156. THE EFFECT OF VITAMIN E DEFICIENCY ON THE VITAMIN A RESERVES OF THE RAT

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In early work in this laboratory on vitamin E deficiency in the rat a basal diet was used in which vitamin A was supplied as cod liver oil (*ca.* 1000 I.U. per g.). The oil was given at the rate of about 70 mg. per rat weekly. Since from the condition of some of the animals it was suspected that this level was not adequate, livers were taken at autopsy and the vitamin A in them was assayed by the $SbCl_3$ method. The vitamin A reserves were found to be absent or low. It is questionable whether at this comparatively low level of intake of vitamin A any other result was to be expected, but in subsequent experiments the vitamin A allowance was increased to 17 mg. weekly of halibut liver oil (*ca.* 60,000 I.U. per g.). Determinations of liver reserves were continued. The results of these determinations, which have been reported in preliminary papers [Moore *et al.* 1939; Moore, 1939], are now given in detail. They indicate that in deficiency of vitamin E the vitamin A reserves of the liver may be much reduced.

Opportunities arose in other experiments to compare the vitamin A reserves of rats having diets low in protein, with or without simultaneous deficiency of vitamin E. In contrast with the marked and consistent reduction of the vitamin A reserves in vitamin E deficiency the differences ascribable to protein deficiency were irregular and slight, and never passed beyond the range of difference in body weight found in the groups receiving normal and low protein diets.

The efficiency of the conversion of carotene into vitamin A in avitaminosis-E was also investigated and a slight reduction in the amount of vitamin A formed was found under the experimental conditions adopted.

Experimental

Rats were killed by coal gas after being kept for varying periods on a diet deficient in vitamin E (light white casein 25%, cane sugar 50%, lard 10%, salt mixture 5%, dried yeast 10%). The animals used as positive controls received orally either a concentrate of vitamin E from wheat germ oil supplied by Glaxo Laboratories, Ltd., or a solution in arachis oil of *dl*-α-tocopherol or its acetate kindly presented by Roche Products, Ltd. At autopsy the livers were digested with alkali, the oils extracted with ether and alcohol, and their vitamin A was determined by the $SbCl_3$ method [Davies, 1933]. To convert blue units into I.U. the approximate factor of 0·6 has been used [Moore, 1937].

Vitamin A allowance. Except where otherwise stated vitamin A was given orally by pipette as one drop (17 mg.) of halibut liver oil per rat per week. The oil was kindly supplied by Allen and Hanburys, Ltd., and contained 60,000 I.U. of vitamin A per g. The vitamin A intake as halibut liver oil was therefore about 1000 I.U. per week. In addition the rats given wheat germ oil concentrate probably received small amounts of carotene from this source. No attempt was made to

identify carotene in the concentrate, but a colorimetric determination of the total epiphasic pigments indicated a maximum carotene content of about 2000 I.U. per g., or 100 I.U. in the weekly allowance. Since the epiphasic pigments may have included artifacts and other pigments inactive as provitamins it seems probable that this estimate is unduly high.

The effect of wheat germ oil concentrate on vitamin A reserves (Exp. 1, Table 1). Groups of female albino rats were restricted to the diet deficient in vitamin E at a mean initial weight of 68 g. and were killed 56 weeks later. The deficient rats were by then in a state of advanced avitaminosis-E, with severe paralysis of the hind legs and deep brown uteri [Martin & Moore, 1936; 1938; 1939]. The positive control animals received 50 mg. of wheat germ oil concentrate daily and were in good health.

Table 1. *The storage of vitamin A by rats with and without vitamin E (wheat germ oil concentrate)*

	Exp. 1							
	− vitamin E				+ vitamin E			
	Wt. of rat g.	Wt. of liver g.	I.U. of vitamin A		Wt. of rat g.	Wt. of liver g.	I.U. of vitamin A	
			Total	Per g. liver			Total	Per g. liver
	126	6·0	6,000	1000	215	12·0	19,000	1600
	145	7·5	9,000	1200	210	9·0	26,000	2900
	150	10·8	15,000	1400	237	12·0	30,000	2500
	144	10·4	15,000	1400	223	13·5	30,000	2200
Means	141	8·7	11,300	1250	221	11·6	26,300	2300

The vitamin A reserves of the rats given vitamin E were invariably much higher than those of the rats deficient in vitamin E. The positive control group had a mean total vitamin A reserve more than twice as great as that of the deficient group. The reserve per g. of liver for the control group was almost twice as much as the corresponding value for the deficient group.

