CHAPTER 8

TYROSINE LEVEL IN THE SERUM AS AN INDEX OF THYROID ACTIVITY IN STURNUS ROSEUS, DURING PRE MIGRATORY PERIOD

Thyroid hormone, which is known to influence the oxidative processes in the cells of several tissues, regulates the rate of metabolism in the body. Apart from its profound action on the metabolism of fat, carbohydrate and protein, thyroid hormone is also known to exert its influence on fertility and reproduction, normal renal function, vitamins and even other endocrine glands (Rall et al.,1964). Because of its marked effect on several physiological processes the thyroid activity in the migratory as well as non-migratory species of birds during different seasons and phases of life cycle has been extensively studied (Davis and Davis,1954; Oakeson and Lilley,1957; 1960; Grosvenor and Turner, 1960; Shellabarger et al.,1961; George and Naik,1964; John,1967).

Several phenomena in birds were reported to be thyroxine induced. Thus, it was shown that thyroxine has a stimulatory effect in producing "Zuguruhe" or the migratory restlessness (Wagner,1930; Merkel,1937; 1938; 1958). Similarly there were suggestions that the thyroid hormone influences moult (Hohn,1950), reproductive activity (Benoit,1950; Maqsood,1952) and migration (Farner,1955). There are several reports that show increased activity of this gland in birds during cold seasons (Oakeson and Lilley,1957; 1960). However, the role

of thyroid in the migratory activities is still not clearly established. The fact, whether thyroid hyperplasia or increased production of thyroxine occurs or not, prior to migration, itself was in the domain of uncertainty. Recently, histological studies of George and Naik (1964) on the migratory starling, Sturnus roseus and of John (1967) on the Wagtails, Motacilla alba and M.flava, proved beyond doubt that thyroid hyperplasia does occur in the premigratory period in these birds. Estimation of protein bound iodine in the thyroid gland (George and Naik,1964) also supported this. On the basis of histological studies, they suggested that, the sudden release of colloid two or three days prior to migration, together with accompanied neurosecretory discharge (George and Naik,1965) and adrenocortical hyperactivity (Naik and George,1963; 1965a) might induce the migratory restlessness and subsequently trigger the migration itself. However, whether the accumulation of thyroid secretion in the gland was accompanied by a gradual release of the hormone into the blood throughout the length of premigratory period before its total release just prior to migration could not be ascertained from their histological studies. Determination of thyroxine or other factors that may denote a higher concentration of thyroxine in the blood would be necessary to show such phenomenon.

Recently, Sós et al.(1961) and Levine et al.(1962) both independently observed that the plasma tyrosine level showed an increase in patients with thyrotoxicosis and hyper-

thyroidism. Since then, a number of studies on the tyrosine metabolism have been reported in patients with thyroid diseases (Malmon et al.,1964; Rivlin and Levine,1963; Rivlin et al.,1965). Malamos et al.(1966)and Siersback-Nielsen (1966) conclusively proved that the tyrosine level in the plasma could be taken as an index of thyroid activity.

If the tyrosine level in plasma varies with the activity of the thyroid gland, an increased level of tyrosine could be expected in the migratory bird at the time of increased thyroid hyperplasia. The present investigation on the level of tyrosine in the blood (serum) during the premigratory period was undertaken to shed light on the dynamics of thyroxine and the various physiological changes it brings about as a prelude to the migratory flight.

MATERIALS AND METHODS

The birds were shot in the field and blood samples were collected immediately into test tubes by cardiac punture. The samples were subsequently transfered to chilled containers. They were brought to the laboratory and centrifuged. The pooled serum was then deproteinized with equal volume of 30% TCA. Determination of tyrosine was then carried out by the method of Udenfriend and Cooper (1952).

Two ml. of the deproteinized serum was transfered to a glass stoppered centrifuge tube and 1.0 ml. each of nitroso-naphthol and nitric acid reagents were added. The tubes were

then kept in a water bath at 55°C. After 30 minutes the tubes were then cooled and the content were shaken with 10 ml. chloroform to extract the unchanged nitrosonaphthol. The supernatant obtained after centrifugation at 2000 r.p.m for 5 minutes was transferred to colorimetric tubes and the optical density was measured at 420 mμ in a Klett-Summerson photoelectric colorimeter. The standard containing 0.1 μM tyrosine was prepared in the same manner. The blank containing water and TCA was also processed in the similar manner.

Histological observations were also made on the thyroid gland of the birds from which the serum was collectd.

Birds collected at 5 a.m. (just before awakening period) were used for the determination of tyrosine so as to eliminate the possible addition of tyrosine from the dietary source.

The oxygen consumption of muscle and liver slices was determined using the Warburg respirometer with Krebs ringer-phosphate buffer solution as the medium and atmospheric air as the gas phase, without the addition of any substrates.

RESULTS

The data obtained from various studies such as tyrosine level in the plasma, measurements from the histological preparations of the gland and QO_2 of muscle and liver slices are presented in Table I.

