K.E. Holt, chairman—This report requested that Method Cd 9–57 be revised to make Section B.4 show that potassium acid phthalate is used as a standard rather than sodium carbonate. This new standard is in general use for this method, and the Uniform Methods Committee recommends its adoption.

Report of the Nomenclature Committee, N.B. Knoepfler, chairman—The Nomenclature Committee strongly recommends that prompt steps be taken to encourage the use of chemically-descriptive nomenclature for fatty acids and to avoid the tendency to apply "trade" names in presentations and papers at meetings and in the Journal of this Society. The committee further recommends that, in the interest of educating and familiarizing the membership with proper nomenclature, seminars on the subject be included in programs for future meetings. This report was accepted by the Uniform Methods Committee, and their recommendations approved.

Report of the Statistical Committe, W.E. Link, chairman—The report of the Statistical Committee was accepted by the Uniform Methods Committee, which requests the Statistical Committee to investigate the needs for precision data of the current oil-refining methods.

Report of the Refining Committee, A.E. Blankenship, chairman—The report of the Refining Committee was accepted, and no changes in methods were recommended.

The report from the Executive Secretary's office, listing items distributed by the LaPine Scientific Company, shows these supplies to be in order; they are in sufficient quantities for current needs.

A request from the chairman of the Soybean Analyses Committee of the American Association of Cereal Chemists was received, asking that a joint committee (A.O.C.S.-A.A.C.C.) be established for the development of a standardized Protein Solubility Index Method. The Uniform Methods Committee recommends that an eight-member Liaison Committee be established, consisting of two representatives from each of the societies A.O.C.S., A.O.A.C., I.F.T., and A.A.C.C., with Endre Sipos as chairman. This committee is to study methods currently in use and to establish a uniform method.

Seeing a need that all thermometers be cross-indexed in the Methods H10–55 with A.S.T.M. thermometers, the Uniform Methods Committee requests the Methods editor to make all necessary additions.

J.J. GANUCHEAU
K.E. HOLT
R.J. HOULE
R.A. MARMOR
L.D. METCALF
ENDRE SIPOS
E.M. SALLEE, editor, *ex officio*
R.L. TERRILL
D.L. HENRY, chairman

Electrophoretic Fractionation of Soluble Antigenic Proteins from the Seed of *Ricinus Communis* (Castor Bean)

LAURENCE L. LAYTON, B.T. DANTE, LLOYD K. MOSS,[1] NANCY H. DYE,[1] and FLOYD DeEDS
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A water-soluble, heat-stable protein component of castor seed meal was subjected to paper-strip electrophoresis in buffers of different chemical composition, pH values, and ionic strengths. It was shown that phosphate buffer at pH 7.4 to 8.0 and an ionic strength of approximately 0.05 gave a sharp resolution of castor seed proteins into bands which would bind bromophenol blue. Spies' Allergen CB-1A was shown to be resolved into six or more components at pH 8.0. Each major component band was found to be antigenic by passive cutaneous anaphylaxis in guinea pigs that were sensitized with rabbit antiserum to the crude castor seed protein preparation. Five bands were shown to be allergenic to humans.

The results appear to support earlier observations (1,2,10) that castor bean seed allergenicity to humans may be caused by more than one antigen in Spies' Allergen CB-1A and possibly by other antigens present in the seed but either absent from, or greatly reduced in concentration in, allergen CB-1A.

The water-soluble component of Altschul's active castor seed lipase was resolved into eight component bands, two of which did not appear in the electrophotograms of the heat-processed preparation CB-1A S.R.I.

IN PREVIOUS PAPERS (1,2) are described experiments which indicate that castor bean allergy may be caused by one or more of several antigens contained principally in the seed. The observations were made while the Schultz-Dale technique was used as directed by Coulson (3) for testing the fractions obtained by chromatography of castor seed allergen CB-1A on diethylaminoethyl-cellulose. It was found that uterine strips from guinea pigs that had been sensitized to allergen CB-1A would be discharged or rendered refractory to material from certain chromatogram peaks but would react maximally when challenged with material from subsequent peaks in the same chromatogram.

Such behavior appeared to indicate that the sensitized tissue contained more than one antibody to castor seed antigens and that significant separation of the antigens in fraction CB-1A had been accomplished by chromatography. Subsequent work indicated that certain of the chromatographic fractions were not electrophoretically homogeneous and that certain serial fractions appeared to have been contaminated by traces of material trailing from the preceding peak. Nevertheless the evidence did demonstrate the presence of more than one sensitizing antigen in castor allergen CB-1A and possibly several others in the seed flour and blossoms. These observations and the conclusions of Spies, Coulson, Stevens, *et al.* (4,5,6,7) that castor seed allergenicity is dependent upon a single specific basic protein, combined in different proportions with polysaccharide, suggested the desirability of fractionating the castor seed protein by another technique.

