14238

Fat Oxidation in Experimental Animal Diets.*

DONALD F. CLAUSEN, RICHARD H. BARNES AND GEORGE O. BURR.
From the Division of Physiological Chemistry, University of Minnesota, Minneapolis.

The large number of deleterious effects caused by the ingestion of oxidized fat, which have been reported from numerous laboratories, will be reviewed in a forthcoming publication.[1] In many cases improper stabilization of experimental diets may have resulted in the inadvertent feeding of rancid fats to test animals. Although it is impossible to tell how frequently experimental results have been unknowingly influenced by this factor more care should be exercised by investigators in preventing fat deterioration. The seriousness of fat rancidification was again brought to the attention of this laboratory some time ago when it was found that rats receiving a normal balanced diet containing the pure B vitamins in place of the customary yeast, cod liver oil, and lard, failed to grow and ultimately died. Replacement of the lard with other fats such as hydrogenated vegetable oils, corn oil, and butterfat provided an adequate diet for growth. If the pure B vitamins were replaced by yeast, normal growth resulted. On further investigation it was found that in the absence of yeast, the lard and cod liver oil rapidly became oxidized and exerted a deleterious effect.

As it is a common practice among nutrition investigators to prepare experimental diets and keep them at room temperatures over periods of several weeks it was considered advisable to have some knowledge of the rates of deterioration of fats incorporated in different food mixtures.

Experimental. Dietary mixtures were compounded in 200 g lots as outlined in Table I. These mixtures were placed in glass bottles and stored in the dark at room temperature. Once each week 25 g samples were removed and extracted with ligroin (B. P. 30-60°C.). After removal of the solvent *in vacuo,* peroxide values were determined.[2] These peroxide values in milliequivalents per 1000 g of fat are plotted in Fig. 1, 2, and 3. The peroxide values of the individual fats at the time of mixing were: wheat germ oil, 7.3; cod liver oil, 18.6; steam rendered lard, 2.4; bland (stabilized) lard, 0.3: vegetable (Wesson) oil, 14.2; and butterfat, 0.0.

Results. It is shown in Fig. 1 that the lard in diet mixtures not containing yeast reached the end of the induction period within 2-3 weeks. The stability of this fat was not greatly influenced by the presence of the pure B vitamins, but addition of yeast prolonged the induction period beyond 8 weeks.† When mixed with Cellu Flour (Fig. 3, Diet 19) the induction period of lard was much longer than when it was mixed with other dietary constituents except yeast (Fig. 1, Diet 5). It seems that a prooxidant, probably the salts, must promote oxidation in the food mixture.

In Fig. 2 are given the oxidation curves for several fats mixed in diets containing the pure B vitamins and cod liver oil, with or without wheat germ oil. The induction period of a lard-containing diet without either cod liver oil or wheat germ supplements (Diet 2) was prolonged slightly by the addition of cod liver oil (Diet 7) and to a greater extent by cod liver oil together with wheat germ oil

* This work was aided by a grant from the Rockefeller Foundation.

1 Burr, G. O., and Barnes, R. H., *Physiol. Rev.*, in press.

2 King, A. E., Roschen, H. L., and Irwin, W. H., *Oil and Soap,* 1933, **10**, 105.

† Since the completion of this study György and Tomarelli (György, P., and Tomarelli, R., *J. Biol. Chem.*, 1943, **147**, 515) have presented evidence of the antioxidant properties of yeast and the lack of antioxidant activity of the known B vitamins with the exception of *p*-aminobenzoic acid, in mixtures of corn starch, butter yellow (N,N-dimethylaminoazobenzene), and crude linoleic acid (iodine value 126-140).

