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DISC ELECTROPHORESIS OF TUBER PROTEINS FROM *SOLANUM* SPECIES AND INTERSPECIFIC HYBRIDS*

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Abstract—Separation of tuber proteins has been accomplished by disc electrophoresis. Soluble tuber proteins from twenty-six species of *Solanum* have been partially "resolved". Comparisons of resulting protein patterns were made to detect possible differences between species. Differences, especially in major bands, suggest that the patterns may be species-specific. In some instances, interspecific hybrids appear to have a greater number of bands than either parental species. In addition, there is segregation in the hybrids for major bands present in either parental species. The possibility of using these results as another approach to taxonomic and genetic studies with Solanums is promising. The availability of a large number of tuber-bearing *Solanum* species, interspecific hybrids, and their derivatives representing different ploidy levels provides an excellent source of material for both genetic and taxonomic studies.

INTRODUCTION

PLANT tissues, in contrast to animal sera, are not a convenient source of unbound proteins. New biochemical and serological techniques are being applied to taxonomic problems in higher plants. With the aid of these techniques progress has been made in delineating species differences with individual compounds or patterns of many compounds.[1,2] Endosperm and embryo tissue of maize, for example, are a source of many enzymes. Within inbred lines of maize, the inheritance of esterases and alkaline phosphatase has been demonstrated by Schwartz,[3,4] and the genetic basis for catalase and leucine aminopeptidase was elucidated by Beckman, Scandalios and Brewbaker.[5,6] More important, these enzymes are controlled by co-dominant alleles similar to the alleles controlling certain serum proteins and enzymes which occur in animals. Furthermore, hybrid substances and gene dosage effects are noted both in plant and animal proteins. Thus biochemical and genetic analyses developed for animals can be successfully applied to plants.

Investigations dealing with more than one compound have been facilitated by chromatographic and electrophoretic techniques. For example, extracts of phenolic substances and other compounds from leaves and flowers of *Baptisia* have been chromatographed and used to characterize the species; also interspecific hybrids were identified.[7] Seed extracts of

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[1] R. E. ALSTON and B. L. TURNER, *Biochemical Systematics*. Prentice Hall, Englewood Cliffs, N.J. (1963).
[2] C. LEONE (Ed.), *Taxonomic Biochemistry and Serology*. Ronald Press, New York (1964).
[3] D. SCHWARTZ, *Proc. Natl Acad. Sci. U.S.* **46**, 1210 (1960).
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[5] L. BECKMAN, J. G. SCANDALIOS and J. L. BREWBAKER, *Science* **146**, 1174 (1964).
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[7] R. E. ALSTON and B. L. TURNER, *Proc. Natl Acad. Sci. U.S.* **48**, 130 (1962).

Lathyrus have yielded amino acid patterns which reflect species differences.[8] Immunoelectrophoretic methods have been employed by Hall[9] for the comparison of ryewheat and its parental species; disc electrophoresis also has been used for the direct correlation of these parental proteins and hybrid proteins.[10] Fox, Thurman and Boulter[11] have employed the same method to plant proteins as an aid to taxonomic studies of legumes.

The purpose of the present survey is to evaluate a relatively new technique, disc electrophoresis, for separating tuber proteins obtained from *Solanum* species and to determine if it is feasible to characterize species by their patterns of soluble tuber proteins.

RESULTS

A survey of this type requires a technique which is accurate, rapid, and preferably requires a small amount of protein. Disc electrophoresis using acrylamide gel as a supporting media was selected since it met these requirements.

