dehydroascorbic acid by glutathione and the coupling of glutathione reduction to reduced di- and triphosphopyridine nucleotides by the enzyme glutathione reductase (20) are well known. Through such coupled systems the capacity of tocopherol would be greatly increased.

The normal range for vitamin E in human blood plasma is 0.9–1.9 mg. per 100 ml.; similar values for ascorbic acid and glutathione in whole blood are 0.1–1.3 and 25–41 mg. per 100 ml., respectively. Thus glutathione coupled to tocopherol would increase its lipid antioxygenic capacity. Further, since glutathione is coupled to tissue respiration, its oxidation-reduction capacity is very great.

Prooxidants Other than Hematin Compounds. The lipid prooxidant-antioxidant balance found in nature is often more complicated than that of hematin compounds and tocopherol. White muscle disease in lambs and calves appears to be a good example of a complicated vitamin E deficiency (12,18,19). Lipoxidase, the only known lipid peroxidation catalyst more active than hematin compounds, appears to play an important role in white muscle disease which has not been noted in previous research. Feeds characteristically associated with white muscle disease (12,18,19), red beans, pea vines, and alfalfa, are known to be good sources of lipoxidase (21–23). Lipoxidase-catalyzed lipid peroxidation in the rumen would be an important peroxidative stress.

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Solubility of Linoleic Acid in Aqueous Solutions and Its Reaction with Water[1, 2]

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In a study of stable emulsions of linoleic acid in 0.1M-KH_2PO_4/Na_2HPO_4 buffer solutions prepared by sonic vibrations, the influence of linoleic acid on pH was manifested in buffer solutions of pH 8.00 and decreased gradually till it became negligible in pH 4.50. This change in pH values was due to differences in solubility of linoleic acid in the buffer solutions.

Ultraviolet spectra of soluble linoleic acid in buffer solutions indicated the presence of conjugated dienes, which increased with the increasing of the pH of the system.

Unbuffered aqueous emulsions of linoleic acid had a pH value which ranged between 4.69 and 5.10. Saturated aqueous solutions, obtained by high-speed centrifugation, had concentrations of 15.80 to 16.00 mg. linoleic acid per 100 ml. of D.I. water.

From the solubility data and conductivity values of linoleic acid the apparent classic and thermodynamic ionization constants were calculated to be $6.974 \pm 0.023 \times 10^{-6}$ and $6.905 \pm 0.017 \times 10^{-6}$ at 0.7°C. and $1.730 \pm 0.009 \times 10^{-5}$ and $1.689 \pm 0.007 \times 10^{-5}$ at 25°C., respectively.

The result of the chemical interaction of linoleic acid and water is a saturated hydroxy fatty acid. This acid gave a positive test for glycol groups with periodic acid oxidation test and appeared to be a tetrahydroxy compound with the exact structure unknown.

THE SOLUBILITIES of the members of the normal saturated fatty acid series and of oleic, linoleic, and linolenic acid have long been known with great accuracy in a wide variety of organic solvents. In 1955 Kolb and Brown (13) provided further data on the solubility of fatty acids as a guide to their separation by low-temperature crystallization from organic solvents.

The solubilities in water of the normal saturated fatty acids from caproic to stearic at various temperatures between 0° and 60°C. are reported by Ralston

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[2] American Meat Institute Foundation Journal Paper No. *204*.

and Hoerr (18). No solubility data for the unsaturated fatty acids in water have been reported.

The specific conductivity of caproic, myristic, palmitic, stearic, and oleic acids *per se* were determined at 100–200°C. (14). The values for oleic and stearic acids at 100°C. were 2×10^{11} and 0.6×10^{11} ohm^{-1} cm^{-1}, respectively. The marked increase in conductivity of oleic acid compared with that of stearic acid agrees with the observation that the salts of unsaturated fatty acids are more strongly dissociated and therefore are better conductors than those of the saturated acids of the same chain-length (14).

