The effect of vitamin A on ubiquinone and ubichromenol in the rat, and its relation to the effect of vitamin E

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Ubiquinone was first observed in the organs of vitamin A-deficient rats (Festenstein, Heaton, Lowe & Morton, 1955), and Morton and his co-workers have shown that vitamin A deficiency leads to the accumulation of both ubiquinone and its cyclic isomer, ubichromenol, in the liver of the rat (see, for example, Heaton, Lowe & Morton, 1957; Morton & Phillips, 1959). In an attempt to ascertain whether an increase in ubiquinone is an essential part of the deficiency syndrome, Lowe, Morton, Cunningham & Vernon (1957) examined the livers of vitamin A-deficient fowls and found no such increase in this species. Moore & Sharman (1960), although they agreed that liver ubiquinone concentration is increased during vitamin A deficiency in the rat, suggested that a good deal of the increase is caused by the severe diminution in weight of the liver, and they were unable to find a corresponding increase in the heart. They concluded, on rather limited evidence, that the effect might be limited to liver. Gloor & Wiss (1959a), on the other hand, found that the uptake of mevalonic acid into ubiquinone was increased in vitamin A-deficient rat liver and suggested that vitamin A is concerned at the metabolic level with isoprenoid synthesis. Edwin, Diplock, Bunyan & Green (1961) have shown that the synthesis of ubiquinone and ubichromenol in the rat is markedly influenced by vitamin E, and Green, Diplock, Bunyan & Edwin (1961) have drawn similar conclusions from a study of the rabbit. It was already observed in both these investigations that the concentration of vitamin A in the tissues was not apparently related to the ubiquinone concentration and, in a preliminary communication, Green, Edwin, Diplock & Bunyan (1960) have suggested that much of the effect of vitamin A on ubiquinone concentrations could be explained by simultaneous variations in tocopherol concentrations. The relationship between vitamins E and A is, in fact, so intimate that it is essential to investigate in considerable detail their influence on each other and on ubiquinone.

The effect of vitamin A—uncomplicated by vitamin E—is not easy to study. When the amount of vitamin A in the diet of the rat is increased, the result is nearly always to diminish the concentrations of tocopherol in the tissues, which, as Edwin *et al.* (1961) have demonstrated, nearly always leads to a decrease in ubiquinone. The effect of vitamin E on ubiquinone—free of vitamin A effects—is easier to investigate, since vitamin E in the diet usually increases vitamin A in the tissues; thus, any effect of vitamin E in increasing the ubiquinone concentration must be unrelated to vitamin A, whose postulated effect would be in the reverse direction. Here we describe experi-

ments designed to separate the effects of the two vitamins and to determine whether vitamin A does indeed have an influence on ubiquinone without the intermediary action of vitamin E.

EXPERIMENTAL

Animals, diets and analytical methods

Animals. Male and female rats of the Norwegian hooded strain were used. At the age of 4 weeks they were removed from the stock colony and fed on the various test diets used in the experiments described below.

Vitamin A-deficient diet, AO 1. This was the basal diet described by Diplock, Bunyan, Green & Edwin (1961), with the addition of thiamine, 9 mg/kg, and riboflavin, 19 mg/kg, and with the omission of vitamin A. It contained 25% casein, 50% sugar and 10% glucose.

Vitamin A-deficient diet, AO 2. This diet had the percentage composition: casein, 'low vitamin content' (Genatosan Ltd) 13, rice starch 65, arachis oil 10, dried yeast (Marmite Ltd) 8, salt mixture 4. It was supplemented with α-tocopheryl acetate, 100 mg/kg, and vitamin D, 300 i.u./kg.

Vitamin A- and E-deficient diet, EAO 1. This was the casein–yeast–lard diet, A40, described by Edwin *et al.* (1961), with the vitamin A omitted.

Vitamin A- and E-deficient diet, EAO 2. This was the vitamin E-deficient diet E 10 Y described by Green, Diplock, Bunyan, Edwin & McHale (1961), with the vitamin A omitted.

Analyses. Tissues were analysed for ubiquinone, ubichromenol, α-tocopherol and vitamin A by the method of Diplock, Green, Edwin & Bunyan (1960).

Plan of experiments

As Morton & Phillips (1959) have shown, the time taken to produce vitamin A deficiency in rats varies considerably, individual rats reaching their weight plateaux at times between 20 and 50 days; the exact period cannot be predicted from experiment to experiment. This has been our experience also; the phenomenon produces a peculiar difficulty in these experiments on ubiquinone, one which, at the time, could not have been considered by Morton & Phillips. Green, Diplock, Bunyan, Edwin & McHale (1961) have stated that ubiquinone concentrations in the rat are subject to an unusually large fluctuation, and this fluctuation is rapid and may be cyclical. Variations of 200% are not uncommon. The experimenter who wishes to discover the effect of vitamin A on ubiquinone is therefore faced with two ways of doing it: either he can randomize the animals in groups and kill them all together at what he considers to be the right time (say, when most of them have signs of xerophthalmia or have ceased growing), or, as Morton & Phillips did, he can kill every animal in the vitamin-deficient group at the same clinical stage of deficiency, regardless of time. If the first, provided the groups are large enough, it can be expected that the ubiquinone concentrations would be similar in groups treated alike, but the animals within each group will probably be in different phases of deficiency. If the second, between-group ubiquinone variation is unavoidable and would be largely unknown. We have not

resolved this difficulty, but have carried out experiments in both ways to see whether they lead to substantially the same conclusions.

