The Occurrence of the Chick Pericardial Edema Factor in Some Oleic Acids and Products Derived Therefrom[1]

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A MATERIAL causing the chick edema syndrome has been reported (1, 2, 3, 4) to occur in specific lots of feed-grade animal fats. These fats were reported to contain the edema-producing factor as a trace impurity produced during certain fat-processing operations (2, 3). Birds fed diets containing this unidentified factor develop an edematous condition, characterized by pericardial edema (distention of the pericardial sac with fluid, also termed "hydropericardium") and in more severe cases ascites and gross liver and kidney damage. In the young chicks the initial gross symptoms are characterized by abdominal distention and labored breathing. Manifestations of the pericardial edema factor appear to be limited to poultry (1).

The active pericardial edema-producing factor has not yet been isolated, but certain characteristics of the pericardial edema factor have been reported by Brew *et al.* (2) as follows: a) presumably a hydrocarbon derivative, possibly of cholesterol, b) a molecular weight of about 360, c) associated with fractions which give the Liebermann-Burchard test for sterol residues, d) it can be concentrated by molecular distillation of fats containing the factor. The edema syndrome is not produced by fat *per se* but appears to be related to impurities in certain lots of fats subjected to special fat-processing operations.

F. W. Hill of Cornell University reported to us that high-level feeding to chicks of a sample of a glycerol ester of oleic acid resulted in some mortality with gross symptoms resembling pericardial edema. Our studies have confirmed his observations and furthermore have shown that the pericardial edema-producing factor is present in many samples of commercially-available oleic acid as well as in glycerol esters made therefrom. Molecular distillation of an active oleic acid or of a glycerol mono-ester of an active oleic acid was found to concentrate the pericardial edema-producing factor.

Technique

In this investigation a rapid, sensitive bioassay procedure, developed in our laboratories, was employed (5). The diet used is a modification of that described by Brew *et al.* (2) and consists principally of purified casein, gelatin, glucose, and 16% test fat. Day-old chicks are fed this diet *ad lib.* immediately on arrival. Subgroups are sacrificed at 10, 14, and 21 days. In general, a higher incidence of pericardial edema is observed in the subgroups sacrificed at 10 and 14 days than in the group sacrificed at 21 days. At autopsy, chicks are examined for the appearance of pericardial edema, ascites, and the appearance of complications, such as liver and kidney damage or labored breathing. Bioassay groups consist of 10 or 15 chicks for each sample of fat. Using a weighting procedure described in Table I, a pericardial edema-activity score is calculated. This makes possible a semi-quantitative comparison between the relative activities of various fat samples.

Occurrence

Oleic acid samples (U.S.P.) from four manufacturers were assayed biologically and chemically (acid value and unsaponifiable content). As indicated in Table I, several of the oleic acid samples were strongly active, but others showed little or no activity. The pericardial edema activity scores showed that no correspondence existed between the presence of the pericardial edema-producing factor and the acid value or the content of unsaponifiables.

Tests for the pericardial edema-producing factor in certain commercial samples of glycerol esters (monoglycerides and monodiglycerides) of oleic acid are summarized in Table II. Seven different manufacturers of glycerol esters are represented. The majority of these ester samples were active. Again, there is no correlation between unsaponifiable content and pericardial edema activity.

In four instances, samples of both the oleic acid and the glycerol ester produced therefrom were bioassayed as summarized in Table III. The glycerol esters showed activity only when the corresponding oleic acid was active. With inactive oleic acids or those with low activity the corresponding glycerol ester did not have increased activity. Thus the edema factor was not formed during the manufacture of the glycerol esters.

Concentration Studies

Molecular Distillation of an Oleic Acid. A sample of oleic acid known to contain a moderate amount of the pericardial edema-producing factor was fractionated in a 14-in. molecular still into six fractions as indicated in Table IV. The first and last fractions and a composite of the middle fractions were bioassayed as described above. The first 12% strip cut was entirely free of the pericardial edema-producing factor. The middle cuts, representing approximately 60% of the input fat, were only slightly active. The last 7% fraction showed a concentration of the edema factor with an increased activity over the input oleic acid. Thus the pericardial edema-producing factor was concentrated in the last fraction after the bulk of the oleic acid was removed by molecular distillation.

Distillation of a Glycerol Mono-ester of an Oleic Acid. A sample of a glycerol mono-ester of oleic acid, prepared from an oleic acid known to contain the pericardial edema-producing factor was fractionated into six fractions in a 14-in. molecular still, as described in Table V. The first 10% cut, the last 3% fraction, and an intermediate fraction representing appoximately 60% of the input material were bioassayed as described above. An increased concentration of the pericardial edema-producing factor was observed in the first 10% cut. Some pericardial edema-producing factor was present in the other fractions examined.

Discussion

The material which produces pericardial edema in chicks has been reported previously only in certain lots of fats subjected to special fat-processing operations (1, 2, 3). Therefore the finding that some U.S.P. oleic acids possessed a high degree of activity was unexpected. Feed-grade fats that contained the edema factor characteristically had high unsaponifiable levels ranging above 6% (2). However, in the present study, samples of the more active oleic acids had unsaponifiable levels ranging from 0.2 to 0.7%. This indicates a 10- to 30-fold concentration of the edema factor in the unsaponifiable fraction and suggests that the unsaponifiable fraction of an active oleic acid might be a starting material for isolation of the factor.

