CCIII. THYROXINE AND HYPERVITAMINOSIS-A

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A CONSIDERABLE literature has developed suggesting that the thyroid gland may be specifically involved in the metabolism of vitamin A. An antagonism between thyroxine and carotene was first suggested by Euler & Klussman [1932], who found that superficially the two substances had opposite actions on the growth of rats deficient in vitamin A. Abelin [1933] reported a decrease of vitamin A in the livers of guinea-pigs receiving thyroxine. The administration of thyroxine reduced the amount of carotene in the blood of drakes [Parhon & Werner, 1935]. In human beings suffering from hyperthyroidism, the amount of carotene and vitamin A in the blood was found to be relatively low [Wendt, 1935, 1], comparable with the low amounts found in patients with deranged fat metabolism. Thyroxine administered to rats on a diet low in vitamin A accelerated the depletion of vitamin A reserves, as determined by the onset of ophthalmia [Sure & Buchanan, 1937, 2] or by the vaginal smear technique [Greaves & Schmidt, 1936]. Thyroidectomized rats developed symptoms more slowly. Fasold & Heidemann [1933] found that the milk of thyroidectomized goats was unusually yellow, suggesting failure in the conversion of carotene into vitamin A.

It has also been reported that thyroxine decreases the symptoms of hypervitaminosis-A, and that vitamin A likewise decreases the symptoms of hyperthyroidism [Fasold & Peters, 1932]. However, olive oil or sesame oil could replace vitamin A in counteracting thyrotoxicosis. Vitamin A reduced the basal metabolic rate of rats receiving excess thyroxine [Rappai & Rosenfeld, 1935; Abelin, 1935; Abelin *et al.* 1936; Logaras & Drummond, 1938], and the vitamin also reduced the basal metabolic rate of human patients suffering from hyperthyroidism [Wendt, 1935, 2] along with the amelioration of other symptoms [Wendt, 1936; Dietrich, 1936; Fasold, 1937]. When vitamin A was administered to a child suffering from hyperthyroidism Catel [1938], however, found that the lowered level of vitamin A in the blood was raised, but that the abnormal basal metabolic rate and other symptoms of the disease were not affected.

In the Reid Hunt reaction in mice, excess of vitamin A reduced the protective action of thyroxine against the toxicity of acetonitrile [Fleischmann & Kann, 1936]. In the development of salamander larvae or axolotls both vitamin A and carotene have been reported to counteract the normal effect of thyroxine in stimulating the metamorphosis of these organisms [Fleischmann & Kann, 1936; Rokhlina, 1936].

The administration of vitamin A is said to affect the amount of colloid in the thyroid gland [Sherwood *et al.* 1934] and histological changes in the gland have been attributed both to vitamin A deficiency [Mitzkewitsch, 1934; Coplan & Sampson, 1935] and to vitamin A excess [Votila, 1938]. Apparently vitamin A also counteracts the effects of the thyrotropic principle of the pituitary, since, when large amounts of the vitamin were given, those histological changes in the thyroid gland ordinarily elicited by the hormone failed to appear [Schneider, 1934; Fellingner & Hochstaedt, 1936]. Decreased stores of hepatic vitamin A were observed both in rats and guinea-pigs after the administration of the thyrotropic

hormone [Schneider & Wiedemann, 1934]. Logaras & Drummond [1938], however, found that the administration of thyroxine to rats caused increased liver reserves of vitamin A.