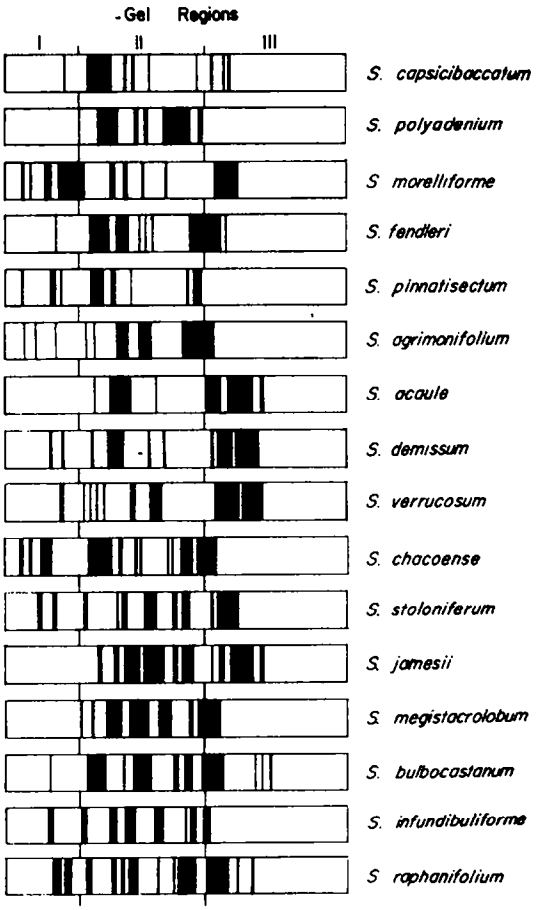

FIG. 2. DIAGRAMS OF PROTEINS PATTERNS OF SPECIES FROM THIRTEEN SERIES.

[8] E. A. BELL, *Biochem. J.* **83**, 225 (1962).
[9] O. HALL, *Hereditas* **45**, 495 (1959).
[10] B. L. JOHNSON and O. HALL, *Am. J. Botany* **52**, 506 (1965).
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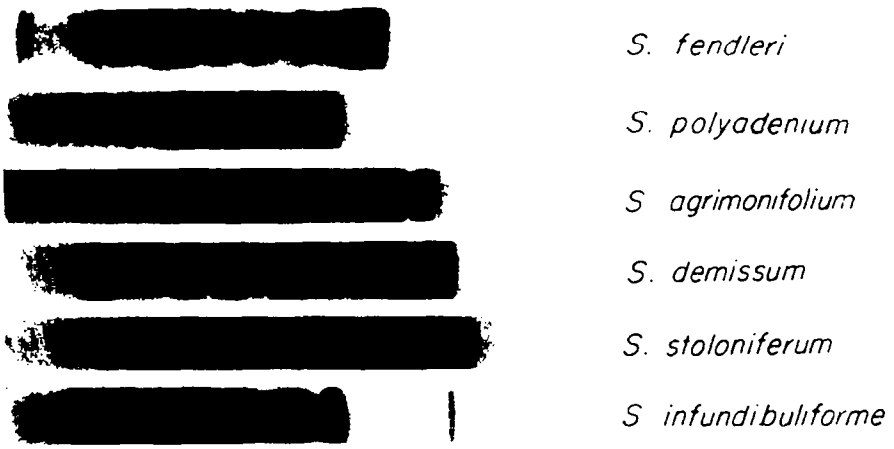

FIG. 1. REPRESENTATIVE GELS WITH SIMPLE TO COMPLEX PATTERNS OF SOLUBLE TUBER PROTEINS.

[facing page 728]

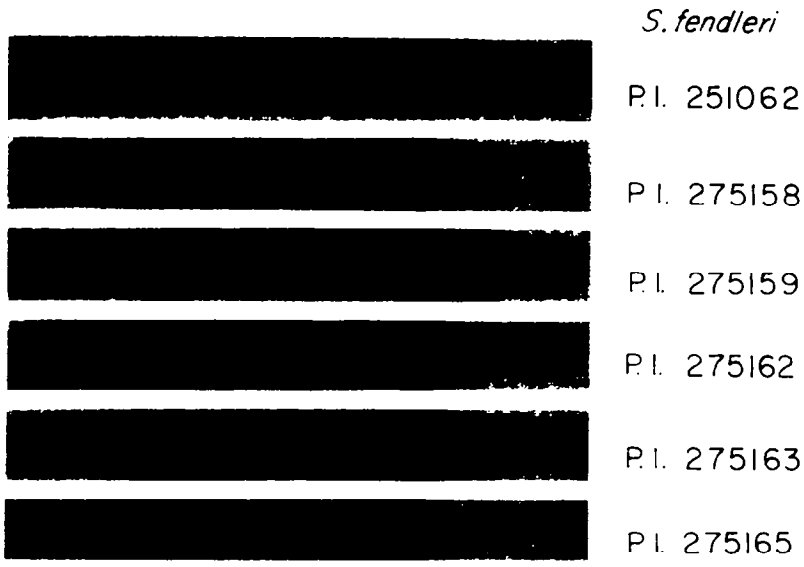

FIG. 4. SIX INTRODUCTIONS OF *S. fendleri* WITH THE SAME PROTEIN PATTERNS, EXCEPT FOR A LIPOPROTEIN.

