Kinetic Investigation into Glucose-, Fructose-, and Sucrose-Activated Autoxidation of Methyl Linoleate Emulsion[1,2]

AHMED FAHMY MABROUK[3] and L. R. DUGAN JR.,[4] American Meat Institute Foundation, The University of Chicago, Chicago, Illinois

Autoxidation of methyl linoleate emulsions in aqueous phosphate buffer solutions in the presence of glucose, fructose, and sucrose has been studied by the rate of oxygen uptake. Oxidation rates increased with increasing concentration of sugars in the system. At comparable molar ratios of sugar to methyl linoleate the rate of oxidation in the presence of fructose was greater than with glucose which, in turn, was greater than with sucrose. Oxidation rates increased with pH until a maximum rate was reached at pH 8.00. There was less conjugation and more CO_2 with fructose than with glucose at a comparable level of oxygen uptake and pH value. This suggested concurrent oxidation of methyl linoleate and sugars; fructose is the most readily oxidized of those studied.

Sugars seemed to be effective only in combination with the resulting methyl linoleate hydroperoxide. The effect of sugars rests in an activation of the decomposition of the linoleate hydroperoxide, and on the acceleration of the autocatalysis.

The activation energy values for the autoxidation of methyl linoleate emulsions in the presence of sucrose, glucose, and fructose are 14.9, 10.6, and 10.6 K. Cal./mol. at pH 5.50; 16.0, 10.8, and 10.4 K. Cal./mol. at pH 7.00; and 14.4, 10.2, and 8.8 K. Cal. at pH 8.00, respectively.

Addition of ascorbic acid to the system at zero time or after 2 hrs. increased oxygen absorption. It appeared that oxidized methyl linoleate caused co-oxidation of the ascorbic acid. Additions of nordihydroguaiaretic acid, propyl gallate, and hydroquinone to the system were ineffective in stopping oxidation when they were added after oxidation had commenced. They reduced effectively the rate of oxidation when added at zero time.

SUGAR IS USED in large quantities in meat curing, although its function in meat processing is not completely understood. Sucrose is the sugar used most extensively in curing meat. Dextrose and corn sugar sirup are used in some meat products. Fructose, glucose, and ribose are reported to be present in fresh meat (5). The content of each varies according to the grade of the meat and the time elapsed after slaughter.

The part played by carbohydrates in oxidative rancidity of fat in meats has not been suitably explained. The deterioration which takes place in dehydrated meat during storage, even in the absence of oxygen, is due to a typical browning reaction between the reducing sugars and the protein and soluble nonprotein fractions of the meat (16, 17, 20). Glycerol has a protective action on codliver oil as its induction period increases (18). In a study of the effect of aliphatic polyhydroxy compounds, such as glycerol, sucrose, glucose, and fructose, on the rate of oxidation of lard in contact with an aqueous phase, it has been shown that these compounds act as weak antioxidants (11, 12). Thompson and Hegsted (22) demonstrated that purified diets, containing dextrose and particularly anhydrous dextrose, turned rancid much more rapidly than diets containing sucrose, as determined by the iodine number of fat extracted from stored samples. In a study of the relation of the browning reaction to storage stability of sugar cookies, it was found that the addition of 5% dextrose to sugar cookies produced a marked browning in the cookies, and these cookies exhibited greater stability to oxidative rancidity than did the cookies in which no browning occurred (6). The antioxidant properties were associated with the presence of reductones formed during the browning reaction. Treating pork trimmings before freezing with a mixture of antioxidants containing 90% dextrose would keep them indefinitely in a palatable condition (7). Recently Spetsig (20) reported that sucrose caused a slight catalysis of the oxidation of methyl linoleate.

Because of insufficient knowledge of the effect of sugars on the oxidative rancidity of foods, the main objectives of this investigation were to study the effect of sugars on the oxidation of methyl linoleate aqueous emulsions with respect to the magnitude of their activity at equimolar concentrations, the effect of the initial pH value of the system, and the effect of temperature. An additional objective was to determine the effect of some antioxidants on the methyl linoleate emulsion-glucose system.

Experimental Procedures

The details of experimental procedures have been given in a previous paper (14), and only exceptions or additions will be mentioned. Each determination was made at least in triplicate.

Materials Used. Methyl linoleate: In order to eliminate the influence of the carboxyl group, methyl linoleate was used as a substrate.

Sucrose, Reagent Grade (General Chemical Division, Allied Chemical and Dye Corporation).

