

THE REGULATION OF PLASMA THYROID HORMONES  
IN POULTRY

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## SUMMARY

The way in which environmental and physiological factors regulate the plasma concentrations of thyroid hormones in birds has been investigated.

The administration of goitrogens in the diets of growing chickens was found to depress the levels of plasma  $T_3$  and  $T_4$  and increase the activity of the thyroid follicular cells.

Daily rhythms in the levels of plasma  $T_3$  and  $T_4$  were found to be inversely related and independent of the circadian system and the pineal gland. The concentration of plasma  $T_3$  increased from its lowest levels at the onset of a light period and was associated with an increase in heart rate and a decrease in the concentration of plasma  $T_4$ . Fasting abolished the daily rhythms in plasma thyroid hormones and was associated with a decrease in the concentration of plasma  $T_3$  and an increase in the concentration of plasma  $T_4$ , while feeding, regardless of the time of day and photoperiod, resulted in an increase in the concentration of plasma  $T_3$  and a decline in plasma  $T_4$ .

Ducks transferred to a long day photoperiod showed an increase in the concentration of plasma  $T_4$  during the refractory period of the breeding cycle which coincided with the post-nuptial moult. The initiation of photorefractoriness and moult in the Willow Ptarmigan were not associated with any marked fluctuations in plasma  $T_3$  and  $T_4$ . In quail the concentration of plasma  $T_4$  responded to changes in daylength while the levels of both  $T_3$  and  $T_4$  were affected by the reproductive state of the bird.

The concentration of pituitary TSH in ducks was affected by changes in thyroid status: goitrogens increased whereas injections of  $T_4$  decreased levels of TSH as measured by immunocytochemistry and bioassay. Injection of TRH into laying hens was shown to increase the levels of both plasma  $T_3$  and  $T_4$ .

Elevated levels of plasma thyroid hormones were found during the first weeks of life, which declined as the birds became sexually mature. The concentration of plasma  $T_3$  increased in broody bantam hens within one or two days after hatch and rose further after the chicks hatched. An injection of prolactin into hens increased the concentration of plasma  $T_3$ .

The stress of repeatedly handling chickens and turkeys resulted in a decrease in the levels of plasma  $T_4$  whereas temperature and dehydration

stress affect the concentrations of  $T_4$  and  $T_3$  principally by a change in food intake.

A reduction in the levels of plasma  $T_3$  induced by thyroidectomy, fasting or by an increase in environmental temperature was associated with a decrease in the rate of heat production. An injection of  $T_4$  into thyroidectomised birds resulted in a sustained increase in the levels of plasma  $T_3$ .

The concentration of plasma triglycerides were found to be positively correlated with the levels of plasma  $T_3$ . Plasma lipoproteins were demonstrated to bind both  $T_3$  and  $T_4$ .

Plasma levels of  $T_4$  were not useful for prediction of subsequent egg production in pedigree hens and neither were the concentrations of plasma  $T_3$  and  $T_4$  related to shell quality as measured by specific gravity and gas conductance. A relationship was established between the levels of plasma  $T_3$  and improper healing of the navel in selected lines of newly hatched chicks.

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No man is an Iland, intire of it selfe;  
every man is a peece of the Continent, a  
part of the maine; if a Clod bee washed  
away by the Sea, Europe is the less ...

John Donne

For my mother

DECLARATION

I hereby declare that the Thesis embodies the results of my own work, and that it has been composed by myself.

Hillar Klandorf

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## OBJECTIVES

The principal objectives of the thesis are to determine in poultry the factors that regulate the concentrations of plasma thyroid hormones and to identify their most metabolically active form. The general approach used to achieve these objectives was to measure the levels of plasma thyroid hormone in different experimentally induced or in naturally occurring physiological states.

The principal objectives of the thesis were pursued by investigating

- 1) the effect of dietary and chemical goitrogens on thyroid function.
- 2) the effect of photoperiod and pinealectomy on the daily rhythms of plasma thyroid hormones.
- 3) the role of the feeding pattern in generating the daily rhythms of thyroid hormones.
- 4) the effect of photoperiodically induced changes in gonadal activity on the levels of thyroid hormones as well as the effect of gonadectomy.
- 5) the factors which affect the secretion of TSH from the pituitary gland: negative feedback relationships and hypothalamic neuro-peptides.
- 6) the relationship between plasma thyroid hormones and reproductive activity as studied during sexual development, the ovulatory cycle and broodiness. In addition the effect of prolactin on the levels of thyroid hormone were investigated.
- 7) the effect of handling, temperature, fasting and dehydratory stress on the levels of plasma thyroid hormone and heat production.
- 8) the relationship between plasma concentrations of  $T_3$  and  $T_4$  and energy metabolism were investigated as well as the ability of thyroidectomised and intact birds to metabolise  $T_4$  to  $T_3$ .
- 9) the role of plasma binding proteins and their relative affinity to thyroid hormones.
- 10) the role of the genotype on thyroid hormone levels and the effects these differences have on egg production, egg shell quality and yolk sac retraction.

### 1.1 General

The thyroid gland is a structure which traps and stores iodide from the blood. Inside the gland iodide is coupled to a large protein and stored. The iodoamino acids are used in the synthesis of thyroid hormones, the most important of which are thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ). For most organisms the supply of iodide is both scarce and highly discontinuous and so the ability of the thyroid cell to draw upon such a reserve when the intake of iodide is low represents an important homeostatic mechanism. The biochemistry of the thyroid gland is thus geared to both retain and to make the most efficient use of iodine.

The thyroid gland releases primarily  $T_4$  into the circulation in response to various stimuli. Other factors regulate the conversion of  $T_4$  into  $T_3$ , the calorigenically active hormone which can act on most tissues to increase metabolic activity and protein synthesis. Thyroid hormone is thus essential for normal growth and development.

### 1.2 Historical perspective

Modern ideas on the function of the thyroid gland originated in the nineteenth century when gross clinical features were recognised as being associated with malfunctions of the gland. Hyperthyroidism was first described by Parry in 1786 and the symptoms categorised by Robert Graves in 1835 (Greene, 1970). The symptoms were a general acceleration of all bodily and mental processes in response to an increase in the rate of cellular oxidations due to the elevated levels of thyroid hormone. Surgical removal of goiters from man and of non-goiterous thyroid glands from animal species led to a deeper understanding of thyroid physiology. Thyroidectomy was found to result in a decrease in oxygen consumption whereas administration of thyroid extract produced the converse effects which resulted in the diagnostic feature of an increase in the excretion of urinary nitrogen (indicative of catabolic processes). Reseverdini and Cochi (1883) were the first to recognise the similarity between the symptoms of myxedema and thyroidectomy and <sup>the</sup> effective treatment of hypothyroidism was first reported by Murray (1891) who noted the restoration of normal bodily functions in response to the administration of sheep thyroid gland extract. Iodine was shown to be a constituent of the thyroid gland in 1896 by Baumann. In the early 1900's Oswald first identified 'thyreoglobulin' as the principal iodinated protein of the thyroid gland and Kendall (1915) isolated 'thyroxin'

in crystalline form from a hydrolysate of thyroid tissue. The name originated as a contraction of thyroxindole because he thought it was a triiodotryptophan derivative. Kendal's proposed structure remained unchallenged until Harrington (1927) removed the iodines by catalytic reduction and identified the new amino acid, thyronine, as the carbon skeleton for the iodoamino acid. Harrington established the structure of thyroxine as 3,5,3<sup>1</sup>,5<sup>1</sup>-tetraiodothyronine (T<sub>4</sub>) and synthesised it from p-methoxyphenol and 3,4,5-triiodonitrobenzene.

#### 1.2.1 Thyroid function in birds

The first studies in the domestic chicken showed that the feeding of desiccated thyroid gland induced a moult which was followed by a regrowth of plumage characteristic of younger birds (Zawadowsky, 1925). The comb and wattle size were increased and egg production was reported to increase as well (Crewe, 1925; Walker, 1925). The relationship between the pituitary and thyroid gland of chickens was first noted by Mitchell (1929). He showed that hypophysectomy led to a decrease in the size of the thyroid gland. Riddle et al. (1935) measured a significant drop in the basal metabolic rate of domestic pigeons after hypophysectomy. Similar findings were also reported in other avian species including the Japanese quail (Woitkewitsch, 1940) and the domestic fowl (Keating et al., 1945). Administration of extracts of anterior pituitary have been shown to directly stimulate the thyroid gland as well as increase the metabolic rate. This effect has been measured in House sparrows (Miller, 1939), domestic ducks (Schockaert, 1932), domestic pigeons (Larianov et al., 1931), quail Coturnix coturnix (Woitkewitsch, 1940), greenfinch Chloris chloris (Kuchler, 1935) and the domestic fowl (Keating et al., 1945). Thyroidectomy retards the growth of chickens (Blivaiss and Domm, 1942), while Blivaiss (1947) has reported abnormal deposition of fat and the onset of obesity in response to removal of the thyroid glands. Chemical and radiothyroidectomy produce a similar effect (Astwood et al., 1955; Wilson and MacLaury, 1961; Winchester and Davis, 1952) which can be reversed by the administration of thyroid hormone (Raheja and Snedecor, 1971; Singh et al., 1968a).

Early studies in mammals showed that treatment of intact animals with thyroid hormone reduced the size of the thyroid gland, and was characterised by a decrease in the size and height of the thyroid epithelium and an accumulation of colloid (Cameron and Carmichael, 1926). A decrease in the TSH content of rat pituitaries treated with thyroid hormone was demonstrated by Kuschinsky

(1933) and Hohlweg and Junkman (1933). Trevorrow (1939) first identified thyroid hormone in the circulation as a small molecule and dispelled the earlier belief that it was thyroglobulin. The major thyroid hormone in blood was subsequently found to be thyroxine after recrystallising the iodoamino acid to constant specific activity (Taurog and Chaikoff, 1948) and by chromatographic methods (Laidlaw, 1949).

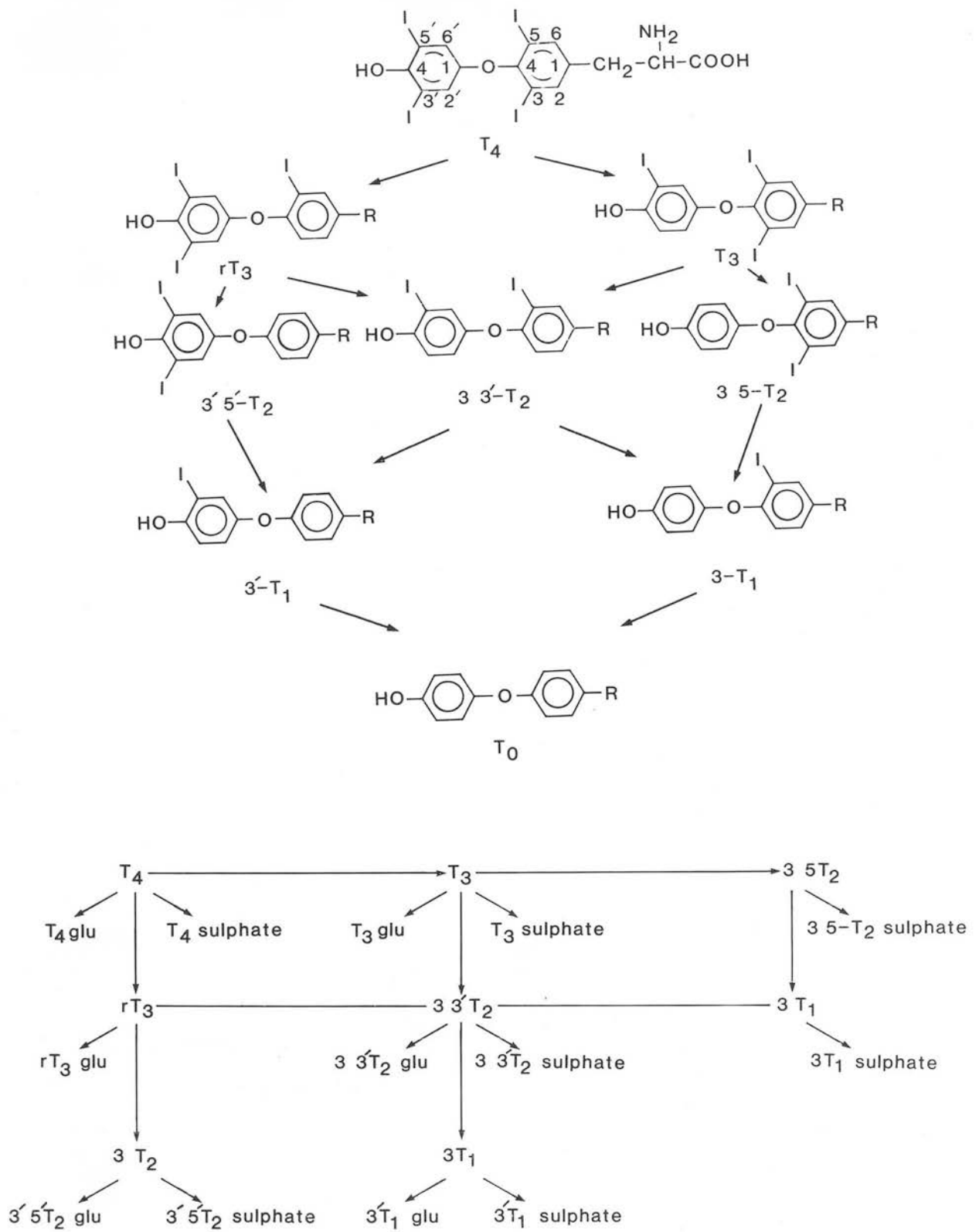
### 1.2.2 Physiological importance of $T_3$ and $T_4$

An important landmark in the history of the thyroid hormones was the identification of 3,5,3'-triiodothyronine ( $T_3$ ) in plasma (Gross and Pitt-Rivers, 1952; Roche et al., 1952). Subsequently this compound was isolated from hydrolysates of the thyroid gland and synthesised (Gross and Pitt-Rivers, 1953).  $T_3$  was soon accepted as the most active thyroid hormone. Injection of  $T_3$  into thyroidectomised hens restored the plumage of the birds to normal and eventually resulted in the resumption of egg laying (Gross and Pitt-Rivers, 1954). Shellabarger and Pitt-Rivers (1958) were the first to identify  $T_3$  and  $T_4$  in the thyroid gland of the chicken. Subsequent studies have found that  $T_3$  does not occur in the thyroid gland of all birds (e.g. cockerel: Rosenberg et al., 1963, 1964; Spotted Munia: Chandola, 1972), and only in trace quantities in the quail and duck (Bayle et al., 1967; Rosenberg et al., 1967).

During the last ten years virtually every iodothyronine which can be derived from  $T_4$  has been reported to exist in the blood or cells of mammals (Fig. 1) and these compounds have been reported to retain varying degrees of biologic activity (Chopra et al., 1978a). This work has shown the way  $T_4$  is metabolised in the peripheral tissues. Various laboratories have studied these deiodination reactions (Chopra, 1978b; Visser, 1930). The results of these investigations question the role of  $T_4$  as a hormone or as a prohormone for the metabolically active  $T_3$ . In the chicken there is some evidence for a direct role of  $T_4$  since Dickstein et al. (1980) has shown in cardiac cells that  $T_4$  can stimulate its own degradation without conversion to  $T_3$ . Current interest thus centres on the possible intrinsic biologic functions of  $T_4$  and  $T_3$  and their metabolites in vertebrates (e.g. Oppenheimer, 1979).

### 1.3 Evolution of the thyroid gland and thyroid hormone

During the course of evolution the thyroid gland has become more



**FIGURE 1** Deiodination (upper portion of figure; modified from Visser, 1980) and metabolism (lower portion of figure; from Robbins and Rall, 1979) of thyroxine (glu = glucuronide).

highly organised. In addition to its endocrine function the thyroid gland may store and distribute iodine for use in other tissues (Etkin and Kim, 1980). For this reason in some species, the gland may be functional even though no endocrine role can be necessarily ascribed. The functional significance of iodine as a component of thyroid hormone is suggested by Frieden (1981) to have evolved as a result of primitive aquatic organisms having ready access to iodide for which trapping mechanisms were developed. Oxidative conditions in the cells favoured coupling of iodine to the tyrosine moieties in proteins and their coupling to iodothyronines, primarily  $T_4$ . Various proteases in the cell and in the circulation could have acted to release iodothyronines from their peptide linkages and into the blood. Carrier proteins may have evolved to solubilise and transport the iodoamino acids and to buffer the tissue against wide fluctuations in plasma levels. In addition the carrier proteins protect the thyroid hormones from being metabolised to inert compounds or lost in the urine. Once the ability to preferentially trap iodine evolved the reutilisation of iodine made available after deiodination contributed to the economy and steady-state biosynthesis of this compound.

Etkin and Kim (1980) have suggested that during the early stages of evolution the hypothalamic-pituitary connection evolved primarily to regulate reproduction and did not control the thyroid gland. The thyroid gland functioned solely to trap and store iodine; only with the evolution of fish did thyroid function become increasingly dependent on the hypothalamo-pituitary axis. The secretions of the thyroid gland could now be regulated by the CNS and respond more readily to changes in the environment. The endocrine role of the thyroid gland arose independently in various lines of evolution and is separate to its fundamental role of sequestering and storing iodine and making it available for cellular function.

Iodinated proteins are found in virtually all of the phyla of invertebrates, except echinoderms and protozoans, as well as in plants and marine invertebrates (Frieden, 1981). The ability to synthesise iodoproteins is not a unique characteristic of the thyroid gland for it is known that various tissues will iodinate proteins. In tunicates and amphioxus the notochord, brachial sac, tunic, gonads and blood are capable of incorporating iodine into proteins (Etkin and Kim, 1980). Thus the presence of organically bound iodine may only be the by-products

of other biochemical reactions occurring in the cell. Alternatively it has been suggested that in marine invertebrates (e.g. algae, corals, molluscs) iodine binding to proteins is associated with hard tissues and may affect the cross-linkage of collagen in such tissues and in many lower vertebrates iodinated proteins are found in the notochord, ovaries and pigment cells (Shaham and Lewitus, 1971).

The thyroid gland exists as a distinct histological structure only in the vertebrates. It occurs as a single organ in mammals, bilateral organs in birds, a single median organ in reptiles and elasmobranchs, and as loosely distributed, unencapsulated follicles in the area of the lower jaw anterior to the heart in cyclostomes, many teleosts and several amphibians. In fishes it occurs at such sites as the head and kidney (Etkin and Gona, 1974).

In evolutionary terms the protochordates, a group of invertebrates, is considered closest to the line leading to modern vertebrates. With the knowledge that the vertebrate thyroid emerges from the mid-ventral floor of the pharynx, the endostyle is considered on morphological grounds to be a homology of the thyroid gland. The organ is a ciliated groove running the length of the pharynx in the mid-ventral line and in several groups of protochordates is able to bind iodine to protein and synthesise  $T_4$  (Barrington and Thorpe, 1965a; 1965b). A similar phenomenon is seen in developing larval lampreys, a primitive living vertebrate. The thyroglossal duct remains open for the duration of larval life, and is connected to the subpharyngeal gland which functions as a thyroid. The secretions of the thyroid drain into the pharynx and eventually are digested in the intestine. Thus it has been suggested that the first thyroid hormones depended on digestive enzymes for the release of thyroid hormone from the protein thyroglobulin (Clements and Gorbman, 1955; Gorbman, 1955).

Iodinated proteins have also been localised in the cnidoblasts (stinging cells) of Hydra, a small freshwater coelenterate (Gorbman, 1974). In the jellyfish, Aurelia aurita, iodide is required to induce strobilation, a stage of development in which sessile polyps metamorphose into the free swimming medusa (Spangenberg, 1967; Silverstone et al., 1977). Silverstone et al. (1978) have suggested that it is likely DIT (diiodotyrosine) that is responsible for this event. Thyroid hormones also stimulate growth and protein synthesis in various poikilothermic vertebrates. In

fish thyroid hormones were shown to increase the incorporation of labelled amino acids into plasma and gill proteins (Narayansingh and Eales, 1975).

In vertebrates, thyroid hormones are involved in aspects of differentiation, development and maturation. One of the best examples is the metamorphosis of the amphibian larval form (Gudernatsch, 1914; Frieden and Just, 1970; Cohen 1970) which will not occur in the absence of thyroid hormones. The development of higher vertebrates is also dependent on thyroid hormones (Legrand, 1979).

#### 1.3.1 Comparative physiology of thyroid function

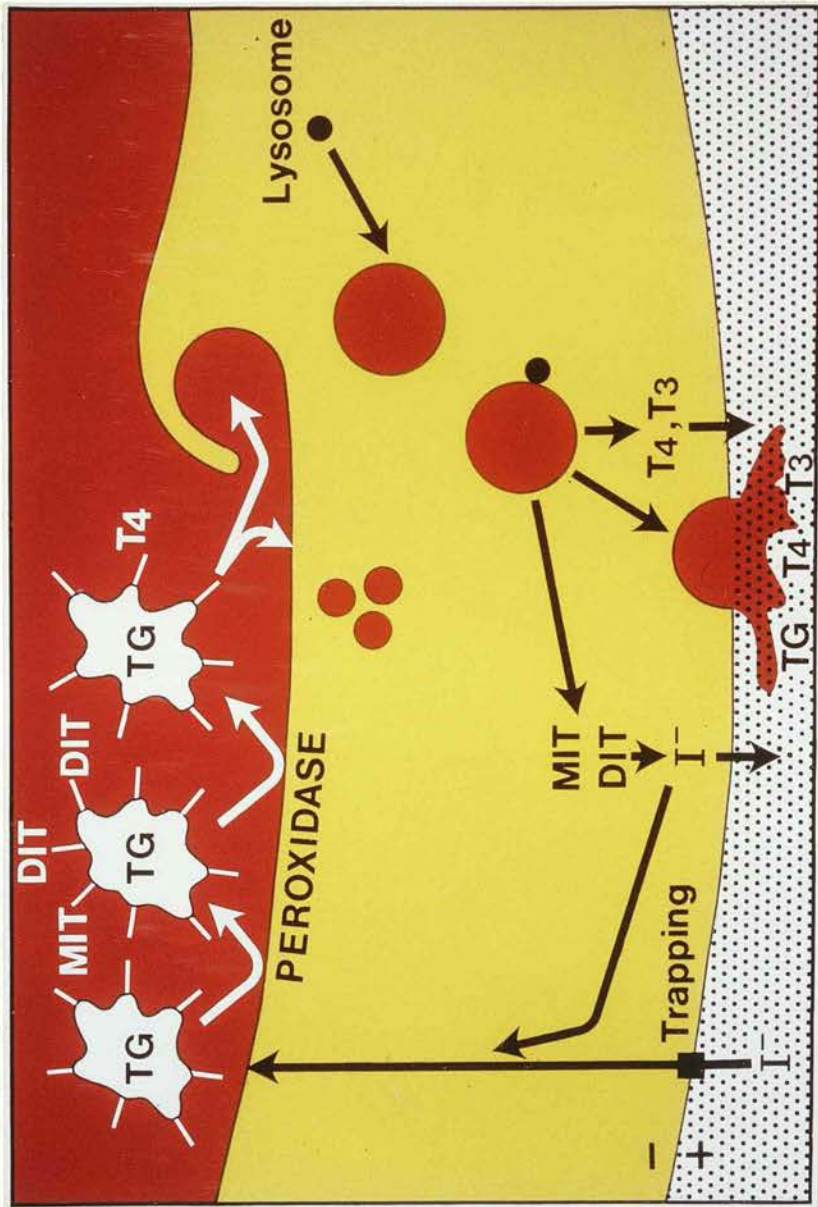
In birds and mammals thyroid hormones play a role in heat production and in the metabolism of carbohydrates, proteins and lipids. Early studies failed to show any effect of thyroid hormones on the general metabolism of poikilothermic vertebrates (Etkin and Gona, 1974) but it was subsequently shown that in amphibians and reptiles, the general metabolic response of the tissues to thyroid hormones is dependent on temperature (Maher, 1965, 1967; Packard and Packard, 1975, Hulbert, 1978). Thus thyroid hormones have a calorogenic effect in lizards when they are at 30°C but have no measureable effect at 20°C. These effects are initiated in the nucleus, but are eventually expressed by alterations in mitochondrial and membrane activity and appear to be reversible (Oppenheimer, 1979; Hulbert 1978).

#### 1.4 Synthesis of thyroid hormone

The synthesis of thyroid hormone proceeds by several steps that are summarised in Fig. 2. Vlijm (1958) was the first to show that the synthesis of thyroid hormone in birds (cockerels) was by the same biochemical pathway as mammals. The first step in the synthesis of thyroid hormone is the trapping of iodide from the extracellular fluid. Iodide can also enter the basal cell membrane from the capillary through diffusion. One of the principal features of the avian thyroid is the prolonged retention of radioiodine after an injection of tracer quantities of  $^{131}\text{I}$  as compared to mammals. This observation has been confirmed in several wild and domestic species of birds (Astier, 1980). In birds fed a low iodine diet or deprived of food the peak accumulation of radioiodine occurs about 3-6 hrs after injection and an elevated level of thyroidal radioiodine is maintained for several days (Newcomer, 1978; Astier, 1980; Davison, et al., 1981).

FIGURE 2      Synthesis of thyroid hormone in the thyroid follicle (modified from Van Herle et al., 1979). A thyroid follicular cell (yellow shading) is depicted facing the follicular space (red shading) and the extracellular space (stippled area).

TG denotes thyroglobulin; MIT, monoiodotyrosine; DIT, diiodotyrosine, + and - indicate the electric charges of the membranes.



Iodide is actively transported against both an electrical and chemical gradient from the basal membrane of the cell into the follicular lumen where it is concentrated (Andros and Wollman, 1970) (Fig. 2). Similarly amino acids enter the thyroid cell from the blood and are synthesised into protein chains on the rough endoplasmic reticulum (Greer and Soloman, 1970). Mannose is added while the molecule is still contained in the endoplasmic reticulum whereas galactose and fucose are added at or near the Golgi apparatus as the prothyroglobulin molecule migrates to the follicular lumen. Lastly sialic acid is added as the final residues to some chains and it has been suggested that the release of thyroglobulin (Tg) into the colloid depends on the presence of sialic acid on the molecule (Monaco and Robbins, 1973). Thyroglobulin is then secreted into the colloid by the fusion of exocytotic vesicles with the apical membrane of the cell (Ekholm et al., 1975).

Once in the cell the iodide mixes with that previously generated in the cell through deiodination of free iodoamino acids liberated during the lysis of the thyroglobulin molecule (Fig. 2). Thyroid iodide transport is dependent on cellular adenosine triphosphate which is supplied by aerobic ATP synthesis in mitochondria or by anaerobic glycolysis (Tylen et al., 1968). Stein and Gross (1964) first showed that iodination occurs at the surface of the microvilli of the thyroid cell. The enzyme, thyroid peroxidase, is located on the apical membrane surface of the cell and catalyzes the oxidation of iodide ( $I^-$ ) to iodine ( $I_2$ ) (Fig. 2). Nagasaka and Hidaka (1980) have found that hypophysectomy in rats causes a marked decline in the activity of the thyroid iodide peroxidase. Injections of TSH restored the activity of the enzyme whereas cyclohexamide blocked this response and so these studies suggest that biosynthesis of the enzyme protein is involved in TSH regulation of thyroidal iodide peroxidase. Thyroglobulin is then secreted into the colloid by the fusion of exocytotic vesicles with the apical membrane of the cell (Ekholm et al., 1975). Thus by restricting iodination to the cell colloid surface those proteins stored in the colloid are iodinated and the likelihood of non-thyroglobulin intracellular proteins being iodinated is minimised. The oxidised form of iodine becomes organically bound to peptide-linked tyrosyl groups in the thyroglobulin molecule to form monoiodotyrosine and diiodotyrosine (Fig. 2).

Iodination occurs on tyrosyl groups already incorporated into

thyroglobulin (Taurog, 1974). Iodoamino acid distribution in thyroglobulin is determined primarily by the degree of iodination in the protein. The high molecular weight of thyroglobulin plays a role in facilitating the formation of thyroid hormone for this factor increases the probability that the number of tyrosine residues per molecule which are iodinated to DIT (diiodotyrosine) are favourably located for coupling into the thyronine structure. Taurog (1974) has shown that the formation of  $T_4$  in thyroglobulin increases progressively with the degree of iodination and that at 150 atoms per molecule, thyroxine reaches a value of more than three residues per molecule of protein. Similar relationships between DIT and  $T_4$  formation were observed by Van Eyl (1967) when thyroglobulin was incubated with molecular iodine. The phenolic group from one DIT molecule becomes attached to the phenolic group of another DIT molecule and so forms one molecule of thyroxine but leaves the alanine side-chain of one DIT still attached to the peptide chain. This causes some increased binding between the peptide chains of Tg which in turn increases the stability of the molecule. Although the formation of  $T_4$  can occur in a wide variety of iodinated proteins thyroglobulin is the most efficient in formation of  $T_4$ , especially at relatively low levels of iodination (Taurog, 1970). The iodotyrosines contained in the thyroglobulin molecule also form some  $T_3$  as well as small amounts of reverse triiodothyronine (De Groot and Niepomniszcze, 1977). Furthermore this pattern of iodination can be shifted in the event of excess iodine intake. High concentrations of iodide inhibit its uptake <sup>in mammals</sup> by the thyroid cell and its binding to proteins in the thyroid (Wolf Chaikoff effect) and decreases the secretion and blood flow in activated glands (Wolf, 1969; Ingbar 1972).

By diffusion and in birds by the stirring action of cilia (Fujita, 1963) thyroglobulin slowly mixes in the colloid; iodination and oxidative coupling reactions occur when the molecule encounters the apex of the cells. In mammals the rate at which this reaction proceeds depends on the level of glandular activity (Bjorkman et al., 1978). Secretion of thyroid hormone first requires the incorporation of thyroglobulin into colloid droplets where secondary lysosomes can act to digest the protein and liberate the thyroid hormone (Fig. 2). The process of ingestion may occur either by phagocytosis or micropinocytosis (Seljelid et al., 1970). The former mechanism accounts for secretion of thyroid hormones after acute thyrotropin stimulation and has been shown to decline with advancing age (Neve et al., 1981) whereas the latter mechanism is thought

to remain at a steady level throughout the life of an animal. Gradual proteolysis of the thyroglobulin in the lysosomes results in the release of the iodoamino acids into the cell. Free MIT and DIT are almost completely deiodinated through enzyme deiodination in the cell. The released amino acids and carbohydrates mix with cellular pools of these precursors while the lysosomes are recycled (Dumont, 1971a, 1971b) and the liberated thyroid hormone diffuse from the secondary lysosomes to the extracellular space. Alternatively the contents of the droplet may be exocytosed and liberated into the extracellular space. Thyroglobulin has been measured in the blood of man (Van Herle et al., 1973) and rats (Van Herle, Klandorf and Uller, 1975) and established as a normal secretory product of the thyroid. Thyroglobulin has not thus far been investigated in the circulation of birds.

The storage of thyroglobulin in the colloid allows the thyroid the capacity to secrete thyroid hormone in excess of the regular metabolic requirements. The colloid represents a reservoir of hormone supply that can maintain a steady secretion for several weeks even if synthesis of new hormones is totally blocked.

### 1.5 Storage and structure of thyroglobulin

Thyroglobulin is a glycoprotein containing approximately 10 per cent by weight of carbohydrates, which include glucosamine, mannose, fucose, galactose and sialic acid. The glycoprotein consists of four polypeptide chains, each with an aggregate of about 5,000 amino acids, yielding a molecular weight of approximately 670,000 and a sedimentation coefficient of 19 ( $S_{20, 0_w}$ ). In the avian thyroid gland this represents 94-95% of the total soluble proteins; this percentage is higher than that found in the lower classes of vertebrates e.g. Reptilia, Elasmobranchia and Cyclostoma, with a range of 40.7% to 90%, but similar to that reported in mammals (Roch et al., 1968). Individual chains may exist in the 6-7S (monomer) or 12S (dimer) form in saline extracts of the gland. Boudouresque-Linerat and Astier (1979) have reported that the 12S component proportionately increases 17% in ducks maintained on a low iodine diet. A closely related iodoprotein, heavier than the 12S thyroglobulin, was first isolated from human and bovine thyroid glands by Salvatore et al. (1965); the iodoprotein was reported to have a sedimentation constant of 27S and a molecular weight of  $1.2 \times 10^6$ . In birds

this protein was found to represent 5-6% of the total protein found in the colloid. The structure was suggested to represent aggregates of 19S molecules or a polymer of thyroglobulin subunits for the molecule contained the same amino acid composition as 19S thyroglobulin and possessed certain common immunochemical determinants.

The thyroglobulin molecule contains approximately 120 tyrosyl residues of which 30 per cent remain uniodinated. The iodotyrosines, which form the most abundant iodinated amino acid components of thyroglobulin, are themselves without physiologic activity (Gross and Pitt-Rivers, 1953). A comparative study of the total amino acid composition of purified Tg in ducks and chickens has revealed that the number of tyrosine residues are significantly higher than what is found in mammalian thyroglobulins (Hoshino and Ui, 1970). As a result in birds, Tg is iodinated 2-to 3-fold more than in mammals (Dauger et al., 1976; Sorimachi and Ui, 1974; Hoshino and Ui, 1970).

Purified avian Tg has been shown to contain the iodotyrosines 3-monoiodotyrosine (MIT) and 3,5-diiiodotyrosine (DIT) with a predominance of DIT. This results in a higher DIT to MIT ratio in the avian thyroid gland than in the mammalian gland (Vlijm, 1958; Sorimachi and Ui, 1974; Astier, 1975) and in a greater generation of  $T_4$ . Thus the presence of  $T_3$  <sup>in the thyroid</sup> in some species of birds remains controversial (Astier, 1980).

In birds the high storage capacity of Tg for iodine may represent an adaptation to periods of low iodine intake in order to maintain a steady secretion of thyroid hormones. This would ensure maximum utilisation of available nutrients from the diet.

#### 1.6 Mechanism of Action

Thyroid hormone exerts an effect on most organ systems in the body and is essential for the maintenance of metabolism. Inadequate levels of hormone during development are most dramatically illustrated by the pathology of cretinism in humans where the lack of thyroid hormone results in mentally underdeveloped dwarfs. Thyroid hormones are required during an early period of development of the CNS for cell maturation and interaction between neurons (Legrand, 1979). Lack of thyroid hormones prevents metamorphosis of tadpoles into frogs (Frieden, 1970) and in birds results in the chick being unable to complete the process of hatching from the egg (Wishart, 1977).

### 1.6.1 Potency of T<sub>3</sub> and T<sub>4</sub>

The relative potencies of T<sub>3</sub> to T<sub>4</sub> in birds is difficult to determine because T<sub>4</sub> is converted peripherally to T<sub>3</sub> (Astier, 1978; Borges *et al.*, 1980). Initial studies in domestic birds showed that T<sub>3</sub> constituted approximately 40% and T<sub>4</sub> approximately 60% of the levels of circulating thyroid hormones (Wentworth and Mellen, 1961). Gilliland and Strudwick (1953) found that T<sub>3</sub> possessed a greater TSH suppressing potency than T<sub>4</sub> whereas Newcomer (1957) and Mellen and Wentworth (1959) observed that T<sub>4</sub> was a more potent inhibitor of goitre-formation in thiouracil-treated birds. This data contrasted with that of Shellabarger (1955) who found that the hormones were equipotent in preventing goitre. Equipotency of T<sub>3</sub> and T<sub>4</sub> in stimulating heart-rate of chickens was shown by Newcomer (1957) as well as being equally effective in counteracting the effects of hypothyroidism on chicken body and comb-growth rates and on liver glycogen. The turnover rate of the two iodochromes is similar and so supported the view that T<sub>3</sub> and T<sub>4</sub> were equipotent (Heninger and Newcomer, 1964) whereas in a previous study Newcomer and Barrett (1960) found T<sub>4</sub> to be a more powerful promoter of oxygen uptake in chick myocardium.

Thyroxine can also be metabolised to the calorigenically inactive reverse triiodothyronine (rT<sub>3</sub>) principally by conversion in the liver (Borges *et al.*, 1980; Chopra, 1978) (Fig. 1). Several investigations have suggested rT<sub>3</sub> blocks the metabolism of T<sub>4</sub> to T<sub>3</sub> (see review, Chopra, 1978) and numerous studies have found an inverse relationship between T<sub>3</sub> and rT<sub>3</sub> levels. A change in the T<sub>3</sub> : rT<sub>3</sub> ratio occurs in the new born period in mammals (Chopra, 1975a; Chopra, 1978) and chicks (Thommes, 1977a), chronic malnutrition (Chopra, 1975b), administration of excess T<sub>4</sub> in mammals (Chopra, 1978) and in birds (May, 1980), acute short term fast (Vagenahis, 1975), anorexia nervosa (Nicod, 1976), a variety of acute and chronic systemic illnesses including renal and hepatic insufficiency (Chopra, 1975b; Burger, 1976; Chopra, 1976; Nicod, 1976) and the period following the administration of glucocorticoids (Chopra *et al.*, 1975c). Furthermore, Chopra (1975) has also measured the concentration of the free (unbound) T<sub>3</sub> and found that in the absence of gross changes in the levels and affinities of the thyroid hormone binding proteins, the levels closely follow the concentration of the total hormone.

In the chicken Premachandra *et al.* (1977) found a positive correlation between the levels of rT<sub>3</sub> and age (from 0-160 weeks); this

suggests that the decline in the rate of lay in older birds may be due to an inhibitory role of  $rT_3$  in the metabolism of  $T_4$  to  $T_3$ . Furthermore the elegant studies of Borges et al. (1980) in the chick embryo support a view that there are developmental changes in the metabolism of  $T_4$ ; formation of  $rT_3$  represents an inactivation pathway that is functional when the requirement for  $T_3$  is lower.

#### 1.6.2 Biochemical and metabolic action: the sodium pump

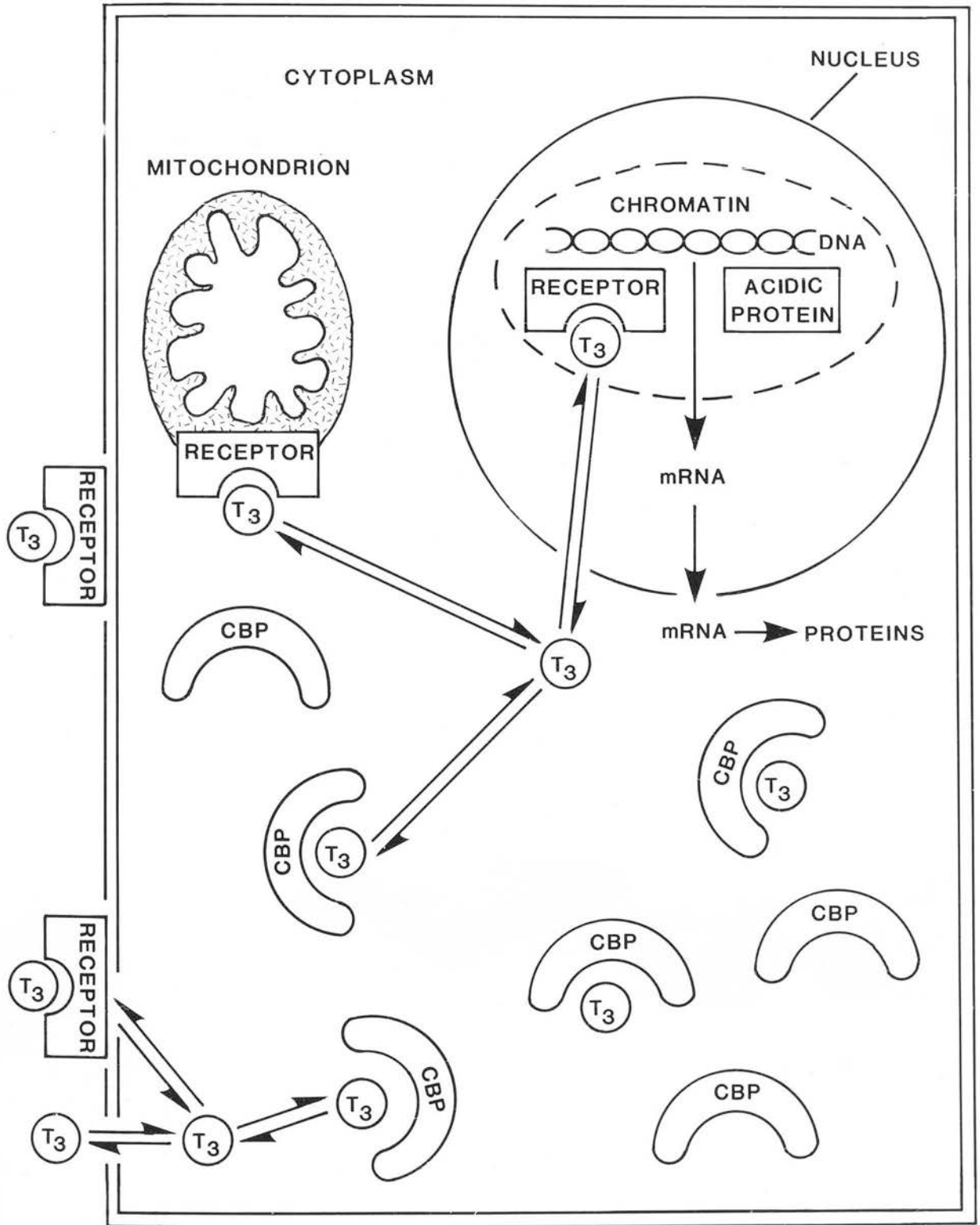
Thyroid hormone has been shown to stimulate the synthesis of cellular proteins in intact animals and in cultured cell populations. Thus, the  $\alpha_2$ -globulin is not detected in the liver of hypothyroid rats but it is synthesised after the administration of  $T_3$  (Kurtz, 1976). Similarly physiological doses of  $T_3$  stimulate the synthesis of GH m-RNA in cultured rat pituitary tumor cells (Seo et al., 1977; Shapiro et al., 1978) as well as the plasma membrane enzyme  $Na^+-K^+$ -ATPase, the "sodium pump" (Edelman and Ismail-Beigi, 1974; Edelman, 1974).

Studies by Ismail-Beigi and Edelman (1970, 1971) suggest that in rat liver and skeletal muscle, increased energy expenditure for active  $Na^+$ -transport could account for approximately 90% of the increase in the oxygen uptake of rats which was induced by treatment with thyroid hormone in vivo. However, subsequent studies have shown this figure to be over-estimated and it is now thought that the maintenance of the sodium and potassium distribution across the plasma membrane by means of the membrane bound  $Na^+-K^+$ -dependent ATPase is unlikely to account for more than 5-10% of the total oxygen uptake (Sestoft, 1980).

#### 1.6.3 Free hormone and interaction with the nuclear genome

The free hormone, that is the  $T_4$  or  $T_3$  not bound to a protein carrier, because of its hydrophobic and lipophilic properties can pass through the cell membrane. In contrast most protein hormones are unable to pass directly through the cell membrane but must first interact with the membrane-bound adenylate cyclase in order to exert an effect.

The exact mechanism of action of thyroid hormones is still controversial but is probably similar to that of the steroid hormones. Free  $T_3$  has been shown to either diffuse through the plasma membrane or bind to a specific receptor in the plasma membrane and be transported into the cell where it is bound by a cytosol binding protein (CBP) (Sterling, 1979) (Fig. 3).



**FIGURE 3** Mechanism of action for  $T_3$  on a target cell (from Sterling, 1979).  $T_3$  within a circle denotes the unbound hormone, CBP cytosol-binding protein.

Instead of being translocated into the nucleus the CBP-T<sub>3</sub> complex is in reversible equilibrium with a minute moiety of intracellular unbound T<sub>3</sub> that can react with high affinity, low capacity receptors in the mitochondrion and nuclear chromatin (Fig. 3).

Thyroid hormones and their analogues are bound by acidic (non-histone) chromatin proteins. There is a positive correlation between the relative binding and biologic activity of the hormones (Lo et al., 1976) as well as the organ distribution of nuclear receptors. The thyroid hormone insensitive tissues (calorigenically inactive), spleen and testes, were found to show no appreciable nuclear binding (Barker and Klitgand, 1952) although binding by brain nuclei, also an inactive tissue, exhibited more than half as many nuclear receptor sites as myocardium. The number of nuclear receptors in pituitary cells is depleted by increasing the concentration of T<sub>3</sub> added to the media (Samuels, 1977). Furthermore a decrease in the number of nuclear receptors has been seen in livers of fasted rats (Schussler and Orlando, 1978; De Groot et al., 1977b) and the decline is related to a decrease in energy metabolism.

#### 1.6.4 Membrane binding and transport

Adamson (1970) showed in chicks a direct effect of thyroid hormone on cell membranes. Addition of thyroid hormone to embryonic chick bone increased the intracellular amino acid incorporation. A similar effect of thyroid hormone has also been observed in the myocardium of the chick embryo. Uptake of <sup>3</sup>H - labelled 2-deoxyglucose was found to be enhanced almost immediately on exposure to near physiological concentrations of T<sub>3</sub> (Segal et al., 1977; Segal and Gordon, 1977); these actions on the cell membranes were not abolished by blockade of protein synthesis using puromycin, cycloheximide or actinomycin D blockage of RNA synthesis. Additionally, studies in the rat have shown that the specificity for thyroid hormone analogues was suggestive of physiologic receptors, for the plasma membrane was able to discriminate between L-T<sub>3</sub> and D-T<sub>3</sub> (Sterling et al., 1977).

#### 1.6.5 Thyroid hormone and catecholamine receptors

Thyroid hormone has been suggested to increase the synthesis of catecholamine receptor sites on cardiac plasma membranes. Banerjee and King (1977) measured an increased number of receptor sites in cardiac

membranes from rats rendered toxic with excess thyroid hormone. In contrast studies by Bilezikian and Loeb (1979) on the nucleated turkey erythrocyte suggest that there is enhanced amplification of the  $\beta$ -adrenergic signal. The physiological responsiveness of hypothyroid turkey erythrocytes to isoproterenol ( $\beta$ -adrenergic agonist) is reduced, and the decreased sensitivity accounted for by a reduction in  $\beta$ -adrenergic receptor numbers (Furukawa et al., 1980).

#### 1.6.6 Action as neurotransmitters

Dratman (1974) has proposed that  $T_3$  and  $T_4$  function as amino acid analogues of tyrosine, thereby modifying the protein and catecholamine pathways of this amino acid. Incorporation of other halogen-substituted amino acids into proteins has resulted in acceleration of protein degradation and secondary stimulation of protein synthesis and turnover (Papaconstantinou, 1967). Furthermore, evidence has accumulated that iodothyronine may enter into the catecholamine biosynthetic pathway and act as precursors for alternate adrenergic neurotransmitters. Dratman et al. (1977) has found  $T_3$  concentrated in peripheral adrenergic nerves and has found thyroid hormone localised and metabolised within synaptosomes. Thus there is sufficient evidence to support the idea that thyroid hormones may serve as neurotransmitters.

#### 1.6.7 Action on mitochondria and oxidative phosphorylation

In view of the crucial role of the mitochondrion in energy metabolism it has been long considered a possible subcellular target for thyroid hormone. The mitochondrion is the site where the energy from the oxidation of foodstuffs is converted to ATP via the process of oxidative phosphorylation. Classically the uncoupling of oxidative phosphorylation was considered to be the principal mechanism of action for thyroid hormone. Uncoupling is the result of an elevation in mitochondrial oxygen consumption without a corresponding increase in ATP synthesis. Thus under conditions where mitochondrial oxidative phosphorylation is coupled, the oxidation of 1 mol  $NADH_2$  to 1 mol  $H_2O$  incorporates 3 mol Pi into ADP per  $\frac{1}{2}$  mol  $O_2$  and so gives a P : O ratio of 3. When uncoupling occurs in mitochondria the P : O ratio is less than 3. Another possible mechanism to explain the inefficient respiratory activity has been proposed by Smith et al. (1964). Thyroid hormones are suggested to

cause uncoupling by stimulating extramitochondrial metabolic pathways which consume nutrients but produce few high energy bonds.

Uncoupling of oxidative phosphorylation in mitochondria was first reported by Martius and Hess (1951) and demonstrated by Hoch and Lipmann (1954). This idea has been generally discarded because the measurement of uncoupling required non physiologic doses of thyroid hormone. Whether the thyroid hormones cause some loosening of coupling in the normal situation remains controversial. The possibility of early, direct thyroid hormone action upon the mitochondria has received considerable support with the discovery of a specific receptor in the mitochondrial membrane (Sterling, 1977; Sterling et al., 1977; Sterling and Milch, 1978). An action on the mitochondrial membrane is appropriate to an effect on oxidative phosphorylation since it has been found that the coupling of electron transport and phosphorylation occurs via a hydrogen ion gradient that is created across the inner mitochondrial membrane (Lehninger, 1975). The binding component exhibited high-affinity, low capacity (saturable) binding sites similar to those reported for the cell nucleus but with an even higher association constant. Furthermore, distribution of the receptor was compatible with its physiological function since it was found in the mitochondrion of organs responsive to thyroid hormone (as measured by oxygen consumption) and not in the nonresponsive tissues, brain, spleen and testes (Roher, 1924; Sterling et al., 1978; Sestoft, 1980). These studies have shown that mitochondria from the brains of neonatal rats possess the specific receptor through the age of 12 days and this corresponds to the neonatal rat-brain capacity to respond to thyroid hormones. These studies have also shown that the more potent synthetic thyroid analogue 3', isopropyl 3,5-diiodothyronine is more tightly bound by the mitochondrial receptors than is T<sub>3</sub> whereas in the brain they are bound equally as well (Sterling, et al., 1977). Physiological doses of T<sub>3</sub> were sufficient to stimulate oxygen consumption in the liver mitochondria of hypothyroid rats and remain effective with cyclohexamide blockage of protein synthesis.

Hulbert (1978) has proposed that the actions of thyroid hormone on the various membranes of the cell are accomplished through the energy released after T<sub>4</sub> is deiodinated to T<sub>3</sub>. In this manner the degree of unsaturation in the membrane fatty acids is altered and, as a result, both the permeability and catalytic activities associated with the

membrane are affected. Alternatively direct hormone action at the mitochondrial level is suggested to involve a conformational change induced in the inner membrane lipoprotein macromolecule which in turn results in enhanced oxidative phosphorylation (Sterling, 1979).

Tissues exposed to elevated levels of thyroid hormone are capable of maintaining a normal concentration of ATP, ADP and inorganic phosphate (Pi), even though there is an increased turnover of energy-rich phosphate and an increased rate of oxygen consumption. Sestoft (1980) has found that the mitochondria rely on an increased capacity for transport of ADP and Pi by increasing the area of mitochondrial membrane per g of tissue and by a change in the translocation of substrates for oxidative phosphorylation.

Thus an effect on mitochondrial energy metabolism and plasma membrane is likely the first measureable effect of thyroid hormone, with an increase in the rate of transcription of genetic sequences and a concomitant increase in the rate of the various ATP-consuming processes occurring soon after. This general view of the mechanism of action of thyroid hormone is fundamental to an appreciation and understanding of the metabolic role of thyroid hormone in birds. These effects are fundamental for normal growth, differentiation and cell maintenance.

#### 1.6.8 Interaction with other hormones

The interactions between thyroid and other hormones are important in the regulation of endocrine functions. Thyroid hormones affect the metabolism of the C<sub>21</sub> steroids, cortisol and aldosterone, and they increase the rate of conversion of androstenedione to testosterone, estriol and estradiol, and of testosterone to dihydrotestosterone (Gordon and Southern, 1977). Other evidence suggests that the actions of other hormones which are mediated by cAMP, such as epinephrine, parathyroid hormone, and glucagon are modified by changes in the plasma levels of thyroid hormones (Guttler et al., 1977).

#### 1.7 Binding proteins

Thyroid hormones, like the steroid hormones, exist in an aqueous solution by means of binding to a carrier protein. The various thyroid hormone binding proteins are synthesised by the liver and are responsible

for the maintenance of a large extra thyroidal pool of thyroid hormone. In birds and mammals only a minute fraction (less than 0.5 per cent as free hormone) is active and in equilibrium with the free intracellular hormone (Davison, 1976). It is evident from studies in birds (El Sayed et al., 1980; Heaf et al., 1980) and mammals (Robbins, 1973) that wide fluctuations in the concentration of binding proteins do not alter the metabolic status of the animal. The free level of hormone in warm blooded vertebrates remains more constant than any other parameter of thyroid function despite variations in the nature and capacity of the thyroid hormone binding proteins. In the absence of binding proteins the extrathyroidal thyroid hormone pool would be completely metabolised in a matter of hours following cessation of hormone secretion. Thus one of the primary functions of the binding proteins is to smooth out any abrupt fluctuations in hormone secretion. The thyroid hormone binding proteins also serve as a means to store iodine. The binding protein imparts macromolecular properties to the circulating iodothyronines by extending their half-life and limiting their loss in the urine (Chan et al., 1972).

#### 1.7.1 Thyroid hormone binding proteins in birds

Early studies in birds have shown that the major classes of proteins that bind the thyroid hormones are the albumins and prealbumins (Farer et al., 1962; Balfour and Tunnicliffe, 1960; Tritsch and Tritsch, 1965; Tanabe et al., 1969; Refetoff et al., 1970). Most of the information about the relative distribution of  $T_4$  and  $T_3$  among the principal binding proteins was derived from studies in vitro utilizing electrophoresis of serum enriched with isotopic-labelled hormones. Gordon et al. (1952) and Larsen et al. (1952) were the first to note in man that  $^{131}\text{I}$ -labelled  $T_4$  migrated in association with an  $\alpha$ -globulin fraction. In most mammals thyroxine-binding globulin (TBG) is the least abundant of the thyroid-hormone binding proteins but it transports 70 to 75 per cent of the total serum  $T_4$  and  $T_3$ . This is due to its high association constant for  $T_4$  and thus the concentration of TBG is the principal factor in determining the concentration of total  $T_4$  in serum as well as the levels of free hormone (Refetoff et al., 1970). Tata and Shellabarger (1959) established that birds lack a specific TBG and that  $T_3$  and  $T_4$  were equivalent in biologic potency unlike the situation in mammals. This

was suggested to be due to a difference in the affinity of the carrier proteins for thyroid hormone between the two species. In mammals  $T_3$  had been shown to possess greater biologic activity than  $T_4$  (Money et al., 1960; Barker, 1955). Sterling et al. (1954) also demonstrated that in man  $T_3$  was cleared more rapidly than  $T_4$  from the circulation while Robbins and Rall (1955) first reported that the carrier proteins had a lower affinity for  $T_3$ . It was later shown by Tritsch and Tritsch (1965) that the relative potency of the two hormones was not related to the intensity of binding to serum proteins.

A role for the  $\alpha$ -globulins in binding thyroid hormones in chicken plasma was established by Davison et al. (1978a) using polyacrylamide gel electrophoresis at pH 7.8. The  $\alpha$ -globulin was found to bind about 10% of circulating  $T_4$  whereas 30-40% of the  $T_3$  was associated with the  $\alpha$ -globulin and an additional 20% with a second binding globulin. Approximately 70% of the  $T_4$  migrated with the albumin fraction (50% in the case of  $T_3$ ) and approximately 20% with prealbumin. Studies of Bhat and Cama (1979) have suggested that even though the concentration of the  $\alpha$ -globulin in the chicken occurs at a concentration lower than what is found in humans it may still be responsible for binding most of the plasma  $T_4$ . In addition prealbumin bound approximately 11% of the circulating  $T_4$  while albumin bound negligible amounts. Thus the  $\alpha$ -globulin may serve as a reservoir for  $T_4$  and maintain the steady-state level while prealbumin may be important in the attainment of equilibrium and maintenance of free  $T_4$  in the normal range (due to its high disassociation rate). Similarly, the budgerigar (Melopsittacus undulatus) contains a thyroid hormone binding protein in the  $\alpha$ -globulin fraction (Robiller et al., 1975). Castay et al. (1972) using immunoprecipitation techniques have found  $T_3$  and  $T_4$  to be principally bound by the  $\alpha$ - and  $\beta$ - lipoprotein components in duck and chicken serum. In these authors view lipoproteins, which are of secondary importance in the binding of thyroid hormone in mammals, are of primary importance in the bird as are such factors as the age and metabolic state of the bird.

The difference in the methodology employed for the measurement of thyroid hormone binding proteins probably accounts for some of the discrepancies in the results between the various laboratories. These studies are also inadequate to explain the rapid peripheral metabolism of thyroid hormone in birds. In general though, the results from these

experiments suggest the evolution of a common mechanism for the transport of thyroid hormone in the circulation of vertebrates. Thus both the activity of the hypothalamo-pituitary-thyroid axis and the levels of thyroid hormone binding proteins are necessary for the maintenance of a steady-state supply of free hormone to the target receptors.

### 1.7.2 Factors influencing the levels of binding proteins

Conditions associated with changes in plasma thyroid hormone binding proteins have been most extensively investigated in man and are classified as being genetic or pathological. The absence of or a decrease in the levels of TEG in some individuals has been shown to be X-linked and although responsible for the abnormal total levels of plasma thyroid hormone do not affect the free level of thyroid hormone (Refetoff, 1979). Pathological conditions which alter levels of binding protein are caused by disease, fasting hormones and drugs (Refetoff, 1979).

