**	20.4± 4.5**	9.6±2.8	9.4±2.8
21	15.1±6.9	18.0±3.2	22.9± 5.7	14.3±4.0	14.4± 5.4	8.2±1.4	9.8±2.6
27	4.9±1.4**	6.8±1.3	6.9± 1.4	6.1±2.2	6.9± 2.6	2.3±0.8	2.3±0.8

* F=fresh; FS=fresh-sonicated; SL=sonicated-lyophilized; R=refrigerated; I=incubated
Each average based on the analysis of 8 to 10 gels

** Significantly different compared with fresh-sonicated extract of the same sex

† Band not present

sonicated extract gave the best separation of protein bands, this extract was used as the basis for comparison. With incubated male extract, the amount of protein in band 7 was significantly reduced when compared with fresh-sonicated extract. Fresh extract also showed significantly less protein in bands 17, 18, and 27. The major quantitative differences in protein from male extracts occurred in band 18 where fresh, refrigerated, and incubated extracts had significantly less protein than the fresh-sonicated extract, while sonicated-lyophilized extract had more. No significant differences were found in the amount of protein in the analyzed bands of sonicated-lyophilized female extracts compared with the amount in fresh-sonicated female extract.

Sex-Related Differences in Electrophoretic Profile :

Comparison of sex-related differences in protein separation were made only with fresh-sonicated extract since this produced the best band separation. A total of 24 and 28 reproducible bands (100 % frequency of occurrence) were found in male and female extracts, respectively (Tables 3 and 4). Of these, bands 1, 4, 13, and 25 were found only in female extract while bands 14 and 28 were characteristic of male extract. It is important to note that (1) although the same amount of protein was added to each gel column (220 μg), and (2) there were no significant differences between male and female extracts in the distribution of protein in the sample, spacer, and lower gels (Table 2), the bands resolved from female extract were noticeably less evident than those from male extract (Fig. 1, B and D ; Fig. 2, B and C).

In the densitometric tracings of male extract (Fig. 4, A and A′), major peaks were found in all gels at bands 8, 11, 14, 17, 18, 21, and 27 while prominent peaks with the female extract were found at bands 7 and 11. Analysis of the heights of these bands showed significantly less protein in bands 8, 11, 17, 18, 21, and 27 of the female extract compared with male extract, but no difference in band 7. Band 14, a major band in the male extract, was not present in the female extract.

Discussion

In their work on molluscan systematics, Davis and Lindsay (1967) pointed out that complex analysis of population differences within a species involves the necessity of an adequate supply of snails which are not always available. This statement also holds true for similar analyses concerning parasites and the acquisition of sufficient amounts of extract can become a greater problem when the parasites, such as *Schistosoma japonicum*, are relatively small. For this reason several methods of increasing the soluble protein content of an extract were investigated in the study reported herein (e.g., sonication and incubation). In addition, since it is not always convenient to make an electrophoretic run immediately after collection of worms, two means commonly used for the preservation of extracts or worms were studied (e.g., refrigeration and lyophilization).

The increase in soluble protein after sonication was probably due to the greater disruption of cells, thus releasing more protein into the extract. Electrophoretic analysis suggested that no major changes were produced in the electrophoretic pattern with regard to the total number of bands found and the position of major peaks, compared with results obtained with fresh extract ; however, there were very obvious advantages in a greatly increased reproducibility of bands and in the sharpness of bands present. The increase in protein with incubation was due possibly to the autolytic breakdown of cells and insoluble protein during the incubation period. Electrophoretic analysis suggested that this breakdown seriously affected the electrophoretic profile. More bands were found than with fresh-sonicated extract ; however, the number of 100 % reproducible bands was greatly reduced (from 24 to 8 in males and 28 to 5 in females). Since the electrophoretic separation of the proteins is dependent on their size, shape, and charge (Ornstein, 1964), even a slight

change in the protein structure during incubation could result in significant changes in the Rf value.

Refrigeration of extracts resulted in a decrease in the total soluble protein, reproducibility, and prominence of bands, especially with female extract. This was due possibly to slow autolytic destruction of some protein and slight changes in the charge on other protein molecules during refrigeration. Lyophilization also decreased the total soluble protein of both extracts. One reason for this was probably the coagulation and/or precipitation of soluble proteins when the extracts were reconstituted with distilled water (Rutkowski and Davis, 1970). Evidence for this was found in all lyophilized extracts since precipitate which was not present in the original extract was observed following the centrifugation after reconstitution of the lyophilized extract. The effect of lyophilization on the electrophoretic patterns of male extract was slight; however, major changes were evident with female extract.