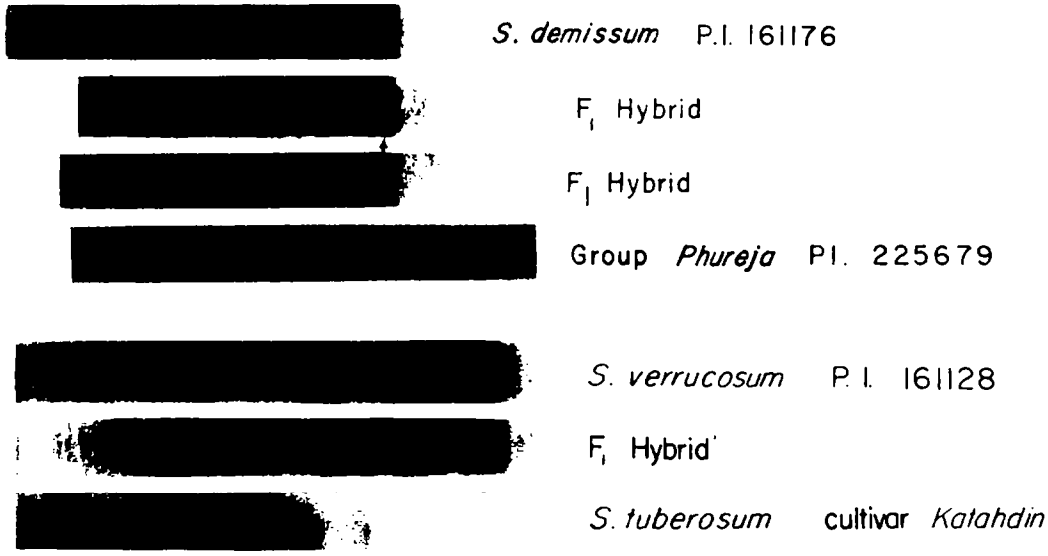

FIG. 5. PROTEINS FROM SPECIES AND F_1 HYBRIDS.
Top—segregration for a major band among the hybrids. Bottom—more bands in hybrid than in either parental species.

Gels are compared by the presence and by the position of the protein bands. Dense, broad protein bands are referred to as "major" bands. These, in contrast to minor bands, are more distinct and their number and location are readily determined for each species. Where minor bands can be clearly differentiated, they are also used. For ease of comparison, the gels are arbitrarily divided into three regions. Gels of selected species representing simple to complex patterns are given in Fig. 1.

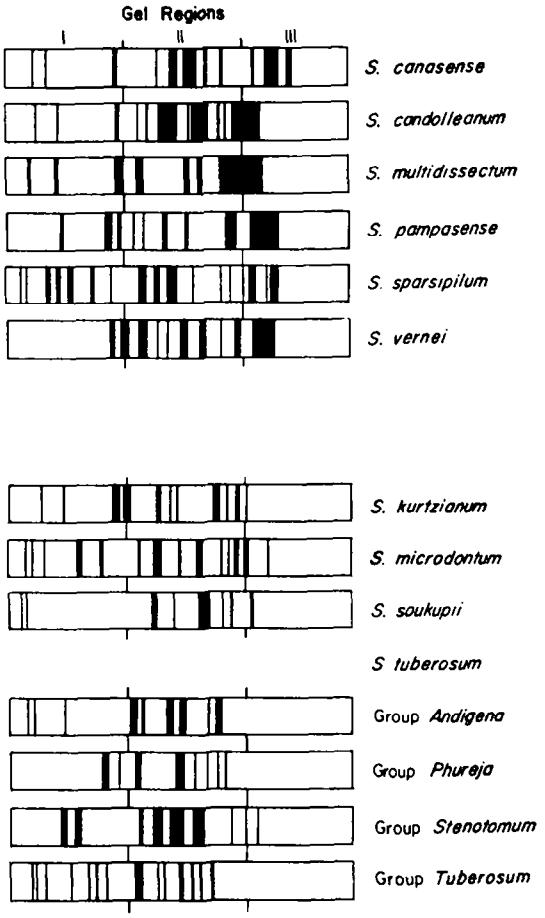

FIG. 3. DIAGRAMS OF PROTEIN PATTERNS OF SPECIES IN SERIES TUBEROSA. NOTE PRESENCE OR ABSENCE OF MAJOR BANDS AT THE BORDER OF II–III.

