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T3 fails to mimic certain effects of T4 in munia birds: physiological implications for seasonal timing

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The comparative effects of equimolar doses of thyroxine (T4) and tri-iodothyronine (T3) were studied on fattening, gonadal status and moulting in thyroidectomized female spotted munia. The effects of suppression of peripheral conversion of T4 to T3 were also studied on these parameters. T4 administration led within 10 days to a significant regression of gonads and mobilization of fat, the response being linear. T3 had no effect on these parameters at any dose level. However, both T4 and T3 were effective, the former to a greater extent in stimulating feather regeneration in the same birds. Inhibition of peripheral monodeiodination of exogenous T4 in thyroidectomized (Tx) birds by iopanoic acid (IOP) did not suppress T4 response, but resulted in greater regression of gonads and fat mobilization; however, IOP alone was ineffective. Long-term suppression of peripheral monodeiodination of endogenous T4 in intact birds with IOP suppressed follicular growth and fat deposition but enhanced moult. Hormonal profiles of T4 and T3 indicated that seasonal moult-inducing, anti-gonadal and anti-fattening effects of the thyroid gland are reflected by circulating T4 but not T3 in the munia bird. It may be concluded that T4 may be more potent than T3 in eliciting certain biological actions and that T3 may not mimic all T4 effects. Contrary to general opinion T4-induced effects need not necessarily be mediated through its prior conversion to T3, giving it an independent hormonal role. This may be a mechanism evolved by passerine birds which are generally known to be iodine-deficient, to preclude the simultaneous occurrence of seasonal events requiring high energy and nutrition, such as moulting, reproduction and migration.

Key words: T4; T3; Seasonal timing; Reproduction; Moulting; Fattening; Peripheral monodeiodination.

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Introduction

Thyroxine (T4) and tri-iodothyronine (T3) are the two major circulating thyroid hormones in vertebrates. Since the demonstration that the major portion of circulating T3 derives from the extrathyroidal conversion of T4, evidence has accumulated indicating that T3 is more potent than T4 in evoking physiological responses, that T3 can mimic all T4 effects, and that inhibition of peripheral conversion of T4 to T3

(by inhibitors like propylthiouracil and iopanoic acid) suppresses the T4 response. These findings, although based exclusively on studies with rats, have led to the current consensus among thyroidologists that T4 is a prohormone that must be converted to its monodeiodinated form T3, before biological activity is elicited (Pitt-Rivers *et al.*, 1955; Oppenheimer *et al.*, 1972; Ingbar and Braverman, 1975; Larsen *et al.*, 1980).

Our studies involving plasma profiles in relation to seasonal physiological events and comparative effects of T4 and T3 in non-mammalian vertebrates have, however, implied that apart from T3, T4 may also have indepen-

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dent hormonal effects (Chandola and Bhatt, 1982; Pathak and Chandola, 1982; Kar and Chandola, 1985; Chandola-Saklani and Kar, 1990). In this paper we present evidence that the gonado-inhibitory and fat-mobilizing effects of thyroxine in a seasonally breeding bird are not mimicked by T3, while those on feather regeneration are, and none of these T4-effects involve prior conversion to T3. The independent roles of T4 and T3 may help in the temporal spacing of reproduction, moulting and fattening, seasonal events in birds known to be influenced by thyroid status.

Materials and Methods

Spotted munia (*Lonchura punctulata*), a locally available passerine bird, was used for the experiments. Ample background information is available on the seasonal cycles of this bird. In nature as well as in captivity this bird breeds in the autumn. Gonads concomitant with a gain in body-weight begin to develop in July, attain peak activity in September and regress in October, followed by complete sexual quiescence in December. Following this, moult is initiated and completed by the following July. Ablation of thyroid gland in this bird abolishes seasonal gonadal regression and seasonal decline in fattening, and arrests moulting. Birds were maintained in laboratory conditions (13 hr light:11 hr darkness at $28 \pm 2^\circ\text{C}$) unless otherwise described. They were housed in wire cages in groups of four and provided with the same diet throughout (pearl millet and water (*ad libitum*)). The following four experiments were conducted.

