A Nutritional Evaluation of Acetostearins in Rats

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Abstract

A modified glyceride, acetostearin, has been studied in feeding experiments with rats using several nutritional and biochemical criteria. As acetostearins are more readily absorbed than the standard fats from which they are prepared, it was possible also to study the effect of high levels of saturated fat on essential fatty acid utilization.

The retarded growth and poor survival of acetostearin-fed animals indicate the possibility of antagonism of the acetostearins to essential fatty acid utilization. The decreased plasma cholesterol levels and elevated liver cholesterol values observed in the acetostearin-fed animals appear to be due mainly to essential fatty acid deficiency. High cholesterol levels in the adrenals show some indication of the involvement of caloric insufficiency in cholesterol metabolism of acetostearin-fed rats. Therefore, the probable explanation of the poor nutritional performance of rats fed acetostearin is a combination of essential fatty acid deficiency and caloric insufficiency.

Introduction

THE AVAILABILITY of the modified glycerides, acetoglycerides, in which acetyl groups have been substituted for one or two of the long chain fatty acids of triglycerides of natural fats and oils has resulted in this nutritional study in which a dual purpose is evident. First, the unique properties of these acetoglycerides (1–6) make them potentially useful in both edible and nonedible products; therefore a study of their nutritional properties is indicated. Secondly, the effect of the saturated fatty acid, stearic, on essential fatty acid utilization and cholesterol metabolism in the rat can better be studied, since acetostearins have been reported to be much better absorbed than the conventional standard fat from which they were prepared (7,8), and therefore more of the saturated fatty acid, stearic, can be introduced into the animal by feeding acetostearins. Evidence exists that high levels of saturated fats may act antagonistically to essential fatty acids (9–12). Also, reports are available to the effect that whereas the ingestion of various unsaturated fats tends to lower plasma cholesterol levels in man and other species of animals, certain fats high in saturated fatty acids tend to raise these levels (13–21).

Experimental Procedures and Data

To evaluate acetostearins for these purposes, various nutritional and biochemical criteria were employed following this general experimental design. Male rats were fed synthetic diets containing acetostearin either as the sole dietary glyceride or in mixtures with cottonseed oil. Various supplements were included in the diet or administered orally to some of the groups receiving only the acetostearins. Control groups received either synthetic diets containing, respectively, no fat, cottonseed oil, and hydrogenated coconut oil, or a commercial rat diet. In some of the control groups the diet was restricted; all other groups, control and experimental, were fed *ad lib.* All animals were weighed at regular intervals. The experiments were terminated after 20 wk, or earlier when survival was poor.

The following nutritional and biochemical criteria were evaluated: growth and survival, digestibility, effect on the requirement for essential fatty acids, and the effect on tissue cholesterol and lipid levels.

Animals. Male albino rats of the U.S.C. colony were used. Animals weighing at least 38 g were selected at weaning and randomly distributed as to weight and litter among the various experimental groups. The rats were individually housed in cages constructed with elevated wire floors to minimize coprophagy.

Acetostearins. USDA-OOP-1035 (AcS-USDA). This product, supplied by the U.S.D.A., So. Utiliz. Res. & Dev. Div., ARS, was prepared by interesterifying hydrogenated cottonseed oil with triacetin in the presence of free glycerol.

DPI-Myvacet, 5-00 (AcS-DPI). This product, prepared by Distillation Products Industries (Eastman Kodak Co.) and supplied by U.S.D.A., consisted of distilled, partially acetylated monoglycerides prepared from hydrogenated lard.

The composition of these acetostearin products, estimated on the basis of random esterification, as well as some of their chemical and physical constants, is shown in Table I. The components listed parenthetically are the major components.

Methyl Linoleate. This ester was obtained from safflower oil by the urea-adduct method of Wells (22). It was prepared by first converting the fatty acids to their methyl ester with methanol and sodium methoxide. The product was washed with water, dried, and dissolved in ethanol. Contaminating saturated and monoenoic esters were then removed as urea inclusion products. After removing the urea, the alcoholic solution was washed with water, dried, and distilled at reduced pressure to isolate the linoleate. The purity of the products obtained by this procedure, based on iodine value determination, was 91–93%.

Hydrogenated Coconut Oil (HCO) I.V. < 1.0
Cottonseed Oil (CSO) I.V. $= 113$.