The faint bands obtained with fresh, refrigerated, and incubated extract, compared with fresh-sonicated or sonicated-lyophilized extract, were probably related to the bands being more defused since analysis of the distribution of protein indicated approximately the same amounts of protein in all lower gel layers regardless of the extract used. This resulted in increased difficulty in the identification of bands present and their location thus increasing variability. As pointed out by Davis and Takada (1969), "variability due to experimental factors involves: (1) resolving faint components in some gels which are only blurs of diffuse protein in others; (2) resolving two bands in some gels which are fused into a single band in others: (3) greater variability around the mean Rf value for a fraction that is particularly faint and thus difficult to measure its location."

Yoshimura (1968) studied the electrophoretic separation of *Schistosoma japonicum* proteins using extracts from lyophilized whole worms with the sexes pooled. The overall electrophoretic profiles which he obtained are very similar to those found in the present study and are easily recognizable as patterns of similar proteins. There are, however, several differences which amply demonstrate the need for exact replication of extraction procedures if direct comparisons are to be made between the results of different investigators (Table 6). First of all, the electrophoretic profiles obtained by Yoshimura appear to be those of male *S. japonicum*, i. e., the male proteins were masking the female proteins in his study. For example, Yoshimura failed to resolve any of the female characteristic bands which were found in the present study such as bands 1, 7, 13, and 25. Female worms of *S. japonicum* are considerably smaller than male worms, thus an extract of pooled pairs will contain proportionately more male protein than female protein. In the present study it has been shown that female extract gave much fainter bands than male extract, even when the same amount of protein was added to each gel column. These findings suggest that it is imperative that sex differences first be established and, if present, that extracts must be made of the sexes separately if valid comarisons are to be made of species or strain variation in protein patterns. Differences between sexes would not necessarily be detected. For example, Davis and Takada (1969) found no sex differences in foot-muscle extract of *Oncomelania*.

The importance of identical extraction procedures is further emphasized by a comparison of the major protein fractionside notified in the two studies. Yoshimura (1968) identified six major bands which, based on the appearance of the electrophoretic profile, would compare to the major bands found at positions 8, 17, 18, 21, and 27 with fresh-sonicated male extract in the present study (Table 6). Four of these bands show good correlation of Rf values within the 0.016 variation accepted as experimental error (Davis and Lindsay, 1967); these were bands 8, 17, 18, and 27 with Rf values of 0.124, 0.439, 0.461, and 0.759, respectively (Yoshimura, 1968), and 0.139, 0.450, 0.476, and

Table 6 Comparison of protein fractions separated in this study with those of Yoshimura (1968)

Present study*			Yoshimura (1968)	
Band no.	Male	Female	Band no.	Pooled
1	—	.008 (100) **	—	—
2	.014 (100)	.018 (100)	1	.026 (100)
3	.034 (100)	.035 (37.5)	—	—
4	—	.048 (100)	1A	.046 (100)
5	.070 (100)	.065 (100)	3	.079 (100)
6	.089 (38)	.081 (100)	—	—
7	.106 (100)	.102 (100) †	—	—
7A	—	.120 (50)	—	—
8	.139 (100) †	.142 (100)	4	.124 (100) †
9	.161 (25)	.164 (100)	5	.159 (100)
10	.177 (100)	.181 (100)	6	.190 (100)
11	.226 (100) †	.228 (100) †	7	.227 (100)
12	.252 (100)	.256 (100)	—	—
13	—	.295 (100)	—	—
14	.317 (100) †	—	8	.289 (100) †
14A	—	.335 (38)	9	.335 (100)
15	.356 (100)	.370 (100)	10	.368 (100)
16	.391 (100)	.398 (100)	—	—
16A	.414 (50)	—	—	—
17	.450 (100) †	.456 (100)	11	.439 (100) †
18	.476 (100) †	.482 (100)	12	.461 (100) †
18A	.496 (13)	—	13	.490 (100)
19	.513 (100)	.516 (100)	14	.524 (100)
19A	—	—	—	—
20	.568 (100)	.556 (100)	15	.557 (100)
20A	—	—	16	.598 (100)
21	.610 (100) †	.601 (100)	16A	.620 (30)
22	.636 (100)	.640 (100)	17	.632 (100) †
23	.674 (100)	.663 (100)	18	.674 (100)
24	.691 (13)	.688 (100)	—	—
25	—	.705 (100)	—	—
26	.731 (100)	.734 (100)	—	—
27	.759 (100) †	.767 (100)	19	.759 (100) †
28	.792 (100)	—	—	
28A	—	—	20	.848 (100)
29	.872 (100)	.862 (100)	21	.888 (100)
29A	.948 (13)	—	—	—
30	.978 (100)	.965 (100)	21A	.957 (40)
31	1.000 (100)	1.000 (100)	22	1.000 (100)