D (+)-Dextrose, C. P. Anhydrous (Pfanstiehl Laboratories Inc.)

Fructose (Eastman Organic Chemicals).

Propyl gallate, commercial.

Hydroquinone, commercial.

Nordihydroguaiaretic acid (N.D.G.A.), commercial.

Ascorbic acid, Reagent Grade (Merck and Company Inc.).

Reagents. Ascorbic acid and antioxidants were freshly prepared in buffer solutions that were saturated with pure nitrogen gas.

Sugars were dried in a desiccator over calcium chloride, carefully weighed, and dissolved in 0.1 M KH_2PO_4/Na_2HPO_4 buffer solutions for the desired pH. These solutions were always freshly prepared for each experiment.

[1] Presented at the 52nd annual meeting, American Oil Chemists' Society, St. Louis, Mo., May 1–3, 1961.
[2] American Meat Institute Foundation Journal Paper No. 215.
[3] Present address: Northern Utilization Research and Development Division, Peoria, Illinois.
[4] Present address: Department of Food Science, Michigan State University, East Lansing, Mich.

Method of Studying Oxygen Absorption. Three ml. of methyl linoleate emulsion containing 0.317 m.mol. linoleate were placed in the main compartment of the Warburg flask. One ml. of sugar solution was placed in the side arm and mixed with the emulsion after gassing with pure O_2 and establishing temperature equilibration. Oxygen gas, bubbled through distilled water to saturate it with water vapor, was used in the Warburg flask for gassing. This precaution reduced water loss to a minimum. To absorb CO_2 from the gas phase, 0.2 ml. of 10% aqueous KOH was pipetted in the center cup of the Warburg flask. To increase the absorption of CO_2 by KOH solution, 2- x 2-cm. filter paper strips were placed in the cup.

Measurement of Carbon Dioxide Formed. The complete curve for the absorption of oxygen could be obtained by one experiment while the amount of CO_2 formed [measured by the method of Umbreit *et al.* (23)] could only be obtained for a particular instance by a separate experiment. It was necessary to perform many experiments over varying intervals of time in order to obtain the data for the construction of curves relating the formation of carbon dioxide to time, oxygen absorption, and pH values.

Data and Discussion

Effect of Sucrose, Glucose, and Fructose on Oxidation Rate of Methyl Linoleate

The oxygen absorption of methyl linoleate emulsions in the presence of 6.3093×10^{-4} mole sugar/mole linoleate at 50°C. is shown in Fig. 1.

The curves in Fig. 1 show clearly that oxidation was accelerated in the presence of sucrose, glucose, and fructose. The calculated rate of oxidation was high in the presence of sugar when compared with autoxidation of methyl linoleate emulsion alone. At comparable molar ratios of sugar to methyl linoleate in the system, the rate of oxidation decreased in the order: fructose > glucose > sucrose.

In the presence of high concentrations of sugars (6.3093×10^{-1} mole sugar/mole linoleate and above) the co-oxidation of sugars appears to cause initial oxygen absorption greater than that accounted for by methyl linoleate autoxidation. At low concentrations of sugars, *i.e.*, $0.7887–6.3093 \times 10^{-4}$ mole sugar/mole linoleate, the amount of oxygen absorption caused by oxidation of sugars did not significantly affect the determination of the rate of linoleate oxidation. During a 5-hr. period the amounts of O_2 absorbed by 1 ml. of 1×10^{-4} M glucose, fructose, and sucrose added to 3 ml. of 0.1 M phosphate buffer of the same pH value, which is equivalent to the highest concentration 6.3093×10^{-4} mole sugar/mole linoleate, were 16, 25, and 12 μl., respectively.

The effect of increased concentrations of the sugars on the autoxidation of linoleate emulsions is illustrated in Fig. 2. This shows that the rate of oxidation increased as the concentration of sugar increased in the system.

