Analysis of Soy Protein Disc Gel Electropherograms[1]

ROBERT L. ANDERSON, Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture,[2] Peoria, IL 61604

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ABSTRACT

Water-extractable, acid-precipitable, and whey proteins from soybeans were examined by disc gel electrophoresis in pH 8.9 tris-glycine buffer at gel concentrations (%T) ranging from 4 to 13. Densitometer tracings of the stained gels were used to construct Ferguson plots (log relative protein mobility [R_M] vs %T), thereby yielding relative free mobilities (Y_o) and retardation coefficients (K_R). Among gels of various %T, 28 protein bands were distinguished in the water-extractable fraction; plots were made for 18. On a single 8.5%T gel, 20 water-extracted proteins were discernible. Acid-precipitable soy proteins differed only slightly from those that were water-extractable. Ferguson plots for these fractions could be conveniently placed into seven groups based upon Y_o. The whey proteins produced five major and 11 minor bands. Twelve of the 16 whey bands produced data for Ferguson plots, which comprised three groups on the basis of K_R. The K_R and Y_o for three of the major whey bands matched those determined for water-extractable soy protein components. The remaining major whey proteins appeared to be charge isomers that were obscured by other proteins in the water-extractable fraction. A band having the K_R and Y_o of soybean trypsin inhibitor was found in all three fractions. The approach used here extends the usefulness of disc gel electrophoresis for comparing various soy protein preparations and for interpreting the effects of different treatments given soy proteins.

A variety of gel electrophoretic conditions have been used to examine soy proteins. These include starch gels containing urea and 2-mercaptoethanol (Puski and Melnychyn 1968, Shibasaki and Okubo 1966) and polyacrylamide gels (Catsimpoolas et al 1968a, Hill and Breidenbach 1974, Larsen 1967, Tombs 1967) with urea and reducing agent (Catsimpoolas et al 1967, 1968a; Eldridge et al 1966; Hill and Breidenbach 1974) or phenol-acetic acid-2-mercaptoethanol-urea (Catsimpoolas et al 1968b).

Gel electrophoretic resolution depends upon gel sieving and charge differences between proteins. The relative position of electrophoresed proteins depends, therefore, upon gel type and concentration, pH, temperature, and buffer composition; urea dissociates some proteins, which may increase the complexity of gel patterns. Drawing unequivocal conclusions regarding protein species identity from gel electrophoresis performed under a single set of conditions is not usually possible.

After electrophoresis at different gel concentrations (%T), Ferguson plots (Ferguson 1964), ie, log relative protein mobility (R_M) as a function of %T, can be constructed from the gel electropherograms. Such plots yield retardation coefficients (K_R) and relative free mobilities (Y_o). K_R is a measure of protein molecular size, and Y_o is related to electrophoretic free mobility and therefore to protein net charge (Chrambach and Rodbard 1971; Rodbard and Chrambach 1970, 1971).

I have electrophoresed water-extractable (WESP), acid-precipitable (APP), and acid-soluble (whey) soybean proteins at different gel concentrations and have used the results to construct Ferguson plots. This analysis of soy protein electropherograms provides a basis for cogent interpretation of experiments involving protein separation and resolution such as are encountered in comparisons of varietal or process-induced differences, protein fractionations, and electrophoresis-based soy protein detection methods.

MATERIALS AND METHODS

Sample Preparation

WESP was prepared by 10:1 aqueous extraction of defatted soybean flakes. The slurry was passed through cheesecloth and the exudate was centrifuged at 34,800 × *g* for 30 min. APP was precipitated from WESP with HCl at pH 4.5, dispersed in water, neutralized, stirred for 30 min, and reprecipitated with acid. Whey proteins and the washed APP were neutralized, and all three samples were dialyzed at 2-4°C against 0.06 *M* tris-HCl pH 6.7 buffer containing 0.01 *M* 2-mercaptoethanol. Most insolubles that formed during dialysis redissolved on warming to room temperature. No detectable decrease in protein concentration was produced by removal of the little remaining precipitate.

Electrophoresis Procedure

Acrylamide, N. N'-methylenebisacrylamide (BIS), N,N,N',N'-tetramethylethylenediamine, ammonium persulfate, and buffer components were weighed out fresh for each gel preparation. Both stacking and separation gels contained 5% sucrose. Using the terminology introduced by Hjertèn (1962), gel concentrations are given as

$$\%T = \frac{\text{g acrylamide} + \text{g BIS}}{\text{100 ml solution}}$$

and crosslinkage (%C) is expressed as

$$\%C = \left(\frac{\text{g BIS}}{\text{g acrylamide} + \text{g BIS}}\right) 100$$

All gels were 3%C.

