TABLE II
Gas Chromatography of Cholesterol Ester and Triglyceride Fatty Acids from Sera of Rabbits on Various Atherogenic Regimens: Experiments 1 and 2

Exp. No.	Acid		Diet							
	No. of carbon atoms	No. of double bonds	Cholesterol–no fat		Cholesterol–solution		Cholesterol–suspension		Normal	
			CE [a]	TG [b]	CE	TG	CE	TG	CE	TG
			%	%	%	%	%	%	%	%
1	14	0	.....	.....	9.4	11.3	.....	13.5	.....	.....
2	14	0	2.4	2.4	.....	.....	7.9	.....	.....	.....
1	16	0	37.0	46.4	15.7	21.9	18.2	33.0	20.8	35.4
2	16	0	20.1	35.9	22.4	29.3	25.2	27.1	23.8	30.2
2	16	1	9.0	3.7	.....	.....	.....	.....	.....	.....
1	18	0	4.9	11.7	1.6	3.6	2.9	5.5	3.8	4.7
1	18	1	43.5	31.5	51.7	28.5	53.0	32.3	34.0	37.9
1	18	2	14.5	10.5	31.5	34.7	25.8	15.7	41.5	21.9
2	18	0	1.4	4.0	3.5	4.3	1.7	3.8	1.7	4.8
2	18	1	42.3	26.6	50.6	44.3	48.6	34.3	38.4	35.4
2	18	2	24.8	27.4	23.5	22.1	16.6	34.8	36.2	29.5
1	% Saturated acids		41.9	58.1	26.7	33.2	21.1	52.0	24.6	40.1
2	% Saturated acids		23.9	42.3	25.9	33.6	34.8	30.9	25.5	35.0
1	% Unsaturated acids		58.0	42.0	83.2	63.2	78.8	48.0	75.5	59.8
2	% Unsaturated acids		76.1	57.7	74.1	66.4	65.2	69.1	74.6	64.9

CE[a] = Cholesterol ester.
CE[b] = Triglyceride.

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_f 20–400 classes of lipoproteins. Thus all the lipoprotein was present as the cholesterol-rich S_f 0–20 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_f 0–20 class (5). The effect of free fatty acids on serum lipid composition in both normal and cholesterol-fed animals merits further scrutiny.

Acknowledgment

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REFERENCES

1. Turner, K. B., J. Exper. Med., *58*, 115 (1933).
2. Meeker, D. R., Kesten, H. D., and Jobling, J. W., Arch. Pathol., *20*, 337 (1935).
3. Bruger, M., and Fitz, F., *ibid.*, *25*, 637 (1938).
4. Weinhouse, S., and Hirsch, E. F., *ibid.*, *30*, 856 (1940).
5. Kritchevsky, D., Moyer, A. W., Tesar, W. C., Logan, J. B., Brown, R. A., Davies, M. C., and Cox, H. R., Am. J. Physiol., *178*, 30 (1954).
6. Bortz, W. M., Larsen, N. P., and Civin, W. H., Arch. Pathol., *66*, 218 (1958).
7. Kritchevsky, David, Moyer, A. W., Logan, J. B., and McCandless, R. F. J., Arch. Biochem. Biophys., *59*, 526 (1955).
8. Trinder, P., Analyst, *77*, 321 (1952).
9. Horning, M., Williams, E., and Horning, E., J. Lipid Research, in press.
10. Schwenk, E., Stevens, D. F., and Altschul, R., Proc. Soc. Exper. Biol. Med., *102*, 42 (1959).
11. Evans, J. D., Riemenschneider, R. W., and Herb, S. F., Arch. Biochem. Biophys., *53*, 157 (1954).
12. Kritchevsky, David, Moyer, A. W., Tesar, W. C., McCandless, R. F. J., Logan, J. B., Brown, R. A., and Englert, M., Am. J. Physiol., *185*, 279 (1956).
13. Nishida, T., Takenaka, F., and Kummerow, F. A., Circulation Research, *6*, 194 (1958).
14. Swell, L., Flick, D. F., Field, H. Jr., and Treadwell, C. R., Am. J. Physiol., *180*, 124 (1955).
15. Rona, G., Chappel, C. I., and Gaudry, R., Can. J. Biochem. Physiol., *37*, 479 (1959).
16. Kelsey, F. E., and Longenecker, H. E., J. Biol. Chem., *139*, 727 (1941).
17. Clement, G., Clement, J., and Louedec, A., Arch. Sci. Physiol., *8*, 233 (1955).
18. Jones, H. B., Gofman, J. W., Lindgren, F. T., Lyon, T. P., Graham, D. M., Strisower, B., and Nichols, A. V., Am. J. Med., *11*, 358 (1951).