TABLE I.
Dietary Mixtures.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Casein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Salts	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Yeast	+							+											
Pure B Vitamins		+				+	+		+	+	+	+	+	+	+	+			
B Vitamins minus Choline			+																
Choline				+															
Cellu Flour																	+	+	+
Mineral Oil															+	+			
Wheat Germ Oil						+		+	+		+		+		+		+		
Cod Liver Oil						+	+	+	+	+	+	+	+	+	+	+	+	+	
Type of Fat	S. lard	S. lard	S. lard	S. lard	S. lard	S. lard	S. lard	S. lard	B. lard	B. lard	Veg. oil	Veg. oil	B. fat	B. fat	—	—	S. lard	S. lard	S. lard

When present in a given mixture the ingredients were added per 100 g of diet in the amounts specified below: Casein (Land O'Lakes, 100 mesh) 20 parts; sucrose (pure cane sugar) sufficient to make a total of 100 g; salts (modified Hubbell, Mendel and Wakeman[3] $CaCO_3$, 543; $MgCO_3$, 25; $MgSO_4 \cdot 7H_2O$, 16; NaCl, 690; KCl, 112; KH_2PO_4, 212; $FePO_4$, 20.5; KI, 0.08) 4 parts; yeast (Anheuser Busch strain G) 6 parts: pure B vitamins (thiamin, 1 mg; riboflavin, 375 μg; nicotinic acid, 2 mg; pyridoxine, 500 μg; pantothenic acid, 2.8 mg; choline, 200 mg; 2-methyl 1-4 naphthoquinone, 40 μg); B vitamins minus choline (the above vitamins without choline); choline, 200 mg; Cellu Flour (Chicago Dietetic Supply House) sufficient to make a total of 100 g; mineral oil (heavy U.S.P.) 18 parts; wheat germ oil (Viobin) 1 part; cod liver oil (U.S.P.) 2 parts; steam lard (Hormel) 18 parts; bland lard (Swift's, contains some hydrogenated fat and 0.1% gum guaiac) 18 parts; vegetable oil (Wesson oil) 18 parts; butterfat (commercial butter melted and centrifuged to separate fat) 18 parts.

(Diet 6.) Bland lard which contains the antioxidant gum guaiac, had a definitely prolonged keeping time, but was not appreciably affected by the inclusion of wheat germ oil (Diets 10 and 9). The induction period of the butterfat diet was prolonged to some extent by wheat germ oil (Diets 14 and 13) but

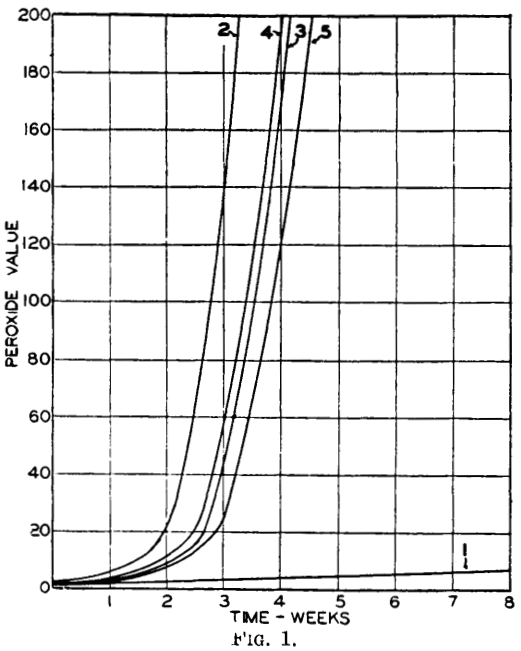

FIG. 1.

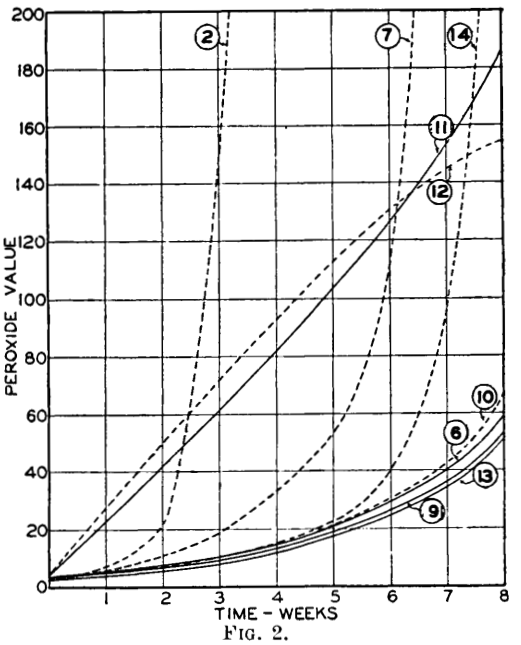

FIG. 2.