In the chicken the binding power of plasma proteins changes with age (Davison et al., 1978) and in quail Glover et al. (1980) and El Sayed et al. (1980) have found changes in the concentration of thyroid hormone binding proteins at the time of hatch and in relation to the reproductive cycle. Bhat and Cama (1978) have shown that in the chicken administration of  $T_4$  increases both the turnover and the rate of synthesis of prealbumin. Thus even though a change in the total plasma  $T_3$  and  $T_4$  concentrations parallels a change in the levels of binding proteins, the concentration of free hormone tends to remain constant.

### 1.7.3 Measurements of half-life

In comparison with mammals the half-life of thyroid hormone in birds is relatively short. This is due in part to the low concentration of a specific high-affinity carrier for thyroid hormone as well as a lowered affinity of the binding proteins for thyroid hormones (Astier, 1980). Davison (1978b) reported that the turnover of  $T_4$  was 206 minutes when the total plasma  $^{125}\text{I}$  was employed as an estimate of  $^{125}\text{I} T_4$  and 138 minutes when the hormonal fraction was extracted on a cation-exchange resin. Thus the presence of a non-hormonal iodinated protein (Astier, 1975b; Davison, 1976b) interfered with the measurement of turnover rate. Previous estimates of plasma thyroid hormone turnover are comparable to

these measurements (Singh et al., 1967; Astier, 1975).

The turnover of  $T_4$  in chickens exposed to a cold temperature is significantly increased within one day of exposure and decreased when the birds are maintained at an elevated temperature (May et al., 1974). These changes reflect different rates in the metabolism of thyroid hormone and suggest that they may be due in part to changes in binding protein concentration due to concomitant changes in food intake (Shetty et al., 1979). Thus the role of binding proteins in regulating the peripheral levels of thyroid hormone is not completely understood and is an area of research that requires more investigation.

### 1.8 The measurement of thyroid hormones

Techniques for measuring the activity of the thyroid gland in birds have been initially developed for the study of mammalian thyroid physiology. These methods included histometric and gravimetric studies, measurements of peripheral effects, plasma and glandular iodine content, radiometric uptake, thyroid secretion rate, chromatographic and competitive protein binding assays (review Assenmacher, 1973).

Measurement of protein bound iodine (PBI) in the blood of mammals correlated with thyroid activity, but in birds was found not to fluctuate with changes in thyroid state (Mueller and Hardy, 1957; Astier, 1973). Astier (1973) also showed that the levels of PBI did not reflect either the concentration or the physiological responses of thyroid hormone. PBI was found to be precipitable by TCA but was not extractable with n-butanol and so was termed "non-hormonal iodinated protein" (NHIP) and thus was responsible for the overestimation of hormonal iodine in birds. Similarly Davison (1976b) reported that  $PB^{125}I$  overestimated levels of plasma  $^{125}I-T_4$  in immature chickens. Although iodinated proteins have been isolated from mammals (Desai et al., 1974), high levels of NHIP have not been found and so this feature further distinguishes thyroid function between birds and mammals.

The application of radioimmunoassay systems for the measurement of the levels of thyroid hormone in plasma represents an important step in the study of thyroid function in birds. The procedure permits a reliable determination of the total level of hormone as well as the assay of large numbers of samples (Seth et al., 1976).

The levels of thyroid hormone in birds are different to those measured in mammals: the concentration of plasma  $T_4$  is lower while the concentration of plasma  $T_3$  tends to be higher (review Astier, 1980). This is due to the different types and affinities of the thyroid hormone binding proteins in birds and mammals (1.7.1). The levels of thyroid hormone also depend upon the physiological state of the bird and the conditions under which it is maintained. Furthermore because the half-life of thyroid hormones in birds is shorter than in mammals (1.7.3) any change in the production is expressed by rapid changes in the levels of plasma hormone.

### 1.9 Hypothalamic-pituitary-thyroid axis

In birds the pituitary-thyroid relationship was first noted by Mitchel (1929) who showed that hypophysectomy leads to a decrease in the size of the thyroid gland. Sectioning the hypophysial portal vessels also results in a reduction in the weight of the thyroid gland (Rosenberg et al., 1967) and it was concluded that the activity of avian thyrotropes are partially dependent on hypothalamic control. Comparable studies in mammals have shown that hypophysectomy results in a decrease in metabolic rate, food intake, heart rate and body temperature. Riddle et al. (1935) measured a significant drop in the basal metabolic rate of pigeons after hypophysectomy. Similar findings were also reported for other avian species including the Japanese quail (Woitkewitsch, 1940) and the domestic fowl (Keating et al., 1945).

#### 1.9.1 Anatomical relationships

The anterior lobe of the pituitary gland receives all of its blood supply through the portal veins; thyrotrophin-releasing hormone, which stimulates thyrophin release from the pituitary gland, passes through the median eminence and into the capillary network which leads to the portal vascular system. This relationship has been shown by studies involving hypothalamic lesions and pituitary transplants (Dodd et al., 1971, Kanumatsu and Mikami, 1969; Bayle, 1980).

The thyrotrophic activity of the adenohypophysis is only moderately altered after hypothalamic-hypophysial disconnection in birds and so suggests a relative functional autonomy of the ectopic

thyrotrophic activity with respect to the hypothalamus (Thompson et al., 1981; Bayle et al., 1980). In ducks (Assenmacher and Tixier-Vidal, 1963; Rosenberg et al., 1967), chickens (Ma and Malbandou, 1963), pigeons (Bayle et al., 1966) and quail (Bayle and Assenmacher, 1967) this procedure did not significantly reduce thyroid  $^{131}\text{I}$  uptake and moderately decreased the  $\text{PB}^{131}\text{I}$ . Egge and Chiasson (1963) lesioned the median eminence of white leghorn hens with no noticeable effect on thyroid activity (no atrophy of the thyroid gland) and in the duck Bayle (1969) also found that electrolytic destruction of the median eminence had little effect on thyroid function. Lesions in the supraoptic and ventrolateral nucleus were found to increase thyroid  $^{131}\text{I}$  uptake and release (Chiasson, 1966; Gehrman, 1968) and increase the content of pituitary TSH (Egge et al., 1975). Furthermore lesions of the anterior hypothalamus, including the ventrolateral nuclei, were shown to result in a lowered disappearance rate of  $\text{T}_4$  from the circulation of the Japanese quail (McFarland et al., 1966) while Takahara et al. (1967) found that lesions of the basal anterior hypothalamus result in thyroid atrophy. The reasons for the lack of agreement between these results and those of Egge and Chiasson (1963) are not clear.

Studies by Kanumatsu and Mikami (1969; 1970) have attempted to locate the sites controlling the release of TSH by using hypothalamic lesions and pituitary tissue transplants in the region of the anterior hypothalamus. These studies suggested that the anterior hypothalamus is a site of production of TRH. The levels of plasma thyroxine in quail are reduced by hypothalamic-hypophyseal disconnection although there seems to be a progressive recovery in thyroid function with increasing intervals of time after surgery (Herbute et al., 1979; Pinat et al., 1980; Moukayi et al., 1980).

Surgical removal of the olfactory bulbs in chickens increases oxygen consumption, the number of active follicles in the thyroid gland and thyrotropic cells of the pituitary (Robinson et al., 1977abc). The increase in thyroid activity was followed by a compensatory increase in food intake and was not associated with an increase in body weight. Addition of 0.1% PTU to the diet of bulbectomised birds abolished the hyperphagia and caused a significant decline in oxygen intake (Robinson et al. 1977a). Lesions in the medial basal hypothalamus of bulbectomised cocks result in the disappearance of the thyroid stimulation (Robinson

et al. 1977b). Thus TRH secretion takes place in or via the basal medial hypothalamus and the olfactory bulb may have an inhibitory role on thyroid activity.

Lesioning the septal nuclei causes an increase in thyroid activity which is accompanied by hyperphagia without development of obesity (Robinson et al., 1978). The TSH content of the chicken pituitary is increased after lesions in the septal-mesencephalic tract (Egge et al., 1975) and the  $^{131}\text{I}$  uptake and release from the thyroid gland is also increased (Gehrmann, 1968).

### 1.9.2 Biochemical and physiological evidence for feed-back loops

Greer (1951) first provided evidence for a neural factor that could stimulate the release of TSH. This evidence was based on studies showing that hypothalamic extracts stimulate TSH release while hypothalamic lesions depress thyroid function. Further support came from electrophysiological studies (Harris and Woods, 1958; D'Angelo and Synder, 1963; Guilleman, 1963) which determined that the effect was due to a hypothalamic peptide. Eventually Guilleman et al. (1965) isolated TRF from ovine hypothalami and Schally et al. (1966) from porcine hypothalami and identified its chemical structure as proglutamyl-histidyl-propyl-amide (Boler et al., 1969; Burgus et al., 1969, 1970; Nain et al., 1970). Bowers et al. (1970) was the first to synthesize a tripeptide which could function as effectively as native TRF in bioassays.

The avian hypothalamus was also shown to contain immunoreactive TRH (Jackson and Reichlin, 1974); TRH was also measured in the median eminence region and in the olfactory bulb.

In mammals studies with tritium-labelled synthetic TRH have shown a specific receptor site on the cell membrane of the thyrotrope (Grant et al., 1972). Once bound to the receptor in the pituitary gland TRH activates the adenyl cyclase system which results in the release and synthesis of TSH (Dannies et al., 1976).

The nuclei of pituitary cells contain specific nuclear binding sites for  $\text{T}_3$  (see review, Oppenheimer, 1979); these sites are of high affinity but low capacity and  $\text{T}_4$  does not compete as well for them. Thus the interaction of  $\text{T}_3$  with the nuclear pituitary receptor may be responsible

for the negative feedback effect of thyroid hormone on the pituitary gland and regulation of the peripheral concentration of thyroid hormones in order to maintain levels essential for cellular metabolism and for growth and development.

Identification of cells producing TSH in the avian pituitary has been accomplished through the use of surgical and chemical techniques. Thyrotropes are basophilic and stain positively with periodic acid-Schiff reagent and with alcian blue and have been identified in Japanese-quail (Tixier-Vidal et al., 1968, 1972, see review 1973) and in chickens (Payne, 1944; Mikami, 1969). Thyroidectomised quail injected with  $T_4$  show a decrease in the size of the thyrotropes (Tixier-Vidal et al., 1972) and so these findings support a negative feedback effect of thyroid hormone on pituitary function in birds.

### 1.9.3 TRH and other pituitary hormones

Studies in vitro have shown that the secretion of TSH from the chicken pituitary is increased by low levels of TRH while in higher doses TRH can induce the secretion of prolactin (Bolton et al., 1977; Scanes, 1974; Chadwick and Hall, 1975). In turkeys Burke (unpublished observations) has shown that an injection of TRH results in an increase in the levels of plasma prolactin. Furthermore administration of TRH in birds markedly increases the concentration of plasma growth hormone (GH) although the degree of response declines with the age of the bird (Harvey et al., 1981).

### 1.10 Effect of thyroid hormone on growth and intermediary metabolism

The effects of thyroid hormone on growth in intact birds have been found to be both dose and age dependent. Singh et al. (1968) measured an increase in growth rate of 3 week-old chickens treated with  $T_4$  whereas Parker (1943) and Herbert and Bronson (1957) recorded only a slight acceleration of growth in their thyroprotein-fed adult chickens. Once a certain dose level is exceeded thyroid hormones are found to decrease growth by increasing the process of catabolism (Singh et al., 1968). King and King (1973) found that PTU-induced hypothyroidism caused a retardation in body growth which was reversible with supplemental doses of  $T_4$ . This study also showed a non uniform response to thyroid hormone in the various muscle tissues. Some studies which suggest that feeding

a supplementary diet containing thyroid hormone and which effect thyroid function and growth rate are subject to criticism for neither the level of food intake nor the endogenous hormone concentration are measured. The relationship of these parameters to growth rate and thyroid function thus limits any interpretation of the results (e.g. Summer and Leeson, 1977).

#### 1.10.1 Plasma protein levels

In chickens elevated levels of thyroid hormone are associated with lowered plasma protein levels. When plasma thyroid hormone levels are depressed by inclusion of the goitrogen methimazole in the diet plasma protein levels are elevated (Nangia *et al.*, 1975). In chickens fed methimazole the synthesis and turnover of the various enzymes and proteins are affected as well as the availability of dietary protein (due to depressed intestinal absorption).

#### 1.10.2 Plasma carbohydrate levels

Carbohydrate metabolism is affected in part by the levels of thyroid hormone, in addition to the activities of the pancreas and adrenal glands. In chickens chemical or radiothyroidectomy have been found to result in an increase in the concentration of liver glycogen (Snedecor, 1971) and Ensor (1970) has shown that thyroidectomy results in hypoglycaemia in ducks. Changes in the concentration of liver glycogen are noted within several days after the onset of the anti-thyroid treatment (Snedecor, 1968) with a complete reversal of these effects found after withdrawal of the goitrogens. A decrease in the concentration of liver glycogen is one of the first responses measured after an injection of  $T_4$  or  $T_3$  (Raheja and Snedecor, 1971); an effect of  $T_3$  was manifested earlier whereas  $T_4$  was effective for a longer period of time. The induction of the glycogenolytic enzyme glucose-6-phosphatase by thyroid hormone was concluded to be the rate-limiting step in this pathway. Raheja and Linscheer (1978) suggested that the influence of thyroid hormone on carbohydrate metabolism is modulated by the carbohydrate and fat composition of the diet whereas Arrondo *et al.* (1978) hypothesised that the  $T_3$ -induced depletion of liver glycogen in chickens was caused by activation of pre-existing enzyme molecules.

### 1.10.3 Plasma lipid levels

Thyroid hormone is intimately involved in lipid metabolism. Benoit and Bagdanovich (1937) found thyroidectomy to raise, and adeno-hypophysial extract to lower, the fatty acid cholesterol and phospholipid content of fowl plasma. An increase in fat deposition was measured in chickens and turkeys (Blakely and Anderson, 1949) made hypothyroid with thiouracil (Andrews and Schnetzler, 1946). Evans *et al.* (1961) confirmed these observations in chicks fed either thiouracil or thyroid powder. Similarly radiothyroidectomy in chickens leads to obesity and elevated serum cholesterol levels (Mellen and Wentworth, 1962). These observations were confirmed in birds made hypothyroid with methimazole whereas the reciprocal effect was found in chickens treated with thyroxine. In turkeys addition of thyroxine to the drinking water causes a decrease in the levels of total serum lipids and cholesterol whereas the induction of hypothyroidism resulted in the opposite effect (Bilezikian *et al.*, 1980).

### 1.11 Thyroid and reproduction in birds

The thyroid gland plays an essential role in the development and maintenance of reproductive activity and secondary sexual characteristics in birds. The effect of a decrease or increase in the levels of thyroid hormone on reproductive function is dependent on various factors including the age, sex and species of the bird as well as environmental conditions (e.g. photoperiod, pattern of food intake, diet, temperature). As a result there are complicated effects of thyroid hormones on the reproductive activity of birds.

Injection of thyroxine into fully grown (somatically mature) drakes results in an increase in testes growth accompanied by spermatogenesis (Aron and Benoit, 1934) whereas this treatment is not effective in the immature male. Generally ducks and chickens require thyroid hormone for normal gonadal development (Greenwood and Blythe, 1942; Assenmacher, 1973) whereas in certain Indian finches thyroid hormone and short days inhibit while thyroidectomy and long days stimulate gonadal activity (Thapliyal, 1969; Thapliyal and Chandola, 1978; Chandola and Thapliyal, 1973; Assenmacher, 1973). Thyroidectomy in both the drake and the domestic chicken leads to a regression in testes size and a cessation of spermatogenesis (Assenmacher, 1973). These effects can be reversed

in male and female chickens by feeding dessicated thyroid in the diet (Greenwood and Chu, 1939); growth of the comb was induced in both sexes and egg production was initiated in the females. In the chicken high doses of thyroid hormone impair fertility while low doses may increase the concentration of sperm in seminal fluid (Wilworth et al., 1954). Woikewitsch (1940) observed that sexual maturity in the young fowl was delayed by thyroidectomy and that such treatment resulted in the atrophy of the testes of adult cockerels; a comparable regression in the ovaries of hens was not seen. In hens thyroidectomy delays gonadal maturation (Blivaiss, 1947) and in mature ducks ovarian weight is reduced after feeding the goitrogen thiouracil in the diet (Berg and Bearnse, 1951). Hypothyroidism in young cockerels caused gonadal regression (Blivaiss and Domm, 1942) and retarded the development of the comb, wattles and spurs (Andrews and Schnetzler, 1946; Shaffner and Andrews, 1948; Kumaron and Turner, 1949).

#### 1.11.1 Seasonal variation

In most species of wild birds thus far studied there is a relationship between thyroidal and gonadal functions. Physiological and morphological data have been used to illustrate this relationship, however the precise nature of this relationship differs between species.

##### 1.11.1a Ducks and pigeons

These species are characterised by a reciprocal relationship between the levels of plasma androgens and thyroid hormones. In the duck thyroidectomy inhibits the testicular response to the seasonal increase in day-length (Benoit, 1936; Assenmacher and Tixier-Vidal, 1962; Jallageas and Assenmacher, 1974). Photostimulated pigeons treated with thyroid extract show a marked inhibition of ovarian growth (Clavert, 1953). In Peking ducks and Teal levels of plasma thyroxine increase gradually through the reproductive period and peak at the end of the breeding season (Jallageas and Assenmacher, 1974, 1979; Jallageas et al., 1978). It was suggested that the increased levels of plasma  $T_4$  are important in the termination of seasonal breeding. In support of this view the administration of thyroxine to sexually active birds resulted in gonadal involution. These studies suggested that the increased plasma  $T_4$  levels at the end of the breeding season may

increase metabolic rate and the clearance rate of testosterone and possibly gonadotropins (Assenmacher, et al., 1975; Jallageas and Assenmacher, 1979). Thus the high levels of  $T_4$  may help maintain low levels of plasma gonadotropins and gonadal steroids at the end of the breeding season.

#### 1.11.1b Indian finches

In the male Baya finch (Ploceus philippinus), male weaver bird and quail thyroidectomy prevents the seasonal development of the gonads (Thapliyal and Gang, 1969; Peczely et al., 1980; Chaturvedia and Thapliyal, 1980). Once the gonads have developed thyroidectomy prevents the development of the post-nuptial refractory phase and the birds remain in full breeding activity throughout the year (Thapliyal and Gang, 1967; Thapliyal and Chandola, 1972).

Thyroidectomy of the nonmigratory Indian finches Lal Munia (Estrilda amardaus) and Spotted Munia (Lonchura punctulata) leads to gonadal recrudescence in both sexes which remain indefinitely active once developed. Administration of thyroxine resulted in a significant reduction in body and gonad weight and termination of the reproductive phase in both thyroidectomised and intact birds at any time during the year (Thapliyal et al., 1968). Thyroidectomised finches also show a seasonal increase in body weight identical to that measured in intact birds but fail to show a decrease later in the reproductive season (Thapliyal, 1969). Body and gonad weight of juvenile thyroidectomised birds increased earlier in the breeding season and did not regress after reaching maximum weight. In juvenile Spotted Munia of both sexes repeated injections of  $T_4$  had no effect on either the weight or histology of the gonads although photostimulated growth of the gonads of both sexes were inhibited when the birds were made mildly hyperthyroid (Thapliyal, 1969).

Thyroidectomy of either male Black-headed Munia and male Lal Munia initiates gonadal recrudescence, extends the active phase of the reproductive period and shortens the regressive phase (Thapliyal and Pandha, 1967). Gonadal redevelopment in thyroidectomised birds was not affected by daylength (Thapliyal and Chandola, 1972) although the gonads of Black-headed Munia will increase in response to an increase in daylength.

#### 1.11.1c European starling and Canada goose

Thyroidectomy of starlings in the summer prevents the regression of the testes in the fall (Woitkewitsch, 1940; Wieselthier and van Tienhoven, 1972). In the Canada goose the levels of plasma  $T_4$  remain elevated throughout the breeding season and did not decline when the gonads were developed (John and George, 1972).

#### 1.11.1d Summary

The evidence that has been thus far accumulated does not support any unifying hypothesis about the role of the thyroid gland in avian reproduction. In tropical and subtropical Indian finches it seems that the thyroid gland is a more important factor than photoperiod in controlling the period of reproductive activity. The timing of the decrease in thyroid activity is associated with the onset of gonadal recrudescence and factors such as food and temperature may be regulating this cycle of development. Alternatively in ducks and teal it is changes in daylength which determine the breeding period. The annual increase in plasma concentrations of  $T_4$  is associated with a marked decline in gonadal function. In general the studies of endocrine cycles in thyroidectomised or gonadectomised birds show a close interaction between the thyroid and sexual cycles which tend to be inversely related.

#### 1.12 Commercial aspects of thyroid hormone in egg production and shell quality

Production of eggs of suitable quality and quantity for the consumer remains one of the major problems of the egg industry. Improvements are continually being made through improved breeding, lighting, nutrition, management and an understanding of the composite biochemical and physiological factors involved. Commercial flocks now contain birds capable of producing eggs at a high rate of lay (70% or more) over prolonged periods of time (14-16 months) (Britton, 1980). Generally egg shell quality is maintained for the first half of the laying period through meeting nutritional requirements but subsequent to this time an increasing percentage of eggs laid are poor in shell quality.

Shell quality is determined by shell thickness as well as shell

porosity; numerous studies have implicated a role for thyroid hormone in the decline in these shell characteristics as well as in egg production. A decrease in the concentration of thyroid hormone is suggested to act by inhibiting or reducing the synthesis of the enzyme carbonic anhydrase which results in shell thinning (Jeffries, 1975). Additionally it is suggested to act by affecting calcium absorption from the gut (Cooke, 1973). Although a reduction in food intake contributes to a decline in shell quality and production, Cook (1973) stated that it cannot solely account for the observed decline.

#### 1.12.1 Shell quality

A seasonal decline in shell quality during the summer months is thought to be due to the associated reduction of thyroid activity in response to the elevated temperature (Bennion and Warren, 1933; Warren and Schnepel, 1940; Wilhelm, 1940; Wilson, 1949; Premovitch and Chiasson, 1976). This finding correlates with studies that have demonstrated a decrease in the thyroid secretion rate associated with the increase in ambient temperature (Reineke and Turner, 1945; Turner, 1948; Huston, 1962ab; Hahn, 1966). Addition of thiouracil to the diet of chicks caused a reduction in shell thickness (Gabuten and Shaffner, 1954) while complete thyroidectomy had been previously shown to reduce shell weight (Taylor and Burmester, 1940) and in turkeys hypothyroidism results in a complete cessation of egg laying (Bilezikian et al., 1980). Conversely an increase in the levels of plasma thyroid hormone produced by the addition of iodinated casein (thyroprotein) to the diet caused chickens to lay eggs with thick shells (Berg and Bearse, 1951; Gabuten and Shaffner, 1954). Addition of thyroxine to the drinking water of turkeys both markedly reduced the rate of lay and resulted in incompletely calcified eggs being laid (Bilezikian et al., 1980), although Asmundson and Pinsky (1935) found this treatment to increase the shell weight of the experimental hens. These diets have been suggested to exert their effect by increasing the amount of calcium absorbed from the gut (Cooke, 1973). Although additional calcium intake is beneficial in improving the egg shell quality of aging layers even high levels of intake have been shown not to prevent the age-related decline in quality (Peterson, 1965). Thus the age-related decline in the plasma levels of thyroid hormone (Davison, 1976; Newcomer, 1978) may, in part, be responsible

for the decline in shell quality.

Further support for this idea comes from the studies of Hurwitz and Griminger (1962) who found that the season of hatch also influenced the rate of shell decline. A depression in shell quality was observed after 20 weeks of production in birds hatched in the spring and maintained at a constant 15-21°C temperature. Birds hatched in the winter did not show a decline when maintained under similar conditions. Cqmpos et al. (1960) studied the influence of a rapid rise in temperature upon shell quality and reported a marked decrease in shell quality in the subsequent experimental period. An increase in environmental temperature results in a decrease in food consumption (Cooke, 1973) which results both in a decrease in dietary calcium and a decline in the thyroid secretion rate. In a later study these authors found that exposure of birds to a temperature of 31°C during the day and 0°C at night did not result in a decrease in shell quality, presumably mediated by the cold related stimulation of the thyroid secretion rate and food intake (Cqmpos et al., 1962). Mueller (1961) also found that a diurnal temperature change resulted in improved shell quality when compared to birds maintained at a constant elevated temperature.

#### 1.12.2 Egg production

Egg production has been shown to decrease if the thyroid glands are removed from laying hens and to increase again if the operated birds are treated with thyroid hormones (Taylor and Burmester, 1940). Similarly administration of the goitrogen thiouracil to the diet results in a decline in egg production (Berg and Bearse, 1951). Thus it has been suggested that the decreased rate of egg production in summer, in hens experiencing seasonal changes in temperature, may be due to a deficiency of thyroid hormones in the blood caused by high temperatures (Turner et al., 1945; Thornton and Moreng, 1959). Alternatively an increase in the levels of thyroid hormone has been found to produce the opposite effect (Crewe, 1925). Turner et al. (1945) observed that addition of protamone to the diet of laying hens improved egg production, although subsequent studies could not confirm these experiments (Hutt and Gowe, 1948; Wilson, 1949; Bearg and Bearse, 1951). This may have been due to a higher concentration of thyroprotein in the diet which would have reduced the rate of lay. Alternatively thyroprotein may have only been

effective in increasing egg production of low-producing birds and so would have no effect on high-producing hens (Booker and Sturkie, 1950). Furthermore as laying hens age the secretion of thyroid hormones decreases and so it has been suggested that the decrease in egg production in old layers is due to decreased thyroid activity (Turner *et al.*, 1945; Turner, 1948). This suggestion was supported by the observation that thyroid hormones stimulate egg production in aging layers (Turner and Kempster, 1946; Moore and Rees, 1948). Finally a relationship has been suggested between the thyroid secretion rate and the rate of lay in birds of the same age. Thus, hens laying two-egg sequences were found to have a lower rate of thyroid secretion than in hens laying four-egg sequences (Booker and Sturkie, 1950).

### 1.12.3 Egg Weight

Egg weight has also been found to be associated with abnormalities in thyroid function. Both thyroidectomy (Taylor and Burmester, 1940) and thiouracil (Berg and Bearse, 1951) which depress the levels of plasma thyroid hormones, as well as addition of thyroproteins or iodinated caseins to the diet (Asmundson and Pinsky, 1935; Wilson, 1949) which increase the concentration of plasma thyroid hormones, cause a reduction in the weight of eggs laid. Similarly egg size has been reported to be influenced by season (Jull, 1924; Cunningham *et al.*, 1960) and by the age of the hen (Atwood, 1928; Clark, 1940). Jeffery (1941) has suggested that egg weight is the result of the interaction of age, environmental temperature and the body weight of the bird. Finally the time of oviposition has been shown not to have a direct effect on egg weight (Choi *et al.*, 1981) although the weight of the shell was found to increase as the eggs in a sequence were laid later in the day.

## MATERIALS AND METHODS

### 2.1 Animal housing and husbandry

The description of the birds listed in this section will be limited to general observations while details for a particular experiment will be given in the Results and Discussion sections.

#### 2.1.1 Chickens

Commercial broiler and White Leghorn chicks were purchased from commercial breeders and maintained either on a 14L : 10D photoperiod or on an experimental lighting pattern in brooders. Food (chick starter mash, SAI Ltd.) and water were provided ad libitum.

At 3-4 weeks of age, birds were transferred to a commercial type windowless hen-house and caged individually or in groups and exposed to the required lighting pattern. Full access to food (SAI layers mash or pellets) and water was allowed except prior to surgical thyroidectomy where food was withdrawn for at least 16 hrs and during the experiment where the effect of fasting on thyroid function was investigated.

Birds of all strains under investigation were initially reared and maintained at the Agricultural Research Council's Poultry Research Centre at King's Buildings. For some experiments, groups of birds were brought to the outstation at Roslin and maintained in identical conditions for the duration of the particular experiment.

#### 2.1.2 Bantams

Adult bantam hens aged between 18 and 24 months were kept in floor pens (4 x 2m) or individual battery cages with free access to food (SAI layers mash) and water and exposed to a 14L : 10D photoperiod. Birds housed together in floor pens were provided with nest boxes and the eggs collected each day.

#### 2.1.3 Ducks

Aylesbury, Mallard and Khaki Campbell ducklings were maintained on a lighting schedule of 14L : 10D photoperiod with food (layers starter mash) and water freely available. The birds were either reared in wire

cages or in floor pens. The mallards were hatched from eggs collected at Loch Leven while the Aylesbury and Khaki Campbell ducks were obtained when they were day-old from commercial sources.

#### 2.1.4 Grouse

Male Willow ptarmigan (Lagopus lagopus lagopus) were reared from eggs laid in captivity at the Department of Arctic Biology, University of Tromsø (Mr. K.A. Stokkan). The birds were exposed to natural changes in daylength while housed in individual cages. The birds were given free access to food (pellet diet formulated for game birds) and water and the diet was supplemented with heather shoots.

#### 2.1.5 Turkeys

Turkey hens (B.U.T. 666) were placed in individual pens (2.5 x 1.5m) containing a large nest box when they were 20 weeks of age. The birds were maintained on a shortday photoperiod (6L : 18D) until they were 30 weeks of age when the photoperiod was increased to 18h light/day.

#### 2.1.6 Quail

Fertile eggs were collected from a colony of quail (Coturnix coturnix japonica) and incubated at King's Buildings. Once the chicks had hatched they were reared on a photoperiod of 6L : 18D until the sexes could be distinguished by the feather pattern on the breast.

### 2.2 Collection of blood samples

#### 2.2.1 Venepuncture

Normally blood samples were taken from the brachial vein using a 2½ or 5ml syringe rinsed with heparin (5,000 iu/ml, Pularin Evans Medical) before use. This technique enabled up to 12 serial samples to be taken at 2 hr intervals from both of the brachial veins. Blood was withdrawn within 2 minutes of removing a bird from its cage.

#### 2.2.2 Decapitation and cardiac puncture

Blood samples from small or young birds were obtained by decapitation and the blood collected in heparinised beakers. The birds were injected

with 200 units of heparin (0.2 ml) 20 min prior to decapitation in order to minimise the formation of clots. In one experiment quail were serially sampled over a period of time by inserting a needle directly into the ventricle and removing 2 ml of blood.

### 2.2.3 Catheters

This technique was utilised in order to minimise the stress-related effect of handling the birds on the concentrations of plasma thyroid hormones. A local anaesthetic was injected i.m. before the brachial artery was exposed. Catheters were threaded approximately 6 cm towards the heart before the wall of the vessel was sutured to the tubing. The catheter was filled with heparinised saline (1 : 250 dilution of heparin, 5,000 iu/ml in 0.9% saline). The catheter tubing was threaded behind the wing and placed over a pulley in the centre of the cage and then inserted on a 5 ml syringe placed on the outside of the cage. Tension on the tubing was maintained by adding weights between the pulley and the syringe and so allowed the birds to freely move about the cage without interference from the tubing. The catheter was flushed several times during the day as well as on the morning of the experiment with heparin saline (1 : 500 dilution or 125 iu/ml). The first 0.5 ml of blood was always discarded before collection of the sample. In this manner repeated blood samples could be taken from the same bird over a period of up to a week.

### 2.3 Radioimmunoassay methods

The radioimmunoassay procedure was a modification of the method described by Seth et al. (1976).

#### 2.3.1 Preparation of thyroid hormone free plasma

Plasma free of thyroid hormone was prepared by a modification of the method of Mitsuma et al. (1972). Plasma was collected from broiler cockerels and grouse and was stripped of thyroid hormones by incubation with prewashed Norit A activated charcoal (Sigma Chemical Corp.) (40g/100 ml for 48 hr at 4°C). The slurry was centrifuged once at 2,500 rpm for 30 minutes and twice at 20,000g for 20 minutes. The supernatant was filtered through a 12 um filter (average pore size) using an amicon

ultrafiltration apparatus to remove any charcoal fines. The procedure is successful in removing over 99% of the  $T_3$  and  $T_4$  from plasma and does not alter the total protein concentration, pH, or the  $T_4$ -binding capacity. The thyroid hormone-free plasma was used to prepare standard curves for the thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) radioimmunoassays in order to equalise protein concentrations between incubates containing samples and standards.

### 2.3.2 Preparation of standards

#### i) Triiodothyronine

L-triiodothyronine free acid was obtained from Sigma Chemical Co. Ltd., Surrey, England. A stock solution was prepared by dissolving 10 mg  $T_3$ , molecular weight 651, in 20 ml of alkaline propylene glycol: water (1 : 1).

Solution A: Dilute 1.0 ml stock  $T_3$  to 100 ml Sorensen's sodium glycinate buffer, 0.1 mol/l, pH 10.5 containing gelatin (2 g/l) and sodium azide 0.1 g/l ( $7.7 \times 10^3$  nmol/l).

Solution B: Dilute 100  $\mu$ l A to 1 ml in thyroid hormone free plasma.

Solution C: Dilute 100  $\mu$ l B to 5 ml in charcoal treated plasma.

Working standards: Dilute solution C in thyroid hormone-free plasma as follows:-

<u>Standard Number</u>	<u><math>T_3/T_4</math> free serum</u>	<u>Solution C</u>	<u><math>T_3</math> ng/ml</u>
1	10.00	0.00	0
2	9.75	0.25	25
3	9.50	0.50	50
4	9.00	1.00	100
5	8.50	1.50	150
6	8.00	2.00	200
7	7.00	3.00	300
8	4.00	6.00	600
9	-	10.00	1000

Standard solutions were stored in aliquots of 500  $\mu$ l at  $-70^\circ\text{C}$  until required for assay.

## ii) Thyroxine

L-thyroxine sodium salt pentahydrate was obtained from Sigma Chemical Co. Ltd., Surrey, England. A stock solution was prepared by dissolving 18 mg  $T_4$ , in 20 ml of alkaline propylene glycol-water (1 : 1).

Solution B: Dilute 0.1 ml stock  $T_4$  standard to 5 ml in charcoal treated plasma.

Solution C: Dilute 0.2 ml B to 10 ml in charcoal treated plasma (400 nmol/l).

### Working standards

Dilute solution C in charcoal extracted plasma as follows:-

<u>Standard Number</u>	<u><math>T_3/T_4</math> free plasma</u>	<u>Solution C</u>	<u><math>T_4</math> ng/ml</u>
0	20.00	0.0	0.00
1	19.875	0.125	1.9
2	19.75	0.25	3.9
3	19.50	0.50	7.5
4	19.00	1.00	15.0
5	18.00	2.00	31.0
6	17.00	3.00	47.0
7	16.00	4.00	62.0

The standard solutions were stored in aliquots of 1 ml at  $-70^{\circ}\text{C}$  until required for assay.

### 2.3.3 Routine assay procedure

The radioimmunoassay procedure is that described by Seth et al., (1976). The solid phase antiserum to  $T_4$  and  $T_3$  was prepared by Dr. Seth and Dr. Beckett of the Department of Clinical Chemistry, Royal Infirmary, Edinburgh.

Reagents: L-  $^{125}\text{I}$  thyroxine and tri-  $^{125}\text{I}$ iodothyronine were obtained from the Radiochemical Centre, Amersham, England. The solid-coupled antibodies were stored in assay buffer [Sorensen's sodium glycinate buffer, 0.1 mole/l, pH 10.5 containing gelatine (2g/l) and sodium azide (0.1 g/l) at a dilution corresponding to a 1 : 100

dilution of the whole antiserum]. Stock antibody suspension was diluted to give working suspensions corresponding to at least 30% of the zero standard. The antibody was dispensed from a stirred suspension. For the  $T_4$  assay 25 mg of 8-anilino-1-naphthalene sulfonic acid (ANS, Sigma Chemicals Ltd.) and  $^{125}I-T_4$  (25 mg) were dissolved in assay buffer and diluted to 25 ml. This solution was made within 24 hrs before use and stored at  $4^{\circ}C$ . For the  $T_3$  assay, ANS (31 mg) and  $^{125}I-T_3$  (600 pg) were dissolved in assay buffer and diluted to 25 ml. This solution was made within 24 hrs before use and stored at  $4^{\circ}C$ . Both ANS and the highly alkaline buffer functioned to reduce the association of thyroid hormones to the plasma binding proteins.

An antibody wash solution (0.1 mol/l sodium chloride containing 0.06% BRIJ) was prepared and stored at  $4^{\circ}C$  until required. Both the assays were carried out in polystyrene round bottom tubes (84mm x 14.5mm, W. Sarstedt, England).

Assay procedure: The reagents, samples, standards were dispensed with a Compu-pet 100 (William R. Warner & Co.).

<u><math>T_3</math> Radioimmunoassay</u>		<u><math>T_4</math> Radioimmunoassay</u>	
Sample or $T_3$ Standard	50ul	Sample or $T_4$ Standard	100ul
ANS plus $^{125}I-T_3$	150ul	ANS plus $^{125}I-T_4$	200ul
Solid-coupled $T_3$ antibody suspension	<u>500ul</u>	Solid-coupled $T_4$ antibody suspension	<u>500ul</u>
Total volume	<u>750ul</u>	Total volume	<u>800ul</u>

The incubates were mixed on a vortex mixer and allowed to stand at  $4^{\circ}C$  for at least 36 hours.

The antibody-wash solution (2.0 ml,  $4^{\circ}C$ ) was added with a repeating syringe dispenser.

The tubes were centrifuged at 3,000 rpm for 15 minutes and the supernatants decanted by smartly inverting the tubes.

The tubes were counted on a Wallac 8000 GAMMA Counter (L.K.B. Instruments Ltd., Surrey) for a time sufficient to accumulate 10,000 counts in the total count tube.

#### 2.3.4 Data analysis

For all the experiments described in this thesis, potency estimates, together with their 95% confidence limits were calculated using a computer program developed by Rodbard and Lewald (1970) and modified as a radioimmunoassay data processing program package by the Animal Breeding Research Organisation, Edinburgh. The computer program was designed to linearize the standard curve using a logit-log transformation. The calibration line was used as the standard curve for determining potency estimates and 95% confidence limits for the control and unknown samples. The program also provided an estimate of the minimal detectable dose (measure of the sensitivity of the assay and defined as the smallest hormone concentration for which the response is different from the "0" standard). The relative simplicity of the radioimmunoassay procedure enabled all the samples of a particular study to be assayed together.

The minimal detectable doses of  $T_4$  and  $T_3$  were 0.18 and 0.13 ng/ml, respectively. The  $T_4$  assay had an intraassay coefficient of variation of 4.6% while the corresponding value for the  $T_3$  assay was 5.9% ( $n=20$ )

#### 2.4 Surgical techniques

##### 2.4.1 Thyroidectomy

Commercial broiler hens were surgically thyroidectomised when they were 7 weeks of age. The birds were fasted overnight in order to minimise interference from the crop during the surgical procedure. Sodium barbitone anaesthesia was infused through a cannula inserted into a leg vein. The area in the region of the crop was plucked and an incision was made with an electric-cauterizer. Each thyroid gland was exposed and gently teased away from its site on the jugular vein using forceps and cotton wool. The glands were normally removed intact and the immediate vicinity cauterised in order to eliminate any remaining thyroid cells. The area was sprinkled with aureomycin powder and the skin sutured together before the birds were returned to recovery cages (14L : 10D,  $30 \pm 0.2^\circ\text{C}$  with a relative humidity of 70%). After the experiment had ended the thyroidectomised birds were killed and the completeness of the surgery was confirmed by gross examination.

#### 2.4.2 Pinealectomy

Pinealectomy was carried out as described by Kobayashi (1968). Commercial broiler hens (7-10 days old) were given an i.m. injection of equithesin (2.5 ml/kg).

The success of all pinealectomy operations was checked at the end of each experiment. The birds were killed by cervical dislocation and the gross morphology of the region was examined. Histological examination was carried out on 20% of the birds, those birds with pineal fragments were excluded from the results. Sham-operated control birds in which the pineal was exposed but not removed were also included as part of the study.

#### 2.4.3 Gonadectomy

Quail (26-30 days of age) and ducks (18-22 days of age) were anaesthetised with equithesin and surgically castrated. Each bird was laid on its right side, the top leg and wing were fastened and an incision was made between the last two ribs. The ribs were kept apart using a retractor. After perforating the abdominal air sacs the immature gonadal tissue was removed using forceps. The region was cauterised in order to destroy any remaining gonadal tissue. The ribs were sutured together and the area sprinkled with aureomycin. Each bird was then turned over to its left side and the remaining gonad removed in an identical fashion. Success of the surgery was determined at the termination of the experiment and birds with any visible remaining tissue were excluded from the study.

### 2.5 Chemical manipulation

#### 2.5.1 Goitrogens

i) Twenty-four broilers (day-old) were divided equally into two groups. Broiler rather than White-Leghorn-type hens were chosen because of their greater growth rate. Group one was fed a broiler's mash containing 15% heat treated Span 2 rapeseed meal (Blair and Scougal, 1975). The diet consisted of maize (15%), wheat (42.8%), herring meal (10%), soya meal (10%) rapeseed meal (15%), limestone (0.5%), methionine (1%), vitamin mix (0.25%), mineral mix (0.25%), calcium phosphate (1.5%), sodium chloride (0.3%) and vegetable oil (3.4%). The

TABLES 1 AND 2

TABLE 1 - Composition of Control and Rapeseed diets

INGREDIENTS	CONTROL	RAPESEED
Maize	15.0 <sup>+</sup>	15.0 <sup>+</sup>
Wheat	46.6	43.7
Herring	10.0	10.0
Soya	23.0	10.0
Rapeseed	-	15.0
Limestone	0.7	0.5
Methionine	0.1	0.1
Dical	1.5	1.5
Oil	2.3	3.4
Mins/Vit/Salt	0.8	0.8

<sup>+</sup> Expressed as per cent of diet

TABLE 2 - Feed Analysis of Control and Rapeseed diets

	CRUDE PROTEIN	ENERGY	Ca	P	M+C	LYS
Control	23.5	12.76	1.00	0.74	0.95	1.47
Rapeseed	23.3	12.65	0.97	0.73	0.95	1.37

control group was fed a layers starter mash and it is listed in Table 1.

In a subsequent series of experiments day-old broiler cockerels were fed a 15% rapeseed diet calculated to contain an equivalent amount of crude protein and energy content as the control broilers mash. The composition of both diets and their respective feed analysis are listed in Table 1 and 2.

ii) Broiler cockerels were fed from hatch on either a control layers starters mash (Table 1) or an identical diet that contained 0.1% methimazole.

iii) Broiler pullets were fed from hatch on an iodine deficient growing ration (Table 3) formulated at the Poultry Research Centre by Dr. W. Dewar. The control starters mash consisted of barley (10%), maize (30%), wheat (24.5%), herring meal (5%), soya meal (22%), dried grass (5%), limestone (0.58%), calcium phosphate (2.17%), vitamin mix (0.25%), mineral mix (0.25%) and sodium chloride (0.25%). Unfortunately the cost of the vitamin free casein prohibited an experimental diet supplemented with iodine to be fed to control birds. The palatability of the experimental diet was not determined but it was observed that the birds fed the iodine deficient diet ate less than the birds fed the control diet.

#### 2.5.2 Implants

Fourteen Khaki Campbell domestic drakes received a subcutaneous implant of silastic tubing (Dow Corning Ltd., i.d. 0.062 in., 10 cm long) containing testosterone (Sigma Chemical Co. Ltd.) when they were 8 weeks of age. In this manner a sustained release of hormone could be maintained for at least a period of up to 2 weeks.

#### 2.5.3 Injections

i) Alternatively an elevated concentration of hormone could be maintained by a daily i.m. injection. Fourteen Khaki Campbell domestic drakes were given daily injections of thyroxine (80 ug, 0.2 ml, 0.9% saline) for two weeks .

ii) Six thirteen-month-old Brown Leghorn hens (body weight, 1.5-1.8 kg) were injected with 100 ug TRH in saline (Sigma Chemical Co.)

in 0.5 ml s.c. in the leg near the femoral vein.

iii) Six laying and six incubating bantam hens were injected i.v. in the brachial vein with bovine prolactin (NIHPB9) (200 ug in 0.5 ml saline).

#### 2.5.4 Salt water

The acute and chronic response to ingestion of salt water was investigated in Aylesbury ducks. Immature ducklings were transferred from drinking fresh water (FW) to salt water (SW) (0.1 or 0.2M). Salt water was placed in plastic reservoirs and connected to the water system of the cages.

### 2.6 Telemetry and calorimetry

#### 2.6.1 Heart rate

Six 12-week-old birds reared on an 8L : 8D photoperiod and 6 reared on a 14L : 10D photoperiod were implanted with radiotelemetry transmitters, two days before the onset of an experiment. These experiments were done in collaboration with Dr. I.J.H. Duncan and Dr. J.H. Filshie. The transmitters were designed to transmit electrocardiograms (ECG) with a distinct QRS complex suitable for analysis (Filshie et al., 1980).

In a second series of experiments done with the collaboration with Dr. J.H. Filshie heart rate was determined in commercial 7-week-old hens implanted with transmitters and exposed to various experimental photoperiods.

#### 2.6.2 Calorimetry

Daily variations in heat production were measured using the open-circuit, automated, multicalorimeter system described by Lundy et al. (1978). These experiments involved the collaboration of Dr. M.G. Macleod. The birds were trained to accept the calorimeters as a familiar environment by putting them into the apparatus for 3 d before the first measurements were made. Alternatively in a second study birds were given training sessions of several hours for a week. Daily variations in heat production were first measured when the birds were given free access to food and water. The birds were then removed from the calorimeters and deprived

of food for 24h before being returned to the calorimeters for measurement of fasting heat production.

Metabolic rate (heat production: H, kJ) was calculated from measurements of oxygen consumption and carbon dioxide production by the following formula.  $H = 16.20 O_2 + 5.00 CO_2$ .

## 2.7 Statistical analysis

Normally statistical analysis was carried out using unpaired and paired Students t test. Studies that were carried out over extended periods of time were treated by analysis of variance. Linear regression analysis was carried out on the relationship between thyroid hormone concentrations and rates of egg production as well as between measurement of thyroid hormones and shell quality. The significance of the cyclic changes in plasma thyroid hormone levels was initially calculated by fitting the data to theoretical sinusoidal curves (Halberg *et al.*, 1972). Statistical advice and certain statistical analyses were done by Mr. R. Morley-Jones.

## 2.8 Bioassay for measurement of pituitary TSH

TSH was measured using a modified bioassay procedure as described by Lamberg (1953). The adenohypophyses from each group of control or experimental ducks were bisected into rostral and caudal lobes and each type of lobe was pooled, weighed and homogenised in 1 ml of 0.9% saline. One day-old male broiler chicks were injected s.c. above the right <sup>breast</sup> muscle with 0.1 ml of homogenate at a dose approximately equivalent to one lobe of an adenohypophysis. Between 8 and 10 chicks were injected with the material from each pool of rostral or caudal lobes. Ovine TSH (NIH-TSH-S8) at doses of 0, 2, 4 and 8ug equivalents were similarly injected s.c. (0.1 ml/chick) into 10 chicks at each dose level. Three hours after the hormone and control injections, each chick was injected s.c. with 2.5 uCi of <sup>32</sup>P (Radiochemical Centre, Amersham) above the left breast muscle in 0.1 ml saline. Two hours later the thyroid glands were removed from each chick, weighed and dried overnight at 37°C. The radioactivity in the thyroids was counted using PC5 (Packard) scintillation fluid (5 ml) on a Phillips β counter. The TSH concentrations of the adenohypophysial homogenates were determined by comparison with the dose response curve.

Since there was not sufficient material to assay the homogenates at more than one dose level it was not possible to calculate 95% confidence limits for the estimates of TSH concentration.

#### 2.9 Immunohistological determination of pituitary TSH

The sections of adenohypophyses from control or experimental ducks were stained using the peroxidase-antiperoxidase complex unlabelled antibody method (Steinberger et al., 1970). The procedure was performed by Dr. Mona El Tounsy.

#### 2.10 Radioimmunoassays of plasma LH, testosterone and free T<sub>4</sub>

Plasma LH and testosterone were measured using the radioimmunoassays by Follett et al. (1972) and Williams and Sharp (1978) respectively and performed by Mr. Ron Wilkie.

Free T<sub>4</sub> in the plasma was measured according to Sharp and Klandorf (1981).

TABLE 3 - Basal mix for iodine free diet

<u>Ingredients</u>	<u>Per cent</u>
Starch	48.8
Maize Oil	5.0
Cellulose	10.0
Casein [vitamin-free]	25.0
Arginine base	0.25
Choline chloride	0.40
Magnesium carbonate, basic light	0.26
Calcium carbonate	2.4
Calcium hydrogen orthophosphate	5.25
Vitamin mix	0.76
Mineral mix	0.76
Sodium chloride	0.45
Potassium chloride	0.70

### 3.1 Effect of goitrogens

Administration of goitrogens results in an inhibition of growth and development, an increase in mortality and an increase in the size and activity of the thyroid gland (Spence et al., 1933; Romanoff and Laufer, 1956; Degroot, 1979). Principally these compounds act either to inhibit iodide transport into the thyroid [potassium perchlorate ( $KClO_4$ ), thiocyanate ( $KSCN$ )] or to inhibit the binding of iodide to tyrosine residues in thyroglobulin [methimazole (tapazole), thiourea, thiouracil, methylthiouracil, propylthiouracil, carbimazole, mercaptamidazole]. Additionally propylthiouracil and iopanoic acid and, to a lesser extent, methimazole, partially inhibit the peripheral deiodination of thyroxine to triiodothyronine (Obregon et al., 1980) although methimazole has been shown to be ineffective in this respect in cockerels (Tanabe et al., 1967). Acute administration of large doses of iodide <sup>in mammals</sup> inhibit thyroid hormone synthesis and transport, whereas administration over a long period of time leads to an "escape" from the inhibition of thyroid synthesis (Thomas and Manhinney, 1973). In rats chronic exposure to excess iodide leads to hypertrophy and hyperplasia of thyroid follicular cells and a decrease in the levels of plasma  $T_4$  (Collins and Capen, 1980).

Natural goitrogens consumed in the diet are suggested to play a role in endemic goiter in many parts of the world. For example the cyanogenic glucosides are hydrolysed by glucosidases and release free cyanide which can be metabolised to thiocyanate (Ermans et al., 1972). Common sources of the cyanogenic glucosides include sorghum, cassava, sweet potatoes and maize. Thioglucosides are metabolised in the body to thiocyanides and isothiocyanates and are contained in various plants including cabbages, brussel sprouts, cauliflower, turnips and rutabagas.

Various insecticides and weedkillers sprayed over crops raise the possibility of domestic and wild birds ingesting contaminated food-stuffs (Cook, 1973; Petersen, 1965). These agents were shown to affect thyroid function and were found responsible for the decreased fecundity of some birds of prey. Various species have been found to lay eggs with thin shells and as a result are not successfully incubated. Thus the possibility of weedkillers, such as aminotriazole which is known to be a potent goitrogen in domestic fowl, contaminating poultry diets

is of serious concern to the poultry industry (Wishe et al., 1979).

The presence of glucosinolates in rapeseed meal has raised doubts about the extent to which it can be safely used as a source of vegetable protein in poultry diets. Rapeseed is a source of vegetable oil for human consumption, with the meal remaining after removal of the oil used as a protein supplement for farm animals instead of soyabean and fishmeals. Thyroid hypertrophy was the first specific effect to be recognised (Pettit et al., 1944; Turner, 1948; Clandinin and Bayley, 1960; Jackson, 1969; Clandinin et al., 1966), and has been shown to be caused by the conversion of glucosinolates (thioglucosides) to aglucones, namely isothiocyanates, thiocyanates, nitrites (which impair liver function) and inorganic thiocyanate (Papass et al., 1979). Furthermore rapeseed meal produces a 'fishy' taint in eggs laid by some strains and increases the incidence of liver-haemorrhage and mortality in laying hens (Pearson et al., 1978; Papass et al., 1979a). Despite the enlargement of the thyroid glands in birds fed rapeseed meal their rate of growth is depressed much less than that of birds fed the well-characterised goitrogens such as methimazole (Wilson and MacLaury, 1961; Singh et al., 1968). Studies have shown that the etiology of the goitrogens on performance is different for the addition of thyroxine to the diet can reverse the depression in growth rate in birds fed methimazole (Singh et al., 1968) but not in birds fed rapeseed meal (Kratzer et al., 1954; Summers and Leeson, 1977). Furthermore Akiba and Matsumoto (1976) have shown that the metabolism of iodine in rapeseed fed chicks is not affected by changes in the absorption of iodine from the gastrointestinal tract and is suggested to be due to a specific change in thyroid function.

The following studies evaluated the change in the concentration of plasma thyroid hormones in growing cockerels fed a diet containing methimazole, a diet low in iodine and a diet containing rapeseed meal. These data were correlated with plasma measurements of thyroid hormones in order to further define the pathogenesis of the thyroid lesion produced by feeding rapeseed meal. The effects on the histology of the thyroid gland was determined, as well as the ability of the liver of birds fed rapeseed meal to metabolise thyroxine to triiodothyronine.

### 3.1.1 Methimazole

Broiler cockerels were fed from hatch on either a control layer's

starters mash (Table 1) or an identical diet that contained 0.1% methimazole. The birds were reared in brooders until they were 5 weeks of age and then were transferred to group cages until the end of the study. Food and water were provided ab libitum. Blood samples were taken weekly from the brachial vein for determination of thyroid hormones, beginning weekly from the third week after hatch until the birds were 10 weeks of age. Measurements of body weight were taken weekly beginning with the second week after hatch.

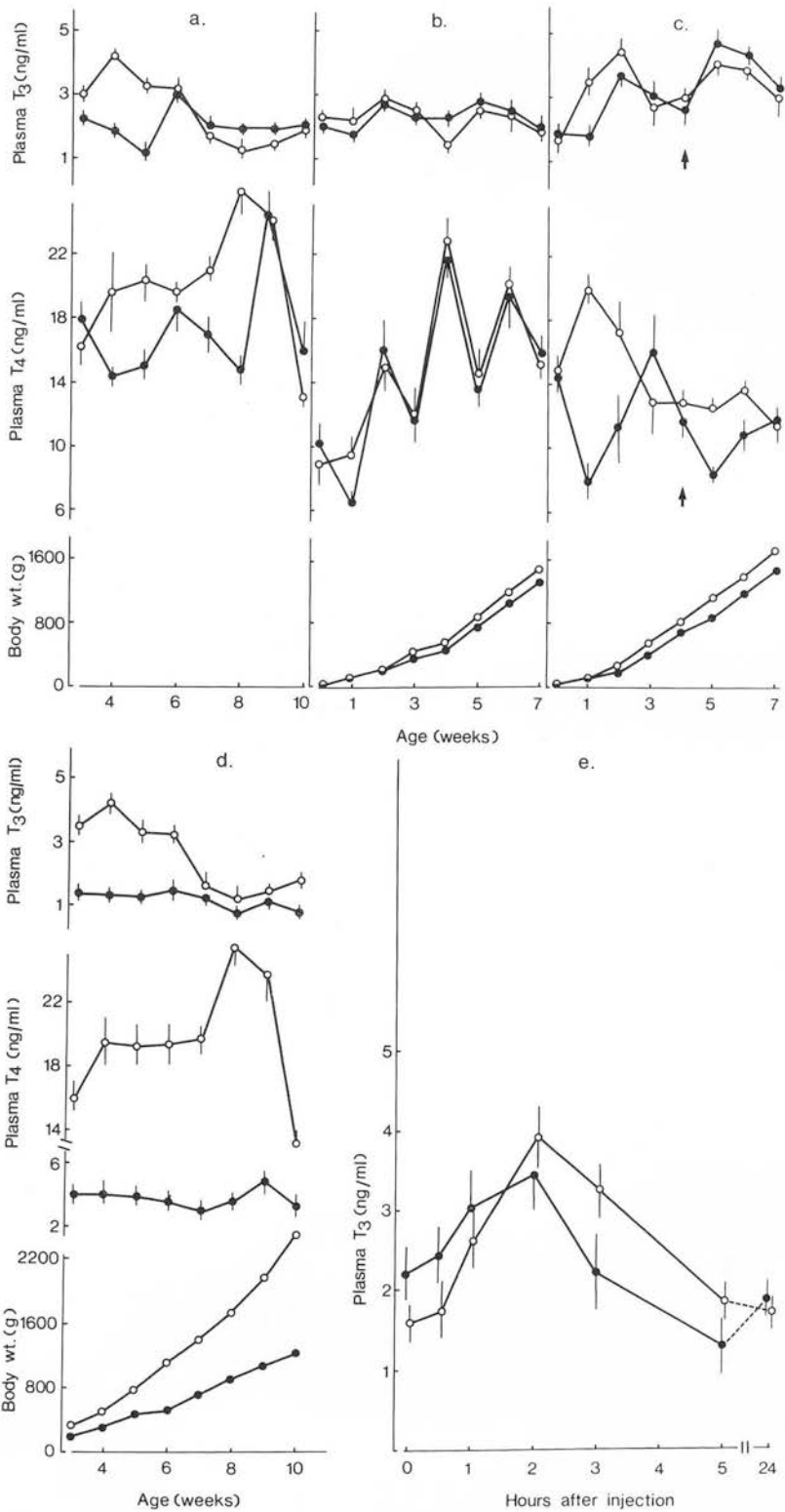
The birds were killed by cervical dislocation at 10.5 weeks of age and the weight of the pituitary gland (pars distalis) and thyroid gland was determined. The thyroids were preserved for histological examination in Bouin's fluid. The fixed tissues were embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin.

The unpaired Student's t-test was used for statistical comparison of the controls with the treatment groups.

The effect of the 0.1% methimazole diet on body weight of the growing chicks is shown in Fig 4d. Body weight of the methimazole group was lower than the control fed birds within one week after hatch and this difference became more pronounced as the study progressed.