Exp. 2, Table 2. Rats were given the diet deficient in vitamin E at a mean weight of 37 g. and were killed 33 weeks later. Control animals received weekly doses of wheat germ oil concentrate. In parallel experiments similar groups of

Table 2. *The storage of vitamin A by rats with and without vitamin E (wheat germ oil concentrate) and with normal and reduced protein allowances*

		Exp. 2							
		− vitamin E				+ vitamin E			
		Wt. of rat g.	Wt. of liver g.	I.U. of vitamin A		Wt. of rat g.	Wt. of liver g.	I.U. of vitamin A	
				Total	Per g. liver			Total	Per g. liver
Normal casein		170	9·0	2000	130	179	9·2	12,000	1300
		163	10·6	1500	140	204	10·6	15,000	1400
		153	7·9	1500	190	185	9·8	15,000	1500
	Means	162	9·2	1400	153	189	9·9	14,000	1400
Low casein		95	5·5	600	110	112	9·1	12,000	1300
		66	4	600	150	110	6·6	12,000	1800
		80	5·2	750	140	(88)*	(3·1)*	(7,500)*	(2400)*
	Means	80	4·9	650	133	111	7·8	12,000	1550

* Died after 203 days; value excluded in taking mean.

rats received a modification of the usual basal diet in which the caseinogen was reduced to 3%, the difference in energy value being made up by additional sugar.

The findings at autopsy were as follows: *Normal casein+vitamin E*: uteri white, good intraperitoneal fat reserves. *Normal casein−vitamin E*: uteri brown, sparse fat reserves. *Low casein+vitamin E*: rats undersized, white uteri, sparse fat reserves. *Low casein−vitamin E*: rats undersized, small brown uteri, very sparse fat reserves.

The mean vitamin A reserves, both total and per g., were about 10 times greater in the groups given vitamin E concentrate than in the deficient groups. Casein deficiency had a relatively small effect of doubtful significance. The group given a normal allowance of casein without vitamin E had total reserves about twice as great as those given a low allowance of casein without vitamin E. When the reserves are calculated per g. of liver this difference disappears.

Exp. 3, Table 3. This was in principle a repetition of Exp. 2. The rats were kept on a slightly modified diet deficient in vitamin E and also low in vitamin A (light white casein 25%, rice starch 50%, lard 20%, salts 5%+dried yeast 15%) from lactation until they were about 10 weeks old and had a mean weight of 124 g. They were then divided into groups, transferred to the standard vitamin E-free diet and treated exactly as in the preceding experiment. The findings at autopsy after a further 32 weeks were as follows: *Normal casein +vitamin E*: large fat reserves, uteri white. *Normal casein−vitamin E*: sparse fat reserves, uteri brown. *Low casein+vitamin E*: sparse fat reserves, uteri white. *Low casein−vitamin E*: very sparse fat reserves, small brown uteri.

Table 3. *The storage of vitamin A by rats with and without vitamin E (wheat germ oil concentrate) and with normal and reduced protein allowances*

		Exp. 3							
		−vitamin E				+vitamin E			
				I.U. of vitamin A				I.U. of vitamin A	
		Wt. of rat g.	Wt. of liver g.	Total	Per g. liver	Wt. of rat g.	Wt. of liver g.	Total	Per g. liver
Normal casein		164	8·4	1200	140	249	12·5	15,000	1200
		164	10·0	1800	180	238	12·0	15,000	1300
		164	8·8	1800	200	264	13·2	18,000	1400
	Means	164	9·1	1600	173	250	12·6	16,000	1300
Low casein		125	8·4	1200	140	156	9·6	7,500	780
		107	8·2	1500	180	149	9·0	7,500	840
		115	8·0	1500	190	132	8·0	9,000	1100
	Means	116	8·2	1400	170	146	8·9	8,000	906

The vitamin A reserves were again much lower in the deficient groups than in those given vitamin E. The casein allowance had no effect on the vitamin A reserves in the groups deficient in vitamin E. In the groups given vitamin E the total reserves were about twice as great with a normal casein allowance as with a low allowance, but this difference disappeared when the results were calculated per g. of liver.