By many of the workers mentioned above the existence of a specific inter-relationship between vitamin A or carotene and thyroxine has been accepted as an established fact. However, there is a certain amount of evidence which appears to contradict this view. For example, analysis of the livers of human beings suffering from "thyroid diseases" showed an increase in vitamin A content [Moore, 1937] rather than a decrease, as might be expected if a true antagonism existed between thyroxine and vitamin A. Four cases of exophthalmic goitre had reserves of 180, 300, 375 and 375 I.U. per g. of liver, as compared with the median reserve in accidental death of 220 I.U. per g. Wolff [1932] reported a similar increase in hepatic vitamin A in the same disease. (Mean for five cases of exophthalmic goitre 210 I.U. per g., for accidental death 147 I.U. per g.) Furthermore, hyperthyroidism is accompanied by excessive excretion of calcium [Parhon, 1912; Heath *et al.* 1925; Aub *et al.* 1929], and in hypervitaminosis-A there is also a negative calcium balance [Harris, 1933] which proceeds until the bones are weakened to such an extent that spontaneous fractures occur [Bomskov & Seeman, 1933; Collazo & Rodriguez, 1933; Davies & Moore, 1934]. It is difficult to visualize a true antagonism between two agents producing in this instance similar physiological responses.

Finally, several groups of workers [Cowgill & Palmieri, 1933; Sure & Buchanan, 1937, 1; Drill & Sherwood, 1938; Peters & Rossiter, 1939] have shown that large amounts of vitamin B₁ protect rats against loss in weight caused by excessive doses of thyroxine.

These findings at least indicate that caution is necessary before the inter-relationship between vitamin A and thyroxine is regarded as specific. The experiments described below were done to test the question of the interrelationship of vitamin A and thyroxine when both are given in doses so large as to be toxic, but not rapidly fatal. Under these conditions no interrelation could be established.

EXPERIMENTAL

(i) *The effect of excessive amounts of thyroxine and high vitamin A alone and combined in adult rats*

The effect of large doses of thyroxine was studied in rats receiving excessive amounts of vitamin A. In the first experiments adult stock female rats, approximately one year old, were used. They had received a mixed diet rich in vitamin A and carotene, and at the commencement of the experiment had very high reserves of vitamin A (*ca.* 30,000 I.U.). They were divided into four groups of 8 rats each, and were fed on a basal ration low in vitamin A previously used in this laboratory [Davies & Moore, 1935]. The dry ingredients consisted of "light white" casein 200, sugar 450, salt mixture 50 and yeast 125 (total 825). 82 parts of the dry ration were mixed with 30 parts of an oily solution containing 298 g. of coconut oil and 2 g. of radiostol (vitamin D). The high fat diet was used to facilitate the absorption of vitamin A. The rats were grouped as follows.

Group I received the low-vitamin A diet plus an excess of vitamin A. The vitamin was given as a concentrate from halibut liver oil, prepared by the British Drug Houses, Ltd. It was dark brown, viscous, and smelled of ionone. The absorption band at 328 m μ was narrow and intense, $E_{328\text{ m}\mu}^{1\%} = 732$. If the absorption of pure vitamin A, $E_{328\text{ m}\mu}^{1\%} = 1800$ [Mead, 1938] our concentrate contained 40% of vitamin A by weight. For administration the concentrate was diluted

with 4 parts of arachis oil and fed by dropper, each rat receiving 4 drops daily. Since each drop weighed 25 mg. the rats received $25 \times 4 \times 20\% = 20$ mg. of concentrate daily, equivalent to 8 mg. of vitamin A. Solutions of the concentrate were made up weekly and were stored in a refrigerator. For our purpose it was not feasible to feed the vitamin A mixed in the basal ration, because of the inequality of food consumption between individuals and between groups.

Group II received the basal diet plus 8 mg. of vitamin A daily plus 0.6 mg. of thyroxine injected subcutaneously three times per week. The thyroxine (Hoffmann-La Roche) was dissolved in *N*/100 NaOH, neutralized with a few drops of *N*/100 HCl until the first appearance of cloudiness in the cold. It was diluted to a concentration of 2 mg. per ml. The solutions were made up fresh each week; they were stored in the cold and were warmed to 37° before injection.

Group III received the basal diet only.

Group IV received the basal diet plus thyroxine.

The basal ration was fed *ad libitum* in all cases, and the food intake of the various groups was recorded.