Diets. Composition of the diets appears in Table II. In the preparation of the fat-free diet, the fat soluble vitamin mixture was "dry-diluted" with

TABLE I

Composition and Constants of Acetostearin Products [a]

Component or Constant		USDA	DPI
		Wt %	Wt %
Monoglycerides	(monostearins)	14.6	20
Diglycerides	(distearins)	5.4	.....
	(monoacetomonostearins)	42.0	50
Triglycerides	(diacetomonostearins)	29.9	30
	(monoacetodistearins)	7.5	.....
	(tristearins)	0.6	.....
Free Fatty acids (% C-18)		0.8	1.0
Acetyl content (%)		10.5	11.9
Iodine value		1.3	0.9
Saponification value		287.0	297.0
Hydroxyl value		111.0	118.0
Melting point (C)		44.1	55.4

[a] Data furnished principally by the So. Utiliz. Res. & Dev. Div., U.S.D.A.

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TABLE II
Composition of Diets

Component	Per cent Dietary Fat [g]						
	0	5	10	15	20	30	50
Sucrose	71.62	64.58	58.57	52.55	45.51	31.48	4.39
Casein, commercial [a]	20.00	22.00	23.00	24.00	26.00	28.00	33.00
Fat	.....	5.00	10.00	15.00	20.00	30.00	50.00
Salt mixture [b]	4.00	4.00	4.00	4.00	4.00	5.00	6.00
Cellufloure [c]	4.00	4.00	4.00	4.00	4.00	5.00	6.00
Choline [d]	.200	.220	.230	.240	.260	.277	.327
Water sol. vitamin mixture [e]	.157	.173	.180	.188	.204	.218	.258
Fat sol. vitamin mixtures [f]	.024	.024	.024	.024	.024	.024	.028

[a] Commercial Casein; H. B. Fuller Co., Los Angeles, Calif.
[b] Wesson Modification of Osborne-Mendel Formula (Science, 75, 339 (1932); General Biochemical, Inc., Chagrin Falls, Ohio.
[c] Solka-Floc; Brown Co., San Francisco, Calif.
[d] U.S.P., Merck & Co., Rahway, N. J.; Choline content of diet represents approximately 55 mg/100 cal.
[e] Contains: 38.57% p-aminobenzoic acid, 31.88% inositol, 12.75% ascorbic acid, 4.59% thiamine hydrochloride, 3.82% niacin, 3.82% calcium pantothenate, 1.73% riboflavin, 1.72% pyridoxine, 0.64% folic acid, 0.32% menadione, 0.16% biotin, and 40 mcg.% cobalamine; Merck & Co., and Nutritional Biochemicals Corp.
[f] Contains: 50% by weight of Nopsol (100,000 I.U. of Vitamin A and 20,000 I.U. of Vitamin D per g; Nopco Chemical Co., Harrison, N. J., and 50% by weight of alpha tocopherol; Nutritional Biochemicals Corp.
[g] *Dietary fat used was either the acetostearin (AcS), cottonseed oil* (CSO), mixtures of these two fats (AcS + CSO), or hydrogenated coconut oil (HCO). The fat free diet (FF) contained no added fat.

sucrose. In all other diets, these mixed vitamins were added to the liquid or liquefied fat. This was then incorporated into the major portion of a dry-mix consisting of the sucrose, casein, and salt mixture. The choline and the water soluble vitamin mixture were each incorporated into the remainder of the dry-mix by dry dilution. This was then combined with the dry-mix containing the fat.

Supplement Administration. Where employed, methyl linoleate was administered orally using a tuberculin syringe. For animals receiving the equivalent of 100 mg/day of the ester, 0.4 ml were given twice a week on separate days; for those receiving twice this amount, this volume was administered four times a week. In all instances, the amount of fatty acid ester used for supplementation during the

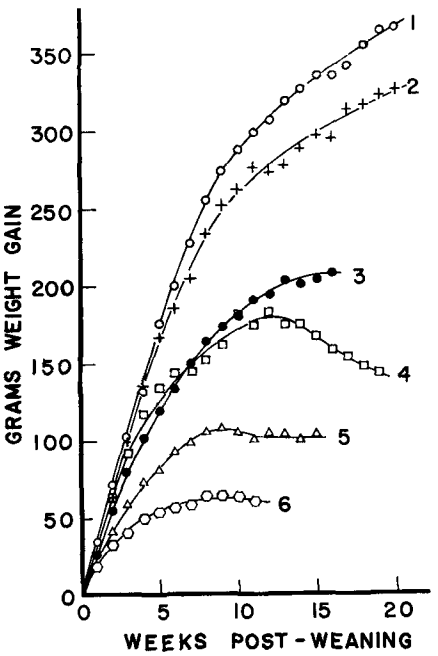

FIG. 1. Effect of type of dietary fat on growth of male rats. Curve 1, 30% cottonseed oil; Curve 2, Purina rat chow; Curve 3, fat-free diet; Curve 4, 30% hydrogenated coconut oil; Curve 5, 30% acetostearin-U.S.D.A.; Curve 6, 30% acetostearin-DPI.