Solubility of a fatty acid in water is accompanied by dissociation of the molecule into ions in a manner similar to inorganic acids. Dissociation of fatty acids in aqueous solutions occurs to a much lesser degree than that of strong mineral acids. Measurements of dissociation constants of monocarboxylic acids indicate a small decrease in dissociation with an increase in molecular weight; however direct comparisons are rather difficult because of the limited solubilities of the higher members of the series in water (9,12,25,26). Concerning the dissociation constants of the members of the fatty acid series, Conant (2) has observed that it is probable that all fatty acids have the same acid strength within the significance of the experimental results (± 0.5 pK unit).

The solubility of stearic acid in water at 35° and 50°C. is 1.17×10^{-5}N and 5.77×10^{-5}N, respectively (3). From the solubility and conductivity values of stearic acid hydrosol the dissociation constants of stearic acid were found to be 1.7×10^{-6} at 35° and 2.6×10^{-6} at 50°C. (4).

Schauenstein and Biheller (20) reported that aqueous solution of linoleic acid showed a pH of 4.6–5.1. Saturated solutions obtained by partial evaporation of dilute solutions had concentrations of about 4.2 g. acid/100 ml. of water. The semicrystal solid obtained by evaporation to dryness of the saturated solution contained 10.27% and 1.05% conjugated dienes and trienes, respectively. Later Schauenstein *et al.* (21, 22) concluded that ethyl linoleate as well as linoleic acid reacted with water, giving a mixture of esters and acids with considerable hydroxyl content and a water solubility of 0.5–0.7%. This mixture was separated into three fractions by paper chromatography. The first fraction was unsaturated, contained no hydroxyl group, and was insoluble in water; the second fraction was saturated, contained one OH-group, and was water-soluble while the third one was rich in OH groups and was water-soluble.

Since a wide variety of studies, such as fat absorption, autoxidation, and catalytic oxidation in aqueous systems, require accurate information about the solubility of linoleic acid in water and buffer systems, a knowledge of the pK of linoleic acid would be of appreciable value. Furthermore it would be valuable to learn whether there is a reaction between linoleic acid and water.

Consequently it was decided to study the acidification of buffer solutions by linoleic acid and to determine the amounts of acid soluble in each buffer system and in deionized carbon dioxide and ammonia-free water, to determine the equivalent conductance of linoleic acid solution in water and to calculate its ionization constant, and, further, to demonstrate the chemical interaction of linoleic acid with water.

Materials and Methods

Materials. Linoleic acid used in this work was obtained from the Hormel Institute, Austin, Minn. It had an iodine value of 181.0 (theoretical value 181.03) and a conjugated diene content of 0.12%. The water used was deionized CO_2- and NH_3-free (D.I.). It had a specific conductance of about 0.36×10^{-6} and 0.81×10^{-6} $ohm^{-1}cm^{-1}$ at 0.7° and 25°C., respectively. Potassium phosphate monobasic and sodium phosphate dibasic, both Merck Reagent Grade, were used in preparing the buffer solutions. The buffer solutions were prepared by mixing 0.1M-KH_2PO_4 solution with 0.1M-Na_2HPO_4 solution until the desired pH was reached. This was measured with a glass electrode, using a Leeds Northrup pH meter, Model No. 76631.

Preparation of Emulsions and Saturated Solutions. Linoleic acid (0.298 g.) was emulsified with 10 ml. of 0.1M-KH_2PO_4/Na_2HPO_4 buffer solution by sonic waves. The diameter of the oil-phase particles in the emulsion ranged from 2–4 microns. The emulsions were prepared in polyethylene bottles in an atmosphere of nitrogen. After measuring the pH, each emulsion was transferred to a polyethylene centrifuge bottle and blanketed with nitrogen. The emulsions were centrifuged at 14,000 r.p.m. until no oil droplets could be seen under the microscope. Centrifuging was carried out at 44°F. (6.7°C.) to minimize autoxidation. The resulting aqueous solutions were siphoned and filtered through two analytical filter papers No. 597, Schleicher and Schuell Company Inc., New York, N.Y. Two 100-ml. aliquots of the aqueous solutions were lyophilized at 100–120 μ. Hg. over-night in a Stokes Freeze Dryer, Model No. 2003-F-2. Free linoleic acid content of each sample was extracted with diethyl ether while the acid present as soap was extracted after acidification with HCl solution. The ether extracts were thoroughly washed with D.I. water, dried over anhydrous sodium sulfate, and filtered. The ether was evaporated at 37°C. in an atmosphere of nitrogen. The acid content of each sample was determined gravimetrically, also by titration with 0.01N-KOH solution.