The morphology of the feathers of the methimazole group was markedly affected. Development of the barbules was impaired and plumage changes were incomplete. The birds exhibited the short-leg dwarfism, associated with abnormal fat deposition (Blivaiss, 1947). Similarly Wilson and MacLaury (1961) describe an increase in the incidence of perosis in birds fed methimazole at a dosage greater than 0.008 per cent.

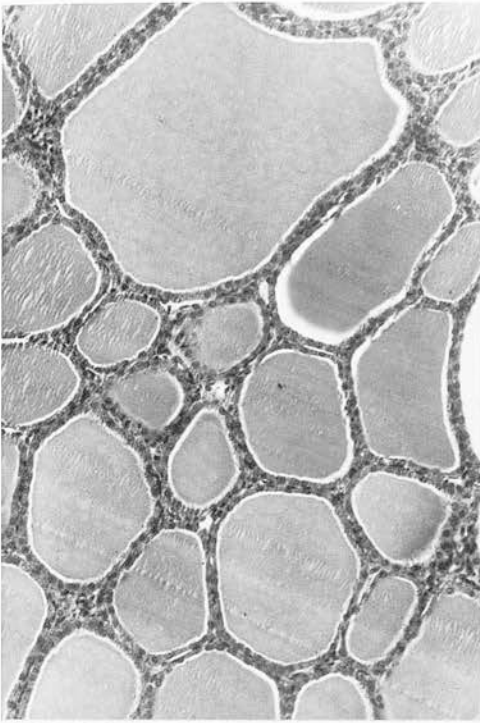
At 10 weeks of age the ratio of the thyroid gland weight to body weight was significantly greater ( $p < 0.001$ ) in birds fed methimazole ( $0.187 \pm 0.031$ ) than birds fed the control diet ( $0.018 \pm 0.001$ ). Thyroid glands of methimazole fed birds were composed of follicles lined by high cuboidal or columnar cells (Fig. 5). Follicular lumens were reduced in size or had collapsed and numerous reabsorption lacunae were seen in the follicles with colloid. The histological picture of the thyroid gland suggests that the secretion of TSH from the pituitary gland is increased. In support of this view the pituitary gland of ducks fed methimazole were found to contain significantly increased concentrations of TSH (3.5.1).



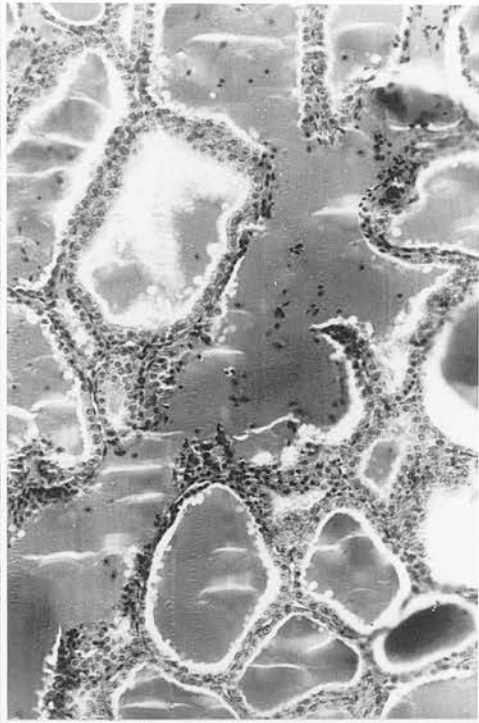
**FIGURE 4**

Changes in the concentrations of plasma  $T_3$ ,  $T_4$  and body weight (Figs. 4a-c) in broiler cockerels fed a diet containing rapeseed meal (solid circles) or a control diet (open circles) during the first weeks of life. The arrows on Fig. 4c represents the time groups of birds were transferred to a lower temperature. Fig. 4e depicts the generation of plasma  $T_3$  after an i.m. injection of 25  $\mu$ g thyroxine.

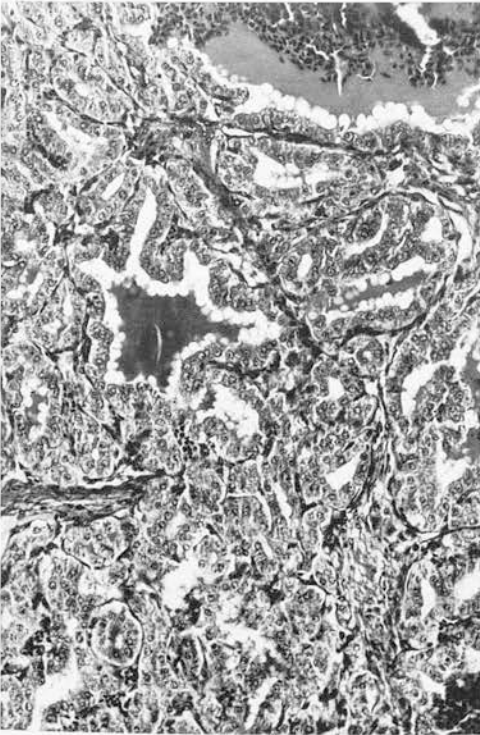
Fig. 4d represents the effect of the inclusion of 0.1% methimazole (closed circles) in the diet of broiler cockerels on the levels of plasma  $T_3$ ,  $T_4$  and body weight.



CONTROL



RAPSEED



METHIMAZOLE



METHIMAZOLE

FIGURE 5 Effect of feeding 15% rapeseed meal, 0.1% methimazole or a control diet on the histology of the thyroid gland of 10-week-old broiler cockerels, X 340.

There was a marked effect of the methimazole diet on the concentrations of plasma  $T_3$  and  $T_4$  in the birds between 3 and 10 weeks of age (Fig. 4d). The concentrations of plasma  $T_3$  and  $T_4$  were significantly depressed ( $p < 0.001$ ) throughout the period of study (for weeks 7, 8 and 9 the concentration of  $T_3$  in methimazole fed birds was depressed at the 0.05 level). The effect of methimazole on plasma thyroid hormones and growth was during the time when the requirement for thyroid hormone is greatest (Bobek *et al.*, 1977; Borges *et al.*, 1980). The resultant reduction in growth and development is evidence for the importance of thyroid hormones in birds during this period.

The decrease in the concentration of plasma  $T_3$  may also have been due to a reduction in food intake (3.3) for Wilson and MacLaury (1961) have shown that food consumption is decreased when the concentration of methimazole is fed at levels above 0.001 per cent. Furthermore a permissive effect of thyroid hormone on growth was not evident for these authors also determined that the feed conversion was not affected as measured by the ratio of food intake: weight gain. Thus although the primary lesion exerted by methimazole is on the synthesis of thyroid hormone, as evidenced by the histology of the thyroid glands, other factors may also be important in determining the physiological responses.

### 3.1.2 Low iodine

Twenty-five broiler pullets were distributed into 2 groups. Group 1 consisted of 10 birds fed the standard Poultry Research Centre (PRC) growing mash. Group 2 comprised 15 birds fed on an iodine deficient growing ration. The layer's starting mash consisted of barley (10%), maize (30%), wheat (24.5%), herring meal (5%), soya meal (22%), dried grass (5%), limestone (0.58%), calcium phosphate (2.17%), vitamin mix (0.25%), mineral mix (0.25%) and sodium chloride (0.25%). The composition of the iodine deficient diet is given in Table 3. Birds were removed from a group if they developed severe bodily impairments. The experiment was terminated when the birds were 7 weeks old with 9 birds remaining in each group. Measurements of body weight were recorded at 2-week intervals beginning at the third week after hatch. A single blood sample was taken on the last day of the experiment.

The birds were killed by cervical dislocation and the thyroids

removed and preserved for histological examination in Bouin's fluid. The fixed thyroid tissue was embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin.

Birds fed a diet low in iodine showed a severe decrease in weight<sup>gain</sup> that became increasingly pronounced with development (Table 4). Qualitatively the birds fed a low iodine diet were smaller in appearance and more poorly feathered than the controls, although there was considerable variation between birds. The fringed feathers characteristic of impaired thyroid function (due to faulty barbulation) gradually appeared as the juvenile plumage replaced the chick feathers. By the seventh week of the study the birds resembled the radiothyroidectomised birds described by Blivaiss (1947) and Mellen and Wentworth (1962). They were squat in appearance, obese and had shortened limb bones.

TABLE 4 - Effect of a low iodine diet on growth  
Mean body weight (grams)  $\pm$  S.E. at various ages

	15 DAYS	28 DAYS	44 DAYS
Controls	260.2 $\pm$ 6.8	631.6 $\pm$ 16.6	1383.6 $\pm$ 71.3
L.I. Diet	174.2 $\pm$ 12.9	356.2 $\pm$ 47.9	678.8 $\pm$ 117.6

TABLE 5 - Effect of a low iodine diet on the concentration  
of plasma thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ )  
at 47 days of age

	$T_4$ (ng/ml)	$T_3$ (ng/ml)
Controls	23.3 $\pm$ 2.3	1.63 $\pm$ 0.18
L.I. Diet	28.2 $\pm$ 3.5	1.16 $\pm$ 0.26

The levels of thyroid hormone in the blood of the birds fed the low iodine diet did not reflect the gross physical changes in morphology. The concentration of plasma  $T_4$  was not appreciably affected although they tended to be non significantly elevated in comparison to control fed birds whereas the concentration of  $T_3$  was marginally depressed in

comparison to controls (Table 5). Turkeys fed a low iodine diet showed a decline only in the levels of  $T_3$  (Bilezikian et al., 1980) whereas Newcomer (1978) found the levels of plasma thyroid hormone to be unaffected by a low iodine diet except for an initial transitory decline in  $T_4$  and  $T_3$ . Thereafter there was a tendency for the levels of plasma  $T_3$  to be lower whereas the concentration of plasma  $T_4$  was unaffected. Rats and mice fed an iodine-deficient diet for 4 weeks showed a decrease in the concentration of  $T_4$  whereas the levels of  $T_3$  were elevated (Collins and Capen, 1980; Deneff et al., 1981). This effect is not consistent though, for some studies reported a decrease in plasma  $T_3$  in response to a chronic iodine deficiency (Volpert and Werner, 1972) or no effect on plasma thyroid hormone concentrations (Riesco et al., 1977). Although rats respond to the chronic administration of a low iodine diet with an increase in the size of the thyroid gland (Collins and Capen, 1980), the birds in this study failed to show an increase in the weight of the thyroid. Similarly Rogler et al. (1961a and b) fed hens a low iodine diet for 4 months and did not find a change in thyroid size even though the histological picture of the thyroid revealed a hypertrophied and hyperplastic tissue with little colloid remaining in the follicular lumen.

After the birds had been on the experimental diets for 7 weeks the histological picture of the thyroids was examined. In the thyroids of control birds the follicles were oval and regular in shape (Fig. 6). The large intact colloid centre was surrounded by a layer of flattened cells which varied in height from squamous to cuboidal. In contrast the thyroids of birds fed a low iodine diet showed evidence of hypertrophy and hyperplasia of the follicular epithelial cells and contained irregularly shaped follicles whose colloid centre was narrow and occasionally completely collapsed (Fig. 6). Although all of the follicles appeared to have responded to iodine-deficient diet the size of the follicular cells was variable. Squamous cell types were infrequently found whereas cuboidal and columnar epithelium predominated along the follicular wall. In those follicles with colloid there were numerous absorption lacunae present. Deneff et al. (1980) observed in mice that iodine deficiency resulted in a similar histological picture; the thyroid gland showed evidence of hyperplasia, the colloid was shrunken and in some cases the follicular structure had collapsed. Similar morphological changes were

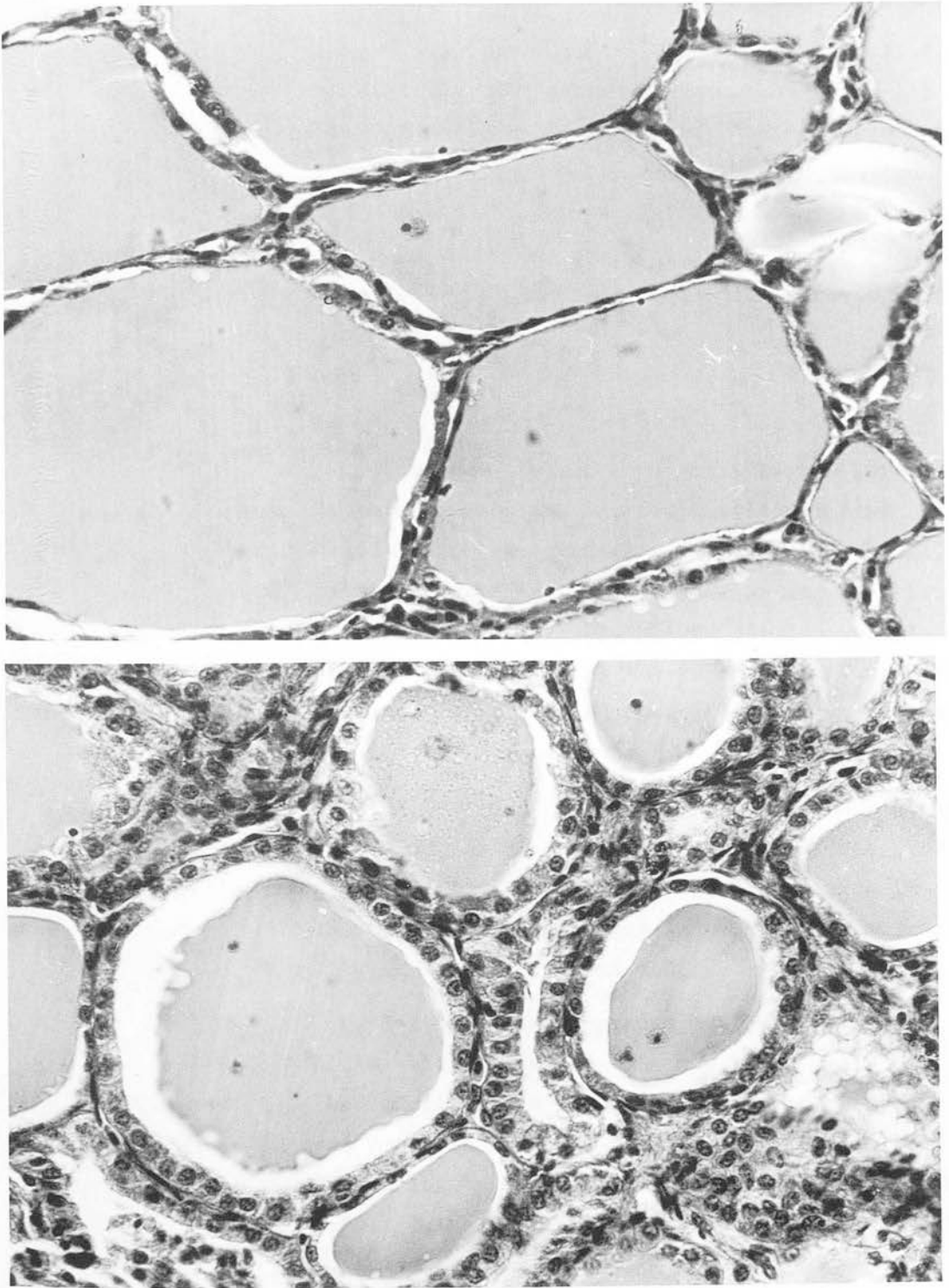


FIGURE 6 Effect of feeding a control (upper portion of Figure) or a low iodine diet on the histology of the thyroid gland of 7-week-old broiler pullets. X 540.

also noted in the increased height of the epithelium as well as an increase in vascularisation.

The histological picture of the thyroid gland is suggestive of an increase in the secretion of TSH. Newcomer (1978) has reported similar findings and in addition reported that goitre formation in birds fed a low iodine diet is transitory and that the half life and turnover rate of  $^{131}\text{I}$  is significantly decreased in these birds (see also Davison et al., 1981). Rats fed a diet low in iodine show a significant increase in the concentration of plasma TSH (Van Herle et al., 1975) which returns to normal levels within 3 days after addition of iodide to the drinking water (Fukuda et al., 1975).

### 3.1.3 Rapeseed

Experiment 1: Twenty-four one day old broiler cockerels were divided equally into 2 groups. Group one was fed a broilers mash containing 15% heat treated Span 2 rapeseed meal (Blair and Scougall, 1975) (Table 1). The layers starter mash is the same as was used for the controls in the low iodine study (3.1.2). Blood samples were taken weekly from the brachial vein beginning from the third week after hatch until the birds were 10 weeks of age. Measurements of body weight were recorded weekly beginning with the second week.

The birds were killed by cervical dislocation at 10.5 weeks of age and the weight of the pituitary, thyroid and liver was recorded. The thyroids were weighed and preserved for histological examination in Bouin's fluid. The fixed tissues were embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin.

The thyroid glands of the birds fed rapeseed meal had large, coalesced masses of colloid which contained both red blood cells and epithelial cells which had proliferated into the follicular space (Fig. 5). Follicles that were not ruptured usually contained a large amount of colloid and showed little activity as evidenced by the absence of absorption lacunae. Follicular cell size varied but cells of the low cuboidal type were predominant.

At 10 weeks of age the body weight of the birds fed the rapeseed meal was not significantly different from the birds fed the layers

starter mash (Table 6). The failure to note a decrease in body weight as reported by others (March et al., 1975; Summers and Leeson, 1977) in birds fed rapeseed meal was due to the composition of the two diets. The control birds were fed a starter's mash for laying type hens whereas the diet containing rapeseed meal (Blair and Scougall, 1975) was formulated to obtain a maximum rate of growth in broilers. The combined thyroid weight of the birds fed the rapeseed meal was significantly elevated over that of the birds fed the control diet (Table 6). There was no change in either the pituitary or liver weights in the two groups (Table 6).

In the control birds there was a marked difference in the pattern of secretion of  $T_4$  and  $T_3$  during the period of study. The concentration of plasma  $T_4$  reached a peak concentration when the birds were 3 weeks of age and fell thereafter whereas the concentration of plasma  $T_3$  reached a peak at 4 weeks of age and gradually declined to a stable level by 7 weeks of age (Fig. 4).

In birds fed the rapeseed meal the concentration of plasma  $T_4$  was only marginally depressed ( $p < 0.05$  at weeks 4, 5 and 8) and so suggests that the impairment of thyroid function in growing birds is not severe. Furthermore in laying hens the inclusion of rapeseed meal in the diet has been shown not to affect the levels of plasma  $T_4$  (Papas et al., 1979b; Smith et al., 1979). However the inclusion of rapeseed meal in the diet caused a significant decrease in the concentration of plasma  $T_3$  in birds of between 3 and 5 weeks of age ( $p < 0.001$ ; Fig. 4). The concentration of  $T_3$  had returned to control levels by 6 weeks and remained marginally depressed ( $p < 0.05$ ) between 7 and 9 weeks of age. Similarly Davison et al. (1981) measured a decrease in the concentration of  $T_3$  in immature hens fed rapeseed meal but recorded an increase in the levels of plasma  $T_4$ . Alternatively in laying hens the concentration of plasma  $T_3$  and heat production were not significantly different in birds fed a rapeseed diet (Smith et al., 1979).

The decrease in the levels of  $T_3$  in birds fed rapeseed meal may, in part, be due to a reduction in food intake (3.3) as compared to controls in addition to the goitrogenic properties of the feed (Davison et al., 1981). Alternatively the elevated levels of  $T_3$  in the control birds may have been due to the composition of the diet which was formulated to

provide a greater metabolisable energy content and thus may have stimulated the generation of  $T_3$ . Thus in the following study diets were formulated to contain an equivalent protein and energy content. The birds were sampled from the day of hatch in order to see how these changes are manifested and at 4 weeks of age the birds were administered a physiological dose of  $T_4$  in order to determine if the ability to generate  $T_3$  is impaired in rapeseed fed birds.

Experiment 2: Thirty-two day old broiler cockerels were divided into two groups. Group one was fed a 15% rapeseed diet that had been calculated to contain an equivalent amount of crude protein and energy content as the control broiler's mash. The composition of the two diets are listed in Table 1. The rapeseed meal used in Experiments 2 and 3 are different from the one used in the first experiment and so could have different "goitrogenic" properties due to the processing and contents of the meal.

A blood sample was obtained from each bird by cardiac puncture until the birds were 3 weeks of age. The first sample was taken when the chicks were day old and were subsequently taken until they were 7 weeks old. Birds that died as a consequence of the sampling procedure were not included in the tabulation of results. At the time the birds were sampled a measurement of body weight was taken.

At 4 weeks of age the birds were divided into 4 groups of 6. The birds from both the rapeseed and control fed groups were injected i.m. with either physiologic saline or 10 ug of  $T_4$  in physiologic saline (0.5 ml). A blood sample was taken before the injection and again at 0.5, 1, 2, 3, 5 and 24 hours.

At 8 weeks of age the birds were killed by cervical dislocation and the weight of the thyroid glands from each of the birds was measured.

The feed analysis of the broiler mash and rapeseed diet is listed in Table 2. The respective diets were found to contain an equivalent level of crude protein and the measured energy content was also similar. The body weight of the birds fed the rapeseed meal was depressed in comparison to control fed birds (Fig. 4b) and this difference became more pronounced with development. This observation confirms the results of other laboratories (March et al., 1975; Summers and Leeson, 1977). Additionally the weight of the thyroid glands of the rapeseed fed birds

were heavier than the control birds ( $0.38 \pm 0.04$  vrs  $0.15 \pm 0.1g$ ,  $p < 0.001$ ).

In the control birds the concentration of plasma  $T_3$  remained fairly stable during the experiment except for one sample taken at the fourth week which was significantly <sup>( $p < 0.05$ )</sup> depressed (Fig. 4b). The concentration of plasma  $T_4$  tended to increase in the weeks after hatch, although the values were more variable than those observed in Experiment 1 (Fig. 4b). The inclusion of rapeseed meal in the diet did not markedly affect the pattern of secretion of  $T_3$  or  $T_4$  in comparison to control fed birds except for a decrease in the concentration of plasma  $T_4$  one week after hatch.

The response of rapeseed and control fed birds to an injection of 10 ug of  $T_4$  is shown in Fig. 4e. In both groups the maximum concentration of  $T_3$  that was measured occurred 2 hrs after the injection (3.8). Subsequently the concentration of  $T_3$  gradually declined and reached baseline levels within 5 hrs. These results are not consistent with the idea that the livers of rapeseed fed birds are deficient in their ability to metabolise  $T_4$  into  $T_3$ . The concentration of plasma  $T_3$  in rapeseed fed birds injected with physiologic saline was significantly depressed at 3 and 5 hrs after the injection ( $p < 0.01$ ) and returned to control levels by 24 hr.

The depressed concentrations of plasma thyroid hormones measured during development in the control fed birds suggested the possibility of a stress effect in response to sampling by cardiac puncture (3.7). In the following experiment groups of birds were decapitated during the first 3 weeks of life and an additional group was subsequently sampled at weekly intervals until they were 7 weeks of age. When the birds were 4 weeks of age the birds were prematurely transferred to a lower temperature in order to stimulate thyroid function.

Experiment 3: One-hundred day-old broiler cockerels were divided into 2 groups. The respective diets for each of the groups was the same as that described for Experiment 2.

For the first 4 weeks 10 birds from each group were injected with heparin (2.2.2) and decapitated. Subsequently an additional group was sampled weekly from the brachial vein, beginning with the fourth week after hatch and continued until the experiment was ended 3 weeks later. When

the birds were 4 weeks of age they were transferred from the brooders (26.7°C) to individual cages (17.8°C). A measurement of body weight was taken at weekly intervals from the time of hatch.

The effect of the rapeseed diet in depressing body weight was confirmed in the present study (Fig. 4c).

The levels of plasma  $T_3$  in control and rapeseed fed birds were similar except for the first and second week after hatch where birds fed the rapeseed diet had significantly lower levels of  $T_3$  (Fig. 4c). One week after the birds were transferred to the lower temperature both groups showed an increase in the concentration of plasma  $T_3$  and these elevated levels were maintained for the following 2 weeks. By the seventh week after hatch the levels of  $T_3$  had decreased although they were elevated in comparison to the value measured in Experiments 1 and 2. These results suggest that transfer to the lower temperature increased food intake and concomitantly increased the production of  $T_3$  (3.3). This view is supported by the finding that the concentration of plasma  $T_4$  was depressed in comparison to Experiments 1 and 2 while the highest levels of  $T_3$  in the rapeseed fed birds were measured at this time (Fig. 4c) (3.3).

In the rapeseed fed birds the concentration of plasma  $T_4$  tended to remain depressed in comparison with control fed birds. The levels were significantly lower ( $p < 0.001$ ) one week after hatch in agreement with the results from the previous experiment. These results suggest that rapeseed meal is exerting a direct effect on the production of  $T_4$  as evidenced from the histological picture of the thyroid glands. Because the metabolic requirements for thyroid hormone are greatest in the growing chick, any decrease in the levels of plasma thyroid hormones results in a compensatory hypertrophy of the thyroid gland (Clandinin *et al.*, 1966; Davison *et al.*, 1981). Thus the effect of rapeseed meal on the levels of plasma thyroid hormones and on growth are transitory and compensated for by an increase in the size of the thyroid gland.

#### 3.1.4 Summary

Diets containing rapeseed meal were found to induce goitre formation in broiler cockerels and decrease the rate of body growth but did not result in a sustained alteration in the concentration of plasma thyroid

hormones. An effect of rapeseed meal on the levels of plasma thyroid hormones was most pronounced during the first weeks after hatch, the levels of plasma  $T_4$  and occasionally plasma  $T_3$  tended to be depressed. Although rapeseed meal can impair liver function there was no evidence of such damage in the present study as suggested by its ability to metabolise  $T_4$  into  $T_3$ . Enlargement of the thyroid gland in rapeseed fed birds was suggested to be in response to an increase in the levels of plasma TSH due to impaired production and release of  $T_4$  from the thyroid gland. Histologically the compensatory increase in the size of the thyroid glands was due to the accumulation of large amounts of intra-follicular colloid. The effect of the rapeseed meal on body weight and general morphology of the birds was not as severe as compared to birds fed 0.1% methimazole or a diet low in iodine. Cockerels fed methimazole had severely depressed levels of thyroid hormone ( $p < 0.001$ ) and enlarged thyroid glands due to increased cell size and number. Although birds fed a diet low in iodine possessed thyroid glands of similar histological appearance the effect on the levels of thyroid hormone were not so pronounced. The levels of  $T_4$  were similar to controls whereas the concentration of plasma  $T_3$  were only marginally depressed. It is suggested that the decrease in body weight and the levels of plasma  $T_3$  are due, in part, to a decrease in food intake associated with the dietary administration of goitrogens.

TABLE 6 - A comparison of body and organ weights in ten-week-old broiler cockerels fed rapeseed meal or a control diet

TREATMENT	Body wt (g)	Combined thyroid wt (g)	Liver wt (g)	Pituitary gland wt (mg)
15% heat treated rapeseed meal	2445.0 ±127.8	0.439 <sup>a</sup> ± 0.043	64.5 ± 2.5	9.31 ± 0.52
Control diet	2564.5 ± 46.6	0.217 ± 0.034	64.2 ± 3.5	9.57 ± 0.57

<sup>a</sup> Indicates means that are significantly different from control mean at the .05 level as determined by an unpaired Student's t-test.

### 3.2 Effect of photoperiod and pinealectomy on daily rhythms of plasma $T_4$ , $T_3$ and heart rate

Daily variations in the concentrations of thyroid hormones in rats are thought to be generated by a circadian rhythm in TSH secretion (Rook et al., 1979; Jordan et al., 1980). A circadian rhythm in the secretion of TSH has also been reported in man (Chan et al., 1978; Weeke et al., 1978); the levels of TSH are highest at night and peak in advance of the increase in thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ). The levels of both  $T_4$  and  $T_3$  were lowest during the night and highest during the day. Alternatively, in man, the daily variations in the levels of serum thyroid hormones may be accounted for by cyclic postural movements of fluids into and out of the vascular compartments (De Costre et al., 1971). In birds there are pronounced daily rhythms in the concentration of plasma  $T_3$  and  $T_4$  which are inversely related, the concentration of plasma  $T_4$  increased during the dark period and decreased during the light period whereas, conversely, the concentration of plasma  $T_3$  increased during the day and fell at night (Newcomer, 1974). These observations suggest that the concentration of plasma  $T_3$  is more directly related to metabolic rate than is that of  $T_4$  since it increased during the light period when the birds would be active and decreased at night when they would be sleeping. In order to investigate this hypothesis further, changes in metabolic rate, as indicated by changes in heart rate, have been related to changes in the levels of plasma thyroid hormone in sexually immature female chickens held on 24-h (14L : 10D) and 16-h (8L : 8D) lighting regimens. The 16-h lighting cycle was selected because it seemed unlikely that it would entrain any circadian rhythms which might be involved in thyroid activity or metabolic rate. This investigation formed part of a preliminary study to confirm the observations of Newcomer (1974) and to determine if there is a circadian element responsible for these daily changes in thyroid hormone.

The daily rhythmicity in the activity of the pineal gland may affect the secretion of pituitary hormones including TSH (Reiter, 1974). Niles et al., (1979) has suggested that in the rat the pineal may be involved in the entrainment of the daily rhythm of TSH secretion by the lighting cycle. Removal of the superior cervical ganglion which blocks pineal function in rams, has been shown to block daylength induced changes in the concentration of plasma  $T_3$  and  $T_4$  (Lincoln et al., 1980).

The pineal hormone, melatonin, has been shown to depress the levels of plasma thyroxine in hamsters (Vriend et al., 1977) while in rats pinealectomy causes an increase in the thyroid secretion rate (Ishibashi et al., 1966) and thyroid hypertrophy (De Fronzo and Roth, 1972). Similarly in quail Oishi and Lauber (1974) have found pinealectomy to increase thyroid weight. Binkley et al. (1971) reported in house sparrows exposed to constant darkness, pinealectomy caused a decrease in the amplitude of the daily rhythm in body temperature patterns. Since thyroid hormones are thought to be involved in the maintenance of body temperature (Arieli and Berman, 1979) it seemed that in this study pinealectomy may have interfered with thyroid function.

The purpose of the second preliminary study was to determine the possible role of the pineal gland in the regulation of thyroid function in the chicken by measuring changes in the concentrations of plasma  $T_4$  and  $T_3$  over a 24 h period in intact and pinealectomised birds transferred to constant darkness.

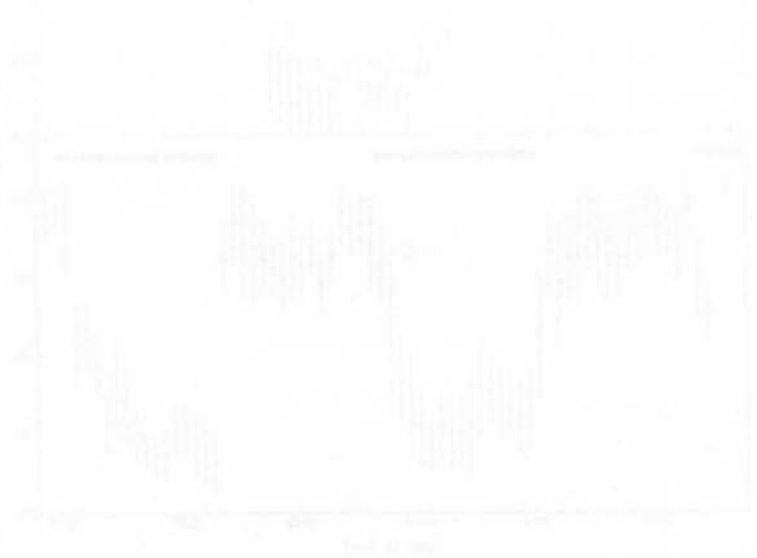
The principal study was undertaken in the chicken to investigate the way in which the daily photoperiod regulates the daily rhythms in plasma levels of  $T_4$  and  $T_3$  and to determine whether these rhythms are influenced by the activity of the pineal gland. Ahemeral lighting cycles of 21 and 27 h were included in the study to obtain evidence for the possible circadian nature of the rhythms. Under these conditions it would be expected that significant phase advances and delays would be measured. Additionally the persistency of the rhythms in constant conditions was investigated in birds transferred to either constant light or dark. Daily changes in heart rate were measured as an indicator of changes in metabolic activity.

### 3.2.1 Preliminary studies; 14L : 10D, 8L : 8D and heart rate

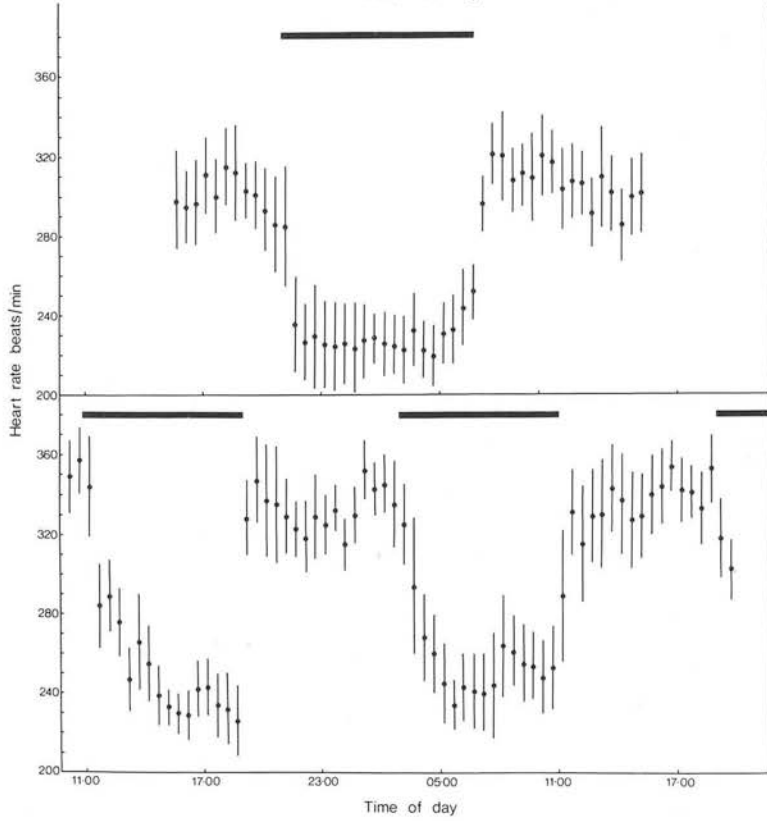
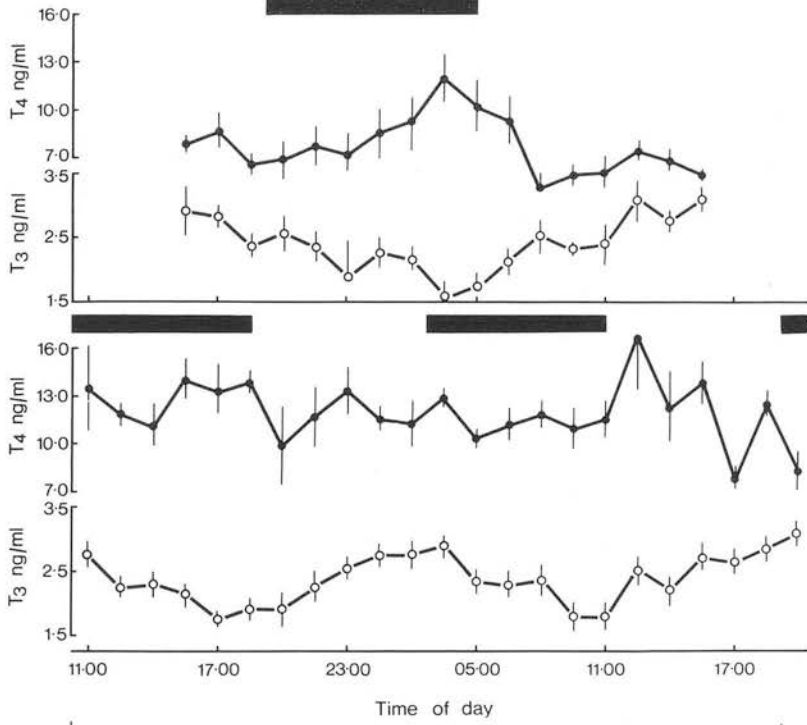
One day-old White Leghorn female chicks were obtained commercially and maintained on 14L : 10D <sup>and 8L : 8D</sup> lighting regimens. The birds were reared in separate cages and allowed free access to food and water. At 12 weeks of age the birds were decapitated in groups of 6 at intervals of 1.5 h. Six birds from the 8L : 8D treatment and 6 from the 14L : 10D treatment were implanted with radiotelemetry transmitters (2.6.1) in order to use the measurement of heart rate as an index of metabolic activity.

FIGURE 7

Variations in the concentrations of plasma  $T_4$  and  $T_3$  (upper panel) and heart rate (lower panel) in 12-week-old sexually immature female chickens during a 24 h cycle of 14L : 10D and a 16 h lighting cycle of 8L : 8D (upper panel). Each point represents mean ( $\pm$  SEM) hormone level in 6 chickens killed at the times shown. In the lower panel each point represents the mean ( $\pm$  SEM) number of heart beats per minute. The horizontal bars represent the periods of darkness.



DIURNAL RHYTHMS OF THYROID HORMONES  
IN 12 WK-OLD PULLETS. (n=6)



The concentration of plasma  $T_3$  showed marked variations during the 24- and 16-h lighting cycles (Fig. 7). A comparison with changes in heart rate shows that the level of  $T_3$  increased during the periods of light when the heart rate rose and decreased during the periods of darkness when the heart rate was depressed (Fig. 7).

There was a clear cyclic variation in the concentration of plasma  $T_4$  during the 24- but not during the 16-h lighting cycle (Fig. 7). In the birds held on a lighting pattern of 14L : 10D, the concentration of plasma  $T_4$  increased towards the end of the dark period at the time the heart rate was depressed (Fig. 7). Although  $T_4$  levels did not show clear cyclic changes in birds held on 8L : 8D, the highest values were observed towards the end of, or just after, the dark period (Fig. 7).

The significance of the cyclic changes in  $T_4$  and  $T_3$  levels was calculated by fitting the data to theoretical sinusoidal curves (Table 7). The fit of each set of the appropriate  $T_3$  data to sinusoidal curves with 24- or 16-h periods was highly significant ( $p < 0.001$  for both sets of data). The fit of the  $T_4$  values observed during the 24-h cycle to a theoretical sinusoidal curve was less satisfactory but still significant ( $P < 0.04$ ) while  $T_4$  values observed during the 16-h cycle did not vary significantly ( $P < 0.2$ ) as a sinusoidal function (Table 7). Irrespective of the period of the theoretical sinusoidal curves, the crests of the curves (the acrophases) tended to occur about 8 h after the onset of darkness for the  $T_4$  data and about 8 h after the onset of light for the  $T_3$  data (Table 7).

TABLE 7

Characteristics of Sinusoidal Curves Fitted by the Least Squares Method to Best Describe Changes in the Concentrations of Plasma Thyroxine and Triiodothyronine in 12-Week-Old Female Chickens<sup>a</sup>

	14L : 10D		8L : 8D	
	Thyroxine	Triiodothyronine	Thyroxine	Triiodothyronine
Level (ng/ml)	8.58	2.39	11.99	2.36
Amplitude (ng/ml)	0.28	0.49	0.79	0.47
Acrophase (hr) from the onset of darkness	7.83	19.19	8.54	15.00
From the onset of light	21.83	8.99	0.54	7.00
P	0.04	0.001	0.20	0.001

<sup>a</sup> Chickens were sampled every 1.5 hr during # 24-hr (14L : 10D) and 16-hr (8L : 8D) lighting cycles. The level is the intercept of the theoretical curve; the amplitude is the maximal deviation of the theoretical curve from the level and the acrophase (hours expressed as a decimal fraction) is the time at which the maximal positive deviation of the curve from the level occurs. The value of P is used to test the null hypothesis that the amplitude is equal to zero.

These observations, in agreement with those of Newcomer (1974), support the view that  $T_3$  and  $T_4$  are regulated by different mechanisms. Further, they show that by manipulating the photoperiod it is possible to regulate the occurrence and duration of periods of increased heart rate and to correlate these periods with increased levels of plasma  $T_3$ . The assumption that heart rate is correlated with metabolic rate is

supported by the observation that there is a diurnal variation in metabolic rate in chicks held on a lighting period of 14L : 10D with an increase occurring during the light period (Lundy et al., 1978; 3.7.2). The correlation between heart rate and the concentration of plasma  $T_3$  supports the view that  $T_3$  is the active thyroid hormone which reflects changes in metabolic rate. The same conclusion was drawn by Bobek et al. (1977) who found that the increase in oxygen consumption observed in chicks during the first and second weeks after hatch was correlated with the concentration of plasma  $T_3$  but not with that of plasma  $T_4$ .

A daily rhythm in the secretion of several pituitary hormones has been observed in mammals (Weitzman et al., 1975). Similarly in the chicken there is a nocturnal increase in the concentration of plasma leutinising hormone (LH) (Scanes et al., 1978) and of plasma corticosterone (Beuving and Vonder, 1973). The increase in plasma corticosterone may reflect an increase in the secretion of corticotropin (ACTH). In man and the rat the concentration of plasma TSH increases during the period of sleep (see introduction to the chapter) and it is possible that the increased concentration of plasma  $T_4$  observed at the end of the dark period and in this study was due to a nocturnal increase in the secretion of TSH. This possibility cannot be investigated until a radioimmunoassay for avian TSH is developed.

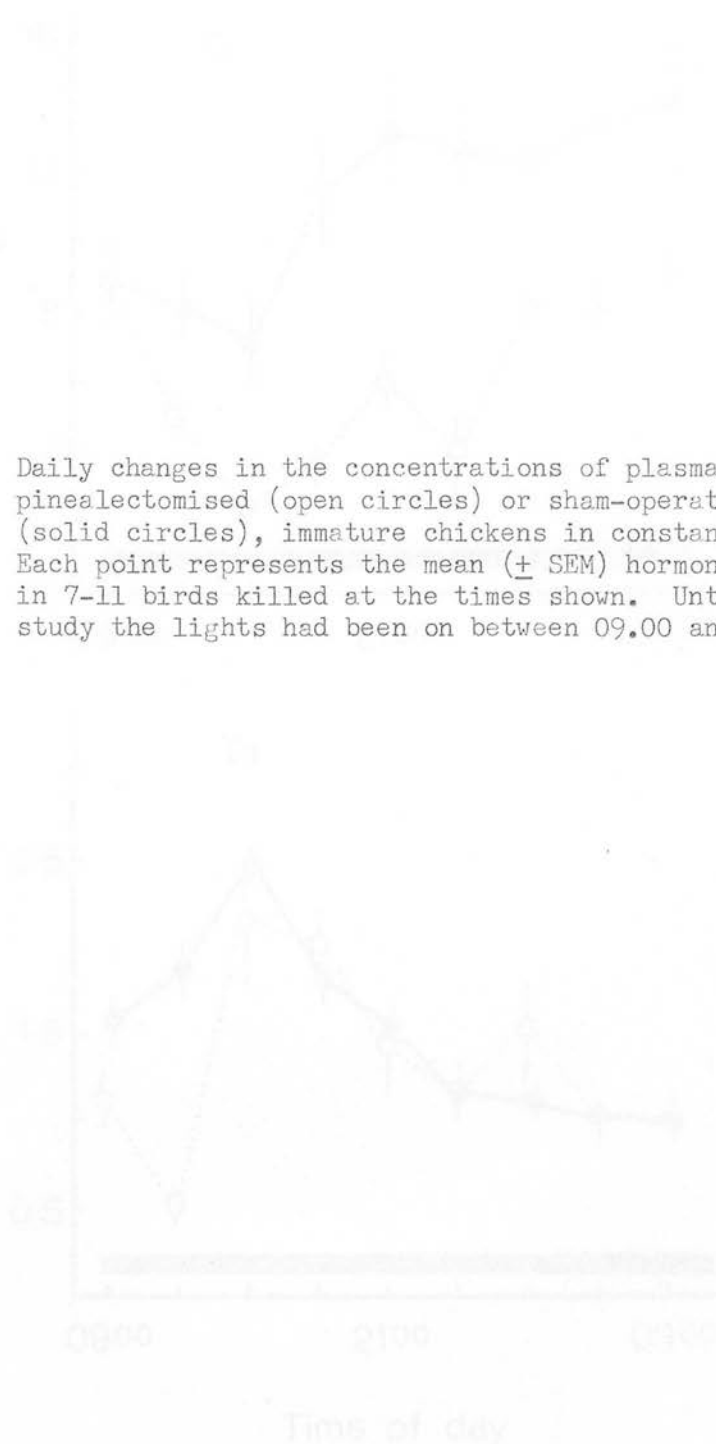
### 3.2.2 Preliminary studies: effect of pinealectomy

Male chickens (Thorner 909) were maintained on a lighting pattern of 16 h light and 8 h darkness (lights on from 09.00 to 01.00) in a floor pen with food and water available ad libitum. Between 7 and 14 d of age one third of the birds were pinealectomised (2.4.2), one third was sham-operated and the remainder were kept as intact controls. At 28 d of age groups of pinealectomised, sham-operated and control birds were removed from the pen every 3 h for 24 h and decapitated within 2 min of being handled. Blood was collected into heparinised beakers. On the day of the study the lights were not switched on at 09.00 and the first group of birds were killed at this time. The remaining groups were killed during the following 24 h of darkness.

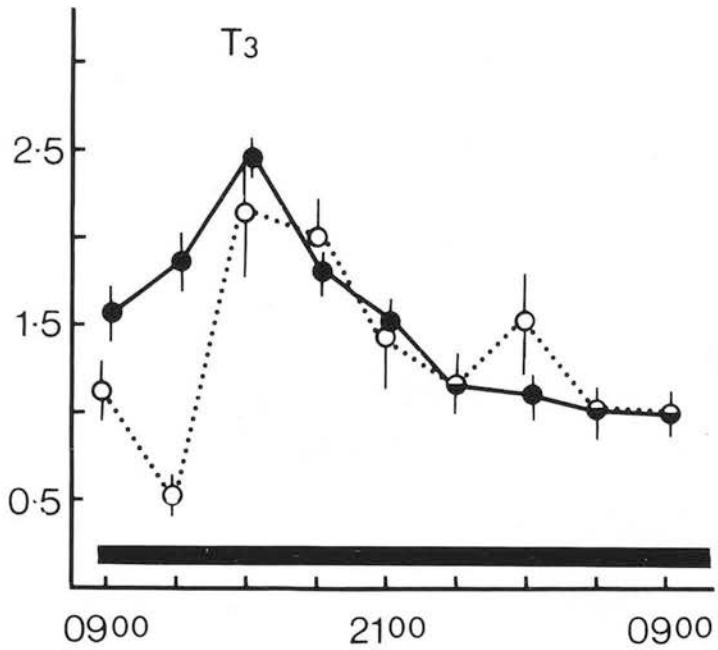
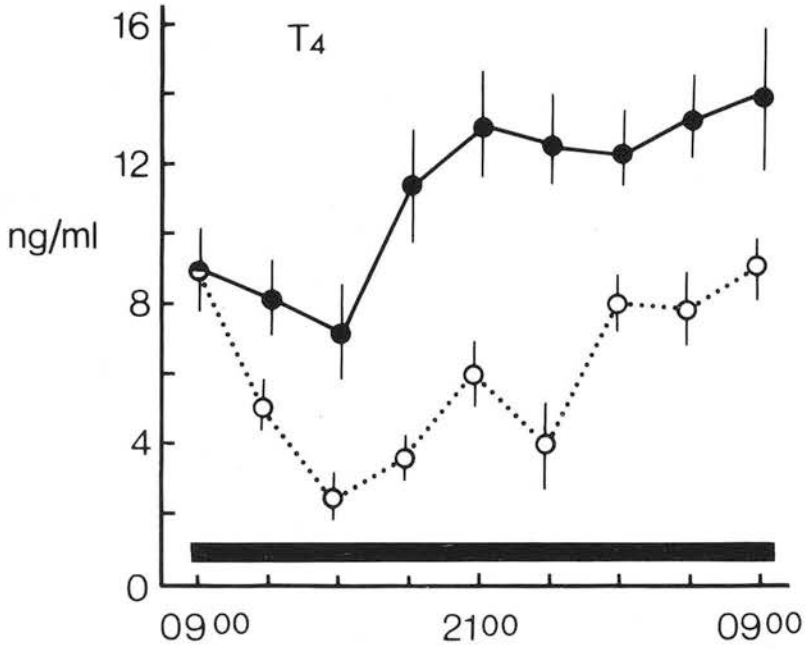
The concentrations of plasma  $T_4$  and  $T_3$  in the sham-operated and

EFFECT OF PINEALECTOMY ON  $T_4$  AND  $T_3$  IN IMMATURE CHICKENS TRANSFERRED FROM LIGHT TO DARKNESS

FIGURE 8 Daily changes in the concentrations of plasma  $T_4$  and  $T_3$  in pinealectomised (open circles) or sham-operated/control (solid circles), immature chickens in constant darkness. Each point represents the mean ( $\pm$  SEM) hormone concentration in 7-11 birds killed at the times shown. Until the day of study the lights had been on between 09.00 and 01.00 h.



EFFECT OF PINEALECTOMY ON T<sub>4</sub> AND T<sub>3</sub> IN CHICKENS  
TRANSFERRED FROM LD TO DD



Time of day

control groups were not significantly different and so the data from these birds were combined. There were no significant differences in the concentration of plasma  $T_3$  between the pinealectomised and control groups (Fig. 8) except at 12.00 h ( $p < 0.001$ ). In contrast, the concentration of plasma  $T_4$  was significantly higher in the controls than in the pinealectomised birds at all times during the 24 h study except in the first group of birds killed at 09.00 h (Fig. 8,  $p < 0.02$ ). The tendency for the concentration of plasma  $T_4$  to increase and for the concentration of  $T_3$  to decrease during the study is consistent with the persistence of an underlying diurnal rhythm of thyroid hormone secretion (Newcomer, 1974; 3.2.1).

The results of the study suggest that in the chicken, the pineal gland has a stimulatory effect on the secretion of  $T_4$  while the production of  $T_3$  appears to be independent of pineal function.

### 3.2.3 Effect of transfer from 14L : 10D to continuous light or darkness

Commercial female broilers (D. B. Marshall Ltd., Newbridge, Edinburgh) were reared on a lighting cycle of 14 h light/day and given free access to food and water. They were maintained in groups until they were 5 weeks of age and then were transferred to individual cages and exposed to various lighting patterns. Blood samples were taken over 24-54 h periods when the birds were 7 weeks old. During the periods when blood samples were taken the ambient temperature varied from 22.9°C at night and 23.4°C during the day.

At 7-10 days of age the birds were divided into groups which were left intact, pinealectomised or sham-pinealectomised. Pinealectomy was carried out as described in section 2.4.2.

Blood samples were taken every 3 h in each experiment in which the birds were exposed to lighting regimens of (1) 14 h light : 10 h darkness : 14 h light : 10 h darkness (14L : 10D : 14L : 10D), (2) 14L : 10D : 24D, (3) 14L : 10D : 24L, (4) 24L : 24L. In each experiment there were 3 groups of birds: each group comprised 8 unoperated controls, 6 sham-pinealectomised birds and 8 pinealectomised birds. Blood samples were taken once from each group during a period of 24 h in order to minimise the stressful effects on thyroid hormone levels of repeated

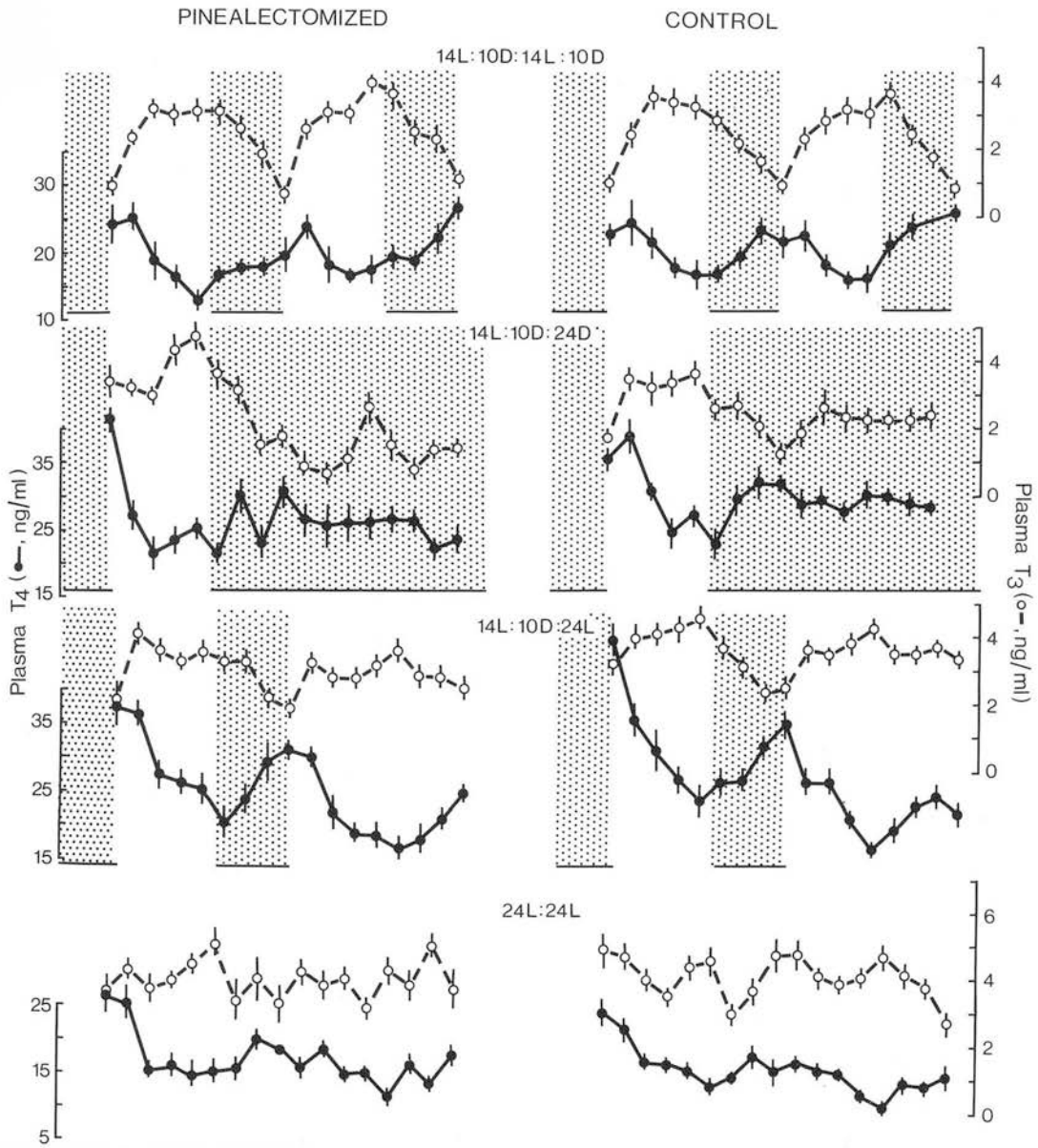


FIGURE 9

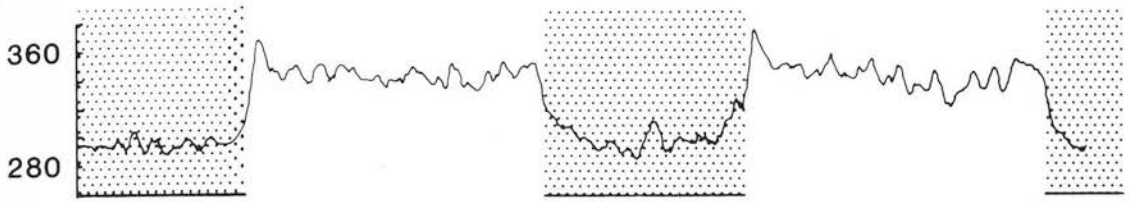
Changes in the concentrations of plasma T<sub>4</sub> (closed circles) and T<sub>3</sub> (open circles) in 7-week-old, intact and pinealectomised female broilers exposed to 14 h light, 10 h darkness (14L : 10D) and sampled for two cycles or transferred from 14L : 10D to continuous darkness for 24 h or to continuous light for 24 h or 48 h. Each point represents the mean ( $\pm$  SEM) concentration of hormone in different groups of 8 pinealectomised and 8 unoperated control birds. Each group was sampled for a second time 24 h later and the first group was sampled for a third time after a further 24 h in some experiments. The concentrations of thyroid hormone in the 6 sham operated control birds were essentially the same as in the unoperated controls and have been omitted for clarity. The stippled areas represent the hours of darkness.

FIGURE 10

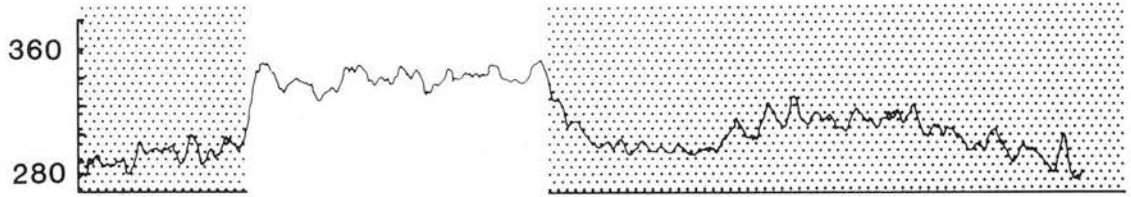
Variations in heart rate in 7-week-old female broilers exposed to 14 h light, 10 h dark (14L : 10D), prolonged darkness (14L : 10D : 24 D), prolonged light (14L : 10D : 18L), ahemeral lighting cycles of 11L : 10D and 14L : 13D, long (20L : 4D) and short (4L : 20D). In each study the birds had been entrained for at least 2 weeks to the first lighting cycle. The stippled areas represent the periods of darkness. The data from 4 hens are shown in each experiment and have been smoothed by an unweighted 5 point average of 5s periods.



