Vitamin A Deficiency and the Ubiquinone and Substance SC Contents of Rat Liver: The Time Factor

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Lowe, Morton & Harrison (1953) found that vitamin A deficiency in rats results in marked changes in the adrenal glands and in the accumulation in the liver of two substances having characteristic ultraviolet-absorption spectra. Heaton, Lowe & Morton (1957) referred to these substances as SA and SC, and the former is now known as ubiquinone (for references see Morton, 1958). Heaton *et al.* (1957) made a detailed comparison of tissues from rats exhibiting avitaminosis A with those of control animals. Ubiquinone was found to occur in many organs but substance SC was detected in normal animals only in kidney, liver and intestine and then in very small amounts. A sharp rise occurred, however, in the amounts of both ubiquinone and substance SC in the livers of rats suffering from frank vitamin A deficiency. Green, Lowe & Morton (1957) subjected rats to the double hazard of vitamin A deficiency and a high intake of dietary cholesterol. The latter neither diminished nor enhanced the rises in liver ubiquinone and substance SC observed in uncomplicated vitamin A deficiency. A hypothesis that the accumulation of these two new substances was a necessary part of the deficiency syndrome was tested on fowl and on guinea pigs. Lowe, Morton, Cunningham & Vernon (1957) found in fowl that the ubiquinone and substance SC of liver did not rise in vitamin A deficiency and Cunningham, Morton & Phillips (unpublished work) observed that the ubiquinone content of guinea-pig liver was also unresponsive to vitamin A deficiency. Such notable differences between species make it necessary to investigate further the effects shown in the rat. Thus it seems desirable to determine at what stage in the development of avitaminosis A the changes occur in storage or metabolism of ubiquinone and substance SC. In the earlier work (Heaton *et al.* 1957) the rats deprived of vitamin A were not killed until there was manifest xerophthalmia and a severe decline in body weight.

In the present work food consumption by individual rats was measured throughout the period of experiment. There is general agreement that vitamin A deprivation reduces appetite but quantitative information is lacking on the rate at

which food consumption falls off as the deficiency syndrome develops. Sampson, Dennison & Korenchevsky (1932) found that during the period when the growth of deprived rats slows to a halt the decrease in appetite is only moderate. Anorexia can be no more than a partial explanation of the diminishing growth. Indeed it seemed that (in relation to body weight) the deprived rats ate rather more than rats on a complete diet. This continued until a late stage when the frankly deficient rats exhibited a marked loss of appetite. Patterson, McHenry & Crandall (1942) observed that with vitamin A-deprived animals on a weight plateau for 3 weeks the food consumption declined linearly with time from 16 to 7 g. of diet/rat/day.

EXPERIMENTAL

Examination of tissues. Livers were saponified as described in previous papers (Mervyn & Morton, 1959) and the unsaponifiable extracts were chromatographed on alumina partially deactivated to Brockman grade 3. Elution by means of light petroleum containing increasing proportions of diethyl ether yielded in succession fractions rich in hydrocarbons, in ubiquinone, in sterol and the substance SC.

Animals. Albino rats were purchased from A. Tuck and Sons, Rayleigh, Essex and at about 30 days of age they were placed on a diet with the following percentage composition: sugar, 65; casein (ethanol-extracted), 18; arachis oil, 5; dried brewer's yeast, 8; salt mixture (Hubbell, Mendel & Wakeman, 1937), 4. Food and water were given *ad lib.*

Experimental plan. The rats (59 males, 59 females) were given the deficient diet for varying lengths of time. Two groups of 15 animals were killed 5 days after weaning, two groups of 10 were killed 11 days after weaning and two groups of 10 were killed 20 days after weaning. These groups are designated as the 5-day, 11-day and 20-day groups respectively. The remaining 48 animals (24 males and 24 females) were housed in individual cages from weaning. Daily food consumption for the 48 animals was measured by difference. A weighed quantity of the diet was measured out each morning and the amount left over after 24 hr. was weighed after recovery of spilled food from filter paper placed about 1 in. below the wire-mesh floor of the cage.

