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Lecithin in Oil-in-Water Emulsions¹

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OIL-IN-WATER EMULSIONS have been undergoing extensive clinical investigation for intravenous alimentation. The stabilizing agent in most of the emulsions that have been tested was a purified preparation of soybean phosphatides (3). These preparations, even after so-called purification, are complex mixtures of phosphatides and other materials. The presence of such impurities and the variation in their composition from one preparation to another may contribute at times to some of the adverse reactions observed on administration of these emulsions (9). This study was undertaken therefore to investigate the use of highly purified phosphatides, principally lecithin, in emulsion preparations. A small scale emulsion stability test was developed for the small quantities of highly purified fractions made available for this purpose. Highly purified lecithin was found to be an inefficient emulsifier producing emulsions particularly unstable to auto-claving, a requisite procedure in the sterilization of emulsions for use in intravenous alimentation.

Various materials were added back to the purified lecithin to determine their influence on emulsification. Such materials included fatty acids, carbohydrates, other phosphatides, and combinations of these materials. Addition of fatty acids improved considerably the emulsification properties of the lecithin preparations, but in no instance did any of the emulsifiers equal in their emulsification properties that of the

soybean phosphatide preparations ordinarily used in emulsification.

Experimental

Purified Lecithin

Lecithin fractions were obtained from egg yolk by chromatographic purification procedures using an alumina column as described by Hanahan *et al.* (4, 5). These were found to contain trace impurities which were indicated to be in part lysolecithin (2, 7) and probably also sphingomyelin (1, 6). In subsequent purification employing silica column procedures similar to that described by Lea *et al.* (7), lecithin fractions were obtained which, by the chromatographic strip technique (2), analyzed as only one component having the same R_f value as an authentic sample of synthetic lecithin.³

Emulsion Evaluation Procedure

A small-scale test was developed to evaluate lecithin and mixtures as emulsifiers. The procedure consisted of dispersing in a Virtis homogenizer (Model No. 6-105) the requisite amount of emulsifier formulation in 85 parts (42.5 g.) of 5% dextrose solution containing 0.3 parts (0.15 g.) of Pluronic F-68⁴ for about one minute at one-half full setting on the Virtis variaec. Then at slow speed 15 parts (7.5 g.) of Wesson Oil⁵ (15 wt.-% of the total emulsion) were added, and the speed was increased over a three-minute period to maximum for the homogenizer (about 13,000 r.p.m.). After five minutes at this speed a sample of about 10 ml. was transferred to a 15-ml. centrifuge tube, stoppered, mechanically

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shaken (about 600 strokes/minute) for one hour, centrifuged (1,800 r.p.m.)⁶ for one hour, and finally the volume of separated oil was read to the nearest 0.05 ml. The physical stability or "stability index" of the emulsion is obtained by calculation of the percentage of oil remaining emulsified.

Stability Evaluations

Purified lecithin. Emulsion stability tests were made with both "chromatographically" purified and the repeatedly precipitated but "unchromatographed" egg lecithin. This latter material is the precursor for the "highly purified" lecithin and consists of about two-thirds lecithins and one-third cephalins (5). To evaluate a particular "lecithin" the solvent was removed by vacuum in an all-glass rotor-evaporator from a requisite amount of alcoholic-phosphatide solution, and the dried lecithin was dispersed in the dextrose solution, as described previously.

Other phosphatide preparations. Several tests, for comparison, were conducted, using a number of commercial and laboratory phosphatide preparations.

Purified lecithin with additives. Since "highly purified" lecithin was obtainable only through lengthy and tedious chromatographic procedures, most of the evaluation tests with additives were made by using the purified but "unchromatographed" egg lecithin, *i.e.*, the precursor to the "highly purified" lecithin. Evaluations were made with added fatty acids, carbohydrates and their derivatives, other phosphatides, some amino compounds, and some combinations of these substances. In making an evaluation, the additive was weighed into a small glass-stoppered test tube, then an alcohol solution containing the required amount of phosphatide was introduced, and the whole was brought into solution by slight warming (about 50°C.) or by use of additional inert volatile solvent. Evaporation yielded the lecithin-additive mixture, which was then used in the standard evaluation procedure.