The technique of paper-strip electrophoresis is a simple procedure, in which there need be little or no loss of material since the constituent proteins are spread out into a "spectrum" or "profile" on a paper strip. Each component band of the electrophoretic profile may be cut out and tested for specific antigenic properties. By varying the conditions of ionic

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strength, pH, time, temperature, electric current, voltage, and choice of buffering salts, the strip electrophoretograms that resulted from a variety of combinations of conditions are available either as permanent records or as records together with samples for biological testing. By the use of a continuous-flow-curtain electrophoresis apparatus one may fractionate samples that weigh several grams and with these fractions determine the chemical properties and immunological behavior of the component proteins.

Experimental

Materials Used. A sample of castor allergen CB-1A was supplied by J.R. Spies of the Eastern Utilization Research and Development Division. This material was prepared by the "-1C method," where carbonate rather than H_2S was used to remove the excess lead ion (4,5). This antigenic preparation was coded CB-1C E.U.

The soluble, heat-stabile castor seed protein (CB-1A S.R.I.) was prepared under contract at Stanford Research Institute as follows. Castor seed flour (500 g.) was suspended in boiling 80% aqueous ethanol (2 liters) and mechanically stirred in the boiling solvent for 10 min. The suspension was filtered by suction through a large coarse, sintered-glass funnel, and the solids were resuspended in 1 liter of boiling 80% ethanol for an additional 10 min. and filtered. The bright-yellow solutions fluoresced blue at 365 mμ. They were combined and evaporated *in vacuo* to a paste (53.8 g.). The paste contained antigenic material and nonspecific irritants, one of which was previously shown to be ricinoleate. It occurred as the calcium salt, alkali metal salts, and free ricinoleic acid, in all three of which forms it elicited a histamine-like contraction in the Schultz-Dale test on the uterine horns of nonsensitized guinea pigs (8).

The extracted castor seed flour was mechanically stirred for 4 hrs. with 3 liters of distilled water, sedimentable material was separated by centrifugation, and the supernatant solution was decanted into a 5-liter Erlenmeyer flask and autoclaved for 10 minutes at 100°C. After it was cooled and refrigerated over-night at 5°C., there was no evidence of a coagulum or precipitate. The solution was concentrated *in vacuo,* then adjusted to a concentration of 80% ethanol. Two precipitate fractions formed; the first, Fraction A, formed immediately and was a fine, light-tan material weighing 11.5 g.; the second, Fraction B, formed more slowly and was a granular paste weighing 33.7 g. Fraction B was dissolved in a minimum volume of distilled water, and the solution was boiled gently for 10 min. to remove ethanol. After the solution was cooled to 5°C., it was centrifuged at 16,000 r.p.m. in the SS-1 head of a Servall centrifuge to assure a clear solution. It was then freeze-dried (lyophilized in 10-ml. portions in Vir-Tis artery tubes. Very fluffy, white flakes of crude protein were produced in approximately 70% recovery from Fraction B. The resultant material was found to be extremely soluble, dissolving rapidly in water. The product thus obtained had the following physical constants.

Ultraviolet Spectra

$E^{1\%}_{1\,cm.}$ 3.38, $\lambda_{max.}$ 276 mμ in water;

$E^{1\%}_{1\,cm.}$ 4.35, $\lambda_{max.}$ 293 mμ in 0.1 N. NaOH.

Optical Activity

$[\alpha]^{30}_{D}$ −44.4° (C, 1.0; water).

Anal.

(Freeze-dried sample.) Found: C, 41.5; H, 7.23; N, 15.5; S, 1.75. This material was coded CB-1A S.R.I.

Castor Seed Lipase. A sample of partially-purified active castor seed lipase was obtained from A.M. Altschul of the Southern Utilization Research and Development Division. This sample had been prepared from homogenized, unheated castor endosperms by separating the castor cream that contained the oil and by removing the fat by ether extraction. The active lipase preparation had been dialyzed against water and dried at reduced temperature. Without any additional treatment the lipase was suspended in distilled water (3 mg. per ml.) and stirred for half an hour. The insoluble material (probably the active lipase) was removed by centrifugation. The clear supernatant solution from 3 mg. of the lipase was lyophilized and adjusted to 0.02 ml. of solution for each strip of the electrophoresis run.

