Metabolic effects of vitamin E and selenium

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In spite of the vast literature that has accumulated on vitamin E during the 40 years since its discovery, most writers and speakers on the subject habitually preface their remarks by emphasizing the confusion that exists in this field. Thus Mason (1944), in the introduction to an extensive review, said of the literature that 'much of it is contradictory, some of it merely baffling', words that have been repeated many times since. In retrospect, the particular difficulties in our understanding of the role of vitamin E can be seen to stem from several factors. Two of these are of especial import. There is, firstly, in spite of the great body of work on vitamin E, still no clue as to its primary site of action (if indeed one exists); and, secondly, the complex interrelationships that exist between vitamin E and other nutrients, such as unsaturated fats, vitamin A and minerals, have undoubtedly contributed more than is usual to the contradictory findings in this field. The discovery by Schwarz & Foltz (1957) that selenium is an essential trace element for the rat and is active in preventing the onset of liver necrosis in rats on diets deficient in vitamin-E and Factor 3 has therefore—apart from its intrinsic importance—particular significance. It not only dispels at least some of the confusion that has existed, but begins to clarify certain aspects of the role of vitamin E itself. Although, in its wake, the discovery has brought new problems, study of the metabolic relationships between Se and vitamin E may offer the best chance in the immediate future of answering some of the most vexing questions in this field. Thus it now seems possible to deal with nutritional states in which the effect of vitamin E is studied as a single nutritional factor—there is no doubt that in the past much work was bedevilled by the existence of partial deficiencies of Se as well.

A number of metabolic changes are known to occur in vitamin E-deficient animals and, in recent years, they have received particular study. Far less is at present known about the metabolic effects of Se deficiency, but it is of great value to compare what is known. It is advantageous, first, to consider the best-studied condition in which these two factors play a part, dietary necrotic liver degeneration in the rat. This condition is now known to be caused by a deficiency of both vitamin E and Se and can be prevented by either substance. Even before Se was implicated, however, it was known that liver slices taken from rats in the latent phase of the disease showed a metabolic defect and were incapable of maintaining normal respiration (Chernick, Moe, Rodnan & Schwarz, 1955). It was shown that this biochemical lesion was associated with the prenecrotic state and could be prevented by either α-tocopherol or Se in the diet. Subsequently it was shown that α-tocopherol, administered to the anaesthetized rat by intraportal injection 30 min before the liver was excised, was just as effective in preventing the respiratory decline (Rodnan, Chernick & Schwarz, 1956). Green, Edwin, Bunyan & Diplock (1960) showed that Se, administered as sodium selenite, was ineffective when given intraportally, although Co^{2+} ions, which were apparently not effective in the diet, prevented appreciable respiratory decline,

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if given intraportally. None of these substances, however, was found to be active in vitro, when added to the slice medium. Subsequently, however, Schwarz, Mertz & Simon (1959) found that a water-soluble metabolite of α-tocopherol, tocopheronolactone, is active in vitro in this system. It is, in fact, the only tocopherol derivative (in acid and in lactone form) with this activity, although its activity is shared by some, but not all, synthetic antioxidants, chiefly *N*,*N*′-diphenyl-*p*-phenylenediamine (DPPD). Subsequently, it was found that respiratory decline could be observed also under suitable conditions in rat-liver homogenates and isolated mitochondria. This decline, however, could not be prevented by either Factor 3 or Se in the diet, but only by vitamin E. In vitro, the decline could be reversed with minute amounts of DPPD, with certain other substances such as ethylenediamine tetraacetate (EDTA) and Mn^{2+} ions, with tocopheronolactone and menaphthone, but not with free α-tocopherol, even in excessive amounts (Corwin & Schwarz, 1959, 1960; Schwarz, 1961*a*). It is difficult to obtain a clear picture from these studies either of the sequence of events in liver necrosis or of the way in which the activities of Se and α-tocopherol are related. Several points emerge, however, from the work of Schwarz and his colleagues. First, they offer considerable evidence that a tocopherol metabolite, rather than tocopherol itself, is concerned with arresting the respiratory-decline effect. Secondly, although certain antioxidants are very active, in vivo and in vitro, in preventing respiratory decline, their effect cannot readily be correlated with their antioxidant properties. Thus methylene blue, by intraportal injection, is highly active in the oxidized state but is quite inactive in the leuco form, and Schwarz (1961*a*) has recently confirmed that α-tocopherol is itself ineffective in vitro. The active substances might all be related by possessing quinonoid structure. Although it is not yet possible to integrate all these effects into a plausible and connected hypothesis, one construction to put on the results might be as follows. As already indicated, it is possible that a metal or metals might be implicated in the respiratory failure of vitamin E-deficient slices and homogenates. Schwarz (1961*b*) suggests that maintenance of normal respiration depends on maintenance of free SH groups in the sensitive oxidase systems. By shifting their equilibrium more towards the disulphide forms, quinones could eliminate attack by inhibitory metals. In the absence of quinonoid substances, there is an accumulation of oxaloacetic acid, which may be primarily responsible for the decline by inhibiting succinic dehydrogenase. However, the chief weakness of this scheme is that it does not explain either the potency of minute amounts of Se in preventing the macroscopic lesions of liver necrosis, or why dietary Se is ineffective in restoring α-ketoglutarate oxidation in tissue homogenates.