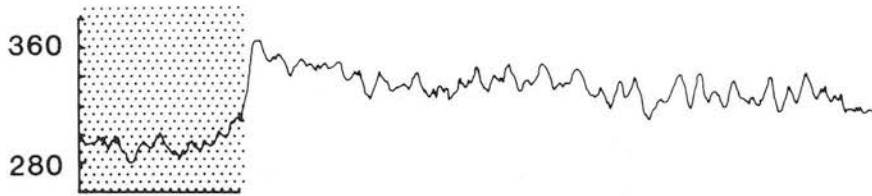
14L:10D:14L10D



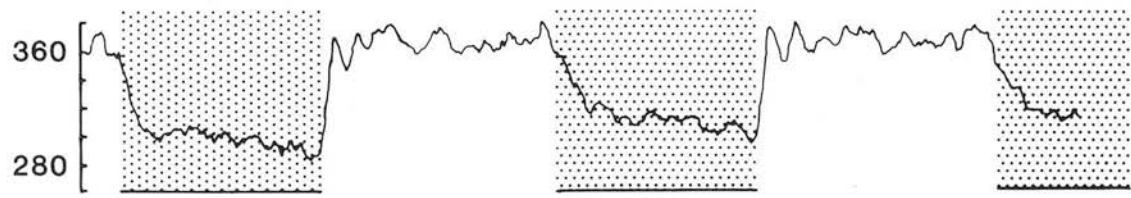
14L:10D:24D



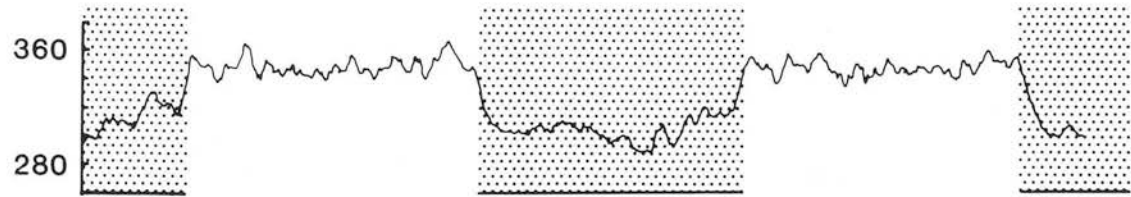
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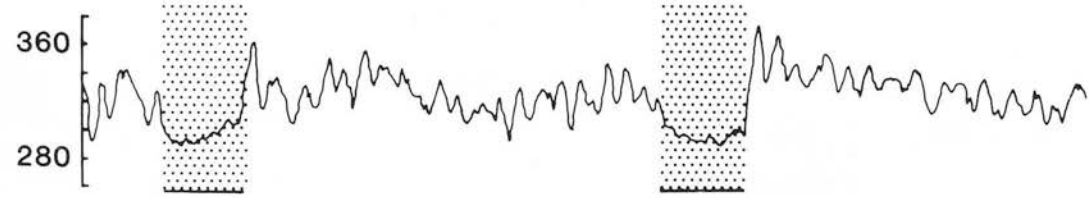
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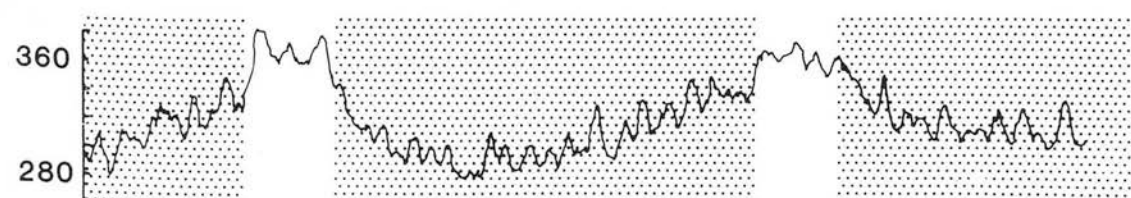
14L:13D:14L:13D



4D:20L:4D:20L



4L:20D:4L:20D



Heart rate (beats / min)

Time

handling (3.7.1). The birds were exposed to a lighting cycle of 14L : 10D for 2 weeks before blood samples were taken. In Experiment 4, the first blood sample was taken immediately after the lights were switched on. Heart rate (2.6.1) was measured in 4 intact birds during each of the experimental lighting regimens except for birds exposed to 24L : 24L.

Significant ( $p < 0.001$ ) variations in the levels of plasma thyroid hormones were observed in intact and pinealectomised birds exposed to 14L : 10D (Fig. 9). The rhythms and levels of thyroid hormones in the pinealectomised birds were not different from those measured in intact controls (Fig. 9). In intact and pinealectomised birds the plasma concentration of  $T_3$  and heart rate (Fig. 10) increased at the onset of the light period with levels of  $T_3$  reaching their highest values immediately before the onset of darkness. Levels of  $T_3$  and heart rate fell at the onset of darkness and concentrations of  $T_3$  were lowest at the beginning of the light period. The concentrations of  $T_4$  were inversely related to those of  $T_3$ . Plasma levels of  $T_4$  fell during the light period and were lowest at the onset of darkness. They then increased during the dark period to reach their highest values just before, or immediately after the beginning of the light period.

After birds were transferred from 14L : 10D to either continuous darkness or light the daily rhythms in the concentrations of  $T_4$  and  $T_3$  were rapidly dampened (Fig. 9). A 24 h rhythm in heart rate also disappeared in birds exposed to continuous light but a low-amplitude rhythm in heart rate appeared to persist in birds exposed to constant darkness (Fig. 10). The plasma concentrations of  $T_3$  were higher in birds exposed to continuous darkness (Fig. 9). Conversely, the plasma levels of  $T_4$  were depressed in birds exposed to continuous light and increased in those exposed to constant darkness.

#### 3.2.4 Ahemeral, long and short day lighting cycles

The protocol in these experiments is the same as is described for the previous section. Blood samples were taken every 3 h for each of the following lighting regimens (1) 11L : 10D : 11L : 10D, (2) 14L : 13D : 14L : 13D, (3) 4L : 20D, (4) 20L : 4D. In each of these experiments the birds had been exposed to the experimental lighting schedule for 2 weeks.

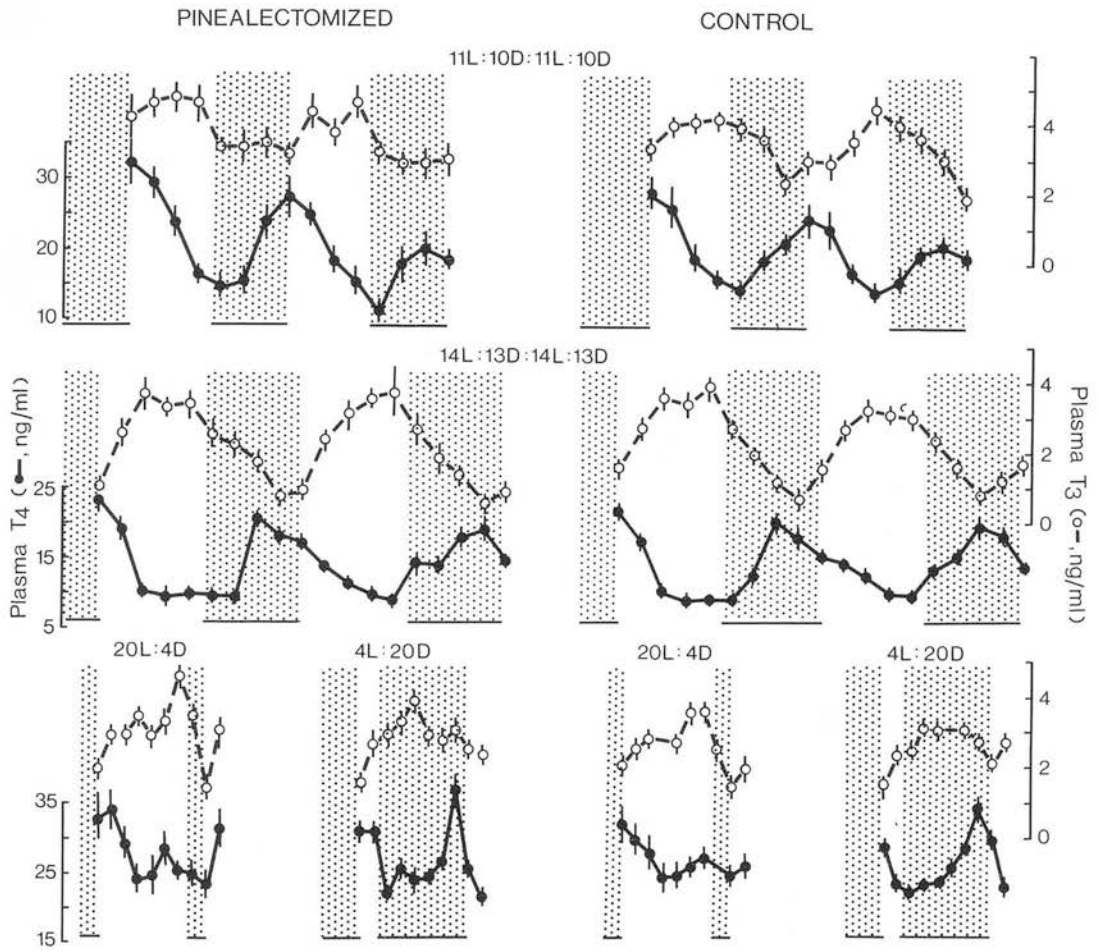
The cyclic change ( $P < 0.001$ ) in the plasma concentrations of  $T_3$  and  $T_4$  in birds exposed to ahemeral cycles of 14L : 13D and 11L : 10D or the daily cycles of 20L : 4D and 4L : 20D showed a similar reciprocal relationship to that observed in birds exposed to 14L : 10D (compare Figs. 9 and 11). Thus, in birds held on 11L : 10D, 20L : 4L and 4L : 20D, the levels of  $T_4$  tended to be highest, and levels of  $T_3$  to be lowest, at the beginning of the light period (Fig. 11). However, in birds exposed to 14L : 13D, the plasma concentrations of  $T_4$  reached peak values before the beginning of the light period and before plasma levels of  $T_3$  had decreased to their lowest levels (Fig. 11). Another modification in the daily pattern of thyroid hormone levels was observed in birds exposed to 4L : 20D. In these birds, plasma levels of  $T_3$  did not fall at the onset of darkness but continued to increase while the levels of  $T_4$  remained depressed (Fig. 11). However, the heart rate fell at the onset of darkness as in other experimental groups (Fig. 10). Concentrations of plasma  $T_3$  fell during the latter half of the 20 h dark period while the levels of  $T_4$  increased steeply (Fig. 11).

In birds exposed to 20L : 4D, the plasma level of  $T_3$  and heart rate were increased throughout the light period while the concentration of  $T_4$  was depressed. During the 4 h dark period, heart rate and levels of  $T_3$  fell steeply (Figs. 10 and 11). The levels of  $T_4$  tended to reach their highest values at the beginning of the light period (Fig. 11).

In birds held on lighting cycles with long dark periods (14L : 13D and 4L : 20D) heart rate began to increase towards the end of the dark period in anticipation of the onset of the light period (Fig. 10). This anticipatory increase was not associated with an increase in the plasma levels of  $T_3$  or a fall in the levels of  $T_4$  (Fig. 11).

No differences were seen between the patterns of change in plasma levels of thyroid hormones in intact or pinealectomised birds exposed to long or short days or to the two ahemeral lighting cycles.

This study has confirmed the reciprocal relationship between the daily rhythmic variations in the plasma concentrations of  $T_4$  and  $T_3$  in the chicken (3.2.1). These rhythmic changes appear to be a direct consequence of the lighting pattern since they do not persist in birds exposed to constant darkness or light for 24 h or more. In the hen, the thyroid glands store  $T_4$  and contain only small quantities of  $T_3$



**FIGURE 11** Changes in the concentrations of plasma  $T_4$  (closed circles) and  $T_3$  (open circles) in 7-week-old, intact and pinealectomised female broilers exposed to ahemeral lighting cycles of 11 h light, 10 h dark (11L : 10D) and 14 h light, 13 h dark (14L : 13D) and sampled for two cycles. Other birds were exposed to long (20L : 4D) or short (4L : 20D) days and samples were taken for 27 h. Each point represents the mean ( $\pm$  SEM) concentration of hormone in different groups of 8 pinealectomised and 14 control birds. Each group was sampled for a second time 21 or 27 h later, respectively, the first group was sampled for a third time at the beginning of the third cycle. The stippled areas represent hours of darkness.

(Astier, 1975b). It is thus possible that the  $T_4$  in the peripheral circulation is derived directly from the thyroid glands while the  $T_3$  is generated peripherally from  $T_4$ . Peripheral monodeiodination of  $T_4$  and  $T_3$  occurs in the liver in the chicken (Borges *et al.*, 1980; 3.8.2) and this process may be stimulated by feeding. The increasing plasma levels of  $T_3$  observed after the onset of darkness in birds exposed to 4L : 20D may thus be due to the birds feeding in the dark. Broiler hens have been observed to feed in the dark when the daylength is less than about 6 h (Cherry and Barwick, 1962). The observation by Shimada and Koide (1972) that heart rate and plasma levels of  $T_3$  decrease in fasting hens suggested that changes in plasma  $T_3$  and heart rate may be correlated. However, this suggestion is not supported by the present study since the increase in heart rate observed in birds exposed to 14L : 13D, 4L : 20D and 24D in anticipation of dawn was not associated with an increase in plasma levels of  $T_3$ . Further, the decrease in heart rate at the onset of darkness in birds exposed to 4L : 20D occurred while plasma levels of  $T_3$  were still increasing.

The view that daily changes in the plasma levels of thyroid hormones in the hen are due to changes in the peripheral conversion of  $T_4$  to  $T_3$  seem to be at variance with observations in mammals (see Introduction to this section) which suggest that such changes may be due to a circadian rhythm in the secretion of TSH. In the present study the apparent disappearance of daily rhythms in plasma levels of thyroid hormones after exposure to continuous dark or light argue against a significant role of the circadian system in the regulation of thyroid function in the hen. However, it remains possible that the secretion of  $T_4$  from the thyroid glands is not constant but is regulated by a circadian rhythm in the secretion of TSH, although most of the daily changes in levels of plasma  $T_4$  can be adequately explained as being due to changes in the rate of peripheral monodeiodination to  $T_3$ .

The failure to confirm the preliminary observation that pinealectomy depresses the plasma concentration of  $T_4$  in chickens exposed to constant darkness (3.2.2) cannot be explained. However, the absence of an effect of pinealectomy on thyroid function seems to have been established since rhythms of plasma concentrations of  $T_4$  and  $T_3$  were identical in intact and pinealectomised birds exposed to several different lighting

patterns. This finding is in agreement with other studies on the chicken which show that pinealectomy has no effect on plasma levels of  $T_4$  and  $T_3$  (Cogburn and Harrison, 1980; Osol et al., 1980).

### 3.2.5 Summary

The effects of different lighting patterns and of pinealectomy on the daily rhythms of plasma  $T_3$  and  $T_4$  were investigated in sexually immature female chickens. The birds were transferred from a schedule of 14 h light : 10 h darkness (14L : 10D) to 24 h darkness, to 24 h light or to 48 h light. Additionally, rhythms of thyroid hormone levels were investigated in birds exposed to ahemeral lighting cycles of 8L : 8D, 11L : 10D and 14L : 13D and to long (20L : 4D) and short (4L : 20D) days. Heart rate was measured during some of the experimental lighting cycles as an indicator of changes in metabolic activity.

In each of the lighting cycles the concentration of  $T_3$  began to increase from its lowest value at the onset of the light period. This increase was associated with an increase in heart rate and a decrease in the concentration of  $T_4$ . In birds exposed to 8L : 8D, 14L : 10D, 11L : 10D, 14L : 13D and 20L : 4D, concentrations of plasma  $T_3$  and heart rate fell at the onset of darkness. In birds exposed to 4L : 20D, heart rate also fell at the onset of darkness but levels of  $T_3$  continued to increase. Exposure to continuous light or darkness caused the daily rhythms in the levels of thyroid hormones to disappear. Pinealectomy had no effect on levels or on changes in the concentrations of thyroid hormones in any of the experimental lighting cycles.

These observations suggest that the inversely related daily rhythms in plasma  $T_3$  and  $T_4$  are not of circadian origin and may be due to changes in another mechanism which could regulate the rate of peripheral monodeiodination of  $T_4$  to  $T_3$ .

### 3.3 Effect of feeding pattern on diurnal rhythms

The ability of a bird to adapt to a change in food and energy intake is fundamental for its survival. Thyroid function and its contribution to energy metabolism is regulated by the level and composition of the food eaten. Diet-induced changes in the concentrations of plasma thyroid hormones were first found in studies of long-term

overfeeding in man (Sims et al., 1973). In these studies of weight gain in normal individuals, more calories were required to maintain the excess weight than what was predicted and was found to be due to an increase in the levels of plasma  $T_3$ . Importance of the composition of diet on concentrations of  $T_3$  have been shown by substituting carbohydrate for fat in the diet. In these studies the levels of  $T_3$  were found to progressively increase (Danforth et al., 1979). Spaulding et al. (1976) has found that hypocaloric diets containing no carbohydrate mimic the fall in plasma  $T_3$  found during starvation. Additionally it has been reported that the fasting induced decrease in the concentration of  $T_3$  and increase in  $rT_3$  was reversed when carbohydrate or a mixed diet was provided (Vagenakis et al., 1975) but not after fat was refed (Azizi, 1978). In birds refeeding after a long fast results in a marked increase in the levels of  $T_3$  (Brake et al., 1980).

In mammals the total concentration of free and bound thyroxine tends to remain constant (Jung et al., 1980). Fasting results in a decrease in thyroid hormone binding proteins and in concentrations of plasma  $T_3$  (Shetty et al., 1979). In order to account for the reduction in the levels of plasma  $T_3$  Liewendahl and Helenius (1976) have suggested that the increase in plasma free fatty acids induced by fasting interferes with the binding of thyroid hormone to plasma binding proteins. This effect would be greater on the concentration of  $T_3$  due to its lower affinity to plasma binding proteins. Secondly starvation results in a decrease in the production of  $T_3$  and an increase in the levels of  $rT_3$  (Azizi, 1978). Principally the increased  $rT_3$  levels are mainly due to decreased elimination rates whereas the decrease in serum  $T_3$  is due to a decrease in the peripheral conversion of  $T_4$  to  $T_3$ , (Vagenakis et al., 1975). In chickens fasting results in a decrease in the concentration of plasma  $T_3$  and an increase in the concentration of plasma  $T_4$  (May 1978, 3.7.2) whereas ducks fasted for a period of 17 d resulted in an equivocal decrease in thyroid activity (Astier et al., 1978).

Therefore, in the first study the effect of fasting on the levels of plasma  $T_3$  and  $T_4$  were investigated in the domestic duck (Anas platyrhynchos). In order to minimise the stress related changes in thyroid hormone concentration due to handling the birds were bled at intervals of 12 h for 36 h (3.7.1).

In chickens exposed to cycles of light and dark, levels of plasma  $T_3$  increase during the light and decrease during the dark periods while the converse changes occur in the levels of plasma  $T_4$  (Newcomer, 1974; 3.2). Since poultry normally feed only during the light period (Savory, 1976, 1980) and daily variations in levels of plasma thyroid hormone disappear in fasted hens and in hens maintained on continuous light, the concentrations of plasma  $T_3$  and  $T_4$  in chickens may be regulated by the feeding pattern. In the second study, this possibility was investigated by measuring daily variations in plasma  $T_3$  and  $T_4$  in hens with access to food limited to the light or dark periods.

The relationship between food intake and the regulation of plasma thyroid hormones in poultry was more closely examined in the third study. Birds maintained on continuous light and given free access to food and water do not show any daily variations in the levels of plasma  $T_4$  and  $T_3$  (3.2). Groups of birds were maintained on continuous light and given access to food either for 2 h in the morning or for 2 h in the afternoon. The variations in plasma thyroid hormones were compared to birds given free access to food or to birds fasted for the previous 24 h. In this manner the effect of restricting food availability to a limited period of the day on the daily rhythms of plasma thyroid hormones could be determined.

### 3.3.1 Ducks fasting

Aylesbury ducklings between 7 and 8 weeks of age and reared on a photoperiod of 14L : 10D (lights on 07.30-21.30 hrs) with food and water freely available were divided into 2 groups. The first group were fasted for 36 hrs, while the second control group were provided food and water ad libitum. A blood sample from each bird was taken prior to food withdrawal (20.30 hrs) and at 12 hr intervals for the following 36 hr.

In ducks deprived of food for 36 h the concentration of plasma  $T_4$  did not differ significantly from the levels in the controls during this period (Fig. 12). Contrarily the concentration of plasma  $T_3$  in birds deprived of food was significantly reduced 24 hr after the onset of the fast ( $p < 0.001$ ) and remained depressed for the remainder of the study (Fig. 12). There were no significant variations in either the

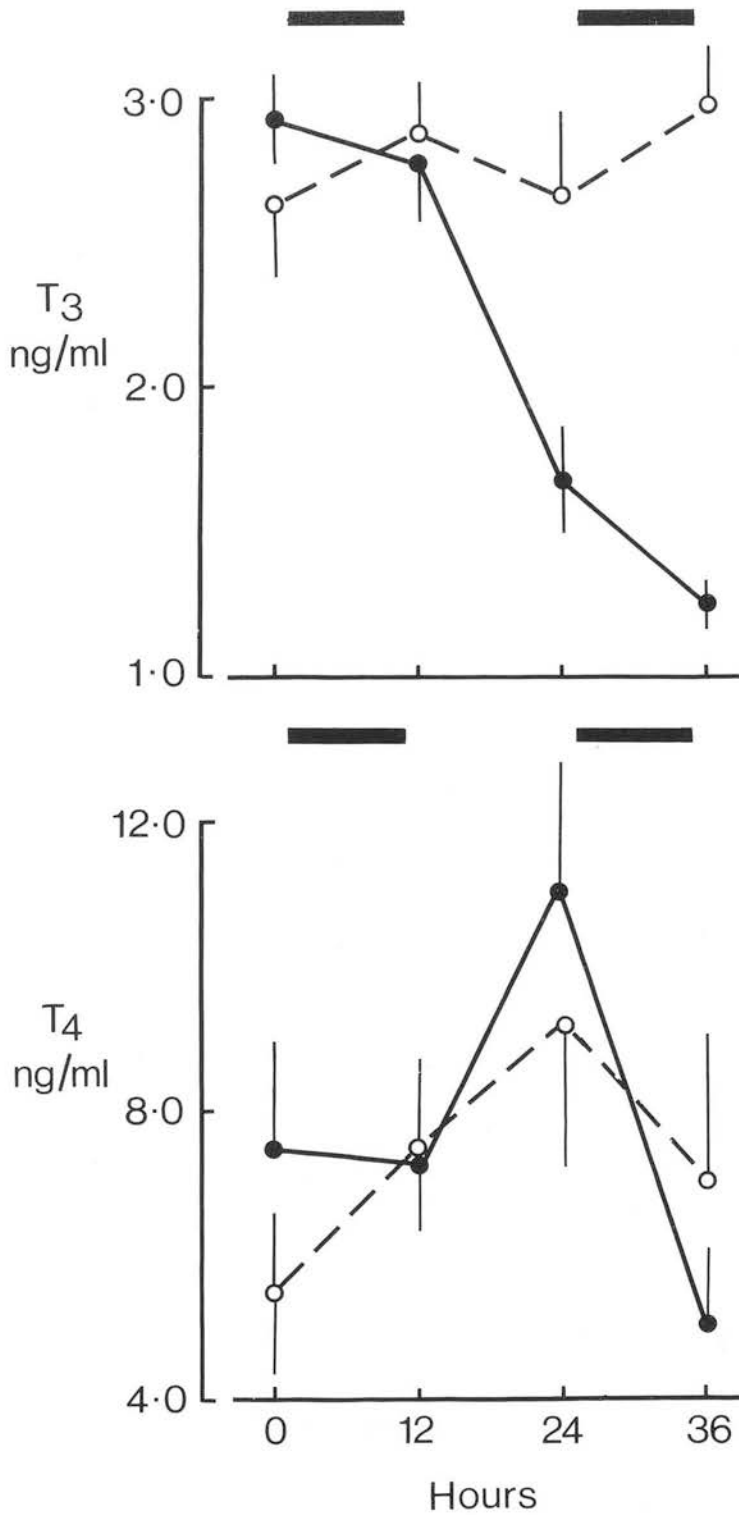


FIGURE 12

Changes in the concentration of plasma T<sub>3</sub> and T<sub>4</sub> in fed (open circles) and fasted (closed circles) 7-week-old<sup>4</sup> Aylesbury ducklings exposed to 14L : 10D. The first blood sample was taken prior to withdrawal of the food troughs. Each point represents the mean ( $\pm$  SEM) concentration of hormone in 10 birds deprived of food for 36 h.

concentration of  $T_3$  or  $T_4$  in ducks given free access to food and water.

In the present study fasting was found to abolish the daily changes in the levels of plasma  $T_3$  previously reported in ducks (Harvey et al., 1980). In mammals, Croxson et al. (1977) found that fasting abolished the diurnal rhythm in the secretion of TSH and suggested that the intake of food was the primary signal for the cycle of TSH release. In quail it has been shown that the daily variation in the release of TSH is affected by food intake (Almeida and Thomas, 1981) which in turn affected the pattern in the levels of both  $T_4$  and  $T_3$ .

This is the first report on the effect of fasting on blood  $T_3$  concentrations in ducks although similar results in fasted chickens have been reported (May, 1978). Low serum  $T_3$  concentrations are also found in neonatal chickens that have never been fed (King et al., 1977).

In contrast to the marked effect of fasting on the concentration of plasma  $T_3$  in ducks, there was not a consistent effect of food withdrawal on the levels of plasma  $T_4$ . Chandola and Thapliyal (1974) also found that 24 or 48 h of starvation had no effect of thyroidal  $T_4$  levels in Spotted Munia, although May (1978) observed an increase in the concentration of plasma  $T_4$  in acutely (16 h) fasted chicks. In the Peking duck, Astier et al. (1978) reported an inhibitory effect of fasting on the concentration of plasma  $T_4$  in birds starved for 17 days, but at the time these birds were bled the levels of  $T_4$  in control birds was not determined. Additionally Astier et al. (1969a, b) have also reported that the rate of  $T_4$  metabolism in fasted ducks is less than in fed birds. Thus the absence of rhythmic variations in the levels of plasma thyroid hormone in fasted birds suggests a relationship between these parameters.

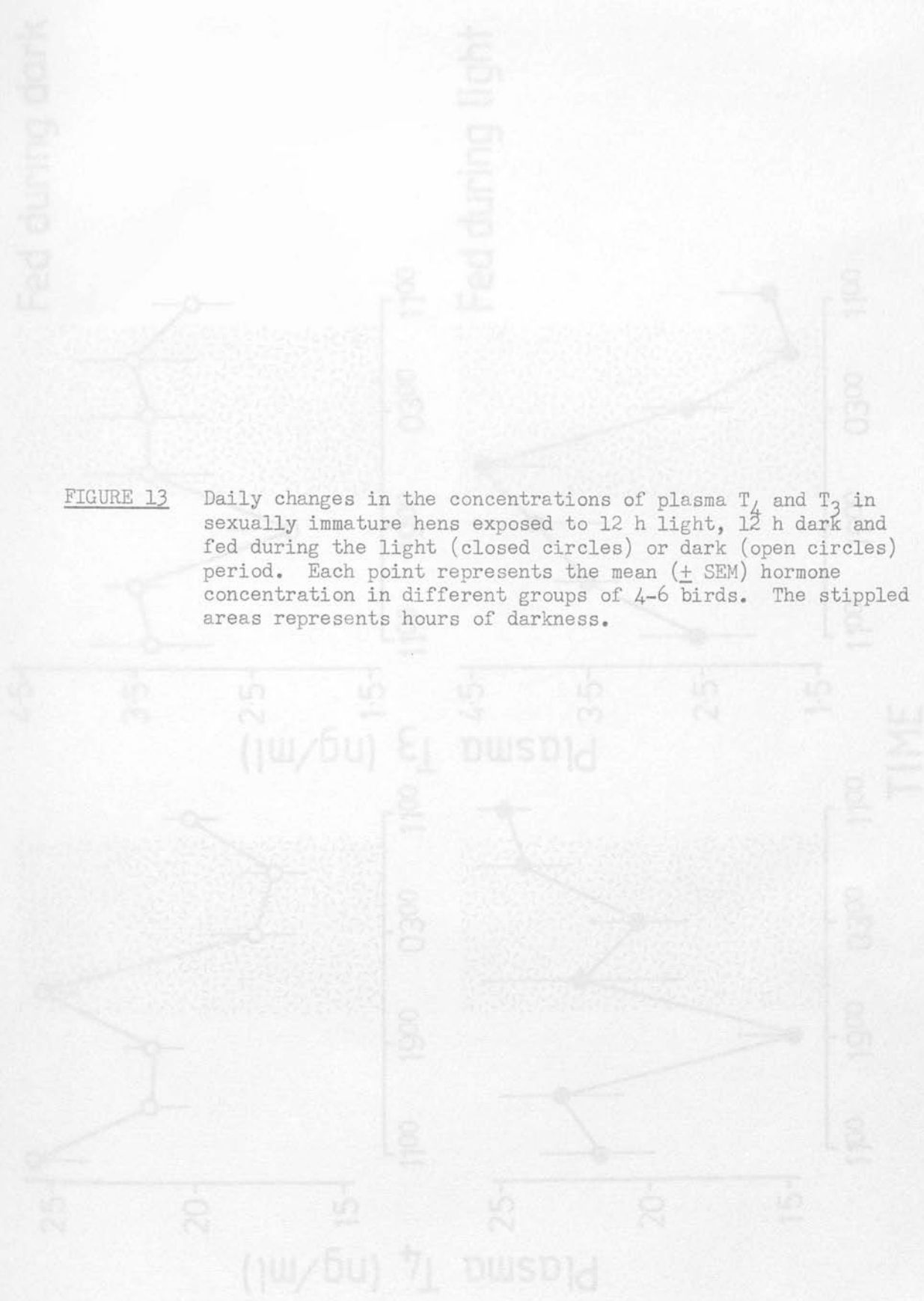
### 3.3.2 Reverse feeding

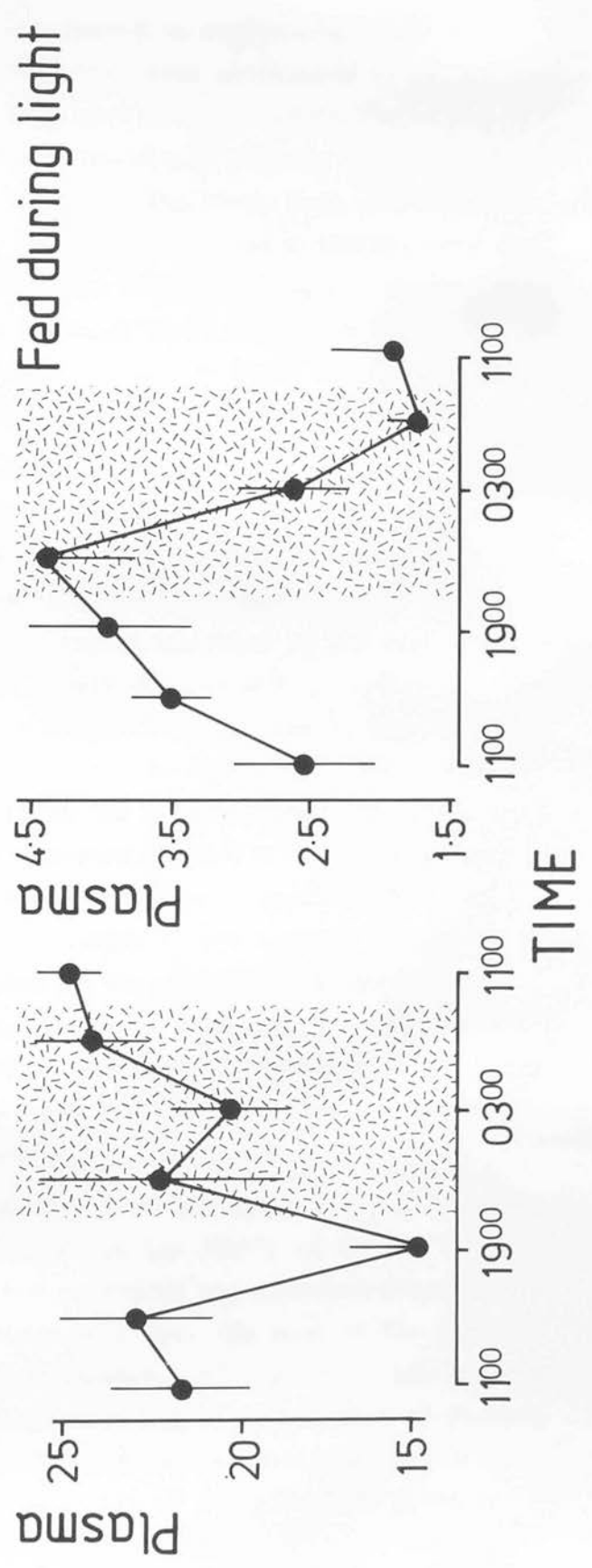
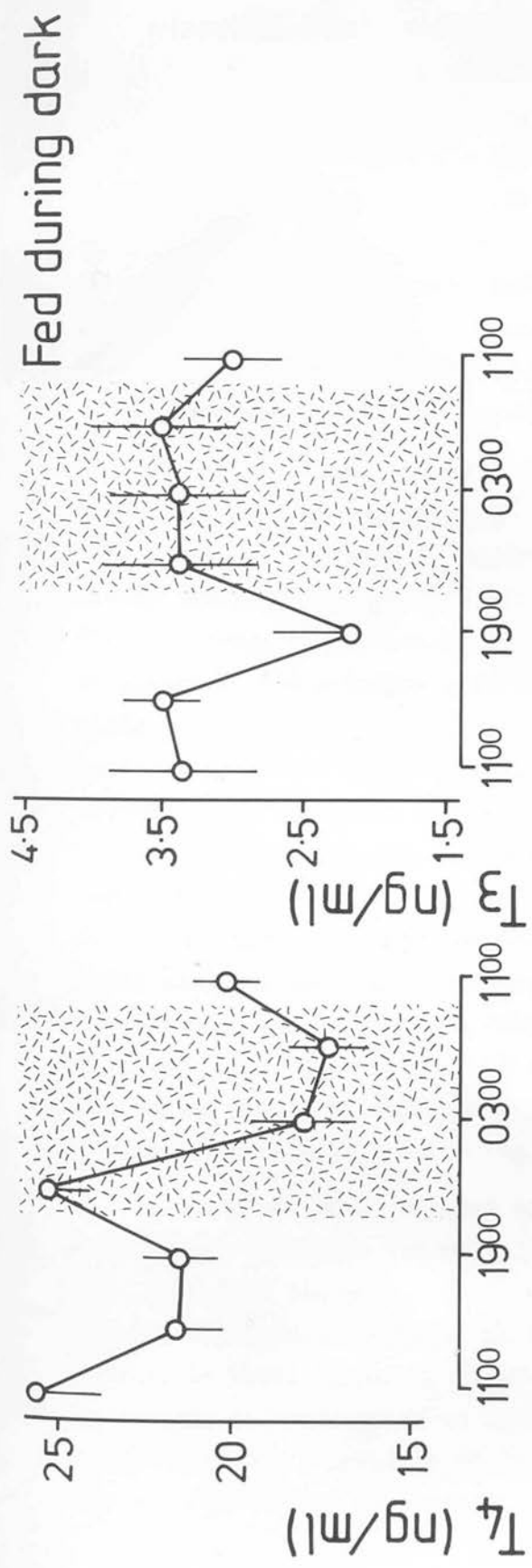
White Leghorn hens were reared on a lighting pattern of 14L : 10D with food and water freely available and were transferred to individual cages when they were 8 weeks-old. When they were 16 weeks-old, the lighting pattern was changed to 12L : 12D; one group of 36 hens and another of 32 were given food only during the dark or light periods respectively with water remaining freely available. The troughs containing food were removed or replaced just before the lights were switched on or off. Casual observations were made, using a small

torch, on the feeding behaviour of the hens fed during the dark period. After the hens had been on the experimental regime for 2 weeks, blood samples were taken every 4 h for 24 h from 4-6 hens from each group: no hen was sampled more than once in 24 h. In this way, any depression in the levels of thyroid hormones which might have been caused by repeated handling (3.7.1) was minimised. Ten hens from each group were weighed when the experimental feeding regime was begun and again 2 weeks later.

The increase in body weight in 10 hens fed during the dark period ( $0.08 \pm 0.01$  kg) was not significantly different from that ( $0.11 \pm 0.02$  kg) in 10 hens fed during the light period. Differences in concentrations of plasma thyroid hormone levels in hens fed during the dark periods were therefore unlikely to be due to differences in total food consumption. The hens fed during the dark period took between 4 and 5 days to learn to eat in the dark. They fed immediately after the lights were switched off and just before the lights came on and were not normally seen to feed at other times during the dark. In contrast, the hens fed during the light period ate intermittantly while food was available. In birds fed during the light period, the daily changes in the concentrations of plasma  $T_4$  and  $T_3$  were similar to those in hens with food freely available and exposed to similar cycles of light and dark (3.2). Thus during the light period concentrations of plasma  $T_4$  fell while those of  $T_3$  increased: the converse changes occurred during the dark period (Fig. 13). In hens fed during the dark period these characteristic daily rhythms were disrupted (Fig. 13). This observation is best illustrated by reference to the levels of plasma thyroid hormones towards the end of a 12 h fast, at 19.00 and 07.00 in hens fed during the dark and light periods respectively. In both groups, the end of the fast was associated with high levels of plasma  $T_4$  and low levels of plasma  $T_3$  irrespective of the lighting pattern (Fig. 13). At these times, the differences in the concentrations of plasma  $T_4$  and  $T_3$  between groups were highly significant ( $p < 0.001$ ). The fact that the daily rhythms in concentrations of plasma thyroid hormones were not fully reversed in the two feeding regimes may reflect differences in the size of 'meals' and the times at which they were taken. The feeding pattern thus plays an important role in the regulation of the daily changes in the concentration of plasma thyroid hormones in the chicken.

**FIGURE 13** Daily changes in the concentrations of plasma  $T_4$  and  $T_3$  in sexually immature hens exposed to 12 h light, 12 h dark and fed during the light (closed circles) or dark (open circles) period. Each point represents the mean ( $\pm$  SEM) hormone concentration in different groups of 4-6 birds. The stippled areas represents hours of darkness.



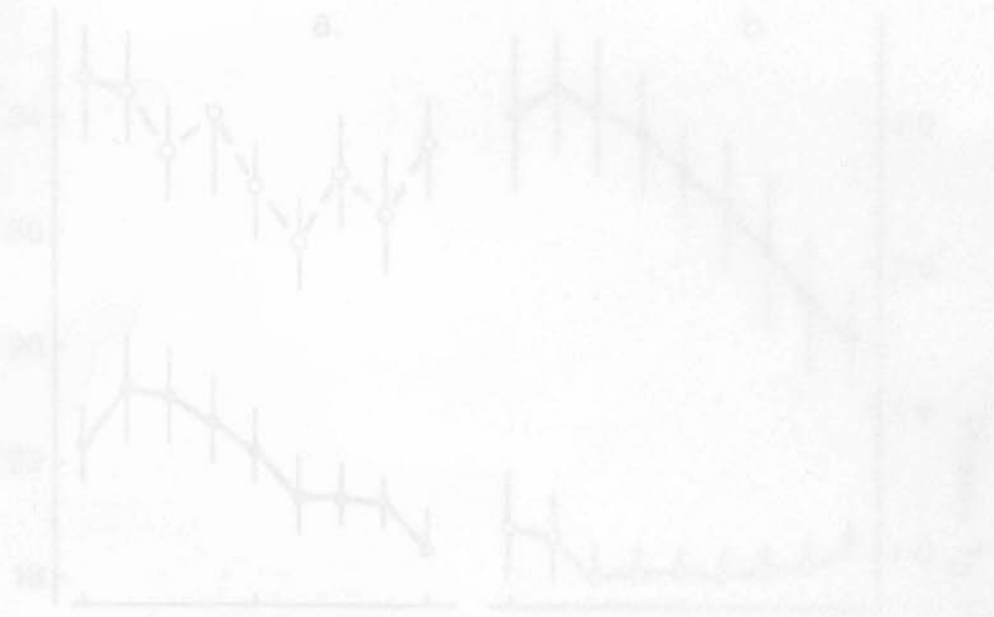


### 3.3.3 Controlled feeding times

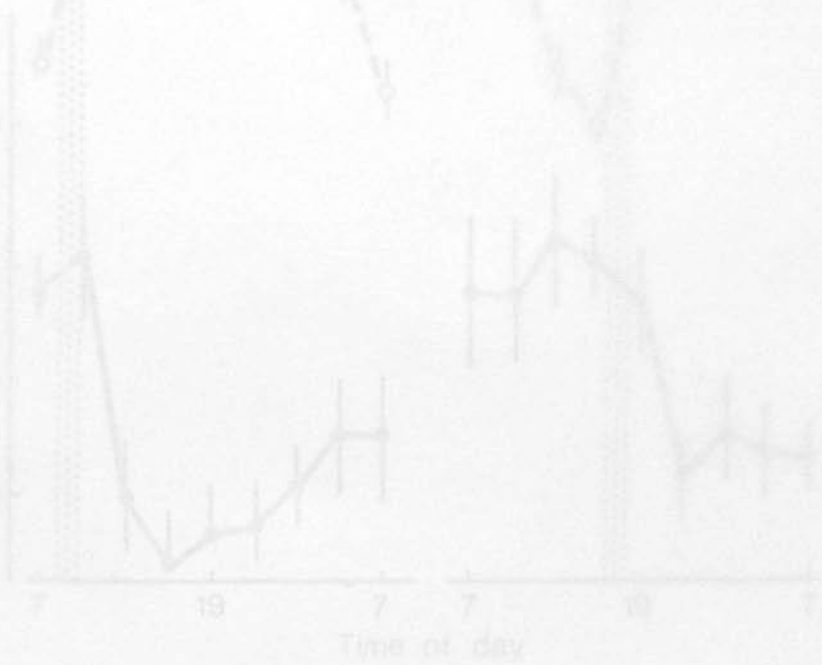
Commercial female broilers were reared on continuous light and given free access to food and water. They were maintained in groups until they were 5 weeks old when they were transferred to individual cages. Groups of birds ( $n = 10$ ) were fed either between 08.00 and 10.00 or between 16.00 and 18.00 while the remaining birds were provided food ab libitum. When the hens were 7 weeks old food was withdrawn from one of the groups of birds given free access to food for 24 h. Blood samples were subsequently taken from each group of birds every 3 h for 24 h. The birds sufficiently filled their crops within the first 30 min of access to food and did not eat during the remaining time food was available.

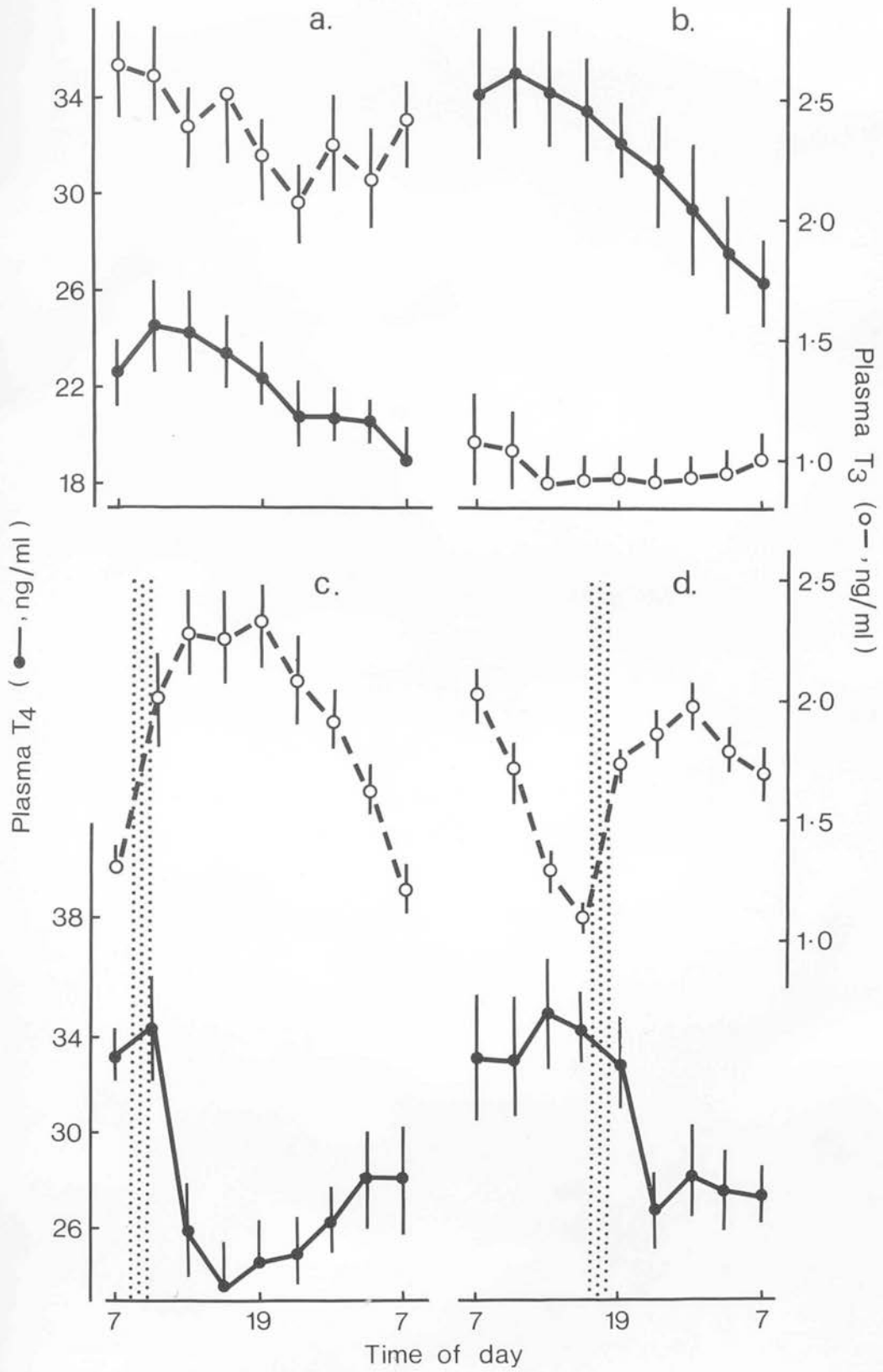
Daily variations in the levels of plasma  $T_3$  and  $T_4$  were not seen in birds provided food ad libitum or in birds fasted for 24 h. The concentration of plasma  $T_4$  tended to decline in birds provided free access to food although this effect was more pronounced in birds fasted for 24 h (Fig. 14). Fasted birds showed an increase in the concentration of plasma  $T_4$  and a decrease in the levels of plasma  $T_3$  ( $p < 0.001$ ). In birds fed at 08.00 or 16.00 the concentration of plasma  $T_3$  was lowest immediately before birds were given access to food. The levels were significantly increased ( $p < 0.001$ ) in the sample taken immediately after the food was removed and remained elevated for 6-9 h before declining to basal levels immediately before the next feeding time. In birds fed at 08.00 and 16.00 the concentration of plasma  $T_4$  was highest before the birds were fed and remained elevated in the sample taken immediately after the food was withdrawn (Fig. 14). The levels of  $T_4$  began to fall only about 3 h after the levels of  $T_3$  had begun to decrease ( $p < 0.001$ ). Thereafter plasma  $T_4$  remained depressed and did not return to the basal levels measured prior to feeding.

These experiments support the view that in chickens the pattern of food intake regulates the daily rhythms in the levels of plasma  $T_3$  and  $T_4$ . Levels of plasma  $T_3$  and  $T_4$  were depressed and elevated respectively immediately before the time the birds were fed. As soon as the birds started to feed, plasma  $T_3$  began to increase. Plasma  $T_4$  did not respond so rapidly to feeding and so suggests that the primary effect of feeding is to alter the production of  $T_3$ . Changes in the levels of plasma  $T_4$  may reflect changes in feedback sensitivity of the pituitary gland to



**FIGURE 14.** Changes in the concentration of plasma  $T_3$  (open circles) and  $T_4$  (closed circles) in 7-week-old female broilers in constant light and (a) given free access to food; (b) fasted for 24 h; (c) fed between 08.00 and 10.00; (d) fed between 16.00 and 18.00. The stippled area represents the period food was available to the birds. Each point represents the mean ( $\pm$  SEM) of 10 birds.





the concentration of plasma  $T_3$ . Thus prior to feeding the levels of plasma  $T_3$  are low and so the secretion of TSH and  $T_4$  would be expected to be maximal at this time. These conclusions support the concept that  $T_3$  rather than  $T_4$  is the metabolically active hormone (Gross and Pitt-Rivers, 1952; Pittman and Pittman, 1974).

#### 3.3.4 Summary

The effect of different patterns of food intake on the levels of plasma  $T_4$  were investigated in sexually immature ducklings and chickens. Food was removed from a group of ducklings and blood samples were obtained at intervals of 12 h for 36 h. Fasting resulted in a significant decrease in the levels of plasma  $T_3$  ( $p < 0.001$ ) while the concentration of plasma  $T_4$  remained unchanged. In a second study the possibility that the concentrations of plasma  $T_3$  and  $T_4$  in chickens may be regulated by the feeding pattern was investigated by measuring daily variations in plasma  $T_3$  and  $T_4$  in hens with access to food limited to the light or the dark periods. The nocturnal decrease in the plasma levels of  $T_3$  could be prevented by restricting food intake to the dark period. In both groups of birds the end of a 12 hr period of food withdrawal was associated with high levels of plasma  $T_4$  and low levels of plasma  $T_3$ . At these times, the differences in the concentrations of plasma thyroid hormones were highly significant ( $p < 0.001$ ). Pronounced daily rhythms in the concentrations of plasma  $T_3$  and  $T_4$  were observed in birds reared on continuous light and fed each day either between 08.00 and 10.00 or between 16.00 and 18.00. The levels of plasma  $T_3$  and  $T_4$  were again depressed and elevated respectively immediately before the birds were fed ( $p < 0.001$ ). As soon as the birds started to feed, regardless of the time of day, plasma  $T_3$  levels began to increase and continued to rise for up to 6 h after removal of food. The levels of plasma  $T_4$  did not respond as rapidly to feeding but began to fall steeply about 3 h after the levels of  $T_3$  had begun to increase. Birds reared on continuous light and either provided food ad libitum or fasted for 24 h did not show daily changes in the levels of plasma  $T_3$  and  $T_4$ . These experiments support the view that the inverse daily rhythms in the levels of plasma  $T_3$  and  $T_4$  are regulated by the pattern of food intake and that the primary effect of feeding is an increase in the peripheral mono-deiodination of  $T_4$ . Consequently plasma levels of  $T_3$  increase and

levels of  $T_4$  decrease. The converse changes may occur at the onset of darkness or when feeding stops. Changes in the levels of  $T_4$  were secondary to changes in the levels of  $T_3$  and may, in part, be due to the negative feedback suppression in the release of pituitary TSH by plasma  $T_3$ .

#### 3.4 Relationship between photoperiod, gonadal activity and photorefractoriness

In birds exposure to long days is thought to directly stimulate the hypothalamo-hypophysial-thyroid axis while also increasing the production of gonadal steroids which depresses thyroid activity. In support of this view studies in the duck have shown that castration results in an increase in the levels of circulating TSH (Benoit and Aron, 1931) and an increase in the number of TSH producing cells (thyrotropes) in the pituitary gland (Tixier-Vidal and Benoit, 1962). Furthermore in the duck castration has also been shown to stimulate the uptake of radioiodine by the thyroid glands (Tixier-Vidal and Assenmacher, 1961a, b; Jallageas and Assenmacher, 1972) while in the duck and quail castration increases the concentration of plasma  $T_4$  (Assenmacher et al., 1975; Jallageas and Assenmacher, 1979; Peczely et al., 1979; 3.5.1). Administration of testosterone reverses these effects (Jallageas and Assenmacher, 1972; Assenmacher et al., 1975; Peczely et al., 1979) and depresses thyroid function in intact ducks and quail exposed to short days (Jallageas and Assenmacher, 1972; Assenmacher et al., 1975).

Long days also stimulate thyroid function as evidenced from studies which have shown a seasonal variation in thyroid morphology of the starling (Burger, 1938) and duck (Hollwich and Tilgner, 1963) and from the finding that exposure to long days stimulates the development of thyrotropes in quail (Tixier-Vidal et al., 1968). However, in intact birds the stimulating effect of increasing day length on thyroid function is likely masked by the concomitant increase in the levels of gonadal steroids. This may explain the transitory increase in thyroid function in quail, as measured by an increase in plasma protein-bound-iodine, which was observed after photostimulation (Bayle and Assenmacher, 1967; Follett and Riley, 1967).

Thus the removal of the inhibitory effects of the gonads might allow

the direct effects of daylength on thyroid function to be fully expressed. Jallageas and Assenmacher (1972) found that in castrated ducks the uptake of radioiodine by the thyroid glands was depressed after photostimulation. Alternatively, in castrated ducks exposed to seasonal changes in daylength the level of plasma  $T_4$  tended to be directly related to the photoperiod (Jallageas and Assenmacher, 1979). In gonadectomized quail levels of plasma  $T_4$  were increased after photostimulation in males but not in females (Peczely et al., 1979, 1980) while in Spotted Munia long days have been shown to stimulate thyroid function directly (Chandola and Thapliyal, 1973).

The first study was carried out to gather more substantial evidence for the effect of daylength on the avian hypothalamo-pituitary-thyroid axis. Changes in concentrations of plasma  $T_4$  and  $T_3$  were measured in intact and gonadectomized male and female quail after transfer from short (6L : 18D) to long (18L : 6D) days and upon return to short days. Plasma LH was also measured to show that the birds were responsive to changes in daylength. After it became apparent that photostimulation resulted in a transitory increase in plasma  $T_4$ , an additional experiment was undertaken to determine whether this was associated with an increase in plasma free thyroxine ( $FT_4$ ). An assessment of the levels of plasma  $FT_4$  was thought to be important because it is the principal hormone released from the thyroid gland and represents the physiologically active fraction of total thyroxine (Robbins and Rall, 1960). Plasma  $FT_4$  concentrations reflect the concentration of total circulating  $T_4$  and the concentrations of the various thyroid hormone binding protein including thyroxine-binding prealbumin in the quail (El-Sayed et al., 1980).

The mechanisms responsible for the development of photorefractoriness in birds are still poorly understood and may differ between species. In species which show photorefractoriness the levels of LH and FSH increase upon exposure to long days, but then decrease dramatically after 50-80 days and so are unable to maintain the gonads in breeding condition. The quail does not represent a classic photorefractory species and remains fully mature for up to 3 years when held on photostimulatory daylengths (Follet, 1980). Several studies on the hormonal interaction between the testes and the thyroid gland in the duck have led to the

suggestion that an increase in levels of plasma  $T_4$  plays a role in the development of photorefractoriness (1.11.1a). The ratio of  $T_4$  to testosterone in plasma has also been suggested to be a factor in stimulating moult (Jallageas and Assenmacher, 1974, 1979). Thus, in ducks, the administration of  $T_4$  depressed testicular function in sexually mature drakes (Jallageas and Assenmacher, 1974; Assenmacher *et al.*, 1975) while plasma  $T_4$  levels rose to an annual maximum at the end of the breeding season and were elevated during the photorefractory period (1.11.1a). In the Peking drake, thyroidectomy of breeding birds delayed the seasonal fall in levels of plasma LH and testosterone from June until October (Jallageas and Assenmacher, 1979). However, similar studies on the starling (Wieselthier and Van Tienhoven, 1972) and the mallard (Haase and Paulke, 1980) showed that thyroidectomy of breeding birds did not prevent the development of photorefractoriness.

Measurement of seasonal changes in plasma thyroid hormones in Barheaded Geese (Dittami, 1981), Canada Geese (John and Gerge, 1978), Lesser Snow Goose (Campbell and Leatherland, 1980) and Ruffed Grouse (Garbutt *et al.*, 1979) do not provide clear evidence for an increase in plasma  $T_4$  during the development of photorefractoriness. These studies suggest that plasma  $T_4$  levels are high during gonadal growth but that they are also influenced by temperature.

Thus the relationship between daylength and the development of photorefractoriness has been more closely investigated in the Mallard duck and the Willow ptarmigan. The purpose of the study was to establish whether the levels of plasma thyroid hormones increase as the birds go out of breeding conditions. Any such increase may, as has been suggested for the duck, be involved in the development of photorefractoriness. In order to avoid the confounding effects of seasonal changes in daylength, reproductive function was stimulated by exposing birds held in light-proof rooms to various photostimulatory daylengths. Exposure to 14 hrs light/day delays the development of photorefractoriness in Willow ptarmigan (Stokkan *et al.*, 1981) while exposure to constant light would be expected, as was found in the chicken (May, 1978; 3.2.3) to abolish the daily rhythms in the levels of plasma  $T_3$  and  $T_4$ . Changes in the levels of plasma LH were also measured to assess the reproductive condition of the birds.

