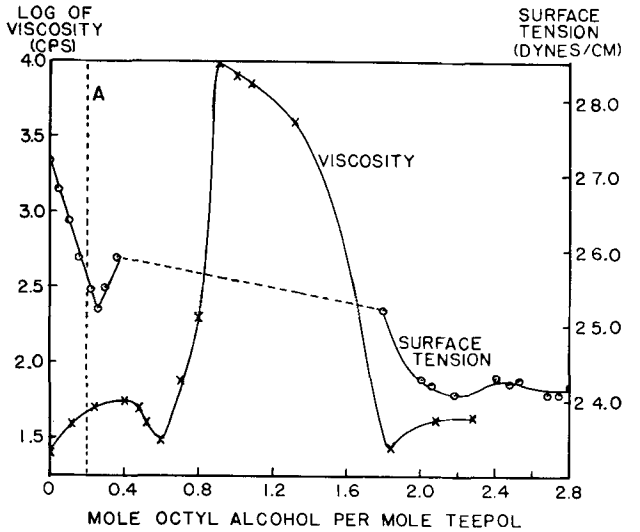

FIG. 3. Effect of n-octanol upon the viscosity and surface tension of 36% Teepol at 25° C. (2)—estimated values. A is the concentration corresponding to the first appearance of liquid crystals.

amined experimentally in sufficient detail to justify inclusion here. With sucrose monotallowate there was no viscosity minimum possibly due to the inherently high viscosity of aqueous solutions of sucrose monotallowate alone. The viscosity curve for octanol shows a secondary maximum at the point where liquid crystals appear, followed by a minimum, and then a large increase in viscosity to a maximum, with further additions.

Discussion

It has been shown that water-soluble nonionic surfactants, similar to their water-insoluble counterparts, form liquid crystals in aqueous solutions of sodium dodecylbenzene sulfonate. However, with essentially the same hydrophobic group, considerably higher molar concentrations of the soluble additives were required for the first appearance of liquid crystals.

The data show that the additions of nonionic polar compounds to solutions of sodium dodecylbenzene sulfonate result in substantial alterations in the properties of these solutions. The changes observed provide background information that may be helpful in the formulation of liquid detergents. Speculations of a more fundamental nature have not been presented, because of the complexities of these systems. There is insufficient knowledge of the micellar properties in concentrated solutions of even the most simple systems. When such information becomes available, these data may provide greater insight into the effect of nonionic polar compounds on micellar properties in concentrated solutions.

Acknowledgment

This investigation forms part of a program of applied research sponsored by the National Renderers Association. It was originally presented before the Division of Colloid and Surface Chemistry of the American Chemical Society.

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[Received May 1, 1961]

Passive Cutaneous Anaphylaxis in the Detection of Seed Antigens of *Ricinus Communis* (Castorbean).

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The phenomenon of passive cutaneous anaphylaxis in guinea pigs was shown to be applicable in the laboratory determination of residual antigenicity in fractionated and chemically treated castor-seed proteins. Six protein fractions obtained by paper-strip electrophoresis of castor-seed protein were shown to be antigenic.

Castor-seed meal which had been cooked 20 minutes at 100°C. in $Ca(OH)_2$ solution at pH 12.4 was shown to retain some of its original antigenicity, while meal cooked for 32 minutes under the same conditions of pH and temperature would not elicit the P.C.A. reaction in guinea pigs sensitized with rabbit antiserum to castor-seed protein.

WHEN BLOOD from an allergic human is transfused into a non-allergic human the recipient frequently becomes temporarily allergic to the antigen responsible for hypersensitivity in the donor (1). If blood serum from the allergic indi-

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vidual is injected into the skin of a non-allergic volunteer the skin of the recipient may become sensitized in the immediate area of the serum injection. These phenomena are referred to as "passive sensitization." The passively sensitized skin sites will often exhibit an inflammatory reaction with wheal formation when injected directly into the sensitized site with a minute quantity of the substance(s) causing allergy in the serum donor (2). This particular direct local application of the phenomenon of passive cutaneous anaphylaxis (P.C.A.) is referred to as the "Prausnitz-Küstner" test, or the "P K" test. The P K test has been used extensively as a diagnostic aid in determining the identity of antigens responsible for allergies, and is especially useful in the allergies of infants and young or debilitated children.