Expt 1. This experiment was a study of vitamin A deficiency. Twenty rats (ten of each sex) were fed on the deficient diet, AO 1, for $7\frac{1}{2}$ weeks, by which time growth had ceased in most of them and their mean weight was about 20 % lower than in a control group of rats (three of each sex), which were fed on AO 1 supplemented with 4 i.u. vitamin A/g for the experimental period. Xerophthalmia was not yet observable, and the deficiency state was therefore not advanced. At 72 h and again at 48 h before all the animals were killed, five of each sex from the deficient group were given, orally, 800 i.u. vitamin A palmitate in olive oil. The animals were killed by breaking their necks, and hearts, livers and kidneys from each group were removed and pooled for analysis.

Expt 2. This experiment was another study in vitamin A deficiency. Fourteen rats (seven of each sex) were divided into two groups. One was fed on diet AO 1 and the other on the same diet supplemented with vitamin A, as above, for 11 weeks. They were then killed, and hearts, livers, brains and kidneys removed for analysis.

Expt 3. This experiment was a study of combined vitamins A and E deficiency. Twelve male rats, in four groups of three, were fed on the doubly deficient diet, EAO 1, for 20 weeks. In order to ensure that they did not die of vitamin A deficiency before advanced vitamin E deficiency was reached, 200 i.u. vitamin A were given to each rat after 2 and again after 3 months on the diet. At 72 and 48 h before they were all killed, group 1 rats were given 500 i.u. vitamin A palmitate, group 2 rats 5 mg DL-α-tocopheryl acetate, and group 3 rats the doses of both vitamins A and E, all orally. Hearts livers and kidneys were removed for analysis.

Expt 4. This experiment was another study of combined deficiency. Six female rats were fed on the doubly deficient diet, EAO 1, for 12 weeks. During the week before they were killed, two rats were given, orally, 20 mg α-tocopheryl acetate, and another two were given 7000 i.u. vitamin A palmitate, each substance in three spaced doses. Hearts, livers and brains were removed for analysis.

Expt 5. This experiment was designed to study combined vitamins A and E deficiency in rats, but, in contrast to Expt 4, each rat in the vitamin A-deficient groups was killed at what was judged to be the same stage of vitamin A deficiency (see Morton & Phillips, 1959). Weight loss was used as the clinical criterion: it should be noted that our rats do not lose weight owing to vitamin E deficiency, and hence the same criterion could be used for animals deficient in both vitamins. Forty-five male rats were fed, from 4 weeks of age, on the vitamin E-deficient diet, E 10 Y, for 3 months. Five rats (group 1) were then killed and their tissues analysed. The remaining rats were divided into eight groups of five and fed on the doubly deficient diet, EAO 2, for the remainder of the test. They received treatments as shown at the top of page 138.

Thus, of the eight groups, only groups 3 and 4 were not on a vitamin A-deficient diet. Of the other six, four were deficient in both vitamin E and vitamin A. The rats in these six groups were weighed individually until they ceased to gain weight. Each rat was then weighed every day until its weight had fallen to $7\frac{1}{2}$ % below the mean weight it had attained at the deficiency plateau. When this weight was attained, the rat

Group no.	Dietary supplement	Single dose before death
2	None	None
3	Vitamin A palmitate (10 i.u./g)	None
4	Vitamin A palmitate (10 i.u./g) + α-tocopheryl acetate (0·1 %, w/w)	None
5	None	10 mg α-tocopheryl acetate
6	None	2000 i.u. vitamin A orally*
7	None	1000 i.u. vitamin A intravenously†
8	α-Tocopheryl acetate (0·1 %, w/w)	None
9	α-Tocopheryl acetate (0·1 %, w/w)	2000 i.u. vitamin A orally*

* Given as a solution of vitamin A palmitate in olive oil 24 h before death.

† Given as a Tween 80 (polyoxyethylene sorbitan mono-oleate, Atlas Powder Company, Delaware, U.S.A.) emulsion of vitamin A alcohol in isotonic saline, 60 min before death.

was killed: rats in groups 5, 6 and 9 were given their single doses of either vitamin A or E 24 h before death, and rats in group 7 were given their intravenous injections 60 min before death. After being killed, each animal was dissected at once, and the tissues were stored at $-20°$ until dissection in each group was complete. The rats in groups 3 and 4 were killed when the last rat in the other groups had been killed. Although this was not entirely satisfactory as a strict control, since the rats in the other groups were being killed over a considerable period of time, we could devise no better scheme.

