CHAPTER - V

DISCUSSION

D I S C U S S I O N

Thyroid hormones are essential for growth and metabolism. But the actual mechanism of action of the thyroid gland is not yet clearly known. However it is understood that hundreds of enzymes are activated by the thyroid hormones. These hormones are also responsible for the protein synthesis and are also responsible for the metabolism of almost all vitamins.

Among the fat soluble vitamins vitamin A is under a typical biphasic control exerted by the thyroid hormones. Both in hypothyroidism and hyperthyroidism the patients are defective in dark-adaptation. In hypothyroidism, there is accumulation of carotene in blood due to non-conversion of carotene to vitamin A. Thyroid hormone is necessary for this conversion and its administration in hypothyroidism restores the conversion to normal levels.

Patients with hypothyroidism frequently have a characteristic yellow pigmentation of certain areas of

the skin (Mandelbaum et al, 1942). In early experimental studies, decreased absorption of carotene by the intestine has been reported in thiouracil-induced hypothyroidism (Cama and Goodwin, 1949). Storage of vitamin A in the liver in such situation may also be reduced (Johnson and Baumann, 1947; Kishore et al, 1971). On the other hand, excess vitamin A is reported to cause hypothyroidism, reduction of metabolism, reduction of growth and metamorphosis (Logaras and Drummond, 1938; Sadhu and Brody, 1947 and Ahmad et al, 1980).

In the present work, the transport and metabolism and also some of the functions of carotene, retinol, retinal and retinoic acid have been studied in relation to thyroid status.

Transport and metabolism of carotene in thyroid disorders :

In the present series, it was found that the level of carotene in hypothyroid human subjects was distinctly higher ($P < 0.001$) and in hyperthyroid subject it was significantly lower (P 0.001) (Table-II).

After oral administration of beta-carotene in normal, hypothyroid and hyperthyroid subjects the levels of beta-carotene in serum was studied and interesting observations were obtained. Twenty four hours after oral administration the level of beta-carotene were increased to about 3 - 4 folds in all subjects of all the groups. Again after 72 hours

the levels were markedly reduced in control and hypothyroidism, but the rate of reduction in hypothyroid subjects were comparatively very slow. But the results remained statistically highly significant ($P < 0.001$) when compared to pre-treatment levels. After 120 hours the levels came down to normal pre-treatment levels in all subjects except in the hypothyroid subjects. In these patients the levels remained very high which was statistically highly significant ($P < 0.001$) when compared to the pre-treatment levels (Table- IV). Literatures are almost silent regarding such observations.

In animal experimentations, no beta-carotene was found in fasting rat intestine. But 24 hours after oral administration of beta-carotene it remained mostly in the contents of the intestine. Very small amount was found in the mucosa and traces were found in the muscle layers. After oral administration of beta-carotene no significant alteration of vitamin A was observed in the serum.

The vitamin A content of the liver was increased in all the experimental conditions. But the enhancement was higher in control and hyperthyroidism ($P < 0.001$) and lower in hypothyroid rats (Table-VII).

That the thyroid gland might be involved in the metabolism of carotene was first observed by Von Noorden in 1907

when he found carotenemia to be associated with metabolic disturbances. Afterwards Wendt (1935) noted that patients with Grave's disease had very low serum vitamin A levels, even though their intake of carotene was equal to the normal individual. Similarly Wohl and Feldman (1939) observed that hypothyroid patients often showed a poor dark adaptation, and concluded that thyroid gland was in some way responsible for carotene metabolism. Excamilla (1942) and Mandelbaum et al (1942) observed a carotenemia associated with myxoedema and both the conditions had the tendency to clear up under treatment with thyroid substance.

Likewise, laboratory experiments have pointed out towards the conclusions that a functioning thyroid gland is necessary for the animal to convert carotene into vitamin A. Kunde (1926) observed xerophthalmia in thyroidectomized rabbits that had been fed an adequate diet containing enough carotene for the vitamin A requirement of the animals. Fasold and Heidemann (1933) reported that vitamin A in the milk was decreased and carotene content was increased in thyroidectomized goats. Drill and Truant (1947) showed that carotene failed to prevent xerophthalmia in thyroidectomized rats, although vitamin A supplementation was found to prevent this lesion.

Abedin (1933) conversely reported that thyroxine may

affect the metabolism of carotene and vitamin A, but guineapigs rendered hyperthyroids by administration of thyroid substance could not metabolize carotene or store vitamin A as did the normal animals.

From our experiments it has been noted that intestinal contents of carotene after oral administration of beta-carotene was very high in hypothyroid rats in this series (Table-VII). It may be due to less absorption or due to less excretion in comparison to normal and hyperthyroid animals, because it is known that hypothyroid status reduces the intestinal motility. No such observation of beta-carotene metabolism in thyroid disorder was reported earlier.

