Unsaponifiable Constituents of Rat Tissues in Relation to Vitamin K Status

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This paper is concerned with the effects of vitamin K deficiency on minor constituents of the unsaponifiable matter from selected rat tissues.

A first approach has been to produce a conditioned vitamin K₂ deficiency by adding to the diet sufficient sulphadiazine to exert a strong bacteriostatic effect on the flora of the gut. This experiment was performed twice, and in each case the sexes were studied separately. When the sulphadiazine-fed animals had become hypoprothrombinaemic they were killed and the liver, kidney and intestinal tissues were removed. These and control tissues were saponified and the unsaponifiable fraction was studied.

A second approach was to place all the animals on a basal diet free from vitamins K₁ and K₂. The control animals were given this diet plus a small amount of menadione (2-methyl-1:4-naphthoquinone, vitamin K₃) and the deprived animals were given the basal diet plus 1% of sulphadiazine to inhibit bacterial synthesis of vitamin K₂. After 21 days all the animals were killed and various tissues were examined.

A third approach was to incorporate in a normal diet for 26 days the vitamin K antagonist dicoumarol and to compare animals so treated with control animals on the unsupplemented stock diet.

A fourth approach was to maintain rats for 45 days on a vitamin A-deficient diet until the reserves had been heavily depleted. Some animals were then killed and the tissues examined. The remaining rats were divided into two groups, one of which was given vitamin A and the other sulphadiazine. In this way it was hoped to show any effects due to the double stress of vitamin A deficiency and vitamin K deficiency. Particular attention was given to ubiquinone concentrations.

METHODS

Animals. Weanling hooded rats reared in the Department were used.

Animal diets. (a) The stock diet of cubes (diet no. 4, British Extracting Co., Bromborough, Cheshire) was ground to a powder and fed as such. (b) Sulphadiazine (1%)

or dicoumarol (L. Light and Co. Ltd.; 0.003%) was added to the powdered diet and thoroughly mixed. (c) A basal diet free from vitamin K₁ had the following percentage composition: sucrose 73; casein (vitamin-free), 18; arachis oil 3; cod-liver oil (1000 i.u. of vitamin A/g.) 2; salt mixture (Hubbell, Mendel & Wakeman, 1937) 4. To this diet was added a vitamin mixture (2 g./kg. of diet) consisting of (g.): calcium pantothenate 0.835, *p*-aminobenzoic acid 0.167, thiamine hydrochloride 0.0835, biotin 0.0015, choline chloride 32.326, folic acid 0.0167, inositol 16.163, nicotinic acid 0.167, pyridoxine 0.067, riboflavin 0.167. (d) A basal diet free from vitamin A contained (%): sucrose 65; casein (vitamin-free) 18; Marmite 8; arachis oil 5; salt mixture (Hubbell *et al.* 1937) 4. Vitamin mixture was added as above (2 g./kg. of diet).

Determination of prothrombin time. The effectiveness of the sulphadiazine supplement in producing vitamin K₂ deficiency was determined by measuring the prothrombin time, following the method of Quick (1937, 1940). Plasma from the blood of stock colony rats was diluted with aq. 0.85% sodium chloride solution to give concentrations of 10, 20, 30 and 50% (v/v) of plasma. A thromboplastin activity curve was obtained (Bacto thromboplastin, Difco Laboratories). Blood was obtained from four animals in each group (2.7 ml., by heart puncture) and to it was added 0.1M-sodium oxalate (0.3 ml.). After centrifuging, the plasma was at once drawn off and the clotting time was determined within 6 hr. (see Tárnoky, 1958).

Examination of tissues. The methods of saponification, extraction of unsaponifiable matter and chromatography on acid-washed alumina partially deactivated to Brockman grade 3 have been described in earlier papers (cf. Mervyn & Morton, 1959). The substances under study were: (i) a hydrocarbon fraction very feebly adsorbed on alumina and exhibiting an inflexion near 260 m μ . It is probable that the weight of this fraction is a measure (in the main) of saturated hydrocarbons and that the intensity of absorption at 260 m μ is a measure of unsaturated material; (ii) ubiquinone (for references see Morton, 1958); (iii) substance SC (Lowe, Morton & Harrison, 1953; Heaton, Lowe & Morton, 1957); (iv) vitamin A; (v) cholesterol and 7-dehydrocholesterol; (vi) substance SF or 3-formylindole (Morton & Fahmy, 1958).