the cottonseed oil diet which had a steady peroxidation from the start was not protected (Diets 12 and 11).

In the absence of other fats the mixture of cod liver oil and wheat germ oil oxidized much

[3] Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.

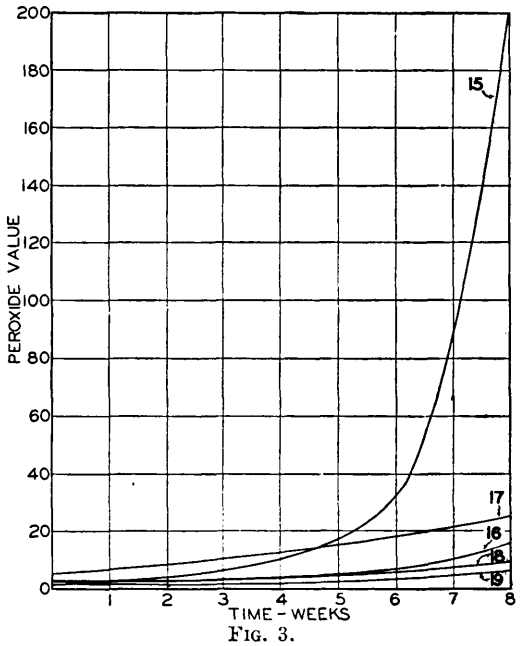

FIG. 3.

more rapidly than cod liver oil alone (Fig. 3, Diets 16 and 15).

Data which are not included in the graphs showed that after a 6-weeks storage of yeast-containing diets at room temperature (Diet 8 with other fats substituted) none of the following fats had developed peroxide values above 15: lard Swift's Silverleaf, lard Cudahy-Rex, lard Swift's-Bland with gum guaiac, corn oil (Mazola), hydrogenated vegetable oil (Crisco), or butterfat.

Discussion. The rancidification of lard in a diet which did not contain yeast was very rapid. With the addition of cod liver oil and wheat germ oil the rate of accumulation of peroxides was considerably less. However it is well known that both of these oils are themselves unstable and that cod liver oil rapidly destroys vitamin E in mixed diets. It is certainly not good practice to incorporate these 2 fat soluble vitamin sources in the diet mixture, especially when yeast is not present. For purposes of controlling nutrition factors it is frequently advocated that yeast supplements be fed separately from the rest of the dietary mixture. When this method of yeast administration is not necessary a prolonged keeping time of the food fat and a probable protection of readily oxidizable vitamins is afforded by the direct incorporation of the yeast in the diet. It is also advantageous to employ other methods of stabilization. Lard containing 0.1% gum guaiac had a somewhat longer induction period. Storing of diets at low temperatures is to be recommended. Diet No. 2 which contains lard and the pure B vitamins had a peroxide value of 12 after approximately 3 weeks in an ice box at a temperature of 12°C. When stored at room temperature for the same time the lard of this diet had a peroxide value greater than 100.

The variation in the keeping quality of different fats and the influence of other diet components on the stability of fat makes careful control of rancidification imperative in nutrition studies. The inadvertent destruction of dietary essentials and the possible independent toxicity of rancid fat are factors that might confuse the interpretation of many diet experiments.

Summary. Studies on the keeping quality of fats in different food mixtures have indicated certain prooxidant and antioxidant characteristics among the dietary components. The relative keeping time of fats added to various dietary mixtures is given and the importance of careful preservation of experimental diets is stressed.