The effect of tocopherol on vitamin A reserves (Exp. 4, Table 4). The rats were given the modified diet deficient in vitamin E from weaning until attaining an age of about 8 weeks. One drop of halibut liver oil weekly was given during

this period. The rats were then transferred to the standard vitamin-E free diet, 6 groups of 2 animals each being formed. Four groups were given weekly doses of 3, 1, 0·3 and 0·1 mg. of tocopherol dissolved in arachis oil. The remaining groups received 50 mg. of arachis oil and 50 mg. of wheat germ oil concentrate respectively. For reasons of convenience some of the rats were killed 24 weeks and some 29 weeks after the commencement of the administration of tocopherol.

Table 4. *The effect of graded doses of* dl-α-*tocopherol on the storage of vitamin A by the rat*

Exp. 4

Weekly dose of vitamin E mg.	Duration of exp. weeks	Wt. of rat (initial and final) g.	Wt. of liver g.	Colour of uterus	Vitamin A reserve I.U. Total	Vitamin A reserve I.U. Per g. liver
Tocopherol 3	24	106–198	10·8	White	12,000	1100
	24	129–219	10·2	White	15,000	1500
				Means	13,500	1300
Tocopherol 1	29	95–196	10·3	White	12,000	1200
	29	122–230	12·7	White	15,000	1200
				Means	13,500	1200
Tocopherol 0·3	29	109–229	10·4	Brown	6,700	650
	29	125–240	13·8	Brown	7,500	550
				Means	7,100	600
Tocopherol 0·1	29	113–198	8·7	Brown	3,700	430
	29	121–231	10·8	Brown	6,000	560
				Means	4,850	495
Nil	24	128–175	11·6	Brown	3,300	280
	24	112–169	11·8	Brown	6,000	510
				Means	4,650	395
Wheat germ oil concentrate 50	24	132–221	10·0	White	15,000	1500
	24	113–208	9·8	White	15,000	1500
				Means	15,000	1500

The uteri of rats in the groups given 3 and 1 mg. of tocopherol or 50 mg. of wheat germ oil concentrate weekly were normal in colour. The uteri of rats given 0·3 and 0·1 mg. of tocopherol or plain arachis oil were brown, and the vitamin A reserves for these groups were only ¼ to ½ of those found in the groups receiving adequate allowances of vitamin E.

It may be noticed that the reserves of the rats given wheat germ oil concentrate were not significantly greater than those of the groups given 3 and 1 mg. of tocopherol. This affords evidence that the ability of wheat germ oil concentrate to promote the storage of vitamin A, as demonstrated in the present and preceding experiments, was due to the vitamin E which it contained and not to the presence in it of unsuspectedly large amounts of carotene, or to its contamination with vitamin A.

The effect of tocopherol on the conversion of carotene into vitamin A (*Exp. 5, Table 5*). The rats were kept on the modified vitamin E-deficient diet from weaning until attaining an age of about 16 weeks. Six groups of 4 rats each were then kept on the standard vitamin E-deficient diet. Three groups were given 1 mg. of tocopherol per rat weekly, the other groups received no vitamin E. This treatment was continued for 98 days. During the last 18 days of this period doses of 1, 0·5 and 0·25 mg. of carotene were given to paired groups with and without tocopherol.

The carotene was purchased from The British Drug Houses, Ltd., and dissolved by warming in lard. A total volume of 1 ml. of carotene solution and lard was given mixed with a little solid diet before the main ration was fed; it was always completely eaten. Since the carotene was not highly purified and showed some fading during the period of the administration the amounts of pigment actually received by the rats must have been smaller than those calculated. Any effects due to this cause must, however, have been equalized between the paired groups at each level.