The ultraviolet spectra of each sample was recorded by using a Beckman DK-2 recording spectrophotometer in the region 340 to 220 mμ.

To demonstrate the chemical interaction of linoleic acid with water, 0.5 g. of linoleic acid was added to one liter of freshly distilled D.I. water, saturated with nitrogen in polyethylene bottles and shaken for three hours at 44°F. (6.7°C.). The emulsions were centrifuged in capped centrifuge bottles at 6.7°C. The resulting clear solution was filtered and freeze-dried as stated above. The residue was purified by crystallization from ethanol, and the crystals were stored under vacuum in a desiccator painted black.

Preparation of Sodium and Potassium Linoleate. Sodium linoleate solution was prepared by adding the equivalent quantity of 0.01N NaOH solution from a microburette to a weighed quantity of linoleic acid in a volumetric flask and completing to the mark with D.I. water. A number of successively dilute solutions of known concentrations (*i.e.,* 0.01N-0.0005N) were prepared from the initial solution. The prepared solutions were transferred to clean polyethylene bottles and kept under a blanket of pure nitrogen. A series of potassium linoleate solutions ranging in concentration from 0.01N to 0.0004N were prepared in the same way.

Measurement of Conductivity. Conductivity bridge, Model RC16B1 (Industrial Instruments Inc., Cedar Grove, N.J.), was used in this study. The cell constant was calculated from the specific conductances of KCl solutions and resistance. Conductivity measurements were made upon each solution at 0.7° and 25°C. The conductivity of each solution was determined three times. The values were not altered by standing for 30 min.

Characterizing Determinations. Unsaturation was determined by catalytic hydrogenation according to Colson's micromethod (1). The hydroxyl content was estimated quantitatively, using the semi-micro method of Ogg, Porter, and Willits (17). The presence of vicinal hydroxyl groups was detected by a periodic oxidation test (10). J.J. Fric Polarimeter, Research Model, was used for determination of the optical rotation with a sodium arc as a source of monochromatic radiation. The neutralization equivalent was determined potentiometrically according to the method described by Niederl and Niederl (15). The crystals were dispersed in potassium bromide and pressed to a clear disk. An infrared spectrum of this disk was made by using a Perkin-Elmer infrared spectrophotometer.

Results and Discussion

Solubility. The solubilities of linoleic acid in 0.1M phosphate buffers and its apparent solubility in D.I. water are listed in Table I. The reproducibility of the results are shown by the following examples: a) for D.I. water at 6.7°C. the experimental results gave a value of 0.0158 g. ± 0.0002 g. acid per 100 ml. of water as an average of seven separate determinations; b) for phosphate buffer pH 8.00 at 6.7°C. the solubility was 0.0597 g. ± 0.0003 g./100 ml. for five determinations.

TABLE I

Solubility of Linoleic Acid in D.I. Water and 0.1M Phosphate Buffers at 6.7°C.

Solvent	Linoleic acid content mg./100 ml. solution		Percentage of free linoleic acid
	Total	Free	
A-Phosphate buffer of:			
pH 4.50	1.77	1.74	98.31
pH 5.00	5.10	4.95	97.06
pH 5.50	6.92	6.71	96.94
pH 6.00	25.50	22.97	90.08
pH 6.50	31.00	27.56	88.90
pH 7.00	60.00	51.59	85.98
pH 8.00	76.00	59.72	78.58
B-D.I. water:			
pH 7.18	16.00	16.00	100.00
pH 7.38	15.80	15.80	100.00

The values for other buffer solutions at a given temperature were reproduced similarly in an average of 3–5 separate determinations with each buffer solution.