### 3.4.1 Quail: effect of photoperiod and gonadectomy

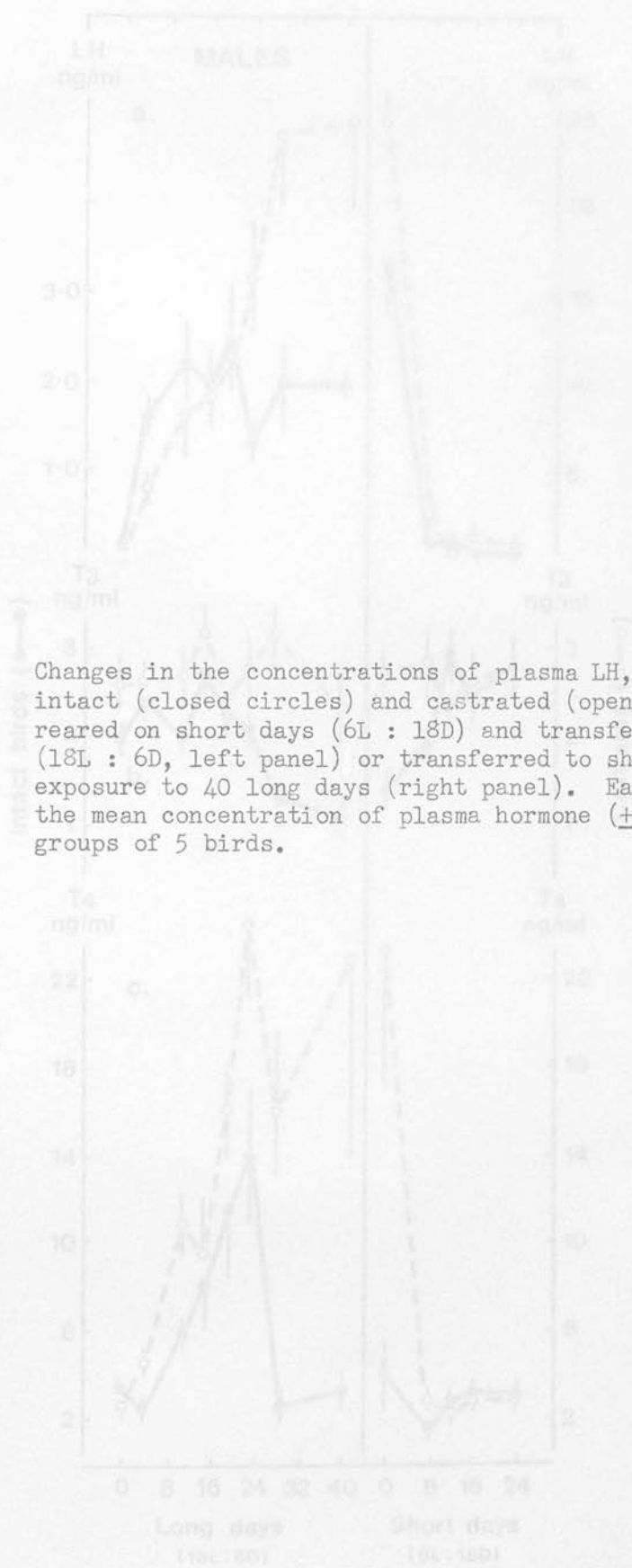
Experiment 1: 40 male and 40 female quail were reared from hatch while exposed to 6 h light/day, and at 26 d of age the photoperiod was increased to 18 h light/day. Groups of 5 birds of each sex were decapitated and blood was collected at 0, 4, 11, 15, 19, 24, 28, and 40 d after photostimulation (2.2.2). All blood samples were collected between 3 and 5 hrs after the beginning of the photoperiod. Gonad and body weights were recorded for each bird.

The concentration of plasma LH increased steeply in intact and gonadectomised quail of both sexes after the photoperiod was increased from 6- to 18 h/day (Figs. 15 and 16). The initial values in intact males and females, were  $0.11 \pm 0.02$  and  $0.12 \pm 0.02$  ng/ml and, after 4 days of exposure to 16L : 8D had increased significantly ( $P < 0.001$ ) to  $1.56 \pm 0.21$  and  $0.68 \pm 0.20$  ng/ml respectively. Thereafter LH levels increased to a maximum of  $2.42 \pm 0.7$  ng/ml at day 19 in the males and  $0.99 \pm 0.6$  ng/ml at day 15 in the females.

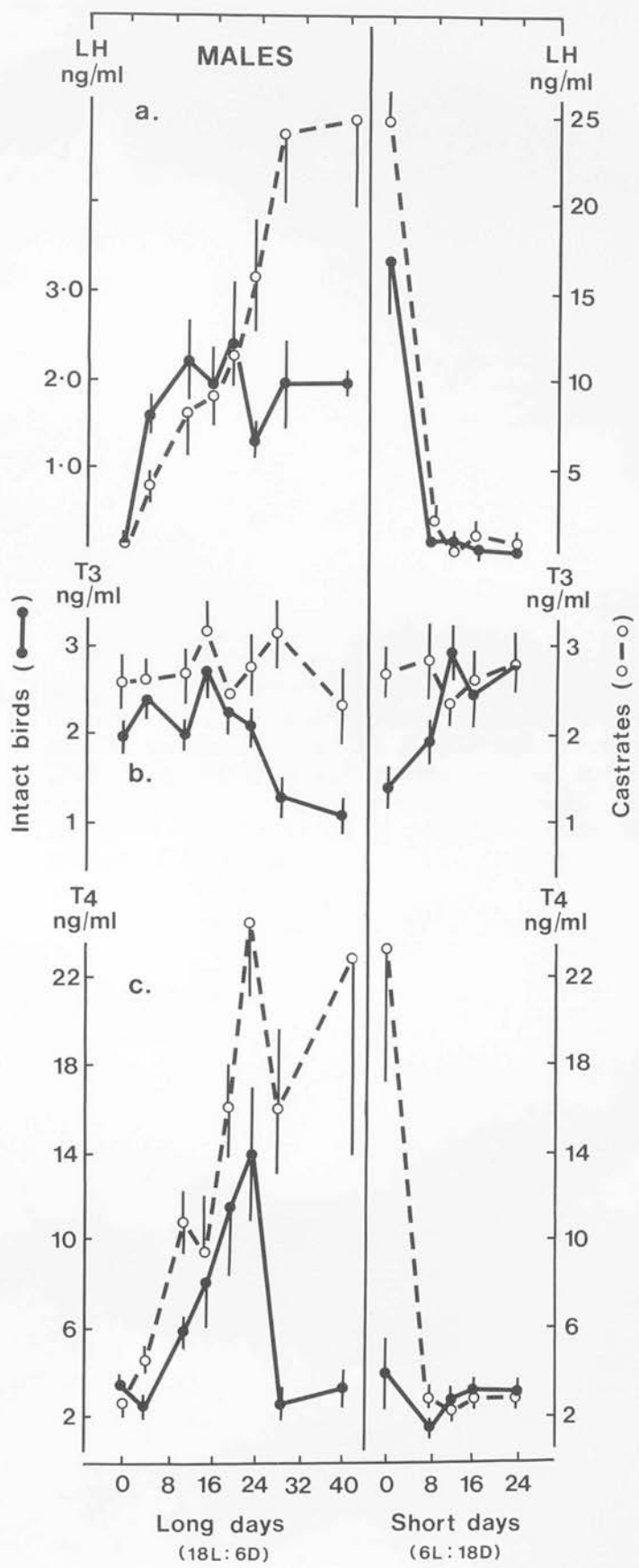
After 0, 4, 11, 15, 19, 24, 28, and 40 days of photostimulation the combined testis weights were  $8.1 \pm 1.1$ ,  $25.6 \pm 4.4$ ,  $259.8 \pm 27.3$ ,  $636.6 \pm 93.1$ ,  $1194.4 \pm 129.9$ ,  $2205 \pm 184.6$ ,  $2676.0 \pm 409.2$  and  $3595 \pm 186.7$  mg. The corresponding ovarian weights were  $23.1 \pm 2.43$ ,  $29.6 \pm 2.08$ ,  $79.7 \pm 7.40$ ,  $94.0 \pm 22.3$ ,  $202.0 \pm 32.6$ ,  $2216.8 \pm 1242.9$ ,  $1728.0 \pm 942$  and  $4888.3 \pm 228.9$  mg.

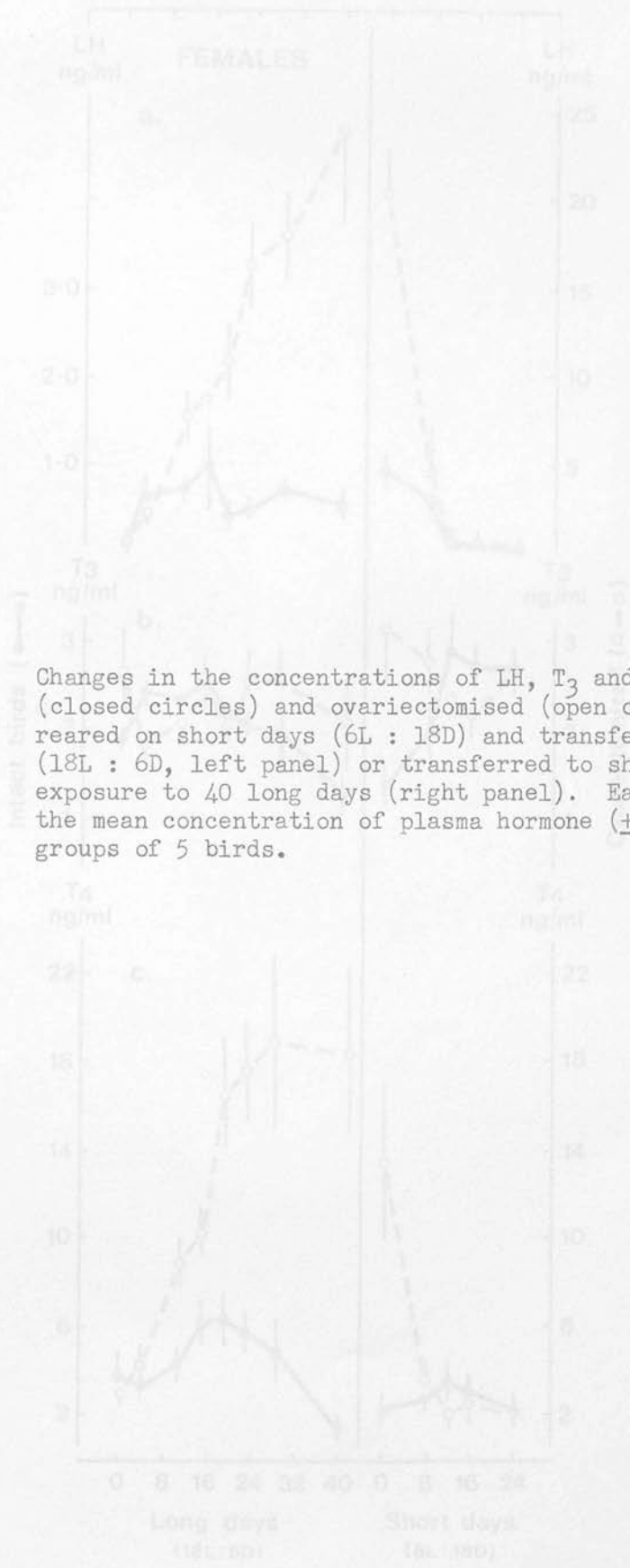
The concentrations of plasma  $T_3$  increased from  $1.96 \pm 0.16$  to  $2.40 \pm 0.16$  ng/ml ( $P < 0.01$ ) in males and from  $1.85 \pm 0.15$  to  $2.48 \pm 0.09$  ng/ml ( $P < 0.01$ ) in females during the first 4 days of photostimulation (Figs. 15 and 16). Thereafter  $T_3$  levels remained slightly elevated until, after 19 days of photostimulation, they began to fall progressively. After 40 days of photostimulation  $T_3$  levels in males and females were  $1.10 \pm 0.14$  and  $1.32 \pm 0.14$  ng/ml respectively and were significantly lower ( $P < 0.001$ ) than the corresponding values at day 0 of the study (Figs. 15 and 16).

The concentrations of plasma  $T_4$  did not change significantly in either sex during the first 4 days of photostimulation (Figs. 15 and 16). After 4 days of photostimulation,  $T_4$  levels started to increase progressively to reach a peak of  $14.06 \pm 3.61$  ng/ml at day 24 in the males, and  $6.48 \pm 1.79$  ng/ml at day 19 in the females. This increase

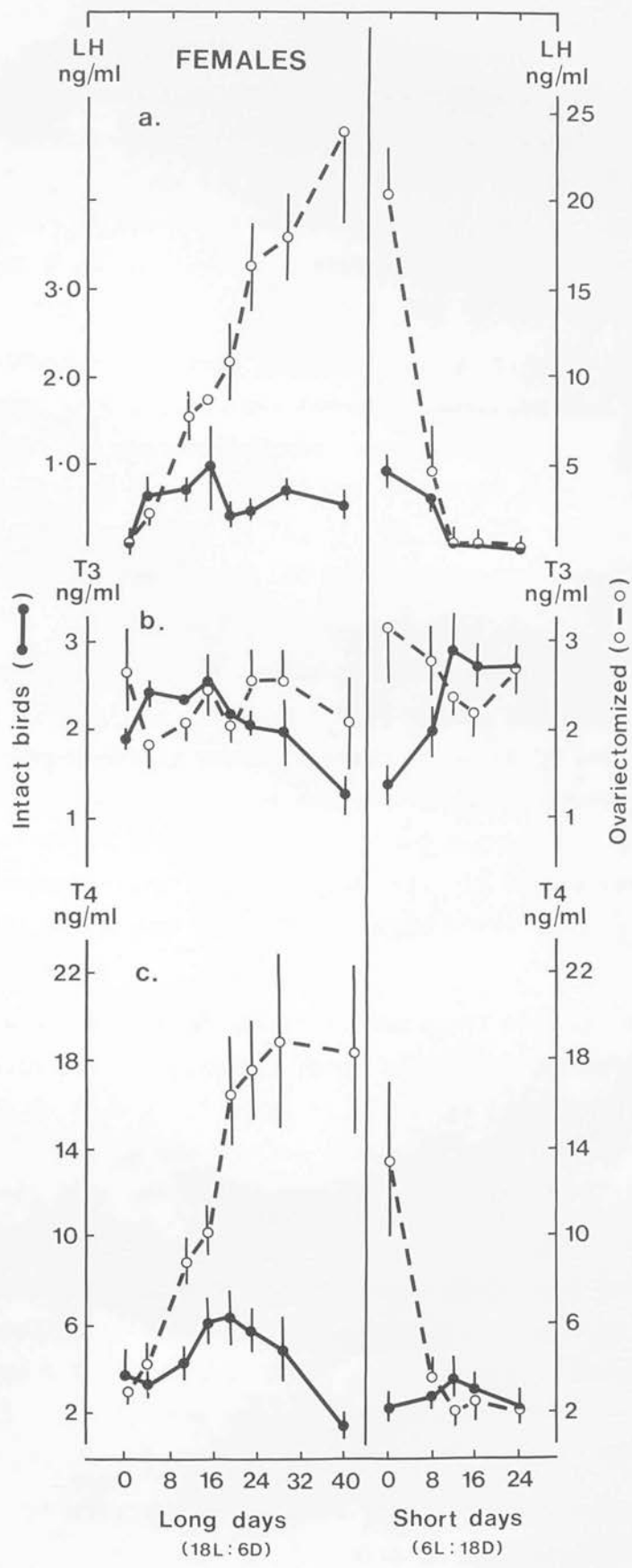


**FIGURE 15** Changes in the concentrations of plasma LH, T<sub>3</sub> and T<sub>4</sub> in intact (closed circles) and castrated (open circles) quail reared on short days (6L : 18D) and transferred to long days (18L : 6D, left panel) or transferred to short days after exposure to 40 long days (right panel). Each point represents the mean concentration of plasma hormone ( $\pm$  SEM) in different groups of 5 birds.





**FIGURE 16** Changes in the concentrations of LH, T<sub>3</sub> and T<sub>4</sub> in intact (closed circles) and ovariectomised (open circles) quail reared on short days (6L : 18D) and transferred to long days (18L : 6D, left panel) or transferred to short days after exposure to 40 long days (right panel). Each point represents the mean concentration of plasma hormone ( $\pm$  SEM) in different groups of 5 birds.



was highly significant ( $P < 0.001$ ), although the peak value in the female was significantly lower ( $P < 0.01$ ) than in the males. After 24 days of photostimulation in both sexes  $T_4$  levels fell steeply and by day 40 were  $3.39 \pm 1.33$  and  $1.57 \pm 0.24$  ng/ml in males and females respectively. In the males the levels of plasma  $T_4$  were not significantly different at 0 and 40 days of photostimulation but in the females they were significantly lower ( $P < 0.01$ ) at day 40 than at day 0.

Experiment 2: 40 male and 40 female quail were reared from hatch while exposed to 6 h light/day and were castrated when they were 28 or 29 d of age. The photoperiod was increased to 18 h light/day when the birds were 30 d of age and blood was collected as before from groups of 5 birds of each sex at 0, 4, 11, 15, 19, 23, 28, and 42 d after photostimulation. The success of the surgery was confirmed at postmortem examination.

The initial plasma LH levels in the castrated and ovariectomised birds were  $0.20 \pm 0.04$  and  $0.13 \pm 0.01$  ng/ml; thereafter values increased progressively reaching about 25 ng/ml in both sexes after 40 d of photostimulation. The concentrations of plasma  $T_3$  in castrated and ovariectomised birds did not show any significant trend with time after photostimulation (Figs. 15 and 16). In both sexes, the concentration of plasma  $T_3$  was higher in gonadectomised birds than in birds with fully developed gonads.

The concentration of plasma  $T_4$  increased steeply in gonadectomised birds of both sexes during the first 24 days of photostimulation from  $2.82 \pm 0.11$  to  $24.8 \pm 3.2$  ng/ml in the males and from  $3.16 \pm 0.43$  to  $17.8 \pm 2.56$  ng/ml in the females (Figs. 15 and 16). Thereafter  $T_4$  levels remained very high and almost an order of magnitude greater than in sexually mature birds.

Experiment 3: 20 male and 20 female quail were reared from hatch while exposed to 18 h light/day, and at 40 d of age the photoperiod was decreased to 6 h light/day. Blood samples were collected as before from groups of 5 birds of each sex at 0, 8, 12, 16, and 24 d after transfer to short days. Gonad weights were recorded for each bird.

In chronically photostimulated intact and gonadectomised quail of both sexes, the concentration of plasma LH fell steeply within 12 d after the photoperiod was decreased from 18 to 6 h/day (Figs. 15 and 16). The

initial values in intact males and females were  $3.37 \pm 0.64$  and  $1.07 \pm 0.12$  ng/ml and after 12 d exposure to 6 h light/day had decreased to  $0.20 \pm 0.03$  and  $0.22 \pm 0.04$  ng/ml respectively.

After 0, 8, 12, 16, and 24 days of transfer from 18 to 6 h light/day, the combined testis weights were  $2314.0 \pm 376.6$ ,  $2072.0 \pm 266.5$ ,  $387.6 \pm 113.2$ ,  $117.2 \pm 23.7$  and  $42.5 \pm 8.3$  mg. The corresponding ovarian weights were  $4438 \pm 549.3$ ,  $3982.0 \pm 533.4$ ,  $164.4 \pm 15.04$ ,  $78 \pm 8.2$  and  $59.0 \pm 9.5$  mg.

The concentration of plasma  $T_3$  increased from  $1.42 \pm 0.75$  to  $2.94 \pm 0.28$  ng/ml ( $P < 0.001$ ) in males and from  $1.44 \pm 0.29$  to  $2.92 \pm 0.41$  ng/ml ( $P < 0.001$ ) in females during the 12 days after sexually mature birds were transferred from 18 h to 6 h light/day (Figs. 15 and 16). Thereafter the concentration of plasma  $T_3$  remained at these elevated values while the birds remained on short days. After sexually mature birds were transferred from 18 h to 6 h light/day, the concentrations of plasma  $T_4$  remained depressed and showed no significant variations.

Experiment 4: 20 male and 20 female quail were reared from hatch while exposed to 6 h light/day and were gonadectomised at 30 d of age. The photoperiod was then increased to 18 h light/day, and after a further 40 d the photoperiod was decreased to 6 h light/day. Blood samples were then collected at days 6, 12, 16, and 24 after transfer to short days as for group 3. The success of the surgery was confirmed in post-mortem examination.

In chronically photostimulated gonadectomised quail of both sexes, the concentration of plasma LH fell steeply within 12 d after the photoperiod was decreased from 18 to 6 h/day (Figs. 15 and 16). The initial LH levels in the castrated and ovariectomised birds were  $25.02 \pm 1.79$  and  $20.48 \pm 2.58$  ng/ml; after 12 d of exposure to 6 h light/day they had decreased to  $0.65 \pm 0.22$  and  $0.63 \pm 0.21$  ng/ml respectively.

The concentrations of plasma  $T_3$  in castrated and ovariectomised birds did not show any significant trend with time after transfer from long to short days (Figs. 15 and 16) whereas the concentrations of plasma  $T_4$  fell steeply in gonadectomised birds of both sexes within 12 days of being transferred from long to short days (Figs. 15 and 16).

Experiment 5: 10 male and 10 female quail were reared from hatch while exposed to 6 h light/day and transferred to 18 h light/day at 30 d of age. Blood samples were taken from each bird by heart puncture (1 ml) at 0, 20, and 60 d after photostimulation.

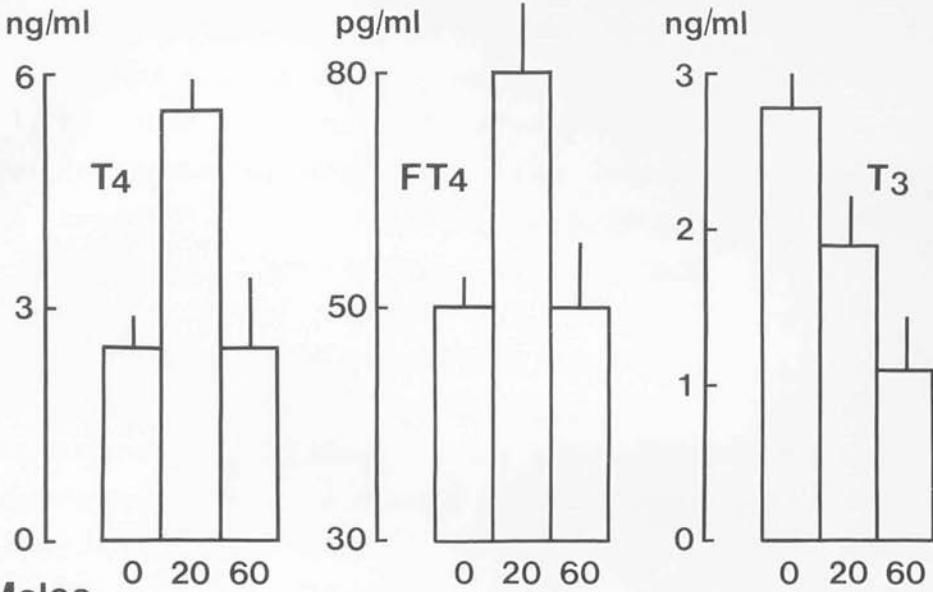
In serially sampled birds, the concentration of plasma  $T_3$  fell progressively between days 0 and 60 days of photostimulation (Table 8) whereas the concentration of plasma  $T_4$  increased significantly between 0 and 20 days of photostimulation in males and females. Thereafter  $T_4$  levels fell in both sexes and after 60 days of photostimulation were not significantly different from those at day 0 (Table 8). The changes in the concentrations of total plasma  $T_4$  were directly related to changes in the concentration of plasma  $FT_4$  (Table 8; Fig. 17). Thus, levels of  $FT_4$  increased significantly between 0 and 60 days of photostimulation from  $56.3 \pm 3.2$  to  $75.2 \pm 8.0$  pg/ml and from  $50.5 \pm 3.9$  to  $66.0 \pm 5.1$  pg/ml in males and females respectively. Thereafter levels of  $FT_4$  decreased after 60 days of photostimulation and were not different from those at day 0.

TABLE 8 - Changes in concentrations of plasma  $T_4$ ,  $FT_4$ , and  $T_3$  in serial samples taken from quail after transfer from short (6L : 18D) to long (18L : 6D) days

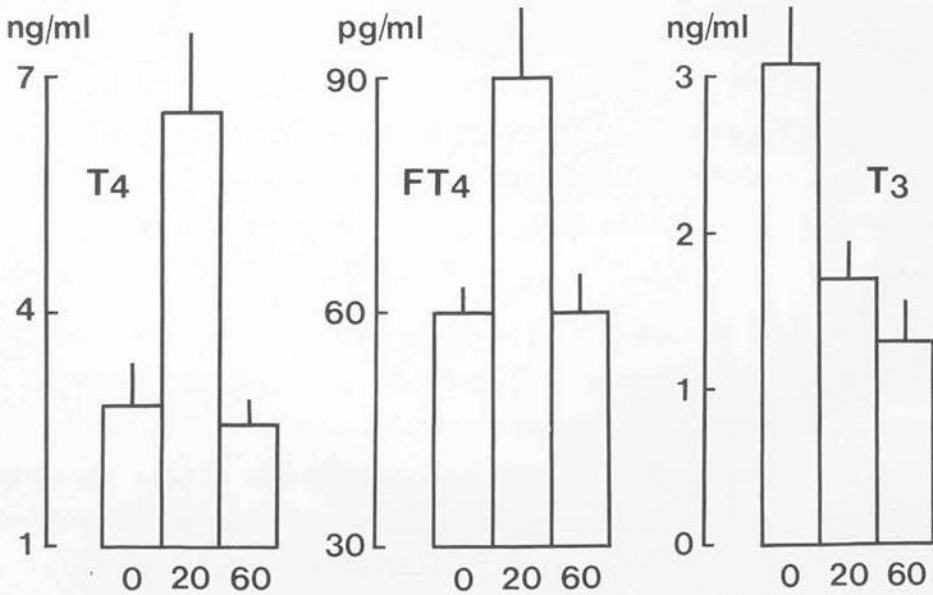
	Days after transfer to 18L : 6D	$T_4$ (ng/ml)	$FT_4$ (pg/ml)	$T_3$ (ng/ml)
Females (n = 6)	0	$2.5 \pm 0.3$	$50.5 \pm 3.9$	$2.8 \pm 0.2$
	20	** $5.5 \pm 0.6$	* $66.0 \pm 5.1$	** $1.9 \pm 0.3$
	60	$2.5 \pm 0.8$	$55.8 \pm 14.0$	** $1.1 \pm 0.3$
Males (n = 8)	0	$2.8 \pm 0.5$	$56.3 \pm 3.2$	$3.1 \pm 0.4$
	20	** $6.6 \pm 1.0$	* $75.2 \pm 8.0$	** $1.7 \pm 0.2$
	60	$2.6 \pm 0.2$	$72.8 \pm 11.0$	** $1.3 \pm 0.3$

\* $\underline{p} < 0.05$ ; \*\*  $\underline{p} < 0.01$  compared with day 0, by paired Student's t test.

### Females



### Males



Days

**FIGURE 17** Changes in the concentrations of plasma T<sub>4</sub>, FT<sub>4</sub> and T<sub>3</sub> in serial samples taken from quail after transfer from short (6L : 18D) to long (18L : 6D) days.

These observations support the view that in the quail thyroid function is regulated by an interaction between daylength and gonadal steroids (see Introduction to this section). In the absence of the gonads, levels of plasma  $T_4$  but not of  $T_3$ , increased after photostimulation and decreased after transfer back to short days, closely following the pattern of LH secretion. This suggests that the production of  $T_4$  is dependent on daylength-induced changes in the secretion of thyrotrophin and thyrotrophin releasing factor (TRF). In support of this view, injections of synthetic TRH stimulate the production of  $T_4$  in birds (3.5.2). The suggestion (Peczely et al., 1980) that the stimulating effect of long days on  $T_4$  secretion in gonadectomised quail is sex dependent (occurring in males but not in females) was not confirmed in this study. Peczely et al. (1980) based their suggestion measurements of  $T_4$  in blood samples taken after 14 days of photostimulation, whereas the results of this study suggest that more than 24 days are required before  $T_4$  levels in intact and ovariectomised birds are different (Fig. 16).

The observation that the concentration of plasma  $T_3$  was not affected by changes in daylength in gonadectomised birds, despite the large changes in the concentration of plasma  $T_4$ , supports the view that plasma thyroid hormones are regulated by different mechanisms (3.8.2) and that the principal factor regulating the generation of  $T_3$  in birds may be the level of food intake (3.3).

The transient increase in levels of plasma  $T_4$  seen in intact male and female quail supports the view that thyroid function increases during the initial stages of photoinduced gonadal growth but falls thereafter (Bayle and Assenmacher, 1967; Follett and Riley, 1967). This increase may be important for gonadal maturation since thyroidectomy partially or completely inhibits photoinduced gonadal growth in several avian species including the Japanese quail (Thapliyal and Garg, 1969; Jallageas and Assenmacher, 1973; Peczely et al., 1979, 1980). Further, gonadal growth is facilitated by treatment with moderate doses of  $T_4$  in ducks and domestic chickens. Since free rather than bound concentrations of plasma thyroid hormones are believed to be biologically important (Robbins and Rall, 1960; Chopra et al., 1980) any specific role that  $T_4$  may have in the induction of gonadal growth might be expected

to be mediated by an increase in plasma  $FT_4$ . The observation that  $FT_4$  increased during photoinduced gonadal growth in both sexes (Fig. 17) supports this possibility.

The fall in the concentration of plasma  $T_4$  and  $T_3$  after 24 days of photostimulation occurred when the gonads were almost fully developed. At this time, as has been shown in the male quail (Follett and Maung, 1978; Follett, 1980), the concentrations of gonadal steroids would have just increased to adult values. Since plasma  $T_4$  levels did not fall in gonadectomised birds after 24 days of photostimulation, it is possible that the depression in concentrations of plasma thyroid hormone in intact birds was caused by high concentrations of gonadal steroids. This conclusion is supported by the finding that injections of testosterone depresses thyroid function in ducks and quail (see Introduction to this section).

Thyroid hormones are transported in the blood of birds by several binding proteins of which thyroxine-binding prealbumin (TBPA) is one of the more important (1.7; 3.9). This protein is synthesized in the liver and in intact quail its secretion is inversely related to seasonal changes in the photoperiod and hence to gonadal development (El-Sayed *et al.*, 1980). It is therefore possible that high levels of plasma gonadal steroids may inhibit TBPA production during the breeding season and that when the gonads regress, this inhibitory effect is lifted to allow the plasma concentrations of this protein to increase. The depression in the concentrations of plasma  $T_3$  and  $T_4$  after 24 days of photostimulation may thus reflect a decrease in the concentration of plasma thyroid binding hormones induced by the inhibitory action of gonadal steroids on the liver. After sexually mature quail were returned to short days, the reverse changes may have occurred resulting in an increase in the carrying capacity of the blood for thyroid hormones and hence an increase in plasma  $T_3$  (Figs. 15 and 16). The absence of an increase in plasma  $T_4$  after transfer to short days confirms the different control mechanisms involved in the production of  $T_3$  and  $T_4$ . As is shown by the steep fall in plasma  $T_4$  in gonadectomised birds transferred from long to short days, the hypothalamic drive on the pituitary-thyroid-axis in intact birds exposed to the same conditions, would be reduced. Thus, the production of  $T_4$  would be so low that the increase in plasma thyroid binding proteins would not result in an increase in total  $T_4$  levels.

### 3.4.2 Mallard ducks and Willow ptarmigan: effect of photoperiod and photorefractoriness

Experiment 1: Mallard duck eggs were incubated and hatched during the last week of April 1977. The birds were reared indoors in light proof rooms and exposed to conditions of simulated natural light until September when the photoperiod was decreased to 6L : 18D. The short day photoperiod was maintained until February, 1978. At this time the photoperiod was increased to 20L : 4D. Blood samples were taken from a wing vein every week, beginning 3 weeks after photostimulation for 20 weeks. Samples were taken between 3-6 h after the lights came on.

In Mallard ducks transferred from short days to long days the concentration of plasma  $T_4$  was observed to increase at the end of the breeding period while the levels of plasma LH and testosterone were falling (Fig. 18). The concentration of LH and testosterone declined between 6 and 7 weeks after photostimulation and was associated with an increase in the levels of plasma  $T_4$  which peaked between 12 and 14 weeks after the daylength was increased. Thereafter the concentration of plasma  $T_4$  declined even though the daylength remained long. During this period there was a moderate increase in the levels of plasma LH and testosterone (Fig. 18).

These observations confirm the reciprocal inhibitory patterns in the levels of plasma thyroid and gonadal hormones and support the view that plasma  $T_4$  may be important in the development of photorefractoriness in ducks (Assenmacher *et al.*, 1975; Jallageas *et al.*, 1978; Jallageas and Assenmacher, 1979). Alternatively the increase in plasma  $T_4$  may be due to an increase in the levels of thyroid hormone binding proteins in response to the decline in gonadal steroids. In support of this view the concentration of  $T_4$  in the first sample taken after photostimulation was elevated while the concentration of plasma testosterone was low. Further gonadal development was associated with a decrease in the levels of  $T_4$ . Thus, like the situation in the quail (3.4.1) there may be a direct effect of light on the hypothalamo-hypophysial-thyroid axis which is masked by the production of gonadal steroids.

Experiment 2: Male Willow ptarmigan reared in captivity while exposed to natural changes in daylength were transferred in groups of 6, in mid-January from short days (6L : 18D) to 14L : 10D, 18L : 6D or

MALE MALLARDS ON 20L 4D FROM FEBRUARY

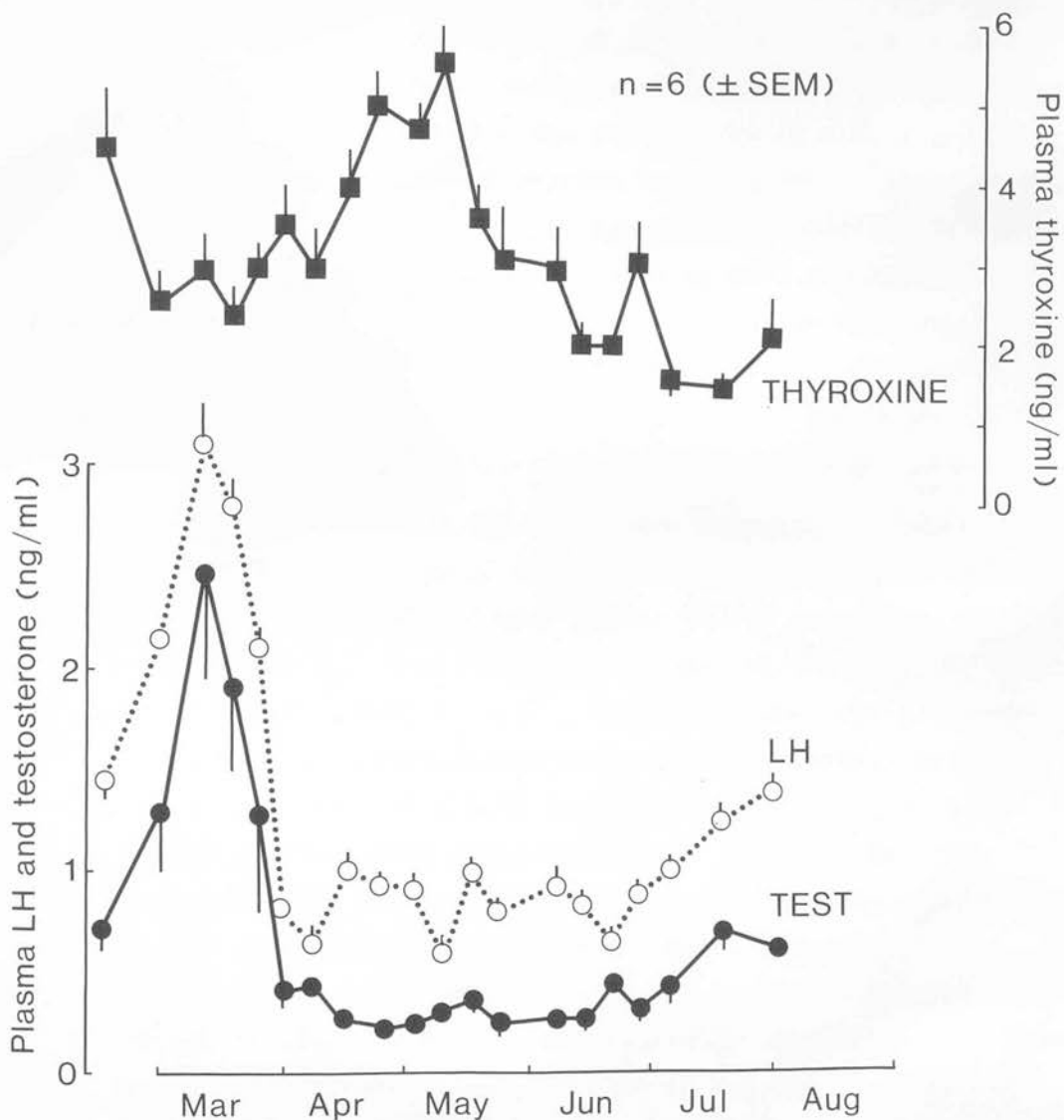


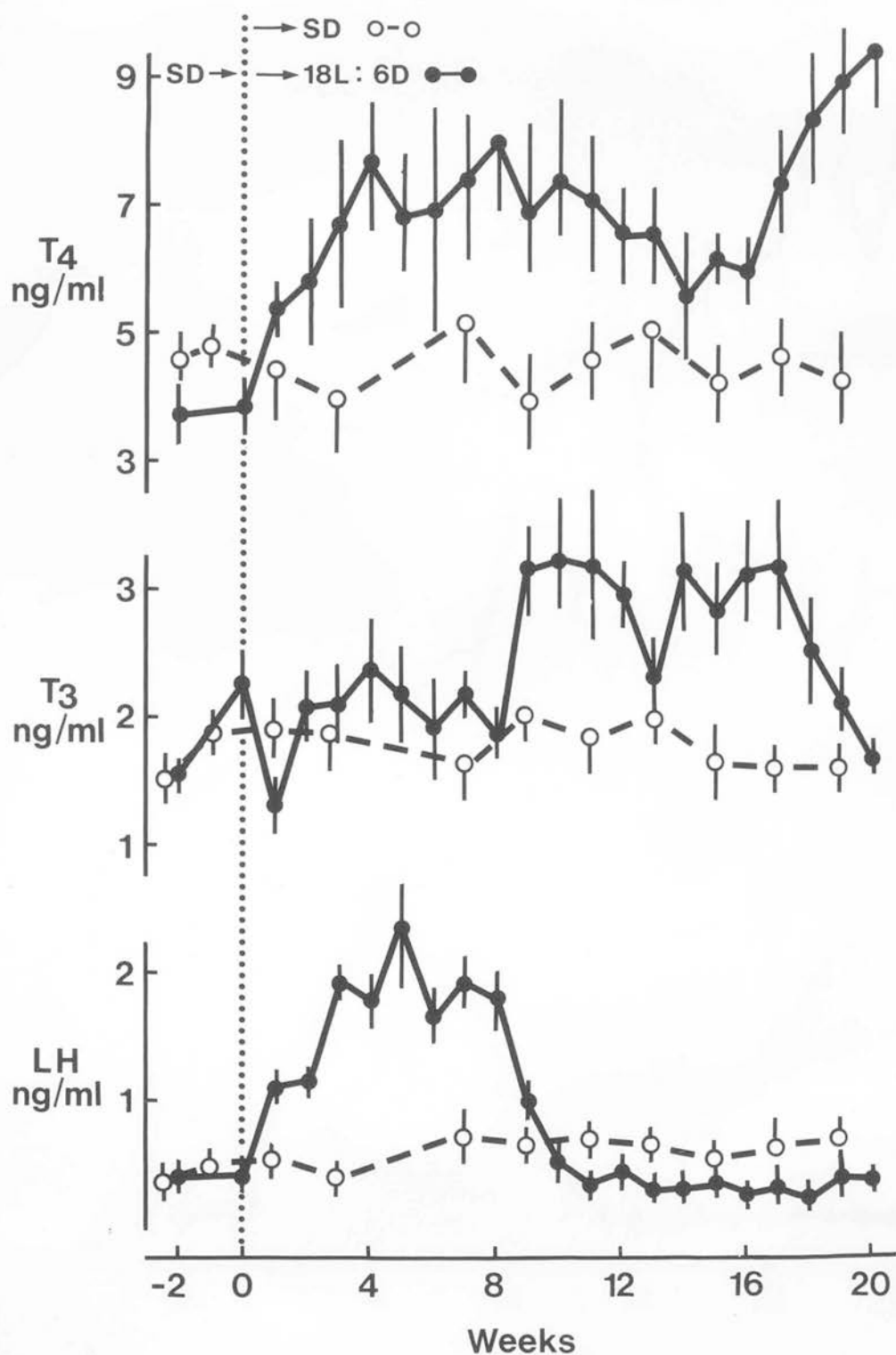
FIGURE 18 Changes in the concentrations of plasma  $T_4$ , LH and testosterone in male mallards transferred from short days (6L : 18D) to long days (20L : 4D) from February. Each point represents the mean concentration of plasma hormone ( $\pm$  SEM) of 6 birds.

to continuous (LL) daylengths. A control group of birds (n = 5) were maintained on a 6 h daylength throughout the study. Blood samples were obtained every week 3-6 hours after the lights came on for 20 weeks after photostimulation.

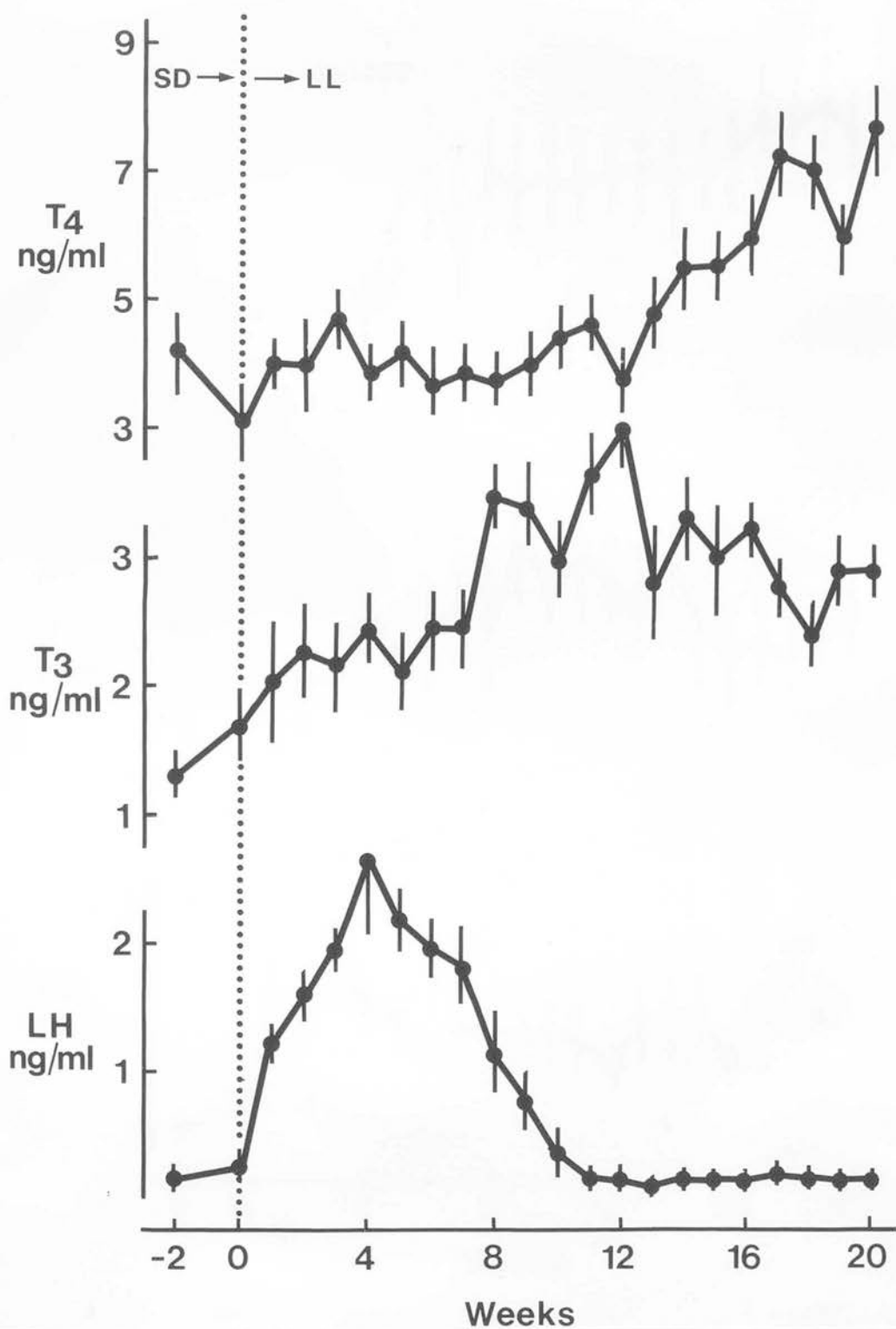
The increase in the concentration of plasma LH observed after transferring Willow ptarmigan from short days to the 3 stimulatory photoperiods, with one exception was not associated with a significant change in the concentrations of thyroid hormones (Figs. 19-21). The one exception was the steep increase in plasma  $T_4$  observed in birds transferred to 18L : 6D (Fig. 19). A week by week comparison of  $T_3$  and  $T_4$  levels in the 3 groups showed that the only major difference was in the levels of  $T_4$  in birds exposed to constant light. In these birds  $T_4$  levels were significantly lower ( $P < 0.05$ ) than in the other groups between weeks 5 and 13 after transfer to long days (Figs. 19-21).

The first evidence that the birds were becoming photorefractory was seen after 9 and 8 weeks of exposure to 18L : 6D and LL respectively, when a fall in the concentration of plasma LH was associated with an increase in plasma  $T_3$ . Thus, in birds exposed to 18L : 6D,  $T_3$  levels increased from  $1.88 \pm 0.23$  to  $3.15 \pm 0.34$  ng/ml ( $P < 0.01$ ) between 8 and 9 weeks after photostimulation while in birds exposed to LL,  $T_3$  levels increased from  $2.43 \pm 0.31$  to  $3.48 \pm 0.28$  ng/ml ( $P < 0.01$ ) between 7 and 8 weeks after photostimulation (Figs. 19 and 20). This increase in plasma  $T_3$  and fall in plasma LH was not associated with any change in the concentration of plasma  $T_4$  (Figs. 19 and 20). The concentration of plasma  $T_3$  remained elevated for 9 and 5 weeks in birds exposed to 18L : 6D and LL, respectively. Thereafter,  $T_3$  levels began to fall with a concomitant increase in the levels of plasma  $T_4$  (Figs. 19 and 20). This change in the ratio of levels of plasma thyroid hormone levels was not correlated with any change in the concentrations of plasma LH, which remained depressed and characteristic of the photorefractory state.

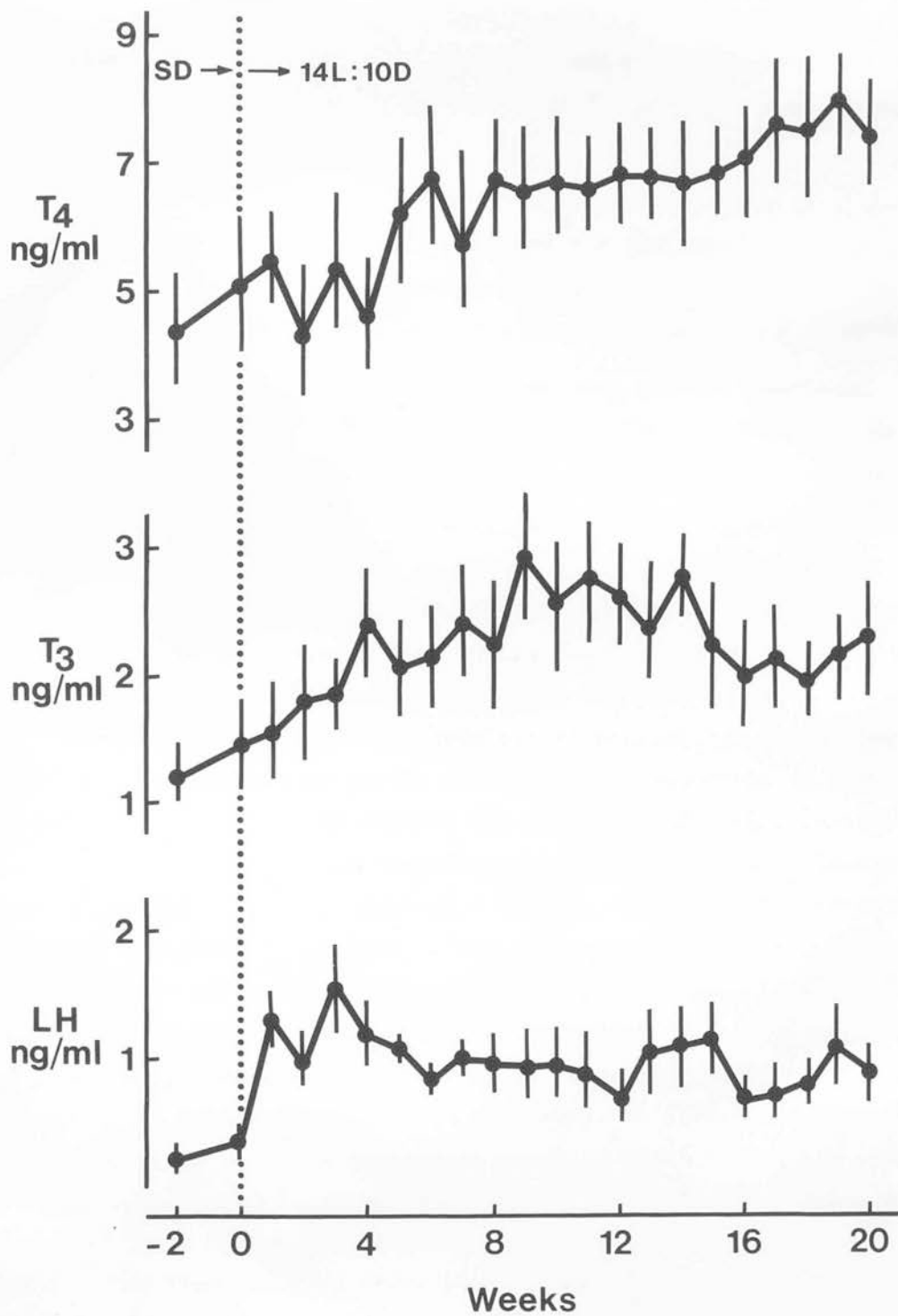
In Willow Ptarmigan exposed to 14L : 10D, plasma LH levels remained elevated during the 20 week study and the concentrations of plasma  $T_3$  did not increase as steeply as in birds exposed to LL and 18L : 6D at any point during this period (Fig. 21). The highest levels were observed between 9 and 14 weeks after transfer to 14L : 10D which corresponded



**FIGURE 19** Changes in the concentrations of plasma T<sub>4</sub>, T<sub>3</sub> and LH in 6 photosensitive male Willow Ptarmigan transferred from short days (6L : 18D) to a 18 h (18L : 6D) daylength (closed circles). Five control birds were maintained on the 6 h daylength throughout the study (open circles). Each point represents the mean ( $\pm$  SEM) hormone level in serial samples taken from the birds.



**FIGURE 20** Changes in the concentrations of plasma T<sub>4</sub>, T<sub>3</sub> and LH in photosensitive Willow Ptarmigan transferred from short days (6L : 18D) to continuous light. Each point represents the mean ( $\pm$  SEM) hormone level in serial samples taken from 6 birds.



**FIGURE 21** Changes in the concentrations of plasma T<sub>4</sub>, T<sub>3</sub> and LH in photosensitive Willow Ptarmigan transferred from short days (6L : 18D) to a 14 h daylength (14L : 10D). Each point represents the mean ( $\pm$  SEM) hormone level in serial samples taken from 6 birds.

with the period during which  $T_3$  levels were at their highest in birds transferred to LL and 18L : 6D (Figs. 19 and 20). There was no evidence for an increase in plasma  $T_3$  at this time in control birds exposed to 6L : 18D (Fig. 19).

After 4 weeks exposure to 14L : 10D, concentrations of plasma  $T_4$  increased and remained slightly elevated for the rest of the 20 week study (Fig. 21). The concentration of plasma  $T_4$  in control birds exposed to 6L : 18D did not change significantly during the study (Fig. 19).

The temperatures to which the birds were exposed increased progressively from a daily range of 4-6°C at the beginning of the study to 10-12°C at the end.

In the Willow ptarmigan, the onset of photorefractoriness, the condition where long days are no longer able to maintain reproductive function, was not associated with an increase in the concentration of plasma  $T_4$ . It thus appears that unlike the previous study in the duck, an increase in plasma  $T_4$  levels in the Willow ptarmigan plays no role in the development of photorefractoriness. In the Willow ptarmigan the difference in the pattern of  $T_4$  production in birds exposed to the 2 lighting regimes (LL and 18L : 6D) which resulted in the development of photorefractoriness, illustrates how  $T_4$  levels are more dependent on the photoschedule than on reproductive condition. The increase in plasma  $T_4$  observed after birds were transferred from short days to 18L : 6D is consistent with the view that long days directly stimulate the hypothalamo-hypophysial-thyroidal axis (3.4.1). Alternatively the pattern of food intake may regulate the levels of plasma  $T_4$  in Willow ptarmigan and this would be influenced by the photoperiod; in chickens exposed to constant light, the daily rhythm of plasma  $T_4$  levels disappear and mean  $T_4$  levels are depressed (May 1978, 3.2.3). These factors may explain why  $T_4$  levels did not increase for 12 weeks after transfer from short days to constant light.

The mechanism responsible for the rise in plasma  $T_4$  and the associated fall in plasma  $T_3$  after the birds had been exposed to LL for 12 weeks and to 18L : 6D for 16-17 weeks is unknown. The only situation in which plasma  $T_4$  and  $T_3$  levels change in this way is when there is a reduction in metabolic rate (3.7.2). Such a reduction could be caused by an increase in ambient temperature or by a reduced moult, and might be

expected to be accompanied by reduced food intake. Unfortunately, food intake was not measured in the present study although it has been observed to fall from the annual maximum during the breeding season to low levels in the later summer in Willow ptarmigan exposed to natural lighting (Hansson, 1982). The difference in the timing of the increase in plasma  $T_4$  in the birds exposed to LL and 18L : 6D and an absence of an increase in control birds seems to rule out a temperature effect. Further, the variations in ambient temperature encountered during the course of this study were within the thermoneutral zone (West, 1972). Although birds exposed to 18L : 6D and LL were moulting continuously throughout the study, new flight and tarsal feathers had regrown and the rate of moult was reduced towards the end when plasma  $T_4$  levels increased and  $T_3$  levels fell.

The fall in plasma LH levels, observed as Willow ptarmigan became photorefractory is accompanied by a decrease in levels of plasma testosterone. It is therefore possible that, as has been found in the duck and quail (Jallageas and Assenmacher, 1972; Peczely *et al.*, 1979; 3.4.2), changes in thyroid function at the end of the breeding season in the Willow ptarmigan may be a consequence of testicular regression. The seasonal regression of the testes might therefore, be partly responsible for the post-nuptial increase in levels of plasma  $T_3$ . Such an increase was observed in quail after gonadal regression was induced by a reduction in daylength (3.4.1) and may be caused by a rise in the concentrations of thyroid hormone binding prealbumin (El Sayed *et al.*, 1980). An increase in plasma  $T_3$  was not seen in castrated quail transferred from long to short days (3.4.1). Although the concentrations and affinities of the thyroid hormone binding proteins probably differ between Willow ptarmigan and quail, the general pattern of response to a change in photoperiod is likely similar.

In the quail, testicular regression induced by short days was not associated with an increase in plasma  $T_4$  levels despite the increase in the concentrations of plasma thyroid hormone prealbumin (3.4.1). This lack of increase was suggested to be due to the removal of the stimulatory effects of long daylengths on  $T_4$  production. Similarly, in the ptarmigan and duck testicular regression was not associated with a sustained increase in plasma  $T_4$ . But unless it is postulated that the

hypothalamo-pituitary-thyroidal axis in these species becomes photorefractory, the absence of an increase in plasma  $T_4$  during testicular regression cannot be explained in the same way as in quail since both the ptarmigan and duck were exposed to long daylengths throughout the study.

The absence of a steep increase in plasma  $T_3$  in Willow ptarmigan while exposed to 14L : 10D supports the view that the steep increase in birds exposed to LL and 18L : 6D was caused, in part, by the associated regression of the testes. However, the observation that  $T_3$  levels were slightly elevated between 9 and 14 weeks after transfer to 14L : 10D is suggestive of a photo-induced cycle of thyroid activity which is not dependent on changes in reproductive activity.

