Vitamin A and Isoprenoid Synthesis in the Rat

By A. T. DIPLOCK, J. GREEN AND J. BUNYAN

Walton Oaks Experimental Station, Vitamins Ltd., Tadworth, Surrey

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1. Vitamin A-deficient rats were compared with similar animals given small amounts of vitamin A sufficient for adequate growth and with animals given large amounts of vitamin A. The effects of pair-feeding and feeding *ad libitum* were compared. 2. Ubiquinone and cholesterol concentrations in liver were measured at various stages of the deficiency, and the uptake of radioactive mevalonate and acetate into isoprenoid compounds was studied. 3. Ubiquinone concentrations in liver increased markedly in deficient rats compared with adequate controls, and heavy vitamin A supplementation had a further effect in depressing ubiquinone concentrations. These effects were unrelated to food intake or to the size of the organs. 4. Radioactive uptake into ubiquinone was often greater in deficient livers, especially during the early stages of the experiments, but the effect was not consistent. 5. Cholesterol concentrations were usually higher in deficient livers and these were more affected by the feeding regimen. 6. No consistent effect of vitamin A deficiency or of vitamin A dosage on the incorporation of mevalonate into cholesterol or squalene was found. 7. No evidence has been found for a specific effect of vitamin A on isoprenoid synthesis at the metabolic level.

The concentration of ubiquinone in rat liver rises sharply during vitamin A deficiency (Heaton, Lowe & Morton, 1957), and Morton & Phillips (1959) found that increases in concentration were already evident in weanling rats after only 11 days on a vitamin A-deficient diet and before their weight plateau had been reached. During and after the plateau period, a marked decline in food consumption was observed, and this coincided with still further increases in the concentrations of ubiquinone, ubichromenol and crude sterol in the liver. Phillips (1962) found a significant inverse relationship in rats between hepatic ubiquinone and dietary vitamin A concentrations. Gloor & Wiss (1959*a,b*) and Phillips (1961) found that the relative incorporation of mevalonate into the ubiquinone and squalene of rat liver *in vivo* was increased in vitamin A deficiency and was associated with a relative decrease in the incorporation of mevalonate into the crude sterol fraction. They suggested that a deficiency of vitamin A leads to a metabolic block in the pathway of isoprenoid synthesis between squalene and cholesterol, which results in an increase in ubiquinone synthesis. However, these findings were not supported by adequate identification of the chromatographic fractions used for radioactive counting, nor were precise descriptions of the feeding, or, in some cases, of the state of deficiency, given. Indeed, they cannot be readily correlated with the increases in liver sterol con-

centration during the later stages of deficiency that have been observed by Morton & Phillips (1959), or with the findings of Johnson & Wolf (1960) that synthesis of cholesterol by rat liver *in vitro* is unaffected by vitamin A deficiency in its early stages.

The present work was carried out in an attempt to elucidate some of these effects in more detail. In view of the findings of Morton & Phillips (1959), vitamin A deficiency was studied with two sets of control rats: (a) pair-fed with deficient animals; (b) fed *ad lib*. This seemed to be important, in view of the large differences in food consumption and animal weight that were observed in the later stages of vitamin A deficiency. Both Gloor & Wiss (1959*a*) and Phillips (1961) compared deficient rats with controls supplemented with large amounts of vitamin A. In the experiments described below, we have used control animals supplemented with large amounts of vitamin A and also other controls given just adequate amounts of the vitamin. Studies were made at three clearly differentiated stages of depletion.

EXPERIMENTAL

Animals. Weanling male rats of the Norwegian hooded strain were taken from the stock colony and fed with the experimental diets from 28 days of age.

Diet. The vitamin A-deficient diet (AO2) was described by Edwin, Bunyan, Green & Diplock (1962).