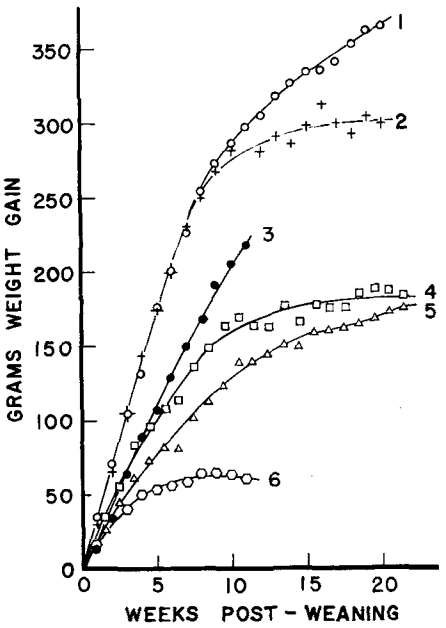

FIG. 2. Effect of concentration of dietary fat on growth of male rats. Curves 1, 2, and 3, 30%, 15% and 50% cottonseed oil, respectively; Curves 4, 5, and 6, 10%, 20% and 30% acetostearin-DPI, respectively.

first four weeks from weaning was half the listed amount. When not in use, this fat was stored frozen.

Growth Studies. These studies were conducted using rats which were placed on the experimental diets at weaning. Water and food were consumed *ad lib.* except in a few experiments in which food was restricted. All animals were weighed weekly.

Digestibility Studies. The procedure used was a modification of that described by Deuel et al. (23). The lipid under test was fed as a component of a standard diet, allowed *ad lib.* Feces were collected during a 9 day period. A 2 day orientation period was employed when the test diet differed from the pretest diet. At the end of this period, feces were transferred to a suitable sieve and vigorously shaken to remove adherent food particles. These, together with spilled food, were weighed, and the record of food consumed during the test period was corrected by this factor. The feces were first air dried, then dried for 12 hr at 60C, ground in a Wiley mill, and finally dried to constant weight in a vacuum oven at 60C.

Total fat consumption was obtained from the weight of food consumed and the lipid content of the diet; total fat egestion was obtained from the fecal output and the lipid content of the feces. Metabolic (endogenous) lipid was estimated on rats fed diets in which sucrose was substituted for fat. Lipids were determined gravimetrically in feces and diets.

The coefficients of digestibility were calculated using the following expression:

$$\frac{\text{fat ingestion} - (\text{fat excreted} - \text{metabolic fat}) \times 100}{\text{fat ingested}}$$

Animal Sacrifice and Tissue Collection. Generally, half the animals in each group were randomly selected and sacrificed after 6 weeks and the remainder after 20 weeks or earlier when survival was poor. For this purpose, animals were anesthetized with sodium pentobarbital, injected intraperitoneally. The abdominal and chest cavities were opened; blood was withdrawn from the heart with a heparinized syringe and transferred to a heparinized test tube. The tissues to be

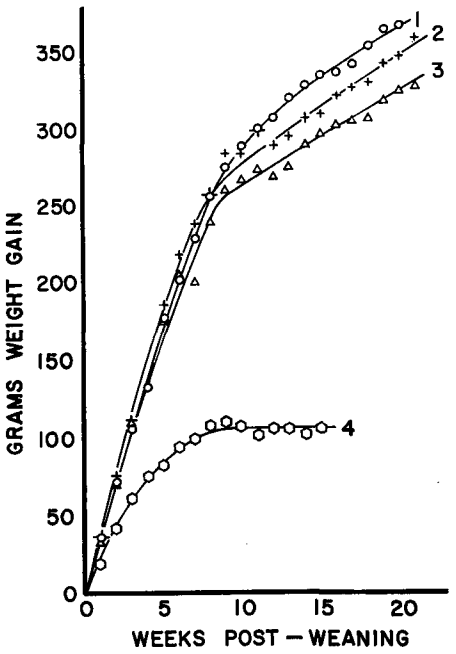

FIG. 3. Effect of mixtures of dietary acetostearin-U.S.D.A. (AcS) and cottonseed oil (CSO) on growth of male rats. Curve 1, 30% CSO; Curve 2, 5% AcS+25% CSO; Curve 3, 15% AcS + 15% CSO; Curve 4, 30% AcS.

studied were removed from every animal, trimmed, and weighed.

Cholesterol was determined on plasma, liver, and adrenals by the Nieft and Deuel modification (24) of the Schoenheimer-Sperry Method (25). Total liver lipids were determined gravimetrically.

Results and Discussion

Weight gain curves are presented in Figures 1–5. For each group the curves represent the weight gain of 10 animals per group up to and including 6 weeks postweaning, and the average of at least 5 animals/group beyond this point. Figure 1 compares the growth response to the various types of dietary fat employed, as well as to the basal fat-free diet. Significantly better growth was obtained with AcS-U.S.D.A. than with the DPI product; however, the performance on both was decidedly inferior to that observed with the other diets. The adequacy of the synthetic diet employed is seen in the favorable comparison of growth on the CSO-containing diet with the growth obtained using the commercial chow diet.