RESULTS

Sulphadiazine added to stock diet

From weaning at the age of 21 days, rats in groups A, C, E and G received the diet of ground cubes and those in groups B, D, F and H the same diet with the addition of 1% of sulphadiazine. It will be seen from Tables 1 and 2 that in both sexes

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the sulphadiazine supplement markedly depressed the final body weight and the weights of liver and kidney. Spleen weights were reduced to as little as 30% of those of the control animals. The prothrombin times indicated very marked decreases in prothrombin concentration as a result of the ingestion of sulphadiazine. It was not necessary to confirm the conditioned deficiency in this way in all the

experiments as the changes in tissue weights were unmistakable. Table 3 shows the results of chromatographic separations and spectrophotometric analyses on liver tissue. The ubiquinone content of liver did not respond to sulphadiazine added to the food. There was no additional mobilization of vitamin A and no significant change in sterol content. The amount of substance SC did not

Table 1. *Comparison of rats kept for 36 days on a diet containing 1% of sulphadiazine, with rats on a normal diet*

Diet	...	...	...	Mean weight (g.)			
				Sulphadiazine added		Normal	
				<i>B</i> (7 males)	<i>D</i> (8 females)	<i>A</i> (8 males)	<i>C</i> (8 females)
Initial				36	40	36	40
Final				72	84	98	101
Liver				3.17	3.84	4.08	4.18
Liver (% of body wt.)				4.44	4.55	4.17	4.14
Kidney				0.40	0.43	0.46	0.45
Spleen				0.14	0.26	0.57	0.71
Intestine				6.06	7.80	7.60	8.40
Prothrombin (% of control)				22	18.5	100	100

Table 2. *Comparison of rats kept for 29 days on a diet containing 1% of sulphadiazine with rats on a normal diet*

Diet	...	...	...	Mean weight (g.)			
				Sulphadiazine added		Normal	
				<i>F</i> (8 males)	<i>H</i> (8 females)	<i>E</i> (8 males)	<i>G</i> (8 females)
Initial				43.6	38.3	39.5	37.3
Final				103	89	157	127
Liver				5.44	4.91	8.58	7.26
Liver (% of body wt.)				5.28	5.52	5.46	5.72
Kidney				0.54	0.47	0.75	0.66
Spleen				0.34	0.27	1.21	0.77
Intestine*				7.0	6.9	9.9	7.7

* Includes in this case stomach and intestine.

Table 3. *Comparison of unsaponifiable constituents from liver of vitamin K₂-deficient rats and control rats*

Group	...	...	...	...	...	Males		Females		Males		Females	
						<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>	<i>H</i>
Sulphadiazine						Nil	1%	Nil	1%	Nil	1%	Nil	1%
Mean liver wt. (g.)						4.08	3.17	4.18	3.84	8.58	5.44	7.26	4.91
Unsaponifiable matter (%)						0.41	0.41	0.56	0.47	0.33	0.38	0.31	0.43
Ubiquinone (μg./g. of liver)						110	148	210	175	116	109	113	115
Substance SC (units/g. of liver)*						5.2	7.3	2.7	3.2		Not measured		
Vitamin A (i.u./g. of liver)						78	116	91	107	19	68	55	75
Sterol (mg./g. of liver)						2.4	2.46	3.26	2.41	2.02	2.12	2.05	2.07
Hydrocarbon (mg./g. of liver)						0.03	0.10	0.13	0.16	0.06	0.12	0.07	0.12
'Petrol 260 mμ'† (units/g. of liver)						2.1	2.4	2.2	2.2	0.81	1.07	1.04	1.03
Substance SF (3-formylindole)‡ (units/g. of liver)						3.06	Trace	2.41	5.14	1.37	0.69	0.81	0.66

* 1 mg. = 96 units (1 unit = $E_{1\text{cm}}^{1\%}$ at 275 mμ × wt. in mg.).

† Eluted from alumina by light petroleum and showing an inflexion at 260 mμ. The unit is $E_{1\text{cm}}^{1\%}$ 260 mμ × wt. of fraction in mg.

‡ 1 unit = $E_{1\text{cm}}^{1\%}$ 282 mμ × wt. in mg.

Table 4. *Comparison of unsaponifiable constituents from kidneys of vitamin K₂-deficient rats and control rats*

Substance SC was not detected in any group. 7-Dehydrocholesterol was present in traces.