In an experiment of this kind, the time at which individual animals reach a given stage of deficiency varies rather widely. All the animals were weighed frequently and those which reached a given stage were taken together. Figs. 1a

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Fig. 1. Each open circle represents a group of rats killed at a definite stage in the growth curve. Circles without letters refer to groups of rats for which individual food consumptions were not measured. Each lettered circle refers to three rats for which individual food intakes were measured daily. (Diagrammatic only.) (a) Male rats; (b) female rats.

and (b) illustrate the manner in which animals were selected for examination. Each letter in the diagrams represents a group of three animals killed and studied as a group. Thus point C represents three male rats killed at 28 days; D represents three female rats killed at 28 days; J represents three female rats killed after 39 days, all three groups without slackened growth. Groups C, D and J can be collectively taken as representing the period a little before the weight plateau. Similarly, A, B, E, H, M and P each represents three rats at the weight plateau. F, I, N, O, T and S each represents three rats declining in weight and approaching the terminal phase of deficiency. Figs. 2 (a) to 2 (d) refer to means for three rats and show how weight curves correspond with food-intake curves.

RESULTS

Weight increases in relation to food consumption.

It is evident from Figs. 2 (a) to 2 (d) that although the rats were all from one colony and were given the same diet, the growth rate varied considerably. A few rats of each sex reached a growth (i.e. weight) plateau after 21 days on the deficient diet (groups A and B) but others required about 37 days (groups M and P). This difference might be the result of differences in liver stores of vitamin A at weaning or possibly differences in metabolic losses of vitamin A.

Occasionally an animal would scatter food so that it was doubtful whether the apparent intake corresponded closely with the amount actually eaten; all such cases were left out of the calculations but very few of the 1800 daily food consumptions measured had to be rejected.

The daily intake of food increased steadily to a maximum which in most cases occurred 11–13 days after weaning. In those animals which reached a plateau in body weight earlier than about 27 days after weaning, food consumption began to fall after about the fourteenth day. Animals which took longer to reach stationary body weight did not begin to lose appetite until about 4 days before the appearance of the weight plateau. From then on they steadily ate less.

Six groups of three animals were maintained on the vitamin A-deficient diet until an unmistakable loss in body weight had occurred. A marked decline in food consumption occurred at or near the end of the plateau period. Appetite waned before growth ceased but an animal was not badly 'off its food' during the variable and some times lengthy period before the terminal stages of the deficiency syndrome. There was a variable lag between loss of appetite and loss of weight. Patterson *et al.* (1942) observed that vitamin A-deficient rats at the plateau stage of the deficiency syndrome contained 6% more body water than pair-fed control rats.

Agnew & Meyer (1956) have thrown light on the loss of appetite shown by vitamin A-deficient rats. In the hypothalamus of normal rats there appears to be an area which acts as a 'satiety' brake, which, in response to metabolic stimuli, inhibits an otherwise constantly acting feed mechanism. By subjecting vitamin A-deficient rats, which had stopped growing, to a hypothalamic injury which destroyed the satiety centre, a weight increase of 70–200 g. occurred. The appearance in many of the rats in the present experiment of loss of appetite and slackened growth after 11–13 days on the vitamin A-deficient diet suggests that an application of the satiety brake may be an early aspect of the deficiency syndrome.

Effects on the liver. Table 1 shows how body weight and liver weight changed during the course of the experiment. In the youngest animals the liver accounted for a larger proportion of the total weight than in the somewhat older animals, but beyond 3 weeks after weaning the proportion became fairly constant and showed no great difference before, during or after the weight plateau. An animal which lost weight suffered a roughly proportionate decline in liver weight.

Table 2 shows how the amounts of hydrocarbon, of ubiquinone, substance SC and sterol in liver tissue varied during the course of the experiment.