The complicated design of this experiment was partly the result of an attempt to cope with the phenomenon already mentioned, i.e. the apparently insuperable difficulty that the time of onset of vitamin A deficiency varies among rats in a group and that ubiquinone concentrations fluctuate rapidly. The experiment was planned after the results of the previous four experiments had been considered, in an attempt to answer the following questions more clearly.

(1) Was the fairly small effect of vitamin A on ubiquinone solely due to interaction with vitamin E concentrations or could a more specific effect be detected? In this experiment, therefore, rats were used in two blocks, vitamin A-deficient only and vitamin A- and E-deficient. In order to minimize the effects of vitamin A on vitamin E, we used an exceptionally high dietary supplement of vitamin E in groups 8 and 9 (only vitamin A-deficient) and also in group 4.

(2) If it proved impossible to separate the effects of vitamins A and E when they were given in the diet because of their inevitable interaction, could the effect of vitamin A be demonstrated (as can the effect of vitamin E) by the use of our previously described techniques of oral or intravenous dosing before death? To this end, certain groups of rats were given such doses of vitamin A, and other rats received vitamin E.

Expt 6. This experiment was another study of simple vitamin A deficiency, designed to compare the effects on ubiquinone at three stages of deficiency. Twelve male rats were divided into four groups and fed on diet AO 2. Rats in group 1 were killed when each had just reached a weight plateau, those in group 2 when each had lost $7\frac{1}{2}$% of its mean plateau weight and those in group 3 when each had lost 20% of its mean plateau weight (see Morton & Phillips, 1959). Rats in group 4 were each given 5 mg thiouracil/day orally from the time they first reached their weight plateau throughout the rest of the experimental period.

Expt 7. One male and one female rat were fed on the deficient diet, AO 2, until their clinical signs of vitamin A deficiency had advanced as far as was judged possible. The male rat was killed on the 69th day of deficiency, the female on the 88th day.

RESULTS

Expt 1. The results are summarized in Table 1. Ubiquinone and ubichromenol levels were found to be considerably higher in the vitamin A-deficient livers than in the control livers, although the concentration of ubiquinone in the former was much less than was observed by Morton & Phillips (1959). The tocopherol content of the deficient livers, however, was nearly double that of the controls. Administration of vitamin A before death did not affect the concentration of either ubiquinone or ubichromenol in the liver, nor did it affect the tocopherol concentration. In heart and kidney, the concentrations of ubiquinone, ubichromenol and tocopherol were not increased during deficiency, nor did oral administration of vitamin A have an effect on the heart. In kidney, vitamin A administration produced a small decrease in the ubiquinone concentration, which may perhaps have been due to the small decrease in tocopherol concentration.

Table 1. *Expt* 1. *Effect of vitamin A on ubiquinone, ubichromenol and α-tocopherol concentrations in the rat*

(Number of rats in each group at death and their mean weights: (*a*) controls, 6, 188 g; (*b*) deficient, 10, 157 g; (*c*) deficient, dosed, 10, 180 g)

Organ	Mean organ (wt g)	Group	Ubiquinone ($\mu g/g$)	Ubichromenol ($\mu g/g$)	α-Tocopherol ($\mu g/g$)
Heart	0·85	Control	179	14	33·9
	0·70	Vitamin A-deficient	162	14	34·2
	0·75	Deficient, dosed	165	13	34·0
Liver	8·71	Control	116	51	28·2
	7·74	Vitamin A-deficient	159	70	44·2
	9·31	Deficient, dosed	160	75	44·4
Kidney	1·56	Control	—	13	22·9
	1·50	Vitamin A-deficient	55	11	21·6
	1·59	Deficient, dosed	42	10	18·2

Expt 2. In this test, the rats were older and were depleted for a longer period. The results are given in Table 2. The ubiquinone concentrations in heart, liver and kidney were all increased by vitamin A deficiency, but ubichromenol concentration was higher only in the deficient liver. In brain, ubiquinone concentration decreased in vitamin A deficiency, but so did that of tocopherol in this organ. The changes in ubiquinone concentration in all four tissues could be correlated with the tocopherol changes.