Results. All animals receiving either thyroxine or excess of vitamin A, or both, showed an immediate decrease in weight (Fig. 1) and a decreased food intake (Table I). There was no evidence that the thyroxine counteracted the effects of hypervitaminosis-A, or vice versa. Animals receiving thyroxine plus high amounts of vitamin A showed the greatest decrease in food intake of all groups and the most rapid loss in weight. The effects of excess of vitamin A and of excess of thyroxine therefore appeared to be additive rather than antagonistic in this experiment.

Symptoms of hypervitaminosis-A appeared in Group I (vitamin A alone) and Group II (vitamin A with thyroxine) between the fifth and tenth days of administration of the vitamin concentrate, confirming previous observations [Drigalski, 1933; Davies & Moore, 1934]. The animals were limp and emaciated. Their hair was rough and their posterior parts were smeared with urine. They sat in a hunched position. The eyes were swollen and in a few cases encrusted. There was loss of hair about the mouths, which were sore and inflamed, as if the vitamin concentrate were irritating. Four out of 6 rats in Group II (vitamin A with thyroxine) died during the first 9 days. The remainder died on the seventeenth and twenty-eighth days. No rats died in any of the other groups. Rats receiving the high vitamin A diet showed symptoms of hypervitaminosis throughout the experiment, but survived. Rats receiving the basal diet were normal in every way; rats receiving the low vitamin A diet plus thyroxine showed no gross symptoms other than a steady continuous decrease in weight.

Four animals in Group I (vitamin A) were killed on the tenth day, and their livers were found to contain from 60,000 to 150,000 I.U. of vitamin A as measured by the Carr & Price [1926] test. The livers of animals of Group II, which had died from the combined effects of thyroxine and hypervitaminosis-A, contained similar amounts of vitamin A, the values ranging from 60,000 to 180,000 I.U.

There was, therefore, no significant difference in the vitamin A contents of the two groups of animals. Thyroxine failed to decrease the amount of vitamin A stored in the liver under these conditions. Rats which survived for more than 30 days on the high vitamin A diet showed from 150,000 to 360,000 I.U. per liver.

A curious effect of thyroxine on the food intake of rats was noted—both in the presence and absence of excess of vitamin A. The injection of thyroxine markedly decreased the food intake for the first 10 days of administration, after which there was a sudden increase in food intake as compared with controls on the same diet (Table I). These results have been duplicated repeatedly. They clarify

certain inconsistencies in the literature, inasmuch as thyroid has been reported both to increase [Schafer, 1912; Hewitt, 1914, 2] and to decrease food intake [Hewitt, 1914, 1; Kojima, 1917].

Fig. 1. Exp. 1. The additive action of thyroxine and excess vitamin A. Effects of excessive vitamin A and thyroxine separately and combined on the body weight of rats. Curves for rats in Group I, which were killed after 10 days for vitamin A determinations, are not shown.

Table I. *The effect of excess thyroxine and vitamin A upon the food intake of rats*

	Food intake per rat per day (g.)			
	I High-vitamin A diet	II High-vitamin A + thyroxine	III Control diet	IV Control diet + thyroxine
First 5 days	3.8	1.9	9.0	5.3
Second 5 days	6.4	3.0*	7.0	4.6
Third 5 days	7.5	—	6.5	12.6
Fourth 5 days	4.9	—	5.8	11.5
Fifth 5 days	6.7	—	5.5	12.2
Sixth 5 days	6.0	—	6.8	13.8

* Only 2 out of 6 alive.

(ii) *Thyroxine and high vitamin A in growing rats*

The failure to demonstrate an antagonism between thyroxine and excess of vitamin A in adult rats led us to attempt similar experiments with young, growing animals, similar to those used by Fasold & Peters [1932]. Young rats, 4 weeks of age, 50–60 g. in weight, were given 2 drops daily of the vitamin A solution as before (= 10 mg. B.D.H. concentrate daily = 4 mg. vitamin A per rat per day). The basal diet was the same high fat ration used in the preceding experiment. Group food intakes were recorded.