Results and Discussion

The results obtained on application of the physical stability test to emulsions of known stability properties (Table I) correlates quite well with tests made

TABLE I
Physical Stabilities of Control Emulsions Containing 0.3 Parts Pluronic F-68, 15 Parts Wesson Oil, 85 Parts of 5% Dextrose and 1.2 Parts of Added Emulsifier

Added emulsifier	Physical stability of large-scale preparations ^a	Ml. oil separated per 10 ml. of emulsion	Stability index
Drumulse 536 R ^b	Very good	0.06	96
Preparation of soybean phosphatides currently used in clinically tested I.V. emulsions	Good	0.06-0.14 ^c	91-96 ^c
None	Poor	0.8	50
Polyoxyethylene lauryl alcohol	Bad	1.1-1.2	25-32

^a Prepared in this laboratory in commercial type of homogenizing equipment.

^b Product of the E. F. Drew Company.

^c Range of five determinations on four preparations.

on a larger scale with the commercial type of homogenizing equipment. A "good" emulsion separated only about 0.1 ml. of oil per 10 ml. of emulsion

³ This sample was kindly supplied by Erich Baer, University of Toronto.

⁴ An ethylene oxide-polyoxypropylene surface-active agent made by the Wyandotte Chemicals Corporation.

⁵ A specially selected, refined, bleached, and deodorized cottonseed oil prepared by the Southern Cotton Oil Company.

⁶ A table top centrifuge, International Clinical Centrifuge No. P 3411, was used for this purpose.

(stability index of about 94% or better) whereas a "poor" or "bad" emulsion separated some 10 times this amount of oil (stability index of about 50% or less). Reproducibility was good, as indicated by a range of 91 to 96 in stability index for five determinations, using different preparations (Table I).

Results of the evaluation tests given in Table II

TABLE II
Physical Stabilities of Emulsions Containing 0.3 Parts Pluronic F-68, 15 Parts Wesson Oil, 85 Parts 5% Dextrose and 1.2 Parts Phosphatide Preparation

Phosphatide preparation	Ml. oil separated per 10 ml. emulsion	Stability index	Apparent physical stability
1. "Chromatographed" egg lecithin	0.87	46	Poor
2. "Unchromatographed" egg "lecithin"	1.10	32	Poor
3. "Chromatographed" egg lecithin + "chromatographed" egg cephalins ^a	0.19	88	Fair
4. "Unchromatographed" egg "lecithin" + "chromatographed" egg cephalins ^b	0.29	82	Fair
5. Animal cephalin ^c	0.14	91	Good
6. Purified soybean currently used in clinically tested I.V. emulsions	0.06-0.14	91-96	Good
7. Ethanol-soluble fraction of 6	0.22	86	Fair
8. Ethanol-insoluble fraction of 6	0.27	83	Fair
9. Defatted, carbohydrate-reduced soybean ^d	0.15	91	Good

^a Contained 0.9% lecithins and 0.3% cephalins.

^b Contained 1.0% lecithins and 0.25% cephalins.

^c Supplied by Nutritional Biochemicals Corporation.

^d Supplied by the American Lecithin Company Inc.

indicate that the purified egg lecithins are inefficient emulsifiers, exhibiting stability index values of less than 50%. Included in this table are results obtained with several other phosphatide materials for comparison with the egg lecithin results. It is noted that the emulsion made with a commercial preparation of soybean phosphatides has the highest stability index whereas "additionally treated" soybean phosphatide samples, *i.e.*, essentially purer phosphatide materials, exhibit lesser values. This apparently indicates that, in general, the emulsifying power of the phosphatides decreases as its "purity" increases.