Glass tubes, 110 mm × 6 mm id, were cleaned with H_2SO_4-dichromate at 90-95°C and rinsed successively with distilled water, dilute NH_4OH, and distilled water before use. Polymerization in the glass tubes yielded gels about 75 mm long.

The pH 8.9 tris-HCl buffer system of Davis (1964) was used, and the chamber buffer containing 5 drops of bromophenol blue per 500 ml was added to the upper chamber after the tubes containing polymerized polyacrylamide were in place. Sample solutions containing 50-150 μg protein and 5% sucrose were layered onto each stacking gel beneath the chamber buffer. Current was maintained at 1 mA per gel until the tracking dye had entered the separation gel, at which time the current was increased to 3 mA per gel (360-390 V). Electrophoresis was continued until the tracking dye had migrated 6 cm from the top of the separation gel (1-1.5 hr). As the tracking dye in each gel reached the 6-cm mark, the tube was closed off with a rubber cap until all tracking dye fronts in the apparatus had reached the mark. The gels were stained with 1% amido black in 7% acetic acid for 45-60 min at 20-25°C and destained for three days by diffusion into daily changes of 7% acetic acid at 20-25°C.

Ferguson Plots

Developed gels were scanned at 600 nm with a Gilford linear

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[2]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

transport system. Gel concentrations were incremented by only 0.5–1.0 %T, producing minimal pattern changes from scan to scan; the same band was, therefore, easily located on successive electropherograms in the sequence. The position of each band in relation to the tracking dye front was measured on the electropherogram, and the log of the ratio (band migration distance/tracking dye migration distance) was plotted as a function of %T; the slope yielded K_R, whereas the ordinate intercept antilog equaled Y_o. Two replicates with WESP yielded average deviations from the mean of 0.0013 and 0.062 for K_R and Y_o, respectively. Single replicates were done with APP and whey.

RESULTS AND DISCUSSION

An 8.5% T gel contains 20 WESP bands (Fig. 1), the most of any single gel, although 28 WESP bands can be distinguished among various gels ranging from 4 to 13% T. Even 28 bands probably do not represent complete resolution. Human blood serum is known to contain 100–200 proteins but only about 30 can be detected on a single gel (Kapadia et al 1974).

APP yields a disc gel electrophoretic pattern (Fig. 2) very similar to that obtained for WESP. Differences occur among the minor proteins (bands 9–15, Fig. 1). In addition, a shoulder that migrates just ahead of band 28 becomes evident in Fig. 2. Ascertaining which protein bands of Fig. 1 are absent from Fig. 2 is not possible. The bands evident in Fig. 1 probably contain several proteins, and the apparent differences may result from either a change in the relative amounts of the various components or the complete removal of

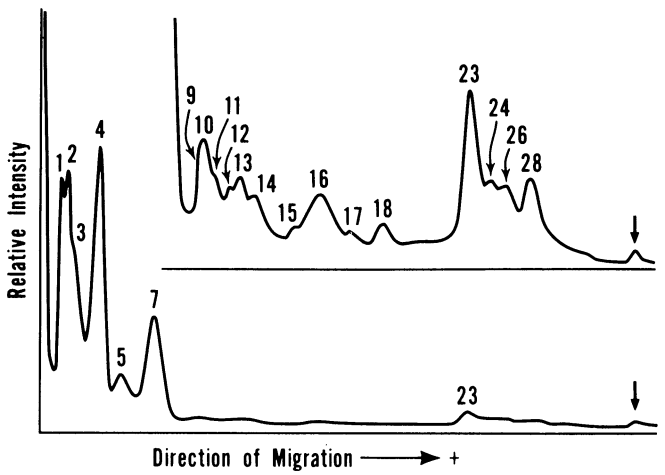

Fig. 1. Densitometer scan of water-extractable soybean proteins gel electropherogram. 50.6 μg protein sample. 8.5% gel concentration, 3.0% crosslinkage. Arrow indicates tracking dye position. Inset: tenfold scale expansion of area directly below.

