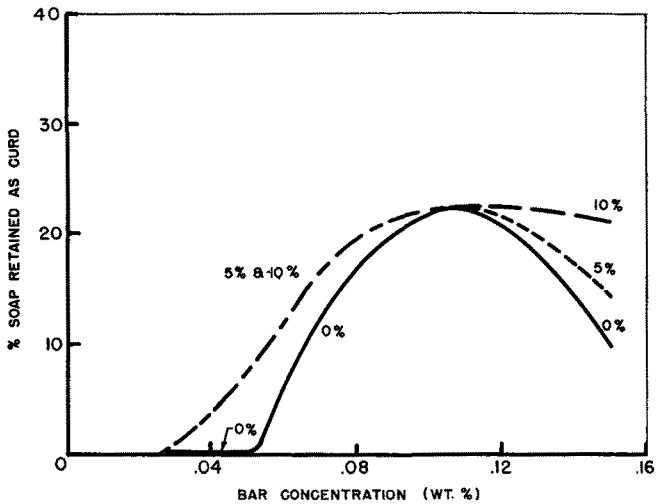

FIG. 3. Effect of added electrolyte on lime-soap dispersion by non-ionic. Dispersion of 95% soap/5% dispersant in the presence of 0%, 5%, and 10% NaCl, based on dispersant concentration.

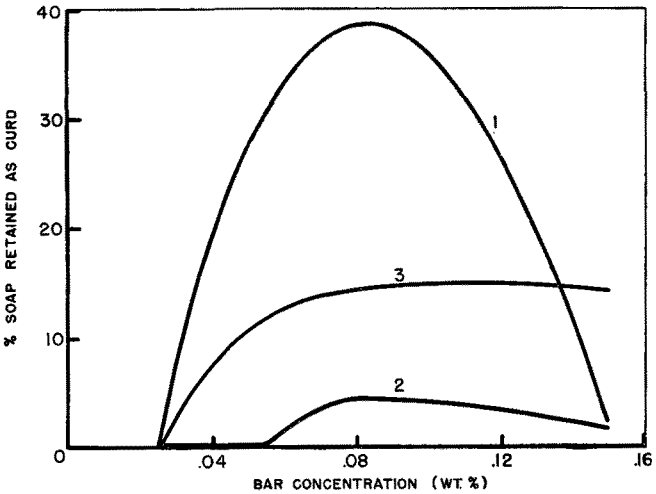

FIG. 4. Effect of coco/tallow ratio on self-dispersion of soda soaps. *1.* 25% coco/75% tallow. *2.* 35% coco/65% tallow. *3.* 50% coco/50% tallow.

tent since this results in a preponderance of more soluble short chain and unsaturated lime soaps being formed. However it is to be noted that the composition containing 35% coco is considerably better in lime-soap dispersing power than any of the others. This is a somewhat surprising result, but it has been borne out by observation in practical use tests.

There is also evidence that the effect of structure on lime-soap dispersion can also be demonstrated with this test. For example, in a series where a fixed hydrophobic group is modified by a hydrophilic group of varying activity (*e.g.*, a series of polyoxyethylene derivatives), it can be shown that optimum dispersion is to be found at quite specific hydrophilic-hydrophobic ratios.

Summary

A new titrimetric method for the measurement of lime-soap dispersion is described. This method differs from previously reported ones in that it is a practical method, *i.e.*, it measures lime-soap dispersion for formulations over a range of water hardness. It is specifically designed for the study of toilet-bar formulations but may be applied to any soap-dispersant formulation. This method also makes manifest the coagulant (as opposed to dispersant) properties of anionic agents at high use concentrations, which may explain why dispersants reported satisfactory by previous tests have been found unsatisfactory in practice. The test is also capable of demonstrating the effect of composition of the formulation and the structure of the dispersant on the lime-soap dispersion.

