CXXX. VITAMIN A AND THE THYROID

By GEORGE LOGARAS¹ AND JACK CECIL DRUMMOND

From the Department of Biochemistry, University College, London

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THERE is a considerable amount of evidence indicating that the action of thyroxine on metabolism is antagonized by vitamin A or by carotene. The effect was first observed by Euler & Klusmann [1932], who found that carotene partly protected rats from losing weight on the administration of thyroxine. They also obtained indications that thyroxine reduced the storage of vitamin A and carotene in the liver. Small numbers of animals were used in these experiments and it is unfortunately true of many other publications on this subject that the experimental procedure can be adversely criticized on similar grounds; Abelin [1933], for example, used only two guinea-pigs in each group when he studied the effect of thyroxine on the fate of carotene injected intraperitoneally. Nevertheless, a survey of the literature as a whole leaves the impression that a case has been made out for the view that there is antagonism between the actions of vitamin A and thyroxine.

Some of the more significant observations may be noted. Chevalier & Baert [1934] found that the basal metabolic rate of rats tended to rise on vitamin A depletion and to fall below normal when large doses were given. Abelin [1935, 1] showed that appropriate dosage with the vitamin will counteract to a marked extent the influence of thyroxine on the B.M.R., and his observations were confirmed by Rappai & Rosenfeld [1935]. Fasold & Peters [1933] found that they could relieve symptoms of thyroid overdosage by administering vitamin A and *vice versa*. Fleischmann & Kann [1936] found that vitamin A antagonized the effect of thyroxine on acetonitrile poisoning. Perhaps the most striking claims are those which record improvement in patients suffering from thyrotoxicosis after treating with large doses of vitamin A [Wendt, 1935; Dietrich, 1936].

In the course of a study of the factors influencing storage of vitamin A in the liver and its mobilization it was necessary to investigate the influence of the thyroid, particularly because there is a certain amount of evidence, not entirely convincing, it must be admitted, in the literature that thyroxine administration causes a depletion of the vitamin A reserves [Abelin, 1933; Fasold & Peters, 1933; Wendt, 1935 etc.]. The opportunity was taken to make some studies of the influence of thyroxine and vitamin A on the metabolic rate, using sufficient animals to yield significant results.

EXPERIMENTAL

Young rats of 68–80 g. weight from a homozygous strain (Glaxo-Wistar) were divided into three groups. All were fed on a basal "A-free" diet consisting of casein, 10; meat-meal, 10; wheat-flour, 59.5; yeast extract (yeastrel), 8; Hubbell *et al.* [1937] salt mixture No. 351, 2.5; and olive oil 10 parts. One group, 32 in number, served as a control. The 35 animals of another received twice weekly a dose of 35 I.U. vitamin A, which was estimated to be a little more than their requirements for growth, whilst the third group, consisting of 37 rats, received 3000 I.U. daily.

¹ Holder of a Rockefeller Foundation Fellowship.

Table I. *Basal metabolism ml. O₂/kg./min.*

All figures represent the mean of triplicate estimations. The figures in brackets are the reference numbers of individual animals

Group	Vitamin A-free diet				10 i.u. vitamin A daily				3000 i.u. vitamin A daily			
	(a)	In- jections*	(b)	% rise	(a)	In- jections*	(b)	% rise	(a)	In- jections*	(b)	% rise
Control	(73) 21.3	7	26.23	23.1	(52) 20.6	7	28.42	37.9	(2) 23.33	7	29.11	24.8
	(94) 21.78	9	31.16	46.2		8	28.94	40.4		7	28.16	20.7
Thyroxine: (a) before, (b) after		11	33.05	55.1						8	26.82	15.8
	(74) 22.6	7	25.93	14.7	(68) 23.3	7	33.58	44.1		9	26.04	14.1
		11	37.62	66.4		11	38.0	63.1	(3) 22.64	7	24.41	7.6
	(100) 21.44	7	27.15	26.1	(69) 20.66	7	35.08	69.8		9	28.29	24.9
		9	34.10	58.6		9	31.24	51.2	(15) 23.24	11	31.72	39.9
		11	35.90	67.4						7	27.25	17.2
										9	29.09	25.2
Dinitrophenol: (a) before, (b) after, 5 mg. per kg.	(93) 21.95		32.38	47.5	(80) 20.48		28.12	37.3	(a)		(b)	% rise
	(95) 22.87		31.64	38.1	(67) 21.82		32.56	49.2	(28) 22.88		35.14	53.5
	(101) 22.78		34.32	50.6	(62) 23.38		30.08	28.6	(16) 22.48		30.87	37.3
Dinitrophenol: (a) before, (b) after, 10 mg. per kg.	(93) 21.95		41.82	90.4	(67) 21.82		46.87	114.8	(17) 23.01		40.00	73.8
	(101) 22.78		41.15	80.7					(28) 22.88		44.97	96.5
									(16) 22.48		49.32	119.3

* Injections of 100 γ.