* Fresh-sonicated extract

** Mean Rf value; the percntage of experiments in which the fraction was resolved is shown within parentheses

† Designates major bands

0.759, respectively (present study). The remaining two bands (14 and 21), however, did not correlate. Their respective Rf values were calculated as 0.289 and 0.632 (Yoshimura, 1968) and 0.317 and 0.610 (present study). In the present study, an Rf value of 0.632 would indicate that this protein fraction was band 22 (Rf value 0.635 for sonicated-lyophilized male extract) rather than band 21. However, since Yoshimura's study used extract of lyophilized whole worms and the present study a lyophilized extract of male worms, plus the fact that the present study showed differences in electrophoretic profile based on extracion procedure, it is not possible to say if these two peaks are the same fraction with a different Rf value or different fractions which were prominent in different experiments.

The above findings all emphasize the necessity of carefully controlled experiments if valid comparisons are to be made between the electrophoretic profiles of different organisms. Sibley (1960) has stated that it is reasonable to assume that electrophoretically identical peaks in different species of the same genus result from proteins nearly identical in basic structure since this structure in genetically determined; however, Davis and Lindsay (1967) pointed out that this must be proven further. Sibley's statement suggests that electrophoretic differences between species might be minor and with strain differences within a species be even less. The findings of Davis and Lindsay (1967), Davis and Takada (1969), and Davis (1969), in fact have shown that population differences may be present within a species, although these differences were small compared with the overall similarities of the electrophoretic patterns. The finding of pattern differences, and the statement of Davis and Lindsay (1967) that "glassware cleanliness, care in gel preparation, storage, handling, and shelf life of chemical solutions can cause differences between laboratories and even between work done at different times in the same laboratory", coupled with the variation reported herein using different extraction procedures, all point out that, although disc electrophoresis offers a valuable tool for the study of the degree of similarities and differences of species or strains, great care must be taken if such results are to be meaningful and valid.

Summary

The effects of several extraction procedures on the disc electrophoretic separation of proteins of male and female *Schistosoma japonicum* were investigated. Extracts used were fresh, fresh-sonicated, sonicated-lyophilized, refrigerated, and incubated. The best separation and reproducibility of protein fractions was obtained with fresh-sonicated extract for both male and female worms. With fresh extract separation of the protein fractions was reasonably good; however, reproducibility (i.e., 100 % frequency of occurrence of bands) was lower than that obtained with fresh-sonicated extract. Lyophilization of the extract produced changes in electrophoretic pattern compared to fresh-sonicated extract. Both refrigeration and incubation of the sonicated extract resulted in obvious major changes in band separation, reproducibilty, and sharpness, especially with female extracts.

A comarison of the electrophoretic patterns between male and female worms, using fresh-sonicated extract, showed definite differences between the sexes. With male extract 30 bands were resolved, 24 of which were present in all gels examined. Female extract produced 31 distinct bands of which 28 had a 100 % occurrence frequency. Of the 100 % reproducible bands, four were found only with female extract and two solely with male extract. Seven prominent peaks were identified with male extract and two with female extract, one of these common to both sexes. Quantitative analysis of the relative amounts of protein in major bands showed that less protein was present in female extract compared with the protein values obtained using male extract.