The effect of varying the concentration of dietary fat is assessed in Figure 2. The early growth response of the group which received 50% CSO was inferior to that of groups fed lower levels of this lipid. When AcS-DPI was fed at levels lower than 30%, growth was improved.

Figure 3 indicates the effect of feeding mixtures of AcS and CSO at a total dietary concentration of 30%. In ratios of 1:5 and 1:1 of acetostearin to cottonseed oil, growth responses were obtained which closely approached that obtained with CSO alone. However, animals grow poorly when fed the acetostearin product as the sole dietary fat.

The effects of oral supplementation and dietary inclusion of methyl linoleate on the growth of male rats which received AcS-DPI are shown in Figure 4. The results of linoleate supplementation of a group fed the fat-free (FF) diet are also shown. Administration of linoleate, amounting to 100 mg/rat/day, to AcS-fed animals produced identical growth

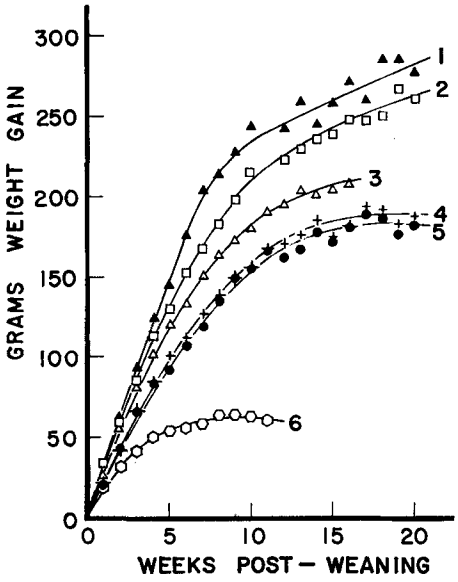

FIG. 4. Effect of methyl linoleate supplementation on growth of male rats fed dietary acetostearin-DPI (AcS) or a fat-free diet (FF). Curve 1, 25% AcS + 5% linoleate; Curve 2, FF + 200 mg linoleate/rat/day; Curve 3, FF; Curve 4, 30% AcS + 200 mg linoleate/rat/day; Curve 5, 30% Acs + 100 mg linoleate/rat/day; Curve 6, 30% AcS.

responses with both types of AcS; growth was not substantially improved in AcS-DPI rats by increasing the linoleate supplement to 200 mg. However, the growth of animals which received 5% dietary linoleate and 25% AcS was superior to either of the orally supplemented groups, since at this 5% level approximately 500 mg of linoleate per day and 5% less AcS, were consumed. The *increment* in growth resulting from the administration of 200 mg of linoleate per day to animals receiving the fat-free diet was less than that obtained for the AcS-fed group.

Growth curves for male rats fed restricted daily amounts of either the FF diet or the 30% CSO diet are compared in Figure 5 with the curve for the group fed AcS-DPI *ad lib.* at the 30% dietary level. It should be noted that all groups receiving restricted amounts of the FF diet reached weight maximum and then lost weight similarly to the AcS-fed group. These maxima for those receiving the FF diet were reached after shorter time periods than was

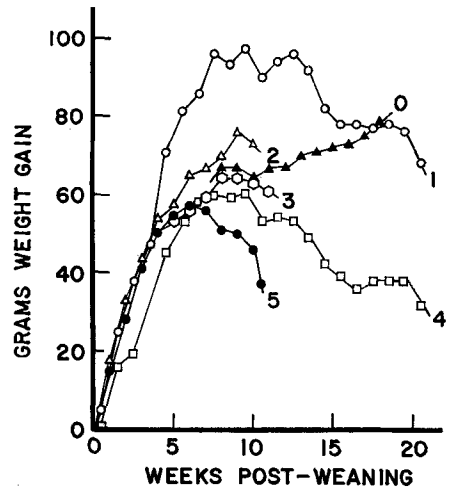

FIG. 5. Effect of caloric restriction on growth of male rats. Curve 0, 30% cottonseed oil diet (CSO), 3.7 g/day; Curve 1, fat-free diet (FF), 8.0 g/day; Curve 2, 30% CSO diet, 4.0 g/day; Curve 3, 30% acetostearin diet, *ad lib.*; Curve 4, FF diet, 5.0–6.0 g/day; Curve 5, FF diet, 5.5 g/day.