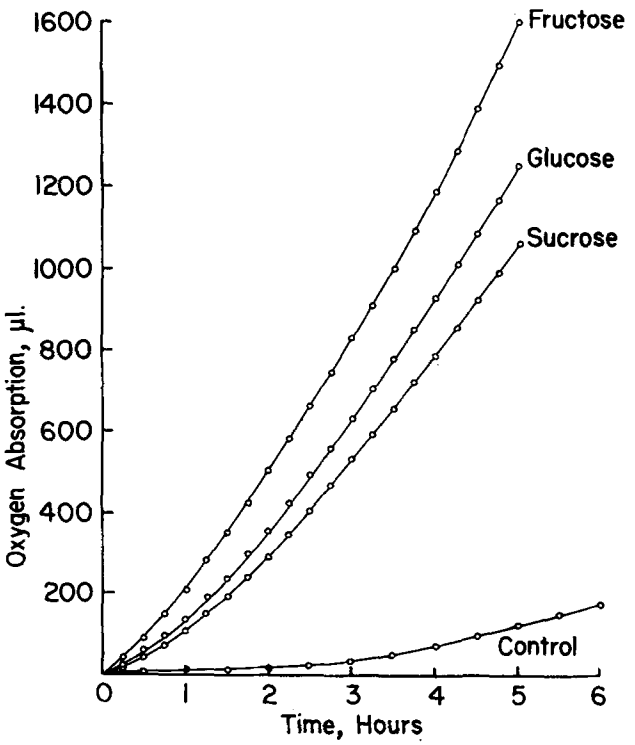

FIG. 1. Oxygen absorption by methyl linoleate emulsions in the presence of 6.3093×10^{-4} mole sugar/mole linoleate, 50°C. Initial pH 7.00.

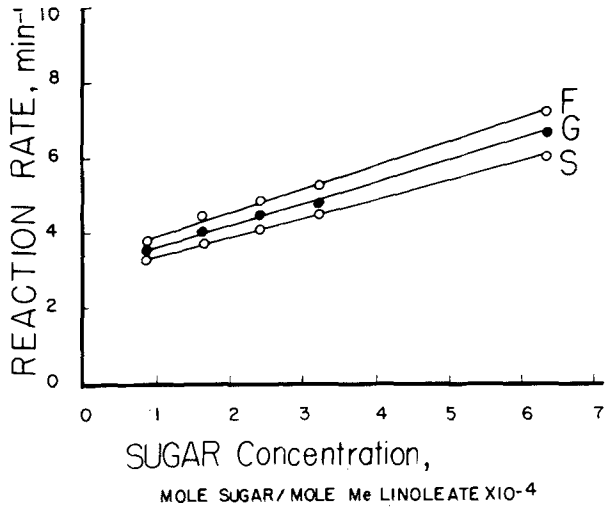

FIG. 2. The effect of increasing concentration of sugars on the oxidation rate of methyl linoleate emulsions, initial pH 7.00, 50°C. F—Fructose, G—Glucose, S—Sucrose.

In the presence of $0.7887–6.3093 \times 10^{-4}$ mole sugar/mole methyl linoleate a straight-line relationship exists between the rate of oxidation of linoleate and sugar concentration in the emulsion. Deviation from the linear relationship at higher concentrations of sugars (6.3093×10^{-1} mole sugar/mole linoleate and above) may result from the change in the pH as co-oxidation of the sugars in the system proceeds. Another factor to be taken into account is the change in solubility of oxygen in the reaction mixture as the concentration of sugar was increased. Measurements on a variety of glucose and sucrose solutions show that a fall in oxygen solubility of about 15 and 17%, respectively, is to be expected as the concentration is doubled (15). Such an effect was reported in a study of the oxidation of ferrous perchlorate by molecular oxygen (4). The very small concentrations of sugars used (1 ml. of 2.5×10^{-6} to 1.0×10^{-4}M sugar added to 3-ml. of emulsion) would minimize the effect of the fall in oxygen solubility.

TABLE I

Reaction Rate Constants of Methyl Linoleate Autoxidation in Aqueous Emulsions of Different pH Values in the Presence of Sugars (6.3093×10^{-4} mole sugar/mole Methyl Linoleate) 50°C.

Sugar	$K \times 10^{-3}$ min.$^{-1}$		
	pH 5.50	pH 7.00	pH 8.00
Sucrose	3.33	6.00	6.33
Glucose	3.41	6.60	6.83
Fructose	3.56	7.16	7.51

Table I indicates that, as the pH of the emulsion increased, the oxidation rates markedly increased. The oxidation rates were the highest at pH 8.00.

Co-oxidation of Sugars During Autoxidation of Methyl Linoleate Emulsions

During oxidation of methyl linoleate-sugar systems (Fig. 3) it was observed that the change in the pH exceeded what could be accounted for by the carbon dioxide formed. Co-oxidation of the sugars yielded acidic compounds, not yet identified, which probably contributed to the decrease of the pH. Furthermore the amount of CO_2 formed increased as oxidation proceeded.