Experiment 1

Experiment 1 investigated the effects of T4 and T3 on the gonadal status, fattening and feather regeneration in thyroidectomized birds.

In the first week of September 60 adult female birds were deplumed and surgically thyroidectomized under ether anaesthesia. Thyroidectomized birds were given $30 \mu\text{Ci } ^{131}\text{I}$ i.p. as carrier-free NaI to ensure complete destruction of thyroid tissue. Two weeks later the thyroidectomy was confirmed by the absence of regenerated feathers on each deplumed breast. Prior to the experiment, $500 \mu\text{l}$ blood samples were drawn from 10 birds at random to ensure that T4 and T3 were undetectable in the plasma. Birds were weighed and randomly divided into seven groups of eight to nine birds each. Their fat deposition was recorded by a scoring method described by King and Farner (1959). Briefly, body fat was assigned an arbitrary value from 0 (no fat) to 6 (full fat deposition). The diameter of the largest ovarian follicle was measured

in situ by laparotomy under ether anaesthesia. Groups I, II and III received 0.37, 0.74 and 1.48 nM of L-T4 per day per bird (sodium salt, Sigma Chemical Company, St Louis, MO) respectively, and groups IV, V and VI received the same equimolar doses of T3 (sodium salt) in 0.1 ml of 0.9% (w/v) alkaline saline over 10 days. Group VII received vehicle and served as the control group. The right breast of each bird was examined daily and the total number of feather papillae was noted. The feather count (expressed as the number of papillae/cm²) was obtained by placing a thin wire ring of known diameter along the centre of the feather tract and counting the number within. Four counts were taken for each bird. Twenty-four hours after the last injection birds were weighed, fat-scored and the ovarian follicle size measured *in situ* under ether anaesthesia.

Experiment 2

Experiment 2 investigated the effect of inhibition of peripheral conversion of T4 to T3 on gonadal status and fattening in thyroidectomized birds. Thirty-five adult munias were thyroidectomized as in experiment 1 and used 2 weeks later. Five groups were established, each containing six to eight birds. Group I was administered i.m. with 1.0 mg iopanoic acid (IOP, Wintraub)/day per bird. Iopanoic acid was dissolved in 0.05 mol NaOH/l followed by the addition of 0.2 mol HCl/l to lower the pH level as much as possible without insolubilization of the contrast agent. The final volume was made up with distilled water (pH 7.5–7.8). Group II received vehicle i.m. and i.p. and served as the control group. Group III was injected with $0.5 \mu\text{g}$ T4/bird/day. Groups IV and V, in addition to $0.5 \mu\text{g}$ T4, received 0.5 mg and 1.0 mg IOP/bird/day, respectively. Twenty-four hours after the last injection (10th) birds were weighed, fat-scored and the diameter of the largest ovarian follicle was measured as described in Experiment 1.

Blood samples were obtained, centrifuged and the plasma deep frozen at -20°C for subsequent T4 and T3 estimation by radioimmunoassay (RIA).

Experiment 3

This experiment investigated the effect of endogenous inhibition of extrathyroidal conversion of T4 on gonadal status, fattening and moulting in intact birds.

Twenty-four intact first-year birds were divided into three groups of six to eight birds each. Prior to experiment, their body-weight and ovarian follicular size were measured. Simultaneously moulting status was also recorded. A moult score of primary wing

feathers was recorded for each bird according to the scoring system of Newton (1966). Feathers were assigned a value from 0 (old) to 5 (new, fully grown feathers). With nine primaries per wing the maximum score was 90. Group I was injected i.m. with 0.5 mg IOP and group II with 1.0 mg IOP each day. Group III received the vehicle (alkaline saline) and served as the control. Observations on moult status (expressed as moult score), fat score and follicular diameter *in situ* were made on days 30 and 75 of treatment.

Experiment 4

Seasonal T₄, T₃ profile in relation to moulting, breeding and fattening were investigated.