Acidification of Buffer Solutions and D.I. Water by Linoleic Acid. Table II shows the pH values of both linoleic acid emulsions and saturated solutions at 6.7°C. Figure 1 shows the change in pH units of the emulsion *versus* the pH of the buffer used. The pH value of both emulsions and their corresponding aqueous solutions were constant and did not change when left for seven days in an atmosphere of nitrogen at 6.7°C.

TABLE II

pH Values of Linoleic Acid Emulsions and Saturated Solutions (0.298 g. of Linoleic Acid/10 ml. 0.1M Phosphate Buffer)

pH Values	Emulsion		Saturated Solution	
	pH	Change	pH	Change
A-Phosphate buffer				
4.50	4.44	−0.06	4.45	−0.05
5.00	4.91	−0.09	4.90	−0.10
5.50	5.40	−0.10	5.40	−0.10
6.00	5.88	−0.12	5.91	−0.09
6.50	6.32	−0.18	6.32	−0.18
7.00	6.68	−0.32	6.67	−0.33
7.18	6.78	−0.40	......	......
7.50	6.87	−0.63	......	......
7.86	6.95	−0.91	......	......
8.00	6.92	−1.08	6.90	−1.10
B-D.I. water				
7.18	4.69	−2.49	7.00	−0.18
7.38	5.10	−2.28	7.16	−0.22

The data above support the concept that long-chain fatty acids contribute hydrogen ions to aqueous solutions at pH greater than 5.00. The change in pH values was less in the case of buffer solutions with a pH value less than 6.00 but increased gradually till it reached a maximum at pH 8.00. This decrease in pH value as the buffer became more alkaline probably resulted from the formation of linoleate soaps. This trend holds in the case of oleic acid emulsions of 0.1M-Na_2HPO_4/citric acid buffer solutions (19). A study of the change in pH values of buffer solutions, water, and the amount of oleate soaps present has been reported (23).

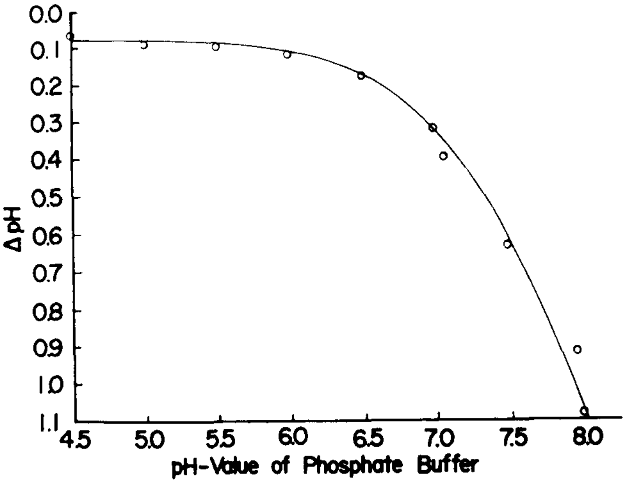

FIG. 1. Change in pH units of linoleic acid emulsions vs. the pH of phosphate buffer solution used.

Table III shows that methyl linoleate has no effect on the pH of 0.1M phosphate buffer solutions. This supports the concept of linoleate soap formation in alkaline buffer when linoleic acid is used.

TABLE III

pH Values of Methyl Linoleate Emulsions (0.287 g. of Methyl Linoleate/10 ml. 0.1M Phosphate Buffer)

pH value of phosphate buffer	pH of emulsion	Change
4.50	4.50	0.00
5.00	4.98, 5.00	0.00 to −0.02
5.50	5.48, 5.50	0.00 to −0.02
6.00	5.94	−0.06
6.50	6.50	0.00
7.00	6.95	−0.05
7.18	7.18	0.00
7.50	7.50	0.00
8.00	7.94	−0.06

Ultraviolet Spectral Characteristics of Soluble Linoleic Acid in Buffer Solutions. Table IV shows the molecular extinction coefficient of soluble linoleic acid in buffer solutions. The readings were made by using Spectro Grade methanol as a solvent.