A considerable number of evaluations were conducted to determine the effect of certain additives on the emulsifying power of egg lecithin. Listed in Table III are results with those additives considered likely components of "lecithin" preparations, namely fatty acids, carbohydrates, and cephalins. It is observed that the addition of these substances to the purified egg lecithin resulted in increased emulsifying powers for the lecithin-additive mixtures. Although the increase was not substantial in most cases, additions of fatty acids and cephalins produced emulsions with stabilities approaching that observed with "good" emulsion formulations. In the case of fatty acid the best emulsion stability was obtained when some 25-30% oleic acid was incorporated in the lecithin. Increasing the fatty acid content or varying the lecithin quantity gave less efficient emulsifiers. However this optimum fatty acid requirement (about 0.34% in the whole emulsion) is greater than the 0.1% indicated as permissible in oils used as solvents for drugs for intravenous administration (8). Several combinations of these three additives were evaluated with both the "unchromatographed" and the "chromatographed" lecithin. Although the results indicated better emulsion stabilities than experienced with a single additive in similar concentration, they

TABLE III

Effect of Added Fatty Acid, Carbohydrate, and Cephalin on the Physical Stabilities of Egg Lecithin Emulsions Containing 0.3 Parts Pluronic F-68, 15 Parts Wesson Oil, 85 Parts 5% Dextrose, and Egg Lecithin-Additive Combinations in the Amount Shown

% Egg lecithin and additives in emulsion				Ml. oil separated per 10 ml. of emulsion	Stability index
Egg lecithin	Oleic acid	Glucose	Animal cephalin		
1.2 ^a	1.10	32
1.2 ^a	0.08	1.07	34
1.2 ^a	0.17	1.03	36
1.2 ^a	0.24	0.67	58
1.2 ^a	0.27	0.22	86
1.2 ^a	0.34	0.08	95
1.2 ^a	0.56	0.11	93
2.4 ^a	0.12	0.41	75
0.6 ^a	0.17	0.62	61
None	0.34	0.77	52
1.2 ^a	0.30 ^b	0.81	50
1.2 ^a	0.30	0.71	56
2.4 ^a	0.24	0.41	75
2.4 ^a	0.60	0.30	81
1.2 ^a	0.12	0.12	0.31	81
2.4 ^a	0.12	0.24	0.27	83
1.0 ^a	0.28	0.46	72
1.2 ^a	0.08	0.46	72
2.4 ^a	0.12	0.44	73
0.6 ^a	0.60	0.22	86
None	1.2	0.14	91
None	0.32	1.2	0.14	91
1.2 ^a	0.06	0.06	0.56	65
1.2 ^a	0.11	0.12	0.35	78
1.2 ^c	0.87	46
1.2 ^c	0.34	0.44	73
1.2 ^c	0.30	0.48	70
1.2 ^c	0.15	0.58	64
1.2 ^c	0.06	0.30	0.42	74
1.2 ^c	0.06	0.06	0.60	63

^a "Unchromatographed" "lecithin."

^b Inositol used in place of glucose.

^c "Chromatographed" "lecithin."

fell short of the high stability index values for good emulsions.

Additional evaluations were conducted, employing a number of amino and hydroxy compounds as additives which might be possible components of phosphatide materials. Results of these tests are listed in Table IV and include tests with amino acids, hydroxy compounds, and various carbohydrate derivatives. Most showed only slight improvement in emulsifying power over the lecithin, but some, such as ascorbyl palmitate, gave interesting results. However ascorbyl palmitate without lecithin did not produce an emulsion.

In addition to physical stability, an emulsion intended for intravenous alimentation must also be

TABLE IV

Effect of Added Hydroxy- and Amino-Compounds on the Physical Stabilities of Egg Lecithin Emulsions Containing 0.3 Parts Pluronic F-68, 15 Parts Wesson Oil, 85 Parts 5% Dextrose, and "Unchromatographed" Egg Lecithin-Additive Combinations as Indicated

% Egg lecithin and additive in emulsion		Ml. oil separated per 10 ml. of emulsion	Stability index
Lecithin	Additive		
1.2	0.12-gelatin	0.35	78
1.2	0.12-urea	0.62	61
1.2	0.12-dl-phenylalanine	0.66	59
1.2	0.12-dl-isoleucine	0.72	55
1.2	0.12-dl-serine	0.79	51
1.2	0.12-dl-glutamic acid	1.04	35
1.2	0.12-glycerol	0.63	61
1.2	1.20-glycerol	0.37	77
1.2	12.00-glycerol	0.54	66
1.2	0.12-dipalmitin	0.73	55
1.2	0.06-cholesterol	0.66	59
1.2	0.12-lactic acid	1.03	36
1.2	0.30-gluconic acid	0.84	48
1.2	0.30-galacturonic acid	0.40	75
1.2	0.12-galacturonic acid	0.31	81
1.2	0.10-ethyl glucoside	0.44	73
1.2	(0.12-ethyl glucoside + 0.12-galacturonic acid)	0.24	85
1.2	0.10-alpha methyl glucose	0.48	70
1.2	0.30-ascorbyl palmitate	0.21	87
None	1.20-ascorbyl palmitate	No emulsion	0