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日本住血吸虫蛋白のディスク電気泳動像におよぼす抽出方法の影響について

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Schistosoma japonicum の雌虫および雄虫につき，種種の方法を用いてタンパク質の抽出をおこない，これらの方法が，ディスク電気泳動法によるタンパク質の分離パターンにおよぼす影響を検討した．

虫体抽出物としては，新鮮虫体をホモジナイズし直ちに遠沈した上清（試料-Ⅰ），新鮮虫体のホモジネートを超音波処理し遠沈した上清（試料-Ⅱ），超音波処理上清を凍結乾燥したもの（試料-Ⅲ），超音波処理上清を4°Cで1日保冷したもの（試料-Ⅳ），および超音波処理ホモジネートを遠心に先立ち37°C に時間インキュベートし遠沈した上清（試料-Ⅴ）を用いた．

雌雄いずれの場合においても，試料-Ⅱにおいて，最もよい分離がみとめられまた再現性も優れていた．試料-Ⅰを用いた場合，タンパク質の分離は良好であつたが，再現性において試料-Ⅱに劣つていた．試料-Ⅲを用いた場合は，試料-Ⅱとは異つた分離パターンがみとめられた．試料Ⅳ，Ⅴにおいては，タンパクの分離再現性バンドの鮮明度に明瞭な差がみとめられたが，このことは雌虫試料においてとくに著しかつた．

試料-Ⅱを用いて行なつた雌雄間の分離パターンの比較では，性による明瞭な差がみとめられた．雄においては，全実験例を通じ30種のバンドがみとめられたが，その中24種がすべての例にみとめられた．雌においては，31種のうち28種がすべての例にみとめられた．100％の再現性のみとめられるバンドのうち，4種は雌のみに，2種は雄のみにみとめられた．雄虫には7種の，雌虫には2種の主要バンドがみとめられたが，これらのうちの1種は両性に共通であつた．

主要バンドにおけるタンパク質量を総タンパク量と比較した場合これらの相対量は雌虫における方が雄虫におけるより低かつた．(H.O.)

Explanation of Figures

Fig. 1 Prints of stained disc electrophoretic gels of *Schistosoma japonicum* proteins. "Blank" gel containing no protein (A′); fresh-sonicated male and female extract (B and C, respectively); refrigerated male and female extract (D and E, respectively).

Fig. 2 Densitometric tracings of disc electrophoretic gels. "Blank" gels containing no protein (A and A′); fresh-sonicated extract of male and female *Schistosoma japonicum* (B and C, respectively).
U=upper or sample gel layer; S=spacer gel layer; L=lower gel layer.

Fig. 3 Densitometric analysis of electrophoretic patterns of adult *Schistosoma japonicum*. Fresh-sonicated extract of male worms (A and A′) and fresh-sonicated extract of female worms (B and B′).

Fig. 4 Densitometric analysis of electrophoretic patterns of adult male *Schistosoma japonicum*. Fresh extract (A and A′) and sonicated-lyophilized extract (B and B′).

Fig. 5 Densitometric analysis of electrophoretic patterns of adult male *Schistosoma japonicum*. Refrigerated extract (A and A′) and incubated extract (B and B′).

Fig. 6 Densitometric analysis of electrophoretic patterns of adult female *Schistosoma japonicum*. Fresh extract (A and A′) and sonicated-lyophilized extract (B and B′).

Fig. 7 Densitometric analysis of electrophoretic patterns of adult female *Schistosoma japonicum*. Refrigerated extract (A and A′) and incubated extract (B and B′).