Apparatus and Methods for Paper-Strip Electrophoresis and Evaluation of the Electrophoretograms. The fractionation apparatus used in this investigation was the Durrum hanging strip-paper electrophoresis cell Model R, manufactured by Spinco Division of Beckman Instruments Inc. Spinco's Model RD-2 Duostat was used as the power supply unit. The method of analysis employed was that described in Spinco's Technical Bulletin No. TB 6050A with several modifications. Whatman 3 MM filter paper strips (3.0 x 30.6 cm.) were utilized as the supporting medium. Each electrophoresis was run for 24 hrs. at a constant current of 10 ma. The protein sample applied to each strip was either 0.50 mg. or 1.0 mg. in 0.01 or 0.02 ml., respectively, of doubly distilled water. The electrophoretograms were stained with bromophenol blue dye (Spinco B-4). The stained electrophoretograms were evaluated for protein-dye intensity by a Spinco direct integrating Analytrol Model RB densitometer, using the 500 mμ filters and the B-5 cam. This technique produces records with a linear relationship to dye concentration. Protein samples weighing 0.5–1.0 mg. were found to give satisfactory separation of components and kept the densitometer tracing below the 14-cm. mark on the chart.

In electromigration there are many factors which must be taken into account. Of these, the most important is the environment, principally the buffer, in which the migrant is placed. Proteins exist in aqueous solutions as electrically-charged entities, and the magnitude and sign of the charge depends upon number and types of ionizable groups present in the protein and on the pH and ionic strength (μ) of the buffering medium. Furthermore certain buffer ions themselves are capable of binding or complexing with the protein and thus affect the migration.

Other factors which contribute to differences in the electrophoretic behavior of the components of a complex material include: duration of migration, type of supporting medium, wetness of paper, temperature, evaporation, electric current flow, and potential gradient. The Schleicher and Schuell 2043A-mgl. paper, which is the type recommended for the technique and

equipment used in Spinco's "Procedure B" specifically designed for serum proteins, was unsatisfactory for this particular study on castor seed proteins. The densitometer response was not great enough for a good quantitative or qualitative interpretation. Whatman 3 MM paper, a thicker paper, was tried and was found to be satisfactory.

The buffer was applied at the mid-line of the strip and allowed to run freely and evenly onto the strip. An equilibration time of one-half hour, instead of the 15 min. specified in Spinco's "Procedure B," elapsed before the sample was added. At the termination of each run the strips were quickly and carefully removed from the cell and immediately dried 30 min. in an oven set at 130°C.

All experiments were performed at an ambient temperature of 20°C. ± 2°C., and a draft cover was used to reduce any convection currents and more or less to stabilize the cell temperature. Since the Spinco cell, Model R, is an enclosed chamber, loss of water by evaporation from the paper strips is minimized. In the course of a run, equilibrium in evaporation-condensation is reached and is maintained nearly constant by the use of the draft cover, thus avoiding change in ionic strength.

Tests for Antigenicity. In several electrophoretic fractionations run in phosphate buffer at pH 7.4 and $\mu = 0.05$, alternate electrophoretic strips were stained with bromophenol blue and the intervening strips were left unstained. Bands corresponding to the stained bands were cut from the unstained strips, and corresponding bands were pooled and extracted with a physiological salt solution. Each group of pooled extract was tested for antigenicity by passive cutaneous anaphylaxis tests (9) in guinea pigs, which had been passively sensitized by intracutaneous injections of rabbit antisera to CB-1C E.U. and CB-1A S.R.I., respectively. Each pig was also injected in two other sites with control serum from an unsensitized rabbit, hence each pig served also as its own control.

Results and Discussion

In Table I are voltage readings that were observed during electrophoretic runs made under the conditions of pH, buffer, and ionic strength (μ) indicated.

TABLE I

Values for pH, Buffers, Ionic Strengths (μ), and Resultant Voltage Readings for the Castor Seed Protein Electrophoresis Runs Conducted at a Constant Current of 10 ma. for a Duration of 24 Hours, Using Whatman 3MM Paper Strips

pH	Buffer[a]	Ionic Strength (μ)	Volts		
			Initial	Intermediate[b]	Final
3.0	Citrate	0.092	145	.....	92
5.0	Citrate	0.209	65	.....	51
5.0	Citrate	0.102	110	.....	78
6.0	Phosphate	0.056	114	.....	89
7.4	Phosphate	0.051	290[c]	.....	190[c]
8.0	Borate[d]	0.004	292[d]	.....	>500[d]
8.0	Borate	0.094	136	118	131
8.0	Phosphate	0.097	89	.....	66
8.0	Phosphate	0.048	170	.....	102
9.0	Borate	0.114	122[c]	106[c]	172[c]
11.0	Phosphate	0.158	71	.....	54
11.0	Phosphate	0.079	128	.....	84
11.0	Phosphate	0.048	202	.....	130

[a] All buffers are standard buffers or dilutions thereof and were prepared according to directions given in W.M. Clark's "The Determination of Hydrogen Ions," pp. 192–220 (The Williams and Wilkins Company, Baltimore, Md., 1928).