No. of animals ...	Expt. 1		Expt. 2	
	16	15	16	16
Sulphadiazine in diet	Nil	1%	Nil	1%
Mean kidney wt. (pair; g.)	0.91	0.83	1.40	1.02
Kidney constituents				
Unsaponifiable (%)	0.72	0.65	0.52	0.52
Hydrocarbon (mg./g.)	0.12	0.15	0.04	0.025
Ubiquinone (μg./g.)	81	81	100	72
Vitamin A (i.u./g.)	8.3	4.2	Trace	Trace
Sterol (mg./g.)	4.7	4.2	3.9	3.6

Table 5. *Comparison of unsaponifiable constituents from intestines of vitamin K₂-deficient rats and control rats*

7-Dehydrocholesterol was detected in each case.

Group ...	Males		Females		Males		Females	
	A	B	C	D	E	F	G	H
Sulphadiazine	Nil	1%	Nil	1%	Nil	1%	Nil	1%
Mean wt. of intestine (g.)	7.6	6.1	8.4	7.8	9.9	7.0	7.7	6.9
Unsaponifiable matter (%)	0.37	0.36	0.39	0.32	0.31	0.41	0.40	0.4
Ubiquinone (μg./g.)	47	48	39	53	41	41	54	43
Sterol (mg./g.)	2.31	2.57	2.44	2.36	2.45	2.59	2.53	2.62
Hydrocarbon (mg./g.)	0.033	0.053	0.019	0.064	0.051	0.029	0.192	0.109

Table 6. *Comparison of control rats and vitamin K₂-deficient rats*

Overall average values are given.

	Liver		Kidneys		Intestine	
	Control	K ₂ -deficient	Control	K ₂ -deficient	Control	K ₂ -deficient
Mean wt. of tissue (g.)	6.03	4.34	1.16	0.92	8.42	6.94
Unsaponifiable matter (%)	0.403	0.422	0.617	0.580	0.370	0.372
Ubiquinone (μg./g.)	137	137	91	76	45	44
Vitamin A (i.u./g.)	61	92	8.3	4.2	Not detected	
Sterol (mg./g.)	2.43	2.27	4.29	3.90	2.43	2.54
Hydrocarbon (mg./g.)	0.07	0.12	0.09	0.08	0.07	0.06

Table 7. *Comparison of rats on a vitamin K-deficient diet with 1% of sulphadiazine for 21 days with control rats given menadione*

The basal diet was diet (C) described under Methods. For groups A and B menadione (5.3 mg./kg.) was added. Substance SF (3-formylindole) was detected in all cases.

Group ...	Vitamin K ₂ -supplemented		Vitamin K-deficient (sulphadiazine added)	
	A	B	C	D
Sex	Male	Female	Male	Female
Survivors (no. out of 8)	8	8	7	5
Mean initial wt. (g.)	32	32	31	33
Mean final wt. (g.)	89.8	81.5	46.6	52.2
Mean liver wt. (g.)	4.68	4.22	2.85	3.29
Liver constituents				
Unsaponifiable matter (%)	0.308	0.33	0.405	0.375
Ubiquinone (μg./g.)	107	112	127	118
Vitamin A (i.u./g.)	69.6	64.2	26.0	28.8
Sterol (mg./g.)	2.13	2.20	2.21	2.26
Hydrocarbon (mg./g. of liver)	0.42	0.38	0.21	0.12

change. Indeed the only indication of change is a small apparent increase in the hydrocarbon content of the unsaponifiable matter. Tables 4 and 5 show a similar lack of response to conditioned vitamin K₂ deficiency in the minor constituents of kidney and intestine unsaponifiable matter. Table 6 presents the overall picture for both sexes in the two experiments. It is clear that the depression in tissue weight is not paralleled by any marked changes in unsaponifiable constituents.

Sulphadiazine added to a diet deficient in vitamin K₁

In this experiment the rats in groups A and B grew normally, but the vitamin K-deficient rats increased in weight only very slowly. Three females and one male died and were found at post-mortem examination to show haemorrhages. The experiments were therefore terminated after 21 days. Table 7 shows in the deficient animals a very marked retardation of growth and small livers. The liver weight expressed as a percentage of body weight rose, however, from 5.2 to 6.3. Examination of the liver unsaponifiable fractions showed that the vitamin A reserves had been substantially reduced in the vitamin K-deficient animals. If the findings are calculated in terms of i.u./liver, the average vitamin A content fell from about 300 to 85 in the vitamin K-deficient animals. There was no such decrease in ubiquinone content and the sterol content expressed as mg./g. of liver was unchanged. The menadione-supplemented animals showed a somewhat higher content of liver hydrocarbon.

