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.

REFERENCES

- Deuel, H. J., jun. (1957). In *The Lipids*, vol. 3, p. 746. New York and London: Interscience Publishers Inc.
- Gloor, U. & Wiss, O. (1959). *Arch. Biochem. Biophys.* **83**, 216.
- Heaton, F. W., Lowe, J. S. & Morton, R. A. (1957). *Biochem. J.* **67**, 208.
- Hubbell, R. B., Mendel, L. B. & Wakemam, A. J. (1937). *J. Nutr.* **14**, 273.
- Lowe, J. S., Morton, R. A. & Harrison, R. G. (1953). *Nature, Lond.*, **172**, 716.
- Martin, A. J. P. & Moore, T. (1939). *J. Hyg., Camb.*, **39**, 643.
- Mason, K. E. (1940). *Amer. J. Physiol.* **131**, 268.
- Mason, K. E. & Harris, P. L. (1947). *Biol. Symp.* **12**, 450.
- Mervyn, L. & Morton, R. A. (1959). *Biochem. J.* **72**, 106.
- Moore, T. & Wang, Y. L. (1943). *Biochem. J.* **37**, i.
- Moore, T. & Wang, Y. L. (1947). *Brit. J. Nutr.* **1**, 53.
- Morton, R. A. (1958). *Nature, Lond.*, **182**, 1764.

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

BY W. E. J. PHILLIPS* AND R. A. MORTON
Biochemistry Department, The University of Liverpool

(Received 29 April 1959)

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

* Seconded from the Chemistry Division, Department of Agriculture, Science Service, Ottawa, Canada.

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

pituitary gland and the liver. The adrenal lipid had 91.8 % of its potential vitamin A activity as pre-formed vitamin A and only 3.8 % as provitamin A (β -carotene). Lowe *et al.* (1953) observed that in vitamin A deficiency the phospholipid content of rat adrenals was reduced and the histological results indicated that the residual phospholipid was confined to the zona glomerulosa. The disappearance of phospholipid from the rat zona fasciculata may be significant since this zone is often thought to be the site of glucocorticoid formation. Wolf, Lane & Johnson (1957) and Wolf, Wagle, van Dyke & Johnson (1958) showed that vitamin A deficiency impaired the synthesis of glucocorticoid hormones. It could therefore be argued that vitamin A deficiency led to partial 'chemical adrenalectomy' with diminished glucocorticoid formation (or secretion) and consequent lowering of glyconeogenesis.

Clark & Colburn (1955) found that the administration of cortisone to normal or adrenalectomized rats brought about a rapid depletion of the stores of vitamin A in liver and kidney. The stress of semi-starvation also resulted in a decline in the liver stores of vitamin A, but the effect was less than that caused by cortisone. The hyperactivity of the adrenals under stress thus played a part in the loss of liver vitamin A. Bodansky & Markardt (1951) observed that Reichstein's compound L reduced the concentration of vitamin A in rat blood. Adrenaline did not have this effect.

The foregoing considerations suffice to show that vitamin A deficiency influences adrenal function. Heaton, Lowe & Morton (1957) described how vitamin A deficiency brought about marked increases in substance SA (ubiquinone) and substance SC contents of rat liver. Morton & Phillips (1959*a*) have confirmed this and have shown that the ubiquinone content rises steadily as the deficiency develops. The rise in substance SC is a late manifestation of deficiency. It was therefore desirable to investigate whether any changes in tissue ubiquinone levels occur in the normal and vitamin A-deficient rat after adrenalectomy.

METHODS

Animals. Hooded or albino rats reared in the University were used.

Diets. Two vitamin A-deficient diets were used. (a) Casein (vitamin-free) 18 %, starch 65 %, arachis oil 5 %, salt mixture (U.S.P.XIII) 4 %, Marmite 8 %. A vitamin supplement (Morton & Phillips, 1959*b*) was added (2 g./kg. of diet). (b) Casein (vitamin-free) 18 %, sucrose 65 %, dried brewer's yeast 8 %, arachis oil 5 %, salt mixture 4 %. In experiments with stock-colony animals Diet no. 4 (British Extracting Co., Bromborough, Cheshire) was used.