In carotene tolerance test, as may be called, it was found that after 120 hours the carotene level in serum was very high after oral dose (Table-IV). No such early report is also available. From these set of experimental observations it can be concluded that carotene metabolism is sluggish in the absence of thyroid hormone both in animal and human beings.

Transport and metabolism of vitamin A in thyroid disorders :

Vitamin A in serum :

Both in hypothyroid patients and in hypothyroid animals the serum level of vitamin A was higher than normal and in

hyperthyroid disorders it was lower (Table- I, III & VI). These observations are in agreement with others (Cama and Goodwin, 1940; Walton et al, 1965; Smith and Goodman, 1971; Smolle et al, 1983).

The thyroid hormones exert selective and specific effect on vitamin A metabolism at several distinct sites. Intestinal absorption, transport in serum, uptake by tissue, intracellular transportation, storage and excretion all are affected in thyroid disorders. To pronounce merely that thyroid hormones increase the vitamin utilization would be a gross over-simplification.

Metabolism of vitamin A is also perhaps affected in the retina leading to defective dark adaptation in both hypo-and hyperthyroid conditions (Walton et al, 1968; Pearlman and Crescitelli, 1971).

Estimation of serum retinol after oral administration of vitamin A was done in human subjects. It was found that after oral administration of vitamin A the serum level of retinol was increased to a very high level after 24 hours and gradually came down almost to normal after 120 hours in all subjevts except in hypothyroid disorders. In hypothyroids, the level still remained at about 1½ fold high than pre-administration

level. It was perhaps due to less utilization and / or mobilization of vitamin A in hypothyroidism. This vitamin A tolerance test was first reported by the present investigators (Mandal and Ghoshdastidar, 1985a); otherwise the literature is silent in this part. From these observations of the utilization or tolerance tests of vitamin A in thyroid disorders it can be presumed that there is definite alteration of systemic function caused by malutilization of vitamin A.

In normal subjects vitamin A level is increased after oral administration. This was reported also by others (Srikantia and Reddy, 1970; Willett et al, 1983). Srikantia and Reddy (1970) reported very high level of serum vitamin A after oral dose which is in agreement with the present observation.

Vitamin A in liver :

In the present series of experiment, it was observed that the vitamin A content of the liver in hypothyroid rats was highest when compared to normal and hyperthyroid animals. But 24 hours after oral administration the liver vitamin was increased by 10 to 20 folds in different experimental animals (Table - VI).

Before the experiments the liver vitamin A was 6.42 I.U., 8.08 I.U. and 3.50 I.U. per gram of tissue in

control, hypothyroid and hyperthyroid condition respectively, while after oral administration of vitamin A the vitamin A content of the liver was increased to 63.28 I.U., 78.03 I.U. and 64.17 I.U. respectively (Table- VI).

Vitamin A in testis :

In testis the vitamin A content was 2.00 I.U. in normal, 1.92 I.U. in hypothyroid animals and 2.15 I.U. in hyperthyroid animals but after oral administration the contents were increased to 3.08 I.U., 2.40 I.U. and 2.64 I.U./gm of tissue respectively in normal, hypothyroid and hyperthyroid animals (Table-VI). This increment in hypothyroids is significantly lower when compared to normal or hyperthyroid animals.

Vitamin A in Kidney :

Almost similar effect was found in the kidney. But the amount of vitamin A in kidney is slightly higher than testis (Table-VI).

Vitamin A in Lung :

In the lungs also the vitamin A was increased after oral administration, but the increased accumulation was comparatively poorer in hypothyroidism (Table-VI).

Such report of the variation of vitamin A in testis, lung or kidney after oral administration in thyroid disorders is not available in literature although the variation in the vitamin A in liver was reported earlier by Johnson and Baumann (1947).

Vitamin A in Intestine :

In the intestine huge quantity of vitamin A remains in the contents 24 hours after oral administration, although no vitamin A could be found in the intestine before oral dose (Table-VI).

Vitamin A in subcellular fraction :

The mean liver retinol was estimated in subcellular fraction (Table-XI). The cytosol contained the highest amount of retinol in euthyroid, hypothyroid and hyperthyroid animals. The amount of retinol was increased markedly in microsome and cytosol in hypothyroid animals. These may be due to less utilization. But in the mitochondria the amount is less than normal which may be due to less metabolism in the mitochondria leading to lesser permeability of the mitochondrial membrane. However in experimental hyperthyroidism the retinol was increased in the mitochondria perhaps due to increased permeability of the mitochondrial membrane, but

there was no significant change in microsome while it was decreased in cytosol perhaps due to excess utilization in the presence of excess thyroid hormone.