Table II shows the amounts of carbon dioxide formed in the methyl linoleate-sugar systems. There was a significant increase in CO_2 formed in the presence of fructose in comparison with that in the presence of glucose or sucrose. In the presence of sucrose the amount of CO_2 formed at pH 5.50 was about two and one-half times that formed at pH 7.00. This may be caused by inversion of sucrose to monosaccharides which, on oxidation, would yield more CO_2. Increasing the sugar concentration also increased the change in the pH but did not cause an increase in the amount of CO_2 formed when comparison was made on equimolar concentrations. Fig. 3 shows that concurrent oxidation of sugars was taking place at all stages of methyl linoleate autoxidation.

Hydrogen peroxide is a well-known catalyst for the oxidation of sugars (10). The slow rate of oxidation of sugars by molecular oxygen is probably the reason why this reaction has been investigated far less than the corresponding oxidation by catalysts in neutral and alkaline aqueous solutions (1, 3, 8, 9, 24, 26, 27).

From these studies it appears that sucrose, glucose, and fructose had an accelerating effect on the autoxidation of methyl linoleate emulsions. It is not possible to define a mechanism from the data acquired since the system evidently becomes quite complex as soon as oxidation begins. Experimental results indicated that oxidation of sugars was taking place. Whether sugars were being oxidized initially by the oxygen, by methyl linoleate hydroperoxide, or by radicals from initial oxidative attack on methyl linoleate is not clear. In any case the system involved a number of oxidizing and reducing substances. If sugars or their oxidation products were oxidized by a radical mechanism, they then could contribute to the number of free radicals in the system and thus cause an acceleration of oxidation rates. In an oxidizing system containing both a sugar and methyl linoleate the contribution of any specific component becomes difficult to delineate, thus beclouding the validity of any proposed mechanism. It is obvious however that both sugars and methyl linoleate are involved in the over-all oxidation of the systems studied.

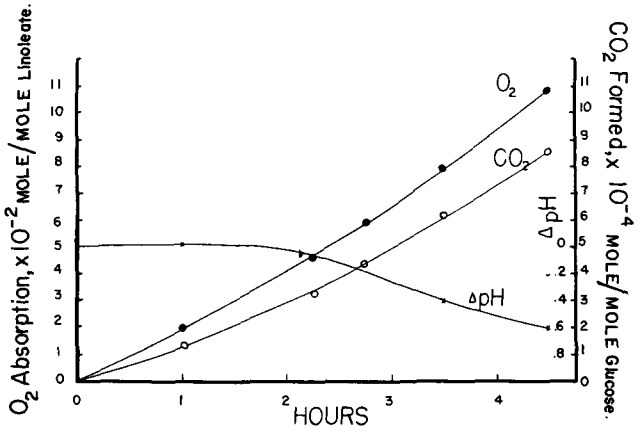

FIG. 3. Oxygen uptake by methyl linoleate emulsions autoxidizing in the presence of 6.3093×10^{-4} mole glucose/mole linoleate, carbon dioxide formation, and pH changes related to time. Initial pH 7.00, 50°C.

TABLE II

Carbon Dioxide Formed from the Oxidation of Methyl Linoleate-Sugar Systems at 50°C. After a Period of 4.5 Hours

Sugar concn. (mole/mole methyl linoleate)	CO_2 Formed, $\times 10^{-3}$ mole/mole Sugar			
	Initial pH: 7.00			Initial pH: 5.50
	Glucose	Fructose	Sucrose	Sucrose
0.6309	1.3625	2.1900	0.4563	1.1350
1.2618	.....	1.7299	0.2902	0.6029
1.8927	.....	1.5179	0.2530	0.4836
2.5237	.....	1.2388	0.2176	0.4408

Activation Energy for the Oxidation of Methyl Linoleate Emulsions with Added Sucrose, Glucose, and Fructose

A plot of the logarithm of the reaction rate constants *versus* the reciprocal of the absolute temperature was linear and permitted calculation of the activation energy as shown in Table III. With hydrogen ion concentrations, the activation energy can also be expected to vary a little. With glucose a slight variation was observed in the activation energy values while with fructose this variation was manifested at pH 8.00. With sucrose the activation energy value was decreased by 10% at pH 8.00 and by 7% at pH 5.50 in comparison with that at pH 7.00. The explanation for the lower activation energy values at pH 5.50 and 8.00 is that, at these two hydrogen ion concentrations, inversion of sucrose to hexoses was accomplished, as was also the conversion of fructose to glucose, which, in turn, decomposed.