Tuber proteins from twenty-six species representing thirteen taxonomic series were separated by disc electrophoresis. Diagrams of the resulting gels, exclusive of the Tuberosa series, are given in Fig. 2. When only major bands are considered *S. capsicibaccatum* Cardenas has one of the simplest patterns, one band in region II. The two major-band pattern is next in complexity and includes three different species: *S. polyadenium* Greenm. has both major bands in region II; *S. morelliforme* Bitt. et Muench has one in III and one at the I–II border; and *S. pinnatisectum* Dun. has a major band in I and one in II.

S. fendleri A. Gray has two bands in region II and one at the border of regions II and III. *S. agrimonifolium* Rydb. also has three major bands, two in II and one at the II–III junction. The distribution of bands in *S. acaule* Bitt., *S. demissum* Lindl., and *S. verrucosum* Schlectd. is similar with two major bands in region III and one in region II.

Many species possess more complex patterns. A four major-band pattern is found in *S. chacoense* Bitt., including one in region I. *S. stoloniferum* Schlectd. et Bchi. has several major bands in II and one in III. *S. jamesii* Torr., *S. megistacrolobum* Bitt., *S. bulbocastanum* Dun., *S. infundibuliforme* Phil., and *S. raphanifolium* Card. et Hawkes have the most complex patterns. Although the number of major bands is larger, these gel patterns still can be differentiated on the basis of location of major bands and by distribution of minor bands.

The Tuberosa series is represented by ten species: *S. canasense* Hawkes, *S. candolleanum* Berth., *S. kurtzianum* Bitt. et Wittm., *S. multidessectum* Hawkes, *S. pampasense* Hawkes, *S. microdontum* Bitt., *S. soukupii* Hawkes, *S. sparsipilium* (Bitt.) Juz. et Buk., *S. vernei* Bitt. et Wittm. and *S. tuberosum* L. (Group Phureja), (Group Andigena), (Group Tuberosum), (Group Stenotomum). Many differences occur between species in this series. Nearly as much variation is present as exists between species of different series. It is possible, however, to divide the series Tuberosa into two groups depending on the presence of major bands at the border of II and III (Fig. 3).

To demonstrate the constancy of the species-specific proteins, gels from several introductions of a species with a simple pattern (*S. fendleri*) are given in Fig. 4.

The soluble tuber proteins from thirty-four interspecific hybrids were examined. Two main types of differences between hybrids and parents occur. There is segregation among the hybrids (*S. demissum* × Group Phureja) for a major band in region III (Fig. 5). Secondly, more bands are present in certain hybrids (*S. verrucosum* × *S. tuberosum* cultivar Katahdin) than occur in either of the parental species (Fig. 5).

DISCUSSION

Prior to evaluation of data concerning proteins extracted from tubers of species and species hybrids it was necessary to establish a reliable electrophoretic technique for proteins. Knowledge of factors affecting stability of tuber proteins also is required. In initial tests proteins were examined for possible variation due to cold storage of tubers and freezing of protein extracts. For these tests commercial varieties and haploids extracted from them were available from different harvest times and in sufficient amounts for preparation of protein extracts. No effects were noted and therefore the extracts were prepared in the same manner from tubers of the species and species hybrids.

The technique of disc electrophoresis was found to be both accurate and rapid for soluble tuber proteins. This method allows direct protein comparisons to be made between the gels, because each protein migrates in a precise manner according to its isoelectric point and shape and weight of the molecules. There is a band of tracking dye in each gel for use as a reference line.

The twenty-six species representing thirteen taxonomic series and three ploidy levels revealed a diversity of patterns. However, relatively constant species-specific patterns were delineated and appeared suitable for further genetic analyses or for use as taxonomic criteria. Since the species patterns do reveal differences, particularly in major bands, the inheritance of these tuber proteins may be realized. Furthermore, interspecific hybrids are easily obtained.

The major bands in region III appear promising for analysis in some of these hybrids. These proteins may be useful genetic markers especially when they are enzymes or structural proteins.

Disc electrophoresis is well suited for identification of proteins.[12–15] Techniques are available for detection of several enzymes including esterases present in potato tubers.[16]

Another study which seems promising is determining proteins in haploids ($2n=24$) for comparison with the proteins from tetraploid Tuberosum or Andigena parents ($2n=48$). It would be expected that simpler protein patterns will be present in the haploids. There should be differences in haploids extracted from any one tetraploid parent. Furthermore, the haploids have been successfully crossed to many 24-chromosome *Solanum* species, providing abundant materials for future investigations.[17] Genetic studies with proteins at the 24-chromosome level may provide important information concerning species relations and the origin of the cultivated tetraploid potato.