DISCUSSION

The original work (Lowe *et al.* 1953; Heaton *et al.* 1957) on the effects of vitamin A deficiency on ubiquinone (SA) and substance SC levels in rat

tissues was carried out on frankly avitaminotic animals. The ubiquinone content of the livers rose from 120 $\mu\text{g./g.}$ in the control rats to 283 $\mu\text{g./g.}$ in the deficient animals. For stock-colony animals the level was 78 $\mu\text{g./g.}$ and substance SC was just

Fig. 2. In each diagram the full curve is food consumption (g./day) plotted against days on the vitamin A-deficient diet and the broken curve shows body weight (g.) plotted against days. Each group consisted of three animals. These diagrams should be read in conjunction with Fig. 1 and Table 1. The dotted line in (d) refers to body weight in group T for which the food consumption was not recorded fully.

detectable. The level of substance SC in the control animals (with small vitamin A reserves) was 7.19 arbitrary units/g. and in the avitaminotic animals 15.5 units/g. (15.5 units/g. = 170 $\mu\text{g./g.}$ on basis of $E_{1\text{cm}}^{1\%}$, 275 $\text{m}\mu$ = 90).

In the present work (Table 2) the liver ubiquinone level began at 82 $\mu\text{g./g.}$ and rose steadily to a value

of 300 $\mu\text{g./g.}$ at the weight plateau, and still further to 410 $\mu\text{g./g.}$ of liver when the rats were rapidly losing weight. Heaton *et al.* (1957) found that the sterol content of liver rose from 2.03 mg./g. of liver in the stock-colony rats to 2.19 mg./g. in the control rats and 2.78 mg./g. in the avitaminotic rats. In the present series of determinations the rats killed

Table 1. *Effects of vitamin A deficiency in relation to the stage reached in the syndrome*

Group	Sex	Average body wt. (g.)	Liver wt. (as % of body wt.)	Unsap. matter (as % of liver wt.)	Ubiquinone ($\mu\text{g./g.}$ of liver)
Growing					
5-day	M.	46.8	8.15	0.298	85.9
	F.	48.2	7.43	0.306	78.8
	Mean	47.5	7.79	0.302	82.3
11-day	M.	68.8	6.18	0.374	132
	F.	63.5	6.25	0.374	140
	Mean	66.2	6.21	0.374	136
20-day	M.	84.9	5.47	0.404	227
	F.	81.7	5.78	0.402	187
	Mean	83.3	5.62	0.403	207
C	M.	88.7	4.67	0.410	220
D	F.	87.3	4.58	0.430	117
J	F.	103	4.96	0.456	398
	Mean	93.0	4.74	0.432	245
Weight plateau					
A	M.	74.3	4.89	0.455	334
E	M.	82.0	4.89	0.392	245
M	M.	105.0	4.36	0.411	253
B	F.	63.7	4.85	0.553	326
H	F.	84.7	5.28	0.448	313
P	F.	97.0	4.56	0.475	325
	Mean	84.5	4.80	0.456	299
Losing weight					
F	M.	82.0	4.58	0.381	297
N	M.	72.0	4.53	0.615	416
T	M.	81.0	5.42	0.450	413
I	F.	83.5	3.91	0.683	660
O	F.	68.0	3.97	0.574	315
S	F.	71.7	5.06	0.420	355
	Mean	73.0	4.6	0.521	409

Table 2. *Minor constituents of rat liver observed as vitamin A deficiency develops*

	Stage ...	5-day	11-day	20-day	28-day	Weight plateau	Post-plateau
Hydrocarbon fraction ($\mu\text{g./g.}$)		72	62	60	116	184	121
Absorption at 260 $\text{m}\mu$ (units/g.)*		0.78	0.89	1.43	1.54	1.79	2.67
Ubiquinone {	($\mu\text{g./g.}$)	82.3	136	207	245	299	409
	(mg./100 g. body wt.)	0.64	0.85	1.16	1.18	1.44	1.88
Substance SC {	($\mu\text{g./g.}$)†	7.33	6.55	9.32	32.3	32	45.2
	(mg./100 g. body wt.)	0.06	0.041	0.052	0.155	0.154	0.21
Sterol {	(mg./g.)	1.85	2.27	2.26	2.20	2.43	2.81
	(mg./100 g. body wt.)	14.6	14.05	12.6	10.4	11.7	12.7

* The units refer to $E_{1\text{cm}}^{1\%}$, 260 $\text{m}\mu$ for the fraction, multiplied by its weight in mg.