Layton and coworkers (3) have reported that serum from castor-allergic humans will sensitize the skin of the Philippine crab-eating monkey, *Macaca irus,* to castor-seed antigens. They have used P. K tests on passively sensitized monkeys along with direct skin testing on humans in an allergy screening program conducted upon employees of a castor-oil producing factory.

Ovary (4) has described the immunological and physiological phenomena associated with local interactions between antigen and rabbit serum antibodies in the skin of guinea pigs. He suggests that a colloidal dye such as Evans Blue be injected intravenously along with the antigen in order to make easily visible the inflamed area resulting from the antigen-antibody reaction. The dye leakage into the extravascular spaces results from the increased permeability of the minute blood vessels in the inflammed area. In order for the dye leakage accompanying the P.C.A. reaction in guinea pigs to be visible, it is necessary that albino or predominantly white animals be used. (Spotted animals may be utilized provided their skins are unpigmented in the areas to be used for the tests.) Male and female guinea pigs react in the same way and to the same degree in the P.C.A. tests, hence no great effort usually is made to have equal sex distribution.

In passive cutaneous anaphylaxis tests for homologous antibody-antigen reaction in guinea pigs sensitized with rabbit antisera, an interval of three hours should be allowed to elapse after the antiserum injections and before the challenging injection of antigen and dye is made. The 3 hour latency period allows time for necessary antibody attachment to the cells and for the disappearance of the primary inflammation due to the insertion of the needle and the injection of the serum. Sera should be diluted with isotonic saline solution in order that the primary inflammatory reaction due to the serum itself shall have subsided within the period of 3 hours. Satisfactory dilutions for rabbit sera are in the range of 1:4 to 1:100. The limit of dilution permissible will vary directly with the antibody content of the serum, and may be determined by empirical tests upon each sample used.

In this report we describe the application of the phenomenon of passive cutaneous anaphylaxis to the problem of detecting residual native protein antigenicity in castor-seed proteins which have been subjected to chemical and physicochemical treatments.

Experimental

Crude Castor-Seed Protein. Crude castor-seed protein was prepared from fat-free castor seed flour by a method which has been described in a previous paper (5). The crude castor protein, designated by code CB-1A-SRI, was water-soluble and free of the toxic albumin ricin. This protein was subjected to paper-strip electrophoresis (5) in phosphate buffer at pH 7.4 and ionic strength 0.051 for 24 hrs. at an ambient temperature of 20° ± 2°C. Electric current flow was held constant at 10 ma; the potential difference was 290 volts at the beginning of the run and 190 volts after 24 hrs. operation. For each strip the protein sample size was 1.0 mg. dissolved in 0.02 ml. of distilled water. The apparatus used was a Durrum hanging-strip electrophoresis cell. The electrophoretograms were stained with bromphenol blue dye and evaluated with a direct integrating densitometer. An electrophoretogram and the corresponding densitometer chart are shown in Figure 1.

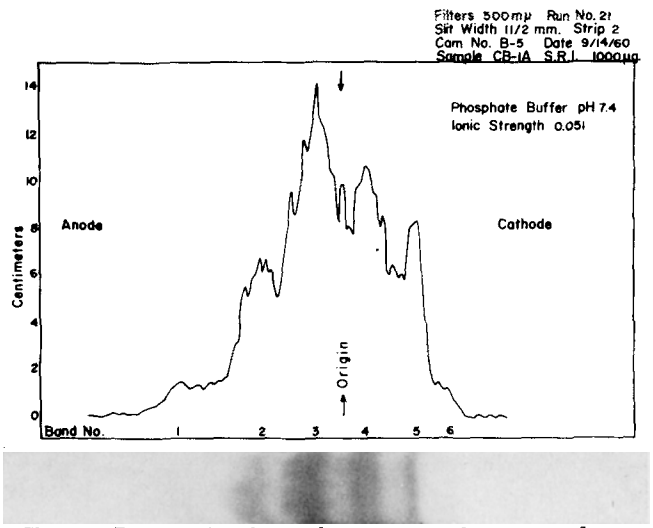

FIG. 1. Paper-strip electrophoretogram of castor-seed protein, CB-1A S.R.I., and the corresponding densitometer chart. The arbitrarily numbered bands are those referred to in the text.