Expt 3. The results are given in Table 3. These rats were much older than those in Expts 1 and 2 when they were killed. The livers of the doubly deficient group

Table 2. *Expt 2. Effect of vitamin A on ubiquinone, ubichromenol and α-tocopherol concentrations in the rat*

(Number of rats in each group at death and their mean weights: (a) controls, 7, 254 g; (b) deficient, 7, 213 g)

Organ	Mean organ wt (g)	Group	Ubiquinone (μg/g)	Ubichromenol (μg/g)	α-Tocopherol (μg/g)
Heart	0·93	Control	179	10	33·7
	0·84	Vitamin A-deficient	213	7	43·5
Liver	13·1	Control	121	31	27·7
	10·1	Vitamin A-deficient	165	55	34·2
Kidney	2·19	Control	38	5	10·4
	1·85	Vitamin A-deficient	60	2	16·4
Brain	1·82	Control	20	4	7·3
	1·83	Vitamin A-deficient	11	5	5·9

Table 3. *Expt 3. Effect of vitamins A and E on ubiquinone and ubichromenol concentrations in the male rat*

(Number of rats in each group at death and their mean weights: (a) controls, deficient in vitamins A and E, 3, 225 g; (b) dosed with vitamin A, 3, 236 g; (c) dosed with vitamin E, 3, 249 g; (d) dosed with vitamins A and E, 3, 247 g)

Organ	Mean organ wt (g)	Group	Ubiquinone (μg/g)	Ubichromenol (μg/g)	α-Tocopherol (μg/g)	Vitamin A (i.u./g)
Heart	1·05	Doubly deficient	180	20	18·9	3·7
	0·99	Dosed with vitamin A	165	15	12·4	4·2
	1·01	Dosed with vitamin E	181	17	20·0	—
	0·97	Dosed with vitamins A and E	153	11	13·8	—
Liver	9·40	Doubly deficient	44	31	3·3	*
	10·5	Dosed with vitamin A	15	13	2·3	25
	12·5	Dosed with vitamin E	72	20	15·8	*
	11·1	Dosed with vitamins A and E	49	7	11·9	12
Kidney	2·16	Doubly deficient	60	21	9·8	8·9
	2·37	Dosed with vitamin A	57	15	4·2	9·1
	2·95	Dosed with vitamin E	64	45	16·8	8·8
	2·31	Dosed with vitamins A and E	58	14	11·2	9·4

* These readings were low and may be misleading, since the animals when killed were in somewhat different stages of vitamin A deficiency (see p. 137).

contained little vitamin A, but, in spite of this, the concentration of ubiquinone was exceptionally low (compare the values in other tables). The ubichromenol level fell within the normal range. Administration of vitamin A depressed the concentrations of ubiquinone, ubichromenol and α-tocopherol markedly, and the concentration of ubiquinone in this group was the lowest so far encountered by us in any sample of rat liver. It seems worthy of note that oral dosage with vitamin A led to little liver storage in these vitamin E-deficient animals. Administration of vitamin E increased the ubiquinone and decreased the ubichromenol concentration in liver. When both vitamins were given together, ubichromenol concentration decreased and that of ubiquinone was increased slightly. In contrast to that in the liver, the ubiquinone concentration in the hearts of the doubly deficient animals was high: dosing with

vitamin A led to lowered tocopherol, ubiquinone and ubichromenol concentrations. In this experiment, dosing with vitamin E did not lead to an increased concentration of ubiquinone in the heart. However, the exceptionally high concentration of tocopherol in the heart of this doubly deficient control group should be noted, compared with that usually found in vitamin E-deficient heart (Edwin *et al.* 1961).

Johnson & Baumann (1948) found that vitamin A depletion was accompanied in the rat by an increase in the vitamin A level of the kidney. Later, Booth (1952) showed that this was a characteristic only of male rats. In our experiment also the vitamin A concentration in vitamin A-deficient kidney was found to be high. Dosing with vitamin A had no significant effect on the vitamin A level in kidney or on the ubiquinone, ubichromenol and tocopherol concentrations in this tissue. Dosing with vitamin E, on the other hand, had no significant effect on the concentration of either vitamin A or ubiquinone, but increased the ubichromenol concentration markedly.

Expt 4. The results are given in Table 4. Only ubiquinone was determined in this test. Vitamin A depressed the concentration of ubiquinone slightly in heart and liver and more markedly in brain. Vitamin E markedly increased the concentrations of ubiquinone in all these organs.

Table 4. *Expt* 4. *Effect of vitamins A and E on ubiquinone concentration in the female rat*

(Each group contained two rats)

Organ	Group	Ubiquinone (μg/g)
Heart	Doubly deficient	46
	Dosed with vitamin A	37
	Dosed with vitamin E	162
Liver	Doubly deficient	54
	Dosed with vitamin A	45
	Dosed with vitamin E	71
Brain	Doubly deficient	8
	Dosed with vitamin A	4
	Dosed with vitamin E	15

Expt 5. Table 5 presents the findings with the rats at death. It should be noted that, although rats in the six vitamin A-deficient groups only lost $7\frac{1}{2}$% of their weight, rats in groups 3 and 4 continued to grow: this accounts for the considerable difference between the mean weights at death of groups 3 and 4 and the others. Another feature of the experiment, which we have been unable to explain satisfactorily, was that the vitamin A-deficient rats appeared, in general, to deteriorate more rapidly than those deficient in both vitamins (see the last column in Table 5). All the vitamin A- and doubly deficient animals exhibited xerophthalmia.