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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.

It would appear that allergy to castor pomace may actually be sensitivity to any one or more of the antigenic components of the pomace, including both pollen and female blossoms.

ALLERGY to castor seed protein has been recognized since 1914 when Alilaire (1) described the allergy and attributed it to sensitivity to the toxic albumin ricin. Ratner and Gruehl (2) showed that ricin was not responsible for the allergenicity of castor proteins, and the classical studies of Spies, Coulson,

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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-stabile, 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 pepsin, 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°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 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 lauryl 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 mμ was continuously recorded by means of a recorder. All the chromatograms were run at

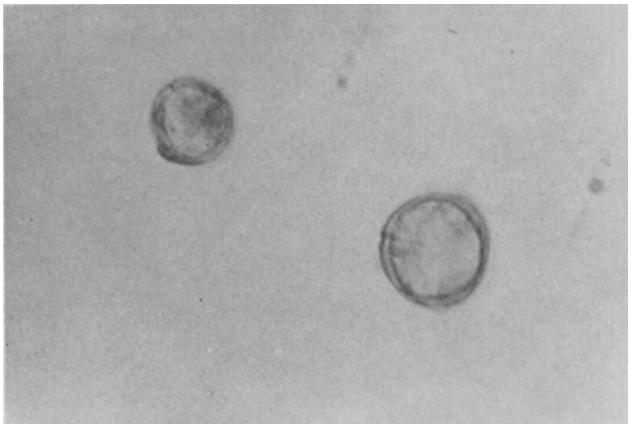

FIG. 1. Castor pollen grains taken from male blossoms.

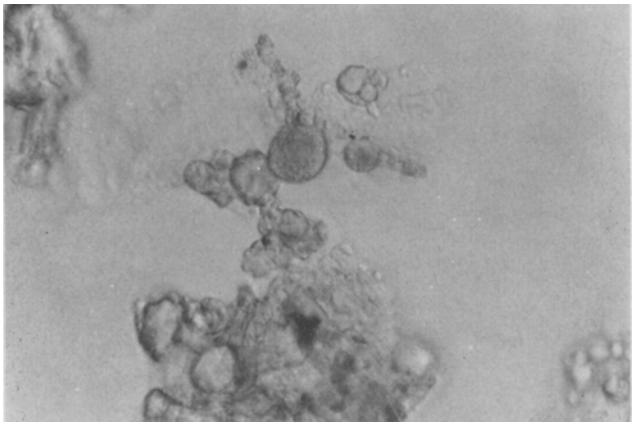

FIG. 2. Sample of dust from commercial castor bean shipment.