In the first week of March, the level of tyrosine in the serum was found to be 18 μg/ml. Thereafter a gradual

TABLE I

Tyrosine level in the serum and the corresponding histological observations of thyroid gland of Rosy Rastor along with the QO_2 of muscle and liver slices during the premigratory period.

Date	No. of birds used	Average body wt. g	Ht. of the follicular cells (μ)	Diameter of the follicle in μ (d)	No. of cells in follicle (n)	Ratio d/n	Tyrosine μg/100	Muscle QO_2	Liver QO_2
MARCH									
1 - 8	3	62	2.98	47.9	17	2.82	18	.....	.....
9 -15	3	66	3.03	46.8	17	2.75	23	0.245*	0.379*
16-23	3	69	3.15	48.3	17	2.84	..	0.237	0.343
24-31	3	74	3.01	48.4	17	2.84	46	.....	.....
APRIL									
1-- 8	3	76	3.4	51.6	17	3.03	68	0.312	0.322
9 -16	2	75	3.6	52.8	19	2.77	65	.....	.....
17-23	3	73	4.3	65.2	22	2.97	72	0.301	0.355
24-30	3	88	1.8	73.7	22	3.35	104	0.308	0.360

* value expressed as μl O_2/ mg wet tissue/ hour

increase was observed. By the first week of April, the tyrosine content reached 68 µg/ml serum. During the next two weeks the level was only slightly increased. But in the last week of April a greater amount of tyrosine (104 µg/ml serum) was recorded.

In the histological preparations of thyroid gland during the last week of April, the d/n (diameter of the follicle/number of cells in the follicles) was found to increase which showed that the thyroid activity was very high. The height of the follicle cells on the other hand was suddenly reduced after an apparent increase from that in the first week of March, denoting a lesser secretory activity in the last week of April.

The oxygen consumption (QO_2) of the muscle slice was found to increase gradually while that of liver slice showed no significant difference.

DISCUSSION

In the present investigation, a gradual increase of tyrosine in the serum during the premigratory period (March-April) was evident (Table I). George and Naik (1964) observed a very low activity of the thyroid gland in the first week of March. The low value of tyrosine (18 µg/ ml.serum) recorded, coincides with the above observation. Since it is evident from the observations of Malamos et al.(1966) and that of Siersback-Nielsen (1966) that tyrosine value in the serum could be used as a parameter to determine the state of thyroid activity, the gradual increase of tyrosine could be taken as indicative of a constantly increasing thyroxine out put from the gland. The

greater amount (104 µg/ml.) in the last week of April, then, could be considered as due to a very high thyroid activity. In the corresponding period a considerable release of colloidal secretion from thyroid gland was observed (George and Naik,1964). The ratio d/n presented in the Table I also agree with the values obtained by George and Naik (1964). The gradual increase of this ratio definitely showed an hyperplasia while the increase in the height of follicular cells could be interpreted as due to higher secretory activity of them. However, in the last week of April the height of these cells suddenly decreased either because of the depletion of colloid or due to the cessation of secretory activity. Taking these facts into consideration, it could be concluded that during premigratory period, the gradual increase in the thyroid hyperplasia was accompanied by a simultaneous release of certain quantities of thyroxine that would correspond to the degree of hyperplasia. A few days prior to migration considerable quantities of the colloid were released from the gland as evidenced by the absence of colloid in the follicles (George and Naik,1964) and a very high value of tyrosine in the serum (the present investigation).

Since the most striking metabolic process in the migratory bird is the enormous build up of fat, it would be but reasonable to consider the affects of thyroid hormone in varied concentration on the metabolism of fat. In all the probability the different levels of tyrosine showed varying concentration of thyroxine in the blood. It was observed that

smaller doses of thyroxine increased the cholesterol synthesis while higher doses decreased it (Scaife and Migicousky,1957; Fletcher and Myant,1960; 1962). Summerizing the various studies on the metabolic effect of thyroxine, Rall et al.(1964) suggested that thyroid hormone in varied concentrations is capable of influencing the same process in different ways. Thus, it influences both the increased synthesis as well as degradation of fatty acids and cholesterol. However, some of the reported variation in the effect of thyroxime could be attributed to other factors. Though, an increased synthesis of cholesterol and fatty acids were observed in rats with hyperthyroidism (Spirites et al.,1953; Ellefson and Mason,1962), inrats given daily doses of thyroxine, no increase in the rate of incorporation of acetate-C^{14} into fatty acids was found (Fletcher and Myant,1958). Variation in the response of certain enzymes concerned with fat synthesis on thyroxine treatment were also reported. An increase in the activities of glucose-6-phosphate dehydrogenase (G-6-PD) (McGuire and Thomkins,1959) and α-glecerophosphate dehydrogenase (GPD) (Ruegamer et al.,1964) were reported in either experimental or pathological hyperthyroidism. However, a fall in the amount of $NADPH_2$ in the liver of thyroxine treated rats was observed (Glock and McLean,1955) which contradict the reported increase of G-6-PD activity. Taking into consideration such conflicting results Myant (1964) concluded that the thyroid hormone may stimulate the fatty acid synthesis in the whole animal, but in the liver slices and cell free fractions from thyroxine ~~treated~~

treated animals a depressed capacity of fatty acid synthesis could be the rule. This depression in vivo experiments may be due to the inadequacy of ATP and $NADPH_2$ (DPNH) regeneration.