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Solubility and Heat Stability of Fat-Soluble Derivatives of Vitamin B_6[1,2,3]

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A WHITE CRYSTALLINE COMPOUND, pyridoxine hydrochloride, has served as a commercial source of vitamin B_6. So-called "fat soluble" derivatives of vitamin B_6 have been prepared by esterifying pyridoxine with the short chain fatty acids, acetic (1, 2, 3, 4, 5, 6) and propionic acid (4). These compounds have two disadvantages for practical use in the fat industry. First, they are completely or almost insoluble in fats. Second, they are readily destroyed by heat at the temperature which is usually applied to frying purposes. Witting *et al.* (7) have recently shown that in certain cases the need for vitamin B_6 is increased when heated fats are included in the diet. Their observations have thus necessitated synthesis of a fat-soluble vitamin B_6 preparation which is stable toward heat.

The synthesis of fat soluble derivatives of the vitamin B_6 group (8) and their biological activity has recently been investigated (9). These long chain fatty acid esters were fully active as a source of vitamin B_6 when tested with rats.

Experimental

Synthesis of Fat-Soluble Derivatives of Pyridoxine. Pyridoxine-5-monopalmitate and the fully

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acylated derivatives of pyridoxine, pyridoxine trihexanoate, pyridoxine trioctanoate, pyridoxine tridecanoate, pyridoxine tripalmitate, and pyridoxine trilinoleate were prepared as reported (8). 3-Palmitoxypyridoxine-4, 5-diacetate [4] was a reaction product of palmitoyl chloride and pyridoxine-4, 5-diacetate in pyridine. The latter compound was prepared, following the procedure reported by Harris *et al.* (10). Pyridoxine triacetate hydrochloride was also synthesized according to the method previously reported (1).

Solubility of Fat-Soluble Derivatives of Pyridoxine in Fats. The solubility of these compounds in winterized olive oil was tested at 3°C. at a level of 1% in most cases. The pyridoxine preparations were dissolved in the oil, and the solutions were placed in the refrigerator at 3°C. for one week. The presence or absence of precipitate was recorded as an indication of solubility.

Heat Stability of Pyridoxine Tripalmitate. Both biological and chemical means were used to show that pyridoxine tripalmitate was stable toward heat.

Biological test (A): approximately 20 mg. of pyridoxine tripalmitate were placed in a small test tube and heated on an oil bath at 205–210°C. (11) for 8 hrs. At the end of this heating period the compound had turned to a black resin-like substance. It was then pulverized, extracted with chloroform, decolorized, and the solvent removed. The residue was dissolved in a few drops of highly hydrogenated coconut oil and administered to a severely acrodynic rat which had been kept on a synthetic diet free of both fat and vitamin B_6 for eight weeks. The change in body weight was recorded.

Biological test (B): thirty mg. of pyridoxine tripalmitate were dissolved in 1 lb. of corn oil and heated at 205–210°C. for 8 hrs. As a control, a pound of corn oil was also heated under the same conditions, and when the oil was cooled, 30 mg. of the tripalmitate were dissolved in it. These oils were incorporated separately into the ration at a level of 10% so that the final concentration of pyridoxine in each diet was 1.5 γ calculated as pyridoxine hydrochloride per gram of feed (Table I). For each group, two of the 35-day-old male rats (average body weight 60.0 g.) were used. They had been kept on a vitamin B_6 deficient diet for two weeks, and then transferred to these diets. The body weight gain was recorded over a period of three weeks.

Chemical test: 100 mg. of pyridoxine tripalmitate were dissolved in 3 ml. of corn oil and heated at 205–210°C. for 8 hrs. The oil was then diluted with 20 ml. of petroleum ether and cooled in the refrigerator overnight; then the precipitate was collected. Recrystallization was repeated twice from 95% ethyl alcohol. The pure white crystals that were recovered weighed 66.3 mg.; they gave a negative ferric chloride test, contained nitrogen, and showed no depression in melting point when mixed with authentic pyridoxine tripalmitate.

Relative Heat Stability of Fat-Soluble Derivatives of Pyridoxine. The relationship between the heat stability and the chain length of the fatty acid moiety which was esterified on the pyridoxine fragment was tested as follows. The fatty acid esters of pyridoxine were dissolved in olive oil at a level of 1% calculated as pyridoxine tripalmitate in most cases. Five ml. of each solution were then heated at 205–210°C. for 8 hrs. Optical density of the heated samples was read over the range of 440 to 700 mμ with a Beckman DU spectrophotometer in 1-cm. cuvettes. Carbon tetrachloride served as a blank as well as a solvent when dilution was required.