Further studies involving hybrid proteins are suggested from these data. An interspecific hybrid may be compared with an artificial mixture of its two parental protein extracts. Any protein not present in the artificial mixture would supposedly be unique to the hybrid.[18] Once genetic segregation was established it would be pertinent to attempt identification of the specific proteins involved, as discussed previously. However, identification of hybrids may be possible without specific knowledge of the proteins involved, since the over-all distribution of soluble tuber proteins should be characteristic of the hybrids and the parents from which they were obtained.

MATERIALS AND METHODS

Tubers of species and hybrids were obtained from the IR-1 (Inter-regional Potato Introduction Project), Sturgeon Bay, Wisconsin.[19] The *Solanum* species and their geographic distribution are given in Table 1.

Selected clones of species with the following Plant Introduction (P.I.) numbers were used: *S. morelliforme* Bitt. et Muench 275221, 275233; *S. bulbocastanum* Dun. 161156, 243506, 243508, 243509, 243511, 275185, 275186, 275190, 275191, 275192, 275196, 275200; *S. jamesii* Torr. 195189, 275168, 275170, 275171, 275172, 275173, 275262, 275263, 275264, 275265, 275266; *S. pinnatisectum* Dun. 184764, 186553, 190115, 259467, 275233, 275234, (184764 × 190115); *S. chacoense* Bitt. 133618, 133656, 133708, 133720, 209411, 217451, 230585, 265576, 275141, (133708 × 133662); *S. capsicibaccatum* Card. 205560, 210036; *S. agrimonifolium* Rydb. 243349, 243352; *S. acaule* Bitt. 175395, 175396, 195160, 205507, 205395, 208879, 210029, 210032, 217450, 230494, 246571, 266381, 275130; *S. demissum* Lindl. 160220, 160229, 160230, 161154, 161175, 161176, 161180, 161366, 161731, 161732, 175411, 186552, 230487, 275210, 275211; *S. verrucosum* Schlechtd. 160228, 161173, 195172, 255543, 275254, 275257, 275261; *S. stoloniferum* Schlechtd. 160224, 160225, 160226, 160372, 161160, 161171, 186544, 186557, 195169, 195195, 205522, 230477, 275245, 275252; *S. fendleri* A. Gray 251062, 275157. 275158, 275159, 275162, 275163, 275164, 275165, 275166, 275167, 283102, 283143; *S.*

[12] B. J. Davis, *Ann. N.Y. Acad. Sci.* **121**, 404 (1964).
[13] L. Ornstein, *Ann. N.Y. Acad. Sci.* **121**, 321 (1964).
[14] O. Smithies, *Biochem. J.* **71**, 585 (1959).
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[18] M. R. Irwin, *Genetics* **24**, 709 (1939).
[19] R. W. Ross and P. R. Rowe, *Wisconsin Univ. Agr. Expt. Sta. Bull.* No. 533 (1965).