In the nuclear fraction no significant change or retinol could be observed.

These observations of subcellular retinol in different experimental conditions could not be compared with others because of scanty literature on this aspect.

Transport and metabolism of retinal in thyroid disorders :

After oral administration vitamin A-aldehyde (retinal) high amount of the drug remained in the intestinal content. Retinol and retinyl ester were also found in the contents only after oral administration of retinal in normal and hyperthyroid animals but not in hypothyroid animals. But in hypothyroid animals traces of retinol only could be detected in the contents (Table-VIII).

In the mucosa and muscles of the intestine retinol and retinal and retinyl esters were detected in control and hyperthyroid rats, while in hypothyroid rats only retinal and retinyl esters could be measured in mucosa (Table-VIII).

In the serum and liver, both the retinyl ester and retinol were increased manyfold in all animals in all the experimental conditions which are statistically highly significant ($P < 0.001$) when compared to those of the animals before oral administration of retinal. Besides, retinal which could not be found normally have been measured in the blood and liver after oral retinal administration (Table-VIII).

Transport and metabolism of retinoic acid :

In the present work it was found that 24 hours after oral retinoic acid administration the content of the intestine reserved high amount of retinoic acid in all experimental animals (Table-IX). The amount was much higher in hypothyroid animals ($P < 0.05$), but in hyperthyroid animals the amount was comparatively lower ($P < 0.001$) than the control group animals.

No retinoic acid could be detected in the intestine of rats before oral dose of retinoic acid.

In intestinal mucosa and muscle and in blood and liver also some amount of retinoic acid could have been detected after the oral administration of the drug (Table-IX).

Reports are scanty regarding the metabolism of retinoic acid in thyroid disorders. So comparable data are not available.

Bliss (1951) and subsequently Zachman and Olson (1961) concluded that the liver alcohol dehydrogenase is responsible for NAD dependant oxidation of vitamin A alcohol to retinene and that is a reversible reaction. Mahadevan et al (1962) demonstrated that alcohol dehydrogenase of rat liver can be separated from another enzyme (retinal oxidase) that catalyses the oxication of retinene to vitamin A acid.

The oxidizing and reducing enzymes for retinal were measured in different subcellular fractions of liver tissues. Both types of the enzymes were increased in all fractions in hyperthyroid animals and were decreased in hypothyroid animals (Table X).

From these observations it is obvious to assume that vitamin A aldehyde metabolism is badly affected in thyroid disorders. Formation of retinoic acid as also retinol is thus decreased in hypothyroidism, and increased in hyperthyroidism (Table-X). So the functions of vitamin A as well as allied enzymes are affected significantly in the thyroid disorders.

Vitamin A acid or retinoic acid was shown to have vitamin A activity and can support the growth in rats (Vandrop and Arnes, 1946). However it could not be detected in liver of rats dosed with the acid (Vandrop and Arnes, 1946; Sharman, 1949). It is also reported that vitamin A acid can replace

vitamin A in general metabolism of the rat except vision (Dowling and Wald, 1960). More recent observations, however, indicated that vitamin A acid failed to maintain reproduction performances in male and female rats in the absence of vitamin A (Thompson et al, 1961 a,b). It is also being increasingly recognized that the classical vitamin A alcohol is not the actual active form of the vitamin for general metabolic need of the animals and the consequences of the opinion is that the active compound might be the retinoic acid.

In hypothyroidism there is alteration of the metabolism of vitamin A and allied compounds with accumulation of vitamin A and carotene in blood with production of symptoms and signs of those of hypo-vitaminosis A (Mandal and Ghoshdastidar, 1985 a,b). Moreover,there is defective formation of retinene from retinol in hypothyroidism (Ingbar and Woeber, 1981). In hyperthyroids there is increased utilization of vitamin A and carotene leading to lower amount of vitamin A and carotene in blood (Mandal and Ghoshdastidar, 1985 a,b). Besides carotenemia and low vitamin A level were reported by others (Walton et al, 1965; Smith and Goodman, 1971). It seems that the vitamin A and allied compounds are utilized more in hyperthyroidism which might be the reason of smooth skin in hyperthyroidism instead of rough skin in hypothyroidism.

Possibly only known function of retinene in animals physiology is in the visual system. Again formation of retinene is affected in hypothyroidism leading to delayed dark adaptation. Besides several other deficiency signs common to both vitamin A or thyroid deficiency are hyperkeratosis, night blindness, ataxia, decreased skeletal growth and maturation, anaemia, impaired sexual development and abortion (Mahadevan et al, 1962; Majia et al, 1979; Scott et al, 1982; Lassiter and Edwards, 1982).