Table 5. *The effect of* dl-*α-tocopherol on the conversion of carotene into vitamin A*

Exp. 5

	−tocopherol					+tocopherol				
	Wt. of rat g.	Wt. of liver g.	Discoloration of uterus	I.U. of vitamin A Total	I.U. of vitamin A Per g. liver	Wt. of rat g.	Wt. of liver g.	Discoloration of uterus	I.U. of vitamin A Total	I.U. of vitamin A Per g. liver
1 mg. of carotene daily	163	7·5	+ + +	600	80	178	9·3	+	825	89
	154	10·0	+ + +	750	75	178	8·8	+ +	825	94
	151	8·0	+ + +	750	94	189	9·0	+	900	100
	168	10·2	+ + +	980	96	167	8·5	0	1050	123
Means	159	8·9		769	86	178	8·9		900	101
0·5mg. of carotene daily	161	9·4	+ + +	225	24	198	8·6	+ +	337	39
	138	8·0	+ + +	225	28	178	7·0	+	410	59
	162	7·5	+ + +	225	30	199	9·8	+	413	42
	168	9·7	+ + +	300	31	161	8·4	0	413	49
Means	157	8·7		244	28	184	8·5		394	47
0·25 mg. of carotene daily	131	7·6	+ + +	38	5	167	7·6	+ +	38	5
	146	7·5	+ + +	75	10	183	8·5	+ +	94	11
	158	7·5	+	113	15	175	7·8	+	132	17
	168	9·4	+ + +	131	14	206	9·9	+	169	17
Means	151	8·0		89	11	185	8·5		108	13

0 = normal. + = slightly discolored. + + = pale brown. + + + = medium, or deeper brown.

The vitamin A reserves determined at autopsy were found to be graded between the groups receiving different levels of carotene without a single instance of overlapping. This applied equally to groups with and without tocopherol. The differences in the vitamin A reserves ascribable to the administration of tocopherol were much less marked than in the previous experiments on the storage of preformed vitamin. Although slight, however, the differences were always in favour of the groups which had received tocopherol. The most marked difference between vitamin A reserves was found in the paired groups receiving 0·5 mg. of carotene daily. The mean total reserve for the group receiving tocopherol was 62% greater than the corresponding value for the untreated group.

In Table 5 it may be noted that in some of the rats which had been given tocopherol the uteri were slightly discolored. This may be attributed to the length of the period of the modified basal diet before the commencement of dosing with tocopherol. A rough superficial correlation between the height of the reserve and the freedom of the uterus from discoloration may be traced, and if data relating to rats with decidedly pale brown uteri are omitted in calculating the means the differences between the corresponding groups are slightly increased.

The storage of vitamin A in rats after the curing of prolonged avitaminosis-E (Exp. 6). Martin & Moore [1939] reported that rats which have been subjected to prolonged deficiency of vitamin E may sometimes be restored to good general condition, but not cured of paralysis, by treatment with large amounts of wheat germ. Two male rats were given the standard diet deficient in vitamin E for 12 months. The animals at this period were in a state of advanced avitaminosis-E, with paralysis. Their body weights were 184 and 180 g. After 60% of wheat germ had been added to the diet for a further 12 months the general condition of the rats had much improved, and the body weights were increased to 220 and 218 g., although the paralysis was little affected. The rats were then killed and found to have total vitamin A reserves of 30,000 and 38,000 I.U. respectively, or 2300 and 2900 I.U. per g. of liver. In the absence of evidence as to the extent of the reserves of companion animals killed before the commencement of the cure, no definite conclusion can be drawn from these results. It appears very probable, however, that treatment with wheat germ restored to normal the power of the liver to store vitamin A. It has been shown in a previous paper [Moore & Rajagopal, 1940] that even the inclusion of 60% of wheat germ in a diet otherwise free from vitamin A does not allow the accumulation of measurable stores of vitamin A in the liver.

Discussion

In all the above experiments in which halibut liver oil was given the vitamin A reserves were always much lower in rats deprived of vitamin E than in animals subjected to exactly the same treatment except for the addition of vitamin E. The degree of disparity varied in different experiments. In Exp. 1 the reserves of rats given vitamin E were about twice as high as those of deficient animals; in Exps. 2 and 3 about ten times, and in Exp. 4 about three times as high. No explanation of these differences in the degrees of disparity can at present be offered. In work by Bacharach [1940] confirming this effect of vitamin E deficiency in lowering the vitamin A reserves the differences noted between the treated and untreated groups were much smaller, amounting to only some 50% difference between means in favour of the treated group. In his work however the rats were kept on the deficient diet for a shorter period.