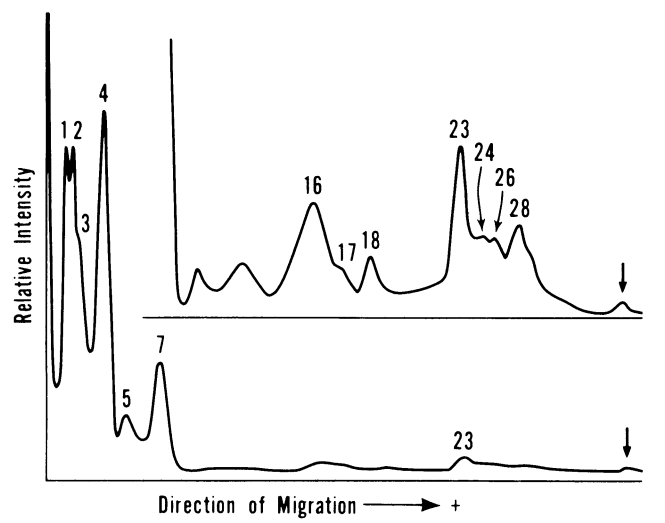

Fig. 2. Densitometer scan of acid-precipitable soybean proteins gel electropherogram. 55.0 μg protein sample. 8.5% gel concentration, 3.0% crosslinkage. Arrow indicates tracking dye position. Inset: tenfold scale expansion of area directly below.

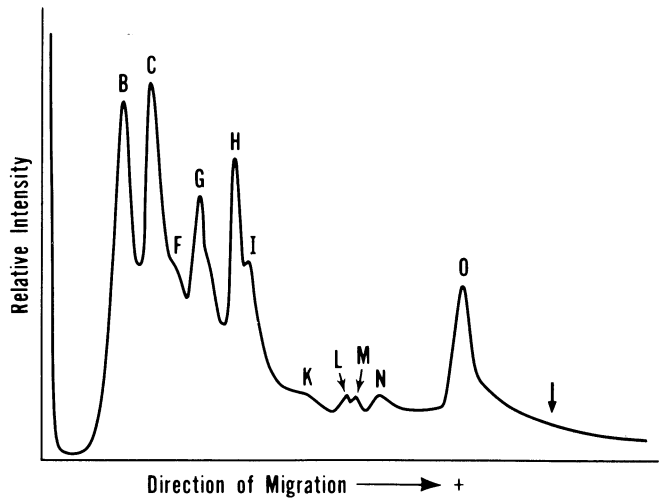

Fig. 3. Densitometer scan of soybean whey proteins gel electropherogram. 50.5 μg protein sample. 8.5% gel concentration, 3.0% crosslinkage. Arrow indicates tracking dye position. Fivefold scale expansion.

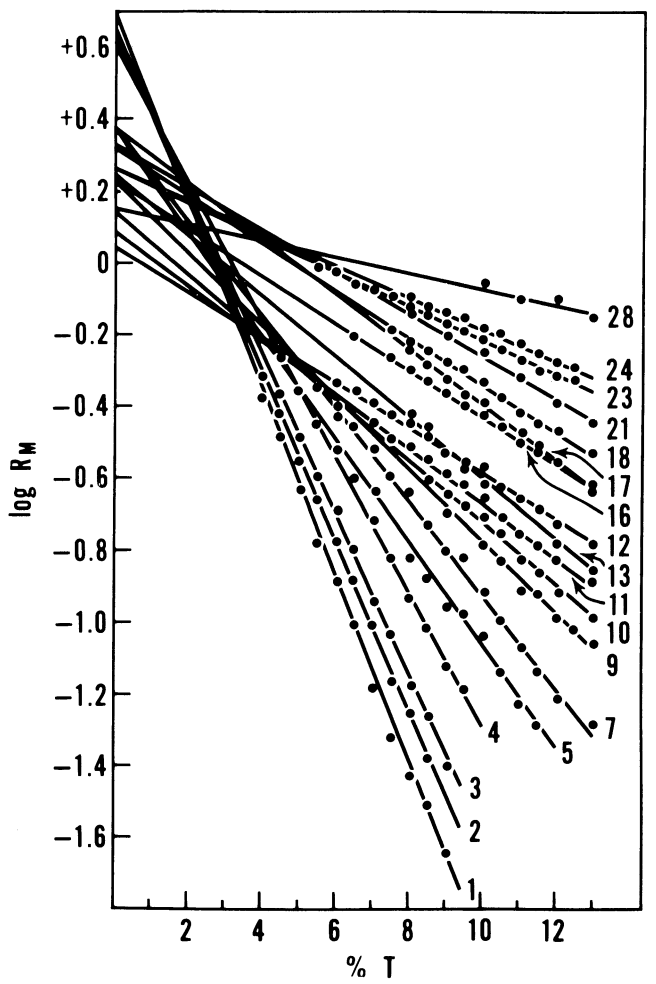

Fig. 4. Ferguson plots of soybean proteins. The numbering corresponds to Figs. 1 and 2.

some of them.