The marked reproducibility of the band structure in the electrophoretograms indicated that corresponding bands in the different strips represented the same components of the protein mixture. The bands were arbitrarily numbered 1 to 6 from left to right (anode) to cathode) for ease of reference. Alternate strip electrophoretograms were stained, and the corresponding bands and areas were cut from the stained and unstained strips respectively. Bands with the same number designation were pooled for extraction of protein and subsequent testing in sensitized animals. Preliminary testing showed that the bromphenol blue stain did not interfere with the P.C.A. test, hence in subsequent runs all of the strips were stained for more precise isolation of the bands.

On the densitometer chart in Figure 1, the height of the curve represents the concentration of protein-bound dye in the corresponding band of the strip-electrophoretogram shown. The areas under the curve represent the total quantity of dye bound by the protein in the corresponding band. The total area under the curve represents the weight of dye-binding protein in the sample, assuming that the components have the same dye binding capacity. (This is not always a valid assumption.)

Alkali-Cooked Castor Meal. Gardner and coworkers (6) have shown that alkaline cooking of castor meal will destroy the antigenic constituents. Spies and coworkers also have suggested that $Ca(OH)_2$ may be a satisfactory base for alkaline inactivation of castor allergens (7). Alkali-cooked castor-seed meal was prepared from domestic commercial castor-seed meal which previously had been hexane extracted for oil removal. The residual pomace, containing approximately 2% fat, was cooked at 100°C. with four volumes of 8% $Ca(OH)_2$ slurry at pH 12.4. Samples were removed after 20 min. and 32 min. They were adjusted to pH 6.8 with phosphoric acid. After low-speed centrifugation, the supernatant solution was decanted and evaporated *in vacuo* to give a 5-fold increase in concentration; each ml. of concentrate was the material extracted from 1.25 g. of pomace containing 430 mg. of protein prior to the cooking. It was determined that 1 to 2 ml. of the clear extract concentrates could be injected intravenously into 300-g. unsensitized guinea pigs without causing anaphylactoid reaction or any other apparent ill effect.

Antisera to Castor-Seed Antigens. Anti-castor serum was prepared from the blood of rabbits which had been receiving intraperitoneal injections of the crude castor-seed proteins over a 6-month period. Serum samples which had been stored frozen for more than a year were found to be immunologically as active as freshly prepared sera. Control serum was prepared from the blood of uninjected rabbits.

Passive Sensitization of Guinea Pigs' Skin. Male and female albino guinea pigs weighing from 200 to 300 g. were purchased in lots of 30 animals each. The abdominal skin of each was freed of hair so that the skin itself was clearly visible. The abdomen was rinsed with 60 percent ethanol and the damp skin marked off with a red wax pencil into six rectangular areas, three on each side of the mid-line. One site in each area was injected with rabbit anti-castor sera or control sera approximately 0.05 ml. of 1:10 dilution was injected into each site. Caution was exercised to prevent the serum from being injected subcutaneously; the immediate formation of a burn-like blister of serum was considered sufficient evidence of intradermal injection. Usually two different sites were injected with each of two rabbit anti-castor sera and two sites with serum from a non-sensitized control rabbit. For preliminary screening, animals were frequently injected with five different antiserum samples and only one control. When only one antiserum was being used—often—three nonadjacent sites were injected with the antiserum and the other three with control serum.