The efficiency of storage of the ingested vitamin A, neglecting the small contribution of carotene in the groups receiving wheat germ oil concentrate, may be calculated as follows:

% of ingested vitamin A found in the liver

	−E		+E
Exp. 1	20		46
Exp. 2 normal casein only	4·2		42
Exp. 3 normal casein only	5		50
Exp. 4 no tocopherol	14	3 mg. tocopherol	40

In the groups given vitamin E the rate of storage of 40–50% points to high efficiency, particularly in view of the prolonged periods during which the earlier doses of vitamin A must have been kept in the livers before they were examined. Without vitamin E the efficiency of storage in Exps. 2 and 3 was very poor. It must be pointed out that both in the present work and in that of Bacharach [1940] the dietary intake of vitamin A was large, and that even in the groups deficient in vitamin E the reserves of vitamin A were not very low in an absolute sense. Experiments at lower levels of dietary intake of vitamin A are obviously needed.

In the experiment on the conversion of carotene the differences between the groups treated and not treated with vitamin E were comparatively small, but greater differences might have been found if the duration of the experiment had been extended and if dosing of the control group had been started earlier.

The cause of the low vitamin A reserves found in vitamin E deficiency is obscure. It may be recalled, however, that the storage of vitamin A in the human liver may be greatly reduced in certain diseases, and notably in chronic nephritis [Moore, 1937]. The association of the reduced power to store vitamin A with the kidney injury which is always found after prolonged deficiency of vitamin E [Martin & Moore, 1936; 1938; 1939] may therefore be significant. The possibility that vitamin A might be lost in the urine of the deficient rats, as in the human subject in chronic nephritis, was investigated, but with negative results. It may be noted, however, that the rat does not seem to excrete vitamin A in its urine in any circumstances, whereas the dog, as I have been able to confirm, excretes the vitamin even when in normal health [Catel, 1938, 1, 2]. The apparent discrepancy may therefore be due to difference of species.

The question of what factors other than vitamin E deficiency may depress the vitamin A reserves is obviously of great interest. Since in the present work it was found that deficiency of protein caused at the most only a slight depression of the vitamin A reserves it is obvious that the phenomenon is not a general non-specific effect of malnutrition. Dann & Moore [1931] failed to detect any marked depression of the vitamin A reserves in rats kept for many weeks on diets deficient in the vitamin B complex. In hens deficient in vitamin K Tomaszewski & Engel [1939] found that neither the vitamin A nor vitamin C reserves were lowered. On the other hand, it is obvious that vitamin E deficiency is by no means unique in depressing the vitamin A reserves. Goerner [1937] has shown that the injection of carcinogenic agents into rats may cause marked reduction in the vitamin A reserves of the liver. The possibility of injury of the liver of the rat in vitamin E deficiency must be kept in mind in view of the observations of Barrie [1939], but no consistent evidence of liver injury was found in the present work.

The reduction of vitamin A reserves in vitamin E deficiency may afford an explanation of the rapid dispersal of the vitamin A reserves in rats observed in one particular experiment by Davies & Moore [1934]. Numerous attempts by the authors to repeat this experiment have given most inconsistent results, and it seems probable that the rapid loss of vitamin A in the experiment reported may have been due to an unsuspected deficiency of vitamin E in the basal diet. Preliminary experiments have indicated that the vitamin A reserves of rats deficient in vitamin E fall rapidly when the animal is restricted to a diet deficient in both vitamins A and E, but detailed publication of the results may await the completion of controlled experiments.

Summary

1. The vitamin A reserves of rats kept for prolonged periods on a diet deficient in vitamin E in which vitamin A was supplied as halibut liver oil were always much lower than those of control animals receiving supplements of vitamin E.

2. After dosing with carotene, the differences between the amounts of vitamin A formed by rats treated and not treated with vitamin E were relatively slight. This may have been due in part to the particular experimental conditions adopted, which resulted in the differentiation between adequacy and deficiency of

vitamin E being less clear cut than in the experiments on the storage of preformed vitamin A.

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