TABLE 1. SERIES, CHROMOSOME NUMBER AND DISTRIBUTION OF TUBER-BEARING *Solanum* SPECIES SAMPLES

Solanum sp.[21-22]	Chromosome number—2*n*=	Series	Samples tested	Distribution
morelliforme	24	Morelliformia	2	C. and S. Mexico, and Guatemala
bulbocastanum	24	Bulbocastana	12	C. to S. Mexico
jamesii	24	Pinnatisecta	11	NW. Mexico, and SW. United States
pinnatisectum	24	Pinnatisecta	7	C. Mexico
chacoense	24	Commersoniana	10	S. Bolivia, Argentina, Paraguay, Uruguay, and S. Brazil
capsicibaccatum	24	Circaeifolia	2	Bolivia
agrimonifolium	48	Conicibaccata	2	S. Mexico to Guatemala
acaule	48	Acaulia	13	Peru, Bolivia, NW. Argentina
demissum	72	Demissa	15	Mexico
verrucosum	24	Demissa	7	Mexico
stoloniferum	48	Longipedicellata	14	C. Mexico
fendleri	48	Longipedicellata	12	NW. Mexico, and SW. United States
polyadenium	24	Polyadenia	2	C. Mexico
infundibiliforme	24	Cuneoalata	2	C. Bolivia to NW. Argentina, and N. Chile
megistacrolobum	24	Megistacroloba	8	S. Peru, and NW. Argentina
raphanifolium	24	Megistacroloba	5	S. Peru
canasense	24	Tuberosa	7	S. Peru
candolleanum	24		1	N. Bolivia
kurtzianum	24		6	W. Argentina
microdontom	24		5	CS. Bolivia, and NW. Argentina
multidissectum	24		5	S. and C. Peru
pampasense	24		3	C. to S. Peru
soukupii	24		1	S. Peru
sparsipilium	24		8	C. Peru, and C. Bolivia
vernei	24		3	NW. Argentina
tuberosum				
Group Phureja	24		7	Venezuela, Colombia, Ecuador, Peru, and N. Bolivia
Group Stenotomum	24		10	S. Peru to C. Bolivia
Group Andigena	48		7	Venezuela, Colombia, Ecuador, Peru, Bolivia, NW. Argentina, few Guatemala, and Mexico
Group Tuberosum	48		6	S. Chile to world-wide

polyadenium Greenm. 175444, 230561; *S. infundibiliforme* Phil. 265867, 283077; *S. megistacrolobum* Bitt. 233124, 233125, 265873, 275147, 275148, 275149, 283083, 283084; *S. raphanifolium* Card. et Hawkes 210048, 210049, 246539, 265862, 265878; *S. canasense* Hawkes 210035, 246533, 265864, 265875, 266385, 283074, 283080; *S. candolleanum* Berth. 243502; *S. kurtzianum* Bitt. et Wittm. 133687, 133695, 175434, 205390, 230584, (133687 × 133695); *S. microdontum* Bitt. 195185, 208866, 218224, 218226, 275151; *S. multidissectum* Hawkes 210042, 210043, 210044, 210051, 210055; *S. pampasense* Hawkes 210046, 275274, 275275; *S. soukupii* Hawkes 230511; *S. sparsipilum* (Bitt.) Juz. et Buk. 210039, 230502, 233693, 234003, 265859, 265869, 275153, 275276; *S. vernei* Bitt. et Wittm. 230468, 230562, 275155; *S. tuberosum* L. Group Phureja 195191, 225671, 225678, 225679, 225682, 225694, 225701; Group Stenotomum 195214, 205526, 230513, 234007, 234011, 234012, 234015, 258909,

[21] K. DODDS, In *The Potato and Its Wild Relatives* (Edited by D. S. CORRELL). Texas Research Foundation, Renner, Texas (1962).

[22] HAWKES, A revision of the tuber-bearing Solanums (2nd ed.); *Scottish Plant Breed. Sta. Rec.* p. 76 (1963).

258910, 258911; Group Andigena 186180, 205622, 214435, 230457, 232057, 243396, 243436; Group Tuberosum cultivars Katahdin, Norland, Ontario, Chippewa, Merrimack, Minn. 20-20-34.

Protein extracts are prepared from tubers in the following manner. Two to three grams of thin tuber slices are soaked in sodium hydrosulphite solution for 0·5 hr, rinsed three times in distilled water, drained, and macerated with a mortar and pestle. The resulting shurry is centrifuged at 500 *g* for 20 min and the supernatant containing the soluble proteins removed. Protein samples are then stored at −25° prior to the electrophoretic run.

The concentration in most samples is sufficient to allow determination of soluble proteins using 0·02–0·04 ml per gel. The standard 7·5% acrylamide gel, spacer gel and sample gel are prepared according to the directions given by Ornstein and Davis.[20] This three-layered gel stacks the proteins at pH 8·3 and runs at pH 9·5. The buffer is tris-glycine of pH 8·3 with bromphenol blue as a tracking dye in the upper reservoir. Model 300 Canalco power source permits twenty-four gels to be run simultaneously. Each gel requires 2·5–5·0 milliamps for a running time of 40–60 min. The gels are removed from their supporting tubes and stained with 1% Amino Schwartz for about 0·5 hr and are destained in 7% acetic acid, by electrophorically removing the free dye with 6·5 mA per gel. The final gels can then be stored indefinitely in 7% acetic acid or photographed for a permanent record.

[20] L. ORNSTEIN and B. J. DAVIS, *Ann. N. Y. Acad. Sci.* **121**, 321, 404 (1964).