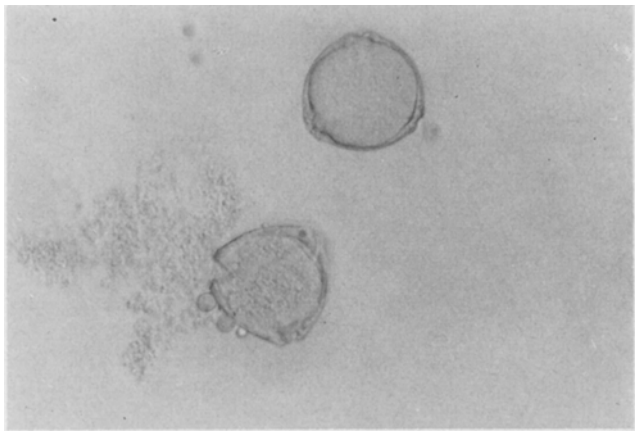

FIG. 3. Castor pollen grains from dust of sample of commercial castor beans. Note surface markings and granules in intact and ruptured grains.

ambient room temperature of 20°–22°C. The castor seed allergens CB-1A was supplied by Dr. Spies. (The detailed description of the preparation of this particular sample of castor allergen will be found in J. Am. Chem. Soc., *65*, 1721, 1943).

Samples of allergen CB-1A ranging in weight from 20 to 35 mg. were dissolved in 2 to 3.5 ml. of 0.02 M glycine solution. Each sample was transferred to the top of a DEAE-cellulose column, and the column was then developed with 0.02 M glycine solution. The castor components not adsorbed passed from the column with the loading and washing solution and gave the first peak of the chromatogram. Washing with 0.02 M glycine solution was continued until 200 ml. had passed through the column. The glycine solution in the mixing flask was adjusted to a volume of 250 ml., the 0.04 M phosphate buffer (pH 6.8) was placed in the assembly, and the ionic-concentration gradient elution was initiated.

The buffer was forced to flow at a rate of 4½ ml. per minute drop by drop, by displacement, into the mixing flask. After 300 ml. of buffer had passed through the column, the first buffer reservoir was replaced by a second containing 500 ml. of acidic buffer, which was 0.1 M with respect to NaH_2PO_4, 0.03 N with respect to HCl, and 0.02 M with respect to glycine. The second buffer was forced to flow in the same manner and rate as the first. When the effluent solution reached pH 3, the elution was discontinued.

Results. A typical chromatogram obtained for 21 mg. of allergen CB-1A is shown in Figure 4. The

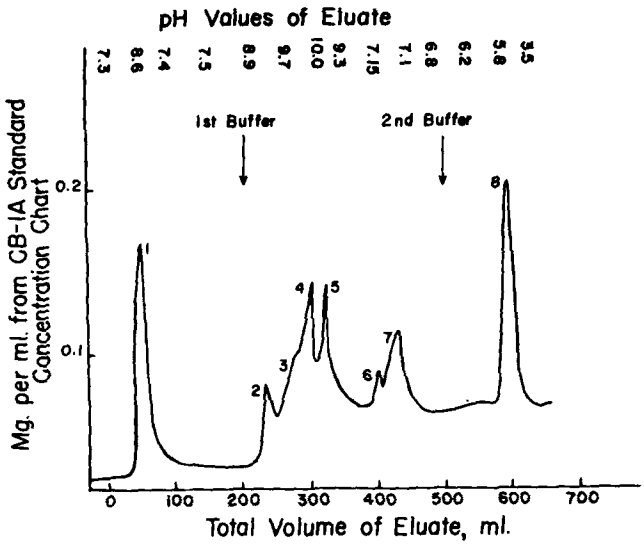

FIG. 4. Chromatogram obtained by diethylaminoethyl-cellulose chromatography of 21 mg. of castor allergen CB-1A.

pH value of the effluent is shown across the top of the chart, the chromatographic curve itself shows relative absorbancy of ultraviolet light at a wavelength of 254 mμ and the ordinate shows equivalent concentration of allergen CB-1A, as read from the curve used for standardizing the ultraviolet absorption monitor.

The antigenicity of each of the fractions represented by the major peaks of the chromatogram of allergen CB-1A was determined by the Schultz-Dale technique, as described by Coulson *et al.* (5,7).