Testing for Antigen by Passive Cutaneous Anaphylaxis. Each skin-sensitized guinea pig was bound, abdomen down, onto a small-animal operating table. The hair was clipped from the foreleg just above the wrist where the cephalic vein can be detected under the skin. The skin was pinched and lifted by the index finger and thumb and cut in a v-shape with one snip of the scissors to expose the vein and its bifurcation over the radius. Ordinarily 1.0 ml. of isotonic saline containing 0.5% Evans Blue dye and an appropriate amount of the material suspected to contain the castor antigens was injected intravenously with a tuberculin syringe and a No. 27 needle. A positive reaction for passive cutaneous anaphylaxis was indicated within two to five minutes by bluing of the skin around the site of antiserum injection; the sites injected with the control serum did not turn blue. The P.C.A. test was shown to be sufficiently sensitive for the detection of 1.0 microgram of antigenic protein injected intravenously. The sensitivity of the test can be extended to 0.1 microgram by microscopic examination of the site from the underside of the skin.

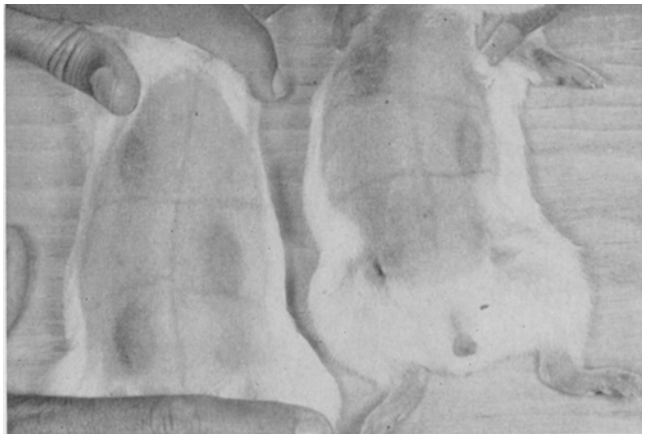

FIG. 2. Passive cutaneous anaphylaxis in guinea pigs. Positive reaction to castor seed-antigen in the sites sensitized with rabbit anti-castor serum is shown by the dark spots. No reaction occurred in sites injected with control serum.

Results

Material extracted from each band of the electrophoretogram was tested in five passively sensitized guinea pigs in the manner described. Thirty passively sensitized guinea pigs were used for testing the bands in this particular group of electrophoretic runs, and it was found that all six of the bands elicited cutaneous anaphylaxis in all the sites sensitized with anti-castor serum.

Figure 2 is a photograph of the actual passive cutaneous anaphylaxis test in the sensitized skin of guinea pigs. Control sites which had been injected with serum from non-sensitized rabbits remained uncolored while sites sensitized with rabbit anti-castor

TABLE I

Local Passive Cutaneous Anaphylaxis Elicited by Cactor-Seed Antigens in Guinea Pigs[a] Sensitized by Intracutaneous Injections of Rabbit Antiserum to Castor-Seed Protein

Antigenic preparation tested	Protein injected (grams)	Passive cutaneous anaphylaxis in sansitized sites[b, c]
Crude castor-seed protein CB-1ASRI	2×10^{-6}	Pale blue 15 mm diameter
Electrophoretogram Band No. 1	2×10^{-6}	Pale blue 15 mm diameter
Band No. 2	2×10^{-6}	Deep blue 15 mm diameter
Band No. 3	1×10^{-5}	Deep blue 20 mm diameter
Band No. 4	1×10^{-5}	Deep blue 20 mm diameter
Band No. 5	1×10^{-5}	Pale blue 20 mm diameter
Band No. 6	2×10^{-6}	Deep blue 20 mm diameter
Meal cooked at 100°C., pH 12.4 for 32 minutes	4.3×10^{-1} as protein	No color (none)
Meal cooked at 100°C., pH 12.4 for 20 minutes	4.3×10^{-1} as protein	Deep blue 20 mm diameter

[a] Animals not sacrificed: intact skins examined and photographed.
[b] The depth of color is proportional to the amounts both of antigen and of antibody injected (amount of dye held constant). The diameter of blue spot is a function of the amount of antibody.
[c] No color developed in sites injected with serum from non-sensitized rabbits.

serum showed pronounced bluing due to the antigen-antibody reaction in the site.

