groups with the highest atheromata. Nishida et al. (13) found a similar trend in chickens fed cholesterol with heated or fresh corn oil. Furthermore the most atherogenic diet produced the lowest serum cholesterol levels although it must be pointed out that even these so-called low levels were considerably above normal serum cholesterol levels. It is probable then that the higher atherogenicity observed for the fat-free diet may be caused by insufficient amounts of the unsaturated fatty acids with which cholesterol is preferentially esterified (16,17), thus retarding normal circulation and metabolism of this sterol. The atherogenicity of the heated fat may be increased by changes affecting the transport of cholesterol and the composition of the beta-lipoprotein. Nishida et al. (13) found that the serum of the chickens fed cholesterol plus heated corn oil was practically free of the $S_1$ 20-400 classes of lipoproteins. Thus all the lipoprotein was present as the cholesterol-rich $S_2$ 20-40 fraction (18). Although we have not done lipoprotein analyses of the sera obtained in this experiment, previous analyses showed that rabbits fed cholesterol in the absence of fat had lower serum lipoprotein levels than those fed cholesterol in corn oil but that a larger proportion of the lipoprotein was present as the $S_2$ 20-40 class (5). The effect of free fatty acids on serum lipid composition in both normal and cholesterol-fed animals merits further scrutiny.

The Complex Nature of Castor Sensitivity

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The castor seed antigen CB-1A, prepared by the basic lead acetate method of Spies et al., has been subjected to column chromatography on diethylaminoethyl cellulose. Antigenic differences between fractions were found by the Schultz-Dale technique, which indicated the possible existence of six antigenic or allergenic components.

Cross-reactions between castor pollen, castor blossoms, and castor seed meal were indicated by the Schultz-Dale method.

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REFERENCES


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Bernton and Stevens (3,4,5) have shown conclusively that the principal allergenic material of the castor seed is distinct from ricin. The latter workers found that the allergenic principle is heat-stable, soluble in basic lead acetate solution, insoluble in 75% ethanol, and nontoxic to unsensitized animals. Furthermore, the allergenic principle was found to be diffusible through collodion membranes, resistant to trypsin, but destroyed by trypsin. These workers have designated their allergenic material as CB-1A, a natural proteose.

Spies and co-workers prepared the allergenic fraction by a method which they had found suitable for the preparation of the cottonseed allergen CS-1A (6). This method involved the extraction of the defatted seed meal with warm water and the treatment of the extract with basic lead acetate to remove the extraneous components. The allergen was found to be in the supernatant solution. After removal of the lead ion as either the carbonate or the sulfide, the solution was reduced in volume and the allergen was precipitated by the addition of ethanol sufficient to make a final concentration of 75% alcohol by volume, and storage at \(-10^\circ C\) for 24 to 48 hrs.

It has been assumed by most workers that allergy to castor seed is acquired only by inhalation of castor seed protein as a finely-divided air-borne dust containing a specific antigen and that subsequent allergic reactions result from contact with the same castor seed antigen.

Coulson, Spies, and Stevens (7) describe a study of what at first appeared to be cross-sensitivity between castor seed and green coffee beans from Central and South America. A possible explanation was that the bags used for transporting the green coffee were used bags and that some of these may have been used previously for sacking castor seeds. The allergic reactions were shown to be caused by dust contaminated with castor seed allergen.

Snell (8) described his own sensitivity to the dust adhering to castor seeds. Similar cases of asthmatic attacks because of handling intact castor seeds and pollen have been described to the authors by Brazilian workers and by several research agronomists. Many of the cases of allergic reaction to castor seem to begin to appear at the time that castor seeds are being unloaded or dumped at the castor oil-processing plants; however the majority of cases appear when the dry, dusty pomace is produced by solvent extraction to remove the last portions of the oil.

The fact that many of the victims have never knowingly been in contact with castor seed pomace and that many of the reported cases of castor allergy appeared several years prior to the general use of solvent-extraction processes indicate that castor seed meats may not have been the sole cause of sensitization. Certain of the earlier cases of castor allergy suggest the possibility of a dry, dusty source of sensitizing allergen other than that derived from fat-free seed pomace. Preliminary tests in our laboratory indicated that crushed or flaked castor seeds would only rarely give rise to air-borne dust without previous removal of the castor oil. The possibility of human allergy to different specific castor antigens and of alternative sources of these antigens led us to conduct sensitivity studies, utilizing castor allergen CB-1A, crude castor seed proteins, castor pollen granules, and female blossoms.

**Experimental**

Several samples of commercial castor seeds were procured from a local processor. The seeds were shaken in a paper bag, and the trash was examined with a microscope. Visual microscopic examination and photomicrographs clearly indicated the presence of considerable quantities of pollen and the dry petals or stigmas of the female blossoms. Many of the pollen grains appeared to be ruptured. Upon closer examination the ruptured grains, when suspended in water, were found to be extruding large numbers of spherical bodies or granules approximating the size of a bacterial cell. We were able to show that the pollen grains are actually capsules with clearly delineated surface sutures or lines along which the grains split open upon the application of a small force, such as the pressure of the forceps moving the coverslip over the pollen grains. Figure 1 shows intact pollen grains from male blossoms; Figures 2 and 3 show samples of the dust from a shipment of commercial castor seeds; ruptured grains and the pollen granules can be seen in Figure 3.

Castor seeds were planted in a secluded part of the laboratory grounds at Albany. When the (yellow) male blossoms appeared, they were harvested by carefully removing the blossoms and storing them in vials. The female blossoms were harvested by taking the entire raceme (stem) to the laboratory, where the distal two-thirds of each pink stigma was removed and collected as the sample.

When several grams of the female blossom stigmas had been collected, they were carefully washed with cold water containing sodium laurel sulfate detergent and with 80% ethanol to remove pollen and other extraneous dust. The pollen grains were removed from the anthers by hand, rinsed once with 80% ethanol, and dried. Samples of stigmas and of pollen were ground in a balanced saline solution in clean new mortars in order to prepare injectable suspensions for sensitization of animals.

Castor allergen CB-1A was provided by J. R. Spies of the Allergen Pioneering Laboratory. A supply of mixed antigens of castor seed was prepared from hexane-extracted, decorticated castor seed meats by extraction with water at 100°C.

**Chromatography of Spies Allergen CB-1A.** Sober, Peterson, et al. (9,10) have utilized diethylaminoethyl-cellulose for the resolution of natural mixtures of proteins, and their work suggested the use of this adsorbent in the study of the antigens and allergens of castor.

The diethylaminoethyl-cellulose columns were prepared as directed by Sober and Peterson, using 50-ml burettes as columns. The burettes were packed under 1½ to 2 p.s.i. nitrogen to a height of 40 cm. The dry weight of the DEAE-cellulose packing was found to be 4.5 to 5.0 g., equivalent to 4.1 to 4.5 milliequivalents of adsorption capacity. These columns permitted a flow rate of 4.5 ml of liquid per minute under a pressure of 1½ to 2½ p.s.i. nitrogen.

The eluate from the column was continuously monitored for hydrogen ion concentration by means of a glass electrode sealed into the system immediately preceding the quartz cell of an ultraviolet absorption meter monitor. Ultraviolet absorbancy at a wavelength of 254 nm was continuously recorded by means of a recorder. All the chromatograms were run at...