Adrenalectomy. Both adrenals in each rat were removed, under ether anaesthesia, in the customary manner through

a midline dorsal skin incision. We are greatly indebted to Professor R. G. Harrison of the Department of Anatomy, who performed all the operations.

Examination of liver tissue. The liver was saponified (in the presence of ethanolic pyrogallol) and the unsaponifiable fraction was chromatographed on alumina partially deactivated to Brockmann grade 3 as described by Mervyn & Morton (1959). The first eluate, obtained with light petroleum alone, contained a small amount of hydrocarbon and exhibited an inflexion at 260 m μ . Ubiquinone was eluted in the fractions obtained with 4-6 % of ether in light petroleum (b.p. 40-60°). The main sterol fractions, eluted by 20 % of ether in light petroleum, were examined for ultraviolet absorption and then acetylated and rechromatographed.

The acetylated sterol fractions consisted in the main of cholesteryl acetate, which was readily eluted by 1 % of ether in light petroleum. A small amount of material (about 1 % of the acetylated fraction) was eluted by 2 % of ether in light petroleum; it showed λ_{max} 280 m μ in cyclohexane, $E_{1\text{cm}}^{1\%}$ approx. 17. Substance SC acetate was eluted by 8 % of ether in light petroleum and showed the characteristic absorption peaks at 275 and 315 m μ and the inflexion at 285 ($E_{1\text{cm}}^{1\%}$ 275 m μ 57). Ether alone eluted free substance SC (λ_{max} 275 m μ with inflexions at 285 and 330 m μ).

Experiment 1. Fourteen female rats were kept on the vitamin A-deficient diet (a) for 45 days from the time of weaning. Seven of the rats were bilaterally adrenalectomized on the forty-fifth day and were then given 0.9 % sodium chloride to drink instead of water. The vitamin A-deficient diet was offered to these animals and to the controls during the next 43 hr. All the animals were then killed (chloroform) and the livers and kidneys were excised and retained for analysis. By the forty-fifth day all 14 animals were in an advanced state of vitamin A deficiency. Three of the adrenalectomized animals died during the last 17 of the 43 hr. after operation, but their livers were included in the material tested.

Experiment 2. Ten male and ten female albino rats were placed at, or very soon after, weaning on the vitamin A-deficient diet (b). In this experiment the aim was to carry out the adrenalectomy after the vitamin A reserves had been almost exhausted but before the deficiency syndrome had become far advanced. By the thirty-third day of experiment growth had slackened or ceased and most of the animals had reached the stage of the weight plateau. Half the animals were then bilaterally adrenalectomized. One operated animal and one control animal died on the third day and their tissues were not used. The 18 remaining animals were killed 72 hr. after the operations. Livers were excised and retained for analysis.

Experiment 3. Twenty male and female rats were given the stock diet from weaning until they were 57 days old. Five of each sex were bilaterally adrenalectomized and maintained for a further 10 days on the stock diet. All the animals were killed and the livers excised on the tenth post-operative day.

RESULTS

Experiment 1. Table 1 summarizes the results. Both the control animals and the adrenalectomized animals were frankly avitaminotic and the post-operative period was short. The hydrocarbon

content of the liver was above the normal value. The ubiquinone content of the livers of the control animals was at least four times the normal amount. In the livers of the adrenalectomized animals it was about three times the normal, but more work is needed to test whether the difference between three times and four times the normal is other than fortuitous. In this experiment the livers from the adrenalectomized animals which died in the later part of the post-operative period were included with those from surviving animals. Because of this the ubiquinone content for the group may have erred on the low side. Perhaps the important point is that the ubiquinone content was at least three times the normal amount. The substance SC content of the liver from both groups was also well above the normal but the superimposition on vitamin A deficiency of the consequences of adrenalectomy did not change the situation. The total liver sterol was, however, raised by about 23 %.