† The basis of this calculation is that $E_{1\text{cm}}^{1\%}$, 275 $\text{m}\mu$ = 90 for the purest substance SC yet obtained.

after 5 days on the vitamin A-deficient diet showed 1.85 mg./g. of liver and 2.2–2.3 mg./g. for the 11- to 28-day periods, and 2.34 mg./g. at the plateau and 2.81 mg./g. of liver when weight was rapidly decreasing. This reproduces the earlier picture.

The content of substance SC in liver rose substantially as the vitamin A deficiency progressed, whether the result was expressed as $\mu\text{g./g.}$ of liver or mg./100 g. body weight. It did not, however, reach in the present experiment the very high value found by Heaton *et al.* (1957). This result may be accounted for if the sharp rise in substance SC is characteristic of the terminal stages of vitamin A deficiency. In the present study the 'post-plateau' animals were not all as severely avitaminotic as those of Heaton *et al.* Table 2 indicates that the liver-hydrocarbon content also rose in vitamin A deficiency. Two criteria for hydrocarbons may be used in this context; one is the weight of unsaponifiable matter eluted by means of light petroleum, and the other is the intensity of absorption at $260\text{ m}\mu$. The former criterion reflects the sum of saturated and unsaturated hydrocarbons and the latter may measure one or more of the unsaturated hydrocarbons. A steady increase in absorption at $260\text{ m}\mu$ occurred as the vitamin A deficiency progressed and a roughly linear relationship was noted between this increase and the rise in ubiquinone content. A rise of 0.1 unit/g. at $260\text{ m}\mu$ was accompanied by an average rise of nearly $20\text{ }\mu\text{g./g.}$ in liver ubiquinone. Since ubiquinone has a $-\text{C}_{50}\text{H}_{81}$ unsaturated side chain, the parallel increase in a free unsaturated hydrocarbon fraction is interesting and possibly significant.

It was in some ways surprising that the phenomena of increases in ubiquinone and substance SC in the livers of rats suffering from avitaminosis A were not reproduced in fowls (Lowe *et al.* 1957) nor in guinea pigs (Cunningham, Morton & Phillips, 1959, unpublished work). The present work shows that whatever the explanation of the species differences, the observations on the rat are consistent with progressive rises in liver ubiquinone and substance SC concentration with increasing time on the deficient diet.

SUMMARY

1. Weanling rats placed on a vitamin A-deficient diet reached a weight plateau after a variable time. Food consumption began to fall after about 14 days in those animals which reached the plateau earlier than 27 days after weaning. Animals which took longer to become stationary in weight began to lose appetite about 4 days before growth ceased. Anorexia became marked only in animals rapidly losing weight.

2. Groups of deprived animals were killed at 5, 11, 20, 28 and 39 days while still growing; other groups were killed at the weight plateau and others at a time when they were losing weight. Chromatographic examinations of liver unsaponifiable matter showed: (a) a rise in sterol content only at or beyond the weight plateau; (b) a rise in substance SC which became marked when growth had slackened or ceased; (c) a progressive rise in ubiquinone; the increase was already definite in the 11-day group and when the phase of declining weight was reached the concentration averaged five times the concentration seen in the group of rats killed 5 days after weaning. The amount of a hydrocarbon fraction showing selective absorption at $260\text{ m}\mu$ also increased steadily with the rising ubiquinone concentration.

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