TABLE III

Digestibility of Acetostearin and other Fats Fed Alone and as Mixtures

Group Number	Weeks Post-Weaning	Coefficient of Digestibility
30% CSO	5	95.4 ± 0.5
30% CSO	12	93.9 ± 0.5
30% HCO	4	92.9 ± 0.3
10% AcS-DPI	7	60.0 ± 1.6
20% AcS-DPI	7	61.2 ± 1.8
30% AcS-DPI	6	53.0 ± 1.8
30% AcS-USDA	5	53.3 ± 0.7
30% AcS-USDA	12	35.5 ± 1.0
30% AcS-DPI + 200 mg Linoleate/rat/day	6	62.5 ± 3.3
15% AcS-USDA + 15% CSO	5	81.6 ± 0.9
15% AcS-USDA + 15% CSO	12	83.8 ± 0.8
25% AcS-DPI + 5% CSO	5	76.6 ± 0.5
30% AcS-DPI [a]	10	53.9 ± 1.8
30% AcS-DPI [b]	20	55.1 ± 2.1
25% AcS-DPI + 5% Linoleate	4	80.5 ± 0.9

[a] Fed during digestibility study. Prior feeding—Purina Chow.
[b] Fed during digestibility study. Prior feeding—fat-free diet.

evident when this FF diet was fed *ad lib.*, as shown in Figure 1. In contrast to the other restricted groups, the one receiving restricted amounts of the CSO diet reached a weight plateau and then proceeded to gain slightly.

The poor growth obtained with AcS fed as the sole glyceride is probably not due solely to the essential fatty acid (EFA) deficiency which develops, since rats fed the HCO or FF diets, also deficient in essential fatty acids, showed better growth. The possibility exists that AcS causes either an interference with utilization of stored essential fatty acids, an increased rate of their depletion, or both. This might account for the observation that the plateau in growth was reached earlier when AcS was fed as the sole fat than when the FF diet was fed. It could also account for the fact that the stimulation of growth observed by supplementing the 30% AcS diet with linoleate was not observed on supplementing a 50% AcS diet with the same amount of linoleate, indicating an increased requirement for essential fatty acids. A more likely interpretation points to a combination of caloric insufficiency and EFA deficiency. In evidence are the observations that: 1) rats fed restricted daily amounts of a FF diet reached a growth plateau earlier than a group fed the FF diet *ad lib.*; 2) rats which had reached a constant weight on the AcS diet evidenced an immediate weight gain when offered a fat-free diet *ad lib.*, despite a continuing EFA deficiency. That the EFA deficiency is partly responsible for the poor growth on AcS is evidenced by: 1) the improved growth upon supplementation with linoleate; 2) the fact that the FF-restricted group reached a maximum weight earlier, and at a lower level, than isocalorically-fed animals receiving the CSO diet. The lack of stimulation by linoleate on the 50% AcS diet might then be explained by the fact that the caloric insufficiency caused by a 50% AcS diet was so great that levels of linoleate higher than 100 mg/day would be needed for growth stimulation to be evident. Also, the growth of rats receiving a mixture of 15% AcS and 15% CSO was comparable to that found in rats receiving 15% CSO alone, therefore either the requirement for essential fatty acids is not increased by the presence of 15%AcS or the 15% CSO diet supplies an overabundance of EFA for the rat.

The weight gained by rats fed 25% AcS plus 5% of either CSO or linoleate, agrees well with the value reported by Mattson (8) for rats fed 20% AcS plus 4% soybean oil.

Mortality. The mortality of animals fed the acetostearin product alone was low in the time period under investigation, except in the group fed 30% AcS-DPI where a 40% mortality was observed at the end of 10 weeks. The substitution of 5% cottonseed oil for 5% of the acetostearin or the addition of linoleate to the acetostearin diet reduced the mortality to 10% in a similar time interval. In most of the other diets where the U.S.D.A. acetostearin was fed, the mortality was 10% or less.

TABLE IV

Comparative Effects of Saturated and Unsaturated Fats and of the Absence of Dietary fat on Tissue Cholesterol and Total Lipid Levels in Young and Adult Rats Fed either *ad lib.* or Restricted Diets