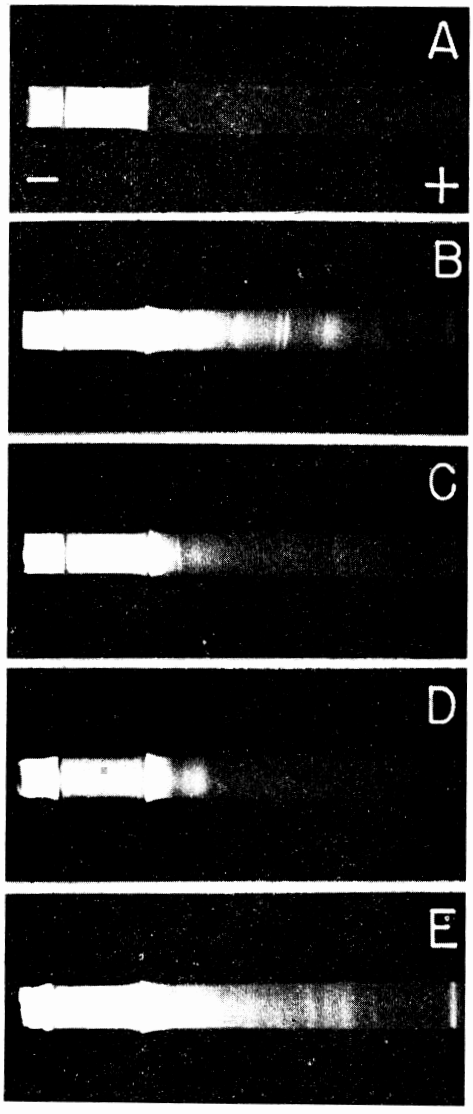

Fig. 1

Fig. 2

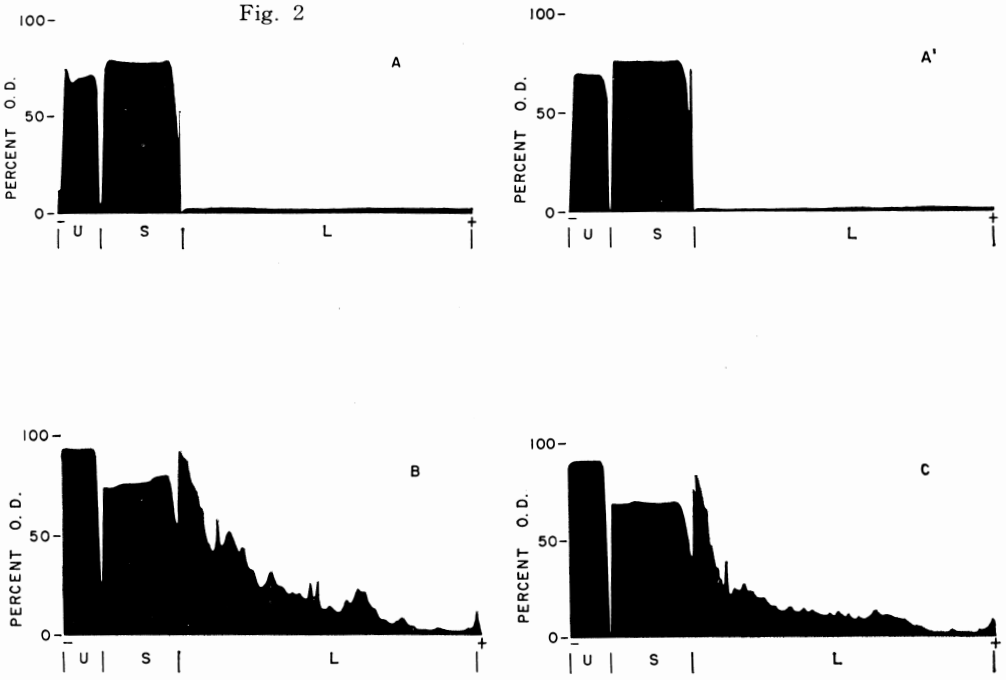