Experiment 2. In this trial both sexes were studied. The animals had used up their stores of vitamin A and were not yet losing weight. They had no xerophthalmia. Table 2 summarizes the results. The sharp increase in the hydrocarbon content of liver which was seen in experiment 1 had not yet

occurred. The ubiquinone content of the livers of the deprived animals was about twice, and of the deprived and adrenalectomized animals about thrice, the normal amount. This was true for both sexes. The substance SC fraction for the female adrenalectomized group was lost but the figures for males in Table 2 showed that the rise in substance SC was only incipient. The sterol content was higher in the adrenalectomized animals. The broad picture is that in rats about to become frankly avitaminotic, adrenalectomy brought the animals nearer, in respect of the criteria considered here, to the very seriously affected animals of Expt. 1. The marked loss of liver weight in the adrenalectomized rats is noteworthy.

Experiment 3. In this experiment all the animals had ample liver stores of vitamin A. Both sexes were studied and all the adrenalectomized animals were alive 10 days after operation. During this period of 10 days the control animals had increased in weight (males by 33 g., females by 21 g.), whereas the adrenalectomized animals remained practically stationary in weight. Table 3 summarizes the results. The control rats had larger livers (expressed as a percentage of body weight) than the adrenalectomized rats. The hydrocarbon content was low

Table 1. *Comparison of vitamin A-deficient rats with adrenalectomized vitamin A-deficient rats*

Seven female hooded rats exhibiting advanced avitaminosis A were in each group.

	Vitamin A-deficient	Adrenalectomized vitamin A-deficient
Mean body wt. day 45 after weaning (g.)	81	81
Mean liver wt. (g.)	3.61	3.59
Liver constituents		
Unsaponifiable matter (%)	0.654	0.632
Hydrocarbon (mg./g.)	0.86	0.85
Vitamin A	Not detected	
Ubiquinone (μ g./g.)	511	397
Substance SC (μ g./g.)	110	105
Sterol (mg./g.)	2.93	3.61

Table 2. *Comparison of vitamin A-deprived rats with adrenalectomized vitamin A-deprived rats*

Animals of both sexes were at or near the growth plateau.

	Vitamin A-deprived		Vitamin A-deprived and adrenalectomized	
	Males	Females	Males	Females
Sex				
No.	4	5	5	4
Mean final body wt. (g.)	100.5	103.2	101.2	92
Mean liver wt. (g.)	4.73	4.88	3.59	3.89
Liver constituents				
Unsaponifiable matter (%)	0.438	0.405	0.459	0.521
Hydrocarbon (mg./g.)	0.22	0.08	0.04	0.12
Vitamin A	Not detected			
Ubiquinone (μ g./g.)	222	212	356	365
Substance SC (μ g./g.)	55	44	70	Lost
Sterol (mg./g.)	2.68	2.15	3.06	3.46

Table 3. Comparison of stock-colony rats on a normal diet with adrenalectomized rats on a normal diet

Adrenalectomy occurred at 57 days of age; all animals were killed 10 days later. The relatively high vitamin A content of liver causes interference with determination of substance SC but there was evidence of no more than trace amounts of substance SC.

	Control		Adrenalectomized	
	Males	Females	Males	Females
Mean initial wt. (g.)	179	124	180	123
Mean final wt. (g.)	212	145	183	127
Mean liver wt. (g.)	12.84	8.78	8.74	6.67
Liver (% of body wt.)	6.06	6.05	4.78	4.78
Liver constituents				
Hydrocarbon (mg./g.)	0.072	0.098	0.089	0.098
Vitamin A (i.u./g.)	151	164	137	216
Ubiquinone (μ g./g.)	110	119	133	130
Sterol (mg./g.)	1.80	2.13	2.23	2.12

throughout. The ubiquinone content rose a little (expressed as μ g./g. of liver) in the adrenalectomized animals, but expressed as μ g./liver it fell a little. This indicates that the ubiquinone content of rat liver was very little affected as a result of adrenalectomy when vitamin A reserves were substantial. The sterol content of liver was also little changed.