Analytical methods. Livers from animals of each group were pooled and minced, and a 10g. sample was taken. This was saponified as described by Mervyn & Morton (1957). One-twentieth of the non-saponifiable fraction was taken for radioactive counting. Another part was used to determine vitamin A by the SbCl_3 reaction. The remainder was dissolved in ethanol-acetone (1:1, v/v) and cholesterol was precipitated as the digitonide. Part of this precipitate was taken for radioactive counting, and part for colorimetric estimation of cholesterol, according to Nieft & Deuel (1948). The supernatant was diluted with water and extracted into ether. The ether extract, after washing with water, was evaporated to dryness and dissolved in light petroleum. This solution was then chromatographed on Decalco F, as described by Diplock, Edwin, Bunyan & Green (1961). Elution with light petroleum (50 ml.) gave a fraction containing all the squalene, which was purified for radioactive counting by reversed-phase paper chromatography. This fraction contained no detectable ubiquinone (absence of material reducible by potassium borohydride) nor was any other radioactive substance isolated from other areas of the paper chromatogram. Whatman no. 4 papers were pre-impregnated with ZnCO_3 containing sodium fluorescein (10 p.p.m.) and then impregnated with liquid paraffin. With aq. 95% ethanol as the mobile phase, a descending run of 6 hr. moved the squalene spot about 10 cm. It was detected by adding pure ubiquinone-45 as a marker (the two substances having identical R_f values in partition systems of this type) and viewing the dry chromatogram under ultraviolet light (λ_{max} , 256 m μ). Further elution of the column with 35% (v/v) diethyl ether in light petroleum (30 ml.) gave the ubiquinone fraction, in which ubiquinone-45 was measured after paper-chromatographic purification in a similar system (Diplock, Green, Edwin & Bunyan, 1960). Further elution of the column with diethyl ether gave an unidentified fraction, which also contained the radioactive label. In one experiment radioactive incorporation into the prenil pyrophosphate fraction was determined by the acid hydrolysis method of Christophe & Popják (1961). In some experiments, another sample of the pooled livers was used for nitrogen determinations by a semi-micro-Kjeldahl method.

Radioactive counting. ^{14}C was counted in the Ekco N 664A scintillation counter or the I.D.L. Tritomat scintillation counter. All counts were monitored for quench by the channel ratio method. Samples were prepared for counting by dissolving in the minimum volume of ethanol or, with the sterol digitonides, methanol (Shapiro & Kritchevsky, 1963). Counting efficiencies were 65–90%, according to the instrument used. Counts were always converted into disintegrations/min.

Experimental plan. Four experiments were carried out. In Expt. 1, male weanling rats were kept with their dams and fed on the laboratory stock diet until they weighed about 40 g. Six rats were divided into three groups of two. Group 1 was fed on the deficient diet AO2, group 2 was fed *ad lib.* on the same diet supplemented with 10 i.u. of vitamin A palmitate/g. of diet, and group 3 was supplemented similarly, but was pair-fed with the first group. After 40 days, each rat was given 1.7 μC of $[2\text{-}^{14}\text{C}]\text{mevalonic acid lactone}$ intraperitoneally and killed 22 hr. later. In Expt. 2, 18 weanling male rats were randomly sorted into three groups of six. Group 1 was fed on the deficient diet AO2, group 2 was fed on AO2 supplemented with 3 i.u. of vitamin

A palmitate/g. of diet and group 3 on the same diet supplemented with 100 i.u./g. After 7 days, three rats from each group were each given 1.45 μC of $[2\text{-}^{14}\text{C}]\text{mevalonic acid lactone}$ intraperitoneally and killed 24 hr. later. After a further 14 days, the remainder of the rats were treated similarly. In Expt. 3, 30 male weanlings were kept together with their dams on the laboratory stock diet until they were 28 days old. They were then randomly sorted into five groups of six and placed on the following regimens: group 1 was fed on the diet AO2; group 2 was fed on AO2, given 10 i.u. of vitamin A palmitate per day orally and pair-fed with group 1; group 3 was given the same supplement, but fed on the diet *ad lib.*; groups 4 and 5 were similar to groups 2 and 3 respectively, except that they were supplemented with 100 i.u. of vitamin A/g. of diet. Each rat was weighed daily. After 14 days on test, two rats from each group were each given 25 μC of $[2\text{-}^{14}\text{C}]\text{acetate}$ intraperitoneally. The remainder of the rats continued on test. Two rats from each group were each given 1.66 μC of $[2\text{-}^{14}\text{C}]\text{mevalonic acid lactone}$ intraperitoneally when two rats of group 1 had reached the plateau stage (no change in weight for 3 successive days). Finally, when the remaining two rats in group 1 had each lost 15–20% of their average plateau weight, the remaining rats of each group were also given 1.66 μC of $[2\text{-}^{14}\text{C}]\text{mevalonic acid lactone}$. All rats were killed 24 hr. after the administration of the radioactive dose. Expt. 4 was similar to Expt. 3, except that dams and their litters were maintained on diet AO2 until the weanlings were 28 days old, when the experiment was started. In this experiment, labelled mevalonic acid lactone was used throughout.