Table 6 shows the effect of deficiency of vitamin A and vitamin E on ubiquinone and ubichromenol concentrations in heart. Considering first the results on heart (Table 5), it was found that both these concentrations had increased considerably during the interval between the 1st and 2nd stages of the experiment (cf. groups 1 and 2), which may have been due to a temporary variation of the kind already discussed

Table 5. *Expt 5. Information about rats used in study of deficiency of vitamins A and E*

(Each group contained five rats)

Group no.	Dietary deficiency	Mean wt at death (g)	Day of experiment on which was killed	
			First rat	Last rat
2	Vitamins A and E	160*	72	118
3	Vitamin E	211	See p. 138	
4	None	226	See p. 138	
5	Vitamins A and E	155*	60	92
6	Vitamins A and E	165*	74	107
7	Vitamins A and E	175*	103	117
8	Vitamin A	150*	77	90
9	Vitamin A	152*	70	80

* Each rat in these groups was killed after it had lost $7\frac{1}{2}$% of its mean plateau weight (see p. 137).

Table 6. *Expt 5. Effect of deficiency of vitamins A and E on ubiquinone, ubichromenol and α-tocopherol concentrations in rat heart and effect of administering single doses of each vitamin before death*

(Each group contained five rats)

Group no.	Treatment	Mean heart wt (g)	Ubiquinone (μg/g)	Ubichromenol (μg/g)	α-Tocopherol (μg/g)
1	None*	—	52	Not detected	1·7
2	None	0·84	169	7	4·7
3	Vitamin A in diet	0·91	136	8	2·4
4	Vitamins A and E in diet	1·08	168	8	33·3
5	Vitamin E orally	0·78	209	18	8·4
6	Vitamin A orally	0·74	138	7	3·8
7	Vitamin A intravenously	0·76	174	9	2·5
8	Vitamin E in diet	0·82	178	11	33·8
9	Vitamin E in diet and vitamin A orally	0·76	184	9	27·1

* Group 1 animals were killed at the beginning of the second stage of the experiment (see p. 137).

and cannot be necessarily attributed to the decline in vitamin A concentration (which was not measured in this tissue). The tocopherol content of heart increased significantly during this period of the experiment. Comparing groups 2 and 3, then, we find that the dietary vitamin A lowered both ubiquinone and tocopherol concentrations, so once again its effect cannot be isolated from that of vitamin E. It is clear, though, from findings with groups 2, 3 and 4, that adequate vitamin E could prevent the decline. Now, comparing groups 5 and 6, we find that an oral dose of vitamin A depressed both ubiquinone and tocopherol concentrations, whereas vitamin E increased both. It should be noted, however, that in group 9, where the animals had sufficient vitamin E, vitamin A, given orally, had no effect on ubiquinone concentration. This absence of effect was confirmed in the important group 7, in which vitamin A alcohol given intravenously also had no effect on ubiquinone concentration.

Table 7. *Expt 5. Effect of deficiency of vitamins A and E on ubiquinone, ubichromenol, α-tocopherol and vitamin A concentrations in rat liver and effect of administering single doses of each vitamin before death*

(Each group contained five rats)

Group no.	Treatment	Mean liver wt (g)	Ubiquinone ($\mu g/g$)	Ubichromenol ($\mu g/g$)	α-Tocopherol ($\mu g/g$)	Vitamin A (i.u./g)
1	None*	—	86	16	0·6	52·2
2	None	5·0	103	56	0·8	1·3
3	Vitamin A in diet	8·2	56	23	0·3	150
4	Vitamins A and E in diet	7·2	82	22	37·6	199
5	Vitamin E orally	5·2	121	71	28·9	1·8
6	Vitamin A orally	5·6	98	41	0·8	68·6
7	Vitamin A intravenously	5·2	106	60	0·8	33·8
8	Vitamin E in diet	5·0	104	36	53·6	—
9	Vitamin E in diet and vitamin A orally	5·8	105	39	40·0	77·4

* Group 1 animals were killed at the beginning of the second stage of the experiment (see p. 137).