About 100 adult female spotted munia were used in this experiment. Fifteen birds were selected at random from this stock every month and blood samples were collected from the brachial vein. After bleeding, samples were centrifuged and plasma deep frozen and stored at -20°C for T₄, T₃ estimation by RIA. Twelve birds were also separated at random from the stock and monthly observations were made on ovarian follicular diameter (*in situ*), fat score and moult score for 1 year. Data of all the experiments were analysed by standard statistical methods following Student's *t*-test and regression analysis wherever required (Fisher, 1963). All birds remained healthy throughout the experiment.

Results

Experiment 1

Comparative effects of T₄ and T₃ on fattening, ovarian follicle diameter and feather regeneration in Tx birds (Fig. 1).

Effect on fattening. (1) *Fat score.* Thyroidectomized (Tx) control birds maintained a high fat score throughout the experiment. Fat score significantly declined in Tx birds treated with T₄ compared to controls at all dose levels ($P < 0.001$, < 0.005 , < 0.001 for groups receiving 0.37, 0.74 and 1.48 nM per day T₄, respectively; control vs treated) and the response was linear ($r = -0.77$, $P < 0.001$). There was no loss of fat in T₃-treated birds at any dose level [P not significant (ns) control vs treated].

(2) *Body weight.* Compared to the Tx controls, body-weight declined in Tx birds receiving T₄ at all dose levels ($P < 0.005$, < 0.005 , < 0.001 for groups receiving 0.37, 0.74, 1.48 nM T₄, respectively). The effect was linear ($r = -0.72$, $P < 0.005$). Body-weight of Tx birds receiving T₃ was not significantly different from that of controls at any dose level (P , ns control vs treated, all doses).

Ovarian follicular diameter. Thyroidectomized controls did not show any significant difference between initial and final values. Follicular diameter was significantly decreased in Tx birds treated with T₄ at all dose levels compared to that in Tx controls ($P < 0.005$,

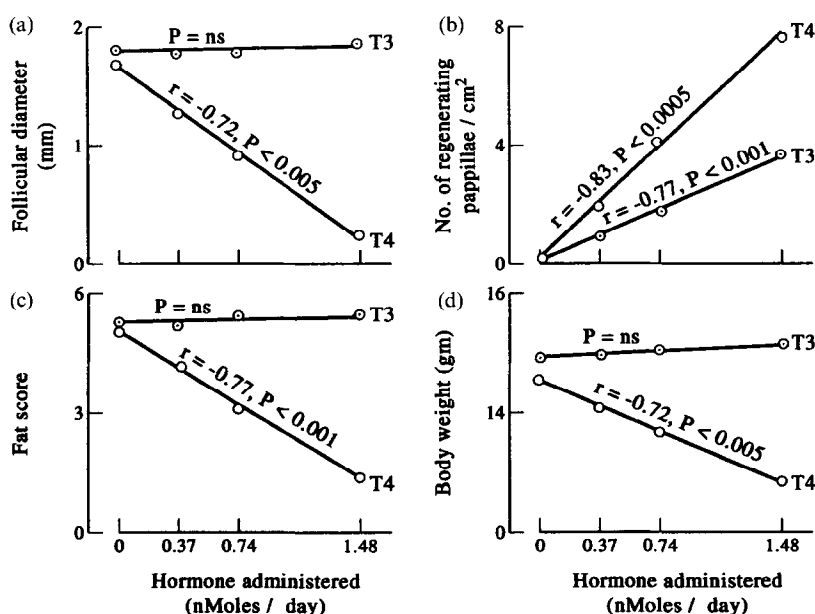


Fig. 1. Experiment 1. Effect of equimolar doses of thyroxine (T₄) and tri-iodothyronine (T₃) in thyroidectomized spotted munia on (a) ovarian follicular diameter, (b) feather regeneration, (c) fat score, and (d) body weight. Values are means \pm SE from eight to nine birds. \circ , Birds given T₃; \circ , birds given T₄.