RESULTS

Expt. 1. The results are given in Table 1. Although these rats had had access to small amounts of vitamin A before weaning, 40 days was sufficient to deplete the livers of the animals in group 1. Their liver weights were correspondingly reduced, and liver protein (mg./g.) values in this group were higher than in the other two groups. The deficient livers contained more unsaponifiable lipid, more cholesterol and considerably more ubiquinone/g. than did the livers of the supplemented groups. There was no effect of pair-feeding on the ubiquinone concentration, but the cholesterol and vitamin A concentrations appeared to be depressed in group 2, as compared with group 3. Radioactive incorporation into ubiquinone was clearly increased in the deficient group, but, in contrast with Gloor & Wiss (1959a,b) and Phillips (1961), there was also a marked increase in incorporation into squalene. The effect on incorporation into cholesterol is not clear in this experiment, for it is apparent that the feeding regimen is the main factor affecting this.

Expt. 2. This experiment was an attempt to study isoprenoid synthesis in the early stages of vitamin A restriction, before growth has been seriously affected. The results are given in Table 2. After 7 days there was already a large difference in the vitamin A content of the livers of the three

groups. There was no difference in the ubiquinone concentrations of groups 1 and 2, but there was a clear depression of the concentration in group 3, which had received the higher vitamin A supplement. Cholesterol concentrations were unaffected. At 21 days, no vitamin A could be detected in the livers of deficient animals, but the ubiquinone concentrations were still similar in groups 1 and 2, and lower in group 3. There was no clear effect either of vitamin A deficiency or of graded supplementation with vitamin A on the incorporation of

mevalonate into ubiquinone; nor did the cholesterol/ubiquinone incorporation ratio show any clear trend. Squalene incorporation showed fluctuations but no trend, and neither did the cholesterol/squalene incorporation ratio.

Expt. 3. Table 3 gives the results of this experiment, which was designed to compare pair-feeding with uncontrolled feeding in more detail and to extend the range of study up to advanced vitamin A deficiency. It will be seen that, because the rats had access to the stock diet before weaning, their weight plateaus were reached relatively late (cf. Morton & Phillips, 1959). Even so, by 14 days there was a depression of the ubiquinone concentration in the livers of rats given the higher vitamin A supplement and fed *ad lib.* Livers from deficient rats that had reached their weight plateau or had lost weight showed the usual elevation in ubiquinone concentration, and there was a progressive diminution in this concentration as the dietary vitamin A supplement was increased. Once again, there was no effect of pair-feeding on ubiquinone concentrations at any stage of the deficiency. The approach of clinical deficiency was generally associated with increases in the concentrations of non-saponifiable lipid and cholesterol.

The interpretation of the radioactive incorporation results is rendered more difficult in this experiment, as the approach of clinical vitamin A deficiency is accompanied by a considerable shrinkage of the liver. To compare the absolute incorporation of one group of animals with that of another, it is probably justifiable to compare radioactivities per gram rather than per total organ, since the protein (mg./g.) values show little change regardless of age or of the stage of deficiency, and the liver weight as a proportion of the total body weight remains practically constant in all

Table 1. *Expt. 1: effect of vitamin A deficiency and pair-feeding on isoprenoid synthesis in rat liver*

Experimental details are given in the text. Group 1 was fed on the deficient diet; group 2 received a supplement of 10 i.u. of vitamin A/g. of diet and was pair-fed with group 1; group 3 was supplemented similarly and fed *ad lib.*

Group ...	1	2	3
Mean rat wt. (g.)	76	91	103
Mean liver wt. (g.)	3.34	5.01	5.13
Unsaponifiable fraction (mg./g.)	7.86	5.59	5.36
Ubiquinone ($\mu\text{g./g.}$)	386	135	139
Ubiquinone ($\mu\text{g./organ}$)	1290	675	715
Cholesterol (mg./g.)	3.72	1.55	2.57
Cholesterol (mg./organ)	12.4	7.75	13.2
Vitamin A (i.u./g.)	0	197	385
Protein (mg./g.) ($N \times 6.25$)	258	189	212
$10^{-3} \times$ Radioactive incorporation (disintegrations/min.)			
Ubiquinone/g.	3.4	0.84	1.0
Ubiquinone/organ	11.5	4.2	5.2
Cholesterol/g.	6.5	1.1	4.6
Cholesterol/organ	21.6	4.4	23.6
Squalene/g.	6.1	2.8	2.0
Squalene/organ	20.5	13.9	10.3

Table 2. *Expt. 2: effect of vitamin A restriction and supplementation on isoprenoid synthesis in rat liver*

Experimental details are given in the text. Group 1 was fed on the deficient diet; groups 2 and 3 were fed on the deficient diet supplemented respectively with 3 i.u. and 100 i.u. of vitamin A/g. of diet.