Five rats received the high vitamin A diet alone; 5 received the high vitamin A diet plus thyroxine. A third group received the basal low vitamin A ration plus

0.2 mg. thyroxine daily, the dose being increased by 0.2 mg. daily according to the scheme of Fasold & Peters until a level of 1.0 mg. per day was reached. The fourth group received the low-vitamin A ration plus injections of water.

The animals receiving the high vitamin A diet only showed some indications of toxic symptoms. Within one week their fur became rough and matted; after 3 weeks the mouths of the animals became sore, growth ceased and a loss in weight followed. All animals in this group survived for 6 weeks, when the experiment was terminated.

Animals receiving the high vitamin A diet plus thyroxine, according to Fasold & Peters, stopped growing after the first week and died between the fourteenth and nineteenth days of the experiment. Instead of protecting the animals against the harmful effects of the vitamin, as claimed, the administration of thyroxine hastened death.

The animals receiving the thyroxine survived the 6 weeks of the experiment. Their rate of growth, however, was less than that of the rats receiving injections of water; they averaged 120 g. in weight at the end of the experiment, compared with 146 g. for the control animals. As with the adult animals, the administration of thyroxine caused an immediate decrease in food intake, followed after one week by a marked increase in food intake as compared with the control group.

DISCUSSION

The evidence which has been advanced to show a relationship between thyroxine and vitamin A has been derived from a field too wide to permit of comprehensive discussion in a paper dealing with only one aspect of the problem. There would seem ample grounds, however, for believing that in the abnormal metabolic states caused by inadequacy or excess of thyroxine, corresponding abnormalities occur in the metabolism of carotene and vitamin A. It is a more open question how far these abnormalities imply a specific relationship between the two substances. Disturbances in vitamin A metabolism occur in other diseases, e.g. in chronic nephritis, in which low liver reserves are usually found, and probably in diabetes, in which the reserves are high [Moore, 1937]. Stringent proof would appear to be necessary before a specific and constant, as opposed to an accidental and occasional, relationship between vitamin A and thyroxine is assumed.

One of the most suggestive points in favour of a specific relationship has been the reported ability of thyroxine to counteract the ill effects of excess of vitamin A, and vice versa, which has been re-investigated with negative results in the present work. It is possible that our failure to observe this effect may have been due to some minor difference between our experimental conditions and those adopted by Fasold & Peters [1932]. In a complicated condition in which the various body tissues come under the toxic influence of two distinct agents it appears that effects might be additive or subtractive according to the nature of the tissues and the dosages employed. The experiments of Logaras & Drummond [1938] in which beneficial effects were produced by large but non-toxic allowances of vitamin A in rats given moderately excessive amounts of thyroxine, represent a limited confirmation of the claims of Fasold & Peters. They do not deal, however, with the question of the neutralization of the excessive doses of vitamin A and thyroxine which given separately would both be highly toxic. While, therefore, our experiments do not rule out the possibility that under some conditions vitamin A and thyroxine may neutralize each other, they suggest that such antagonism is neither as specific nor as universal as hitherto supposed.

The effect of thyroxine in temporarily decreasing and then increasing the food intake is readily explicable if it is assumed that the action of this hormone is

primarily confined to the catabolic side of metabolism. The increase in food intake is presumably a secondary phenomenon and is probably delayed until the organism has had time to adjust itself to the increased metabolic rate.

SUMMARY

When injected into rats receiving excess of vitamin A, thyroxine failed to counteract the effect of hypervitaminosis. Animals receiving both thyroxine and excess of vitamin A ate less, lost weight more rapidly, and died earlier than those receiving either agent alone. The injection of thyroxine produced a temporary decrease in food intake, followed by a marked increase. No evidence was obtained for a specific antagonism between thyroxine and vitamin A.

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