### 3.4.3 Summary

Changes in the concentrations of plasma  $T_4$  and  $T_3$  were measured in intact and gonadectomised quail of both sexes after transfer from short (6L : 18D) to long (18L : 6D) days and upon return to short days. The concentration of free thyroxine ( $FT_4$ ) was also measured in intact birds after transfer from short to long days. In both intact and gonadectomised birds, the concentrations of plasma  $T_4$  but not of  $T_3$  increased after transfer to long days. However, after 19-24 days of photostimulation, the concentrations of plasma  $T_4$  and  $T_3$  began to fall in the intact birds but remained unchanged in the gonadectomised birds. The transitory increase in plasma  $T_4$  observed in intact birds after photostimulation was associated with an increase in concentrations of plasma  $FT_4$ . The decrease in plasma  $T_4$  and  $T_3$  in photostimulated intact birds began when gonadal growth was nearly complete.

The concentration of plasma  $T_3$  in sexually mature male and female quail was lower than in sexually immature birds. In gonadectomised birds of both sexes, the concentration of plasma  $T_4$  fell while that of plasma  $T_3$  remained unchanged after transfer from long to short days. In sexually mature males and females, the transfer back to short days did not change the concentration of plasma  $T_4$  but caused an increase in that of plasma  $T_3$ .

These observations are consistent with the view that in the quail, long daylengths directly stimulate the hypothalamo-pituitary-thyroid

axis and the production of  $T_4$ . The production of  $T_3$  is dependent, at least in part, on the peripheral monodeiodination of  $T_4$ ; this process is regulated by the level of food intake and is not directly dependent on daylength. It is suggested that in sexually mature birds, the concentration of plasma  $T_4$  and  $T_3$  are depressed because of a reduction in the concentration of plasma thyroid hormone binding proteins. The production of these proteins is suggested to be inhibited by gonadal steroids.

Changes in the concentrations of plasma  $T_4$ , LH and testosterone were measured in photosensitive Mallard drakes transferred from a 6 h daylength (6L : 18D) to a 20 hr daylength (20L : 4D). Similarly the concentrations of plasma  $T_4$ ,  $T_3$  and LH were measured in photosensitive Willow ptarmigan transferred from a short day photoperiod (6L : 18D) to 14 h (14L : 10D), 18 h (18L : 6D) or continuous (LL) daylengths. In Mallard ducks the concentration of plasma  $T_4$  increased at the end of the breeding period after the concentrations of plasma LH and testosterone had fallen. In Willow ptarmigan the increase in plasma LH observed after transfer to the 3 stimulatory photoperiods were not associated with any immediate changes in the concentrations of plasma  $T_4$  or  $T_3$  except for an increase ( $P < 0.01$ ) in  $T_4$  levels in birds transferred to 18L : 6D. As indicated by a fall ( $P < 0.01$ ) in concentrations of plasma LH, the birds exposed to LL and 18L : 6D were becoming photorefractory after 8-9 weeks of photostimulation. At this time, the concentration of plasma  $T_3$  but not of  $T_4$  increased significantly ( $P < 0.001$ ) and remained high for 5 and 9 weeks in birds exposed to LL and 18L : 6D respectively. Thereafter, levels of plasma  $T_3$  began to fall concomitant with an increase in the levels of plasma  $T_4$ . In birds exposed to 14L : 10D, LH levels remained elevated during the 20-week study and minor increases were observed in the levels of plasma  $T_3$  and  $T_4$ . These observations do not suggest a major role for thyroid hormones in the initiation of photorefractoriness in the Willow ptarmigan.

### 3.5 Pituitary-thyroid relationships

In mammals the secretion of TSH is influenced by the negative feedback inhibition of thyroid hormones which reach the pituitary gland via the systemic circulation and by two of the hypothalamic releasing

hormones, TRH and SRIF which are released from the median eminence and transported to the gland via the hypothalamic-hypophysial portal vessels. Thyrotrophin releasing hormone (TRH) stimulates whereas somatostatin (SRIF) inhibits the secretion of TSH (Drouin et al., 1976; Hedge et al., 1981). It is uncertain whether  $T_4$  exerts an inhibitory feedback effect on the release of TSH (Obregon et al., 1980). It is likely that it is deiodinated within the pituitary cells to  $T_3$  which inhibits TSH release. Even so  $T_4$  has functioned in the classic sense of a hormone for it has transmitted information from its site of synthesis to the target tissue.

In birds the cells producing TSH in the adenohypophysis have been identified in the rostral and caudal lobes (Tixier-Vidal et al., 1962; review: Tixier-Vidal and Follett, 1973) whereas in others TSH cells were found in the rostral, but not in the caudal lobe (Marchand and Bugnon, 1972, 1973; Marchand et al., 1972, 1980). Each of these studies used classical tinctorial or cytochemical methods to identify cells producing TSH.

In the first study an attempt was made to resolve this uncertainty about the localisation of the TSH cells in the duck and to investigate the relationship between the levels of plasma thyroid hormone and levels of pituitary TSH. In addition the possibility that gonadal steroids affect the concentration of plasma thyroid hormones at the level of the pituitary gland was also investigated.

In the chicken, thyrotrophin-releasing hormone (TRH) stimulates the release of thyroid-stimulating hormone (TSH) from the pituitary gland (Scanes, 1974), the uptake of  $^{32}P$  into the thyroid (Breneman and Rathkamp, 1973) and causes an increase in protein-bound iodine in the blood (Shimada et al., 1973; Newcomer and Huang, 1974). In addition Combest et al. (1978) has shown that TRH increases the activity of ornithine decarboxylase (ODC) in the anterior pituitary. ODC is the rate-limiting enzyme in the polyamine biosynthetic pathway and is known to be stimulated by hormones and drugs which stimulate RNA and protein synthesis. In rats TRH is stored in granules in the cytoplasm of anterior pituitaries and is suggested to play a role in the storage or packaging of TSH (Childs et al., 1981). The purpose of the second study was to examine the effectiveness of TRH in stimulating the chicken pituitary-thyroid axis by directly measuring the changes in plasma  $T_4$  and  $T_3$  concentrations

TABLE 9 - Body, adeno-hypophysial and thyroid weights (means  $\pm$  S.E.M.) in 10-week-old drakes used for the immunocytologic study, after experimental manipulation

Treatment	Body (kg)	Combined thyroid gland (mg)	Adeno-hypophysis (mg)	mg/kg body weight
Controls				
Aylesbury (2)	2.48, 2.48	102, 174	11.1, 12.6	4.50, 5.08
Khaki Campbell (3)	1.75 $\pm$ 0.07	139.3 $\pm$ 4.9	10.7 $\pm$ 0.8	6.2 $\pm$ 0.7
Castrates (5)	1.72 $\pm$ 0.10	152.8 $\pm$ 17.6	14.4 $\pm$ 0.4*	8.5 $\pm$ 0.6*
Methimazole fed (5)	0.52 $\pm$ 0.06***	334.2 $\pm$ 54.5*	5.4 $\pm$ 0.2***	10.8 $\pm$ 0.8**
Castrated, injected with thyroxine (5)	1.66 $\pm$ 0.05	73.2 $\pm$ 5.0***	14.3 $\pm$ 0.8*	8.6 $\pm$ 0.4*
Methimazole fed, implanted with testosterone (4)	0.57 $\pm$ 0.05***	368.5 $\pm$ 67.6*	6.1 $\pm$ 0.3**	11.0 $\pm$ 1.3*

All the birds were Khaki Campbell drakes unless otherwise shown. Statistical comparisons with the Khaki Campbell controls are \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05 (unpaired t test)  
The number of birds are shown in parenthesis

after an injection of TRH.

### 3.5.1 Ducks: localisation and regulation of TSH

One day-old domestic drakes (Khaki Campbell or Aylesbury) were obtained from a local breeder and reared on a photoperiod of 14L : 10D with food and water freely available. The birds were divided into a control group and four experimental groups of 14 or 15 birds. All the drakes in the study were Khaki Campbells except for 12 of the control birds which were Aylesburys.

Castrates: 15 birds were surgically castrated at 3 weeks of age (2.4.3).

Methimazole fed: 14 birds were fed layers starter mash containing 0.1% methimazole from hatch (2.5.1).

Castrates treated with thyroxine: 14 birds were castrated at 3 weeks of age and were given daily intramuscular injections (0.2 ml) of 80 ug thyroxine for 2 weeks before they were killed (2.5.3).

Methimazole fed and treated with testosterone: 14 birds were fed a layers mash containing 0.1% methimazole from hatch. At 8 weeks of age each bird received a subcutaneous implant of silastic tubing (2.5.2) containing testosterone. Five of the birds in this group died prior to the end of the experimental period.

At 10 weeks of age the birds were killed and 4 or 5 of the pituitary glands from each group were taken for immunohistology (2.9) and the remaining 8-10 glands were used to bioassay the TSH content of the rostral and caudal lobes of the adenohypophysis (2.8).

Body weight was not affected by castration or by castration combined with injections of thyroxine but was severely depressed in the 2 groups of drakes fed methimazole (Table 9). The adenohypophyses were heavier in both groups of castrated drakes than in the control birds but in both groups of drakes fed methimazole, the adenohypophyses were about half the weight of those from the controls (Table 9). The weight of the thyroid glands was not affected by castration but was lower in the castrates treated with thyroxine than in the controls (Table 9). The thyroid glands in both groups of drakes fed methimazole were heavier than in the controls (Table 9).

TABLE 10 - Concentrations of plasma hormones (means  $\pm$  S.E.M.) in 10-week-old drakes after experimental manipulation

Treatment	Number of birds	Thyroxine (ng/ml)	Triiodothyronine (ng/ml)	LH (ng/ml)	Androgen (ng/ml)
Controls	15	4.5 $\pm$ 0.27	2.9 $\pm$ 0.23	0.90 $\pm$ 0.10	0.5 $\pm$ 0.09
Castrates	15	5.9 $\pm$ 0.38**	3.1 $\pm$ 0.12	9.50 $\pm$ 0.85***	Not detected
Methimazole fed	14	1.3 $\pm$ 0.23	1.0 $\pm$ 0.05***	4.60 $\pm$ 0.24***	0.3 $\pm$ 0.05
Castrated, injected with thyroxine	13	19.6 $\pm$ 2.33***	2.4 $\pm$ 0.13	6.19 $\pm$ 0.58***	Not detected
Methimazole fed, implanted with testosterone	9	0.7 $\pm$ 0.11***	0.8 $\pm$ 0.04***	5.22 $\pm$ 0.28***	4.5 $\pm$ 0.76***

Statistical comparisons with the controls are \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05 (unpaired t test)

The concentration of plasma  $T_4$  was significantly higher in castrated drakes and in birds injected with thyroxine than in the controls but was depressed in both groups of drakes fed methimazole when compared to the controls (Table 10). However the concentration of  $T_3$  was lower ( $p < 0.01$ ) in drakes fed methimazole and implanted with testosterone than in drakes fed methimazole and not treated with testosterone. The concentration of plasma  $T_3$  was significantly lower ( $p < 0.01$ ) in castrated drakes injected with thyroxine than in castrates not treated with the hormone (Table 10).

The concentration of plasma LH was elevated in each of the experimental groups investigated when compared to the control group ( $p < 0.001$ ). The levels of plasma LH were higher in the castrated drakes than in the castrates treated with thyroxine (Table 10).

Testosterone was not detected in the plasma of castrated drakes. The concentration of androgen was higher in the plasma of drakes fed methimazole and implanted with testosterone than in the control birds or in methimazole fed birds not treated with the steroid (Table 10).

Immunochemical staining was observed in the cytoplasm of cells distributed throughout the rostral lobe of the adenohypophysis, and few or no stained cells were seen in the caudal lobe (Fig. 22). A flange of stained cells in the rostral lobe extended ventrocaudally beneath the caudal lobe. The distribution of cells binding anti-bTSH serum between the rostral and caudal lobes was not affected by experimental manipulation of the gonadotrophic or TSH content of the adenohypophysis (Fig. 22).

Immunochemically stained cells were more closely packed in the rostral lobe of the adenohypophysis in both groups of drakes fed methimazole than in control drakes. Cells reacting with the anti-bTSH serum were more widely dispersed in the rostral lobe of the adenohypophysis in both groups of castrated drakes than in control birds (Fig. 22).

The total amount of TSH was greater in the rostral lobe than in the caudal lobe of the adenohypophysis in all the drakes in the study, irrespective of experimental treatment. The concentration of TSH was also greater in the rostral than in the caudal lobe in the control, castrated and methimazole fed/testosterone implanted birds (Table 11).

TABLE 11 - The TSH content, as measured by bioassay, of the rostral and caudal lobes of the adeno-hypophysis in 10-week-old drakes after experimental manipulation

Treatment	Number of birds	TSH content of rostral lobe		TSH content of caudal lobe	
		per lobe	per mg net weight	per lobe	per mg wet weight
Controls	10	1.94	0.04	0.62	0.02
Castrates	9	1.54	0.03	0.89	0.02
Methimazole fed	9	2.48	0.11	1.22	0.18
Castrated, injected with thyroxine	8	1.35	0.02	1.03	0.03
Methimazole fed, implanted with testosterone	5	3.92	0.11	2.22	0.13

TSH potency, expressed as microgram equivalents of NIH-TSH-S8, was measured using the Lamberg (1953) assay

The total amount and the concentration of TSH in the adenohypophysis was greater in both groups of birds fed methimazole than in the control and castrated groups. The TSH content of the adenohypophysis in castrated drakes was similar to that in the control birds (Table 11).

The mechanisms that regulate the secretion of TSH in the duck are thus similar to those in mammals. The structure of mammalian TSH is composed of 2 subunits,  $\alpha$  and  $\beta$ ; the  $\beta$  subunit is responsible for the hormonal specificity of TSH while the  $\alpha$  subunit, which is identical with the  $\alpha$  subunit of FSH and LH, is responsible for species specificity (Cornell and Pierce, 1973; Vaitukaitis et al., 1976). Antisera raised against the  $\beta$  subunit of bovine TSH (bTSH $\beta$ ) cross react with TSH from other species (Baker et al., 1972; El Etreby et al., 1975, 1977). In the rat, the specificity of the binding of anti-bTSH $\beta$  serum to TSH cells was supported by the demonstration that immunochemical staining was dependent on the TSH content of the pituitary gland (Baker et al., 1972).

The size, staining intensity and distribution of cells binding anti-bTSH $\beta$  serum in the drake was directly related to the TSH content of the adenohypophysis. In both groups of birds fed methimazole, the cells binding anti-bTSH $\beta$  serum appeared to be larger, less intensely stained and more closely packed than in the controls which suggested that they were highly stimulated TSH cells. This view was supported by the observation that in drakes fed methimazole, the TSH content of the adenohypophysis, as measured by bioassay, was high and that as indicated by enlarged thyroid glands, TSH secretion was enhanced. The enlargement of the thyroid glands was consistent with a decrease in the negative feedback action of thyroid hormones on TSH secretion due to a lowering of the concentrations of thyroid hormones in the blood (Table 10; 3.1.1). The elevated level of plasma androgen in testosterone implanted birds confirmed the effectiveness of the implants (Table 10). The finding that immunochemically stained cells in methimazole fed/testosterone implanted birds were regressed is further evidence that the antiserum was specific for TSH producing cells.

The similarity in the appearance of cells binding anti-bTSH $\beta$  serum in the adenohypophyses of castrated and control drakes and the relatively sparse distribution of immunochemically stained cells in the castrates suggests that castration did not have an effect upon the secretory

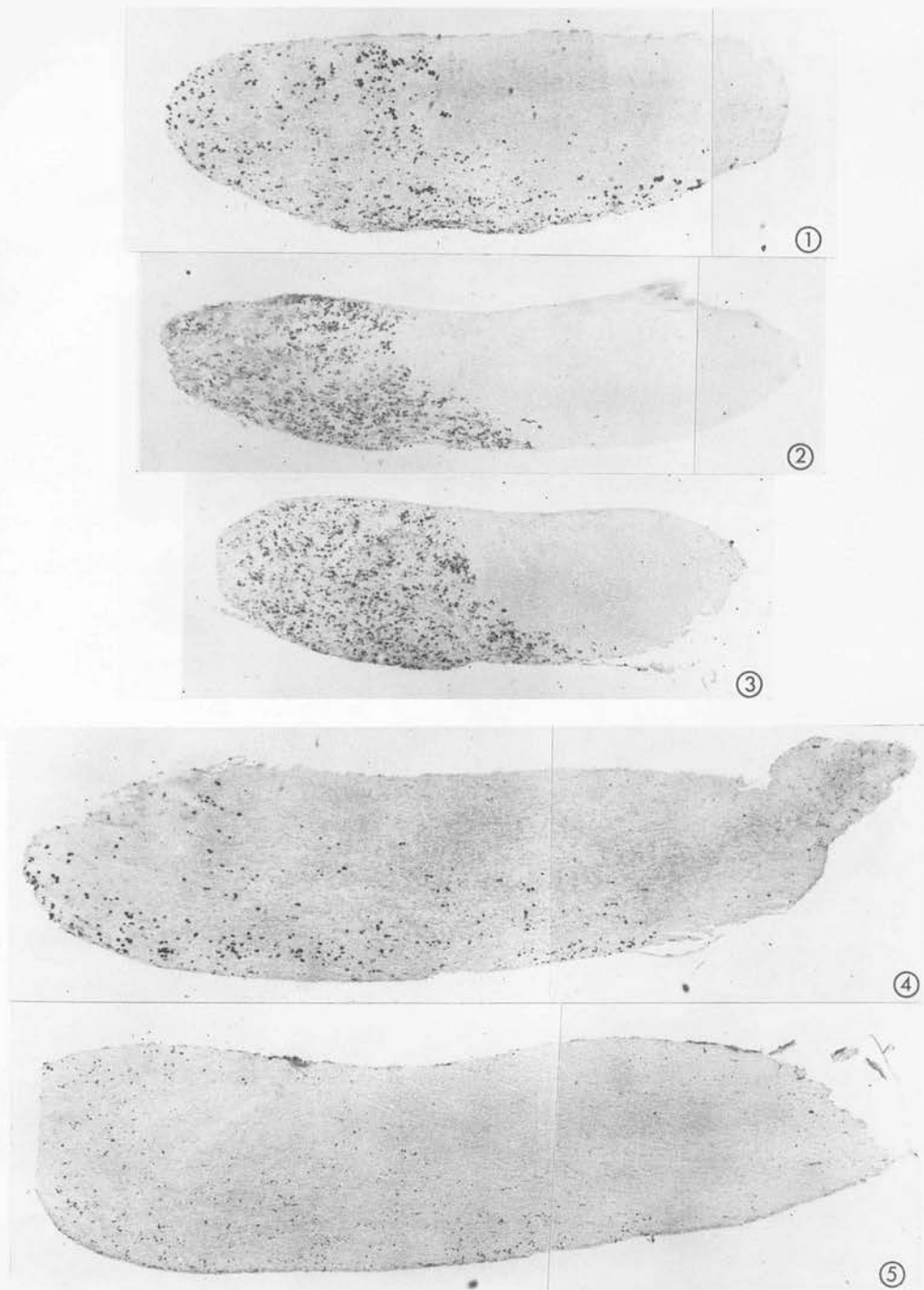


FIGURE 22 Parasagittal sections of adenohypophyses from 10-week-old Khaki Campbell drakes showing cells which bind anti-bovine TSH  $\beta$  serum: (1) section from a control drake; (2) section from a drake fed methimazole; (3) section from a drake fed methimazole and implanted with testosterone; (4) section from a castrated drake; (5) section from a castrated drake injected for 14 days with 80 ug thyroxine/day. X 29.5.

activity, or numbers, of the TSH producing cells. The relatively sparse distribution of stained cells in the castrates when compared with the controls might be due to the proliferation and enlargement of FSH/LH cells. The immunochemical evidence that castration did not increase the activity of the TSH cells was supported by the bioassay data which showed that the TSH content of the adenohypophyses in the castrates was similar to that measured in the controls.

The regressed appearance and poor staining of cells binding anti-bTSH $\beta$  serum in castrated drakes treated with thyroxine indicated that the rate of TSH production by these cells was low. In these birds, as suggested by the low weight of the thyroid glands, TSH secretion was likely depressed. This hypotrophy of the thyroid glands is consistent with an increase in the negative feedback action of thyroxine on TSH secretion due to the elevated level of this hormone in the blood (Table 10).

The occurrence of bioassayable TSH in the caudal lobe in the drake can be accounted for by the imprecise nature of the Lamberg assay and by the ventral flange of the rostral lobe which was included in the caudal portion of the adenohypophysis taken for bioassay (Fig. 22).

The localisation of TSH cells in the rostral lobe of the adenohypophysis confirms the findings of Marchand and co-workers (Marchand et al., 1972; Marchand et al., 1980). Since TSH cells have also been observed to occur in the rostral but not in the caudal lobe of the adenohypophysis in the chicken (Payne, 1944; Tai and Chadwick, 1977), the quail (Mikami et al., 1975; Wada, 1975) and Zonotrichia leucophrys gambelii (Mikami et al., 1969), the localisation of TSH cells exclusively in the rostral lobe may be a general feature of the avian adenohypophysis.

The effect of gonadal steroids and gonadectomy on the levels of plasma thyroid hormones supports the view that they are exerting an effect on the concentration of plasma thyroid hormone binding proteins (3.4.1). Thus testosterone implants would decrease the concentration of binding proteins and concomitantly decrease the levels of plasma T<sub>4</sub> and T<sub>3</sub> (Table 10) whereas gonadectomy would tend to have the reciprocal effect.

The normal levels of  $T_3$  in ducks injected with thyroxine supports the view that the metabolism of  $T_4$  is regulated. Chicks fed iodinated casein have raised plasma levels of  $T_4$  and normal levels of  $T_3$  (May et al., 1980). Thus, as has been suggested in mammals, birds may also possess a homeostatic "wisdom" whereby conversion of  $T_4$  to  $T_3$  is inhibited when catabolic processes are overactive.

### 3.5.2 Effect of thyrotrophin-releasing hormone

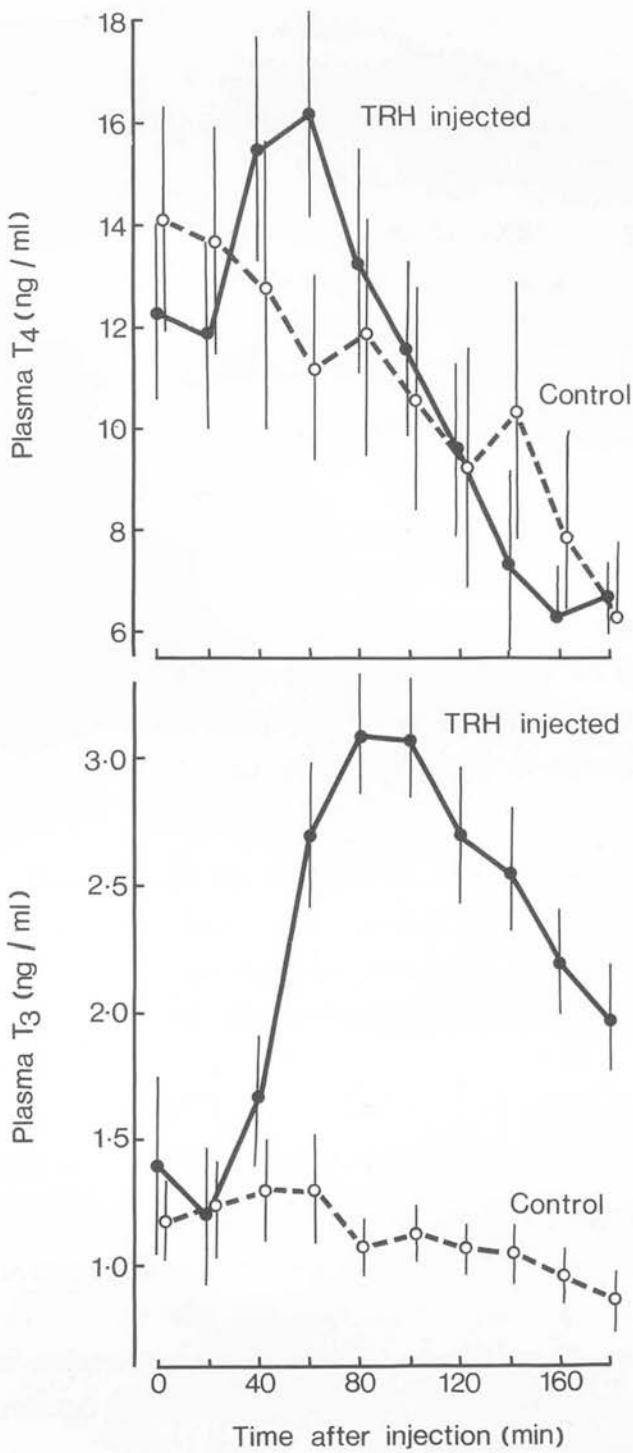
Six 13-month-old laying Brown Leghorn hens (body weight, 1.5-1.8 kg) were kept in individual cages on a lighting schedule of 14L : 10D (lights on at 05.00 and off at 19.00 hr). TRH (Sigma Chemical Co.) was dissolved in a 0.9% saline solution and injected as a dose of 100 ug in 0.5 ml s.c. in the leg near the femoral vein. Injections were given between 13.30 and 14.00 hr.

Sixty minutes after injection of 100 ug of TRH the concentration of plasma  $T_4$  had increased ( $p < 0.05$ ) from a mean preinjection level of  $12.3 \pm 1.7$  ng/ml to a peak of  $16.2 \pm 2.0$  ng/ml (Fig. 23). After the peak level had been reached the plasma  $T_4$  concentration fell progressively and was significantly ( $p < 0.001$ ) lower than the preinjection value 180 min after injection.

Eighty minutes after the injection of TRH the concentration of plasma  $T_3$  had increased ( $p < 0.001$ ) from a mean preinjection level of  $1.37 \pm 0.33$  ng/ml to a maximum of  $3.09 \pm 0.24$  ng/ml (Fig. 23).

It was calculated that the concentrations of  $T_4$  and  $T_3$  reached peak values at  $54 \pm 9$  min (range, 41-64) and  $85 \pm 10$  min (range, 70-95), respectively, after injection of TRH. The mean time difference between the calculated  $T_4$  and  $T_3$  peak was  $31 \pm 2$  min.

An increase in the concentrations of plasma thyroid hormones in response to TRH has also been shown in the lesser snow goose (Anser caerulescens caerulescens), Rouen duck (Anas platyrhynchos), Japanese quail and immature chicken (Campbell and Leatherland, 1979; Kamis and Robinson 1978; Kuhn et al., 1978). The peak concentration of plasma  $T_3$  was found to lag behind that of  $T_4$  in each of the species studied except for the Japanese quail where maximum concentrations occurred at the same time.



**FIGURE 23** Changes in the concentrations of plasma T<sub>4</sub> and T<sub>3</sub> after a s.c. injection of 100 ug of TRH. Each point represents the mean (+ SEM) for 6 hens.

It has been shown in birds that  $T_3$  is derived from a peripheral monodeiodination of  $T_4$  to  $T_3$  (Astier and Newcomer, 1978; Borges *et al.*, 1980; 3.8.2) and that  $T_3$  rather than  $T_4$  is the major biologically active thyroid hormone (3.3.3; 3.7.2; 3.8.2).

The present observations showed that, after an injection of TRH, the levels of plasma  $T_4$  rose to a peak and started to decline before plasma  $T_3$  levels had reached a maximum. This lag between the  $T_4$  and  $T_3$  peaks supports the view that the thyroid gland releases primarily  $T_4$  into the circulation and that  $T_3$  is generated by peripheral deiodination of this  $T_4$  (3.8.2).

The time taken in the hen for the plasma thyroid hormone levels to return to baseline after an injection of TRH was much less than in man (Leaton *et al.*, 1973; Uller *et al.*, 1973). This observation may be explained by the fact that the half-life of thyroid hormones in birds is less than in mammals (Davison, 1978) due to the different types, concentrations and affinities of the classes of binding proteins between the 2 species.

Thus, as has been shown in mammals, the secretion of TSH from the pituitary gland is also under the control of a releasing factor from the hypothalamus which may function to over-ride the normal feedback loop between the pituitary and the thyroid in certain physiological situations (3.3).

### 3.5.3 Summary

The concentration of pituitary TSH in ducks was affected by changes in thyroid status. The goitrogen methimazole increased whereas injections of thyroxine decreased the TSH content in the rostral lobe of the adenohypophysis as determined by bioassay and immunocytochemistry. Cells in the caudal lobe of the pituitary gland did not bind the anti-bovine TSH  $\beta$  serum. Compared with control drakes the immunochemically stained cells of birds fed methimazole were enlarged and more densely packed, while the stained cells in drakes injected with thyroxine were shrunken and less intensely stained.

The effect of gonadal steroids on the levels of plasma thyroid hormones was not mediated at the level of the pituitary gland as

evidenced by unchanged concentrations of pituitary TSH in castrated or testosterone implanted birds.

Injection of the hypothalamic peptide thyrotrophin-releasing hormone into laying hens was shown to increase the levels of both plasma  $T_3$  and  $T_4$ . The concentration of plasma  $T_4$  peaked before that of  $T_3$  and so suggests that the secretion of  $T_4$  is directly under hypothalamic-pituitary control whereas plasma  $T_3$  is generated peripherally from  $T_4$  in the peripheral tissues.

### 3.6 Breeding cycles

An increasing number of studies support the view that there are changes in the pattern of thyroid hormone metabolism during the reproductive cycle in vertebrates (Walker et al., 1980; Nishikawa et al., 1981). These changes appear to reflect the metabolic state of the animal and ultimately are directed to making  $T_4$  an efficient source of the calorigenically active  $T_3$ . The availability of hormone supply is essential for normal development as well as acute responses to metabolic need. In birds the requirement for thyroid hormone is cyclical and dependent on a variety of factors (3.2, 3.3, 3.4). Thus it was of interest to examine several of the well characterised breeding cycles and determine the relationship of thyroid hormone secretion to these events.

Studies in birds have investigated the pathways of  $T_4$  metabolism during the ontogeny of the chick embryo (Thommes and Hylka, 1977a, Borges et al., 1980). In the immature chick embryo the formation of  $T_3$  is conserved by metabolism of  $T_4$  to  $rT_3$  as well as rapid clearance of any  $T_3$  generated. A shift in this pathway occurs at the time of pipping and is associated with sparing  $T_4$  and preserving the  $T_3$  that is formed. During the first weeks of life when oxygen consumption, rate of growth and food intake is greatest the levels of plasma  $T_3$  remain elevated (Bobek et al., 1977; 3.7.1) while the levels of  $rT_3$  are undetectable. An increase in the levels of plasma  $rT_3$  occurs as the birds age (Premachandra et al., 1977). Thus the generation of  $rT_3$  and the associated reciprocal decline in plasma  $T_3$  was suggested to be an effect associated with aging. Alternatively a study in man has concluded that the increase in  $rT_3$  and decline in  $T_3$  with age is a result of disease and not of age

per se (Olsen et al., 1978).

In the first study changes in the concentration of plasma thyroid hormones were more closely investigated in growing cockerels throughout a breeding cycle from the time of hatch until they were sexually mature. Measurements of plasma testosterone were obtained because of its pronounced effects on the concentrations of plasma thyroid hormone binding proteins in birds and mammals (3.4).

The normal ovarian cycle of female chickens maintained under 14 h of light is to lay one egg every 24-30 h for several consecutive days. Normally in hens that lay eggs in a regular clutch ovulation occurs about 30 to 45 min after oviposition (Kadona and Besch, 1980). In birds body temperature displays a circadian rhythm which is related to both oviposition and ovulation (Winget et al., 1965; Kandono and Besch, 1980). At the time of oviposition there is either a peak or a marked increase in body temperature while a smaller increase in body temperature has been recorded with ovulation (Winget et al., 1965). Previous studies have suggested that the temperature rise at oviposition is due to increased musculature activity at the time of oviposition (Sykes, 1953; Winget et al., 1965) or to the presence of a ruptured follicle after ovulation (Bohr and Sheldon, 1977).

The involvement of thyroid hormone in energy metabolism has been established (3.8) and so it was decided to investigate the role of  $T_3$  and  $T_4$  in the metabolic changes associated with the ovulatory cycle of the hen. The birds were maintained on an ahemeral lighting cycle in order to lengthen the time between ovulation and oviposition and thus to determine any contribution of  $T_4$  and  $T_3$  to these events.

A cycle of broody behaviour in the bantam hen consists of incubation and care of the young, and is both initiated and maintained by an increase in the levels of plasma prolactin (Riddle et al., 1935). At the onset of incubation the ovary, oviduct and comb regress (Collins, 1950) and food intake is markedly reduced until after the eggs have hatched when it exceeds the levels found in laying birds (Savory, 1979; Sherry et al., 1980). Similarly the concentration of LH and progesterone decrease at the onset of incubation and remain depressed until the hen stops brooding her chicks (Sharp et al., 1979; Burke and Papkoff, 1980). Thus any effects of gonadal steroids on the levels of thyroid hormone binding

proteins would be expressed at this time for studies in the quail have shown the concentration of plasma  $T_3$  and  $T_4$  to fall in intact but not in ovariectomized birds after photostimulation (3.4.1).

Injections of prolactin induce hypertrophy of the thyroid glands in the Japanese quail (Wada et al., 1975) and immature cockerels (Maiti and Chakraborty, 1980) and in the quail depress the levels of plasma  $T_4$  (Wada et al., 1975). An effect of prolactin on thyroid function has also been observed in other non-mammalian vertebrates. Thus, injections of prolactin cause thyroid gland hypertrophy in the lizard (Anolis carolinensis) (Licht and Jones, 1967), the eel (Olivereau, 1966) and the teleost, Pterophyllum scalare (Osewald and Fiedler, 1968). Further Singh and Singh (1976) found, in the teleost Iteteropneustes fossilis, that prolactin reduced both pituitary TSH levels and the uptake of  $I^{131}$  into the thyroid gland. In another teleost, the rainbow trout (Salmo gairdneri), Milne and Leatherland (1978) found that injections of prolactin increased the concentrations of plasma  $T_3$  but did not affect levels of plasma  $T_4$ .

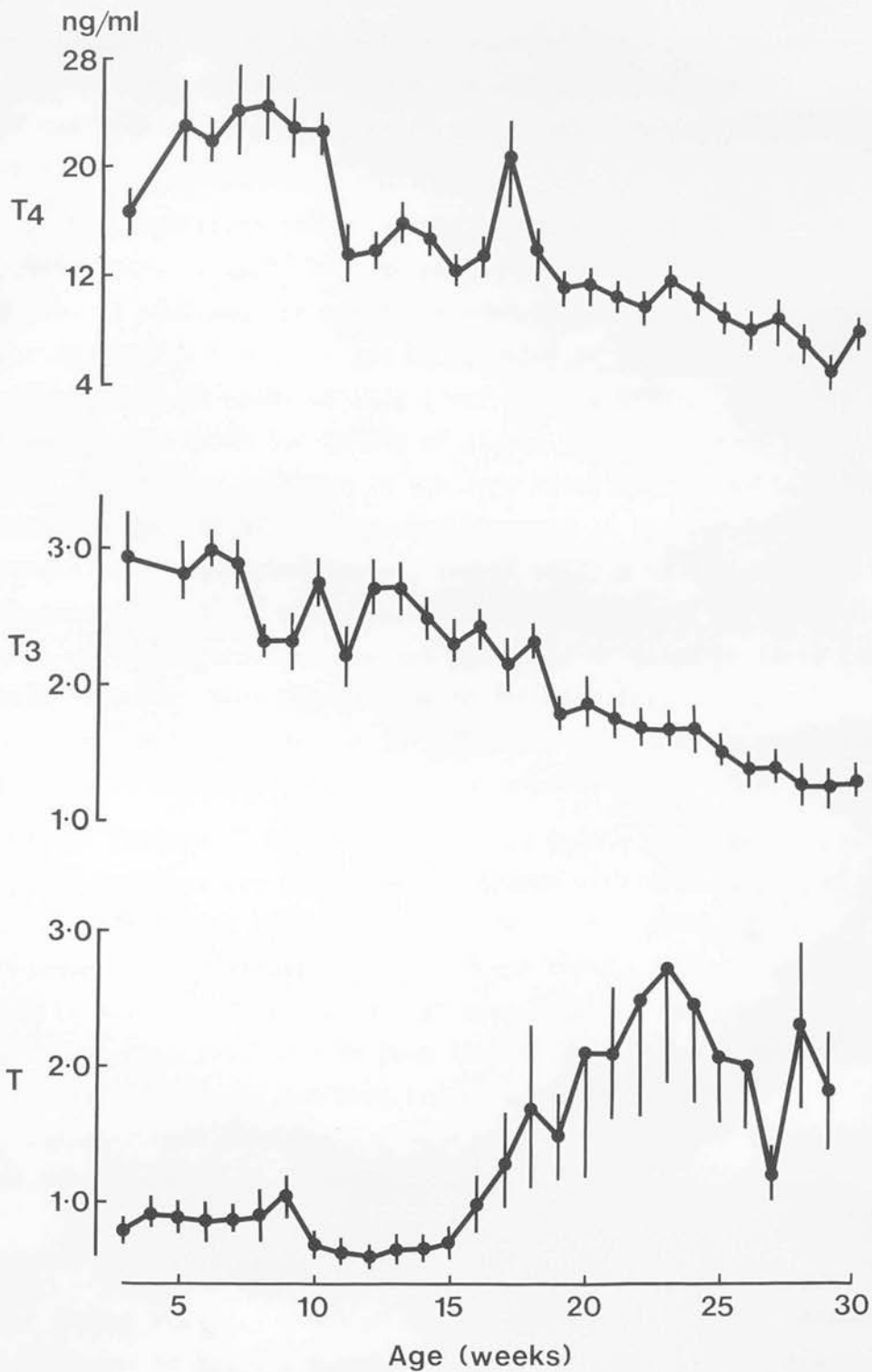
Thus the changes in the levels of plasma prolactin in bantams and turkeys during a cycle of brooding might be expected to affect thyroid function (Sharp et al., 1979; Etches et al., 1979; Burke and Dennison, 1980; El Halawani et al., 1980). In incubating hens prolactin levels increase whereas after the chicks hatch prolactin levels tend to fall.

The present study was designed to examine the changes in thyroid function in laying, incubating and brooding bantam and turkey hens and to investigate the possible regulatory role of plasma prolactin.

### 3.6.1 Sexual development in cockerels

Broiler cockerels from a commercial strain (2.1.1) were sampled at weekly intervals from 3 to 30 weeks of age.

In birds sampled throughout the period of sexual development the levels of plasma  $T_4$  remained elevated until they were 10 weeks of age and thereafter gradually declined for the duration of the study (Fig. 24). The levels of plasma  $T_3$  tended to remain elevated during the time of maximum body weight gain and energy metabolism (Bobek et al., 1977) and similarly declined as the birds aged (Fig. 24). Associated with the



**FIGURE 24** Changes in the concentrations of plasma T<sub>4</sub>, T<sub>3</sub> and testosterone in broiler cockerels from 3 to 30 weeks of age. Each point represents the mean ( $\pm$  SEM) of 8 birds.

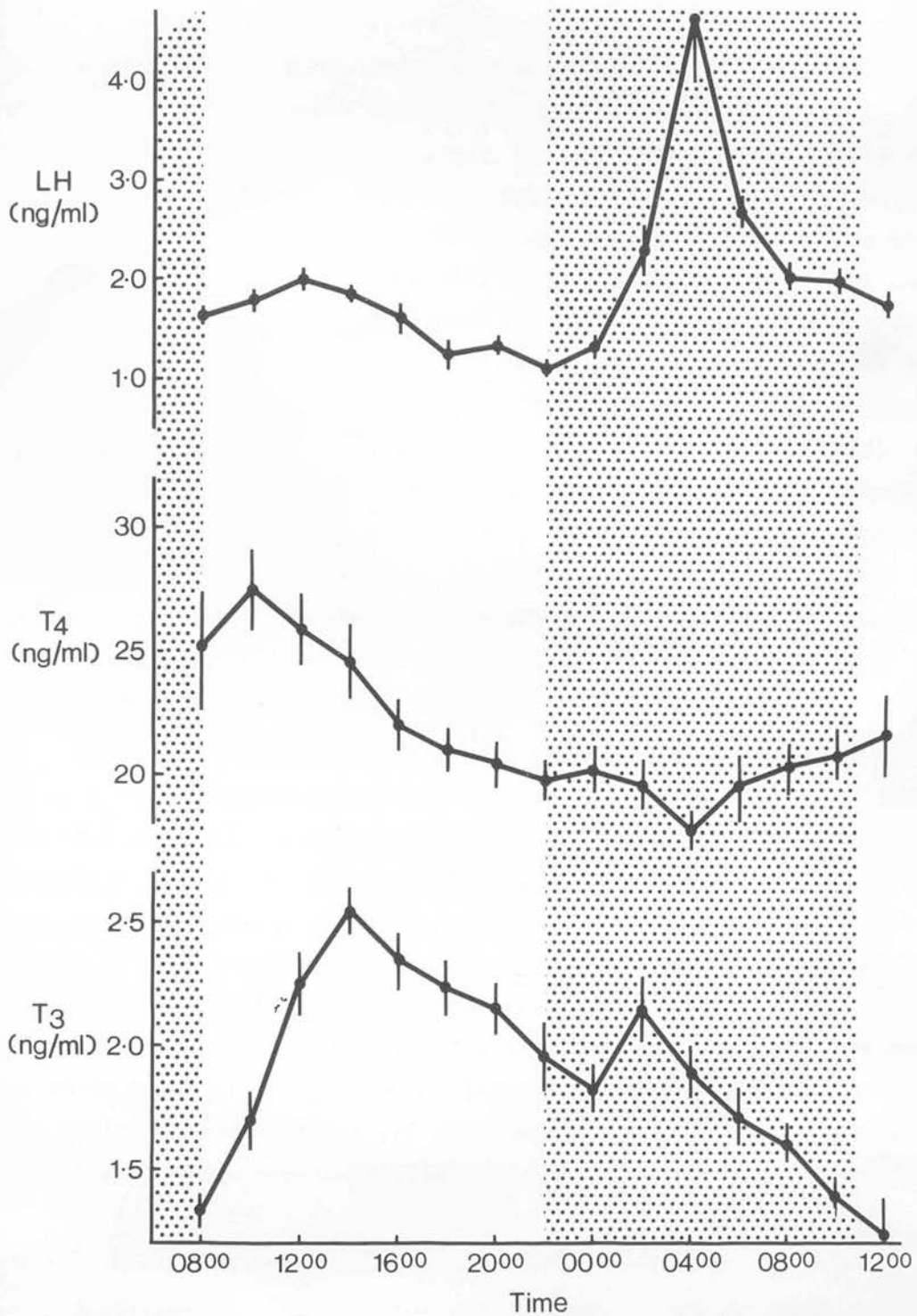
decrease in the levels of plasma thyroid hormones was an increase in the concentration of plasma testosterone (Fig. 24). The concentration of plasma testosterone remained low until the birds were 15 weeks of age and subsequently increase to adult levels during the following weeks.

The possibility that the concentration of thyroid hormone binding proteins may, in part, be responsible for these fluctuations is supported by several studies. In quail the concentration of thyroid hormone binding protein (TBPA) is low at the time of hatch and rapidly increases during the first weeks of life (Heaf et al., 1980). Similarly in the newly hatched chick the levels of plasma thyroid hormone are low (King et al., 1977) and increase in the days after hatch (Davison, 1976a). In quail, the levels of TBPA remains elevated if the gonads do not develop whereas photostimulated gonadal growth results in a prompt decline in the concentration of this carrier protein (El-Sayed et al., 1980) due to an effect of androgens on the synthesis of these proteins from the liver (3.4.1). Thus the decline in both the levels of plasma  $T_4$  and  $T_3$  in cockerels as they mature is suggested to be due, in part, to the increase in androgen synthesis associated with testicular development.

The pattern of age related changes in the concentration of plasma thyroid hormones are in general agreement with previously published studies (Newcomer, 1976, 1978; Davison, 1976b, Bobek et al., 1977). The presence of high levels of plasma  $T_3$  in chicks during the first weeks of life has been directly related to a high rate of oxygen consumption (Bobek et al., 1977) and to food intake (King et al., 1977). Alternatively, studies in man have found an age related decline in  $T_3$  and not  $T_4$  and attribute this finding to a decrease in the turnover of  $T_4$  with age (Nishikawa et al., 1981).

### 3.6.2 Ovulatory cycle in hens

Laying hens (27 weeks of age) were maintained on an ahemeral photoperiod of 14L : 13D and bled ( $n = 24$ ) every 2 h for a complete cycle. The time of lay for each bird was recorded. The concentration of plasma LH was measured in each sample in order to determine the time of the ovulatory surge.



**FIGURE 25**

Changes in the concentration of plasma LH, T<sub>4</sub> and T<sub>3</sub> during the ovulatory cycle of the hen. The birds were exposed on a hemeral lighting cycle of 14L : 13D. Each point represents the mean ( $\pm$  SEM) of the same 11 birds. The stippled area represents the period of darkness.

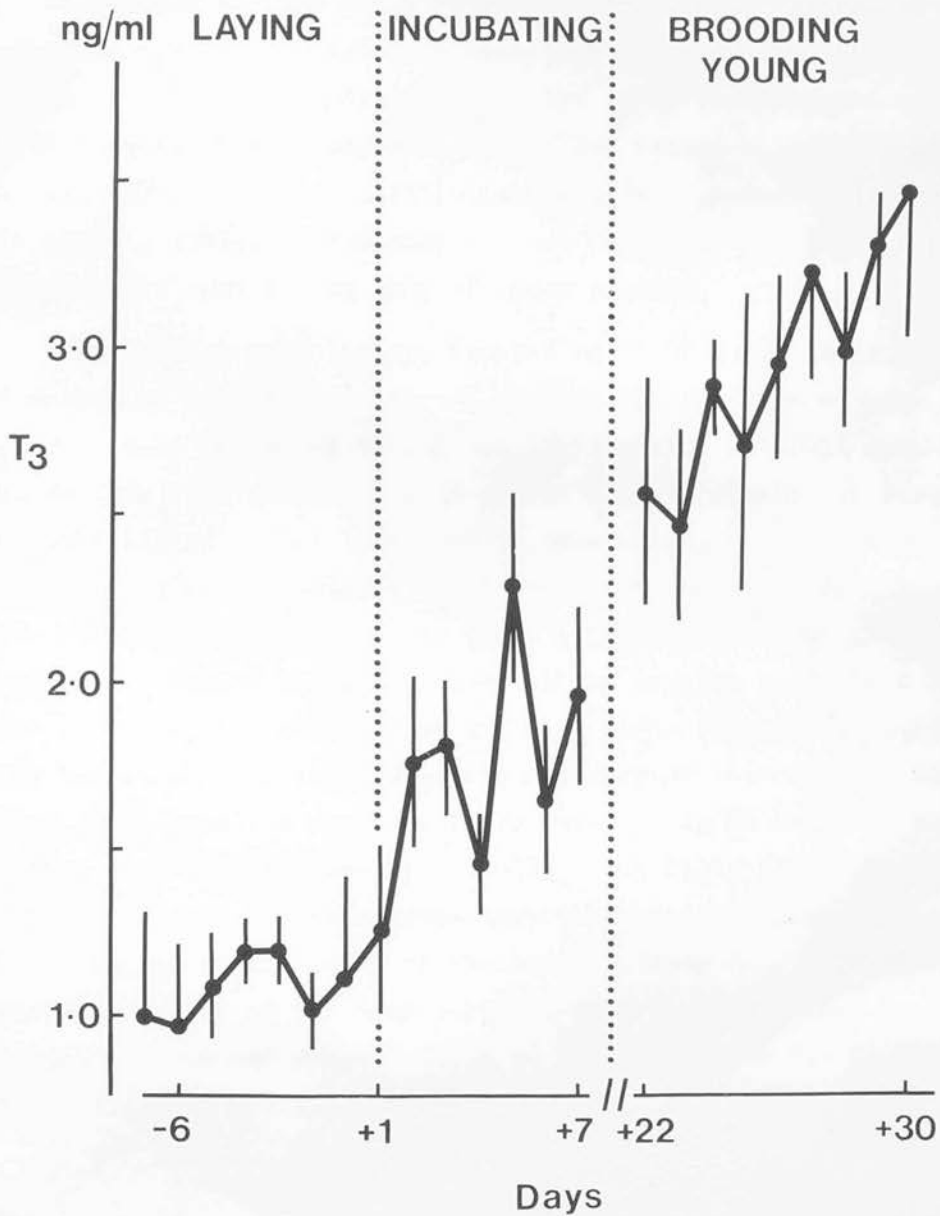
The concentration of plasma thyroid hormones for 11 birds which showed an ovulatory surge 6 h into the dark period are illustrated in Fig. 25. No relationship between the concentration of plasma  $T_4$  and the ovulatory surge of LH was found. The finding that the levels of plasma  $T_4$  were lowest at this time supports the view that  $T_4$  is not physiologically important but rather serves as a prohormone for the metabolically active  $T_3$ . This view is supported by the finding that the concentration of plasma  $T_3$  rose ( $p < 0.01$ ) in each bird 2 h before the ovulatory surge of LH (Fig. 25). The increase in the levels of plasma  $T_3$  prior to the rise in body temperature at the time of ovulation suggests the possibility of a relationship between these events. Plasma  $T_3$  plays a role in heat production in birds (3.7.2) and the pre-ovulatory rise may be physiologically important. Alternatively the timing of the increase in  $T_3$  may only be coincidental and due to intake of food which may be associated with the onset of pre-laying behaviour (Bobr and Sheldon, 1977). This view is supported by the finding that birds will eat during the dark period (3.3.2).

The results of this study do not support a role for either plasma  $T_3$  or  $T_4$  in the increase in body temperature at the time of oviposition. For each of the birds oviposition times occurred up to 6 h after ovulation with most occurring within 4 h. There were no significant changes in the levels of plasma thyroid hormones associated immediately before or at the time of egg laying (Fig. 25). The levels of  $T_4$  were seen to be increasing while the converse changes occurred in the levels of plasma  $T_3$  in accordance with the normal daily pattern in the secretion of these hormones (3.2). Thus a direct role of thyroid hormone during the ovulatory cycle of the hen remains speculative although a permissive function in these events is likely (1.12).

### 3.6.3 Bantam hens: incubation and brooding chicks

Experiment 1: Bantam hens aged between 18 and 24 months were kept in floor pens with free access to food and water and exposed to 14 hrs light/day. Blood samples were taken at the same time of day for one week before and one week after the onset of incubation and again for 9 days after the chicks hatched.

The concentration of plasma  $T_3$  did not vary significantly prior to



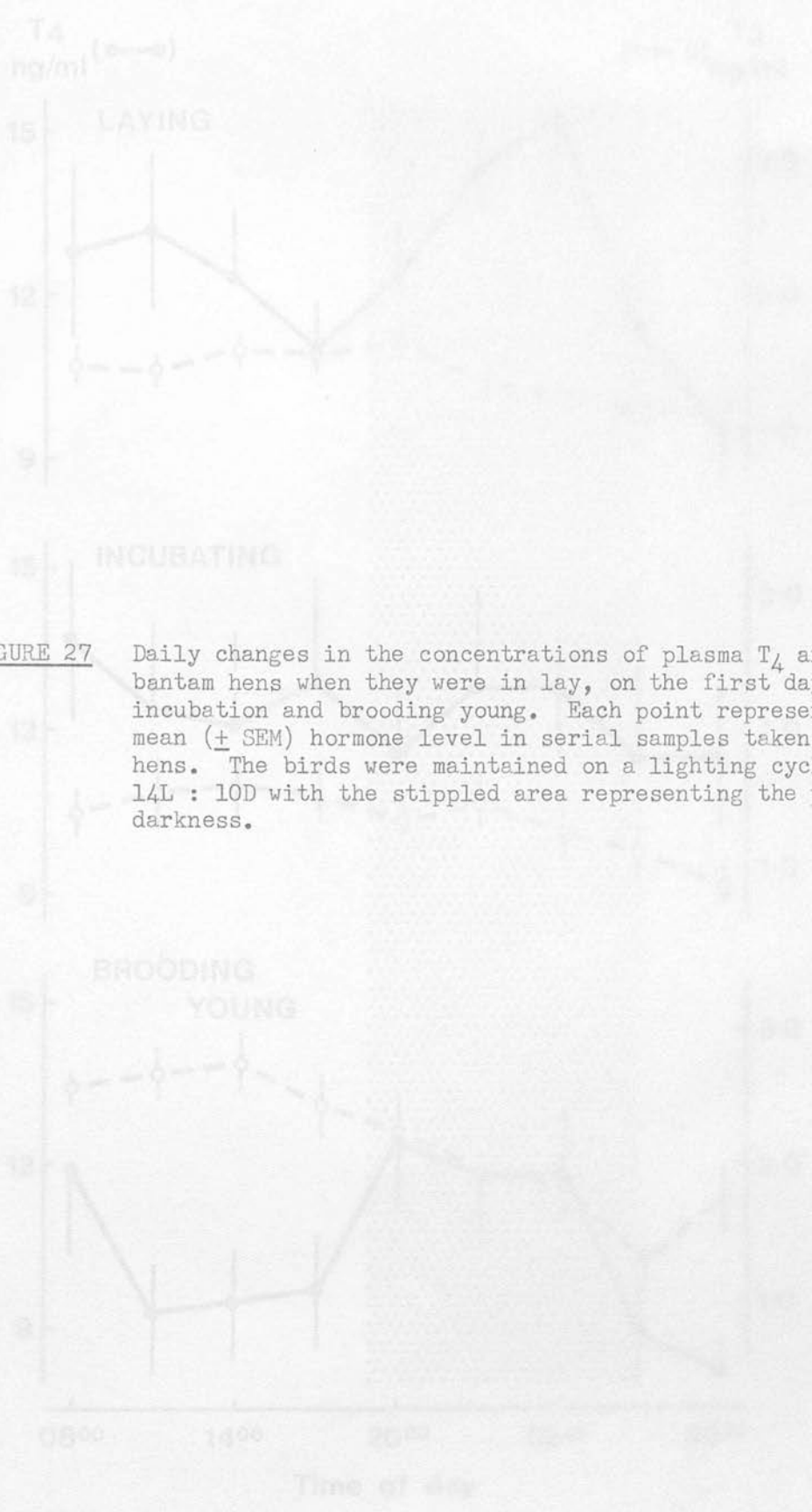
**FIGURE 26**

Changes in the concentrations of plasma  $T_3$  in bantam hens when they were in lay, during the first week of incubation and during the first 9 days the hens were brooding their young. Each point represents the mean value ( $\pm$  SEM) for 6 hens.

the onset of incubation but increased on the first or second day of incubation and continued to increase during the following week (Fig. 26). When the hens began brooding their young, the concentration of plasma  $T_3$  continued to increase.

Experiment 2: The daily rhythms in the concentrations of plasma thyroid hormones in bantam hens were compared when they were laying, incubating eggs and brooding young. The birds were sampled on the first day of incubation when the mean daily food intake significantly drops (Savory, 1979) and the concentration of plasma prolactin is elevated (Lea *et al.*, 1981). Blood samples were taken every 3 h for a period of 24 h from the hens during each of these periods.

When bantams were laying, incubating or brooding their young the concentration of plasma  $T_3$  showed significant daily variations (Table 12; Fig. 27); they increased during the light period and fell during the dark period. The mean levels of plasma  $T_3$  were similar in birds while they were laying and at the onset of incubation, ( $1.42 \pm 0.1$  and  $1.37 \pm 0.2$  ng/ml respectively) but increased almost 2-fold while the birds were brooding their young ( $2.18 \pm 0.1$  ng/ml). The concentration of plasma  $T_3$  tended to be depressed in the samples taken 24 h after the first in laying, incubating and brooding birds (Fig. 27). Significant daily variations in plasma  $T_4$ , with an increase occurring in the dark period, were observed when the birds were laying or brooding young but not when they were incubating eggs (Fig. 27; Table 12). The mean level of  $T_4$  over 24 hrs was lower when they were brooding young than when they were laying or at the onset of incubation (Table 12). The effect of repeated sampling of the same bird appeared to result in a depression in the amplitude and concentration of plasma  $T_4$  over the 24 hr sampling period (Fig. 27; 3.7.1).



**FIGURE 27** Daily changes in the concentrations of plasma  $T_4$  and  $T_3$  in bantam hens when they were in lay, on the first day of incubation and brooding young. Each point represents the mean ( $\pm$  SEM) hormone level in serial samples taken from 7 hens. The birds were maintained on a lighting cycle of 14L : 10D with the stippled area representing the period of darkness.