Expt. stage ...	After 7 days			After 21 days			
	Group ...	1	2	3	1	2	3
Mean liver wt. (g.)		3.03	2.54	2.93	4.63	3.62	5.03
Unsaponifiable fraction (mg./g.)		6.82	6.05	7.06	5.75	6.10	6.44
Ubiquinone ($\mu\text{g./g.}$)		161	180	135	167	152	105
Cholesterol (mg./g.)		2.62	2.09	2.49	3.38	2.86	3.16
Vitamin A (i.u./g.)		2.0	159	1560	0	93.1	2420
Protein (mg./g.) ($N \times 6.25$)		195	182	138	201	206	212
$10^{-3} \times$ Radioactive incorporation (disintegrations/min.) per organ							
Ubiquinone		3.2	3.2	0.97	1.3	2.3	2.4
Cholesterol		9.3	11.9	12.8	8.3	7.6	10.7
Squalene		8.6	8.2	2.4	4.5	6.9	4.5

Table 3. *Expt. 3: effect of three stages of vitamin A deficiency and pair-feeding on isoprenoid synthesis in rat liver*

Experimental details are given in the text. Group 1 was fed on the deficient diet; group 2 received a supplement of 10 i.u. of vitamin A/day and was pair-fed with group 1; group 3 received the same supplement but was fed *ad lib.*; groups 4 and 5 were similar to groups 2 and 3 respectively but received a supplement of 100 i.u. of vitamin A/g. of diet.

Expt. stage	After 14 days					Plateau					Post-plateau					
	Group	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Mean rat wt. (g.)	69	71	69	71	64	187	169	178	189	171	175	207	212	201	216	
Mean liver wt. (g.)	3.33	3.56	3.37	3.15	3.36	6.78	6.90	6.69	7.42	6.56	6.41	8.09	7.57	6.85	8.90	
Unsaponifiable fraction (mg./g.)	5.89	6.26	7.38	6.15	8.04	13.9	8.1	4.61	8.99	8.16	7.95	5.30	5.35	7.34	6.20	
Ubiquinone (μ g./g.)	107	89	107	95	78	132	68	82	54	62	180	87	103	55	49	
Cholesterol (mg./g.)	1.8	1.8	2.2	1.5	1.9	4.8	4.8	3.1	3.6	3.5	6.3	3.6	3.6	2.7	1.9	
Protein (mg./g.)	183	180	172	182	165	191	191	163	193	177	179	143	159	181	181	
Vitamin A (i.u./g.)	1.8	1.7	2.0	309	410	0	72	8	7.27	9.17	0	65	62	1740	1520	
$10^{-3} \times$ Radioactive incorporation (disintegrations/min.)																
Ubiquinone/g.	0.090	0.056	0.58	0.11	0.036	0.25	0.27	0.57	0.45	0.38	0.79	0.35	0.50	0.38	0.55	
Ubiquinone/organ	0.30	0.20	0.20	0.33	0.12	8.45	1.87	3.70	3.30	2.46	5.22	2.81	3.78	2.58	4.94	
Cholesterol/g.	1.22	1.72	1.53	3.68	2.30	3.89	5.35	4.68	5.11	6.11	7.80	5.40	5.20	3.50	4.40	
Cholesterol/organ	4.06	6.13	5.16	11.60	7.74	26.7	36.9	31.2	38.0	40.2	50.0	43.6	39.4	24.0	39.1	
Squalene (g.)	0.12	0.14	0.12	0.35	0.17	6.95	2.60	1.95	2.29	1.81	3.2	2.0	2.2	3.0	2.7	
Squalene (organ)	0.41	0.51	0.40	1.09	0.59	47.0	18.0	13.0	17.0	11.9	20.5	16.2	16.7	23.6	24.0	
• Ether eluate/g.	3.1	5.1	3.56	13.0	6.5	11.3	13.1	12.3	7.2	9.7	12.4	9.5	9.1	5.7	6.4	
• Ether eluate/organ	10.4	18.1	11.7	41.0	22.0	76.6	90.4	82.2	53.5	63.6	79.5	77.0	69.0	39.0	57.0	
• Prenol pyrophosphate/g.	2.4	3.4	3.7	5.3	4.0	0.49	0.37	1.02	0.46	0.40	0.41	0.38	0.56	0.28	0.42	
• Prenol pyrophosphate/organ	8.1	12.2	12.4	16.5	13.3	3.2	2.8	6.8	3.4	2.6	2.6	3.0	4.2	1.9	3.7	
Incorporation ratios:																
(a) Cholesterol/ubiquinone	13.6	30.6	36.0	34.5	65.0	3.1	19.8	8.2	11.4	16.4	9.7	15.6	10.4	9.3	8.0	
(b) Cholesterol/squalene	9.9	11.9	12.8	10.6	13.3	0.56	2.05	2.4	2.25	3.36	2.45	2.7	2.5	1.02	1.63	