Fig. 3

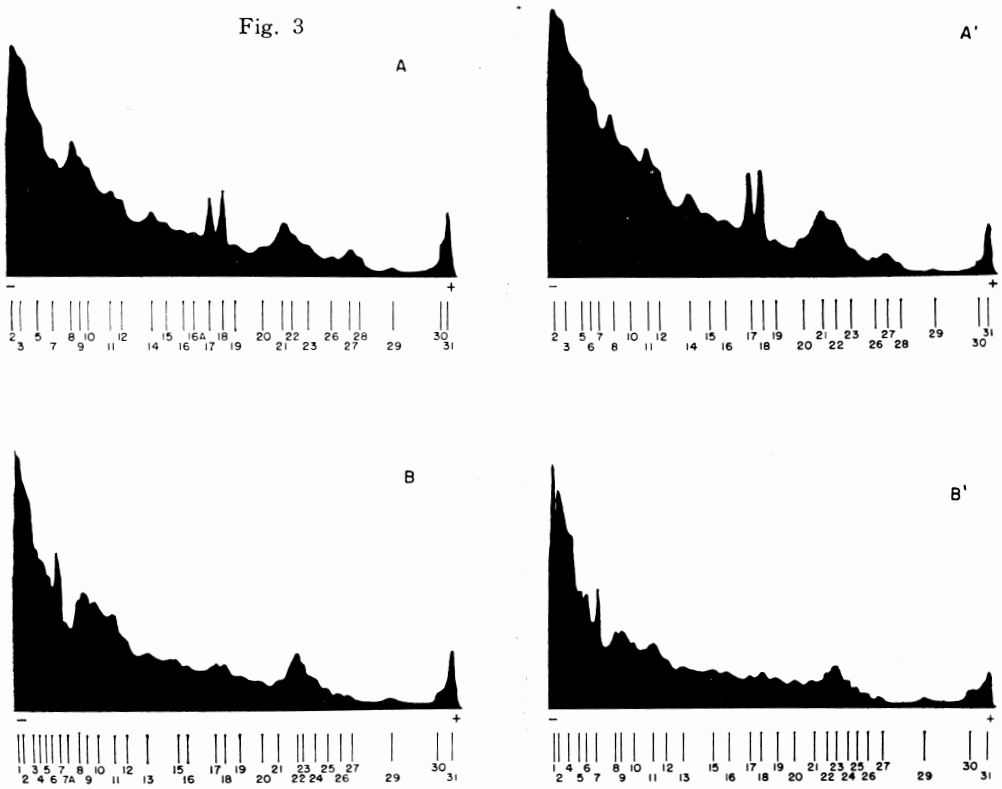

Fig. 4

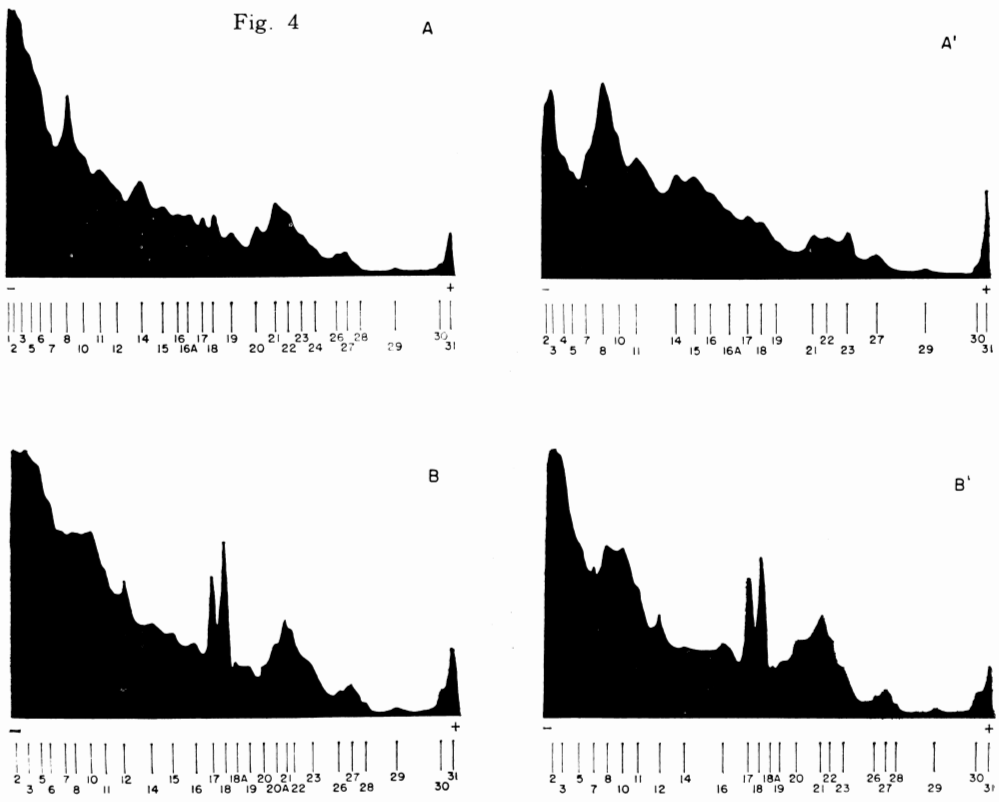