The activation energy values for the autoxidation of methyl linoleate emulsions in the presence of sugars are less than the value for autocatalysis, 18.20 K. Cal./mol. (14).

An absorption maximum in the diene region (2300–2350 Å) in methyl linoleate autoxidized in presence of sugars was observed, accompanied by the appearance of another maximum at 2775 Å. The absorption at 2775 Å increased by the addition of alkali. Ultra-

TABLE III

Activation Energy for the Autoxidation of Methyl Linoleate Emulsions in the Presence of Sugars

Sugar	Activation Energy (K. Cal/mol.)		
	pH 5.50	pH 7.00	pH 8.00
Sucrose	14.9	16.0	14.4
Glucose	10.6	10.8	10.2
Fructose	10.6	10.4	8.8

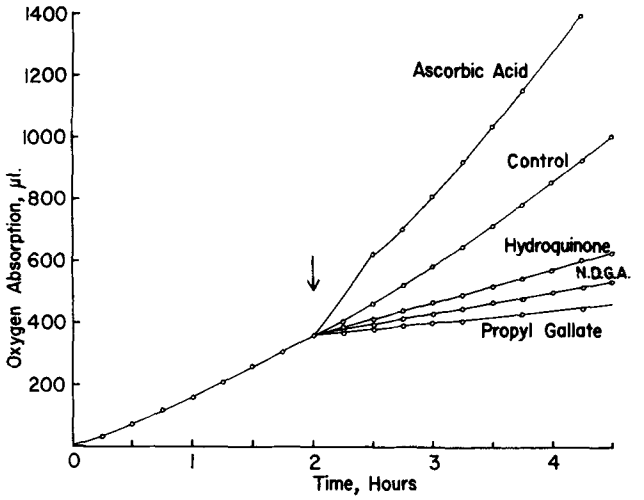

FIG. 4. Effect of added ascorbic acid, N.D.G.A., propyl gallate, and hydroquinone on oxidation of methyl linoleate-glucose systems after 2 hrs. Initial pH 7.00, glucose concentration 6.3093×10^{-4} mole/mole linoleate, 50°C. Concentration of antioxidant 1.25×10^{-3} M.

violet absorption revealed that the conjugated dienes did not account for all the oxygen uptake by the system. At the same level of oxygen absorption a decrease of the percentage of conjugated dienes formed was observed as the concentration of fructose increased.

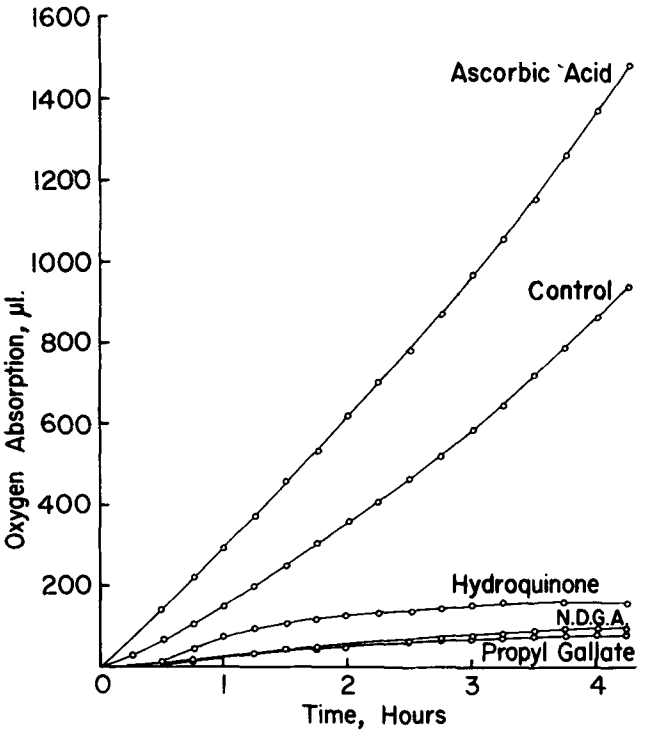

FIG. 5. The effect of added ascorbic acid, N.D.G.A., propyl gallate, and hydroquinone to methyl linoleate-glucose systems at zero time. Initial pH 7.00, glucose concentration 6.3093×10^{-4} mole/mole linoleate, 50°C. Concentration of antioxidant 1.25×10^{-3} M.