groups beyond the 14-day stage (in agreement with Morton & Phillips, 1959).

Radioactive uptake into the ubiquinone fraction was considerably greater in deficient animals that had reached their weight plateau or had passed beyond it, compared with supplemented controls, whatever concentration of vitamin A or regimen of feeding was used for the latter. Incorporation into cholesterol (disintegrations/min./g. of tissue) showed no clear trend. At 14 days, the incorporation increased with vitamin A supplementation, and this was also found when the rats were compared at the plateau period; however, the reverse was the case later, and incorporation into the cholesterol/g. of tissue appeared to be elevated in deficient animals at the post-plateau stage. The small incorporation of radioactivity in the first part of this experiment is due to the fact that labelled acetate was the substrate (see the Experimental section). No essential differences in the pattern of distribution of radioactivity in the compounds studied was found when compared with the results with mevalonate as substrate. In contrast, uptake into squalene was greater in the deficient livers only at the plateau stage. Pair-feeding had little effect on the radioactive incorporation in this experiment. Table 3 gives the ratios of incorporation of ^{14}C into ubiquinone, cholesterol and squalene. They do not support the concept that vitamin A deficiency leads to a metabolic block between squalene and cholesterol.

Table 3 also gives the figures for the radioactive incorporation into the 'ether eluate' fraction from the Decalso column. This fraction was not characterized, but contains hydroxy compounds that do not form insoluble digitonides, and it includes free prenols such as farnesol. Uptake into prenol pyrophosphates is also given in Table 3. No effect of vitamin A deficiency was observed in either of these fractions.

Expt. 4. The chief difference between this experiment and the previous one was in the rate at which the deficiency occurred. Thus the two rats in group 1 reached their weight plateau at 28 and 30 days after being placed on the deficient diet. Pair-feeding markedly affected growth, and the livers from groups 2 and 4 were shrunken and similar in weight to those from group 1. Results are given in Table 4.

At 14 days there was little difference in the weights of the livers from the five groups, nor in the ubiquinone concentrations of rats from groups 1, 2 and 3. However, heavy vitamin A supplementation had, even in this short time, halved the ubiquinone concentrations in groups 4 and 5. Rats killed at the plateau and post-plateau stages illustrated clearly the two effects of vitamin A that were suggested by the previous experiments. The first effect (cf.

Table 4. *Expt. 4: effect of three stages of vitamin A deficiency and pair-feeding on isoprenoid synthesis in rat liver*

Experimental details are given in the text. Group 1 was fed on the deficient diet; group 2 received a supplement of 101 u. of vitamin A/day and was pair-fed with group 1; group 3 received the same supplement but was fed *ad lib.*; groups 4 and 5 were similar to groups 2 and 3 respectively but received a supplement of 1001 u. of vitamin A/g. of diet.