From the data in Table IV it is evident that the increase of the solubility of linoleic acid in buffer solutions is accompanied by an increase in the absorption at 232.5 mμ, indicating the presence of conjugated dienes. The molar extinction coefficient at 277.5 mμ was approximately 196.3 in all samples and did not change after the addition of a 5% alkali solution. This shows the absence of diene ketones, which frequently are noted as secondary products of the autoxidation of linoleic acid.

TABLE IV

Molecular Extinction Coefficient of Soluble Linoleic Acid in Buffer Solutions at 232.5 [a]

Buffer solution	Free linoleic acid	Linoleic acid extract from soaps	Total linoleic acid
pH 4.50	442.00	284.15	396.54
pH 5.00	583.71	599.13	417.86
pH 5.50	505.79	484.93	434.68
pH 6.00	602.95	551.93	417.86
pH 6.50	717.28	500.47	434.68
pH 7.00	870.04	876.38	481.52
pH 8.00	1411.57	1299.39	862.35

[a] The values reported for free linoleic acid and of linoleic acid extract from soaps are from one series of experiments, and those for total linoleic acid are from another series.

Apparent Ionization Constant of Linoleic Acid. As linoleic acid is a weak electrolyte, its equivalent conductance at infinite dilution cannot be obtained directly from conductivity measurements, but may be derived from the Kohlrausch law of independent migration of ions (11), utilizing the following relationship:

$$\Lambda_{0(\text{Linoleic Acid})} = \Lambda_{0(\text{HCl})} + \Lambda_{0(\text{Na Linoleate})} - \Lambda_{0(\text{NaCl})}$$

The classic ionization constant of linoleic acid was calculated from the Ostwald dilution law. The thermodynamic ionization constant was calculated by use of the Debye-Hückel-Onsager equation for uni-univalent electrolytes:

$$\Lambda = \Lambda_0 - (A + B\Lambda_0)\sqrt{C}$$

where Λ is the equivalent conductance at concentration C, Λ_0 is the equivalent conductance at infinite dilution, A and B are constants dependent on the solvent and temperature. The calculated values in this work are as follows:

	A	B
0.7°C.	30.33	0.220
25.0°C.	60.20	0.229

The procedure advocated by Davies (5,6) and used by Dippy and Williams (7,8) was followed for determination of the equivalent conductance of Na linoleate and K linoleate at infinite dilution (Figures 2 and 3).

Using the modified Debye-Hückel equation:

$$\text{Log } K_{\text{Class.}} = \text{Log } K_{\text{Therm.}} + 2A\sqrt{aC}$$

which includes corrections for the activity coefficient of each ion, the thermodynamic ionization constant was calculated. The value of *a* used in this equation is the true degree of ionization. The values assigned for the constant A in water at 0.7° and 25°C. are 0.347 and 0.509, respectively.