Four groups of weanling female albino guinea pigs were sensitized to castor antigens as follows. Each of the 24 animals in the first group was injected i.p. with a saline suspension containing 100 mg. of female blossom stigmas. Each of 24 animals in the second group was injected with 100 mg. of pollen. Each of 24 animals in the third group was injected with 100 mg. of castor seed meal extract, and each of 48 animals in the fourth group was injected with 100 mg. of allergen CB-1A of Spies. The injected animals were allowed at least three weeks for the development of sensitivity. Uninjected guinea pigs were used for controls.

Preparation of Materials for Schultz-Dale Testing. Our preliminary studies had revealed the presence of substances with histamine-like action in castor seed flour, pollen, and blossoms. These irritants, which cause false-positive tests in the Schultz-Dale technique, were found to be easily extracted by hot methanol or ethanol, leaving the residual material free of such nonspecific, histamine-like action. Allergen CB-1A possessed no histamine-like activity.

Castor pollen, female blossoms, and castor seed flour were exhaustively extracted with hot methanol until they were free of histamine-like activity. They were then ground and suspended in saline to give suspensions containing 10 mg. of solid per ml.; allergen CB-1A was used as a 1% solution in saline.

Schultz-Dale Test for Sensitization. Each guinea pig was stunned by a sharp blow at the base of the skull and was then quickly exsanguinated by severing the blood vessels in the neck. The uterus was removed in its entirety and placed in oxygenated Tyrode solution.

Each uterine horn was cut into two segments, a and b, and corresponding segments 1a and 2a from the two horns were each mounted in two separate 40-ml. Schultz-Dale baths and connected to the Kymograph. After obtaining a base line for the behavior of the mounted strips or segments, each was challenged by the addition of a solution or suspension of from two to five mg. of the material to be tested for specific antigen. Uterine strips from control (uninjected) animals were tested alternately with those from the sensitized animals.

The addition of the appropriate sensitizing antigen in sufficient concentration will cause a strong contraction (anaphylaxis) of the smooth muscle of adequately sensitized animals. Control animals (not sensitized to the antigen) will give no anaphylactic reaction. Furthermore, when all of the specific antibody in the tissue has reacted with the specific antigen, no further reaction to that particular antigen can be elicited from the muscle strip.

Results of Biological Tests

The uterine strips from guinea pigs sensitized to allergen CB-1A of Spies were found to react to each of the fractions represented by peaks in the chromatogram. Furthermore, as shown in Table I, the fractions tested in the order of their elution from the column elicited additional contractions from strips which no longer reacted to earlier fractions. This phenomenon could be interpreted only as indicating the presence of several specific antigens reacting with their homologous antibodies in the sensitized tissues.

TABLE I

Effect of Exhaustion of Specific Antibodies in CB-1A Sensitized Uterine Strips by Repeated Challenge of Each Strip with Each Chromatographic Fraction [a] (Peak), in Succession, in the Order of Elution from Column

Material added to bath until no further reaction to material	Schultz-Dale contractions: uteri reacting	Preliminary interpretation of result
Peak No. 1	10/10	Allergen CB-1A antigen present in 1st peak
Peak No. 2	9/10	Second antigen present in 2nd peak
Peak No. 3	9/10	Third antigen present in 3rd peak
Peak No. 4	0/10	4th peak containing third antigen or 3rd peak containing 4th antigen
Peak No. 5	7/10	5th peak containing another antigen
Peak No. 7	10/10	7th peak containing yet another antigen
Peak No. 8	10/10	8th peak containing still a different antigen
Peak No. 6	not tested	(6th peak not on every chromatogram)
Unsensitized (control) uterine strips		
Allergen CB-1A	0/10	Controls not sensitive to CB-1A
Combined fractions from chromatogram	0/10	Chromatography of allergen not contributing any histamine-like effect

[a] Corresponding fractions from five chromatographic runs were pooled in order to have sufficient quantities of antigens for exhaustive challenge of strips.