The liver results (Table 7) show a similar picture, further illuminated by the results of vitamin A determinations in the tissue. A word is necessary here about some of these vitamin A results. In most of the vitamin A-deficient livers taken from mildly deficient animals, we have, contrary to what might be expected from the results of earlier workers, found small but demonstrable amounts of vitamin A and have recorded them (we hope to deal with the reason for this difference in a separate study of analytical methods for vitamin A, now in preparation). A few particular points should be noted from Table 7. From findings for groups 2 and 3 it seems that the increase in both ubiquinone and ubichromenol concentrations can be nearly accounted for, as Moore & Sharman (1959) suggest, by the difference in the organ weight, indicating that, in this stage of deficiency, no biochemical effect is involved. Dietary vitamin E (group 8) did not materially affect the ubiquinone concentration in the liver (as it sometimes does not in this organ). When the livers from groups 3 and 4 were compared, however, it was found that group 4, receiving vitamin A and given the tocopherol supplement, contained rather more ubiquinone, in spite of the fact that there was more vitamin A in the group 4 livers. The livers of animals in groups 5–9 varied only little in weight (they were all on a vitamin A-deficient diet) and, although oral dosing with vitamin E produced a small increase in ubiquinone concentration, vitamin A administration was without effect, despite the fact that a large amount of the vitamin reached the tissue. The absence of effect when the intravenous route was used is particularly to be noted, since Green, Diplock, Bunyan, Edwin & McHale (1961) have demonstrated substantial effects on ubiquinone concentrations when vitamin E or its metabolites are given by this route.

The results for kidney (Table 8) are again similar to those for liver. The effect of dietary vitamin A was to lower the concentration of ubiquinone a little, but this decrease was exactly balanced by the increase in the organ weight: ubichromenol concentration was affected identically. In group 4, however, the addition of vitamin E has

led to a real increase in ubiquinone concentration. Once again, comparison of groups 5–9 shows that vitamin A administration by either route did not affect ubiquinone concentration.

Table 8. *Expt 5. Effect of deficiency of vitamins A and E on ubiquinone, ubichromenol, α-tocopherol and vitamin A concentrations in rat kidney and effect of administering single doses of each vitamin before death*

(Each group contained five rats)

Group no.	Treatment	Mean kidney wt (g)	Ubiquinone (μg/g)	Ubichro-menol (μg/g)	α-Toco-pherol (μg/g)	Vitamin A (i.u./g)
1	None*	—	28	Not detected	0·9	19·8
2	None	1·54	53	5	1·0	Not detected
3	Vitamin A in diet	1·84	44	4	1·1	3·8
4	Vitamins A and E in diet	1·84	56	5	24·2	4·3
5	Vitamin E orally	1·58	69	4	6·1	Not detected
6	Vitamin A orally	1·50	54	4	0·9	7·8
7	Vitamin A intravenously	1·62	48	3	1·0	17·3
8	Vitamin E in the diet	1·54	64	6	35·4	Not detected
9	Vitamin E in diet and vitamin A orally	1·38	64	7	24·2	10·4

* Group 1 animals were killed at the beginning of the second stage of the experiment (see p. 137).

Table 9. *Expt 6. Information about rats used in study of vitamin A deficiency*

Group no.	Rat no.	Initial wt (g)	Weight at death (g)	Day on which killed	Signs
1	1	60	162	45	None
	2	58	188	38	None
	3	48	138	45	None
2	4	51	148	77	Xerophthalmia
	5	49	132	70	Xerophthalmia
	6	51	154	63	Xerophthalmia
3	7	52	146	77	Xerophthalmia, myasthenia
	8	53	142	59	Xerophthalmia, very weak
	9	36	50*	42	Xerophthalmia, gross intestinal inflation
4	10	50	98	60†	Xerophthalmia
	11	51	120	76	Xerophthalmia
	12	46	136	76	Xerophthalmia

* Died, 17 % below its mean plateau weight, and dissected immediately after death.
† Killed when moribund.

Group 1 was killed at the beginning of the weight plateau, group 2 when 7½ % below the weight plateau, group 3 when 20 % below the weight plateau, and group 4 was dosed with thiouracil and killed at the times stated on p. 145.

Expt 6. The results of the previous experiments seemed to suggest the absence of any clear metabolic effect of vitamin A on ubiquinone concentration. One feature of the experiments that still seemed puzzling, however, was that we had not been able to observe the extremely high concentrations of ubiquinone in liver found by Morton & Phillips (1959), even though there were not only differences in the strain of rats used

but also in the diets, particularly in the protein and fat contents. Our rats certainly seemed to reach their weight plateau in approximately the same time as those of Morton & Phillips (see Table 9), but the plateau was nearly always much more protracted. Weight loss when it began, however, was rapid. In this experiment, therefore, we studied three stages of vitamin A deficiency: (*a*) at the onset of growth cessation, (*b*) after $7\frac{1}{2}\%$ weight loss and (*c*) after 20% weight loss. In addition, one group of rats was given thiouracil daily from the time weight ceased to increase until they were killed. It has been known for many years that thyroxine induces vitamin A deficiency (Sure & Buchanan, 1937), and Wiese, Mehl & Deuel (1948) found that thiouracil administration prolonged the lives of rats fed on a vitamin A-deficient diet. The reason for this relationship is still under discussion. We have shown previously that thyroxine administration greatly increased ubiquinone and ubichromenol concentrations in rats, and the magnitude of the effect seemed to us to be closely similar to the effect of vitamin A deficiency, as described by Morton & Phillips. We therefore wondered whether an antithyroid drug, such as thiouracil, would in any way influence the effect of the deficiency on ubiquinone concentrations.