[b] Intermediate voltage readings after approximately 6 hrs. duration are given for those runs where the voltage did not continue to decrease with time. The increase occurring over-night may be caused by polarization effects and the formation of electrolysis products along the electrode wires.

[c] These values are averages of several electrophoreses conducted under identical conditions.

[d] The ionic strength (μ) of this borate buffer was so low that the potential was greater than the 500-volt limit of the Duostat at a constant 10-ma. operation. The run was thus carried out initially at a constant 2.5 ma.; the voltage gradually increased and after 6 hrs. the amperage was lowered to 1.0 ma.

Phosphate buffer (pH 7.4, $\mu = 0.05$), was selected as the starting-point for our investigations. CB-1A S.R.I. (Figure 1) was found to be resolved into six distinct electrophoretic bands and possibly two additional very lightly-stained bands. These latter two bands were only faintly visible to the eye, did not photograph well, and did not give significant readings on the densitometer. Castor preparation CB-1C E.U. separated into two distinct broad bands, one cationic, the other anionic (Figure 2). These bands appeared to possess structure which greatly increased their width; the banded structure in Spies' allergenic preparation, CB-1C E.U., became more distinct in the electrophoretogram ran at pH 8.0 (Figure 3).

Inspection of the electrophoretogram of the water-soluble components of the castor seed lipase preparation suggested that it is qualitatively very nearly

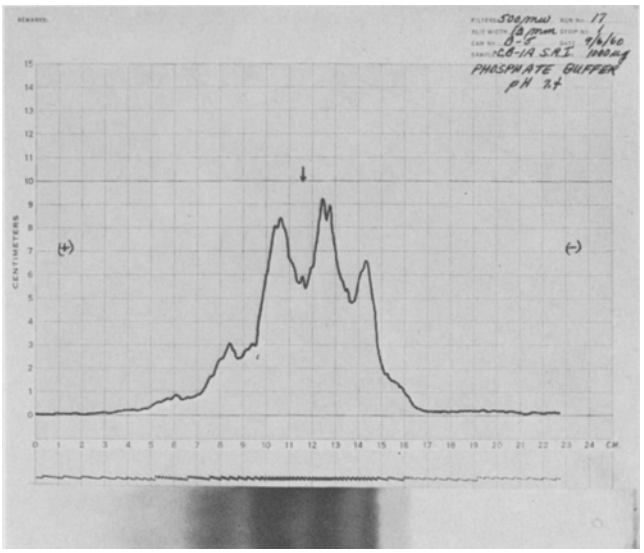

FIG. 1. Electrophoretogram of crude castor seed protein CB-1A S.R.I. in phosphate buffer at pH 7.4 and ionic strength of 0.051.

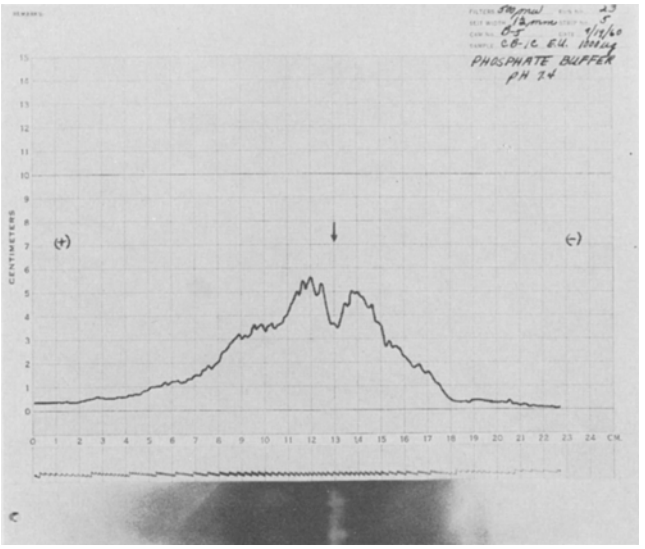

FIG. 2. Electrophoretogram of allergen CB-1C E.U. in phosphate buffer at pH 7.4 and ionic strength of 0.051.

