

sively studied. Quantitation with the selenoamino-acids was rendered difficult by the heterogeneity of the system; none was completely in solution in the substrates used.

Changes in peroxide value were followed in lard samples with and without added selenomethionine and also with and without added peroxides. The results, shown in Table 2, indicate that in the presence of selenomethionine the peroxide values did not rise so high and tended to decrease with time, more so than when either methionine or no additional additive was present. In this sense selenomethionine appeared to be able to decompose peroxides.

The results with selenomethionine differed from those observed with other antioxidants in: (1) they appeared to have no effectiveness in low concentrations; (2) the protection even in large amounts could not be uniformly demonstrated; (3) no protection was afforded to a highly unsaturated oil; (4) some oxidation appeared to occur before the antioxidant effect appeared. These observations may tentatively be explained by assuming that selenomethionine is not itself an antioxidant but that it is oxidized to a derivative which is. Such systems have been described for thio- and dithio-compounds by Barnard *et al.*⁹, and for seleno-compounds by Woodbridge⁶. In the former case the active antioxidants are thought to be the derived sulphoxides, and thiosulphinates.

It is reasonable to suppose that the reactions which lead to the formation of a sufficient amount of a powerful antioxidant so that oxidation stops would be of a complexity such that only at times could this consumption be reached. Thus, if a highly unsaturated oil formed peroxides rapidly, the intermediate antioxidant might be further oxidized before it could stabilize the reaction.

These results give further suggestive evidence that some of the tocopherol-like physiological properties of selenium compounds may be mediated through an antioxidant action⁴. Perhaps the lack of parallelism in their physiological effects is due not only to the differences of availability to different cells of tocopherols compared with seleno-compounds but also to the requirement that specific selenium derivatives, which occur or are synthesized in ways not yet understood, are required to achieve the antioxidant state.

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Occurrence of Mevalonic Acid in Carrots

ALTHOUGH tracer studies in a number of carotenogenic systems^{1,2} have conclusively proved that mevalonic acid is an effective precursor of carotenes, the presence of mevalonic acid has not been demonstrated in any of such systems. In this laboratory an attempt has been made to isolate mevalonic acid from carrots. Previous work^{3,4} has shown that cell-free extracts of carrots can synthesize carotenes from acetate and mevalonic acid.

100 gm. (wet weight) of freshly collected carrots were minced and placed in 100 ml. phosphate buffer (0.1 M, pH 5.8) for about 2 min. in a Waring blender to prepare the homogenate. From the filtered homogenate the proteins were removed by treatment with trichloroacetic acid and the supernatant, after neutralization, was concentrated *in vacuo* to about 60 ml. For isolation of mevalonic acid the concentrated material was acidified with 8 ml. of 10 N sulphuric acid, then extracted with ethyl ether for 8 hr. and the extract treated according to the method of Lynen and Grassl⁵; finally mevalonic acid was determined by reaction with hydroxylamine at pH 7.0 and subsequent colorimetric measurement of the resultant hydroxamic acid by formation of a complex with ferric salts. The quantitative range of mevalonic acid present in carrots varied from 2 to 4 μ moles/100 gm. of carrots (wet).

The dibenzylethylenediamine (DBED) salt of isolated mevalonic acid was prepared according to the procedure described by Hoffman *et al.*⁶; a crystalline salt was obtained which melted at 115° (114–119°).

For further confirmation the mevalonic acid DBED salt was dissolved in minimum amount of water, the pH was adjusted to 10 and the free amine was extracted with ethyl ether. The alkaline solution was readjusted to pH 6.5–7, and then, after concentration, portions were chromatographed according to the method of Yokoyama *et al.*² and the spot of mevalonic acid was identified by spraying with bromophenol blue.

The compound isolated was also confirmed as mevalonic acid by identifying the hydroxamate derivative which was chromatographed by using butanol saturated with water as the solvent system as reported by Knauss *et al.*⁷. The spots were made visible by spraying with 5 per cent FeCl₃.6H₂O in 90 per cent ethanol and 0.1 N hydrochloric acid.

In view of the recent findings of Braithwaite and Goodwin⁸ that mevalonate is more efficiently incorporated than acetate into carotenes of carrots, the occurrence of mevalonic acid in carrots makes the carrot system a valuable tool for further work.

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