With an abundant supply of ATP and $NADPH_2$ through carbohydrate katabolism, effect of thyroxine could be an increased fat synthesis. In this connection, experiments in whole animals could be more reliable, since all other factors that may accompany the thyroid activity could also take part. Merkel (1958) and Schildmacher and Rautenberg (1952) observed an increased rate of fat deposition in birds when small doses of thyroxine were injected. The shift of diet by the Rosy Pastor (to a diet of fruits) during the premigratory period, provides a good supply of carbohydrate (Chapter 1). Thyroid hormone also facilitates increased absorption of carbohydrates (Holliday et al.,1962). In view of these facts, the influence of thyroid hormone on fat metabolism in the Rosy Pastor could be surmised as follows: Low levels of thyroxine could effect an increase in the uptake of carbohydrate from the diets which in turn assure enough supply of glucose for utilization. By activating G-6-PD and other enzymes in the HMP shunt, more $NADPH_2$ (TPNH) are produced. With an increased GPD activity, mitochondria gets sufficient quota of $NADPH_2$ (DPNH) for the maximum efficiency of ATP formation. (c.f. Rall et al.,1964). When ATP and $NADPH_2$ are plenty and insulin is in the optimum level fatty acid synthesis and subsequent incorporation of these into fats could take place. (Further studies on G-6-PD

and GPD in the liver of these migratory birds should be of help to shed more light on such mechanism).

It is increasingly realized that thyroid function has close association with the haematopoietic activity in the marrow. In the Rosy Pastor during premigratory period a marked increase of erythrocyte production was observed (Chapter 5). Since it is evident that the stimulation of erythropoiesis was not by any external factors such as hypoxia, temperature etc., the enhanced red blood cell production, in all the probability might have been induced by some factor like thyroid hormone. Thyroidectomy caused mild anæmia in man (Evans et al.,1964), bone marrow hypoplasia in rabbits (Kunde et al.,1932; Sharpe and Bisgard, 1936), reduced uptake of radioiron by bone marrow and blood (Austoni et al.,1956; 1958) and diminished erythrocyte number in domestic fowl (Domm and Taber,1946). Hyperthyroidism resulted in an increase of erythrocyte production and an increased total erythrocyte mass (Fischer and Cook, 1962; Waldmann et al.,1962) and hyperplasia of bone marrow (Kurimoto,1962). Hence it could be concluded that increased erythropoiesis in Rosy Pastor could be an effect of the thyroid hormone.

Studies on the O_2 consumption of the liver and muscle slices also showed a possible influence of thyroxine on these tissues. A gradual increase in the O_2 consumption (QO_2) of the muscle slices was observed while there was no change in the liver slices. The increased O_2 uptake by muscle could be due to increased thyroid hormone since thyroxine is known to increase

the over all O_2 utilization by influencing enzyme systems and mitochondrial oxidation (Rall et al.,1964). Higher oxygen uptake by the muscle might be due to the increased myoglobin, heme compounds and enzymes in it, since iron in the muscle was found to increase towards the end of April (Chapter 4). Tata et al.(1962) found that the thyroxine increased the mitochondrial hemes and cytochromes. The liver slices on the other hand showed no significant differences in the rate of O_2 consumption during March-April. Perhaps smaller concentrations of thyroxine in the blood may not suffice to induce an increased O_2 uptake by the liver. It is interesting to note that during these periods the liver was actively concerned with lipid synthesis (Chapter 1). Higher concentration of thyroid hormone may bring about an increased lipolysis, at which time the oxidative processes might take place in the liver. However, though high hormonal release was evident in the last week of April, a corresponding increase in the QO_2 of the liver was not seen. This could be explained by the fact that there is a latent period after the administration of the thyroid hormone, before it's influence on the oxygen consumption of tissues become evident (Rall et al.,1964). Thus the increased uptake of oxygen or in other words the oxidative reactions involving lipolysis may set in in the liver, quite a few days after the release of colloid from the gland, perhaps while the migratory flight is well under way.

Action of thyroid hormone in effecting the "Zugunruhe",

could be explained in the light of recent findings that the hormone may act at the neuromuscular junction (Pickens and Lockett, 1961; Schwartz et al.,1960; Lockett and Ganju,1957).

This preliminary study shows that a biphasic effect of thyroid hormone - a low level increasing fat synthesis and a high concentration elevating oxidative processes - could play a significant role in the physiological mechanisms of premigratory preparations.