Table 9 summarizes the findings with the rats at death. The animals in groups 2, 3 and 4 all showed progressive signs of vitamin A deficiency. On examination, the livers of the group 3 and 4 animals were small and grossly fatty, exactly as described by Heaton *et al.* (1957). The rats receiving thiouracil proved rather difficult to deal with. One rat (no. 10) had severe xerophthalmia and had to be killed (when moribund) after 60 days, when it had only lost 10% of its mean plateau weight; another (no. 12) had only lost 11% of its plateau weight on the 76th day and, since it showed no immediate signs of losing any more, was killed. Rat no. 11 in this group was killed on the 76th day, having lost 17% of its plateau weight.

Table 10 summarizes the results of this experiment. In the hearts there was no difference at all in the ubiquinone concentrations during the first stages of deficiency. Even after the rats had lost $7\frac{1}{2}\%$ of their weight, the concentrations were still well within the normal range. When the deficiency was advanced (group 3), however, there was a sharp increase in ubiquinone and ubichromenol concentrations, paralleled by a rise in the tocopherol level; vitamin A could not be detected after the plateau was reached. Once again there was a sharp reduction in the organ weight simultaneously with the increase in ubiquinone concentration, although not nearly enough to account for the increase. In this experiment, relatively high ubiquinone and ubichromenol concentrations were observed in liver even at the plateau weights (compare, for example, the results of Expts 1 and 2). In Expt 5, even in a more advanced stage of deficiency the levels were within the normal range for the rat (50–150 μg/g). We are inclined to believe that the main reason for this difference may be due to the difference in the composition of the diets in Expts 5 and 6. The ubiquinone concentration in liver showed a progressive increase as deficiency advanced and eventually reached the levels described by Morton & Phillips; but the high tocopherol concentrations were exceptional, particularly the extraordinary concentration found in group 1. Kidney showed only a slight increase in ubiquinone concentration during deficiency, not related to the tocopherol content but probably due solely to the progressive decline

Table 10. *Expt 6. Effect of vitamin A deficiency and of thiouracil on ubiquinone, ubichromenol, α-tocopherol and vitamin A concentrations in rat heart, liver and kidney*

(Each group contained three rats)

Group no.	Mean organ wt (g)	Ubiquinone (μg/g)	Ubichromenol (μg/g)	α-Tocopherol (μg/g)	Vitamin A (i.u./g)
			Heart		
1	0·70	120	11	20	Trace
2	0·70	118	9	11	Not detected
3	0·45	268	70	27	Not detected
4	0·61	172	23	26	Not detected
			Liver		
1	5·94	226	135	84	1·6
2	5·19	332	135	41	Not detected
3	3·45	426	138	38	Not detected
4	4·67	350	191	34	Not detected
			Kidney		
1	1·38	74	12	22	Not detected
2	1·15	85	6	16	Not detected
3	0·63	120	6	19	Not detected
4	1·16	86	4	10	Not detected

Table 11. *Expt 7. Effect of extreme vitamin A deficiency on ubiquinone, ubichromenol and α-tocopherol concentrations in the rat*

(One rat of each sex was used)

Sex	Heart			Liver		
	Ubiquinone (μg/g)	Ubichromenol (μg/g)	α-Tocopherol (μg/g)	Ubiquinone (μg/g)	Ubichromenol (μg/g)	α-Tocopherol (μg/g)
♂	—	—	—	380	232	95
♀	307	Not detected	106	193	250	153

in weight of the organ. All the values for kidney were within the normal range, although no vitamin A could be detected and there was no sign of the abnormal values reached in liver. The results with thiouracil are difficult to interpret: clinically there was some evidence that thiouracil inhibited the effect of vitamin A deficiency in terms of weight loss, which is also suggested by the mean weights of the three organs in group 4 rats, which were considerably higher than those from group 3. The apparent reversal of the trend in the ubiquinone values in group 4 animals would seem to be almost wholly accounted for by this weight reversal: differences in the degree of deficiency make comparison difficult, but it certainly seems that thiouracil does not reverse the ubiquinone increase in vitamin A deficiency.

Expt 7. The hearts and livers of the two animals in this experiment, which were in the final stages of vitamin A deficiency when they were killed, contained high concentrations of ubiquinone and ubichromenol. The liver, particularly, was again found to be grossly adipose. The most remarkable feature of the results is undoubtedly the extraordinary tocopherol concentration observed in both tissues. In our experience it is virtually impossible to produce concentrations of this magnitude by giving

vitamin E to rats, however much is given. In fact, we have in the past given rats 100 mg α-tocopherol daily for several weeks without increasing its concentration in heart and liver much above the normal in adequately fed animals. There is no doubt that the concentrations found in Expt 7 must be regarded as pathological.