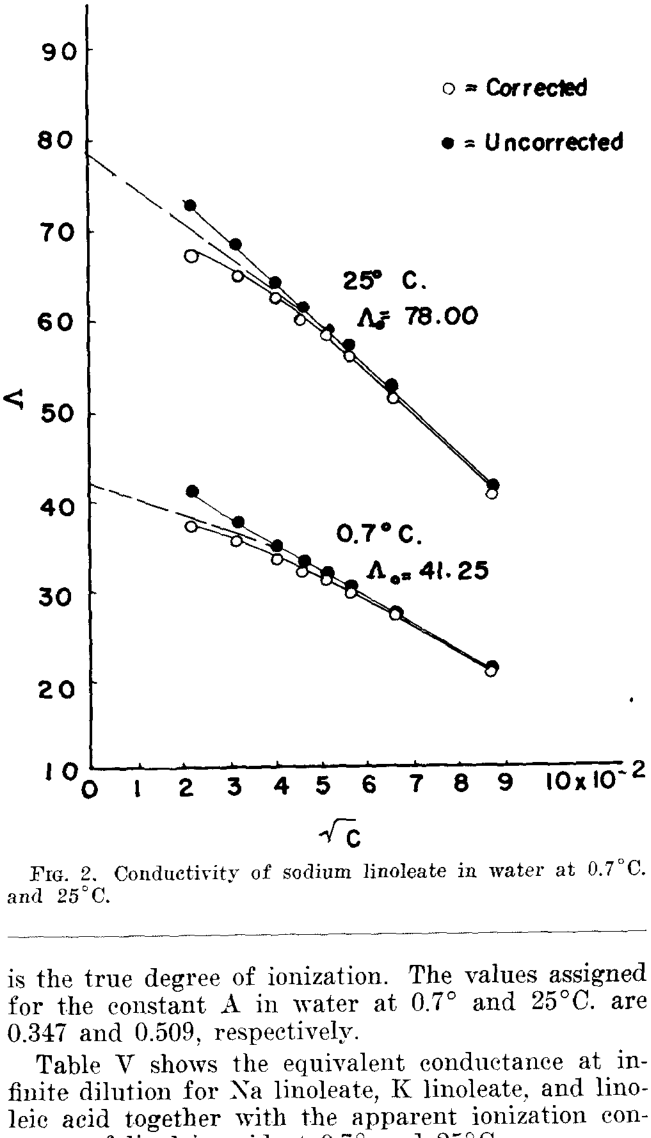

FIG. 2. Conductivity of sodium linoleate in water at 0.7°C. and 25°C.

Table V shows the equivalent conductance at infinite dilution for Na linoleate, K linoleate, and linoleic acid together with the apparent ionization constants of linoleic acid at 0.7° and 25°C.

The values of $\Lambda_{0(\text{linoleic acid})}$ obtained in this work are reproducible as the difference between the $\Lambda_{0(\text{K linoleate})}$ and $\Lambda_{0(\text{Na linoleate})}$ is 23.60 ohm^{-1} cm^2 at 25°C. (theoretical constant difference is 23.60) and 14.25 ohm^{-1} cm^2 at 0.7°C. (theoretical constant difference is 14.40) (11).

TABLE V

Equivalent Conductance at Infinite Dilutions for Na Linoleate, K Linoleate, and Linoleic Acid and the Apparent Ionization Constants for Linoleic Acid

Temperature °C.	$\Lambda_{0(\text{Na linoleate})}$ Ohm^{-1}cm^2	$\Lambda_{0(\text{K linoleate})}$ Ohm^{-1}cm^2	$\Lambda_{0(\text{Linoleic Acid})}$ Ohm^{-1}cm^2	$K_{\text{Class.}}$	$K_{\text{Therm.}}$
0.7	41.25	55.50	257.00	$6.974 \pm 0.023 \times 10^{-6}$	$6.905 \pm 0.017 \times 10^{-6}$
25.0	78.00	101.60	377.10	$1.730 \pm 0.009 \times 10^{-5}$	$1.689 \pm 0.007 \times 10^{-5}$