Expt. stage...	After 14 days					Plateau					Post-plateau				
	1	2	3	4	5	1	2	3	4	5	1	3	5		
Group.....	65	63	66	56	64	103	126	147	120	169	81	169	173		
Mean rat wt. (g.)	3.10	2.92	3.14	2.99	3.65	4.54	4.60	6.82	4.31	8.07	3.39	7.23	6.92		
Mean liver wt. (g.)	215	193	233	148	121	354	192	174	—	98	434	175	78		
Ubiquinone ($\mu\text{g./g.}$)	4.1	3.1	6.1	1750	2000	0	0	0	2700	2750	0	6.7	3950		
Vitamin A (1 u./g.)															
10 ³ × Radioactive incorporation (disintegrations/min.)															
Unasaponifiable fraction/g.	47.6	46.7	58.9	35.2	32.0	36.7	51.0	31.3	43.1	25.1	71.0	32.1	32.7		
Unasaponifiable fraction/organ	148	160	185	105	117	166	234	214	186	203	240	232	226		
Ubiquinone/g.	6.9	5.9	10.4	2.3	3.1	3.2	6.4	5.4	—	1.0	9.7	4.9	5.3		
Ubiquinone/organ	21.4	17.2	32.6	6.9	11.3	14.6	29.0	37.0	—	8.2	32.8	35.4	36.3		
Cholesterol/g.	34.2	24.2	31.7	22.6	19.7	29.0	30.8	21.0	30.8	19.3	40.6	17.5	16.2		
Cholesterol/organ	106.7	71	100	68	72	131	141	103	133	156	137	126	112		
Squalene/g.	4.8	6.8	5.5	6.1	3.1	6.8	10.6	6.9	1.8	7.0	10.3	7.3	8.75		
Squalene/organ	14.8	19.8	17.3	18.2	11.4	30.6	48.7	47.1	30.3	14.4	35.0	52.6	60.5		
Radioactive incorporation distribution in each fraction (% of unsaponifiable fraction)															
Ubiquinone	14.5	12.6	17.7	6.5	9.7	8.8	12.4	17.3	—	4.0	13.7	15.3	16.0		
Cholesterol	71.8	51.8	54.0	64.1	61.5	79.0	60.5	67.2	71.4	77.0	57.4	54.5	49.6		
Squalene	10.0	14.5	9.4	17.2	9.8	18.4	20.8	22.0	16.3	7.1	14.5	22.7	26.8		
Total recovery (% of disintegrations/min. in unsaponifiable fraction)	96.5	81.4	91.2	98.7	90.9	106.3	93.7	106.5	—	88.1	85.6	92.5	92.4		

groups 1, 2 and 3) is due to the vitamin deficiency and is reflected in a change in the liver ubiquinone concentration that is, it should be noted, unrelated to the organ's shrinkage, for it is unaffected by pair-feeding. Since the animals of groups 2 and 3 received just sufficient vitamin A to ensure adequate growth but no detectable liver storage, this effect is not directly related to the concentration of vitamin A in the liver. In groups 4 and 5 a second effect manifests itself, and there is a further marked depression (even at 14 days) of the ubiquinone concentration. This is apparently related to the presence of large amounts of vitamin A in the tissue: it is unrelated to the size of the animal or the weight of the liver.

The radioactive incorporation into the various fractions show considerable fluctuations but no clear trend. The distribution of radioactivity in ubiquinone, cholesterol and squalene as a percentage of the total incorporation into the non-saponifiable lipid is shown in Table 4. Although there is an indication that excessive vitamin A supplementation has suppressed incorporation of ¹⁴C into ubiquinone at the 14-day stage, incorporation into cholesterol and squalene show no such trend. At the plateau and post-plateau periods, vitamin A had no clear effect on the distribution pattern.

DISCUSSION

These results confirm the picture of the effect of vitamin A deficiency on ubiquinone in rat liver as described by Morton & Phillips (1959). The concentrations of ubiquinone we have observed correspond with theirs, when rats of similar age and at a similar stage of deficiency are compared, and we also have found increases in the sterol content of deficient livers. The ubiquinone concentrations found by Phillips (1962) are in general much higher than ours, but were not obtained on chromatographically pure fractions.

Although, in the four experiments described, we have varied the stage of the deficiency, the rate of producing the deficiency, the feeding regimen and the vitamin A supplementation, we have been unable to confirm the results of Gloor & Wiss (1959*a,b*) or Phillips (1961) on the effect of vitamin A on isoprenoid synthesis. Although the measurements of radioactivity were made 24 hr. after administration of the labelled substrate and may therefore partly reflect factors such as further metabolism and mobilization of labelled compounds, Phillips (1961) used the same conditions, and Gloor & Wiss (1959*a*) found their results to be essentially the same even when the radioactive dose of mevalonate was given over 4 days. It would appear from our results that such fluctuations as occur are not specific and are influenced by other factors,

including the feeding regimen. Differences in analytical method may partly account for the differences in the findings; thus Wiss & Gloor (1960) also indicate that if the ubiquinone fraction of rat liver is isolated by paper chromatography, then vitamin A deficiency is not found to affect radioactive uptake into the fraction. Furthermore, our results show that, although a trend may be apparent in one part of an experiment, this can be readily reversed at the later stage, or by a change in conditions, or in a succeeding experiment. Our findings therefore are in general agreement with the conclusions of Johnson & Wolf (1960) that there is no specific effect of vitamin A on isoprenoid synthesis at the metabolic level.

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