Wk on diet	Rat wt	Plasma F [b]	Plasma T [a]	Cholesterol Liver F	Cholesterol Liver T	Adrenal [c] F	Adrenal [c] T	Liver Total Lipid
wk	g	mg%		mg/g		mg/g		%
Rats fed 30% acetostearin diet—*ad lib*								
DPI								
6	99(10)	20	61±6	2.8	3.9±.2	5.7	41	4.8±.3
10	117(8)	28	69±4	2.6	3.3±.1	5.6	40	4.6±.2
13	119(5)	14	68±4	2.4	3.5±.2	5.3	51	4.3±.2
U.S.D.A.								
11	163(9)	18	64±4	2.1	2.9±.1	7.1	45	4.7±.3
Rats fed a fat-free diet—*ad lib.*								
11	240(10)	26	70±4	2.1	3.2±.1	....	36	5.8±.1
20	271(5)	5	46±8	2.7	5.1±.2	4.4	27	6.8±.8
Rats fed a fat-free diet—restricted								
5.0–6.0 g/day								
20	79(5)	14	64±2	2.9	4.9±.4	8.7	66	3.6±.3
5.5 g/day								
10	77(5)	....	73±3	3.0	3.6±.2	7.5	64	2.4±.2
8.0 g/day								
20	114(5)	14	68±1	2.6	4.4±.7	9.7	78	4.8±.8
Rats fed 30% cottonseed oil diet—*ad lib.*								
20	415(7) [d]	13	77±5	2.1	2.9±.1	6.8	38	5.2±.1
24	435(10)	17	82±8	1.8	2.5±.1	3.3	35	4.8±.2
Rats fed 30% cottonseed oil diet—restricted (3.7–4.3 g/day)								
6	94(5)	25	83±6	2.6	3.0±.1	5.9	59	3.1±.2
10	108(5)	30	70±3	2.7	2.9±.1	4.2	50	2.4±.4
20	120(5)	30	77±3	2.4	2.7±.1	....	48	4.4±.1
Rats fed 30% hydrogenated coconut oil diet—*ad lib.*								
6	221(5)	13	55±5	2.5	3.4±.1	2.4	16	6.4±.4
20	220(5)	23	81±2	2.6	3.3±.4	11.0	91	5.8±.4

[a] Free cholesterol.
[b] Total cholesterol. Includes standard error of the mean.
[c] Calculated per wet weight of tissue.
[d] Figures in parentheses are number of rats per group.

Digestibility Studies. Fat digestibility coefficients are shown in Table III. No significant differences in digestibility between the two AcS products were observed. The digestibility coefficients found for AcS when fed as the sole dietary fat are lower than values reported in other studies (2,26) in which AcS was the major, but not the only, fat. When fed as a mixture of 25% AcS-5% CSO, which approximates the conditions of studies reported in the literature, digestibility of the fat mixture approached the published values.

The results indicate that total digestibility decreased with increased dietary acetostearin, when determined on rats fed mixtures of AsS-CSO totaling 30% of the diet. The addition of linoleate to the diet improves digestibility. The decreased digestibility of AcS after 12 weeks as compared to after 5 weeks in animals fed these products as the sole dietary fat from weaning is probably not linked to EFA deficiency of the older animals. Animals in which an EFA deficiency was produced by feeding the FF diet for 20 weeks were able to digest AcS as well as those fed this fat for the shorter period. It is possible that some pathology occurs after the longer period of AcS ingestion, which does not occur on the FF diet. However, it is unlikely that the low digestibility obtained after the shorter period reflects something other than a limited ability to digest AcS when fed as the sole fat. Results from animals prefed Purina were no better and rats on the EFA deficient-HCO diet showed digestibility coefficients as high as those obtained for the CSO diet.

Tissue Cholesterol Levels. Values for the free and

total cholesterol concentration in plasma, liver, and adrenals of rats fed various fats as the sole source of fat in the diet, and of rats fed fat-free diets are shown in Table IV. The values for plasma cholesterol in the group of animals fed the 30% acetostearin resembled those obtained for rats fed the fat-free diet in restricted amounts which indicates that the effect of acetostearin feeding is not only that of essential fatty acid deficiency but of caloric restriction as well. Rats fed the cottonseed oil diets exhibited plasma cholesterol levels which were significantly higher than those of the animals fed the acetostearin diet, although no comparison was possible between the acetostearin and unrestricted cottonseed oil diets at 20 weeks because of the high mortality of animals in the former group. The plasma cholesterol levels of the rats fed hydrogenated coconut oil were low after 6 weeks on diet, but at the end of 20 weeks were similar to the values seen in the animals fed the cottonseed oil. This may be due to the gradual adaptation of the animal to the saturated medium chain fatty acids found in hydrogenated coconut oil. The animals on the fat-free diet had plasma cholesterol levels which showed a marked decrease (from 70–46 mg%) with time. The animals fed the fat-free diet in restricted amounts did not show this significant reduction with time which may be because the observed restriction in growth prevented the depletion of the stored essential fatty acids, as compared to the group where food consumption was *ad lib.*, and EFA depletion was greater due to the increased growth of these animals.

The fraction of the total cholesterol present in the free form was variable, but did not differ among groups ingesting the various fat-containing diets *ad lib.*

The concentration of total cholesterol in the liver was generally only slightly higher in animals fed either the AcS or HCO diet than in those which received the CSO diet. However, it was significantly elevated in rats fed the FF diet for the longer period, whether or not dietary consumption was limited, and it is possible that the same effect would have been found in the AcS group had they been maintained for 20 weeks on the diet.