[4] 3-Palmitoxypyridoxine-4,5-diacetate melted at 76.0–79.0°C. after being recrystallized from absolute methanol. Calcd. for $C_{28}H_{45}O_6N$: N, 2.85. Found: N, 2.48.

Results and Discussion

Solubility of Fat-Soluble Derivatives of Pyridoxine in Olive Oil. The results of solubility tests indicated that pyridoxine trilinoleate was readily miscible with winterized olive oil and did not separate out even when the proportion of the ester to the oil was one to one on a weight basis (Table II). A 0.01% solution of pyridoxine tripalmitate in olive oil remained clear at 3°C.; this concentration corresponds to 10.5 mg. of pyridoxine hydrochloride in 1 lb. of oil. When the concentration increased to 0.1%, precipitation of the ester was observed. The solubility of the ester was improved when two of the palmitoxy groups were replaced by two acetoxy groups. A 1% solution of 3-palmitoxypyridoxine-4, 5-diacetate in the same oil remained clear under the conditions employed. Pyridoxine-5-monopalmitate, pyridoxine trihexanoate, pyridoxine tridecanoate, and pyridoxine trioctanoate were also readily soluble in fats.

TABLE I
Composition of the Basal Ration

	Per 100-lb. ration
Glucose (Cerelose)	68 lbs.[a]
Corn oil	10 lbs.
Casein	18 lbs.
Wesson salts	4 lbs.
Inositol	45.5 g.
Choline chloride	91.0 g.
Niacin	4.5 g.
Thiamine	200 mg.
Riboflavin	400 mg.
p-Aminobenzoic acid	136 mg.
Folic acid	36 mg.
Ca Pantothenate	450 mg.
Biotin	190 gamma
Vitamin A	160 I.U./week
Vitamin D	1.6 gamma/week
Vitamin E	295 gamma/week

Fat-soluble vitamins were dissolved in a highly hydrogenated coconut oil and administered by a medicine dropper weekly.
[a] 78 lbs. for fat-free ration.

Heat Stability of Fat-Soluble Derivatives of Pyridoxine. Pyridoxine is considered to be one of the heat-stable members of the vitamin B complex and was not inactivated when subjected to heating at 100°C. in 5 N sulfuric or hydrochloric acid, or 5 N sodium hydroxide solution (12). A low vitamin B_6 retention in meat after cooking (14–42%) was probably not due to the destruction of the vitamin under such conditions but mainly due to loss of the vitamin by extraction (13). Harris (14) however observed that, when pyridoxine was heated to 120°C. in an aqueous solution for sterilization purposes, polymerization took place, which involved a reaction through the functional groups on the pyridine rings. Since these groups are completely or partially blocked in the fat-soluble derivatives, they would be more stable than the free form of pyridoxine.

The results of biological tests indicated that the pyridoxine tripalmitate was heat-stable. The rat used in the first biological test had been steadily losing

weight for four weeks due to a lack of pyridoxine in the diet. In two days after the administration of the chloroform extract, the body weight increased from 76 to 84 g., and signs of improvement in the dermal lesions were noted. The body weight on the 7th day was 90 g., indicating that the chloroform extract of the heated compound possessed sustained vitamin B_6 activity. In the second biological test the growth rate and the appearance of all the rats were normal. The average weekly body weight gain of the animals which had received the heated ester was 12.8 g. as compared with 10.5 g. for those on the unheated ester. These results indicated that no destruction of the pyridoxine moiety of the fat-soluble derivative took place when dissolved in corn oil and heated to an ordinary cooking temperature. This fact was also proven by the isolation of the unchanged tripalmitate from the heated oil as described.

Since pyridoxine triacetate has been reported to be a fat-soluble derivative of vitamin B_6, the heat stability of this compound was also examined. Both the free base and the hydrochloride were sparingly soluble in corn oil. When the oil which contained either form of pyridoxine triacetate was heated to 205°C., a carbonized mass separated out, indicating that the derivative was destroyed by heat. The oil still showed the presence of some pyridoxine activity when tested with acrodynic rats. The hydrochloride of pyridoxine triacetate however could not be isolated from the oil by a procedure similar to the one applied to the isolation of the tripalmitate, presumably because most of the ester was destroyed after heating at 205–210°C.