The soybean whey electrophoresis profile is shown in Fig. 3. Because of the uncertainty of band identification arising from the dissimilarity between whey and WESP patterns, letters are used for whey band labeling. According to biuret analysis (Layne 1957), 88% of WESP was recovered as APP and 12% as whey; therefore, one would not expect each protein band in the whey profile to be manifest in the WESP electropherogram.

The many proteins present in a soy extract make unambiguous identification of each species difficult. A more penetrating analysis of soy protein disc gel electrophoresis can be achieved by constructing Ferguson plots for as many of the discernible protein bands as possible. The results of such analysis for the WESP and APP bands that provided sufficient data for Ferguson plots are shown in Fig. 4 and Table I. The tabular data are arranged in the order of decreasing Y_o. The band sequence does not follow that found in the gels (Figs. 1 and 2), indicating that adjacent bands on an 8.5% T gel generally have widely different Y_o values. The results of Table I are conveniently placed into seven arbitrary groups based upon Y_o: bands 1–4; 5,7,17; 18,21; 13,23,24; 9,16; 10,28; and 11,12. Band 13 could be grouped either with bands 23 and 24 or with bands 9 and 16, because its Y_o is intermediate between the two groups. Briggs and Mann (1950) observed seven proteins in moving boundary electrophoresis.

The results in Fig. 4 and Table I suggest that before analytical gel electrophoresis, WESP and APP should first be fractionated on the basis of charge under "nonsieving" conditions such as steady-state stacking during preparative electrophoresis in agarose gel. Then the seven Y_o groups would be expected to migrate in order (Table I), with bands 1–4 moving most rapidly and bands 11 and 12 most slowly. Subsequent disc gel electrophoresis of each Y_o group could then be analyzed with Ferguson plots, and because each group would contain fewer proteins, additional minor bands might be observed. Furthermore, gel concentrations could be selected that would yield optimum separation and resolution for each Y_o group as well as more accurate K_R and Y_o values.

Ferguson plot analysis of soybean whey proteins (Fig. 5) presents a different pattern than found for WESP and APP (Fig. 4) in that the slowest bands have the smallest log R_M on extrapolation to 0%T. The Y_o and K_R values for the whey bands are listed in Table II in order of decreasing K_R. The data in Table II fall into three arbitrary groups based upon K_R: bands A, B, C, F, G; K, L, H, I; and M, N, O. Each group contains proteins that apparently differ little in size but have different charges; that is, they resemble charge isomers (Hedrick and Smith 1968). The whey contrasts with the WESP and APP fractions, which exhibit a considerable range of both size and charge among the constituent proteins (Table I); each WESP/APP Y_o group is comprised of components which, having similar charges, behave almost as size isomers (Hedrick and Smith 1968).

Whey bands G, H, K, and O appear on the basis of K_R and Y_o (Table II) to be the same as bands 10, 12, 16, and 23 or 24, respectively, found in WESP and APP (Table I). Hearing et al (1976) report that soybean trypsin inhibitor has K_R equal to 0.046 and Y_o equal to 1.90, which suggests that whey band O and WESP/APP bands 23 and 24 are trypsin inhibitors.

Curiously, APP appears to contain considerable amounts of protein bands 23 and 24 (Figs. 1 and 2), suggesting little loss of these proteins during acid-precipitation. In consonance with this observation, whey band O represents little more than one-fourth of

TABLE I
Free Mobilities (Y_o) and Retardation Coefficients (K_R) for Water-Extractable and Acid-Precipitable Soybean Proteins

Band[a]	Y_o	K_R
1	4.679	0.2574
2	4.365	0.2342
3	4.268	0.2212
4	3.936	0.1866
17	2.427	0.0785
7	2.399	0.1298
5	2.300	0.1424
21	2.149	0.0593
18	2.100	0.0663
23	1.820	0.0472
24	1.820	0.0446
13	1.758	0.0842
9	1.679	0.1002
16	1.679	0.0655
28	1.419	0.0226
10	1.396	0.0867
11	1.227	0.0756
12	1.127	0.0635

[a]The band numbers correspond to those used in Figs. 1 and 2.