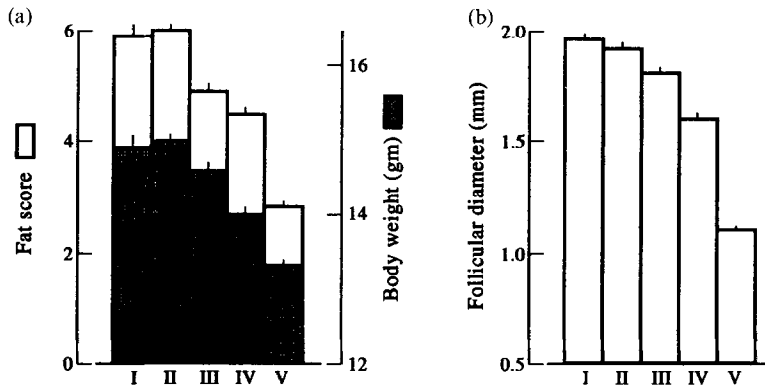


Fig. 2. Experiment 2. Effect of inhibition of peripheral monodeiodination of thyroxine (T4) to tri-iodothyronine (T3) on (a) fat score (□) and body weight (■), and (b) ovarian follicular diameter in thyroidectomized spotted munia. Group I received vehicles, group II iopanoic acid (IOP) alone, group III 0.5 g T4, group IV 0.5 g T4 ± 0.5 mg IOP, and group V 0.5 g T4 + 1.0 mg IOP. Values are means ± SE from six to eight birds per group.

0.001, <0.002 for groups receiving 0.37, 0.74, 1.48 nM T4, respectively), and the response was linear ($r = -0.72$, $P < 0.005$). There was no significant difference in follicular diameter of Tx birds treated with T3 at any dose level compared with that of controls (P , ns).

Feather regeneration. Thyroidectomized control birds did not regenerate feathers. On day 10 the number of regenerating feather papillae was higher in Tx birds receiving T4 compared with those receiving T3 at all dose levels ($P < 0.0005$, <0.0002, <0.0005 at 0.37, 0.74 and 1.48 nM per day, respectively).

Experiment 2

Effect of inhibition of peripheral monodeiodination of exogenous T4 on fattening and gonadal status in Tx birds.

Fattening (Fig. 2a). Fat score and body-weight were significantly less in Tx birds treated with 0.5 µg T4 compared with Tx controls receiving the vehicle only (fat score: $P < 0.001$; body-weight $P < 0.0001$). Fat score and body-weight were significantly less in Tx birds receiving different doses of IOP in addition to 0.5 µg T4 compared with those receiving T4 alone (fat score: $P < 0.005$ at 0.5 mg and $P < 0.001$ at 1.0 mg IOP; body-weight: $P < 0.001$ at 0.5 mg and $P < 0.005$ at 1.0 mg IOP). Fat score and body-weight of Tx birds receiving 1 mg IOP alone were not different from Tx birds receiving vehicle (ns).

Ovarian follicular size (Fig. 2b). Follicular diameter significantly decreased in Tx birds receiving 0.5 µg T4 compared with that in thyroidectomized controls ($P < 0.005$). Follicular diameter significantly decreased in Tx birds receiving different doses of IOP in addition to 0.5 µg compared with those receiving T4 alone ($P < 0.0005$ at 0.5 mg and $P < 0.0005$ at 1.0 mg

IOP). Tx birds receiving IOP alone did not show follicular regression compared with Tx birds receiving vehicle only (P , ns).

Experiment 3

Effect of long-term inhibition of peripheral monodeiodination of endogenous T4 on gonadal status, fattening and moulting in intact birds (Fig. 3a-c).

Follicular diameter. Ovarian follicular diameter in saline injected birds showed consistent growth as birds entered the first reproductive cycle ($P < 0.001$ day 1 vs day 30, $P < 0.0001$ day 1 vs day 75). Both doses of IOP significantly inhibited growth of follicle compared with that in control birds ($P < 0.005$ at both doses of IOP on day 30 and $P < 0.001$ at 0.5 mg and $P < 0.0001$ at 1.0 mg IOP on day 75). Regression analysis of day 75 values showed that the effect of IOP was linear ($P < 0.01$, $r = -0.90$).

Fat score. Control birds showed a gradual increase in fat score ($P < 0.01$ initial vs final). This increase was not shown by IOP-treated birds. Fat deposition in IOP-treated birds was significantly less than that in controls ($P < 0.001$ at 0.5 mg and $P < 0.005$ at 1.0 mg IOP final values on day 75). Regression analysis of day 75 values indicated that the effect of IOP was linear ($P < 0.05$, $r = -0.90$).