DISCUSSION

The experiments described here confirm the original observations of Morton and co-workers that ubiquinone and ubichromenol concentrations increase during vitamin A deficiency in the rat. Certain quantitative differences between our results and those of Morton & Phillips (1959) may have their origin in the differences between the various diets used. The extremely high ubiquinone concentrations reported by Moore & Sharman (1960) are, on the other hand, almost certainly unattainable and may be attributed to the fact that these workers did not subject their extracts to chromatographic separation. Nevertheless, our results confirm and amplify their suggestion that much of the vitamin A effect on ubiquinone is due to a corresponding reduction in the weights of the organs. But this by itself is an oversimplification. It would seem to us that there are three clear aspects of the vitamin A effect and they all reinforce our original opinion as to the absence of any direct metabolic or biochemical interaction between vitamin A and ubiquinone. First, there is the phenomenon of tissue shrinkage, already referred to, which appears to explain at least part of the ubiquinone increases that occur in the early stages of deficiency—more in heart and kidney, but less in liver. Secondly, the experiments described here present substantial evidence that a further part of the increase can be ascribed to a simultaneous increase in tocopherol concentration. In these experiments, almost every change in ubiquinone concentration can be correlated with a tocopherol change, but rarely with a change either in magnitude or direction of vitamin A concentration. The changes in ubiquinone concentration in liver as well as in other tissues during the early stages of vitamin A deficiency are, we believe, wholly due to these two factors.

The third aspect of vitamin A deficiency, which is apparently limited to one or two specific tissues, manifests itself in the later stages of deficiency, when really large increases in ubiquinone and ubichromenol concentrations occur. The picture that emerges, however, is not one of a biochemical involvement, but of a gross pathological condition—the shrunken tissues, the remarkable fatty infiltration, the high sterol contents and, finally, the unusually high tocopherol concentrations imply that some vast disorder of lipid metabolism exists in the affected animals. Johnson & Wolf (1960) have recently enjoined against the danger of interpreting results of experiments on severely vitamin A-deficient animals as having necessarily any bearing on the metabolic function of vitamin A. They give clear examples of how such results may differ from those found in mildly deficient animals and believe that only such animals can throw light on the metabolic function of vitamin A. Thus they criticize the results of Gloor & Wiss (1959 a, b), who found that vitamin A deficiency in the rat led to an increased uptake of labelled mevalonate into ubiquinone and a decreased uptake into cholesterol, and have themselves found that the effect on cholesterol (they did not study ubiqui-

none) was only apparent in severely deficient rats, not in mildly deficient ones. The biochemical results of Gloor & Wiss, in fact, stand in sharp contrast to ours, which suggest that vitamin A has no metabolic role in ubiquinone synthesis, and we have searched for an explanation why it should be so. There is strong evidence that the 'ubiquinone' fraction of lipids obtained by adsorption chromatography on alumina is contaminated with an unknown metabolite, which becomes much more labelled than ubiquinone when labelled mevalonate is incorporated into the non-saponifiable fraction of lipids and can be separated from ubiquinone only by partition paper chromatography. Thus, Olson, Dialameh & Bentley (1961) have described such a substance, and we have had similar results. It is not clear whether or not Gloor & Wiss, who did not record its existence, included it in their 'ubiquinone' fraction. Recently, Wiss & Gloor (1960) have described an experiment on vitamin A deficiency, in which paper chromatography was used to study the ubiquinone fraction: the results of this experiment would appear to contradict the earlier results of these workers and support the findings of our nutritional experiments.

SUMMARY

1. In seven experiments the effect of vitamin A deficiency on ubiquinone and ubichromenol concentrations in the rat has been studied. In some experiments the effect of combined vitamin A and vitamin E deficiency was also investigated.

2. In the early stages of vitamin A deficiency, increases in ubiquinone concentration were observed in liver. Later, similar increases were found in heart, but little change was observed in kidney.

3. There appear to be three main effects on ubiquinone concentration attributable to the vitamin A-deficient state. First, reductions in the size of the various organs lead to an increase in the concentrations of ubiquinone, but not always in the absolute amounts present. Secondly, increased vitamin E concentrations, which nearly always accompany vitamin A deficiency, play a substantial part in increasing ubiquinone concentrations. In the final stages of vitamin A deficiency, the increased ubiquinone and ubichromenol concentrations in heart and liver can be attributed to a pathological disorder of lipid metabolism, characterized by fatty infiltration and exceptionally high tocopherol contents.

4. No evidence for a metabolic role of vitamin A in relation to ubiquinone synthesis was found.

5. Thiouracil administration did not reduce the high ubiquinone concentrations found in the final stages of vitamin A deficiency.

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