Molecular distillation of a fat which contains the factor concentrated this factor in the more volatile fractions (2). Molecular distillation of an active glycerol mono-ester of oleic acid also concentrated the edema factor in the more volatile fractions, but separation from the monoglyceride fraction was not as complete as it was from the higher-

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Sample No.	Cut, %	Analysis		Bioassay Results						Activity ratio[c] pos./total	Activity score,[d] %
				Pericardial edema				Other deaths[b]	Negative		
		Acid value	Unsap. %	Plus death	Plus complications[a]	Plus ascites	Uncomplicated				
TABLE I. Oleic Acids and Their PE (Pericardial Edema) Activity											
1	.....	201.0	0.45	.....	.....	.....	.....	.....	21	0/21	0
2	.....	202.5	0.68	.....	2	1	.....	.....	7	3/21	110
3	.....	201.2	0.77	.....	.....	1	.....	.....	8	1/10	33
4	.....	202.8	0.52	.....	.....	2	.....	.....	8	2/10	60
5	.....	203.0	0.18	1	1	1	.....	.....	6	3/10	133
6	.....	202.5	0.28	.....	4	.....	3	.....	4	7/11	200
7	.....	201.3	0.51	.....	.....	.....	.....	.....	10	0/10	0
8	.....	204.9	0.50	.....	.....	.....	1	.....	8	1/9	22
9	.....	204.4	0.77	.....	3	4	3	.....	.....	10/10	300
10	.....	203.9	0.52	.....	.....	.....	.....	.....	10	0/10	0
11	.....	202.0	0.20	.....	.....	.....	.....	.....	10	0/10	0
TABLE II. Oleic Acid Esters and Their PE (Pericardial Edema) Activity											
1	.....	2.2	.....	.....	3	1	2	.....	0	6/6	320
2	.....	1.6	0.12	.....	.....	1	5	.....	4	6/10	130
3	.....	1.7	0.13	3	4	4	13	.....	6	24/30	230
4	.....	2.2	0.26	.....	.....	.....	.....	.....	11	0/11	0
5	.....	.....	.....	.....	.....	.....	1	.....	13	1/14	14
6	.....	0.5	0.32	.....	.....	2	.....	.....	9	2/11	55
7	.....	0.3	0.63	.....	.....	.....	.....	.....	9	0/9	0
8	.....	2.8	0.5	1	.....	.....	4	3	2	5/10	160
9	.....	4.7	0.49	2	1	.....	3	.....	4	6/10	200
10	.....	7.1	0.35	.....	.....	1	.....	.....	8	1/9	33
11	.....	1.2	0.46	.....	.....	.....	.....	3	6	0/9	33
12	.....	5.5	1.2	.....	.....	.....	2	3	9	2/14	50
TABLE IV. Molecular Distillation of an Oleic Acid (Sample 6, Table I)											
Charge	.....	.....	.....	.....	4	.....	3	.....	4	7/11	200
F-1	11.9	.....	.....	.....	.....	.....	.....	.....	10	0/10	0
F-2	10.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
F-3	29.1 }	.....	.....	.....	1	.....	.....	.....	8	1/9	45
F-4	29.6 }										
F-5	9.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
F-6	7.2	.....	.....	2	1	5	.....	.....	2	8/10	290
r	1.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
TABLE V. Molecular Distillation of a Glycerol Mono-ester of Oleic Acid (Sample 2, Table II)											
Charge	.....	.....	.....	.....	.....	1	5	.....	4	6/10	130
F-1	10.4	.....	.....	3	2	4	.....	.....	1	9/10	350
F-2	9.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
F-3	30.6 }	.....	.....	.....	.....	1	1	1	6	2/9	67
F-4	31.5 }										
F-5	11.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
F-6	2.8	.....	.....	.....	1	2	1	.....	6	4/10	120
r	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

[a] Complications include gross liver and kidney damage or labored breathing.
[b] Early deaths in excess of control groups, PE not observed.
[c] Chicks with positive pericardial edema/total chicks in test.
[d] The "PE activity score" is a numerical index of the severity of the activity. The products of the number of chicks in each category times its weighting factor are summated and divided by the total number of chicks as follows:

$$\text{PE activity score} = 100 \times \frac{5 \times (\text{PE} + \text{death}) + 4 \times (\text{PE} + \text{compl.}) + 3 \times (\text{PE} + \text{ascites}) + 2 \times (\text{PE}) + 1 \times (\text{other deaths})}{\text{Total chicks in test group}}$$

distilling triglycerides. Molecular distillation of an active oleic acid concentrated the edema factor in the less volatile fraction. Since fatty acids, such as oleic acid, distill at temperatures lower than the corresponding monoglycerides, the distillation range of the edema factor appears to lie above that of oleic acid and below that of the glycerol mono-ester of oleic acid.

Summary

A number of samples of commercially-prepared (U.S.P.) oleic acids and glycerol esters of oleic acids were shown to contain a material which produces pericardial edema in chicks. The active material was concentrated from either the oleic acid or the glycerol mono-ester by molecular distillation.

Four of the 11 samples of oleic acid were inactive; this indicates that the pericardial edema is not caused by the fatty acid itself but by an incidental material in the fatty acid.

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TABLE III
Comparison of PE (Pericardial Edema) Activity of Oleic Acids and Their Corresponding Glycerol Esters

Oleic Acid			Glycerol Ester		
Sample No. (from Table I)	PE activity ratio	PE activity score	Sample No. (from Table II)	PE activity ratio	PE activity score
	pos./total	%		*pos./total*	%
5 (75%)	3/10	133 }	2	6/10	130
11 (25%)	0/10	0 }			
5	3/10	133	3	25/31	230
1	0/21	0	4	0/11	0
3	1/10	33	5	1/14	14

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