Moulting. The moult score was significantly enhanced in IOP treated birds compared with that in control birds ($P < 0.05$ at 0.5 mg and 1.0 mg IOP on day 30 and $P < 0.001$ at 0.5 mg and 1.0 mg IOP on day 75). The response was linear ($P < 0.001$, $r = 0.9$ on day 75).

Experiment 4

T4, T3 profile and seasonal moulting, breeding and fattening (Fig. 4).

Circulating T₄ concentration was high during winter months (Nov, Dec, Jan). Significant decrease occurred in spring ($P < 0.001$ Jan vs Feb or Mar) reaching minimum values in summer and autumn months ($P < 0.0001$ Jan vs Jun, $P < 0.005$ Jan vs Sep or Oct). T₄ registered a significant increase in early winter ($P < 0.0005$ Oct vs Nov). Plasma T₃ levels were lowest during winter months, and started to increase in the spring ($P < 0.05$ Dec vs Apr) and reached a maximum during autumn ($P < 0.001$ Jan vs Aug or Sep), followed by a decline the next winter. Fat score was minimal in winter, started increasing in spring and reached a maximum by summer and autumn ($P < 0.01$ Dec vs Feb, $P < 0.01$ Mar vs Jun, $P < 0.001$ Apr vs Sep), followed by a decline the next winter ($P < 0.001$ Sep vs Dec or Jan).

The onset of moulting occurred in the last week of December and was completed by the end of July. Gonadal development commenced

from July, peaked in September and was followed by a decline reaching sexual quiescence by November (data shown elsewhere).

Discussion

Present investigations provide clear evidence that T₃ fails to mimic certain effects of T₄ in thyroidectomized spotted munia. T₄ administration in Tx birds led to a significant regression of gonads and mobilization of deposited fat within 10 days of injection, the response being linear. T₃ had no effect whatsoever on these parameters at any dose level. However, both stimulated feather regeneration in the same birds (Fig. 1). When the extrathyroidal conversion of T₄ to T₃ was inhibited by IOP in Tx birds (Fig. 2) the T₄-induced regression of gonads and fat mobilization were more pronounced. The effectiveness of T₄ action in terms of follicular regression and fat mobilization in both cases increased as the proportion of T₄ to T₃ conversion decreased, reflecting greater availability of T₄ in circulation. IOP alone had no effect on Tx birds, ruling out a direct action of the inhibitor on these parameters. Furthermore, inhibition of T₄ to T₃ conversion by IOP in young birds with growing ovaries suppressed the rate of follicular development and fat deposition with an acceleration of feather moult (Fig. 3). Obviously, the effect of T₄ on these parameters did not involve prior conversion to T₃. These findings are contrary to the effects of T₄ and T₃ on various responses in rats where response to T₃ is often more effective and response to T₄ is invariably mediated through its conversion to T₃ (Escobar *et al.*, 1981). These studies, in addition to our earlier studies with moulting in spotted munia, attribute an independent hormonal role to T₄. It seems that in non-mammalian vertebrates T₄ is often equally, if not more active than T₃ in eliciting a response, e.g. in garden lizard, *Calotes versicolor*. T₄ had a significantly greater effect on scale shedding and gonadal growth, and both these effects were shown to be independent of T₄ to T₃ conversion (Chandola-Saklani and Kar, 1990). In morphological and biochemical studies on the induction of metamorphosis in amphibia, T₄ apart from T₃ was also found to be active, at least in the early phase of metamorphosis (Cohen *et al.*, 1978; Galton *et al.*, 1982). T₄ has been shown to be more effective than T₃ in preventing goiter in thiouracil-treated chickens (Mellen and Wentworth, 1959).