Effect of Antioxidants on the Autoxidation of Methyl Linoleate—Sugar Emulsions

Because of the processing practice of adding ascorbates to cured meats there was a question whether ascorbic acid would have a reducing effect in the methyl linoleate emulsion. The effect of hydroquinone, propyl gallate, and nordihydroguaiaretic acid (N.D.G.A.) was also observed.

Addition of ascorbic acid to the methyl linoleate-glucose system after allowing oxidation to proceed for 2 hrs. caused an increase in oxygen absorption (Fig. 4). When ascorbic acid was present initially, a great increase in total oxidation was observed (Fig. 5).

It appeared that methyl linoleate hydroperoxide caused co-oxidation of ascorbic acid. Similar results were obtained by the addition of ascorbic acid and ascorbates to aqueous fat systems (13, 19, 25).

The addition of N.D.G.A., propyl gallate, and hydroquinone to the linoleate-glucose system, which had been allowed to proceed in oxidation for 2 hrs., was ineffective in stopping the oxidation. Addition of these antioxidants at zero time slowed considerably the oxidation of methyl linoleate emulsions. Hydroquinone was not quite as effective as N.D.G.A. and propyl gallate, possibly because hydroquinone favors oxidation of glucose to D-arabinoic acid by oxygen in aqueous solutions (2).

REFERENCES

1. Binkley, W. W., and Wolfrom, M. L., Advances in Carbohydrate Chemistry, *8*, 291–314 (1953).
2. Dubourg, J., and Naffa, P., Bull. soc. chim. France (1949), 1353–1362.
3. Gabrryelski, M., and Marchlewski, L., Biochem. Z., *265*, 50–57 (1933).
4. George, P., J. Chem. Soc. (1954), 4349–4359.
5. Grau, R., Gunther, H., and Scheper, J., Fleischwirtschaft, *12*, 728–730 (1960).
6. Griffith, T., and Johnson, J. A., Cereal Chem., *34*, 159–169 (1957).
7. Hall, L. A., and Gleason, H. L., (The Griffith Laboratories Inc.), U.S. 2,845,358 (1958).
8. Keyns, K., and Heinemann, R., Ann., *558*, 187–192 (1947).
9. Kuchlin, A. T., Rec. trav. Chim., *51*, 887–913 (1932).
10. Kuchlin, A. T., Biochem. Z., *261*, 411–424 (1933).
11. Lea, C. H., Rept. Food Invest. Bd. (1935), 38–43.
12. Lea, C. H., J. Soc. Chem. Ind., *55*, 293–302T (1936).
13. Lehman, B. T., and Watts, Betty M., J. Am. Oil Chemists' Soc., *28*, 475–477 (1951).
14. Mabrouk, A. F., and Dugan, L. R., Jr., J. Am. Oil Chemists' Soc., *37*, 486–490 (1960).
15. National Research Council of the United States of America, "International Critical Tables of Numerical Data, Physics, Chemistry, and Technology," Vol. 3, p. 272, New York, McGraw-Hill Book Company, (1929).
16. Regier, L. W., and Tappel, A. L., Food Research, *21*, 630–639 (1956).
17. Regier, L. W., and Tappel, A. L., Food Research, *21*, 640–649 (1956).
18. Roller, P. E., J. Phys. Chem., *35*, 3286–3292 (1931).
19. Scarborough, D. A., and Watts, Betty M., Food Technology, *3*, 152–155 (1949).
20. Sharp, J. C., J. Sci. Food and Agric., *8*, 14–20 (1957).
21. Spetsig, Lars-Olov, Arkiv. Kemi, *14*, 527–533 (1959).
22. Thompson, P., and Hegsted, D. M., J. Nutrition, *60*, 361–365 (1956).
23. Umbreit, W. S., Burris, R. H., and Stauffer, J. F., "Manometric Techniques and Related Methods for the Study of Tissue Metabolism," p. 16–18, Minneapolis, Burgess Publishing Company (1949).
24. Warshowsky, B., and Sandstrom, W. M., Arch. Biochem. and Biophys., *37*, 46–55 (1952).
25. Watts, Betty M., and Wong, Ruby, Arch. Biochem., *30*, 110–120 (1951).
26. Whistler, R. L., and Schweiger, R., J. Am. Chem. Soc., *81*, 3136–3139 (1959).
27. Wolfrom, M. L., and Schumacher, J. N., J. Am. Chem. Soc., *77*, 3318–3323 (1955).

[Received May 26, 1961]