From the above results it seems that both vitamin A alcohol dehydrogenase and retinene oxidase, the vital enzymes for vitamin A metabolism, are increased in hyperthyroidism and reduced in hypothyroidism. It seems again that the retinoic acid or some other compounds derived from it is the active form of vitamin A. The growth of the brain and body which is affected in hypothyroidism may be due to inadequate formation of retinoic acid. Many of the deficiency signs of hypothyroidism could have been caused by deficiency of retinoic acid, since retinoic acid can substitute vitamin A for growth and development and for skin formations (Dowling and Wald, 1960). In hypothyroidism there is accumulation of vitamin A and carotene in blood due to non-utilization (Mandal and Ghoshdastidar, 1985 a,b) which might be the reason for the signs of the deficiency of thyroid hormones.

From these observations it appears to be clear that retinoic acid at least in part helps in growth in the absence of thyroid hormone. It is also presumed that in growth of body and brain thyroid hormone works not only by synthesizing protein and activating enzymes but also by producing retinoic acid. It is already reported that growth hormone is increased by the retinoic acid (Nutrition Rev. 1989) and also it was reported that retinoic acid and thyroid hormone act on the same receptor site (Umesone et al, 1988 and Edeo et al, 1989).

The growth of the animals in the hypothyroid group was depressed in the present experiment. This observation is in agreement with the findings of Hughes (1944) and Scow and Simpson (1945). The evidence is clear that the animals given propylthiouracil were suffering from chronic thyroid deficiency. It was also found that growth of the brain was also reduced in them. This is also in agreement with Scow and Marx (1945) and Eayrs and Taylor (1951). In the present work, it was also found that retinoic acid helps growth in the deficiency of thyroid hormone during the treatment with propyl thiouracil. Again in other group of experiments, it was found that retinoic acid help growth of brain and body in hypothyroid animals. Though this growth is lesser than normal but higher than control hypothyroid animals (Table-X & XII).

Another interesting finding was that after oral administration of retinoic acid, the vitamin A levels in the plasma is

lowered (Table-XII). From this it may be assumed that retinoic acid controls the serum level of vitamin A by a feed-back mechanism.

From these observations it seems that thyroid hormone act through the retinoic acid as second messenger. Thyroid hormones act directly on the cellular level and activate the enzymes (Hoch, 1968 a;b). But it may be thought that it also helps growth at least to some extent by producing adequate amount of retinoic acid.

Our observations corroborates and strengthen the previous findings of others. Retinoic acid plays an important role in development (Thaller and Eichele, 1987), cell differentiation (Breitman et al, 1980), and induction of specific gene expression (Petkovich et al, 1984; Moore et al, 1984; Imai et al, 1988). Retinoic acid has been shown to influence cell differentiation and alteration of specific oncogene expression as well as transcriptional activity of several genes in normal cells (Moore et al, 1984; Griep and Deluca, 1986; Ann et al, 1988). Moreover, retinoic acid has recently been shown to induce expression of cellular mRNA which encodes for a protein containing a homeobax sequence domain (LaRosa and Gudas, 1988). Homeobax protein have recently been implicated in specific DNA binding regulating the transcription of growth hormone (Bodner et al, 1988; Ingraham, 1988). Furthermore, Morita et al (1989) reported that

retinoic acid selectively stimulates growth hormone secretion and messenger RNA levels in rat pituitary cells.

Recent studies on the analyses of steroid receptors for the understanding of molecular details of the transcriptional control and the individual transacting factors led to the identification of a super family of regulatory proteins that include receptors for thyroid hormone and the vertebrate morphogen retinoic acid (Evan, 1988).

Moreover, retinoic acid is reported to enhance the growth promoting activity of epidermal growth factor in mucosa $_3$T3 cells (Underwood, 1984) and human fibroblasts in culture (Harper and Savage, 1980). Umesomo et al (1988) described that retinoic acid and thyroid hormone induce gene expression through a common responsible element. Again Bedo et al (1989) postulated that retinoic acid had the ability to regulate growth hormone gene expression.

From the previous statements (Umesomo et al, 1988; Bedo et al, 1989) and the present observations in this series it seems that the growth of the animals can also be mediated by retinoic acid due to the excess production of growth hormone under its influence.

Bedo et al (1989) pointed out that one of the best known conseqeences of vitamin A deficiency is growth retardation.

They hypothesized that this effect may be caused by insufficient production of growth hormone, possibly exacerbated by decreased availability of thyroid hormone (Nutr. Rev. 1989).

Present investigation shows that oral administration of retinoic acid cannot influence growth in the presence of thyroid hormone but in the status of poor activity of thyroid it influences the growth. From these observations it seems that in the presence of thyroid hormone an adequate amount of retinoic acid is formed which helps the growth of the animals. It may be postulated, therefore, that part of the function of thyroid gland is through the retinoic acid formation.