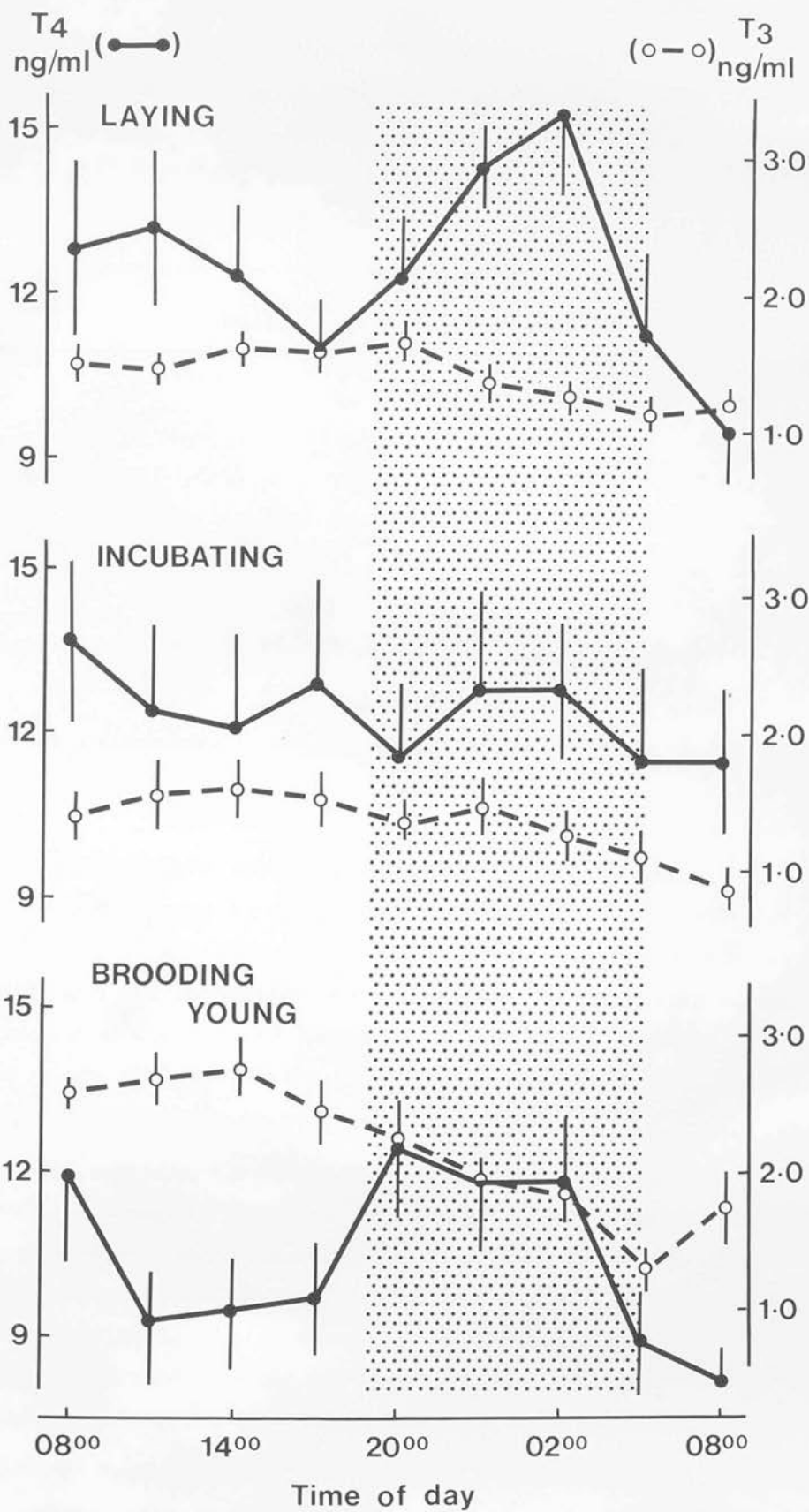


TABLE 12 - Characteristics of sinusoidal curves fitted by the least squares method to describe the daily variations of thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) in laying, incubating and brooding bantam hens (n = 7).

	<u>LAYING</u>		<u>INCUBATING</u>		<u>BROODING YOUNG</u>	
	$T_4$	$T_3$	$T_4$	$T_3$	$T_4$	$T_3$
Level (ng/ml)	12.5	1.42	12.4	1.37	10.5	2.18
Amplitude	1.21	0.21	0.02	0.21	1.37	0.56
Acrophase (hr) from onset of light	23.93	16.56	20.51	16.29	22.61	14.52
P	0.16	$4.13 \times 10^{-5}$	0.999	0.057	0.049	$2.93 \times 10^{-6}$

**Note:**

Bantams were serially sampled every 3 h during a 14L : 10D lighting cycle. The level is the intercept of the theoretical curve; the amplitude is the maximal deviation of the theoretical curve from the level and the acrophase (hours expressed as a decimal fraction) is the time which the maximal deviation of the curve from the level occurs. The value of P (set at the 0.05 level) is used to test the null hypothesis that the amplitude is equal to zero.

An increase in the concentration of plasma  $T_3$  was first noted after the hens began to incubate their eggs. The depressive effect of reduced food consumption on plasma  $T_3$  levels could have been countered either by a stimulating effect of increased plasma prolactin levels (see Introduction to this section) or by an increase in the concentration of thyroid hormone binding proteins caused by the fall in the levels of plasma ovarian steroids (3.4.3).

A decline in the concentration of plasma  $T_3$  has been shown to be correlated with a reduction in the rate of heat production (3.7.2). Thus the decline in food intake during incubation in bantams would also be

expected to lead to a decrease in plasma  $T_3$  and heat production. Such a decrease would be inappropriate in incubating birds since body heat is required to maintain the temperature of the eggs. The elevated levels of  $T_3$  may thus be important in preventing heat production dropping below that required for optimal incubation conditions for it has been shown that incubation temperature critically determines the duration of the incubation period (Romanoff et al., 1938; Decuyper et al., 1979).

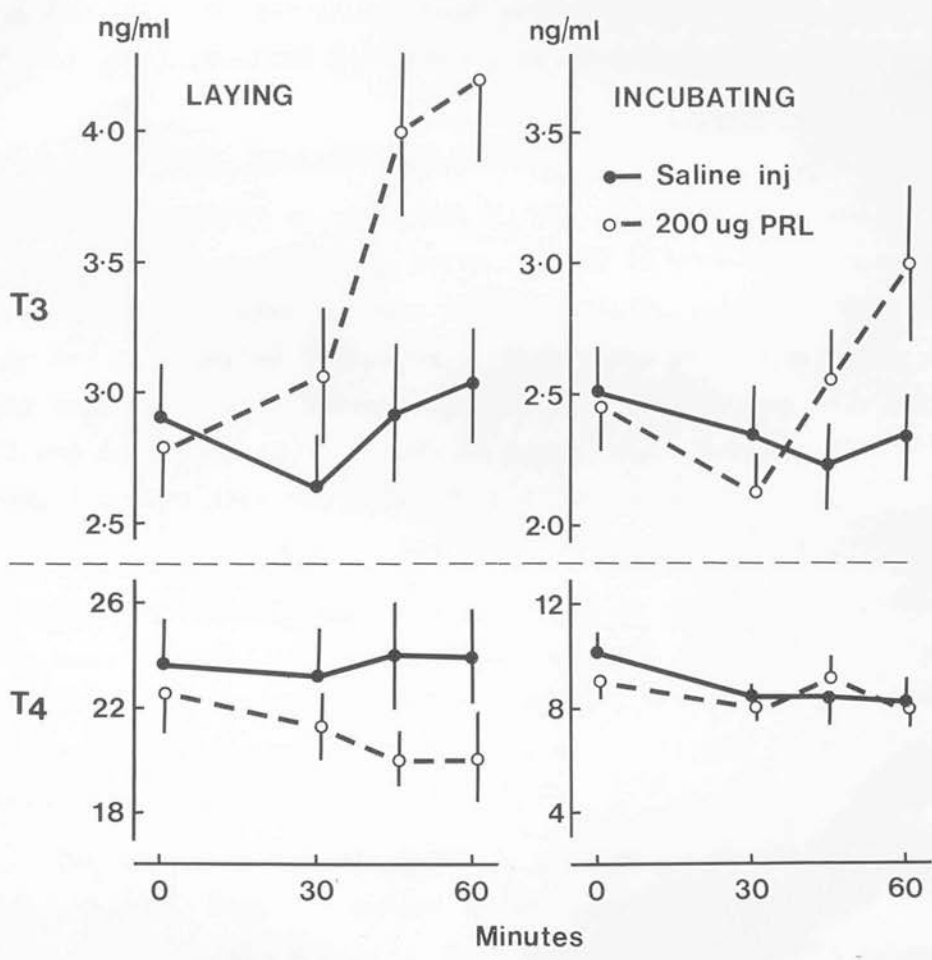
The increase in plasma  $T_3$  levels in bantam hens observed after their chicks had hatched was similar to that observed in hens given food after a prolonged fast (Brake et al., 1979). Savory (1979) has shown that in the bantam, the increase in food intake at the onset of brooding young is associated with the recovery of body weight lost during incubation. At this time food consumption is greater than when the hens were laying. Although the levels of plasma prolactin would have been decreasing at this time, they may be involved in the generation of  $T_3$  in brooding bantams. This possibility has been further investigated in the following study.

### 3.6.3a Effect of prolactin

Bovine prolactin (NIHPB9) (200 ug in 0.5 ml of 0.9% saline solution; TSH content < 0.1 ug %) was injected into 6 laying and 6 incubating bantam hens (day 20, 21 of incubation). Blood samples were taken at 0, 30, 45 and 60 minutes.

Concentrations of plasma  $T_4$  did not differ significantly in either laying or incubating birds after an injection of saline solution or prolactin (Fig. 28). In laying birds circulating  $T_3$  levels were significantly increased ( $p < 0.001$ ) between 30 and 60 minutes after prolactin administration. The concentration of plasma  $T_3$  was not significantly increased in incubating birds after an injection of prolactin ( $p < 0.1$ ). No changes in  $T_3$  levels were observed after an injection of saline solution.

The possibility that prolactin plays a role in maintaining the elevated levels of plasma  $T_3$  in broody bantams is supported by the finding that an injection of prolactin stimulated  $T_3$  production. Since the levels of  $T_4$  did not change after the administration of prolactin the present observations suggest that prolactin might be exerting a



**FIGURE 28** Effect of an I.V. injection of 200 ug bovine prolactin on plasma T<sub>4</sub> and T<sub>3</sub> levels in laying and incubating hens (Day 20, 21 of incubation). Each point represents a mean value ( $\pm$  SEM) for 5 or 6 hens.

peripheral effect on the conversion of  $T_4$  to  $T_3$ . This view is supported by the studies of Milne and Leatherland (1978) who found that in the rainbow trout the concentration of  $T_3$  but not of  $T_4$  increased after an injection of prolactin.

The elevated levels of prolactin in incubating bantams may thus be important in preventing plasma  $T_3$  levels dropping as a result of reduced food intake. In this manner heat production could be maintained at an optimum level required for incubation (Decuyper *et al.*, 1979).

#### 3.6.4 Turkeys: reproductive cycle

The concentration of plasma  $T_3$  was measured in turkey hens during a photoinduced reproductive cycle. Eight 30 week-old turkey hens (2.1.5) were transferred from a short day (6L : 18D) photoperiod to a long day photoperiod (18L : 6D). Four weeks after the onset of lay the hens were artificially inseminated (Dr. P. E. Lake) and each hen was allowed to accumulate a clutch of eggs. Blood samples were obtained every 3-4 days when the birds were 27 weeks of age until they had completed an incubation cycle.

Egg production began between 17 and 26 days after photostimulation. Four hens successfully collected a clutch of eggs (5-21) and began to incubate. The remaining 4 birds came into lay at the same time but did not incubate their eggs. These hens also went out of lay but did not become broody.

The concentration of plasma  $T_3$  in hens on short days ( $1.11 \pm 0.15$  ng/ml,  $n = 34$ ; Fig. 29) tended to decline with transfer to long days ( $0.75 \pm 0.07$  ng/ml,  $n = 18$ ). In a previous study Scanes *et al.* (1979) did not observe a decline in the levels of plasma  $T_3$  after turkeys were transferred from a short day to a long day photoperiod or during the first week of lay. When the birds came into lay the levels of hormone had returned to non photostimulated levels ( $1.10 \pm 0.11$  ng/ml,  $n = 75$ ). The concentration of  $T_3$  in birds that became broody and incubated a clutch of eggs ( $0.75 \pm 0.15$  ng/ml,  $n = 30$ ) tended to be lower than the levels found in birds that did not become broody ( $1.12 \pm 0.13$  ng/ml,  $n = 12$ ). Although there was considerable day-to-day variation in the levels of plasma  $T_3$  for each bird, once the eggs had hatched or were taken away the concentration of plasma  $T_3$  significantly increased

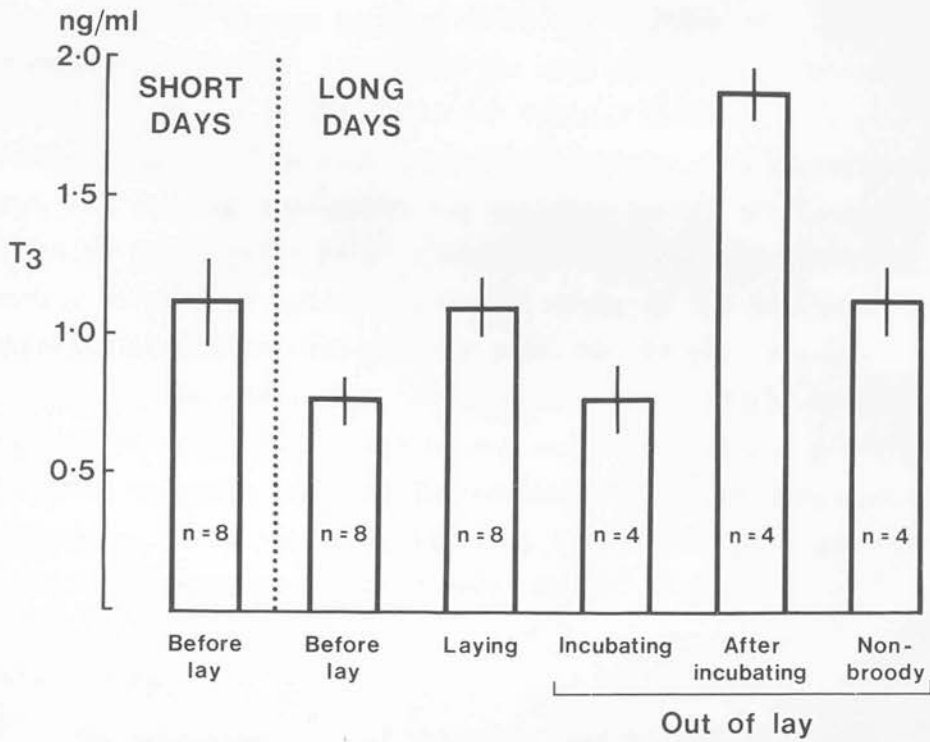


FIGURE 29

Changes in the concentrations of plasma  $T_3$  in turkey hens during a photoinduced reproductive cycle. Serial blood samples ( $n = 34$ ) were taken from 8 birds before transfer from short days (6L : 18D) to long days (18L : 6D). The hens were further sampled before they came into lay ( $n = 18$ ) and while they were in lay ( $n = 75$ ). Four of the birds became broody and were sampled during incubation ( $n = 30$ ) and after the eggs had hatched or were taken away ( $n = 11$ ). The remaining 4 birds also went out of lay but did not become broody ( $n = 12$ ).

( $1.88 \pm 0.07$  ng/ml;  $n = 11$ ;  $p < 0.001$ ). Furthermore this increase was not seen in the 4 birds who were also out of lay but did not incubate eggs ( $1.12 \pm 0.13$  ng/ml,  $n = 12$ ).

The results of this study do not support a significant role for plasma  $T_3$  during a photoinduced reproductive cycle in turkey hens. In addition, unlike the observations made on bantam hens, the levels of plasma  $T_3$  were depressed during the time of incubation and increased only once the eggs had hatched (3.6.3). The decline in the levels of  $T_3$  suggest that the decline in food intake associated with incubation (Savory, 1979; Sherry *et al.*, 1980) was responsible. Furthermore the results suggest that the endocrine response to a cycle of incubation and brooding may be different for various species of birds. A definitive conclusion cannot be made because the bantam hens in the previous study were not sampled throughout the complete period of incubation. In addition the intense genetic selection programmes applied to the domestic turkey versus the relatively "wild" state of the bantam as well as the significant differences in body size, may in part explain the different pattern of response. For these reasons an effect of prolactin on thyroid activity in the turkey may not be as physiologically important. The data suggests that the factor responsible for the increase in the concentration of plasma  $T_3$  in birds which incubated eggs was an increase in food intake once the eggs were removed (3.3.3).

### 3.6.5 Summary

The concentrations of plasma  $T_3$  and  $T_4$  were measured in broiler cockerels from 3 to 30 weeks of age. The levels of thyroid hormones remained elevated for the first 10 weeks of life and then gradually declined as the concentration of plasma testosterone increased. The period of increased levels of thyroid hormone was associated with maximum body weight gain and energy metabolism. The increase in the levels of gonadal steroid as the birds become sexually mature is suggested to result in a decrease in the levels of plasma thyroid hormone binding proteins.

The concentration of plasma  $T_3$  rose before the pre-ovulatory increase in plasma LH and so suggests a possible role for  $T_3$  in the

ovulatory cycle of the hen. No relationship was found between the levels of plasma  $T_3$  and  $T_4$  and the time of lay.

The concentration of plasma  $T_3$  increased in broody bantam hens within one or 2 days after the onset of incubation and rose further once the chicks had hatched. A daily rhythm in the levels of plasma  $T_4$  was not observed in incubating bantams. The possibility that prolactin is involved in the regulation of thyroid function during broodiness was investigated by measuring the concentrations of plasma thyroid hormones after an injection of bovine prolactin. The concentration of plasma  $T_4$  was not affected by the injection of prolactin whereas the concentration of plasma  $T_3$  in laying and incubating bantam hens was significantly increased after 60 minutes. Thus one of the factors which influences thyroid function in broody bantams may be the increase in prolactin secretion, although changes in the pattern of food intake are suggested to be the principal factor regulating the levels of plasma  $T_3$  and  $T_4$ .

### 3.7 Effect of stress

There is considerable interest in animal welfare and the degree of stress a bird must endure when exposed to conventional intensive animal husbandry systems. Methods for estimating these stresses may be inferred by the occurrence of behavioural and physiological changes (Duncan, 1981). These results are thus critical for determination of present and future legislation governing the management of domestic animals.

Measurement of the physiological rather than the behavioural changes to a stress are preferred, for stress responses which have a functional basis in the natural habitat may be distorted in an artificial situation (Murphy, 1978). The measurement of the physiological response to stress is dependent on the duration and type of stress imposed on the animal. For example a peak level of heart rate in birds which are caught and handled is maintained for only a short time even though the particular stress remains constant (Jones *et al.*, 1981). Thus the technique is useful only for the measurement of acute stress in poultry. Furthermore before stress can be measured it is necessary to induce it. Experimentally stressing birds suggests that certain stimuli or manipulations will elicit more or less of a response than others and will in turn be reflected in the secretion and generation of circulating hormones.

In mammals there are many contradictory studies on the effect of stress on thyroid function (review Reichlin, 1966; Dohler et al., 1977; McKenzie, 1979). These are due, in part, to the many diverse influences upon the thyroid axis during and after exposure to a stressful situation. In rats an increase in the concentration of serum TSH after transport stress has been observed in studies of Dohler et al. (1977) although Ducommun et al. (1966) measured a decrease in TSH levels after transport stress. Wong et al. (1977) has shown that the removal of rats from their cages results in an immediate increase in serum TSH which was followed by a decline even though the animals were being moved at the time. Various acute stresses (either, noise, restraint, bleeding) also lead to a marked fall in plasma TSH in rats (Ducommun et al., 1966; Mueller et al., 1976ab; Simpkins, 1978).

The concentration of plasma  $T_3$  has been shown to increase after transport stress while the levels of plasma  $T_4$  remain constant (Dohler et al., 1977). The turnover of plasma  $T_3$  and  $T_4$  are increased during fever and infection (Gregerman and Solomon, 1967) and exercise increases the degradation of  $T_4$  in mammals and birds (Irvine, 1968; Harvey et al., 1982).

In view of the interest in the measurement of stress in poultry the response of thyroid hormone to the stress of repeated handling has been investigated in chickens and turkeys bled over various periods of time. The effect of handling stress on egg production in turkey was also measured because other stresses (e.g. temperature) are known to affect the subsequent rate of lay (Nordstrom, 1973).

Fasting and temperature stress depress heat production (Huston et al., 1962; Shannon and Brown, 1969ab; O'Neil et al., 1971; Richards, 1973; Farrel and Swain, 1977; Lundy et al., 1978) and so might be expected to depress the production of thyroid hormones. Early studies have shown that in the chicken thyroid secretion rate is depressed when the ambient temperature increases (Reineke and Turner, 1975; Hahn et al., 1966) and is enhanced by chronic exposure to cold (Assenmacher, 1973; Arieli and Berman, 1979). In fasted chickens the concentrations of plasma  $T_3$  have been reported to be depressed, while the concentrations of plasma  $T_4$  are increased (3.3.1). Alternatively in a study on forced moulting in laying hens, Brake et al., (1979) found that concentrations

of plasma  $T_4$  fell while those of plasma  $T_3$  remained unchanged after withdrawal of food and water.

In chickens cold stress has been found to result in a transient increase in the concentrations of plasma  $T_3$  and  $T_4$  (Kuhn and Nouwen, 1978), while in quail transfer to a warmer temperature also resulted in an increase in the levels of plasma  $T_3$  even though oxygen consumption was depressed (Bobek et al., 1980).

In view of these conflicting observations in the response to temperature stress the role of thyroid hormones and energy metabolism in the chicken has been re-examined. The daily rhythms in heat production in fed and fasted laying hens were measured in hens held at 20° and subsequently transferred to either 10°C or 32°C. The changes in metabolic rate in the laying birds were related to daily variations in the concentration of plasma  $T_3$  and  $T_4$ .

Birds are also subject to periods of dehydration caused by changes in environment or by the behavioural anorexia associated with incubation (Sherry et al., 1980; Savory, 1980). The response to dehydration would be an increase in the concentration of plasma components and thus the osmotic stress imposed upon the birds might be capable of affecting thyroid function.

In the wild the possession of salt glands endows euryhaline bird species with the capacity for extra-renal salt excretion and thus enables them to adapt to marine and brackish water environments. The osmotic stress of saline adaptation necessitates additional energy expenditure and consequently results in a loss of body weight in adults and in growth retardation in immature birds (Ensor and Phillips, 1972). In mammals thyroid hormones are known to affect osmoregulation (hypothyroidism results in an increase in salt and water retention while hyperthyroidism increases glomerular filtration rate and urine production). Additionally, as thyroidectomy impairs salt gland function in salt loaded ducks (Ensor and Phillips, 1970) alterations in thyroid hormone secretion might be expected to accompany salt water adaptation. Therefore in the third study variations in thyroid hormones were determined in freshwater reared ducklings during acute and chronic exposure to salt water.

3.7.1 Handling and response to bleeding: <sup>in chickens and turkeys</sup> effect on egg production  
in turkeys

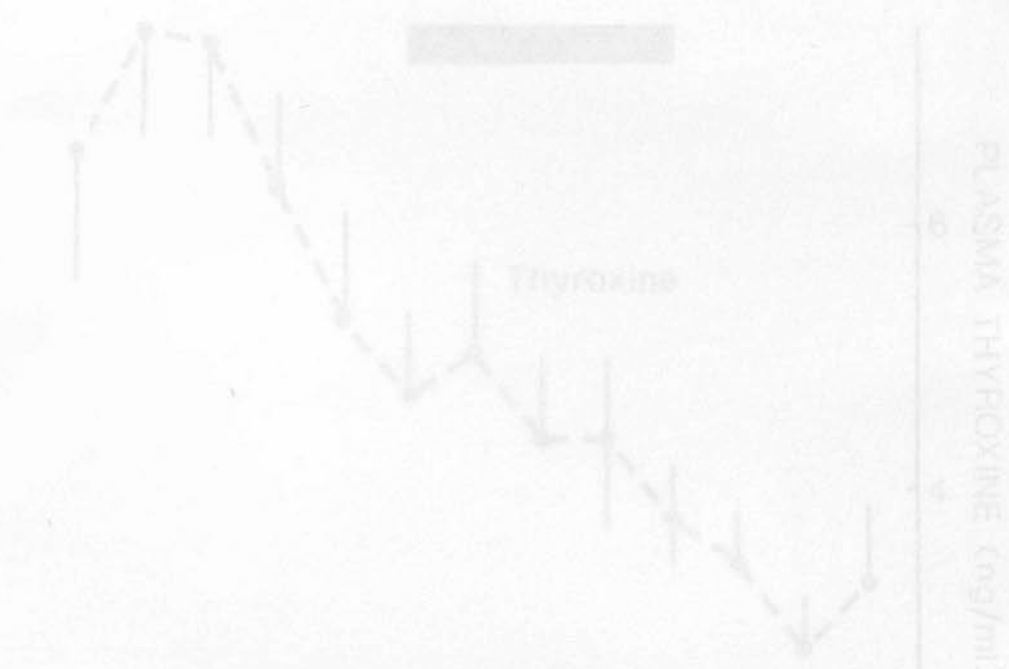
Experiment 1: Six 13 month-old laying Brown Leghorn hens were kept in individual cages and maintained on a lighting cycle of 14 h light/day (lights on at 05.00 and off at 19.00 h). Birds were injected with 1.0 ml physiological saline and blood samples were taken immediately before and then at 20 min intervals for 180 min. The birds were returned to their cages after each bleed and provided food and water ad libitum throughout the experiment.

There was no significant difference in the concentration of plasma  $T_3$  in the birds between 0 and 180 minutes after the onset of handling and bleeding. Contrarily there was a significant ( $P < 0.001$ ) and steady fall in the levels of  $T_4$  throughout the period of sampling (Fig. 23).

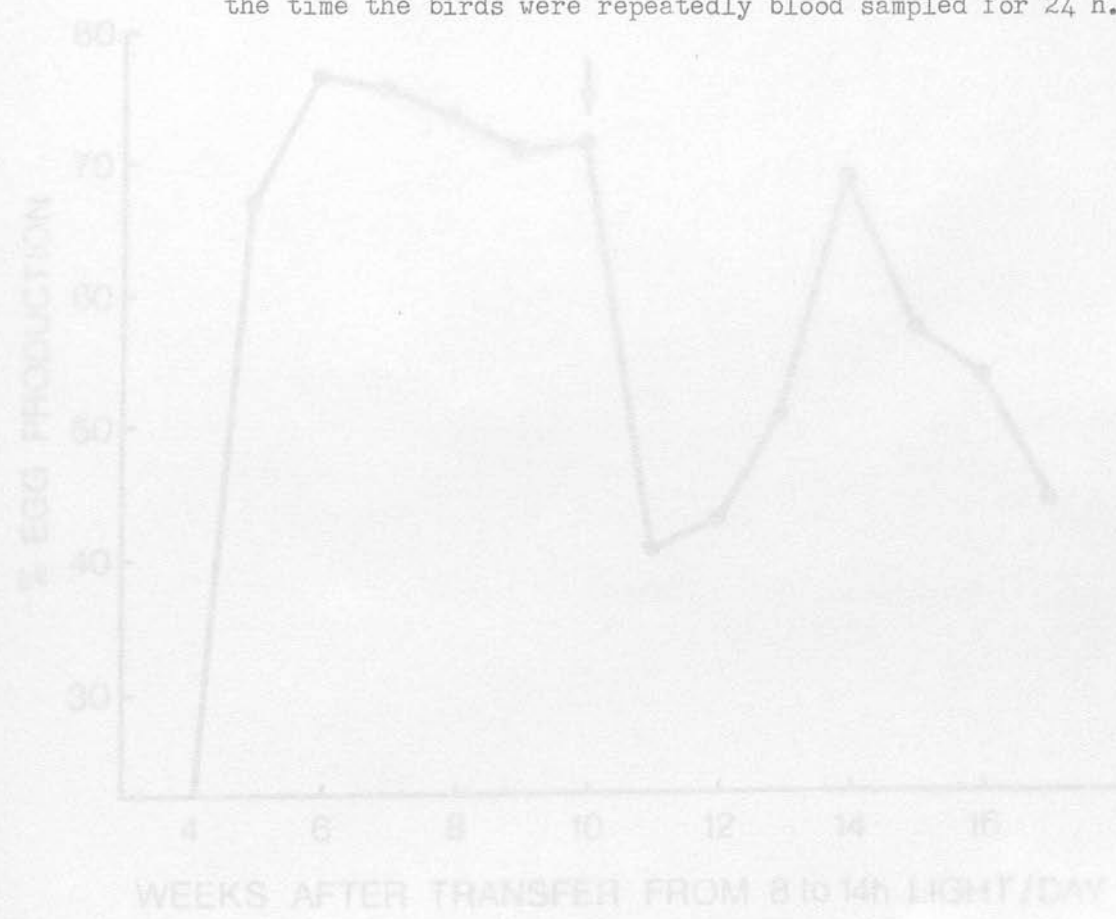
Experiment 2: Turkeys (2.1.5) of adult body weight (8 kg, Ross Super-Midi) were transferred to individual cages at 30 weeks of age and the photoperiod increased from 8L : 16D to 14L : 10D. The birds started to lay 3 to 4 weeks after photostimulation and a daily record was kept of individual egg production. Egg production was maximal at about 70% rate of lay after 10 weeks of photostimulation. At this time 12 birds were bled every 2 h for 24 h.

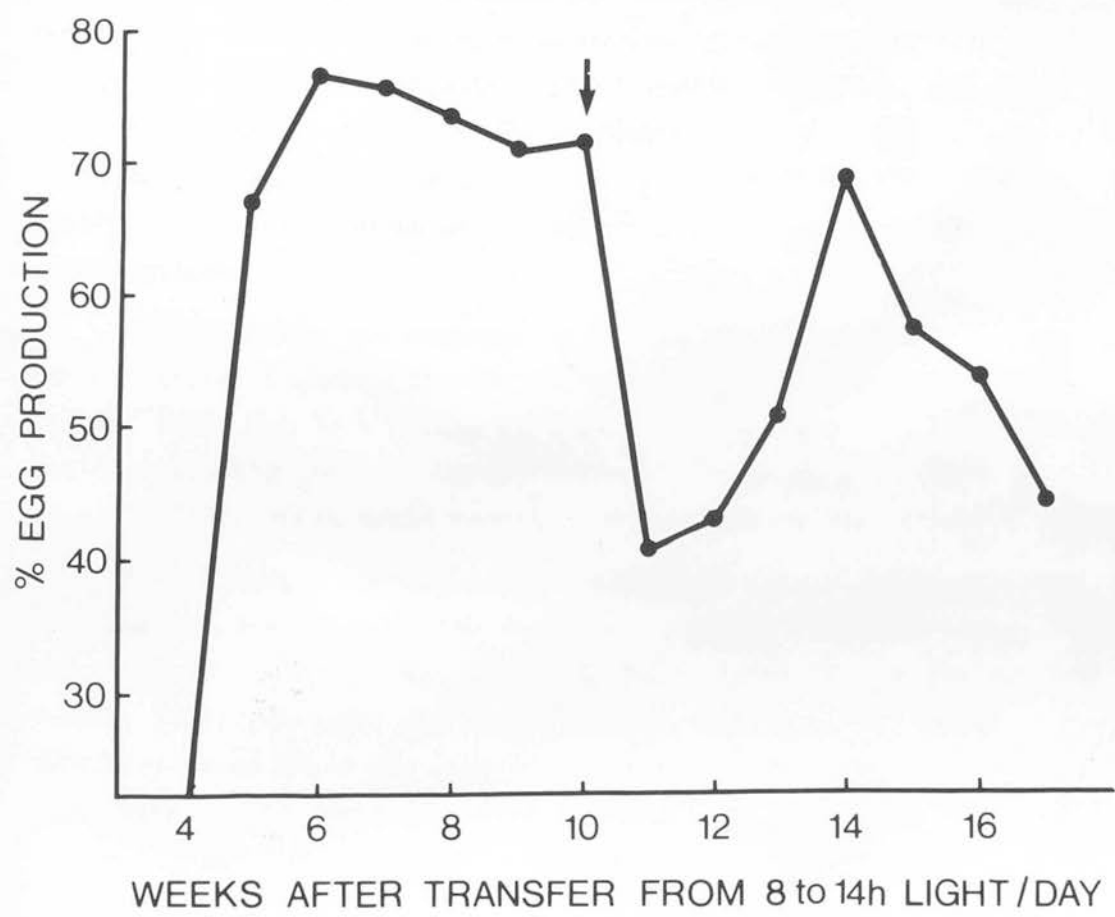
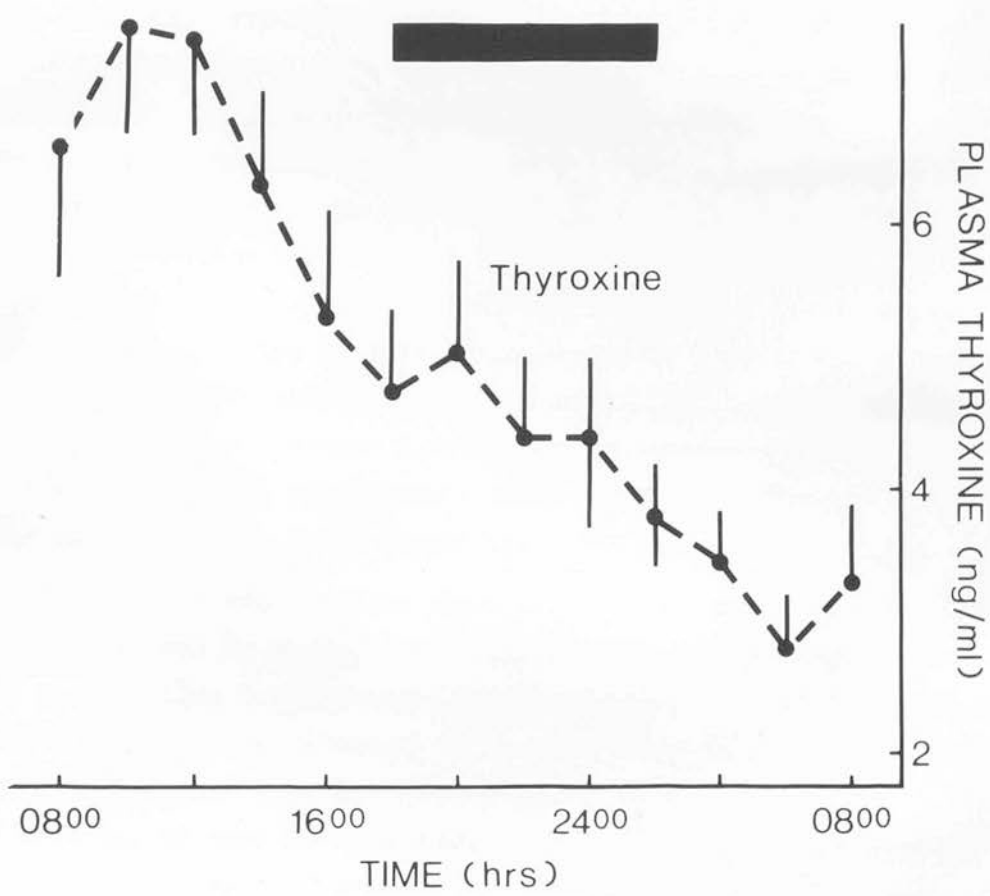
The concentration of plasma  $T_4$  steadily declined throughout the 24 h period of sampling and remained significantly depressed 24 h after the first sample was taken ( $p < 0.001$ ; Fig. 30). The effect of the stress of repeated handling and blood sampling on egg production in turkey poults is seen in Fig. 30. The rate of lay markedly declined from about 70% to 40% egg production and required 3 weeks to return to prestressed levels. At this time egg production was declining as an increasing number became 'broody' and went out of lay. This observation is particular to flocks of turkey and is of major concern to the poultry industry.

The steep decline in the baseline  $T_4$  concentration in chickens and turkeys suggests that the stress of repeated handling and bleeding may have inhibited the secretion of TSH from the pituitary gland. This view is supported by studies on the response to stress in mammals which have measured a decrease in the concentration of plasma TSH (see Introduction



**FIGURE 30** Changes in the concentration of plasma  $T_4$  in adult turkey hens serially sampled during a 24 h cycle of 14L : 10D (upper panel). Each point represents the mean ( $\pm$  SEM) of 12 birds. The horizontal bar represents the period of darkness. Egg production in turkey hens transferred from short days (8L : 16D) to long days (14L : 10D) (lower panel). The arrow represents the time the birds were repeatedly blood sampled for 24 h.





to this section). For example rats which were injected with saline and subsequently bled under ether anaesthesia show a decrease in thyroid activity (Fukuda et al., 1975) while in quail handling results in a decrease in the concentration of plasma  $T_4$  (Bobek et al., 1980). In chickens the decrease in the levels of plasma  $T_4$  was likely not due to diurnal changes since such a steep fall in baseline  $T_4$  over a period of 2.5 hr (Fig. 23)<sup>was</sup> not observed at this time during the light period (3.2). Similarly in turkeys the pattern of secretion of  $T_4$  over the 24 h sampling period is unlike that measured in non stressed chickens maintained under the same photoperiod (Section 3.2.1). In support of this view the concentration of  $T_4$  in the stressed birds had not returned to baseline levels 24 h after the first sample was taken (Fig. 30).

There are several factors which may influence the thyroid hormone response to stress in birds. The possible role of the sympathetic nervous system in mediating these changes in the levels of plasma  $T_4$  cannot be clarified at this time, although in mammals there is an increase in the release of adrenalin from the adrenal gland in response to stress which results, as does an injection of catecholamines, in an increase in the secretion of thyroid hormones (Melander, 1970; Ericson et al., 1970). Furthermore the release of norepinephrine from nerve terminals within the thyroid gland can activate thyroid hormone secretion (Melander, 1977) while dopamine and serotonin are suggested to inhibit the release of TSH (Mueller, 1976a, b). Thus the extent of involvement of the nervous system in the response to acute stress in birds is not clear and requires investigation.

Alternatively the response to stress may be a decrease in the concentration of plasma thyroid hormone binding proteins (Bellabarba et al., 1968) due to a change in hepatic function or to a lowered binding capacity for the thyroid hormones (Adami et al., 1978; Chan et al., 1978), which would result in a decrease in the levels of plasma  $T_4$ .

Pharmacologic and physiologic concentrations of corticosterone decrease the secretion of TSH from the pituitary gland (Reichlin, 1966; McKenzie, 1979) as well as reduce the TSH response to TRH (Wilber and Utiger, 1969; Parmenter and Hedge, 1980). Additionally, in man corticosteroids lower the concentration of plasma  $T_3$  due to an inhibition of its generation from  $T_4$  (Chopra et al., 1975; Robbins,

1981). In birds handling stress elicits an immediate increase in the concentration of plasma corticosterone (Freeman, 1976; Harvey *et al.*, 1980) and thus may play a role in the regulation of the concentrations of plasma thyroid hormones.

The decline in the levels of plasma  $T_4$  during periods of stress does not reflect the increase in energy metabolism of the bird during this time. Rather the results suggest that  $T_4$  serves as a prohormone for generating  $T_3$ , whose concentration is maintained at the expense of  $T_4$ , and supports the view that there are separate mechanisms of control that regulate the levels of plasma  $T_3$  and  $T_4$ . These results demonstrate the lability of the secretion of  $T_4$  in response to handling stress and its usefulness as a physiological indicator of stress in birds.

### 3.7.2 Temperature and fasting stress: relationship to energy metabolism

White-Leghorn hens were reared on a lighting cycle of 14 h light/day at a temperature of 30°C and had free access to food and water. At 12 weeks of age the birds were caged individually at 20°C. Experiment 1 and 2 were done when the birds had been in lay for about 14 weeks.

Daily variations in heat production were first measured with the birds provided free access to food and water. The birds were then removed from the calorimeters and deprived of food for 24 h: they were returned to the calorimeter for a further 24 h with access to water only, for the measurement of fasting heat production. The lighting pattern in the calorimeter was synchronised with that in the bird house.

Experiment 1: The purpose of this experiment was to investigate the response of the thyroid glands to a chronic increase or decrease in ambient temperature. The birds were exposed to 14 h light/day (lights on 04.00 to 18.00) and given free access to food and water. Blood samples were taken between 09.00 and 09.30 on 2 occasions before, and on the day, the ambient temperature was changed to either 10°C or 32°C (relative humidity 70%) and further samples were taken 1, 3, 7 and 10 days thereafter. A group of control hens kept at 20°C were sampled at the same times. The cloacal temperature, measured at 15.30 h, and the amount of food eaten was recorded for each bird.

The concentration of plasma  $T_3$  fell significantly ( $p < 0.01$ ) in laying hens within 24 hr of an increase in the ambient temperature from  $20^\circ\text{C}$  to  $32^\circ\text{C}$  and remained depressed thereafter (Fig. 31). No change was seen in the concentration of plasma  $T_3$  in the control hens held throughout the study at  $20^\circ\text{C}$  and in the birds transferred to  $10^\circ\text{C}$  except for a slight rise 24 hr after the transfer (Fig. 32). In contrast to plasma  $T_3$ , the concentration of plasma  $T_4$  did not change after the increase in ambient temperature (Fig. 31). Daily food consumption decreased ( $p < 0.01$ ) during the 2 d after the ambient temperature was increased but then returned to the level observed in the control birds. Thereafter, daily food consumption fell progressively for the remainder of the study period. There was no consistent change in either the concentration of plasma  $T_4$  or in the daily food consumption during the study period in the control birds. The concentration of plasma  $T_4$  tended to increase between 3 and 7 days after transfer to  $10^\circ\text{C}$  and was associated with a marginal increase in the level of food intake (Fig. 32). Twenty-four hours after the increase in ambient temperature, body temperature increased ( $p < 0.01$ ) from  $41.3 \pm 0.1$  to  $41.6 \pm 0.1^\circ\text{C}$ . Thereafter the body temperature of birds held at  $32^\circ\text{C}$  was not significantly different from that in control birds held at  $20^\circ\text{C}$ . There was no effect of transfer to  $10^\circ\text{C}$  on body temperature.

Experiment 2: The purpose of this experiment was to measure daily changes in heat production in fed and fasted laying hens and to relate them to daily changes in the concentration of plasma  $T_4$  and  $T_3$ . After the last blood sample was taken for experiment 1 the same hens were further subdivided into 6 groups. Three groups, one maintained at  $20^\circ\text{C}$  and the others at  $10^\circ\text{C}$  and  $32^\circ\text{C}$ , were trained for 3 d to become used to the calorimeters (2.6.2). Daily variations in heat production were then measured when the birds were fed and fasted as described above. In parallel with the measurements of daily variations in heat production, blood samples were taken from the remaining 3 groups of hens, held at 10, 20 and  $32^\circ\text{C}$  when they were fed and fasting. The samples were taken 30 min before and at 4, 8, 12 and 24 h after the lights came on.

A daily rhythm of heat production was observed in fed and fasted laying hens kept at  $10^\circ$ ,  $20^\circ$  and  $32^\circ\text{C}$  with the lowest and highest values occurring during the dark and light periods respectively (Figs. 33 and 34).

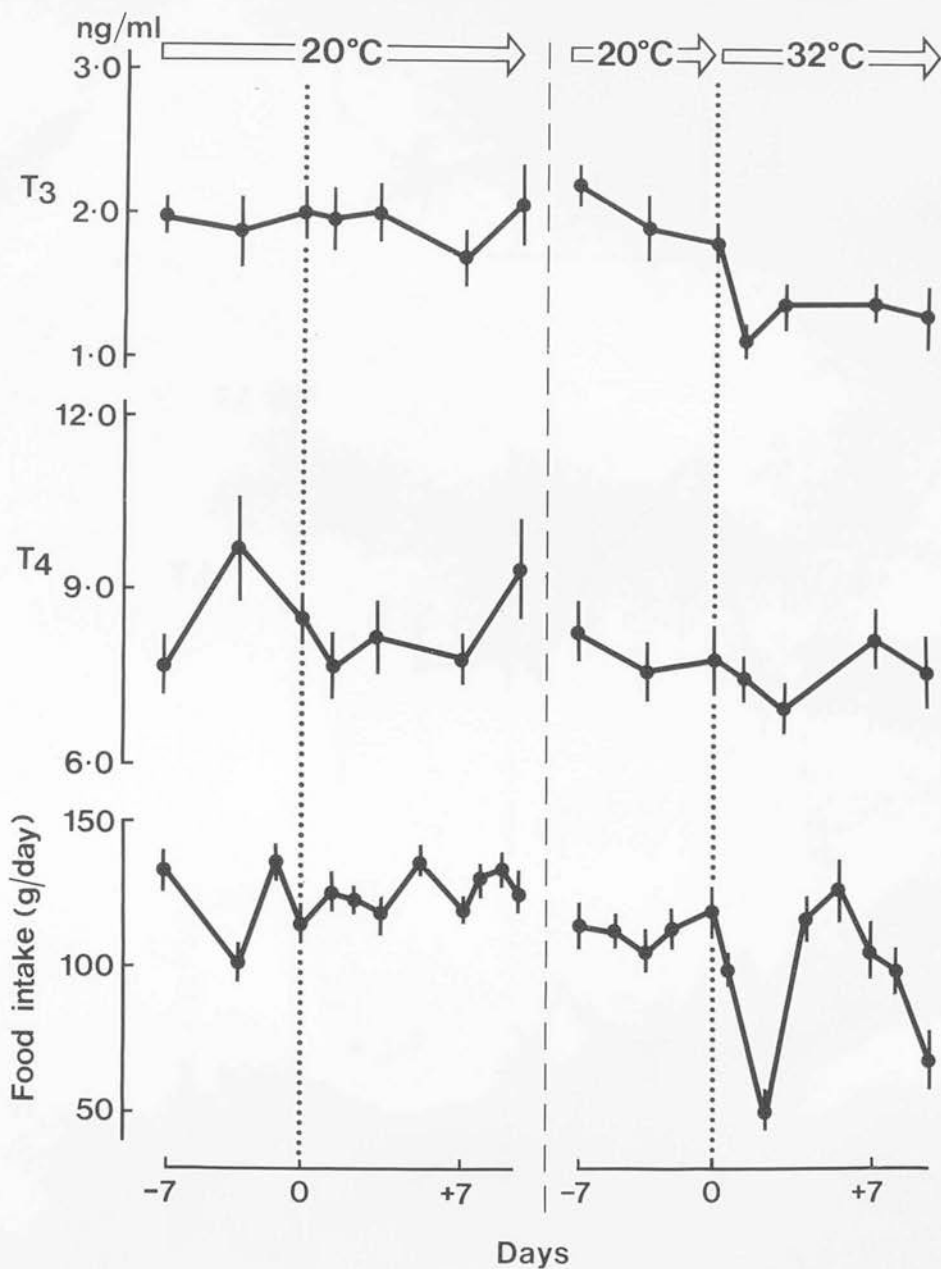
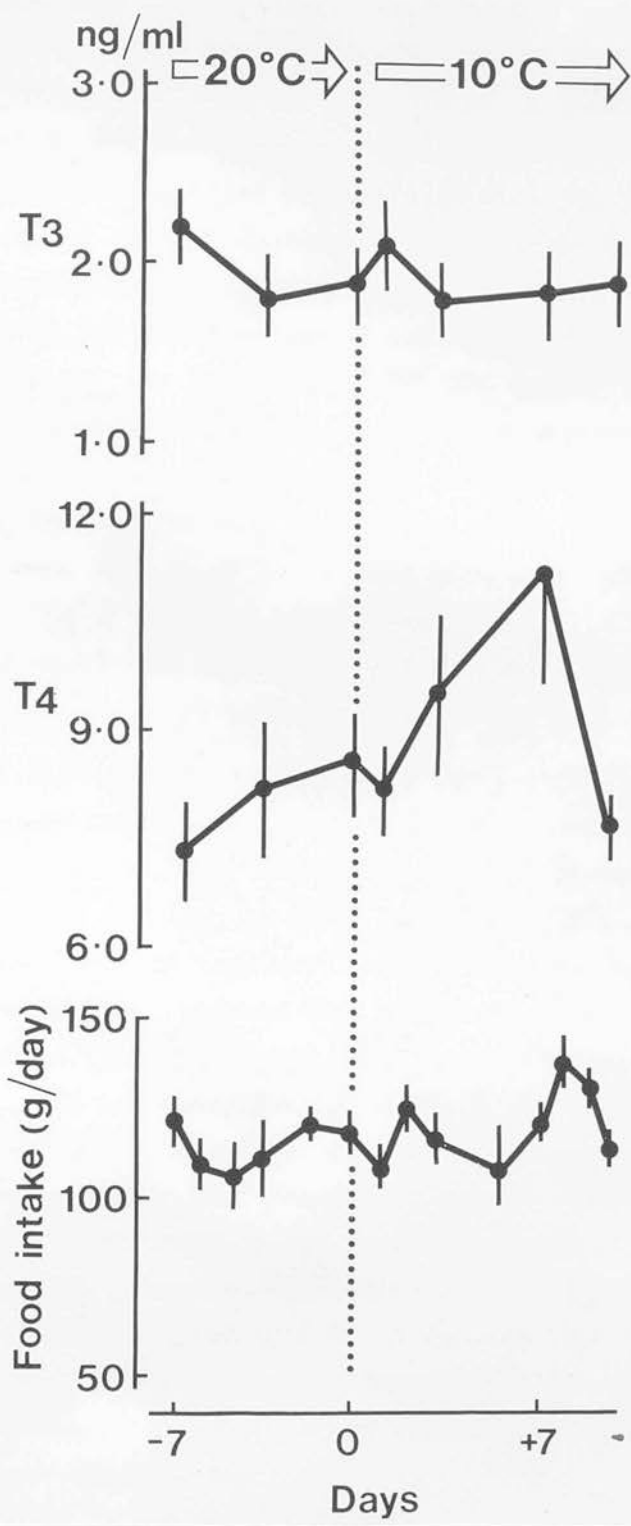


FIGURE 31 Changes in the concentrations of plasma T<sub>3</sub>, T<sub>4</sub> and food intake in 12 laying hens after an increase in temperature from 20° to 32°C (right panel) and in 12 control laying hens maintained at 20°C throughout the study (left panel). The vertical lines represent  $\pm$  SEM.

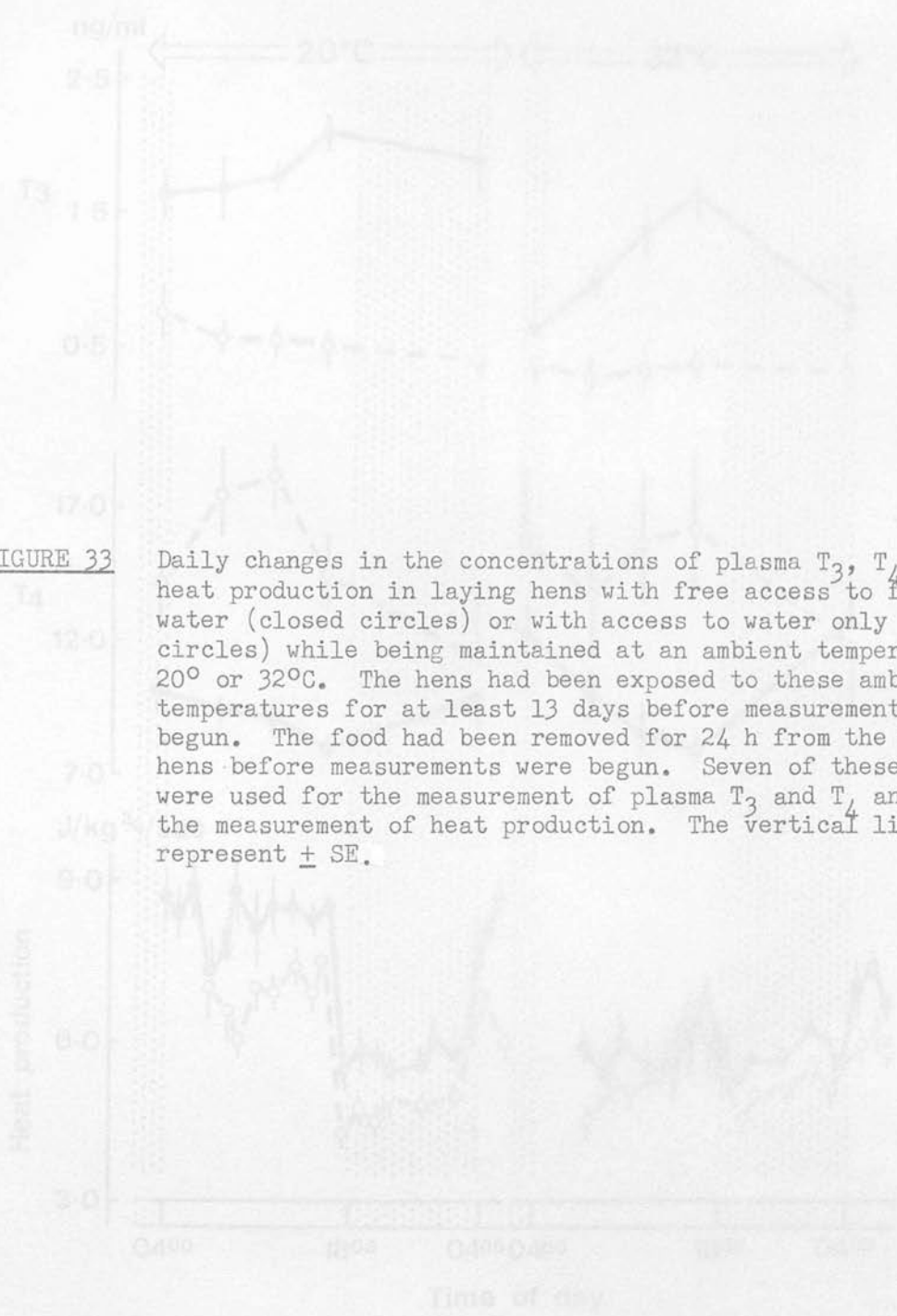


**FIGURE 32** Changes in the concentrations of plasma T<sub>3</sub>, T<sub>4</sub> and food intake in 12 laying hens after a decrease in temperature from 20° to 10°C. The vertical lines represent ± SEM.

The amplitude of this rhythm was greater in hens kept at 10° and 20° than in those kept at 32°. This was because in hens maintained at the lower temperatures the rate of heat production during the light period was about 50% greater than the rate during the dark period (Fig. 34). In hens kept at 32° the rates of heat production at several sampling points during the light and dark periods were the same (Fig. 33). However peaks of heat production occurred at the beginning and end of the light period (Fig. 33). The principal effect of the increase in temperature on the daily rhythm of heat production was to decrease heat production during the light period but not during the dark period. This effect was probably due to a change in food consumption (Fig. 31) and was not seen in birds held at 10° and 20°C. Fasting resulted in a decrease in the rate of heat production during the light and dark periods in hens kept at 20°C. A less pronounced effect of fasting on the daily rhythm of heat production was observed in hens kept at 10° and 32°C. Heat production tended to be depressed during the dark period but not during the light period (Figs. 33 and 34).

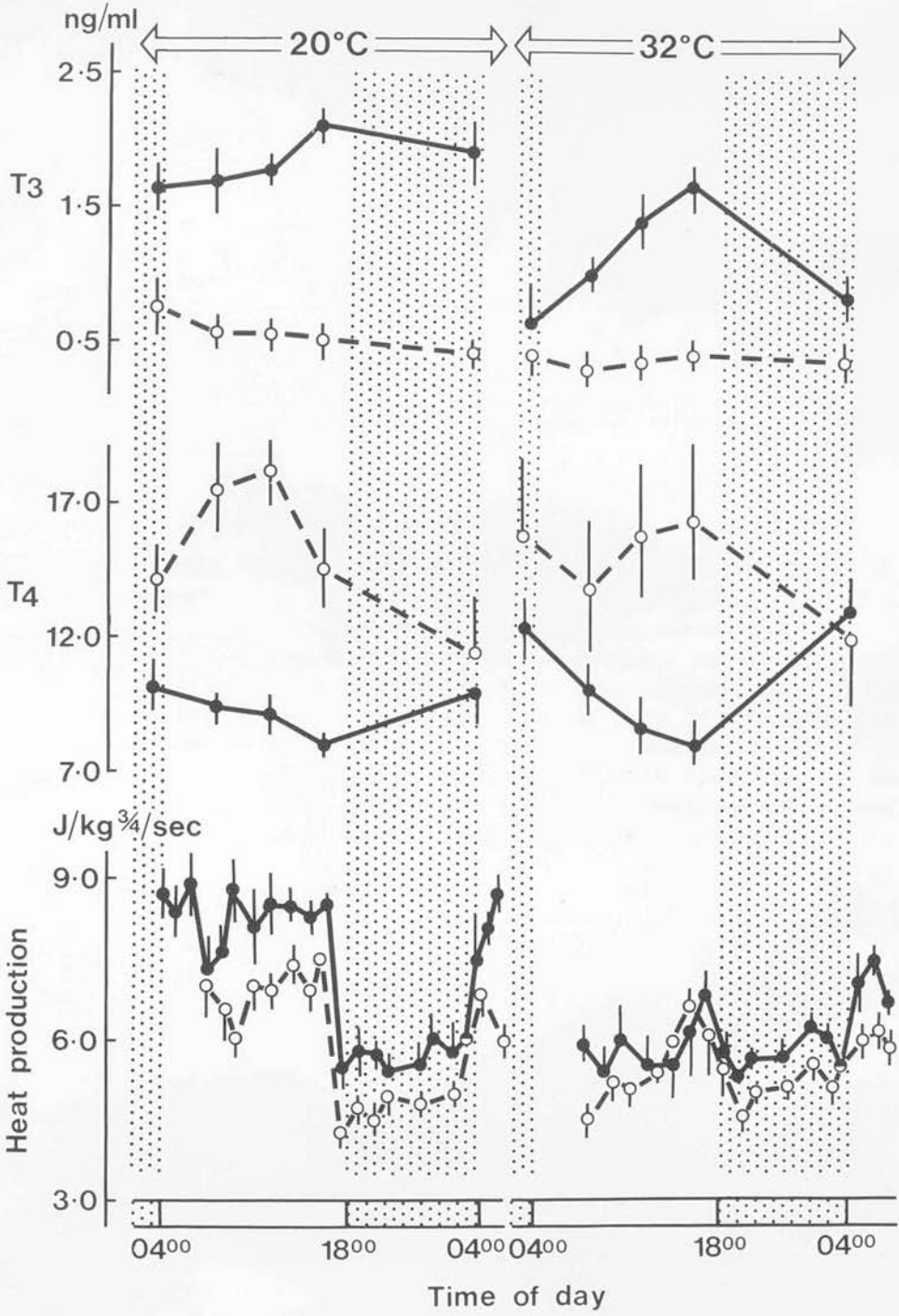
In fed laying hens at each temperature studied, the concentration of  $T_3$  increased during the light period and decreased during the dark period (Figs. 33 and 34). The amplitude of this daily rhythm was greater in birds held at 32° than in those at 10° and 20°. The concentration of plasma  $T_3$  was lower in hens kept at 32°C than in those at 20° and 10°C at all times of day. However this difference was greatest at the end of the dark period ( $p < 0.001$ ) and smallest at the end of the light period ( $p < 0.05$ ). The concentration of plasma  $T_4$  was inversely related to that of  $T_3$ : levels of  $T_4$  decreased and increased during the light and dark periods respectively. The increase in ambient temperature did not result in any significant change in the concentration of plasma  $T_4$  during the light period. However, the concentration of plasma  $T_4$  at the end of the dark period was higher ( $p < 0.05$ ) in hens kept at 32° than in those at 10° and 20°C. This difference resulted in the amplitude of the daily rhythm in levels of plasma  $T_4$  being greater in the hens kept at the higher ambient temperature.