In a number of clinical situations serum T₄ concentrations correlate better than T₃ with clinical data on physiological status, including serum thyroid stimulating hormone regulation, and erythropoiesis (Chopra *et al.*, 1978;

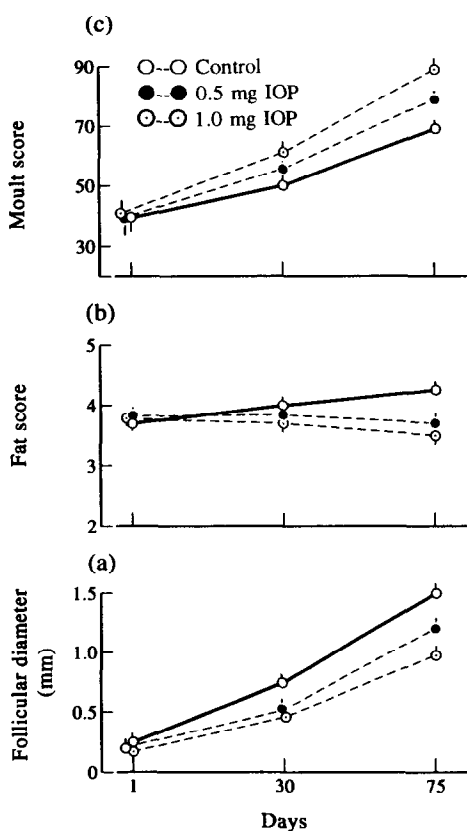


Fig. 3. Experiment 3. Effect of inhibition with iopanoic acid (IOP) of endogenous extrathyroidal conversion of thyroxine (T₄) to tri-iodothyronine (T₃) in intact spotted munia on (a) ovarian follicular diameter, (b) fat score, and (c) moult score. Dotted lines indicate birds treated with 0.5 mg IOP (●) and 1.0 mg IOP (○) and solid line those given alkaline saline (controls; ○). Each point depicts mean values \pm SE from six to eight first-year birds on day 1, 30 and 75 of treatment.

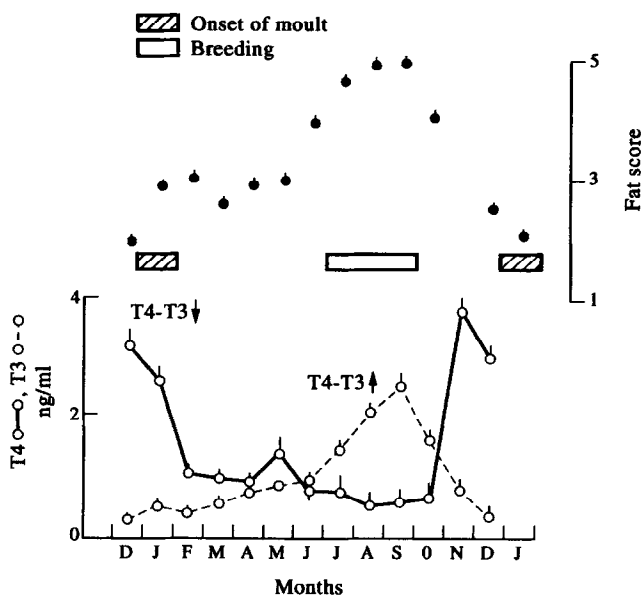


Fig. 4. Experiment 4. Seasonal profile of circulating thyroxine (T4; —) and tri-iodothyronine (T3; ---) levels in adult female spotted munia in relation to breeding (□), onset of moulting (▨) and fattening (●). Each point depicts mean values \pm SE from 12 to 15 birds.

Andreoli, 1981). However, using a potent inhibitor such as IOP these observations were subsequently explained on the basis of an intracellular conversion of T4 to T3 which varies from tissue to tissue (Larsen *et al.*, 1980). Our findings cannot be explained on this basis considering that IOP was used, nor can they be explained on the basis of a quicker turnover of T3 in blood (Refetoff *et al.*, 1970) since in birds, T4 and T3 both bind to pre-albumin and albumin and have similar half lives (Wentworth and Ringer, 1986). Our results suggest two possibilities of T4 actions, either an independent effect in certain situations depending on the responding system, or production of biologically active T4 metabolites other than T3. Studies on subcellular distribution and metabolism are in progress to test these possibilities.