The study of respiratory function leads us logically to another question that has received considerable attention: whether vitamin E is concerned directly with either electron transport or oxidative phosphorylation. Several workers have investigated such a connexion and some attractive hypotheses have been produced. Slater (1961) and Gray, Chisholm & Lee Peng (1960) have discussed some schematic possibilities in terms of possible oxidation–reduction reactions for α-tocopherol. Slater (1961) reported 0·4 μmole α-tocopherol/g protein in a horse-heart sarcosomal preparation.

This concentration is of the same order as that of known components of the respiratory chain, and he considered that the possibility that vitamin E may play a direct part in electron transport could still not be ruled out. In our laboratories (J. Bunyan, 1961, unpublished) we have, however, recently successfully bred rats born of vitamin-E deficient dams given DPPD in the diet and reared them to 5 months of age on a wholly synthetic vitamin E-free diet (only the daily allowance of 100 mg methyl linoleate was from a natural source and it was very highly purified and free from vitamin E). The rats were given adequate amounts of Se to prevent necrotic liver degeneration. They did not develop late-lactation paralysis or dystrophy. They were sterile, smaller than usual, but otherwise healthy. It seems to us, as a result of these experiments, that vitamin E cannot any longer be seriously considered to be an essential component of the electron transport chain, for, on analysis, no tocopherol was detected in the organs of these rats. In view of the quantities involved, it also appears unlikely that Se functions in electron transport. (It must be noted that these Se-dependent, vitamin E-free rats are not 'normal'. At about 6 months of age they develop progressive nervous lesions characteristic of vitamin E deficiency, and these lesions are not preventable by Se.)

Vitamin E has been shown to stimulate the synthesis of a variety of other substances of metabolic importance. The situation with regard to cholesterol is of some interest. Morgulis & Spencer (1936) showed that dystrophic muscle in guinea-pigs contained substantially more cholesterol than did muscle from controls, although liver and kidney from dystrophic animals contained less than did liver and kidney from controls. This has been confirmed in several species by other workers, but cholesterol levels are so dependent on the qualitative and quantitative nature of the dietary fat that contradictory statements occur. The sum total of the evidence, however, leaves little doubt that cholesterol levels are, under certain conditions, affected by vitamin E. The experiments of Gray (1959) on liver homogenates indicate that synthesis is affected, although other work suggests that transport is also implicated in the change in cholesterol levels. Perhaps both effects operate. Gray (1959) has also found total phospholipid synthesis to be influenced by vitamin E levels. We have in several papers described the results of our own experiments, which have shown that ubiquinone levels in the rat and some other species are generally increased by vitamin E and Se. During the past 2 or 3 years, it has been shown by us and other workers that ubiquinone levels are affected by a variety of other factors, which are not only nutritional but include also certain hormones, uncouplers of oxidative phosphorylation and other substances (Edwin, Diplock, Bunyan & Green, 1961; Diplock, Edwin, Green & Bunyan, 1961; Edwin, Green, Diplock & Bunyan, 1960; Aiyar & Sreenivasan, 1961*a*,*b*; Phillips & Morton, 1959; Beyer, Noble & Hirschfield, 1962).

The effect of vitamin E on ubiquinone levels in fact appears to be complicated by several factors, not all of which are understood (cf. the results of Morton & Phillips (1959) who did not find an effect of vitamin E on ubiquinone). Study of the levels of ubiquinone in deficient animals together with a consideration of what is known about its function leave no doubt that the biological role of vitamin E cannot be

directly related to ubiquinone concentrations. In fact the effect on ubiquinone must be considered to be only one result of some more generalized primary action. The position thus appears to be essentially analogous to that of cholesterol, whose synthesis and levels are also influenced by a great variety of metabolic and nutritional factors. We do not yet know whether cholesterol levels are influenced by Se as well as by vitamin E, and it is obviously of some importance to know this. Such a relationship may well exist. There is no indication of how the effect of vitamin E on cholesterol or ubiquinone metabolism may operate. In this connexion, it is particularly intersting to note that Rosecan, Rodnan, Chernick & Schwarz (1955) found the liver slices from rats on a torula-yeast diet (deficient in both Se and vitamin E) utilized acetate less readily than slices from control rats receiving dietary supplements of tocopherol. They found decreased ketogenesis, acetoacetate formation, lipogenesis and carbon dioxide production, and all these metabolic defects could be overcome within 60 min by an intraportal injection of α-tocopheryl acetate. The experiments of these workers were carried out before Factor 3 was identified with organically bound Se and it would be most useful to repeat them with diets deficient in either vitamin E or Se alone. The effect of vitamin E on levels of metabolically active substances is not restricted to the lipid fraction. Dinning, Sime & Day (1955) found a marked increase in the turnover rate of nucleic acids in vitamin E-deficient rabbits and considered this to be a primary metabolic function, although it did not appear to be associated with any difference in nucleic acid levels. The synthesis of ATP and CoA has also been stated to be influenced by vitamin E (Gray *et al.* 1959). It is, at present, not possible to correlate these various metabolic effects either with themselves or with the studies of Schwarz and his colleagues on the disturbances of oxidative processes in Se and tocopherol-induced liver necrosis. They may be secondary effects resulting from failure of such processes; or they may be secondary disturbances of metabolism resulting from a withdrawal of a physiological antioxidant in some primary process elsewhere; or they may be ultimately resolved in terms of a still more deep-seated effect, which may be attributable to either tocopherol or Se or perhaps to both.

All discussions about the function of vitamin E, within the last decade particularly, have turned around the question of its in vivo antioxidant properties and whether these are sufficient to account adequately for its observed physiological effects. Many of the arguments are familiar and need no reassertion. They rest on the following observations and partly but not necessarily logically connected statements: (1) a number of the clinical signs of vitamin E deficiency in animals can be prevented by antioxidants other than α-tocopherol; (2) α-tocopherol is itself an antioxidant; (3) vitamin E deficiency signs are exacerbated by the stress of dietary unsaturated fat; (4) lipid peroxidation is considered to be increased in vitamin E-deficient tissues, as evidenced by in vitro incubation followed by assay of malondialdehyde by the thiobarbituric acid method. According to one hypothesis, vitamin E is considered to function in vivo by trapping the free radicals that are produced during the initial stages of lipid auto-oxidation. It is considered that, in the absence of antioxidants,

the free radicals attack sensitive cellular structures, especially in the mitochondria and lysosomes, leading to various metabolic defects.

The antioxidant theory is valuable, rational and undoubtedly economical. It is also difficult to disprove. The main difficulty is that, although the postulate that vitamin E functions as an antioxidant can theoretically account for many of the observed facts, such a function has been demonstrated only in vitro. Since α-tocopherol is undoubtedly an in vitro antioxidant, the argument involves what appears to be a truism. A number of criticisms can be made of the antioxidant hypothesis. As Caputto, McCay & Carpenter (1961) have said, the thiobarbituric acid test fails to detect lipid peroxidation in vivo in deficient tissues and we have confirmed this finding on many occasions. There is no clear explanation as to why the in vitro production of malondialdehyde should be linked to in vivo peroxidation; the oxidation of fatty acids containing a methylene-interrupted double-bond system has indeed been shown to be preceded by the formation of conjugated olefinic systems (Magee, 1959). It has been shown (Green, Diplock, Bunyan, Edwin & McHale, 1961), moreover, that the in vitro production of malondialdehyde by vitamin E-deficient liver homogenates is prevented by a variety of substances that do not seem to be antioxidants. Especially active is the non-reducing but quinonoid tocopheronolactone; and vitamin A is at least as effective as the antioxidant ubichromenol. Furthermore, the tissues in which peroxidation can be shown to occur are often not those affected by the vitamin E deficiency state. For example, the brains of chicks with encephalomalacia peroxidize as much or even more than the brains from normal chicks.

In any discussion on the role of vitamin E it seems plain that the role of Se must now occupy a central place. The logic of the situation would seem to be that if vitamin E is an antioxidant Se must also be an antioxidant. It is a recognition of this fact that has prompted attempts to discover an antioxidant function for Se. Bieri, Dam, Prange & Søndergaard (1961) have demonstrated that the prevention of exudative diathesis in the chick by Se is indeed accompanied by a decrease in lipid

Table 1. *Comparison of the effects of low dietary concentrations of vitamin E and selenium and high concentrations of 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin) and DPPD on lipid peroxidation and the incidence of exudative diathesis in chicks*

	Percentage decrease of:*		
	Peroxides		Incidence of exudative
Addition to basal diet	Liver	Kidney	diathesis
Vitamin E, 10 p.p.m.	16	0	88†
Se, 0·03 p.p.m.	−44‡	4	67‡
Se, 0·15 p.p.m.	29†	13§	92†
Ethoxyquin, 500 p.p.m.	49‡	75‖	75‡
DPPD, 250 p.p.m.	18	38‡	0

*In relation to controls not given supplements.
†Significantly lower than control value, $P<0.001$.
‡Significantly different from control value, $P<0.05$.
§Significant difference only in Expt 1.
‖Significantly lower than control value, $P<0.01$.

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peroxidation (thiobarbituric acid assay) in certain tissues, especially in liver. Table 1 shows the results of some experiments we have recently carried out (Bunyan, Diplock, Edwin & Green, 1962), which appear to show very clearly the lack of correlation between the activity of Se, vitamin E and antioxidants in preventing exudative diathesis and their effects on lipid peroxidation. Other workers have usually compared the effects of deficient diets with those of diets supplemented with relatively large amounts of vitamin E, much more than is necessary to prevent the deficiency state. In our experiments we used a range of concentrations and studied the effects on lipid peroxidation of the minimal amounts of vitamin E, Se and two antioxidants required to affect the incidence of exudative diathesis. As Table 1 shows, there was no correlation between the two effects. Se at 0·03 p.p.m. in fact increased lipid peroxidation in liver though it reduced exudative diathesis by 67%. DPPD, although it affected peroxidation, was without effect on exudative diathesis. It is especially significant that this concentration of DPPD is completely adequate to prevent encephalomalacia in chicks (Scott, 1962). Since the latter disease is produced only by vitamin E-deficient diets containing considerable quantities of unsaturated fat, its prevention would almost certainly require, by the tenets of the antioxidant theory, increased physiological concentrations of antioxidants. It seems impossible, therefore, for the prevention of exudative diathesis in the chick to be related to an antioxidant function of any of the substances tested. Table 2 illustrates the results of experiments of a similar kind carried out with rats. The animals were reared on a baker's-yeast diet, and we studied the effects of dietary Se, vitamin E and DPPD on

Table 2. *Effects of selenium, vitamin E and DPPD on liver necrosis in rats and lipid peroxidation of tissues*

Expt no.	Addition to diet	Total incidence of liver necrosis	Malondialdehyde in liver (μg/g) after 15 min at 37° (value with standard deviation)
1	None	7/13	9·3±1·0
	Se, 0·05 p.p.m.	0/12	10·4±2·4
	Se, 0·50 p.p.m.	0/11	10·3±1·3
2	None	5/7	32±12
	Vitamin E, 10 p.p.m.	2/7	27± 7
	Vitamin E, 20 p.p.m.	1/7	24± 4
	Vitamin E, 30 p.p.m.	0/7	23± 6
3	None	6/7	30± 6
	DPPD, 8 p.p.m.	3/6	30± 6
	DPPD, 15 p.p.m.	5/7	22± 4*
	DPPD, 30 p.p.m.	3/7	17± 3*

*Significantly lower than control value, $P<0·01$.

the incidence of liver necrosis and liver-lipid peroxidation. It is clear that Se had no effect whatsoever on peroxidation, and vitamin E, at the level required to prevent necrosis, also had no significant effect. DPPD, however, although it did not eliminate necrosis at the levels tested, significantly reduced peroxidation.

It would seem to us, therefore, that though α-tocopherol and, to a much lesser extent, some Se compounds exhibit antioxidant properties these properties cannot

account for their biological role, although with α-tocopherol certain deficiency states involving fat stress could partly involve such properties. Even fat stress, however, is not necessarily related to peroxidation. Schwarz (1954) has shown very clearly that fat stress precipitates liver necrosis in rats, which is entirely prevented by Factor 3 Se, which, as we have shown, exerts no antioxidant effects in this system. There is much evidence that points to a so-far undiscovered metabolic role for a derivative of tocopherol, which is closely paralleled, biochemically, by the role of Se.

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Muscular dystrophy in man: clinical aspects

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Of the many myopathic disorders which occur in man, muscular dystrophy is most secure in its possession of a separate identity. It can be defined as a genetically determined primary degenerative myopathy (Walton, 1961). I shall discuss briefly here the clinical characteristics of the commoner varieties of human muscular dystrophy, and I shall also mention some of the other varieties of myopathy known