TABLE II

Solubility of Various Esters of Pyridoxine in Winterized Olive Oil at 3°C.

Compound	Wt. % of the ester dissolved in olive oil	Precipitation
Pyridoxine Trihexanoate	5.0	—
Pyridoxine Trioctanoate	1.0	—
Pyridoxine Tridecanoate	1.0	—
Pyridoxine Tripalmitate	0.01	—
Pyridoxine Tripalmitate	0.1	+
Pyridoxine Trilinoleate	>50	—
3-Palmitoxypyridoxine-4, 5-diacetate	1.0	—
Pyridoxine-5-monopalmitate	1.0	—

The relationship between the chain length of the esterified fatty acids and the discoloration of the olive oil, in which various pyridoxine preparations were dissolved, was demonstrated (Table III). The results indicated that when the chain length was increased from C_6 to C_{16}, the stability of the ester in oil was progressively improved, and pyridoxine tridecanoate and pyridoxine tripalmitate had almost the same order of stability. The color intensity of olive oil, which was heated in the presence of various fatty acid esters of pyridoxine, was compared tentatively by a "color index," which was a numerical expression obtained by dividing the optical density of the heated oil at 460, 550, 620, and 670 mμ by the optical density of the heated plain olive oil at the same wavelengths. The results indicated that an introduction of one palmitoyl group to pyridoxine markedly improved the heat stability and 3-palmitoxypyridoxine-4, 5-diacetate, and pyridoxine-5-palmitate had a degree of heat stability similar to that of pyridoxine trioctanoate. The heat stability of pyridoxine trilinoleate was also found to be in the neighborhood of the trioctanoate.

TABLE III

"Color Indices"[a] of Heated Olive Oil Containing Various Esters of Pyridoxine

	% in oil[b]	Wavelength, mμ[c]			
		460	550	620	670
Olive oil plus:					
Pyridoxine trihexanoate	1	4.4	6.7	6.5	6.5
Pyridoxine trioctanoate	1	2.1	2.9	2.5	2.4
Pyridoxine tridecanoate	1	1.6	2.2	2.5	2.5
Pyridoxine tripalmitate	1	1.6	2.0	2.0	2.0
Pyridoxine tripalmitate	0.1	1.2	1.4	1.4	1.3
Pyridoxine tripalmitate	0.01	1.1	1.1	1.1	1.0
Pyridoxine trilinoleate	1	2.0	2.6	2.7	2.6
3-Palmitoxypyridoxine-4, 5-diacetate	1	2.3	3.3	3.3	3.2
Pyridoxine-5-monopalmitate	1	2.0	2.9	3.0	2.8

[a] Color index = $\frac{\text{O.D. of heated test oil}}{\text{O.D. of heated olive oil}}$

[b] Percentage refers to the level calculated as pyridoxine tripalmitate.

[c] Beckman DU spectrophotometer with 1-cm. cuvettes. Carbon tetrachloride served as a blank as well as a solvent when dilution was required.

Summary

The heat stability of several fatty acid esters of pyridoxine and their solubility in fats was tested. Short chain fatty acid esters of pyridoxine, such as pyridoxine triacetate, and long chain saturated fatty acid esters of the vitamin, such as pyridoxine tripalmitate were almost insoluble or had a rather limited solubility in fats. An improvement in solubility was observed when pyridoxine was partially esterified with palmitic acid, fully esterified with a long chain unsaturated fatty acid, such as linoleic acid, or when it was esterified with a fatty acid of intermediate chain length (C_6, C_8 or C_{10}). Free pyridoxine and the acetate were destroyed by heat when mixed in a fat and heated at 205–210°C. A profound improvement in heat stability in pyridoxine was noted when one palmitoyl group was introduced, and the ester became completely stable when it was fully esterified with a saturated fatty acid of C_{10} or longer chain length.

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