TABLE II
Retardation Coefficients (K_R) and Free Mobilities (Y_o) for Soybean Whey Proteins

Band[a]	K_R	Y_o
A	0.0879	0.638
F	0.0874	1.296
B	0.0863	0.705
G	0.0857	1.412
C	0.0843	0.912
K	0.0666	1.679
L	0.0661	1.861
I	0.0652	1.296
H	0.0612	1.096
M	0.0529	1.584
N	0.0473	1.514
O	0.0452	1.774

[a]The band designations correspond to those used in Fig. 3.

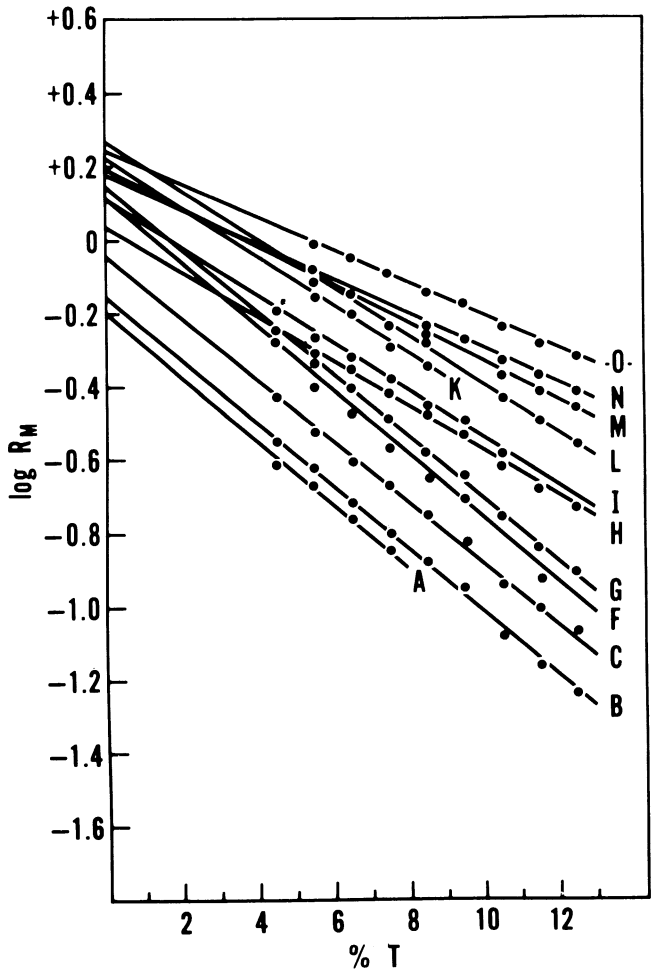

Fig. 5. Ferguson plots of soybean whey proteins. The letter designations correspond to Fig. 3.

WESP band 23 when one takes into account the difference in scale expansions between Figs. 1 and 3 and the greater than 7:1 ratio of APP/whey obtained from WESP. The emphasis in this work has been on analysis of protein band position after electrophoresis, and only tentative conclusions can be made regarding quantities of individual proteins. Nevertheless, the data suggest that a major portion of soybean trypsin inhibitor remains with the washed APP. Others have detected trypsin inhibitor activity in unheated soy protein isolates (Churella et al 1976, Rackis et al 1963, Theuer and Sarett 1970).

Whey bands B and C migrate to approximately the same position in an 8.5%T gel (Fig. 3) as do WESP bands 5 and 7, respectively (Fig. 1). Tables I and II show, however, that the whey bands are different proteins in each case than WESP protein bands 5 and 7, because the K_R and Y_o differ widely between the corresponding whey and WESP bands.

CONCLUSION

The data presented here illustrate the ambiguity associated with disc gel electrophoretic comparisons between soy protein samples at only a single gel concentration. The approach used in the present work extends the usefulness of disc gel electrophoresis for comparing various soy protein preparations and for the detection of minor changes against the background of otherwise complex patterns. Delineation of characteristic soy protein electropherogram differences presents the potential for monitoring changes that occur in a protein fraction as a result of experimental conditions. The appearance or disappearance of a single component in the complex WESP system might be observed even in instances, as is often the case, where the protein component of interest has no biological activity and one has no prior knowledge of its existence.