Results of the present studies also indicate that while the effects of thyroid on gonadal regression and fat mobilization may be brought about directly by T4 alone, without prior conversion to T3, the effects on feather stimulation may involve action of both T4 and T3 independently. It is clear from these findings and from the above discussion that apart from T3, T4 also has a role as a hormone in the submammalian vertebrates investigated. This may have significant implications in the physiological timing of seasonal events. In seasonally breeding animals for survival it is imperative that physiological events requiring high energy and nutrition, such as reproduction, moulting and migratory

disposition, are phased in order to prevent their simultaneous occurrence. Thus, in sedentary spotted munia annual reproductive and fattening cycles are coincident and the moult cycle is temporally spaced (Fig. 4). All three physiological events in spotted munia have been shown to be influenced by thyroid status. In the study of other birds thyroid hormones have also been shown to be associated with one or two, if not all three physiological events (see Follet *et al.*, 1988; Follet and Nicholls, 1985; Assenmacher and Jallageas, 1980; Thapliyal, 1969; Chaturvedi and Thapliyal, 1979; Devi and Lal, 1993; Pant and Chandola-Saklani, 1994; Goodridge, 1978). In spotted munia thyroidectomy abolishes seasonal decline in gonadal activity and fattening (Thapliyal, 1969; Thapliyal and Chandola, 1972) and arrests moult (Pant and Chandola-Saklani, 1993) and replacement therapy reverses the effects. Seasonal moult-inducing, anti-gonadal and anti-fattening effects of the thyroid gland are indicated by the circulating T4 and T3 profiles in this bird (Fig. 4). Initiation of moult occurs in late winter only after T4 levels have attained peak value. As moult progresses into spring T4 levels decline. Gonadal development and fat deposition commence in June–July, attaining a peak in September. This occurs while T4 levels are minimal but T3 maximal. The seasonal variations in circulating T4 and T3 are due to variations in peripheral conversion of T4 into T3 (Pathak and Chandola, 1982; Lakhera and

Chandola-Saklani, 1987). It appears that high circulating levels of T₄, and low T₃, provide the stimulus for moult while simultaneously keeping the gonadal development and fat deposition suppressed. A shift in this pattern towards low T₄ and high T₃ as a result of enhanced peripheral conversion in summer may allow gonadal development and fat deposition to occur. The failure of the increased circulating level of endogenous T₃ to produce an inhibitory effect on gonads and fattening during the breeding period (Experiment 3, Fig. 3) is in keeping with the observed failure of exogenous T₃ to mimic these effects of T₄ in Tx birds (Experiment 1, Fig. 1). This selective lack of response to T₃ may be an adaptation for the effective use of peripheral conversion of T₄ to T₃ as a strategy to separate temporal events. A number of environmental and physiological factors can influence peripheral conversion of T₄ to T₃ (Ingbar and Galton, 1975; Higgs and Eales, 1977; Chandola-Saklani *et al.*, 1989). This would give it a great flexibility and hence a selective value. Obviously a seasonal variation in peripheral conversion to T₄ may be a device to make the required thyroid hormone available, either T₄ or T₃ during different phases of the year.

The thyroid hormones, like the steroid molecule, have remained conservative during the course of evolution, but the uses to which they have been put in different vertebrates, ranging from fish to mammals, or in different tissues of the same organism, are remarkable, a broad spectrum of actions being the characteristic of the thyroid gland. It is, therefore, not surprising that birds, and perhaps other homeothermic seasonal vertebrates, make use of these hormones in the timing of their seasonal physiological events. A direct action of T₄ at the cellular level as well as through its conversion to T₃, thus deriving maximal efficiency from one molecule (T₄), would be in keeping with the diversity of actions and may be a part of thyroid hormone economy in birds (which exhibit all features of iodine-deficiency, e.g. rapid and high thyroidal ¹³¹I uptake and long thyroidal ¹³¹I retention (Assenmacher, 1973; Gorbman and Bern, 1974), and may serve to preclude simultaneous occurrence of mutually incompatible seasonal physiological events. Peripheral conversion of T₄ into T₃, which has been demonstrated in all phyla, may assume great importance in this context, with its role in exercising a subtle organ-specific, action specific metabolic control.

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