In the HCO group, adrenal cholesterol first decreased and then increased greatly when compared with the CSO group. This again may be some effect of medium-chain saturated fatty acids in contrast to the longer chain fatty acids found in the AcS diet. The concentration of total cholesterol in the adrenal was generally increased in AcS-fed rats when compared with those fed CSO. It was also increased in CSO-fed rats and in those which received no fat when the dietary intakes of these groups were restricted as compared with the levels observed in the corresponding groups fed *ad lib.*

The total lipid level in the liver was significantly elevated only in the group fed the FF diet *ad lib.* for the longer period. Adrenal total lipid values were variable and not interpretable.

Table V presents the tissue lipid level of rats fed AcS and supplemented with methyl linoleate. Included are results obtained with animals fed AcS and CSO concomitantly. Supplementation with 200 mg or less of the methyl ester resulted in low levels of plasma total cholesterol. However, when 5% linoleate was included in the AcS diet, the plasma cholesterol concentration was within the range observed for rats fed the CSO diet. Groups fed various

TABLE V

Effects of Methyl Linoleate, and Cottonseed Oil on Tissue Cholesterol and Total Lipid Levels in Rats Fed Acetostearin

Group No.[a]	Wk on diet	Rat wt	Plasma		Cholesterol liver[b]		Adrenal[d]		Liver Total Lipid
			F[b]	T[c]	F	T	F	T	
	wk	g	mg %		mg/g		mg/g		%
Rats fed acetostearin supplemented with linoleate									
16	11	218(10)[e]	....	.....	2.0	2.4±.1	5.3	46	4.0±.2
17	20	219( )	6	49±2	2.1	2.5±.1	6.6	37	4.2±.4
18	20	222(5)	13	54±6	2.3	2.7±.1	7.3	47	4.3±.3
41	20	249(5)	....	82±4	2.3	2.7±.1	6.0	54	4.7±.3
Rats fed mixtures of acetostearin and cottonseed oil									
32	6	235(5)	18	76±5	2.5	3.5±.1	2.0	16	5.3±.5
32	20	322(5)	25	89±5	2.4	2.9±.1	8.0	43	5.2±.1
33	23	431(10)	17	89±4	1.8	2.5±.1	3.2	27	5.2±.2
36	6	230(5)	15	75±3	2.5	3.1±.1	7.9	13	5.5±.2
36	20	264(5)	30	101±5	2.6	2.9±.1	10.2	71	4.4±.2
37	23	398(9)	15	74±6	1.9	2.5±.1	3.3	18	2.6±.1
40	6	213(5)	13	48±4	2.6	3.1±.2	5.8	25	5.6±.5
40	20	323(5)	22	69±4	2.2	3.1±.2	3.5	34	3.2±.5

[a] Group
#16—Diet 30% AcS-USDA + 100 mg linoleate/rat/day
#17—Diet 30% AcS-DPI + 100 mg linoleate/rat/day } orally
#18—Diet 30% AcS-DPI + 200 mg linoleate/rat/day
#41—Diet 25% AcS-DPI + 5% linoleate
#32—Diet 5% AcS-DPI + 25% CSO
#33—Diet 5% AcS-USDA + 25% CSO
#36—Diet 15% AcS-DPI + 15% CSO
#37—Diet 15% AcS-USDA + 15% CSO
#40—Diet 25% AcS-DPI + 5% CSO

[b] Free cholesterol.
[c] Calculated per wet weight of tissue.
[d] Total cholesterol. Includes standard error of the mean.
[e] Figures in parentheses are number of rats per group.

mixtures of AcS and CSO evidenced plasma cholesterol values similar to those of the CSO groups when the dietary concentration of the unsaturated fat was greater than 5%.

The fraction of the total plasma cholesterol present in the free form was independent of the diet.

The cholesterol levels in the livers of rats receiving AcS plus CSO or linoleate were not significantly different from the levels in those fed CSO alone. Also, the concentration of liver total lipid in these AcS-fed groups was similar to the values obtained for those fed AcS or CSO alone.

In adrenals, higher cholesterol concentrations were noted after the 20-week period than after 6 weeks in the groups fed mixtures of AcS and CSO. The adrenal cholesterol of animals fed the AcS-DPI material (Groups 32 and 36) was much higher than that observed in the animals fed the U.S.D.A. product (Groups 33 and 37) for comparable length of time, indicating that the DPI material induced a greater stress condition in the animals than did the U.S.D.A. product.

The similarity of plasma and liver cholesterol values of rats on the AcS and fat-free diets strengthens the possibility that alterations of these values in AcS fed rats are due mainly to essential fatty acid deficiency. This hypothesis is also supported by the findings that a dietary concentration of 5% linoleate is sufficient to maintain "normal" plasma cholesterol levels in rats fed AcS at dietary levels as high as 25%.