LITERATURE CITED

BRIGGS, D. R., and MANN, R. L. 1950. An electrophoretic analysis of soybean protein. Cereal Chem. 27:243.

CATSIMPOOLAS, N., CAMPBELL, T. G., and MEYER, E. W. 1968a. Immunochemical study of changes in reserve proteins of germinating soybean seeds. Plant Physiol. 43:799.

CATSIMPOOLAS, N., EKENSTAM, C., ROGERS, D. A., and MEYER, E. W. 1968b. Protein subunits in dormant and germinating soybean seeds. Biochim. Biophys. Acta 168:122.

CATSIMPOOLAS, N., ROGERS, D. A., CIRCLE, S. J., and MEYER, E. W. 1967. Purification and structural studies of the 11S component of soybean proteins. Cereal Chem. 44:631.

CHRAMBACH, A., and RODBARD, D. 1971. Polyacrylamide gel electrophoresis. Science 172:440.

CHURELLA, H. R., YAO, B. C., and THOMSON, W. A. B. 1976. Soybean trypsin inhibitor activity of soy infant formulas and its nutritional significance for the rat. J. Agric. Food Chem. 24:393.

DAVIS, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121:404.

ELDRIDGE, A. C., ANDERSON, R. L., and WOLF, W. J. 1966. Polyacrylamide-gel electrophoresis of soybean whey proteins and trypsin inhibitors. Arch. Biochem. Biophys. 115:495.

FERGUSON, K. A. 1964. Starch-gel electrophoresis: Application to the classification of pituitary proteins and polypeptides. Metab. Clin. Exp. 13:985.

HEARING, V. J., KLINGLER, W. G., EKEL, T. M., and MONTAGUE, P. M. 1976. Molecular weight estimation of Triton X-100 solubilized proteins by polyacrylamide gel electrophoresis. Anal. Biochem. 72:113.

HEDRICK, J. L., and SMITH, A. J. 1968. Size and charge isomer separation and estimation of molecular weights of proteins by disc gel electrophoresis. Arch. Biochem. Biophys. 126:155.

HILL, J. E., and BREIDENBACH, R. W. 1974. Proteins of soybean seeds. I. Isolation and characterization of the major components. Plant Physiol. 53:742.

HJERTÈN, S. 1962. "Molecular sieve" chromatography on polyacrylamide gels prepared according to a simplified method. Arch. Biochem. Biophys. Suppl. 1:147.

KAPADIA, G., CHRAMBACH, A., and RODBARD, D. 1974. Approaches of macromolecular mapping by polyacrylamide gel electrophoresis. Page 115 in: ALLEN, R. C., and MAUER, H. R. (eds.). Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel. Walter deGruyter and Company: Berlin.

LARSEN, A. L. 1967. Electrophoretic differences in seed proteins among varieties of soybean, Glycine max (L.) Merrill. Crop Sci. 7:311.

LAYNE, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. Methods Enzymol. 3:447.

PUSKI, G., and MELNYCHYN, P. 1968. Starch-gel electrophoresis of soybean globulins. Cereal Chem. 45:192.

RACKIS, J. J., SMITH, A. K., NASH, A. M., ROBBINS, D. J., and BOOTH, A. N. 1963. Feeding studies on soybeans. Growth and pancreatic hypertrophy in rats fed soybean meal fractions. Cereal Chem. 40:531.

RODBARD, D., and CHRAMBACH. A. 1970. Unified theory for gel electrophoresis and gel filtration. Proc. Natl. Acad. Sci. U.S.A. 65:970.

RODBARD, D., and CHRAMBACH, A. 1971. Estimation of molecular radius, free mobility, and valence using polyacrylamide gel electrophoresis. Anal. Biochem. 40:95.

SHIBASAKI, K., and OKUBO, K. 1966. Starch gel electrophoresis of soybean proteins in high concentration of urea. Tohoku J. Agric. Res. 16:317.

THEUER, R. C., and SARETT, H. P. 1970. Nutritional adequacy of soy isolate infant formulas in rats: Choline. J. Agric. Food Chem. 18:913.

TOMBS, M. P. 1967. Protein bodies of the soybean. Plant Physiol. 42:797.

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