Uterine strips from the pollen-sensitized animals were found to give a strongly positive Schultz-Dale test with aqueous extracts of pollen, of female blossoms, and of castor seed flour. Only about 30% of the pollen-sensitized tissues gave a positive Schultz-Dale reaction to CB-1A. Uterine strips from the female blossom-sensitized animals gave strongly positive Schultz-Dale tests when challenged with castor seed flour, with pollen, or with female blossoms. Approximately 50% of the female blossom-sensitized pigs gave a positive Schultz-Dale reaction to CB-1A. The data are presented in Table II.

Those uterine strips sensitized by pollen or by female blossom stigmas, which gave a positive Schultz-Dale test with the castor allergen CB-1A, subsequently gave an additional positive test upon the application of the aqueous extract of castor seed flour and a further positive reaction to either of the respective

TABLE II

Antigenic Interaction Between Castor Bean and Pollen Female Blossoms, and Seed Protein [a] (by Schultz-Dale Technique)

Total number of animals	Sensitizing material	Horn and segment	Material added to bath	Schultz-Dale uterine contraction	Maximum reaction	Minimum or no reaction
10	Female stigmas	1a	2 mg. CB-1A	++++	5/10	5/10
	Female stigmas	2a	2 mg. ground pollen suspension	++++	10/10	0
	Female stigmas	1b	Aqueous extract from 40 mg. meal	++++	10/10	0
	Female stigmas	2b	5 ml. isotonic salt solution	0	0	10/10
10	Castor pollen	1a	2 mg. CB-1A	++++	3/10	7/10
	Castor pollen	2a	2 mg. ground pollen	++++	10/10	0
	Castor pollen	1b	Aqueous extract castor meal	++++	10/10	0
	Castor pollen	2b	5 ml. isotonic salt solution	0	0	10/10
10	CB-1A	1a	2 mg. CB-1A	++++	10/10	0
	CB-1A	2a	2 mg. ground pollen	++++	8/10	2/10
	CB-1A	1b	2 mg. female blossom	++++	10/10	0
	CB-1A	2b	5 ml. isotonic salt	0	0	10/10
10	Castor meal extract	1a	2 mg. CB-1A	++++	8/10	2/10
	Castor meal extract	2a	2 mg. ground pollen	++++	10/10	0
	Castor meal extract	1b	2 mg. female blossom	++++	10/10	0
	Castor meal extract	2b	5 ml. isotonic salt	0	0	10/10

[a] The castor bean meal, pollen, and blossoms used for challenging the mounted strips were first extracted with hot methanol and dried. The dry solids were then suspended in hot, isotonic salt solution, and the cloudy supernatant added to the bath. Control uterine strips from nonsensitized guinea pigs did not contract when treated with these suspensions in the Schultz-Dale test.

sensitizing agents. This fact indicates the presence of additional specific antigens peculiar to the pollen and to the blossoms and not present in the seed protein (CB-1A).

REFERENCES

1. Alilaire, E., Ann. Inst. Pasteur, *28*, 605 (1914).
2. Ratner, B., and Gruehl, H.L., Amer. J. Hyg., *10*, 236 (1929).
3. Spies, J.R., and Coulson, E.J., J. Am. Chem. Soc., *65*, 1720 (1943).
4. Spies, J.R., Coulson, E.J., Chambers, D.C., Bernton, H.S., and Stevens, H., J. Am. Chem. Soc., *66*, 748 (1944).
5. Coulson, E.J., Spies, J.R., Stevens, H., and Shimp, J.H., J. Allergy, *21*, 34 (1950).
6. Spies, J.R., Bernton, H.S., and Stevens, H., J. Allergy, *10*, 113 (1939).
7. Coulson, E.J., Spies, J.R., and Stevens, H., J. Allergy, *21*, 554 (1950).
8. Snell, W.H., Science *59*, 577 (1924).
9. Sober, H.A., Peterson, E.A., *et al.*, J. Am. Chem. Soc., *78*, 756 (1956).
10. Sober, H.A., and Peterson, E.A., Fed. Proc., *17*, 1116 (1958).

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The Effect of Storage Temperature and Antioxidant Treatment on the Chemical and Nutritive Characteristics of Herring Meal

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The effects of storage temperature and antioxidant treatment on the chemical and nutritive properties of herring meal were studied. The following observations were made:

1. Low-temperature (−20°C.) storage promoted more rapid decrease in ether extractability and in iodine value of the ether extract than did storage at 25.5°C.
2. Antioxidant treatment prevented the decrease both in ether extractability and iodine value of the extract.
3. Binding of the lipid into a complex from which the lipid was extractable with acetone only after HCl treatment occurred early in the storage period. After six weeks the amount of lipid in this fraction decreased, presumably as a result of further oxidation and polymerization into more refractory compounds.
4. Pepsin digestibility tests showed that BHT-treated meal contained the lowest amounts of undigestible nitrogen. Meals subjected to −20°C. contained the most undigestible nitrogen.
5. All meals were similar in nutritive value as protein or vitamin-B supplements in chick diets, composed of natural ingredients, even after storage for nine months. As the sole source of protein in a purified diet, the meal kept at −20°C. from time of processing promoted slower growth than the other meals.

HERRING MEAL used in livestock and poultry rations contains a variety of lipids. Commercially-produced herring oil is a relatively unsaturated fat, and the lipids remaining in the press-cake are even more unsaturated (1). Because of the reactivity of the unsaturated fatty components in the press-cake, the chemical and nutritive characteristics of press-cake might be expected to undergo considerable change in the course of processing and subsequent storage of the meal.

Stansby (2) observed that the apparent fat content of fish meal, as determined by ether extraction, decreases even during a short storage period. Almquist (3) reported that in sardine meal the extractability of fat and digestibility of protein decreased during storage and concluded that the changes noted were due to oxidation since they were not observed in samples which were stored in sealed glass ampoules. Meade and McIntyre (4) and Aure (1) reported that the addition of butylated hydroxytoluene (BHT) to menhaden and herring meal, respectively, retarded oxidative changes in the meal.

The following experiments were designed to determine and follow the changes which occur in herring meal stored under different conditions and to ascertain what effect antioxidant treatment might have upon any changes that normally take place in meals under ordinary conditions of storage.

Procedure

The herring meals for the experiment were prepared on December 9, 1958, at a commercial reduction plant. The meal required for the experiment was taken directly from the bagging operation, and portions were treated as follows:

A Stored at 25.5°C. This meal will be referred to henceforth as the "normal" meal.

B Stored at −20°C.

C Spread out on trays to facilitate cooling in a freezer room at −20°C. over-night and then bagged. Stored at 25.5°C.

D Spread out in a freezer room at −20°C. over-night and then bagged. Stored at −20°C.

E Stored at 25.5°C. for one week. BHT [1] was then added at a level of 0.15%. Stored at 25.5°C.

F BHT added immediately to the hot meal at 0.15%. Stored at 25.5°C.

The meals were all stored in polyethylene-lined multiwall paper bags.

Samples of meals A and D were taken for immediate chemical analysis and samples of the remaining meals were withdrawn for analysis 10 days after preparation. All meals were sampled on January 16, March 16, and September 24, 1959, for chemical analysis and biological testing with chicks.

Chemical Tests. Weighed samples of the meals were dried *in vacuo* at 99–100°C. for 5 hrs. They were then extracted with peroxide-free diethyl ether in a Soxhlet apparatus for 20 hrs. Iodine numbers (Hanus) were determined on the ether extracts. The ether-extracted samples were then extracted with acetone. The acetone-extracted samples were next digested with HCl

[1] A finely divided product: 90% <100 mesh, 70% <200 mesh.