The ingestion of saturated fats has been reported to increase the essential fatty acid requirement for the rat as evidenced by increased severity of deficiency symptoms (9–12). These fatty acids are also thought to be required for proper regulation of cholesterol metabolism (27,28). However, the results of this study indicate no greater alteration in plasma and liver cholesterol levels in AcS-fed rats than in those fed the fat-free diet, despite the considerable amounts of saturated fat ingested.

There is some indication that caloric insufficiency may be involved in cholesterol metabolism of AcS-fed animals, in that cholesterol levels in the adrenals were similar to those found in both the CSO and FF groups when dietary intake was restricted.

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Fatty Acids, Fatty Alcohols, Wax Esters, and Methyl Esters from *Crambe abyssinica* and *Lunaria annua* Seed Oils[1]

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Abstract

Crambe abyssinica and *Lunaria annua*, members of the Cruciferae family, have seed oil glycerides containing ca. 55–65% of C_{22} and C_{24} unsaturated fatty acids. Fatty acids were prepared by saponification; fatty alcohols, by sodium reduction of glycerides; liquid wax esters, by *p*-toluenesulfonic acid-catalyzed reaction of fatty acids with fatty alcohols; and methyl esters, by reaction of fatty acids with diazomethane. Solid hydrogenated glyceride oils and wax esters were compared with several commercial waxes. Chemical and physical constants were determined for the seed oils and their derivatives. Position of unsaturation in the *Crambe* fatty acids was determined by gas chromatographic analysis of the permanganate-periodate degradation products. The major dicarboxylic acid was brassylic (C_{13}), proving the docosenoic acid to be erucic.

Introduction

CRAMBE ABYSSINICA Hochst. ex R. E. Fries (Family: Cruciferae) is an annual herb, about 3 ft tall, that produces numerous spherical pods which are one-seeded and indehiscent. Chiefly distributed around the Mediterranean, through western Europe, and in central Asia, *Crambe* may be introduced into the U.S. as a new chemurgic crop because of its potential industrial and feed uses (1,17).

Lunaria annua L. (*L. biennis* Moench) (Family: Cruciferae), commonly called "honesty," is an annual or biannual herb, 2–3 ft tall. It has fragrant pink-purple flowers and is grown chiefly for the ornamental, thin, lustrous septa that are held in the pod margins, like spectacles in their rims (3,5).

Preliminary analyses for oil and fatty acids, reported earlier (9,12), showed that *Crambe* seed oil contains ca. 60% docosenoic among the derived fatty acids, and *Lunaria* ca. 40% docosenoic and 20% tetracosenoic acids. The major acids in *Lunaria* were later shown to be 13-docosenoic (erucic) and 15-tetracosenoic acids (16). The amino acid composition of *Crambe* seed meal was also reported (15).

The present study follows a recent investigation on derivatives of *Limnanthes douglasii* seed oil (11). Selected chemical and physical properties of oil, fatty acids, fatty alcohols, wax esters, and methyl esters derived from seeds of *Crambe* and *Lunaria* are reported. The major acid in *Crambe* is shown by infrared analysis, permanganate-periodate degradation, and gas chromatographic analysis to be *cis*-13-docosenoic (erucic) acid.

Procedure

Materials, Sample Preparation, and Analytical Methods

Crambe seed was obtained from Montana State College, Bozeman, Mont., and *Lunaria* seed from Herbst Brothers, New York, N.Y. Botanical identity was verified by botanists of the Crops Research Division, U.S.D.A., Washington, D.C.

Solvents, reagents, procedures on preparation of

TABLE I

Derivatives from Seeds of *Crambe abyssinica*[a] and *Lunaria annua*[b]

Sample	Yield, %	Acid value	Iodine value	Hydroxyl, %
Oil (dry basis):				
Crambe	28[c]	2.3	90	....
Lunaria	38	2.0	79	....
Fatty acids (from oil):				
Crambe	90	172	93	....
Lunaria	88	170	80	....
Fatty alcohols (from oil):				
Crambe	79	0.0	100	5.8
Lunaria	77	0.0	85	5.2
Wax esters (from acids and alcohols):				
Crambe	88	0.7	98	0.0
Lunaria	92	0.0	87	0.0
Methyl esters (from acids):				
Crambe	100	0.0	88	....
Lunaria	100	0.0	77	....

[a] Seed + pericarp.
[b] Seed + seed coat.
[c] Oil content in *Crambe* seed may vary 25–40%. The average of crops from 9 locations in the U.S. was 32%.

[1] Presented in part at the AOCS meeting in New Orleans, La., 1962.
[2] A laboratory of the No. Utiliz. Res. & Dev. Div., ARS, U.S.D.A.