Dicoumarol added to a stock diet

Ten female weanling rats were divided into two groups having the same aggregate weight. The control animals were given the stock diet of ground cubes *ad lib.* and the others received this diet

supplemented with dicoumarol. The treatment significantly ($P < 0.01$) reduced the rate of growth and the final body weight, but no gross lesions were seen. Table 8 shows that the minor constituents of liver were not very much affected by the treatment. The vitamin A was low even in the control rats, so that the low value for the dicoumarol-treated rats is probably not significant.

Combined vitamin K and vitamin A deficiency

A number of weanling rats were placed on a vitamin A-deficient diet for 45 days. At that stage some were killed and it was confirmed that all the liver reserves of vitamin A had disappeared. Fourteen remaining animals were then divided into two groups. One was given the original diet supplemented with vitamin A (100 i.u./rat/day) and the other group was given the same diet supplemented with sulphadiazine (1%). After 16 days the surviving animals were killed; five of the first group failed to recover after vitamin A therapy and four of the animals receiving sulphadiazine died. Table 9 summarizes the results obtained on the survivors. The rise in ubiquinone content of rat liver in vitamin A

Table 8. *Comparison of rats kept for 26 days on a diet containing dicoumarol (30 mg./kg.) with control rats*

Five female rats were in each group.		
	Control	Dicoumarol-treated
Mean initial wt. (g.)	36	37
Mean final wt. (g.)	117	108
Mean liver wt. (g.)	5.87	6.11
Liver constituents		
Unsaponifiable matter (%)	0.413	0.518
Ubiquinone (μ g./g.)	160	176
Vitamin A (i.u./g.)	25	31.4
Sterol (mg./g.)	2.24	2.39
Hydrocarbon (mg./g.)	0.88	1.72

Table 9. *Comparison of vitamin A-deficient rats with rats suffering from combined vitamin A and vitamin K deficiency and rats given a vitamin A supplement*

Treatment	1	2	3
Group	...	...	...
	Vitamin A-deficient animals killed at 45 days	Vitamin A-supplement given after 45 days	Vitamin A- and K-deficient after 45 days (sulphadiazine supplement given)
Body wt. after 45 days on vitamin A-deficient diet (g.)	81	73	93
Body wt. after 61 days (g.)	—	132	80
Liver wt. (% of body wt.)	4.46	4.54	3.75
Liver constituents			
Unsaponifiable matter (%)	0.655	0.632	0.609
Ubiquinone (μ g./g.)	511	190	660
Substance SC { (units/g.)*	9.85	3.9	12.0
{ (units/liver)*	35.6	23.4	36
Sterol (mg./g.)	2.93	2.75	2.93
Hydrocarbon (mg./g.)	0.86	0.38	0.89
Vitamin A (i.u./g.)	Nil	62	Nil

* See Table 3 for explanation of units.

deficiency continued when the diet lacked both vitamin A and vitamin K but was reversed when vitamin A was provided and the deficiency syndrome corrected. Similarly, the rise in substance SC was reversed. No marked change in sterol content occurred as a result of the double hazard of vitamin A and vitamin K deprivation.

DISCUSSION

The present work is an extension of earlier work on the distribution of ubiquinones (for references see Morton, 1958). These compounds are dimethoxybenzoquinones:

where R is a polyisoprenoid grouping. The ubiquinones of heart and probably of kidney and liver have a $C_{80}H_{81}$ side chain.

