

in protein phosphorus, on electropherograms of sera of oestrogenized and laying birds<sup>4</sup> probably represents a lipovitellin-phosvitin- $\gamma$ -livetins complex in the sense in which this term is used by Joubert and Cook<sup>6</sup>. The 'PP' fraction was originally distinguished by the use of methanolic veronal buffer, but more recently we have been able to distinguish it by zone electrophoresis in aqueous buffer (pH 8.6,  $\mu$  0.05). The second, somewhat more mobile, lipoprotein fraction ("fraction 8" of Vanstone *et al.*)<sup>7</sup> presumably corresponds mainly to lipovitellenin.

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### Basic Substances as Synergists for Fat Antioxidants

ADDED fatty acids decrease the effectiveness of a number of antioxidants in marine and other oils and fats<sup>1</sup>. It has now been demonstrated that many oil-soluble basic substances may act as synergists. For example, a primary amine (octadecylamine, ODA), a secondary amine (proline), and a tertiary amine (triisooctylamine, TOA) provided unexpectedly great protection against oxidation to oils containing added 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (EMQ, 'Santoquin'), whereas in the absence of the last-named they conferred only slight or no protection at the same levels (Table 1).

The effects of the various basic additives differed with each antioxidant. For example, in general they were synergists to 2-tertiarybutyl-4-methoxyphenol (BHA) but had no effect or acted as antagonists to 2,6-ditertiarybutyl-4-methoxyphenol (BHT). They were also more effective with  $\gamma$ -tocopherol than with  $\alpha$ -tocopherol. In this respect they have been found useful in differentiating the various tocopherols with regard to antioxidant effectiveness.

Substrates also modified the respective activities. With the samples of highly purified trilinolein available to us (Hormel Foundation), octadecylamine acted as an antagonist to  $\alpha$ -tocopherol and synergist to  $\gamma$ -tocopherol, whereas in menhaden oil octadecylamine was synergistic with both.

Proline was particularly effective in vegetable oils, probably acting in conjunction with the natural antioxidants. For example, samples of a refined olive oil containing 0.025 and 0.05 per cent added proline had induction periods at 60° C. of 4, 12 and 23 days, respectively.

The synergistic effects of phospholipids and of some amino-acids on phenolic type antioxidants have been recognized for a number of years<sup>2</sup>. It appears likely that these effects may be mediated

Table 1. EFFECT OF MIXTURES OF ANTIOXIDANTS WITH BASIC SYNERGISTS ON MENHADEN OIL AND TRILINOLEIN

Antioxidant	Basic additive	Induction period*—Days (50° C.)	
		Menhaden oil†	Trilinolein
EMQ	None	3	31
	ODA	28	56
	Proline	87	61
	TOA	104	41
BHA‡	Phospholipid‡	33	—
	None	2	12
BHT	TOA	11	15
	None	4	19
$\alpha$ -Tocopherol	TOA	2	12
	None	2	10
	ODA	3	6
$\gamma$ -Tocopherol	Proline	3	18
	TOA	17	7
	None	3	13
	ODA	9	31
None	Proline	25	—
	TOA	36	36
	None	1	1
	ODA	1	1
None	Proline	1	1
	TOA	1	1

\* Induction periods were determined by the gain in weight method recently described (ref. 4). 10-ml. beakers containing 200 mgm. substrate plus 0.5  $\mu$ M of antioxidant and 2.5  $\mu$ M of basic additive were incubated in a draft oven at 50° C., cooled and weighed once daily (to 0.1 mgm.). The end of the induction period was usually readily detected by a rapid gain in weight and arbitrarily fixed at the time at which the weight gain was 1 mgm.

† The menhaden oil was a commercial alkali-refined product treated to remove peroxides as follows: a 10 per cent solution in light petroleum ether was passed through a column of deactivated alumina, and the solvent removed *in vacuo*.

‡ A preparation of yeast lecithin containing 1.89 per cent nitrogen, 4.1 per cent phosphorus, 0.03 per cent amino-nitrogen (D. J. Hanahan).

§ BHA is often designated 2 (or 3)-tertiarybutyl-4-methoxyphenol. The preparation which was used in much of this work contained at least 85 per cent of the 2-tertiarybutyl derivative. Similar results were obtained with the pure 2-isomer.

through the basic groups of such compounds, modified by solubility, heavy-metal chelating, and other properties. With respect to phospholipids, this contrasts with a previous suggestion<sup>3</sup> that the phosphate portion of the molecule was primarily involved.

The progress of this work has been hampered by difficulties in obtaining reproducible runs. The results shown are, however, typical of a number of determinations. Details will be published elsewhere.

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### An Improved Method for preparing Pectate Gels

THE routine use of pectate gel media as an aid to the detection of bacteria causing soft rot of plant tissue has been restricted by the difficulty commonly encountered in their preparation. One of the most useful methods is that of Wieringa<sup>1</sup>, who adopted the double-layer technique, consisting of a plate of calcium agar over which the pectate solution was poured. Diffusion of calcium ions from the agar brought about the formation of a gel.