The vitamin was supplied in the form of a concentrate containing approximately 1,700,000 I.U. per g. A measured volume of a solution of this in olive oil was administered to the animals daily from an "Agla" micro-syringe.

Basal metabolic rate

Basal metabolism determinations were made after the animals had reached a weight of about 150 g. Most of them attained this weight in 30 days. The apparatus and method described by Richards & Collison [1928] were found very suitable for the purpose. After 80 days on the diets the rats in each group were divided into three subgroups. One lot were left as they had been to serve as controls. Another group was given 19 doses of 100 γ of thyroxine spread over 28 days, administration being by subcutaneous injection. The third group received subcutaneous injections of 2:4-dinitrophenol, 11 injections at the level of 5 mg. per kg. and subsequently 4 injections at 10 mg. per kg. Basal metabolism studies were made on these animals, care being taken in the case of those dosed with dinitrophenol to make the determinations about half an hour after an injection. Food was withheld for 18 hr. before a B.M.R. determination was made. Representative results are shown in Table I.

The figures show a clear effect of heavy dosage with vitamin A in counter-acting the effect of thyroxine treatment. No such action was detectable when the intake of vitamin was just sufficient to supply the requirements of the animal. There is no evidence of such an antagonistic action between the vitamin and 2:4-dinitrophenol. This is an additional piece of evidence differentiating the action of thyroxine and that of the thermogenic drugs of the dinitrophenol class. The latter are known to act by influencing directly the oxidative mechanisms of the cells, whereas the precise mode of action of the thyroid hormone still remains obscure.

The results of the B.M.R. estimations are supported by records of the effects of the various treatments on the weight of the animals. The figures in Table II confirm the observations of other workers who have recorded that vitamin A tends to protect against the loss of weight which thyroxine treatment causes.

Table II. *Change of weight in g. in 28 days*

Vitamin A I.U. per day	Control	Thyroxine	Dinitrophenol
Nil	♂ + 7.7 ♀ + 7.1	- 11 - 1.6	- 3.3 + 3.0
10	♂ + 15.7 ♀ + 11.6	- 17.5 + 0.4	+ 13.0 + 8.2
3000	♂ + 21.0 ♀ + 7.0	- 8.6 + 2.6	+ 13.4 + 9.6

The possibility that a response by the adrenal cortex is evoked by prolonged administration of large doses of vitamin A is now being investigated.

Storage of vitamin A in the liver

All the animals employed in this experiment were killed and the reserves of vitamin A in the liver estimated by measurement of the intensity of selective absorption in the ultra-violet at 328m μ . The livers of the animals which had been fed on the "A-free" diet for 110 days were found, as was expected, to contain no measurable amount of the vitamin. The second group of animals, each of which received 10 I.U. vitamin A daily, also showed no detectable

storage, presumably because that amount was just about the minimum required for satisfactory growth.

The third group, dosed with the larger amount of 3000 I.U. per day, showed storage. The estimations are set out in Table III.

Table III. *Rats reared on diet supplying 3000 I.U. vitamin A daily*

I.U. $\times$ 1000 present in whole liver			
	Controls	Thyroxine	Dinitrophenol
♂	75.7	79.2	102.0
	65.5	134.4	105.6
	106.8	103.3	103.3
	64.8	111.0	91.8
	57.0	76.0	88.4
	55.2	72.7	93.8
	—	79.2	78.6
Mean	70.8	93.7	94.8
♀	52.8	80.3	100.8
	64.8	61.5	81.6
	66.6	85.2	66.6
	68.0	77.5	84.0
	33.6	81.3	84.0
	67.2	—	—
Mean	58.8	77.2	83.4

Calculated on the basis of the amount of vitamin A in 100 g. of liver the differences are still apparent.

I.U. $\times$ 1000 vitamin A per 100 g. liver					
Control		Thyroxine		Dinitrophenol	
♂	♀	♂	♀	♂	♀
26.3	35.0	41.8	47.0	35.4	48.2

In spite of the wide variation the differences in Table III are significant and indicate that the stimulation of cellular metabolism, whether caused directly by dinitrophenol or indirectly by the administration of thyroxine, does not accelerate the depletion of reserves of vitamin A in the liver but rather tends to conserve them.

Our results do not support the view that thyroxine increases the utilization or decreases storage of vitamin A [Euler & Klusmann, 1932; Fasold & Peters, 1933; Wendt, 1935].

It is too early to discuss the possible significance of this observation, but, meanwhile, it is of interest to note that Wolff [1932] and Moore [1937] have recorded that the liver reserves in human cases of hyperthyroidism are usually higher than the normal level.

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