Vitamin K_2 (McKee, Binkley, Thayer, MacCorquodale & Doisy, 1939; Binkley, McKee, Thayer & Doisy, 1940), has the structure:

where $n = 5$. Isler, Rüegg, Chopard-dit-Jean, Winterstein & Wiss (1958) have further purified vitamin K_2 and have found its formula to be $C_{46}H_{84}O_2$, so that $n = 6$ and the side chain has 35 carbon atoms. These authors have synthesized vitamin K_2 and a number of lower homologues. Careful determinations of intensities of ultraviolet absorption have shown that the absorption spectrum is uninfluenced by the length of the polyisoprenoid side chain in the sense that the molecular-extinction coefficients are the same through the series (the $E_{1\text{cm}}^{1\%}$ values are, of course, higher for the lower members). It is convenient to select the 248 $m\mu$ absorption peak to illustrate this; if the side chain is C_{10} (geranyl), ϵ_{max} 248 $m\mu = 19\,000$ ($E_{1\text{cm}}^{1\%}$ 617), and if the side chain is C_{35} , ϵ_{max} = 18 900 ($E_{1\text{cm}}^{1\%}$ 292).

Francis, Madinaveitia, Macturk & Snow (1949) and Snow (1952) isolated from a strain of *Mycobacterium tuberculosis* a vitamin K_2 homologue as an uncrystallized oil ($E_{1\text{cm}}^{1\%}$ 248 $m\mu$ 241; C, 85.1; H,

10.27%). Noll (1958), using *M. tuberculosis* (Brevannes), obtained vitamin K_x (m.p. 58–59°; $E_{1\text{cm}}^{1\%}$ 249 $m\mu$ 240; C, 85.24; H, 10.27%), which agreed well with the properties of Snow's compound.

If the molecular-extinction coefficient of vitamin K_x is 18 900, as in vitamin K_2 , its molecular weight should be $18\,900/24 = 787$. Vitamin K_x may be written $C_{11}H_7O_2 \cdot R$, so that $R = 616$ and $C_{45}H_{73} = 613$ ($C_{56}H_{80}O_2$ requires C, 85.65; H, 10.27%). Noll considers that K_x contains seven to eight isoprenoid units, but the $E_{1\text{cm}}^{1\%}$ value points more definitely to a C_{45} side chain with nine double bonds. A hydrocarbon $C_{45}H_{76}$ was isolated from liver by Channon, Devine & Loach (1934) and the formula was confirmed by Dimter (1941, 1942).

The ubiquinones with polyisoprenoid side chains thus resemble the vitamins K of microbiological origin, and the existence of a $C_{45}H_{76}$ hydrocarbon hitherto unrelated to substances of known biochemical interest makes it necessary to explore the possibility of biogenetic or metabolic interrelationships.

The best-known manifestation of vitamin K deficiency is the prolongation of the clotting time of blood, attributed to a reduced concentration of prothrombin (Dam, Schonheyder & Tage-Hansen, 1936), a compound which is made in the liver (Warner, Brinkhous & Smith, 1938; Warren & Rhoads, 1939). According to Quick & Collentine (1950, 1951), vitamin K_1 may be the prosthetic group of an enzyme occurring in liver and concerned in the synthesis of prothrombin, but vitamin K does not itself appear to be an integral part of prothrombin (Dam, Glavind, Lewis & Tage-Hansen, 1938).

Lowe *et al.* (1953) found that vitamin A deficiency brought about in rats a marked increase in two substances, SA and SC, found in small amounts in liver and some other tissues. This phenomenon does not occur in all species. Substance SA has since been called ubiquinone (see Morton, 1958) and co-enzyme Q (Lester, Crane & Hatefi, 1958).

The whole problem of lipid cofactors participating in oxidation processes is being studied in many laboratories. Inasmuch as, in the rat at any rate, ubiquinone levels respond to vitamin A status, it seems necessary to ascertain experimentally whether vitamin K status can also regulate ubiquinone levels in particular tissues.

The simplest method of producing vitamin K deficiency in rats is perhaps the dietary administration of sulphonamides (Black, McKibbin & Elvehjem, 1941; Black, Overman, Elvehjem & Link, 1942; Welch & Wright, 1943). Kornberg, Daft & Sebrell (1944b), however, found that on a diet containing 1% of sulphadiazine neither the caecal contents nor the faeces of rats exhibited vitamin K activity. Sulphamerazine at the 1%

dietary concentration failed to produce hypoproteinaemia in 4 weeks (Mushett & Seeler, 1947). The results of Kornberg, Daft & Sebrell (1944*a*) indicated that sulphadiazine was the most suitable material to administer. In view of the well-known lack of high structural specificity among vitamin K-active substances (Almquist, 1954) and the many unsolved problems of modes of action, the first approach with a dietary sulphadiazine supplement was not by itself adequate or conclusive. Accordingly, a diet was used which lacked vitamin K₁; this could be supplemented by either menadione or sulphadiazine. In this way the vitamin K deprivation would be more stringent and for the controls the replacement would be (in some respects at least) unequivocal. The replacement was effective, but whether the menadione functioned as such or as a precursor for vitamin K₂ was not pursued. The growth-retarding effects of vitamin K deficiency were especially well-marked and the fact that animals which died showed haemorrhagic lesions confirmed the speedy appearance of the deficiency syndrome.