DISCUSSION

Cowie (1949) showed that completely adrenalectomized rats could survive from 8 to 19 days, depending upon their age at operation. All the animals in Expt. 3 survived for 10 days and some might have lived longer. We do not think that the experiment was vitiated by the development of 'accessory' adrenal tissue. Vitamin A deficiency shortened the survival time of adrenalectomized animals. Adrenalectomy hastened the appearance of some of the late manifestations of vitamin A deficiency in animals having no remaining liver store. If, however, the pre-operative deficiency was very advanced the further results of adrenalectomy were much less striking. The third experiment suggests that the rises in ubiquinone and substance SC concentration which occur in vitamin A deficiency are not caused by the partial 'chemical' adrenalectomy of deficiency since total adrenalectomy (surgical) of animals normal in vitamin A status leaves ubiquinone and substance SC at practically normal concentrations in liver. The rise observed by Heaton *et al.* (1957) and confirmed by Morton & Phillips (1959a) is seen even more clearly to reflect vitamin A status in the rat.

SUMMARY

1. The rises in ubiquinone and substance concentration SC which occur in the livers of

vitamin A-deficient rats may be hastened if at an early stage in the deficiency the rats are bilaterally adrenalectomized.

2. If, however, the deficiency syndrome is already far advanced adrenalectomy does not noticeably influence the concentrations of minor constituents of liver unsaponifiable matter.

3. In rats on a stock diet adrenalectomy brought about no striking changes in liver vitamin A (range 137–216 i.u./g. of liver), ubiquinone content (range 110–133 μ g./g.) or sterol content (range 1.80–2.23 mg./g.). Substance SC was not present in more than trace amounts.

REFERENCES

- Bailly, O. & Nettor, R. (1932). *Bull. Soc. Chim. biol., Paris*, **14**, 623.
 Bennet, L. R., Bennet, V. C., Shaver, A. & Gracht, T. (1950). *Proc. Soc. exp. Biol., N.Y.*, **74**, 439.
 Bodansky, O. & Markardt, B. (1951). *J. biol. Chem.* **190**, 83.
 Clark, I. & Colburn, R. W. (1955). *Endocrinology*, **56**, 232.
 Cowie, A. T. (1949). *J. Endocrin.* **6**, 94.
 Davies, A. W. & Moore, T. (1934). *Biochem. J.* **28**, 288.
 Heaton, F. W., Lowe, J. S. & Morton, R. A. (1957). *Biochem. J.* **67**, 208.
 Lowe, J. S., Morton, R. A. & Harrison, R. G. (1953). *Nature, Lond.*, **172**, 716.
 Mervyn, L. & Morton, R. A. (1959). *Biochem. J.* **72**, 106.
 Moore, T. (1957). *Vitamin A*, p. 209. London: Elsevier Publishing Co. Ltd.
 Morton, R. A. & Phillips, W. E. J. (1959a). *Biochem. J.* **73**, 416.
 Morton, R. A. & Phillips, W. E. J. (1959b). *Biochem. J.* **73**, 421.
 Popper, H. & Greenberg, R. (1941). *Arch. Path.* **32**, 11.
 Willmer, J. S. & Laughland, D. H. (1957). *Canad. J. Biochem. Physiol.* **35**, 819.
 Wolf, G., Lane, M. D. & Johnson, B. C. (1957). *J. biol. Chem.* **225**, 995.
 Wolf, G., Wagle, S. R., van Dyke, R. A. & Johnson, B. C. (1958). *J. biol. Chem.* **230**, 979.