heat-stable, that is, it must be unaffected by autoclaving. The more stable emulsions were tested further for heat stability. A number of formulations were homogenized by using the standard test procedure (Virtis homogenizer) and others in a Manton-Gaulin pressure homogenizer (up to 5,000 p.s.i.g.) modified for about 75-ml. batches. These test emulsions were then subjected to autoclaving at 121°C. for 15 min. in sealed bottles prior to the stability test. Results of these tests, Table V, indicated that all formulations separated some oil during autoclaving. Although heat-sensitive, the better formulations approached the stability values exhibited by the "control" soybean phosphatide emulsion.

TABLE V

Effect of Autoclaving on the Physical Stabilities of Emulsions Containing 0.3 Parts Pluronic F-68, 15 Parts Wesson Oil, 85 Parts 5% Dextrose and Egg Lecithin-Additive Combinations in the Quantities Indicated

% Lecithin and additives in emulsion			Ml. oil separated per 10 ml. of emulsion		Stability index	
Egg lecithin	Oleic acid	Other additive	Unautoclaved	Autoclaved	Unautoclaved	Autoclaved
1.2 ^{a, b}	0.33	0.19	0.04	88	97
1.2 ^{a, c}	0.33	0.21	0.19	87	88
1.2 ^{a, c}	0.20	0.3 ^d	0.35	0.29	78	82
1.2 ^{a, c}	0.6 ^e	0.42	0.23	74	86
1.2 ^{b, f}	0.36	0.06	0.05	96	97
0.8 ^{b, f}	0.33	0.4 ^g	0.05	0.13	97	92
0.9 ^{c, f}	0.33	0.3 ^g	0.11	0.15	93	91
0.9 ^{c, f}	0.3 ^g	0.22	0.28	86	83
Control emulsion with soybean phosphatides	1.2 ^h	0.12	Trace	Trace	92	> 98

^a "Unchromatographed" egg "lecithin."

^b Homogenized under pressure (3,000-4,000 p.s.i.g.) in modified Manton-Gaulin homogenizer.

^c Homogenized by rapid stirring in Virtis homogenizer (13,000 r.p.m.).

^d Glucose.

^e Ascorbyl palmitate.

^f "Chromatographed" egg lecithin.

^g Cephalin fraction from the chromatographic purification of egg lecithin.

^h Control emulsion prepared from the basic formulation indicated above but made with 1.2% of a soybean phosphatide preparation currently used in clinically tested I.V. emulsions in place of the egg lecithin.

Summary

A study was made to investigate the use of purified phosphatide in I.V. emulsions. Lecithin isolated from egg yolk and purified by alumina and silica chromatography was analyzed by chromatographic strip techniques as a one-component material. Highly purified lecithin was found to be an inefficient emulsifier. Moreover emulsions containing highly purified lecithin were heat-sensitive. An emulsion physical stability test was developed to evaluate emulsifier formulations containing purified phosphatides for use with small amounts of emulsions (approximately 50 g.). Using this procedure, a considerable number of substances were tested as additives to enhance the purified lecithin's emulsifying power. None were found to be as effective as natural soybean phosphatide, which was used as a control. From these observations it is indicated that pure phosphatides are inefficient emulsifiers and that those phosphatide preparations possessing good emulsifying characteristics are presumably mixtures or complexes of the phosphatides with other substances.

Acknowledgment

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Letter to the Editor

May 6, 1958

AT THE SPRING MEETING of the American Oil Chemists' Society, in April 1958, in Memphis the Statistical Committee received a suggestion that articles of a statistical nature that are submitted to the Journal be reviewed by this committee prior to their publication. Inasmuch as the committee is not represented on the editorial board of the Journal and has no real jurisdiction in this matter, it is obvious, from a practical standpoint, that such a procedure must await implementation by the Journal Committee.