Extract of castor meal which had been cooked for 32 min. at 100°C. with $Ca(OH)_2$ solution at pH 12.4 elicited no passive cutaneous anaphylaxis. The absence of the P.C.A. test in the sensitized sites indicated that all of the antigenic proteins of the seed had been so changed by the treatment that they had lost their original or native antigenicity. This result is consistent with previous knowledge that proteins are relatively quickly hydrolyzed by heating with strong alkali. It is also well known that alkaline hydrolysis of protein yields racemic aminoacid mixtures.

The samples of castor-seed meal which had been cooked for only 20 min. under the same conditions of pH and temperature were found to contain antigenic material which gave a positive P.C.A. test with anti-castor serum. This result may indicate that certain antigens had not yet been attacked during 20 min. or, more probably, that certain partially degraded castor-seed proteins retain some of their original specific antigenicity.

The results obtained in the passive cutaneous anaphylaxis tests are presented in Table I.

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[Received April 18, 1961]

Low Temperature Aminolysis of Methyl Stearate Catalyzed by Sodium Methoxide[1]

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Aminolysis of methyl stearate by both primary and secondary amine catalyzed by sodium methoxide was found to be rapid at 30°C. under anhydrous conditions. With primary amines under optimum conditions (mole ratio to ester: amine, 10; catalyst, 0.12), the minimum reaction times necessary to obtain yields of amide over 90% were: *n*-butylamine, 30 min.; *iso*-butyl-, 1 hour; allyl-, 1.8 hr.; benzyl-, 3.2 hr.; *sec*-butyl-, 16 hr.; ammonia (a heterogenous reaction requiring an optimum triethylamine to ester ratio of 2 ml./g. and a catalyst mole ratio of 0.20) 2 days. Secondary amines reacted rapidly at 30°C. (15 min. to 24 hr. for a 90% yield of amide) when the nitrogen atom was joined into a saturated ring or held at least one methyl group, but very slowly even at 100°C. when the substituent was dialkyl larger than methyl. Uncatalyzed, all reactions were extremely slow.

PART I. PRIMARY AMINE AMINOLYSIS

BASIC CATALYSTS have been used both in preparative and in kinetic studies of the aminolysis of esters. A number of preparative studies of the reaction of amines and esters, using sodium methoxide as the catalyst, showed rapid conversion to amide at high reaction temperatures but, in general, slow conversion at room temperature (1,2,3,4,5,6,7,8). None of these reports presented a study of optimum reaction conditions. In investigations of the kinetics of the aminolysis of esters at low temperatures (9,10,11, 12), a marked increase in the rate of reaction by alkoxide ion has been shown, but practical preparative conditions were not given. In the absence of any catalyst, high reaction temperatures (150°–250°C.) were often necessary to convert esters to amides rapidly and the use of pressure equipment with volatile amines was required (13,14,15,16,17,18,19,20). Studies relating the effect of structure and reactivity of both amines and esters have also been made (14) principally by Day and co-workers (21). Arnett, Miller, and Day (22) studied the effect of structure of various primary amines in reaction with methyl acetate at 25°C. This report revealed that the reactivity of the isomeric butylamines bracketed the reactivity range of most of the amines studied.

From the published results, it appeared that a general process could be developed for the preparation of amides through alkoxide catalyzed aminolysis of esters at a low temperature by a careful investigation of reaction conditions and by observance of rigorously anhydrous conditions to avoid catalyst destruction. Part I describes the results of a study of the sodium methoxide catalyzed aminolysis of methyl stearate by selected, structurally varied primary amines at 30°C. Part II reports the results with secondary amines.

The purposes of Part I are: (1) to report the optimum conditions found for the sodium methoxide catalyzed aminolysis of methyl stearate by primary alkylamines and to describe how these conditions were obtained; (2) to demonstrate that these conditions are applicable to a wide variety of primary amines and esters, and (3) to treat with three special cases. By

[1] Presented in part at the 138th meeting of the American Chemical Society held in New York, N.Y., September 11–16, 1960, and in part at the 52nd meeting of the American Oil Chemists' Society held in St. Louis, Missouri, May 1–3, 1961.