4. Rats which had been on a vitamin A-deficient diet for 45 days after weaning had exhausted their liver reserves of vitamin A, and the liver concentrations of sterol, ubiquinone and substance SC had all risen. One group of such rats was given curative doses of vitamin A; the survivors showed restored growth and diminutions in liver ubiquinone and substance SC concentrations. A similar group received a sulphadiazine supplement which added to vitamin A deficiency a conditioned vitamin K₂ deficiency. The superimposed vitamin K₂ deficiency did not alleviate nor appreciably exacerbate the effects of vitamin A deficiency on liver concentration of sterol, ubiquinone and substance SC.

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Unsaponifiable Constituents of Liver, Kidney and Heart Tissues from Vitamin E-Deficient Rats Compared with α -Tocopherol-Supplemented Rats

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The tocopherols can be converted *in vivo* into *p*-benzoquinone derivatives such as α -tocopherylquinone (I). This makes it necessary to investigate whether there is any simple relationship between tocopherol status and the ubiquinone (II, for

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references see Morton, 1958) content of tissues, because (i) the precise roles of vitamin E and ubiquinone are of interest in the study of lipid cofactors and (ii) the origin of the aromatic portion of the ubiquinone molecule has not been established. The biogenesis of the polyisoprenoid side chain in ubiquinones (e.g. the $-C_{60}H_{81}$ side chain in the animal

		Mean weight (g.)			
		Vitamin E-supplemented		Vitamin E-deficient	
		Males	Females	Males	Females
Body	Initial	42	45.5	37.5	43.5
	Final	260	189	229	183
Liver		10.9	8.5	8.75	8.4
Kidney (pair)		2.11	1.61	1.87	1.58
Heart		0.83	0.63	0.73	0.63
Testes		2.58	—	1.81	—
Uterus		—	0.35	—	0.38
Testes (% of body wt.)		0.99	—	0.79	—
Uterus (% of body wt.)		—	0.185	—	0.207

fractions eluted by 20 % ether in light petroleum a small amount of α -tocopherylquinone was found (λ_{max} 260 and 270 m μ). This fraction also contained cholesterol and 7-dehydrocholesterol. When it was acetylated and rechromatographed the esterified sterols were readily eluted by light petroleum containing a little ether, whereas the α -tocopherylquinone was eluted only by ether itself. Liver storage of vitamin A was either very small or negligible. Substance SC (Lowe, Morton & Harrison, 1953) was not found in more than trace amounts.

Table 2 indicates that the vitamin E-deficient animals were very similar to the control animals in the hydrocarbon, ubiquinone and sterol contents of liver unsaponifiable matter. The occurrence of SG (tocopherol?) and α -tocopherylquinone in the supplemented animals but not in the deprived animals confirms the efficacy of the dietary regimen. The sexes behaved similarly. Table 2 also shows how the kidney and heart tissues showed little difference between the supplemented and deprived animals in hydrocarbon, sterol and ubiquinone content. The analytical work on the testes of the deprived animals was marred by incomplete saponification; there can, however, be

no doubt that neither the ubiquinone nor the sterol contents were appreciably different from those for normal testes.

DISCUSSION

In rats the heart tissue is richer in ubiquinone than the liver or kidney tissue. Deprivation of vitamin E for 127 days made no demonstrable difference to the ubiquinone contents. Heaton, Lowe & Morton (1957) did not detect ubiquinone in rat testes; the present work shows that although the amount is not large ubiquinone is present. There is about 1.2 mg. of ubiquinone in the entire body of the weanling rat; this is less than the amount found in a few selected tissues of the vitamin E-deprived rat 127 days later. It therefore seems that α -tocopherol is neither a precursor of ubiquinone nor is it needed in any enzymic step in the biosynthesis of ubiquinone. The substance SC was previously (Lowe *et al.* 1953; Heaton *et al.* 1957) found to increase in amount in the livers of vitamin A-deficient rats. The substance SC content of the liver of the vitamin E-deprived rats and of the tocopheryl acetate-supplemented controls was negligibly small in both cases.