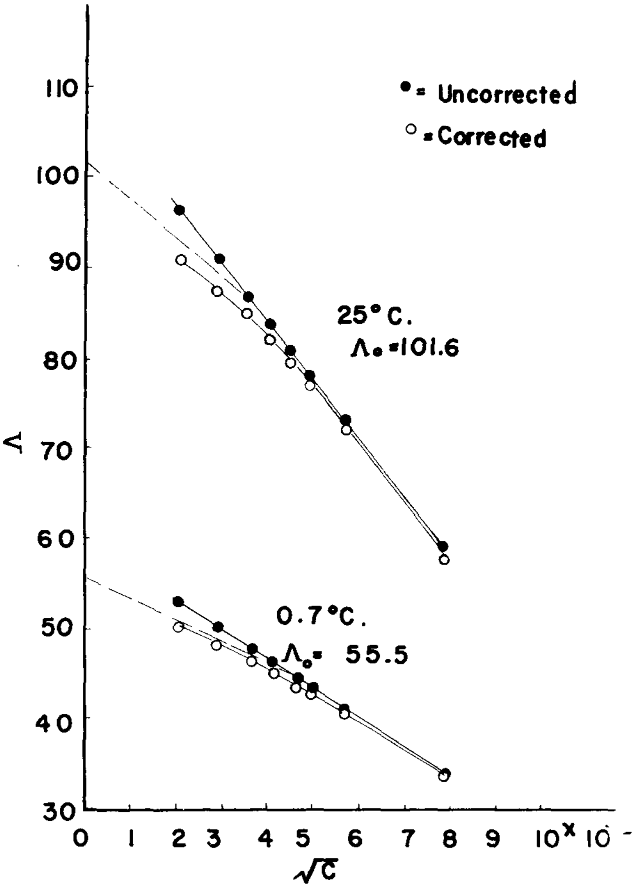

FIG. 3. Conductivity of potassium linoleate in water at 0.7 °C. and 25 °C.

Analysis of the Product of the Chemical Interaction of Linoleic Acid with Water.

Yield	0.0158%
Hydrogen number	0
Hydroxyl value	10.44%
Periodic acid oxidation test	positive
Neutralization equivalent	615.49

From these data it is apparent that linoleic acid reacted with water to yield saturated hydroxy acid or acids. This acid contained vicinal hydroxyl groups since it gave a positive test for glycol groups with periodic acid while the starting material did not react with the reagent. From the neutralization equivalent and the hydroxyl value it may be assumed that the result of the interaction of linoleic acid and water is not dihydroxystearic acid. The optical activity of the acid was too low to be determined with certainty. It must be a complex mixture as linoleic acid contains four asymmetric centers. The position of the OH groups has not been established. The acid absorbed strongly in the ultraviolet region 205–340 mμ but did not show any maximum absorption at any wavelength even after the addition of alkali. This also confirmed the absence of double bonds.

Infrared Spectral Analysis. All position isomers of dihydroxystearic acids show strong absorption around 3400 cm^{-1} and a series of medium strength bands between 1000 and 1200 cm^{-1} corresponding to the O—H stretching vibrations and C—O stretching and/or deformation vibrations, respectively (16,24). Also the 1180 to 1350 cm^{-1} region shows only weak bands with no apparent regularity (24).

Figure 4 shows the infrared spectra of the hydroxy acid. Examination of this spectra showed a strong absorption band at 3375–3390 cm^{-1} corresponding to O—H stretching vibrations. The band at 2880–2900 cm^{-1} is caused by C—H stretching. The presence of chelated hydroxy acids in the sample was indicated by the absorption band at 1608–1612 cm^{-1}.

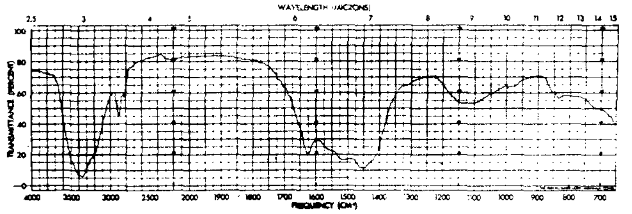

FIG. 4. Infrared absorption spectra of the hydroxy fatty acid obtained by lyophilizing the saturated aqueous solution of linoleic acid.

The infrared spectrum is comparable with that from dihydroxystearic acid. However any acid containing vicinal dihydroxyl groups would give a comparable spectrum. The physical properties, compositional data, and infrared spectrum indicate a compound with a molecular weight approximately twice that of dihydroxy stearic acid but with only one carboxyl group. This would connote a tetrahydroxy compound or compounds in the reaction mixture.

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