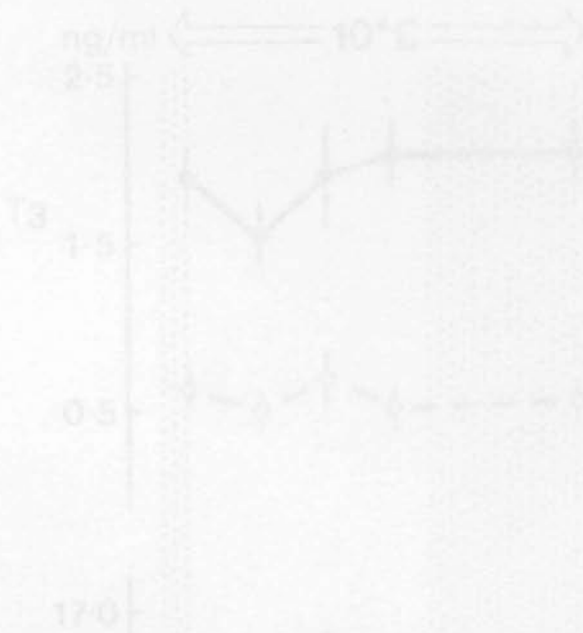
Fasting markedly affected the concentration of plasma  $T_4$  and  $T_3$  in a similar way in hens kept at each temperature studied. The concentration of plasma  $T_3$  was depressed while that of  $T_4$  was increased and the daily



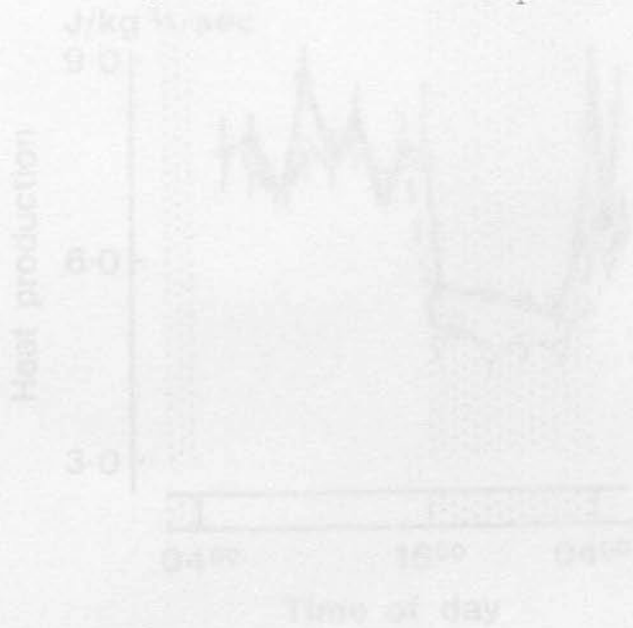
**FIGURE 33**

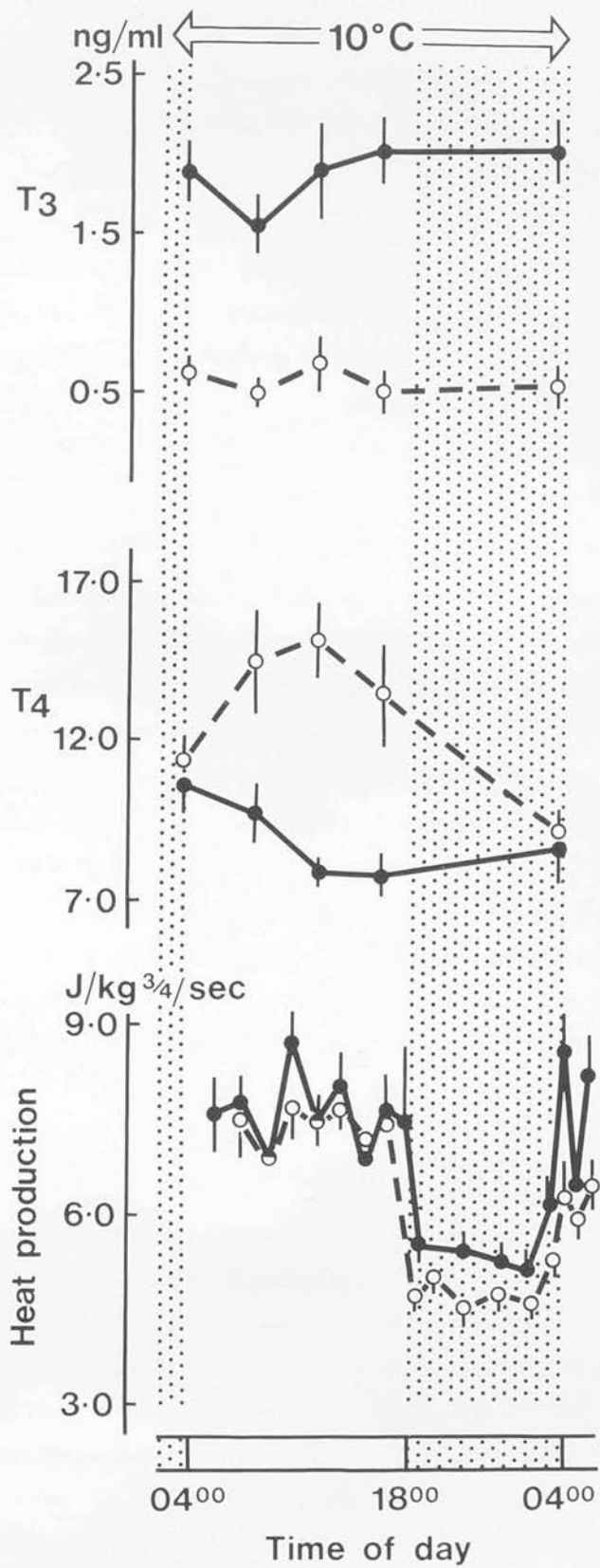
Daily changes in the concentrations of plasma  $T_3$ ,  $T_4$  and heat production in laying hens with free access to food and water (closed circles) or with access to water only (open circles) while being maintained at an ambient temperature of  $20^\circ$  or  $32^\circ\text{C}$ . The hens had been exposed to these ambient temperatures for at least 13 days before measurements were begun. The food had been removed for 24 h from the fasted hens before measurements were begun. Seven of these birds were used for the measurement of plasma  $T_3$  and  $T_4$ , and 5 for the measurement of heat production. The vertical lines represent  $\pm$  SE.





**FIGURE 34** Daily changes in the concentrations of plasma  $T_3$ ,  $T_4$  and heat production in laying hens with free access to food and water (closed circles) or with access to water only (open circles) while being maintained at an ambient temperature of  $10^{\circ}\text{C}$ . The hens had been exposed to this ambient temperature for 2 weeks before the measurements were begun. The food had been removed for 24 h from the fasted hens before measurements were begun. Seven of the birds were used for the measurement of plasma  $T_3$  and  $T_4$  and 5 for the measurement of heat production. The vertical lines represent  $\pm$  SE.





rhythm in the concentration of plasma  $T_3$  disappeared. The concentration of plasma  $T_4$  increased during the light period in birds held at 10° and 20°C ( $p < 0.001$ ) and declined during the dark.

The significant decline in the concentration of  $T_3$  in fasted birds held at 10 and 32°C was not associated with a lowered rate of heat production during the light period: only during the dark period was there a sustained decrease observed. Thus the mean daily rate of heat production is reduced in fasted birds. This finding may suggest a reason for the failure of Acheson and Burger (1980) to observe a relationship between lowered  $T_3$  levels and measurements of heat production; samples were taken only in the early morning and not over a 24 h period.

The fall in the concentration of plasma  $T_3$  after the increase in ambient temperature is in agreement with the findings of May (1978) and Cogburn and Harrison (1980). It could be a consequence of the reduction in food consumption which might lead to a decrease in the peripheral conversion of  $T_4$  to  $T_3$  (3.8.2). Such a mechanism is consistent with the observation that in the Japanese quail (McFarland *et al.*, 1966) and chicken (May *et al.*, 1974) the half-life of  $T_4$  is increased after a rise in ambient temperature. In view of the large body of evidence that thyroid activity is reduced in chickens exposed to high ambient temperatures (see Introduction to this section), it is possible that in the present study,  $T_4$  secretion was reduced when the ambient temperature increased. However, if there was an associated reduction in peripheral monodeiodination of  $T_4$  to  $T_3$ , the reduction in  $T_4$  secretion could have been compensated for by an increase in its half-life.

Removal of thyroid hormones by thyroidectomy results in a decrease in heat production (3.8.1). Similarly, the reduction in plasma  $T_3$  associated with fasting and increased ambient temperature also resulted in a decrease in heat production. These observations suggest that heat production is regulated, at least in part, by levels of plasma  $T_3$ . Since the concentration of plasma  $T_4$  was increased in fasting hens or after an increase in temperature, it seems that  $T_4$  is not directly involved in the regulation of heat production. This conclusion supports the view that  $T_3$  rather than  $T_4$  is the principal metabolically active thyroid hormone.

Although the levels of plasma  $T_3$  were equally depressed at 10, 20 and 32°C in the study on fasted laying hens, the rate of heat production during the light period was much greater when the birds were kept at 10 and 20°C than when they were kept at 32°C (Figs. 33 and 34). It seems that, as was observed in the thyroidectomized hens (3.8.1), changes in heat production may occur independently of changes in the concentration of plasma  $T_3$ .

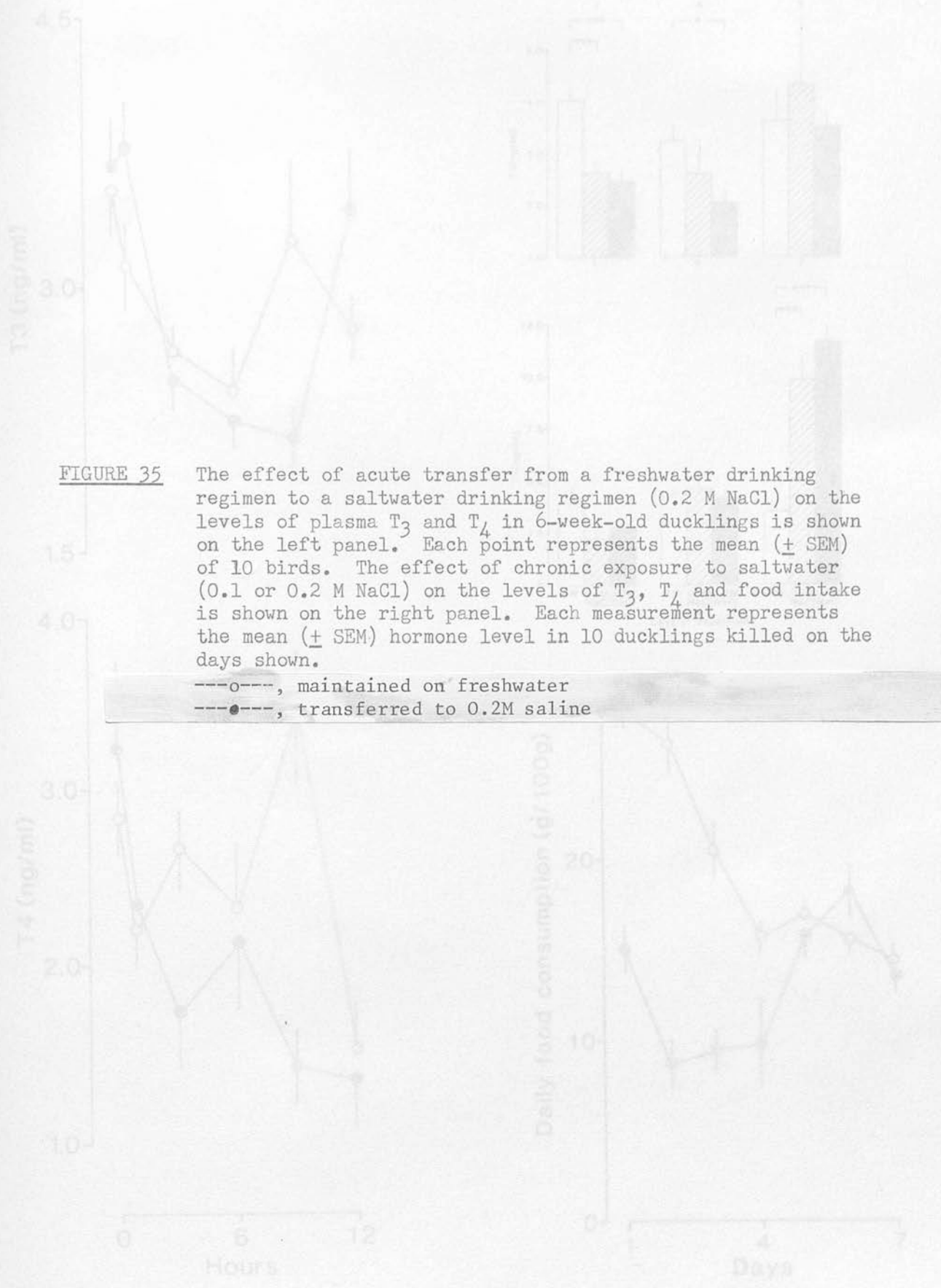
The disappearance of a daily rhythm in concentrations of plasma  $T_3$  and  $T_4$  in fasted hens supports the view that these rhythms are generated by the pattern of feeding (3.3). The decrease in the concentration of plasma  $T_3$  and the associated increase in that of  $T_4$  in the fasted hens is consistent with a reduction in the peripheral monodeiodination of  $T_4$ .

### 3.7.3 Adaptation to salt water: stress of dehydration

Experiment 1: Aylesbury ducklings were obtained commercially as one-day old birds and reared on deep litter under a 14L : 10D photoperiod (lights on 07.30 - 21.30 hrs) with food and water provided ad libitum. When the birds were 6 weeks of age they were transferred to 0.2 M NaCl and blood samples taken before and 3, 6, 9 and 12 hrs afterwards. A control group was maintained on freshwater and bled at the same time.

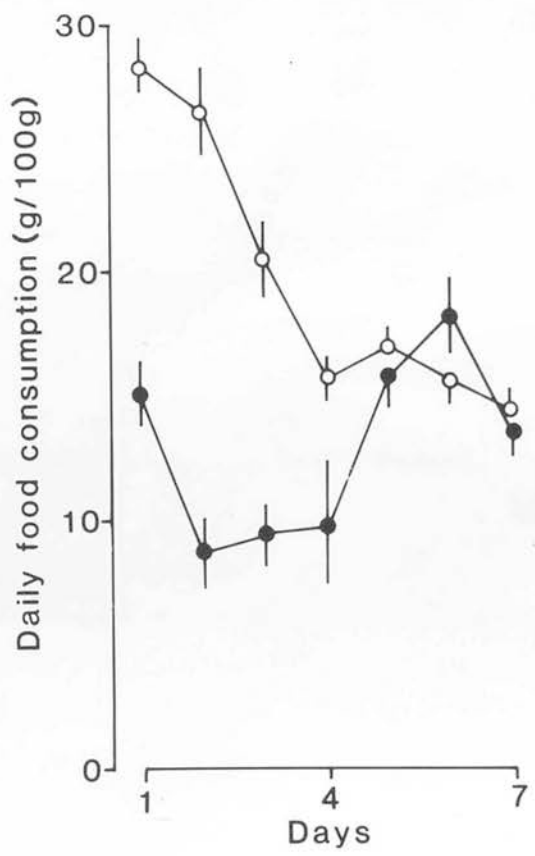
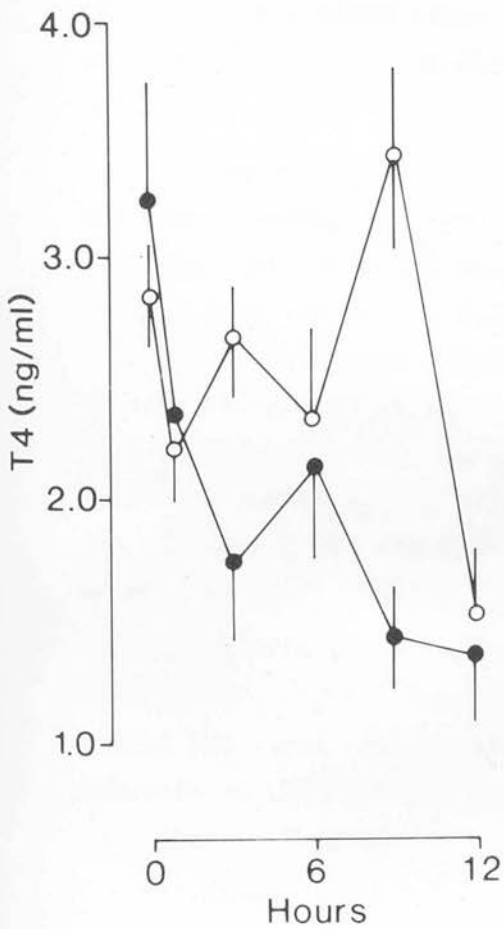
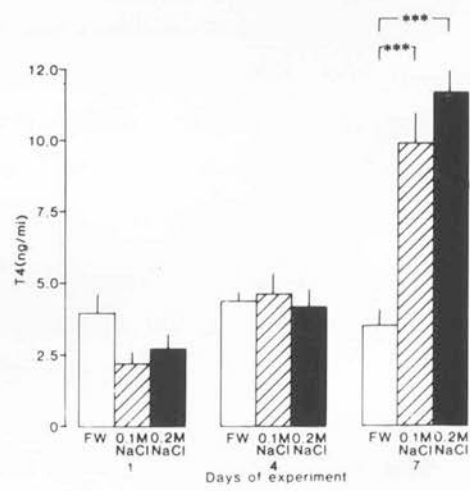
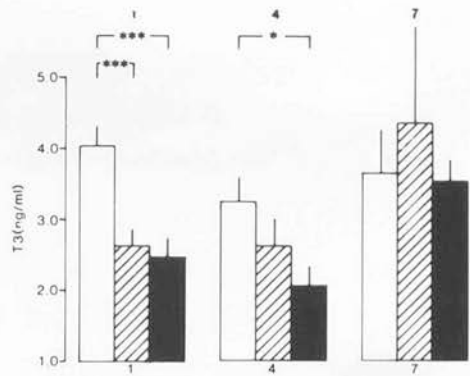
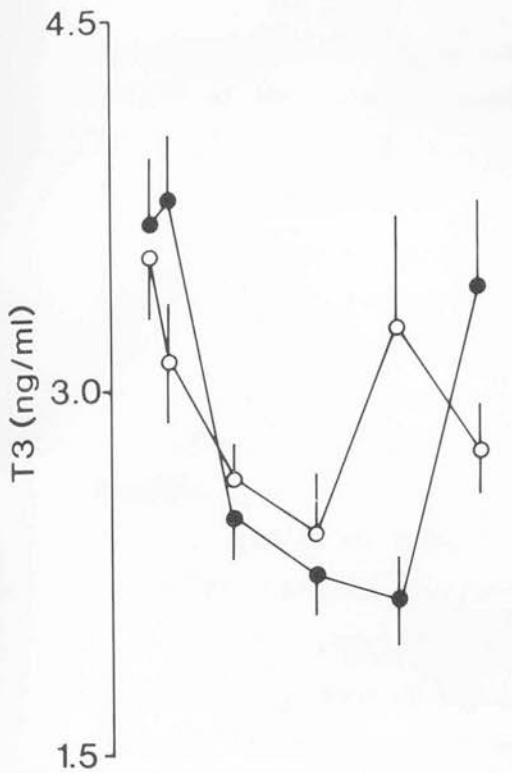
The levels of plasma  $T_4$  tended to remain higher in birds transferred to 0.2 M NaCl (Fig. 35). There was no consistent effect of acute exposure to saline found on the concentration of plasma  $T_3$  as compared to birds maintained on freshwater. The stress of repeatedly handling the birds as well as a reduction in food intake (Fig. 35) was likely responsible for the observed changes in the concentration of thyroid hormones during the acute phase of exposure to salt water.

Experiment 2: Aylesbury ducklings, between 5 to 11 days of age, were transferred to a salt water drinking regimen. In order to minimise any effect due to handling on the levels of plasma hormones blood samples were obtained by decapitation from groups of birds 1, 4 or 7 days after transfer to 0.1 M or 0.2 M saline. Food intake was measured in separate groups of growing birds transferred to 0.2 M saline or maintained on freshwater.



**FIGURE 35** The effect of acute transfer from a freshwater drinking regimen to a saltwater drinking regimen (0.2 M NaCl) on the levels of plasma T<sub>3</sub> and T<sub>4</sub> in 6-week-old ducklings is shown on the left panel. Each point represents the mean ( $\pm$  SEM) of 10 birds. The effect of chronic exposure to saltwater (0.1 or 0.2 M NaCl) on the levels of T<sub>3</sub>, T<sub>4</sub> and food intake is shown on the right panel. Each measurement represents the mean ( $\pm$  SEM) hormone level in 10 ducklings killed on the days shown.

---o---, maintained on freshwater  
 ---●---, transferred to 0.2M saline



The weight of the salt gland increased from  $18.0 \pm 1.8$  to  $33.6 \pm 2.0$  mg/100 g ( $p < 0.01$ ) after one day of exposure to saline and further increased to  $86.5 \pm 4.4$  mg/100 g after 7 days. Similarly the weight of the adrenal glands increased after 1 day from  $26.1 \pm 1.8$  to  $31.7 \pm 1.6$  mg/100 g ( $p < 0.05$ ) and further increased to  $32.4 \pm 2.6$  mg/100 g after 7 days.

The levels of plasma  $T_3$  and the daily food consumption were transitorily decreased between 1 and 4 days after transference to salt water (Fig. 35). After 7 days the concentration of plasma  $T_3$  as well as food intake had returned to the levels measured in birds maintained on freshwater. Contrarily the concentration of plasma  $T_4$  in both groups (3.3.1) exposed to saline remained at the levels in the control birds between 1 and 4 days after transference whereas after 7 days the concentration of  $T_4$  was significantly increased ( $p < 0.001$ ) in both groups (Fig. 35).

Experiment 3: Aylesbury ducks were chronically exposed to saline in order to determine the long term effects on the levels of thyroid hormone. Aylesbury ducks were reared from 5 days of age on 0.1 M NaCl until 25 days of age and on 0.2 M NaCl until they were 7 weeks of age. At this time a blood sample was obtained by decapitation. The effect of chronic ingestion of saline on the weight of various tissues was examined.

The body weight of freshwater birds (g) ( $2452 \pm 29$ ,  $n = 10$ ) was not significantly different from birds reared on saline ( $2378 \pm 59$ ,  $n = 10$ ). Similarly the weight of the adrenal glands (mg/100 g) ( $9.36 \pm 0.34$  vrs  $8.19 \pm 0.35$ ) and liver (g/100 g) ( $4.81 \pm 0.13$  vrs  $3.94 \pm 0.16$ ) were not affected by chronic saline ingestion. The weight of the salt glands in birds exposed to saline were significantly ( $p < 0.001$ ) increased (mg/100 g) ( $15.94 \pm 1.25$  vrs  $48.44 \pm 2.00$ ). The levels of plasma  $T_3$  (ng/ml) ( $2.68 \pm 0.50$  vrs  $2.87 \pm 0.79$ ) and  $T_4$  (ng/ml) ( $3.1 \pm 0.4$  vrs  $4.1 \pm 0.4$ ) were unaffected by long term exposure to saline.

A specific effect of exposure to saline on the levels of plasma thyroid hormones was not evident during the acute phase of adaptation. During the first week of adaptation there is a suggestion that both the increase in the activity of the adrenal glands concomitant with a reduction in food intake are affecting the concentrations of plasma  $T_3$

(3.3; 3.7.1). The hypertrophy of the salt glands in response to drinking saline was not directly dependent on thyroid hormone as evidenced by the decrease in the levels of  $T_3$  during this period.

The increase in the concentration of plasma  $T_4$  found 7 days after transference was not observed in birds chronically adapted to saline. This temporary increase in the levels of  $T_4$  was not due to the temporary reduction in food intake and may, in part, be due to an increase in the levels of plasma TSH at this time. Thus the adaptation to salt water as a model for the stress of dehydration is associated with a sequence of changes in thyroid activity which suggest the effect of several factors of which the initial reduction in food intake is principal.

#### 3.7.4 Summary

The effect of acute and chronic stress on the levels of plasma thyroid hormones was investigated. The baseline concentration of plasma  $T_4$  but not of  $T_3$  in the laying hen appeared to be affected by the stress of repeated handling since it fell significantly ( $p < 0.001$ ) during the course of a 3 h sampling period. Similarly the concentration of plasma  $T_4$  in laying turkeys declined throughout a 24 h sampling period. In response to this stress, the rate of lay in the handled birds was depressed and required 3 weeks before the levels returned to those measured prior to sampling.

The effect of transferring laying hens to a cold or warm temperature on plasma levels of thyroid hormones and energy metabolism was investigated. In birds exposed to a 14L : 10D photoperiod and held at 20°C the amplitude of the daily rhythm in heat production was greater than that in the same birds held at an increased temperature of 32°C. The birds ate significantly less at the higher temperature due to a lowered energy requirement and there was a decrease in the rate of heat production during the light but not during the dark period. Fasting lowered heat production during the light and dark periods when the hens were exposed to 20°C and during the dark period when the hens were held at 10 and 32°C. The mean daily level of plasma  $T_3$  was lower in hens held at 32°C than in those maintained at 10 and 20°C although the amplitude of the daily rhythm was greater in the hens kept at the higher temperature. The mean concentration of plasma  $T_4$  was not increased after exposure to the

higher temperature. The mean concentration of plasma  $T_4$  was not changed after exposure to a change in temperature, although there was an increase in the amplitude of the rhythm in birds held at  $32^{\circ}\text{C}$ . In fasted hens held at each temperature, the concentration of plasma  $T_3$  was uniformly depressed during the light and dark period while the concentration of plasma  $T_4$  remained elevated.

The effect of dehydratory stress on the levels of plasma thyroid hormones was investigated by transferring ducklings adapted to fresh-water to a 0.2 M NaCl drinking regimen. A specific effect of saline was not seen during the first 12 h after transfer. For a period of 4 days the decline in food intake was associated with a corresponding reduction in the levels of plasma  $T_3$ . Thereafter both food intake and plasma levels of  $T_3$  returned to baseline concentrations. The increase in the concentration of plasma  $T_4$  measured 7 days after transference was not maintained in birds chronically adapted to saline.

It was concluded that the decline in the levels of plasma  $T_4$  in response to handling stress may be due to a decrease in the secretion of pituitary TSH whereas temperature and dehydration stress affect the levels of thyroid hormone principally by a change in food intake.

### 3.8 Energy metabolism and peripheral conversion of $T_4$ to $T_3$

The thyroid gland plays a fundamental role in the regulation of metabolism in the bird (3.7.2). Thus, surgical or chemical thyroidectomy results in a decrease in metabolic rate (Winchester, 1939; Mellen and Wentworth, 1962; Davison *et al.*, 1980) whereas heat production is stimulated after the administration of thyroid hormones (McCartney and Shaffner, 1950; Mellen and Wentworth, 1958; Singh *et al.*, 1968; Arieli and Berman 1979). Thyroid function is depressed in fasting birds and is associated with reduced heat production (Shannon and Brown, 1969a, b; O'Neil *et al.*, 1971; Farrel and Swain, 1977; Lundy *et al.*, 1978; MacLeod *et al.*, 1980). The pronounced daily rhythm of plasma  $T_4$  and  $T_3$  (3.2) and their dependence on food availability (3.3.3) suggests that in the absence of either the thyroid or food there should be a pronounced effect on the daily pattern of energy metabolism as well. Previous estimates of heat production in thyroidectomized birds have been limited to single estimates of metabolic rate and do not consider the effect on

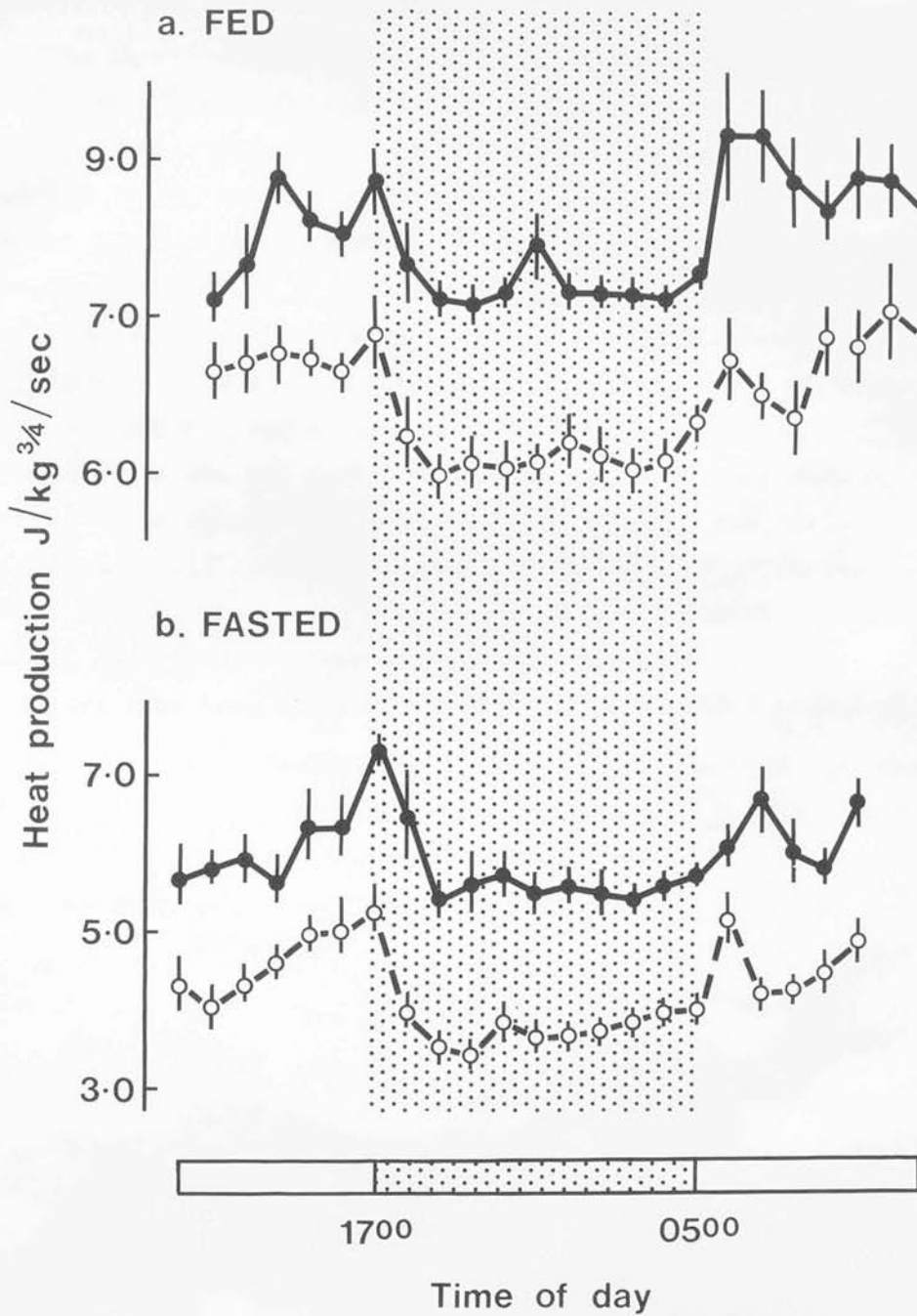
the daily rhythm of heat production. Thus the ability to record sequential changes in energy metabolism with time in the same bird permitted an investigation on the effect of thyroidectomy on the daily rhythm of heat production in fed and fasted immature hens.

The finding of reduced levels of plasma thyroid hormones in thyroidectomized birds led to a study on the capacity of the suspected remaining tissue fragments to respond to handling stress (which causes a decrease in the concentration of plasma  $T_4$  in intact birds; 3.7.1) and to an injection of TRH (which increases the concentration of plasma  $T_4$  in intact birds; 3.5.2). In this manner the possible contribution and responsiveness of these low levels of thyroid hormone to a change in environmental temperature could be assessed.

The conversion of  $T_4$  to  $T_3$  in peripheral tissues has now been repeatedly demonstrated in mammals (Braverman *et al.*, 1970; Fisher *et al.*, 1972).  $T_4$  is thought to have little intrinsic biological activity and to serve primarily as a precursor of  $T_3$  although the fundamental role of  $T_4$  in biological processes is still not fully understood (1.6). Various factors have been shown to regulate the rate of generation of  $T_3$  from  $T_4$ : these include stress, disease, fasting, drugs, goitrogen treatment etc. (3.7). In birds studies have provided evidence for the conversion of  $T_4$  into  $T_3$  in the peripheral tissues during the ontogeny of the chick embryo (Borges *et al.*, 1980) and in thyroidectomized ducks given daily injections of thyroxine (Astier and Newcomer, 1978). Studies on the effect of feeding patterns on daily changes in plasma levels of  $T_3$  and  $T_4$  in chickens suggested that the concentration of  $T_3$  was regulated by the conversion of  $T_4$  into  $T_3$  (3.3). In order to determine how this conversion is manifested the metabolism of  $T_4$  was investigated in thyroidectomized broilers. The generation of  $T_3$  in response to an injection of a physiologic dose of thyroxine was then compared with the response in adult birds.

### 3.8.1 Heat production in fed and fasted thyroidectomized hens

Commercial broiler hens were thyroidectomized (2.4.1) when they were 7 weeks of age. Blood samples were taken from the experimental and control birds before surgery and subsequently at weekly intervals for 2 weeks. Daily variations in heat production were measured 2 weeks



**FIGURE 36** Daily changes in heat production in 5 intact (solid circles) and 5 thyroidectomized (open circles) 9-week-old broiler female hens held at 30°C (relative humidity, 70%) when (a) they had free access to feed and water and (b) after withdrawal of food. The food was removed 24 h before measurements were begun. The vertical lines represent  $\pm$  SE.

after the surgery (2.6.2) with food and water freely available. The birds were then removed from the calorimeter and deprived of food for 24 h; they were returned to the calorimeters for a further 24 h with access to water only in order to measure fasting heat production.

The thyroidectomized hens were lethargic and cold to the touch. Low concentrations of immunoreactive plasma  $T_4$  and  $T_3$  were observed after surgery (Table 13). A daily rhythm of heat production was observed in fed and fasted thyroidectomized hens which was qualitatively similar to that in the control birds (Fig. 36). Heat production fell steeply at the onset of darkness and remained depressed until the beginning of the light period. After the lights came on, heat production increased rapidly to form a small peak; it then began to increase slowly to form a second peak at the onset of darkness. The rate of heat production in the fed thyroidectomized hens was lower than in the fed control birds during the light and dark periods (Fig. 36). This difference could not be accounted for in terms of differences in food intake because the amount of food eaten by the thyroidectomized hens ( $51.0 \pm 13.1$  g) while they were in the calorimeter, was not statistically different from that eaten by the control birds ( $58.4 \pm 10.1$  g).

The effect of fasting the birds for 24 h obviated any possible contribution of food intake on heat production. Fasting lowered the rate but did not alter the pattern of heat production during the light and dark periods in thyroidectomized and control birds (Fig. 36).

Rhythm of heat production observed in intact hens (Schmidt and Roth, 1970; Herman and Kadow, 1973; Lohdy et al., 1973; Hargrave et al., 1976) also occurs in thyroidectomized birds. It is therefore concluded that the rhythm of heat production is primarily associated with changes in locomotor activity and muscle tone (Schmidt and Roth, 1970; Schmidt et al., 1977). The peak in heat production at the beginning and the end of the light period may be caused by the increased activity associated with food-seeking behaviour which occurs at these times (Hargrave, 1976; Hargrave and Harris, 1978). The observation that fasting reduced heat production during the light and dark periods in thyroidectomized birds suggests that a diurnal rhythm of heat production is not entirely dependent on changes in muscle tone. A diurnal rhythm of heat production is observed in intact birds and is associated with changes in locomotor activity and muscle tone.

TABLE 13 - Concentrations of immunoreactive plasma T<sub>4</sub> and T<sub>3</sub> in surgically thyroidectomized 9-week-old broiler hens.

Weeks since surgery <sup>a</sup>	CONTROLS		THYROIDECTOMIZED	
	T <sub>4</sub> (ng/ml)	T <sub>3</sub> (ng/ml)	T <sub>4</sub> (ng/ml)	T <sub>3</sub> (ng/ml)
0	18.0 ± 1.8 <sup>b</sup>	1.92 ± 0.18	24.8 ± 3.3 <sup>b</sup>	1.51 ± 0.26
1	20.1 ± 1.9	2.38 ± 0.30	5.0 ± 0.4 ***	0.14 ± 0.02***
2	16.8 ± 0.7	2.07 ± 0.10	5.8 ± 0.7 ***	0.22 ± 0.01***

a The birds were thyroidectomized at 7 weeks of age and held at 30°C.

b Mean ± S.E., n = 5.

\*\*\* P < 0.001 compared with Week 0.

Thus the depression in heat production in thyroidectomized hens confirms earlier studies (Winchester, 1939; Mellen and Wentworth, 1962; Davison, 1980) and extends these observations to show that the daily rhythm of heat production observed in intact hens (Aschoff and Pohl, 1970; Berman and Meltzer, 1978; Lundy *et al.*, 1978; MacLeod *et al.*, 1980) also occurs in thyroidectomized birds. It is therefore concluded that the rhythm of heat production is primarily associated with changes in locomotor activity and muscle tone (Deighton and Hutchison, 1940; Benedict *et al.*, 1932). The peaks in heat production at the beginning and the end of the light period may be caused by the increased activity associated with food-seeking behaviour which occurs at these times (Savory, 1976; Bhatti and Morris, 1978). The observation that fasting reduced heat production during the light and dark periods in thyroidectomized birds suggests that diet-induced thermogenesis does not require elevated concentrations of thyroid hormone. Spotted munia (*Lunchura punctulata*) represents an exception for neither the intact or

thyroidectomized bird shows a significant circadian rhythm in metabolic rate (Thapliyal et al., 1981). Although food intake has been reported to be depressed in birds after thyroidectomy or treatment with goitrogens (Blakely and Anderson, 1949; Snedecor, 1971; Davison et al., 1980) a similar depression was not found in the present study. This may be due in part to the age of the birds; Davison et al. (1980) reported that birds thyroidectomized at an early age showed a reduction in growth due to reduced food intake. Birds in this study were thyroidectomized at an age when the rate of growth was declining and so the effect would not be as marked. Thyroid hormones are known to be important in the efficient digestion and absorption of ingested nutrients (Kempster and Turner, 1945; Blakely and Anderson, 1949) for Davison et al. (1980) has shown that pair-fed intact birds gain more weight than the thyroidectomized birds.

The finding that very low levels of immunoreactive  $T_4$  and  $T_3$  were present in the blood of the thyroidectomized hens is in agreement with other studies on thyroidectomized birds (Mellen and Wentworth, 1962; Peczely et al., 1980; Davison et al., 1980) and mammals (Obregon et al., 1981). The immunoreactive material could have been generated from non-hormonal iodinated protein (Astier, 1975; Davison, 1976b, 1978) which has been found in the blood of birds. This protein may be iodinated albumin, thyralbumin, which was first found in the hog (Shulman et al., 1955) and subsequently described in other species (Shulman et al., 1957; Stanburg and Janssen, 1962). It has been suggested that thyralbumin could replace thyroglobulin as the precursor of thyroid hormone (Lissitzky et al., 1968; Desai et al., 1977) and thereby generate low levels of thyroid hormones in thyroidectomized animals.

### 3.8.1a Response of thyroidectomized hens to handling and TRH

Commercial broiler cockerels were surgically thyroidectomized at 4 weeks of age (2.4.1). Two and 4 weeks later the birds were injected with 1 mCi  $I^{131}$  and 2 mCi  $I^{131}$  respectively to destroy any remaining thyroid tissue. The experiment was done when the birds were 20 weeks of age. Blood samples were obtained from thyroidectomized and intact birds before being repeatedly handled for a period of 3 h. Blood samples were taken at hourly intervals for 3 h; at this time each bird

was injected with 100 ug of TRH (i.v.) and samples were taken at 30 min. intervals for 90 min. Measurements of the  $T_3$  binding coefficient ( $T_3BC$ ) were estimated by Dr. T. F. Davison (Davison, 1976). The coefficient is obtained from a comparison of the binding properties of the plasma proteins with those of Sephadex G25. An increase in the binding (capacity and/or affinity) of the plasma proteins results in a decrease of the binding coefficient. The free thyroxine index ( $FT_4I$ ) was obtained from the product of the  $T_3BC$  and the  $T_4$  concentration. It is considered to be directly proportional to the concentration of free thyroxine in the plasma (Davison, 1976).

Thyroidectomized birds which were repeatedly handled for a period of 3 h did not show any variations in the levels of either plasma  $T_3$  or  $T_4$  (Fig. 37). The concentration of plasma  $T_3$  but not of  $T_4$  in handled control birds declined and was significantly depressed ( $p < 0.001$ ) within 2 h after the onset of the experiment. Thereafter the levels continued to decline but remained higher than those measured in thyroidectomized birds (Fig. 37). There was no significant effect of handling on the levels of plasma  $T_4$  in control birds, although the levels of plasma  $T_4$  were similar to those measured in thyroidectomized birds.

Thirty minutes after an injection of TRH into control birds the concentration of plasma  $T_4$  had significantly increased ( $p < 0.01$ ) whereas the levels of plasma  $T_3$  were unchanged at this time (Fig. 37). This observation supports the view that the avian thyroid glands contain very little  $T_3$  (Astier, 1975b, 1980). The levels of plasma  $T_4$  continued to increase and were associated with a significant increase in the concentration of free thyroxine ( $p < 0.01$ ; Fig. 37). Similarly the levels of plasma  $T_3$  were significantly elevated 60 min after an injection of TRH ( $p < 0.001$ )<sup>3</sup> and were maximal at the end of the study. There was no effect of TRH on immunoreactive levels of either plasma  $T_3$  or  $T_4$  in thyroidectomized birds (Fig. 37).

There was no significant effect of thyroidectomy on the binding coefficient of  $T_3$  (Fig. 37). The values for both groups are higher than those previously reported except for neonates (Davison, 1976). Thus thyroidectomy does not appear to affect the binding of plasma thyroid hormones to their respective carrier proteins, (Clegg et al., 1959).

The mechanism responsible for the generation of low levels of

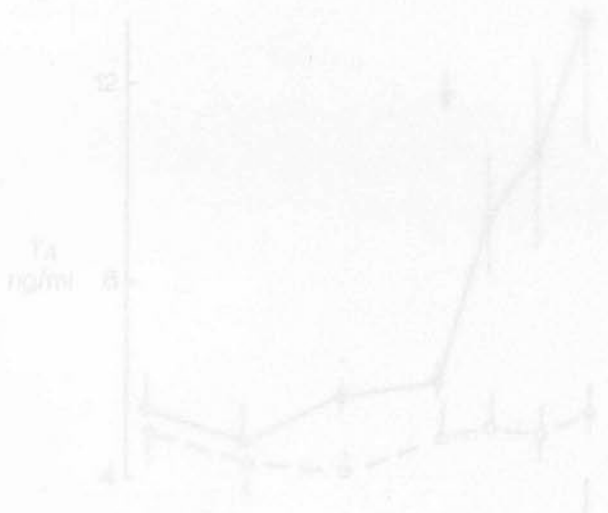
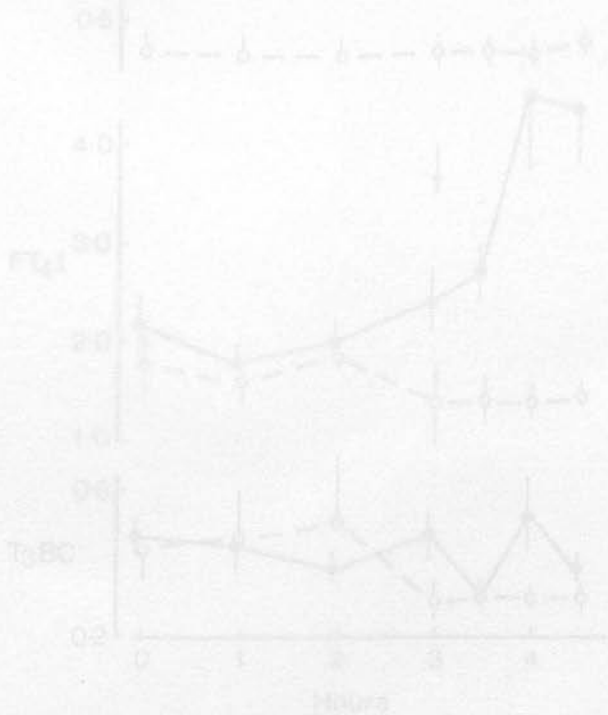
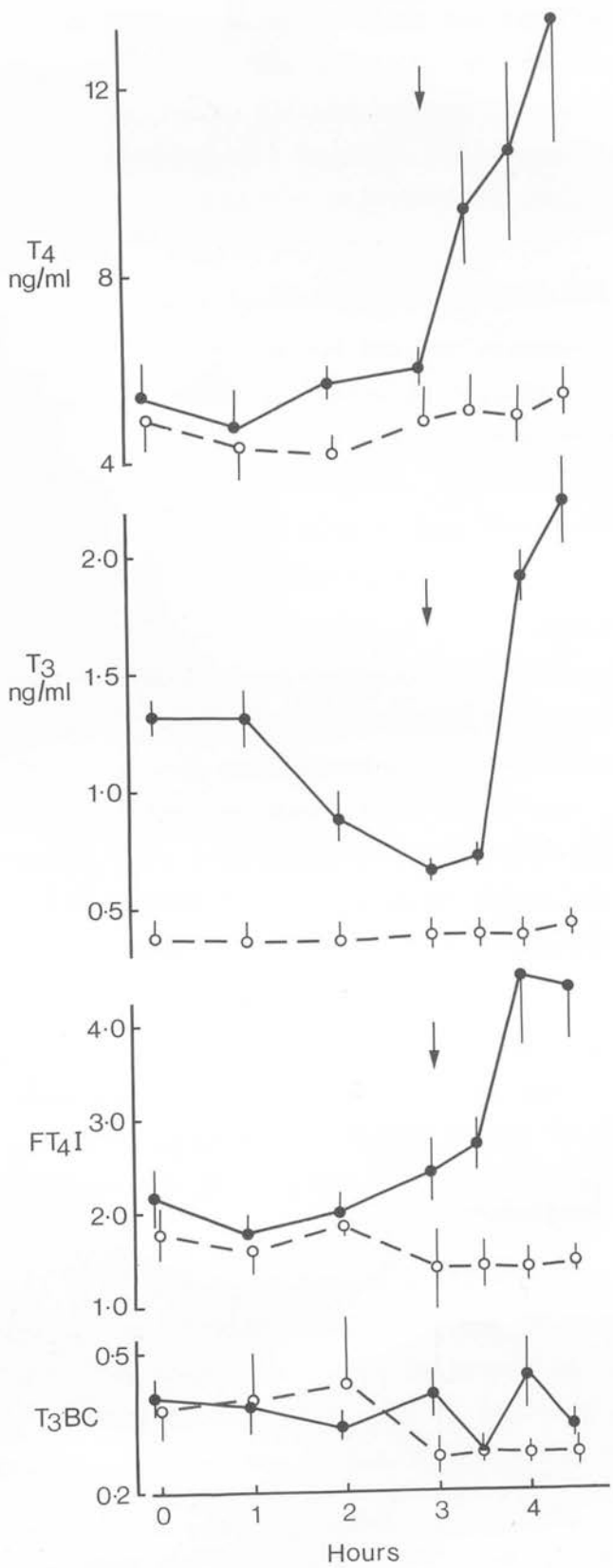


FIGURE 37

Changes in the levels of plasma  $T_4$ ,  $T_3$ ,  $FT_4I$  and the  $T_3$  binding coefficient in thyroidectomized (open circles) and intact (closed circles) 20-week-old broiler cockerels while being repeatedly handled for a period of 3 h. The arrow represents the time each bird was injected I.V. with 100 ug of TRH. Each point represents the mean ( $\pm$  SEM) hormone level for 6 birds.





thyroid hormones in thyroidectomized birds are thus independent of the classic regulatory controls. The inability of the levels of plasma thyroid hormone to respond to TRH-induced TSH stimulation suggests that the birds could not respond to a decrease in temperature and that the source of both  $T_3$  and  $T_4$  is of non thyroidal origin.

### 3.8.2 Conversion of $T_4$ to $T_3$ in thyroidectomized and intact chickens

Experiment 1: Thyroidectomized broiler cockerels (2.4.1.1) were given an i.m. injection of 10 ug thyroxine (0.2 ml per bird in 0.9% saline). Blood samples were obtained immediately prior and 30, 60, 90, 120, 240 and 300 min after the injection. Contamination of the thyroxine preparation (Sigma Chemical Co.) with  $T_3$  has been assessed by Astier and Newcomer (1980) to be not more than 0.13% (0.15 M).

Thirty minutes after an injection of 10 ug thyroxine the concentration of plasma  $T_4$  in thyroidectomized cockerels had increased ( $p < 0.05$ ) from a mean preinjection level of  $4.3 \pm 0.6$  ng/ml to a level of  $6.4 \pm 0.8$  ng/ml (Fig. 38). After the peak concentration had been reached these elevated levels were sustained for the duration of the study. In contrast, the levels of plasma  $T_3$  were seen to steadily increase and reach maximum concentrations 2.0 h after the injection of thyroxine. Thereafter the levels of plasma  $T_3$  remained elevated for the duration of the 6 h period of the study ( $p < 0.001$ ; Fig. 38).

Experiment 2: Immature hens (20 weeks old) were implanted with an arterial catheter (2.5.3) and injected with either 25 ug thyroxine (1 ml in 0.9% saline) or with physiologic saline (1 ml). Blood samples were obtained before and 30, 60, 90, 120, 180 and 360 min after the injection.

The levels of plasma  $T_3$  in intact birds given an i.a. injection of 25 ug thyroxine were maximally elevated within 30 minutes ( $p < 0.001$ ; Fig. 38). Thereafter the levels of  $T_3$  plateaued at an intermediate level for the duration of the study. Birds injected with saline did not show any significant variations in the levels of  $T_3$  throughout the study (Fig. 38).

The finding that the peak concentration of  $T_4$  preceded that of  $T_3$  in thyroidectomized birds is further evidence for the peripheral

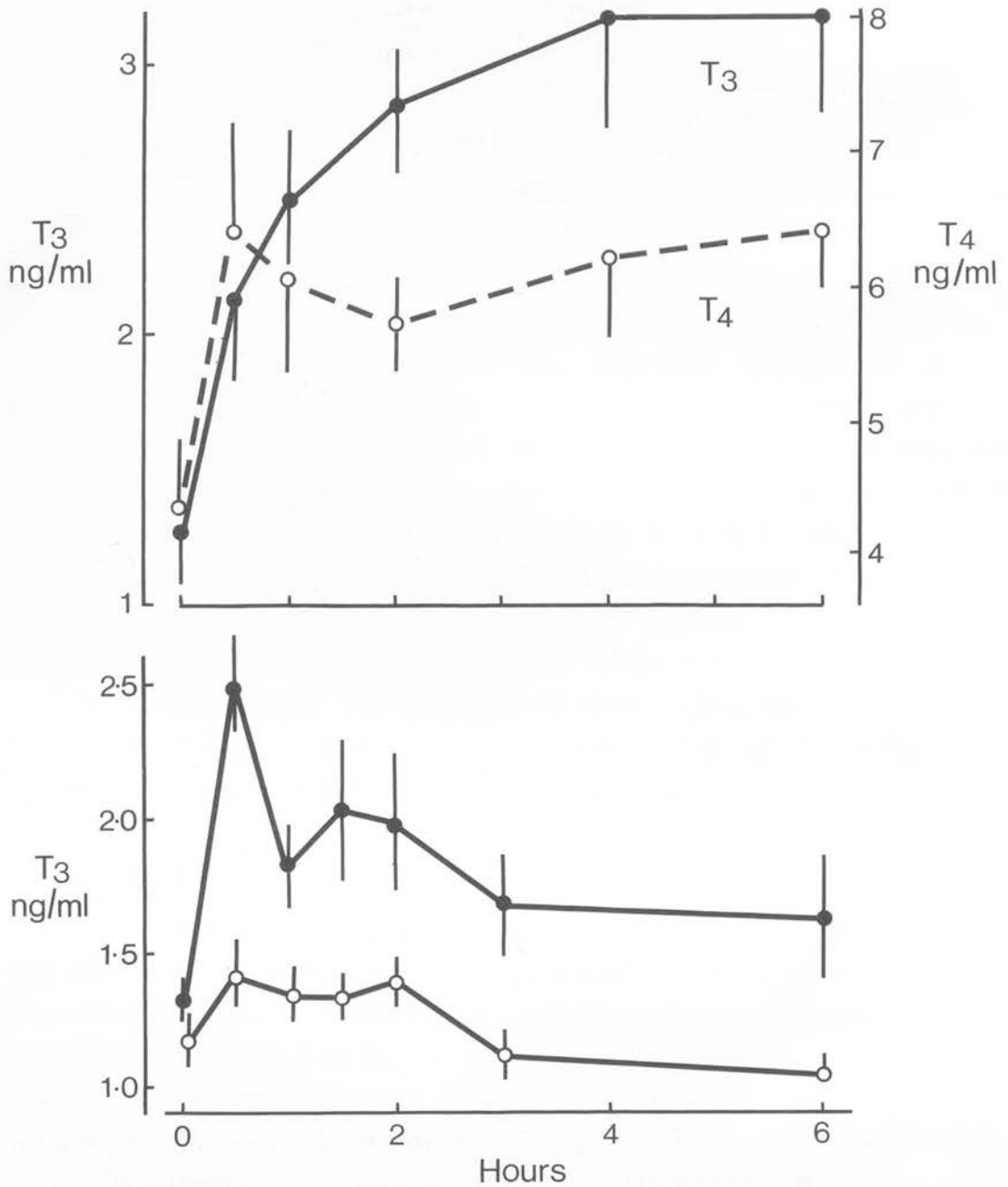


FIGURE 38

Changes in the levels of plasma T<sub>3</sub> (open circles) and T<sub>4</sub> (closed circles) in 20-week-old broiler cockerels after an i.m. injection of 10 ug thyroxine (upper panel). Each point represents the mean ( $\pm$  SEM) hormone level for 6 birds. In the lower panel the changes in the levels of plasma T<sub>3</sub> are shown after an i.a. injection of 25 ug T<sub>4</sub> (closed circles) or physiological saline (open circles). Each point represents the mean ( $\pm$  SEM) hormone level for 8 birds.

conversion of  $T_4$  into  $T_3$ . Furthermore because the distribution space for  $T_3$  in chickens is twice that for  $T_4$  (Singh et al., 1967) the generation of almost equivalent levels of hormone lends further support to this view.

That the bird can convert  $T_4$  to  $T_3$  has been definitely shown by Borges et al. (1980). The mechanism responsible for the generation of  $T_3$  from  $T_4$  has not thus far been elucidated and so it is interesting to consider the pattern of plasma  $T_3$  formation. It appears that the bird is able to regulate the metabolism of  $T_4$ , for although the levels of  $T_4$  remain elevated in the thyroidectomized birds the level of  $T_3$  is seen to plateau and not to further increase. This view is supported by previous studies in which elevated concentrations of plasma  $T_4$  are associated with normal circulating levels of plasma  $T_3$  (3.5.1; May, 1980; Bilezikian et al., 1979). It has been suggested that the peripheral concentration of  $T_4$  can regulate the rate of this reaction. An increase in the concentration of  $T_4$  leads to a reduced conversion rate and so the production of  $T_3$  remains stable over a wide range of  $T_4$  concentrations. Furthermore the response of intact birds to an injection of  $T_4$  was similar to that of the thyroidectomized birds. