SORBITAN MONOSTEARATE METABOLISM
Lack of Deposition upon Chronic Feeding

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A synthetic nonionic emulsifier consisting of the partial esters of stearic acid and the mixed anhydrides of sorbitol (sorbitan monostearate) is used in small concentrations as a food additive. The object of this work was to determine whether the chronic ingestion of this fat-soluble emulsifier would result in the deposition and accumulation of the fatty acid esters of its polyhydric alcohol moiety in the fat stores of the animal body. Rats were fed for 28 days a synthetic diet containing the emulsifier radioactively labeled with carbon-14 in the polyol moiety, and their fat stores were then analyzed for residual radioactivity. The results demonstrated that the fatty esters of the mixed polyol are not deposited or accumulated in the fat stores.

SINGLE-DOSE FEEDINGS to rats of carbon-14–labeled mixed anhydrosorbitol monostearates (sorbitan monostearate) have shown (*2*) that the polyhydric components of this ester are almost completely eliminated within 48 hours, with only 0.05 to 0.1% of the ingested radioactivity remaining in the tissues after 2 to 7 days in a form which could have represented deposited anhydrosorbitol esters. Because such an emulsifier (manufactured and sold by Atlas Powder Co., Wilmington, Del., under the trade-mark Span 60) is used as a minor food adjunct in the human dietary (*1*), it was of interest to determine whether this residue resulted in accumulation in the tissues. Rats were accordingly fed for 28 days a diet containing 0.1% of this ester labeled with carbon-14 in the polyol moiety identical with the material fed in the previous single-dose experiments, and their tissues were analyzed for residual radioactivity.

Methods

Test Diet The experimental diet had the following percentage composition: sucrose, 50; casein, 15; Crisco, 20; yeast, 5; cod liver oil, 5; salt mixture, 5; sorbitan monostearate dissolved in the Crisco, 0.1.

Feeding Procedure Nine adult rats were fed the experimental diet *ad lib.* A control group of four rats was fed the same diet, except that 1.5% of the sucrose was replaced by radioactive uniformly labeled glucose, and the radioactive sorbitan monostearate was replaced by nonisotopic sorbitan monostearate. The diet containing the radioactive sorbitan monostearate assayed 7800 counts per gram, and the radioactive glucose control diet assayed at 2600 counts per gram. The animals were maintained in separate metabolism cages, and a daily record was made of their food intake.

Analytical Procedure At the termination of the 28-day feeding period, the animals were sacrificed, and the carcasses were rapidly frozen and dehydrated by lyophilization. The dehydrated tissues were extracted with hot chloroform. Inasmuch as the sorbitan monostearate is soluble in chloroform, the present studies were confined to this chloroform-soluble fraction. The radioactivity of the chloroform-soluble (crude fat) fraction was determined. The crude fat was saponified with hot alcoholic potassium hydroxide for 6 hours. The alcohol was removed on a steam bath, and the residue was extracted with petroleum ether to remove steroids. The hydrolyzate was acidified and the fatty acids were removed by extraction with petroleum ether, and the radioactive carbon-14 content was determined.

The aqueous residue from the saponification was evaporated to dryness and repeatedly leached with absolute alcohol. The alcohol extract was evaporated to dryness. This fraction contains the water-soluble hydrolytic products of the crude fat, including the glycerol and any deposited anhydrosorbitol polyols. The glycerol was removed by sublimation and its radioactivity was determined by direct count.

In order to confirm the identity of the glycerol and verify its carbon-14 content, the sublimed alcohol was converted to the tribenzoate. Mixed melting point determinations with the esters prepared from the isolated glycerol and an authentic sample of glycerol tribenzoate showed no depression of the melting point. The carbon-14 content of the recrystallized derivative agreed with the direct count

Table I. Ingested C^{14} Found in Crude Fat Fraction (Chloroform-Soluble) and Distribution of C^{14} in Hydrolytic Products of Crude Fat

	Radioactive Anhydrosorbitol Monostearate, %									R.A. Glucose (Controls), %	
Rat No.	1	2	3	4	5	6	7	8	9	10–11	12–13
Crude fat[a]	0.35	0.31	0.35	0.34	0.43	0.41	0.49	0.35	0.49	3.59	3.01
Fatty acids	0.23	0.21	0.25	0.20	0.19	0.29	0.32	0.15	0.25	1.25	1.38
Glycerol		0.03			0.07			0.01		0.93	0.95
Residue		0.04			0.09			0.07		0.54	0.39

[a] Crude fat from each animal was saponified separately for isolation of fatty acids except in radioactive glucose control animals, in which crude fats for 10 and 11 and for 12 and 13 were combined. In isolation of glycerol and residue fraction, corresponding fractions for animals 1, 2, and 3, animals 4, 5, and 6, and animals 7, 8, and 9 were combined.

of the glycerol after making the appropriate correction for the carbon introduced by the benzoate. The carbon-14 content of the nonsublimable unidentified residue, which would contain any anhydrosorbitol polyols if present, was determined. The results are shown in Table I.

Discussion

In confirmation of previous work it was found that a small portion of the carbon-14 fed as anhydrosorbitol esters appeared in the chloroform-soluble crude fat, with the activity distributed in the fatty acids, glycerol, and the nonsublimable residue. The unidentified residue which could be presumed to be the anhydrosorbitol polyols was of the same order of magnitude following the 28-day feeding period as was observed in the previous single dose experiments; this indicated the absence of accumulation. That this unidentified residue is probably not the polyol fraction of the fed ester is suggested by the finding that a corresponding fraction was obtained in even greater amounts from the crude fat extracted from the carcasses of rats fed labeled glucose without labeled anhydrosorbitol esters. The higher percentage of carbon-14 in the crude fat and its fractionated components of the labeled glucose–fed rats as compared with the labeled ester–fed rats is to be expected in view of the known metabolic fate of glucose and the previously demonstrated rapid excretion of the anhydrosorbitol polyols (*2*).

Literature Cited

(1) Pratt, C. D., *Food Technol.*, **6**, 425–30 (1952).
(2) Wick, A. N., and Joseph, L., *Food Research*, **18**, 79–84 (1953).

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FLAVOR AND SOIL TREATMENT

Flavor of Selected Vegetables Grown in Soil Treated With Isomers of Benzene Hexachloride

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Studies were made to determine the effect on flavor of tomatoes, lima beans, potatoes, radishes, and carrots from use of purified gamma, alpha, beta, and delta isomers of benzene hexachloride, a mixture of these isomers, commercial gamma isomer, and lindane as insecticides in growing the vegetables. The results indicate that flavor may be affected by certain formulations of benzene hexachloride; the effect on flavor varies with the formulation, the dosage rate, and the type of vegetable grown.

BENZENE HEXACHLORIDE USED IN THE soil as an insecticide has been found to impart off-flavors to some vegetables. Susceptibility to such contamination of flavor appears to vary with the type of vegetable, the formulation of benzene hexachloride (BHC), the amount used, and the time and method of application (*2–7*).

Studies were started at Beltsville, Md., during the summer of 1948 to ascertain the role played by each of the four major isomers in technical benzene hexachloride when applied to the soil in causing the objectionable flavor or odor of vegetables. As there were indications that some of the isomers caused more off-flavors than others (*6*), further studies were planned.

Reported here are studies on tomatoes, lima beans, and potatoes used during 1949 in tests with the chemically pure gamma, alpha, beta, and delta isomers of benzene hexachloride, and on radishes and carrots used in 1950 in tests with lindane (99.9% gamma isomer). Experiments on potatoes grown in 1950 with lindane were reported by Kirkpatrick *et al.* (*3*). [The name lindane has been established by the U. S. Interdepartmental Committee on Pest Control (*9*) as the common name for the gamma isomer of benzene hexachloride of a purity of not less than 99%.]

The vegetables were grown by the Bureau of Entomology and Plant Quarantine and the quality studies were carried out by the Bureau of Human Nutrition and Home Economics.

General Procedure for Growing Crops

The crops were grown in 3-gallon crocks to prevent any possible contamination by soil movement that might occur if grown in the open fields. The crocks were glazed inside and out. They were 9.25 inches in diameter and 11.25 inches high, inside measurements, and when filled with soil to within 2 inches of the top rim as used, had a capacity of 0.359