The investigation would also have been incomplete without a study of dicoumarol-fed rats. Dicoumarol may act by injuring the liver or as antagonizing vitamin K by competitive inhibition (see Mentzer & Meunier, 1943; Woolley, 1944, 1945). The idea, advanced by Collentine & Quick (1951), that dicoumarol displaces vitamin K in enzyme systems producing prothrombin, fits the observations of Spinks & Jaques (1950) to the effect that ¹⁴C-labelled dicoumarol accumulates mainly in the liver and that its disappearance from the liver is accelerated when vitamin K is administered. The longer the dicoumarol is allowed to remain in the liver the greater its effect in reducing the prothrombin concentration. The mode of action may be a multiple process (cf. Dam, 1948; Sørbye, Kruse & Dam, 1950).

The final step in the present study was to ascertain whether the additional stress of administering sulphadiazine to rats suffering from vitamin A deficiency causes any marked change in the occurrence of ubiquinone, substance SC and other constituents of the tissue unsaponifiable matter.

These tests show that vitamin K status has remarkably little effect on most of the variables under study. Sulphadiazine has a striking effect on the spleen which deserves further study. In the first experiments (see Tables 1 and 2) growth was markedly retarded and organ weights were smaller. In liver, kidney and intestine there was no evidence that vitamin K₂ deficiency had appreciably affected the vitamin A, sterol, ubiquinone, substance SC and hydrocarbons. There was possibly an increase in vitamin A in the liver but this was offset, perhaps, by the reduction in liver weight.

The experiment which aimed at excluding dietary vitamin K₁ as well as inhibiting production of vitamin K₂ (Table 7) also showed striking retardation of growth. Again, the results showed that vitamin K status had little effect on the hydrocarbon, ubiquinone, sterol and substance SF (3-formylindole) contents of liver. In this experiment the decrease in vitamin A content was noteworthy.

The feeding of dicoumarol caused a small but statistically significant (*P* 0.01) reduction of body weight. The minor constituents of liver listed in Table 8 were not much changed, although some increase in hydrocarbon content was indicated.

The final experiment, with vitamin A-deficient rats, showed how the increase in ubiquinone content of liver which results from avitaminosis A is maintained or even enhanced in avitaminosis A and K but reversed by vitamin A supplementation. In the rehabilitated rats the increased weight of liver tended to exaggerate a decline in ubiquinone content, which was none the less real. Similarly, the animals given vitamin A showed less substance SC than those deprived of vitamin A. The additional stress of vitamin K deprivation neither exacerbated nor mitigated the vitamin A-deficiency syndrome as it affects the minor constituents of rat-liver unsaponifiable matter. Therefore despite the fact that ubiquinone and the K vitamins are quinones and that vitamin K₂ shares with ubiquinone a polyisoprenoid side chain, the present experiments disclosed remarkably little biochemical interaction.

SUMMARY

1. Weanling rats kept for 29 or 36 days on a diet containing 1% of sulphadiazine showed as compared with control rats smaller livers, kidneys, intestines and very much smaller spleens. The prothrombin content of plasma was reduced to about one-fifth. Despite the induced vitamin K₂ deficiency the concentrations of ubiquinone, vitamin A, sterol and hydrocarbon in the tissues were but little affected.

2. Weanling rats kept on a diet free from vitamin K₁ and containing sulphadiazine to inhibit bacterial formation of vitamin K₂ were compared with rats on the vitamin K₁-free diet supplemented with menadione. The vitamin K-deficient rats showed retarded growth and decreased liver vitamin A as well as smaller livers. Liver concentrations of ubiquinone and sterol were about the same in the control rats as in the deficient rats.

3. Weanling rats kept for 26 days on a diet containing dicoumarol (30 mg./kg.) differed from control rats slightly but significantly in body weight. No other signs of vitamin K deficiency were seen.

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