The Statistical Committee is nevertheless immediately concerned that the high standard exhibited by previous articles be met by those papers which are clearly statistical or which attempt to make quantitative conclusions. The committee is willing to act in

an advisory capacity in any matter pertaining to statistics and concerning the Society and, in particular, offers its services to contributors to the Journal. We hope, by this letter, to impress upon these contributors the desirability of including adequate statistical treatment of appropriate data and of soliciting statistical aid in such cases. The committee stands ready to review papers on request and, where the need is clearly indicated, will offer recommendations. In this manner we wish to be of service to the Journal and to the Society.

W. E. LINK, chairman
700 Investors Building
Minneapolis, Minn.

A B S T R A C T S . . . R. A. REINERS, Editor

ABSTRACTORS: Lenore Petschaft Africk, R. R. Allen, S. S. Chang,
Sini'tiro Kawamura, F. A. Kummerow, and Dorothy M. Rathmann

• Oils and Fats

The oxidation of unsaturated compounds. IX. The effects of structure on the rates and products of oxidation of unsaturated compounds. F. R. Mayo, A. A. Miller, and G. A. Russell (Gen. Elec. Res. Lab. and Stanford Res. Inst.). *J. Am. Chem. Soc.* **80**, 2500-07 (1958). The relative rates of reaction of some unsaturated compounds with one atmosphere of oxygen have been investigated, using one monomer at a time, and using two monomers at a time (to yield a terpolymer with oxygen). The close correspondence between the two sets of data indicates that the reactivity of the double bond toward a peroxide radical is the principal factor governing the over-all rate of reaction. The organic part of the peroxide radical (M in MO₂) has a small but significant effect on the propagation reactions of the peroxide radical. The products of oxidation of unsaturated compounds are considered, using the data in this report and in the literature.

Studies on seed fats of Cucurbitaceae family. II. The Component fatty acids of *Trichosanthes cucumerina*, Linn. S. A. Patel, S. Bhattacharyya and M. M. Chakrabarty (Univ. College of Science & Technology, Calcutta). *J. Indian Chem. Soc.* **35**, 67-71 (1958). The seed fat of *Trichosanthes cucumerina* contains 11.87% saturated fatty acids, 32.59% oleic acid, 19.83% linoleic acid, and 35.46% conjugated triene acid calculated as α -elaeostearic acid. A considerable amount of arachidic acid was found.

Isolation of methyl monohydroperoxido-9-octadecynoate from the autoxidized methyl 9-octadecynoate. N. A. Khan (East Regional Laboratories, Pakistan Council of Scientific & Industrial Research, Tejgaon, Dacca, East Pakistan). *J. Org. Chem.* **23**, 606-7 (1958). Methyl stearolate was reported to react with oxygen and yield monohydroperoxide with the triple bond intact during the initial stages of autoxidation. Methyl hydroperoxide-9-octadecynoate was isolated as the sole product from autoxidized methyl stearolate.

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Autoxidation of 2-ethyl-1-hexene. K. Morikawa (Yokohama Natl. Univ.). *Bull. Fac. Eng. Yokohama Natl. Univ.* **6**, 87-94 (1957). The autoxidation of 2-ethyl-1-hexene was carried out at 10, 20, 30, 40, 50, and 80°, and the hydroperoxides produced were separated by silica gel-chromatography and decomposed by ferric ions or reduced by sodium acid sulfate to the corresponding ketones or alcohols. (*C. A.* **52**, 8932)

Bear Fat. H. Steger and F. Püschel (Inst. Tierzuchtforsch. h., Dummerstorf, Rostock, Ger.). *Pharmazie* **12**, 821-5 (1957). The body fat, intestinal fat, and kidney fat was examined from 3-year old brown bears (*Ursus arctos*) living on a vegetarian diet in a zoo. In the order of body fat, intestinal fat, and kidney fat, the following properties are: specific gravity (20°/20°) 0.9184, 0.9191, 0.9195; melting point (flowing) 25.7°, 34.2°, 36.6°; (clear) 35.2°, 39.2°, 40.2°; n_{D_20} 51.6, 51.4, 51.4; acid number 0.85, 1.1, 0.76; saponification number 200.0,