Table 2. Comparison of unsaponifiable constituents from tissues of vitamin E-deficient rats with those from control animals given α -tocopheryl acetate

	Vitamin E-supplemented		Vitamin E-deficient	
	Males	Females	Males	Females
Liver				
Hydrocarbon (mg./g.)	0.12	0.19	0.19	0.11
Ubiquinone (μ g./g.)	117	114	110	138
Sterol (mg./g.)	2.07	1.93	2.49	2.05
Substance SG (units/g.)*	3.1	4.1	Nil	Nil
Tocopherylquinone (μ g./g.)	4.1	6.3	Nil	Nil
Kidney				
Unsaponifiable (%)	0.62		0.64	
Hydrocarbon (μ g./g.)	61		74	
Ubiquinone (μ g./g.)	108		118	
Sterol (mg./g.)	3.83		4.03	
(Vitamin A, 7-dehydrocholesterol and 3-formylindole were detected in both batches)				
Tocopherylquinone	Present		Not detected	
Heart				
Unsaponifiable (%)	0.36		0.27	
Hydrocarbon (mg./g.)	0.35		0.14	
Ubiquinone (μ g./g.)	233		224	
Sterol (mg./g.)	1.50		1.34	
7-Dehydrocholesterol	Detected		Detected	
Tocopherylquinone	Trace		Not detected	
Testes				
Unsaponifiable (%)	0.28		0.73	
Hydrocarbon (mg./g.)	0.07		Doubtful	
Ubiquinone (μ g./g.)	25		< 18 > 30	
Sterol (mg./g.)	1.6		2.06	
3-Formylindole	Detected		Detected	
Tocopherylquinone	Trace		Not detected	
7-Dehydrocholesterol	Not detected		Not detected	

* Units represent $E_{1\text{cm}}^{1\%}$ 298 m μ for the SG fraction multiplied by its weight in mg.

The material SG obtained from the tissues of the tocopheryl acetate-supplemented rats is slightly less strongly adsorbed than ubiquinone; it is very similar to α -tocopherol but it may be a metabolite with a slightly changed side chain. The tocopheryl-quinone found in the controls might be a 'detoxication' intermediate or a part of the tocopheryl-quinol redox system.

The effects of vitamin E deficiency on sterol content and hydrocarbon content are unimportant. In considering the results as a whole it seems probable that the biological role or roles of tocopherols are not directly concerned with other unsaponifiable constituents of tissue lipids.

The control rats in the present experiments obtained in the diet about 2.0–2.5 mg. of α -tocopheryl acetate/day. This is at least twice the minimum requirement (Deuel, 1957). Mason (1940) found that in the male rat prevention of irreversible damage to the testes makes it obligatory to provide the vitamin from the age of 40–50 days, whereas the requirement of female rats is not critical until after conception and implantation. In the present experiments the male rats were more responsive than the females to vitamin E deprivation (Table 1), but with respect to liver ubiquinone and sterol both sexes were equally unresponsive.

SUMMARY

1. Two groups (males and females) of weanling rats were given a vitamin E-free basal diet for

127 days with a tocopherol supplement. Two similar groups were given the unsupplemented diet and when killed were vitamin E-deficient.

2. The unsaponifiable fractions from liver, heart and kidney were chromatographed on alumina. Ubiquinone contents of approx. 120, 110, 230 and 25 μ g./g. were found for liver, kidney, heart and testes.

3. The vitamin E status had no striking influence on the amounts of hydrocarbon, sterol or ubiquinone obtained from the different tissues.

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Effects of Vitamin A Deficiency and Adrenalectomy on Ubiquinone and Substance SC in Rat Liver

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Lowe, Morton & Harrison (1953) described changes in the adrenal glands of rats suffering from vitamin A deficiency. In the normal rat the adrenals contain very little vitamin A (Moore, 1957). Bailly & Nettor (1932) found β -carotene in the cortex of ox adrenals but not in the medulla. Davies & Moore (1934) observed that rats given large (but not toxic) doses of vitamin A esters were capable of storing relatively large amounts of vitamin in the adrenal glands (1500 i.u./g. of adrenal tissue). Popper & Greenberg (1941), by fluorescence microscopy, found that the vitamin A of adrenal glands was

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sharply localized in the zona fasciculata even when the animals were hypervitaminotic.

Bennet, Bennet, Shaver & Gracht (1950) exposed the whole bodies of rats to X-irradiation and found absorption of vitamin A alcohol to be apparently unaffected, but 6 hr. after giving the vitamin the adrenals of the control animals contained more vitamin A than those of the irradiated animals. After 10 hr. however, the latter contained more than the former.

Willmer & Laughland (1957) administered β -[14 C]carotene to rats and found that the concentration of radioactivity became highest in the adrenals, followed in decreasing order by the