Fig. 5

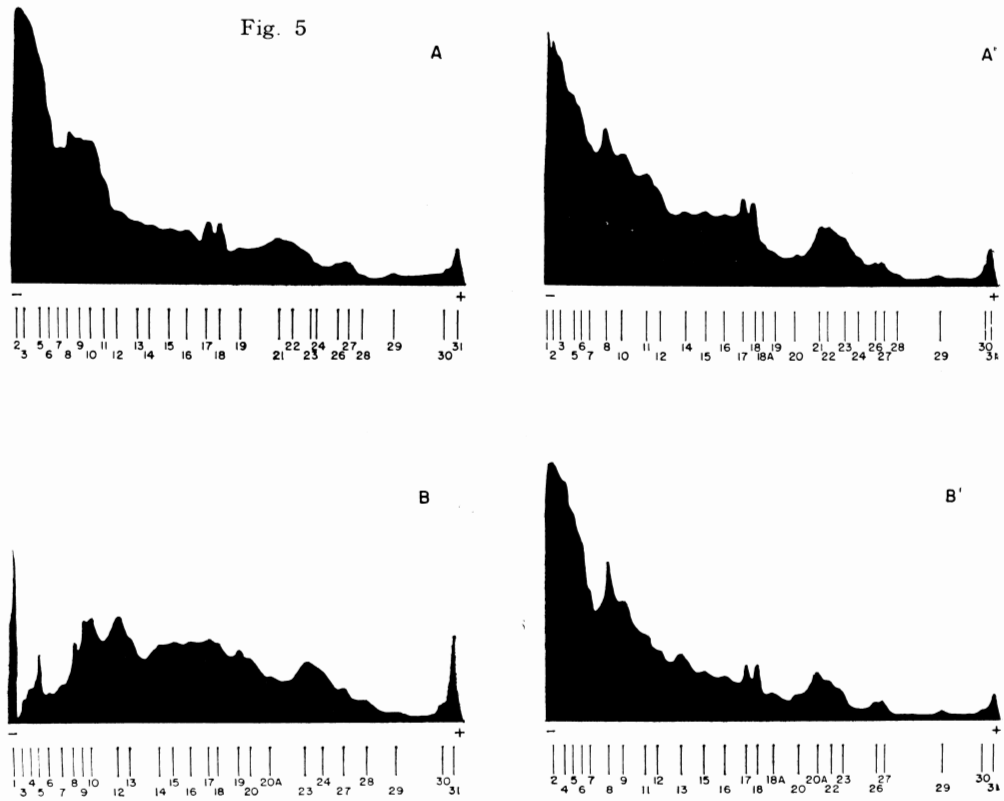

Fig. 6

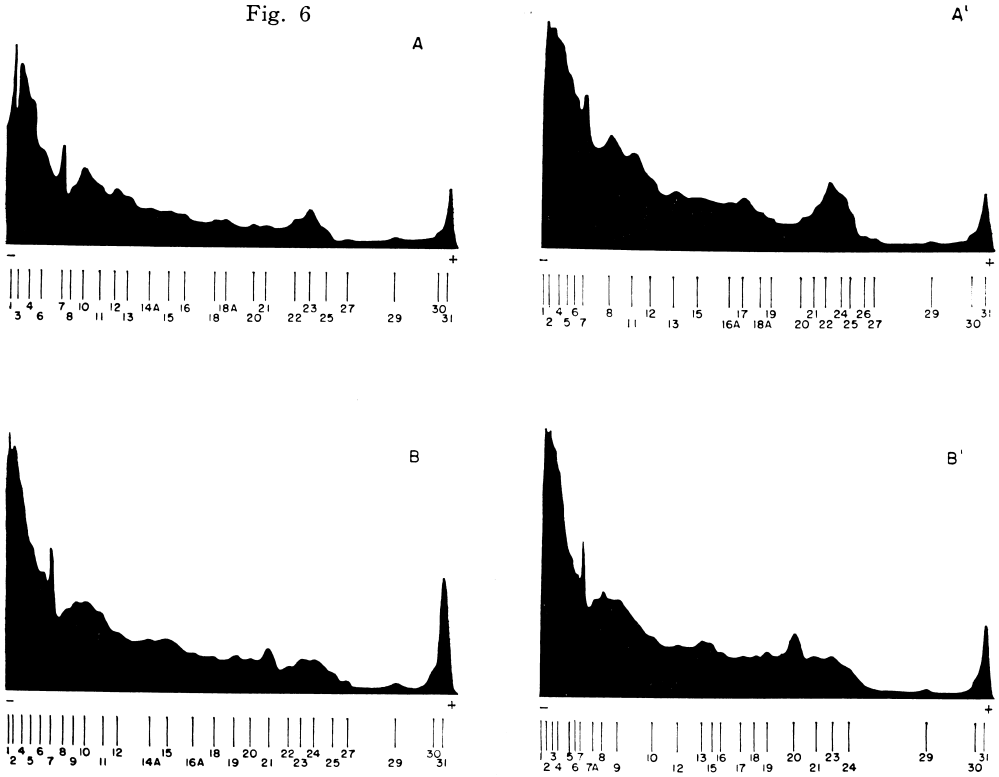

Fig. 7