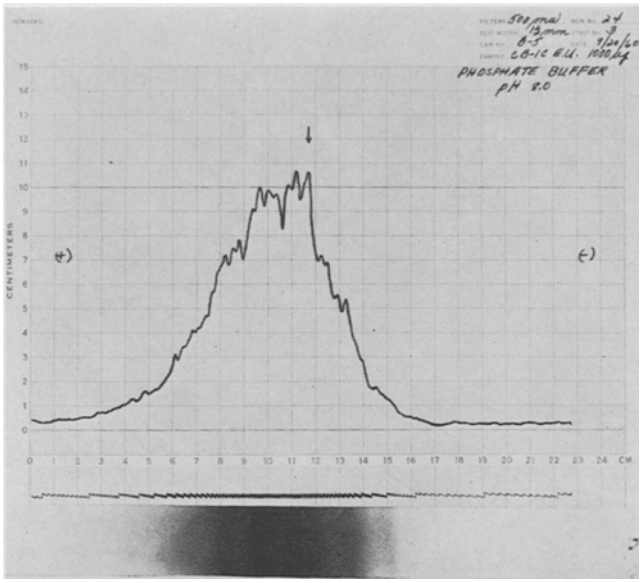

FIG. 3. Electrophoretogram of allergen CB-1C E.U. in phosphate buffer at pH 8.0 and ionic strength of 0.048.

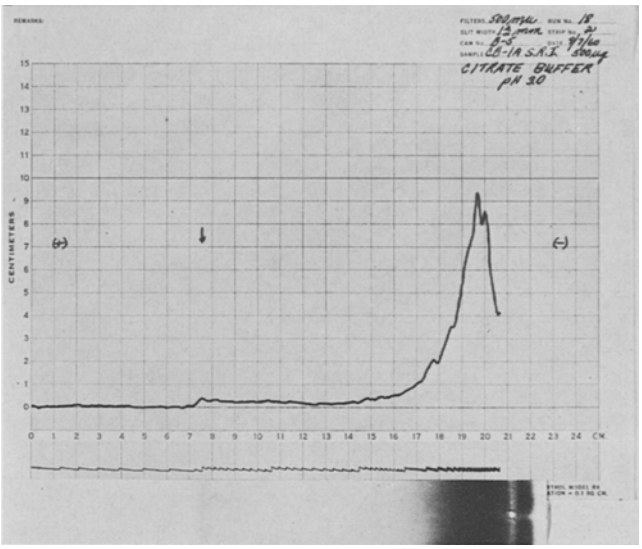

FIG. 5. Electrophoretogram of crude castor seed protein CB-1A S.R.I. in citrate buffer at pH 3.0 and ionic strength of 0.092.

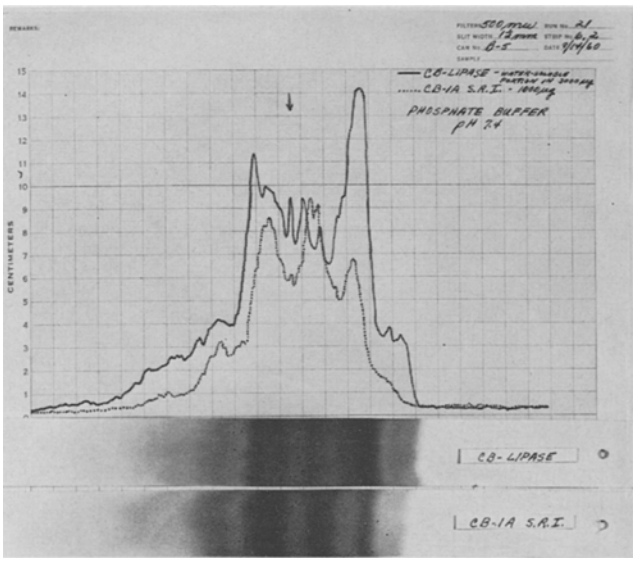

FIG. 4. Electrophoretograms of crude castor seed protein CB-1A S.R.I. and castor seed lipase in phosphate buffer at pH 7.4 and ionic strength 0.051.

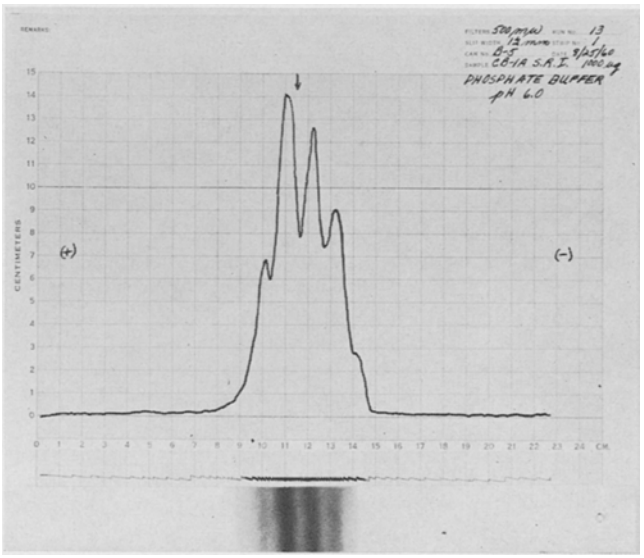

FIG. 6. Electrophoretogram of crude castor seed protein CB-1A S.R.I. in phosphate buffer at pH 6.0 and ionic strength of 0.056.

identical with our crude castor protein, CB-1A S.R.I. The two electrophoretograms and redrawn densitometer charts are shown together in Figure 4, where corresponding bands are aligned and the origins are superimposed in the chart.

The effect of pH upon the electrophoretogram of CB-1C E.U. has been briefly mentioned (compare Figures 2 and 3). At pH 3.0 (citrate buffer, μ =0.092), the three materials under examination traversed a remarkably great distance toward the cathode without resolution; some of the sample probably even passed into the wick. This high mobility indicates that at this pH the proteins bear a relatively-large net positive charge. Figure 5 presents the electrophoretogram and densitometer chart for CB-1A S.R.I., and the behavior of this material at pH 3 was found to be characteristic for the other materials tested.

Upon use of a citrate buffer of pH 5.0 with μ = 0.209, very little, if any, migration from the origin took place during the course of the run. In fact, the band obtained for each material appeared very similar to the ordinary diffusion pattern, which occurred when the material was applied to the strip and left undisturbed for 24 hrs. without any passage of electric current. The citrate buffer was diluted (μ = 0.102), and another run was carried out but only slight migration took place. These results seem to indicate that pH 5.0 is very near the iso-electric point of the proteins. If the complexity of the CB-1A S.R.I. material is considered, this is a surprising situation; it is possible that the components are associated in a complex at this particular pH value. This aspect of the problem is currently being studied.

At pH 6.0 (μ = 0.056) a phosphate buffer produced electrophoretograms with sharp resolution of the com-

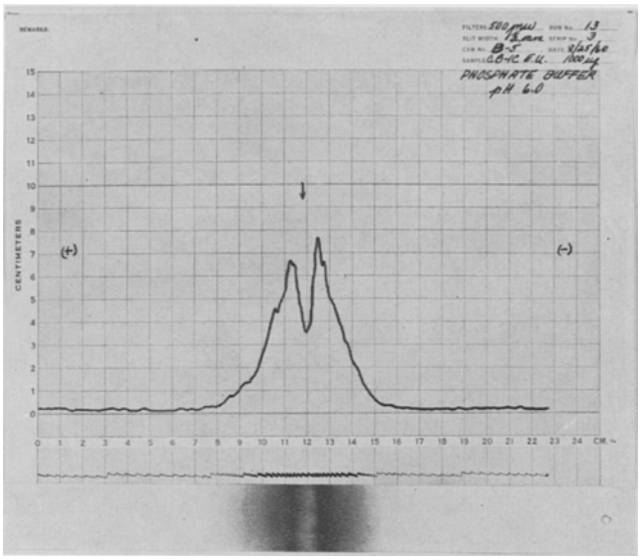

FIG. 7. Electrophoretogram of allergen CB-1C E.U. in phosphate buffer at pH 6.0 and ionic strength of 0.056.

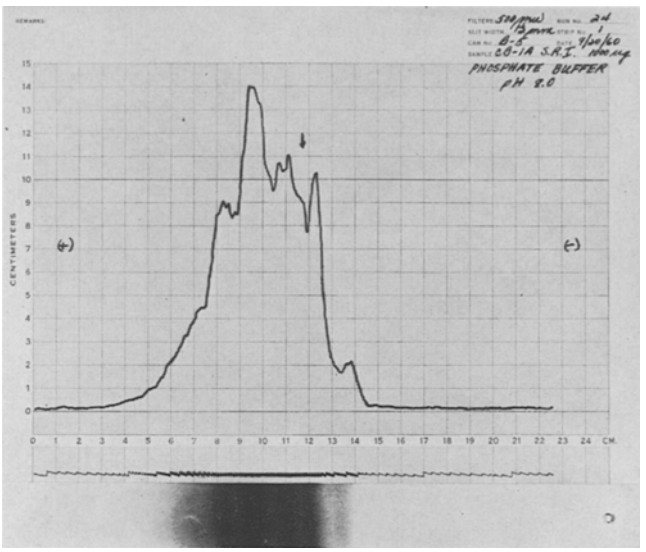

FIG. 9. Electrophoretogram of crude castor seed protein in phosphate buffer at pH 8.0 and ionic strength of 0.048.

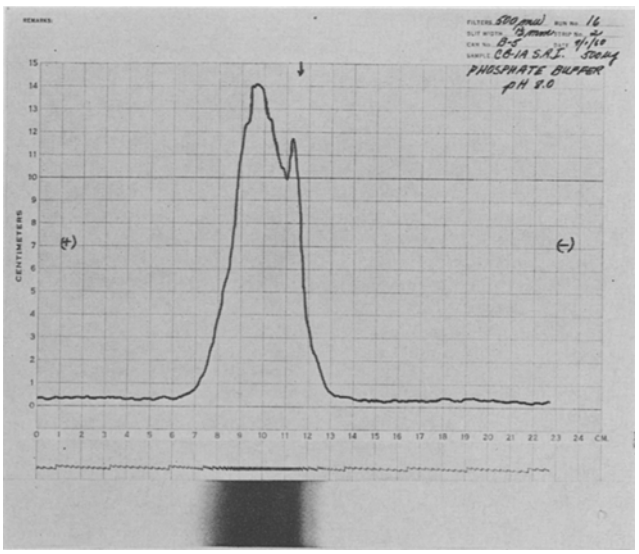

FIG. 8. Electrophoretogram of crude castor seed protein CB-1A S.R.I. in phosphate buffer at pH 8.0 and ionic strength of 0.097.

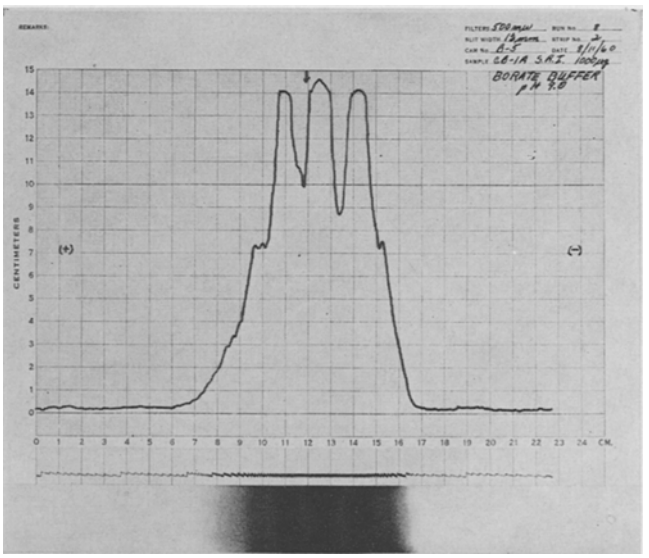

FIG. 10. Electrophoretogram of crude castor seed protein CB-1A S.R.I. in borate buffer at pH 9.0 and ionic strength 0.114.

ponents of CB-1A S.R.I. (Figure 6). It is noteworthy that the migration distances, even at this low ionic strength, were not great since pH 6.0 is not far removed from the apparent iso-electric point of the proteins. Of particular interest was the densitometer scan of the CB-1C E.U. electrophoretogram (Figure 7) with its two broad but distinct peaks separated by a deep valley, which occurred at the origin; corresponding bands can be seen in Figure 6.

A phosphate buffer of pH 8.0, and $\mu = 0.097$ was tested and gave poor separation of components; this electrophoretogram is shown in Figure 8. The buffer was then diluted to an ionic strength of 0.048, which brought about very sharp separation of components (Figure 9). It will be noted in Figure 3 that a banded structure for CB-1C E.U. became apparent at this pH and ionic strength.

Borate buffer of pH 9.0, $\mu = 0.114$ has been reported to produce good resolution of serum proteins, and runs were made to determine what effects occurred with castor seed proteins. The migration distance was not great, but separation into five or six distinct bands was obtained with CB-1A S.R.I. No separation of any significance was obtained on the CB-1C E.U. in this buffer. The borate ion apparently causes artifacts, possibly because of formation of organic borate complexes. This may have been indicated by the anomalous migration of the protein in this buffer. In borate at pH 9.0 the bands moved toward the negative pole (Figure 10), yet the same proteins in alkaline phosphate or NaOH solution migrated toward the positive pole.

Phosphate buffers at pH 11.0 with different ionic strengths were studied; the CB-1A S.R.I. components all moved toward the anode as expected for this pH. The first buffer, $\mu = 0.158$, gave no resolution into bands. Upon dilution to $\mu = 0.079$, bands were detectable, and, upon further dilution to $\mu = 0.048$, the bands were very well separated (Figure 11).

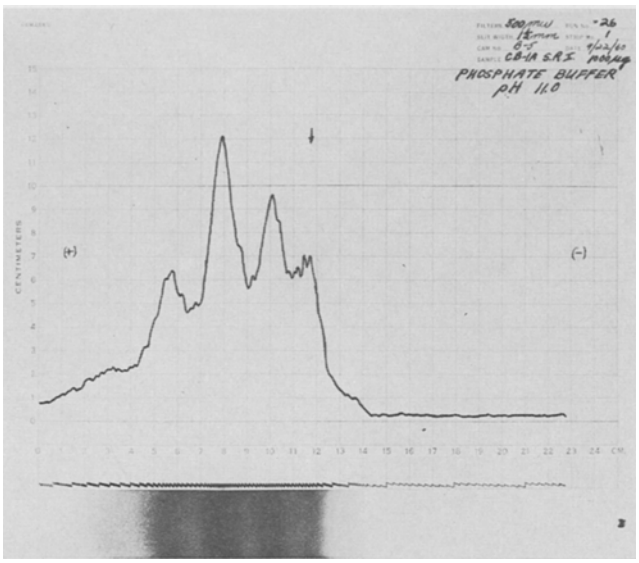

FIG. 11. Electrophoretogram of crude castor seed protein in phosphate buffer at pH 11.0 and ionic strength 0.048.

Spies and coworkers (6) designed their purification procedure on the premise that the castor seed allergen was a basic protein that was combined ionically in various proportions with polysaccharidic carbohydrate. Their stated objective was to free the specific allergenic protein from the acidic polysaccharide in order to determine the role of the polysaccharide moiety. At completion of their electrophoretic purification they combined the contents of the four cells on the cathodic side of the six-cell electrophoresis train in order to to recover the protein relatively free of the acidic component in the anode cells. In this step of the procedure no attempt was made to determine whether or not protein fractionation had occurred.

Electrophoretic procedures used by the authors of this paper utilized different buffers with several different pH values and ionic strengths, hence would be expected to reveal any existing electrophoretic heterogeneity. The banded structure in the strip electrophoretograms was clearly visible, little or nothing was lost, and each and every band was easily available for isolation and immunological testing of the protein.

Each band of the electrophoretograms of CB-1C E.C. and CB-1A S.R.I. ran at pH 7.4, and at pH 8.0 ($\mu = 0.05$) was tested for antigenicity in passively sensitized guinea pigs as described. Each and every band elicited a positive reaction in the passive cutaneous anaphylaxis test. This indicated that all of the dye-binding bands contained antigen.

Similar tests were run on Philippine monkeys passively sensitized with serum from castor bean-allergic humans; these tests indicated that at least five distinct allergenic specificities were affecting allergic humans.

The biological testing techniques used for human allergic sera and the evidence of resolution of the protein into immunologically specific allergenic components will be discussed in separate papers (9,10).

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Sucrose Ether- and Ester-Linked Surfactants

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Several effective nonionic surface-active agents in which a single sucrose moiety is the only solubilizing group were described. Alkylsucroses, prepared *via* sodium sucrate in dimethyl sulfoxide (DMSO) and higher alkyl bromides, are believed to be the first sugar-based surfactants which are at least equivalent to the best anionics and nonionics in cloth detergency on the basis of laboratory data; they were also active lime-soap dispersants. Another ether-linked type, the (3-alkoxy-2-hydroxypropyl) and (2-hydroxyalkyl) sucroses, derived from glycidyl ethers or olefin oxides, although second in detergency to the first type, included exceptional nonionic lathering agents. t-Dodecylbenzylsucrose, from dodecylbenzyl chloride, was also a rather active detergent and lime-soap dispersant. Less active but easily prepared sucrose half-esters from alkenylsuccinic anhydrides were described.

One sucrose moiety in ether-linked surfactants was an effective hydrophile for alkyl hydrophobes at least as large as hexadecyl, but tridecyl derivatives were superior in activity.

Major importance was attached to the choice of solvent, catalyst, and time/temperature factors. Evidence was presented to show that sucrose derivatives were partially degraded by the alkaline conditions required in this work and that this degradation was both detrimental to detergency and initially beneficial to lather in specific cases.

An improved procedure involving the use of DMSO for the alkaline dehydrochlorination of higher alkoxychloropropanols to glycidyl ethers was described.

MANY SURFACE-ACTIVE DERIVATIVES of sucrose and other sugars or sugar derivatives have been described in the literature. These may be divided broadly into two types: simple, in which the saccharide is the only solubilizer, and mixed or complex, which contain additional hydrophilic groups in addition to the carbohydrate. No attempt will be made to summarize this voluminous detail. An excellent recent review (3) has included this field within the broader one of long-chain derivatives of mono- and oligosaccharides.

Among all of these varied products, only simple sucrose mono-esters of fatty acids (17) have received widespread attention as surfactants. These com-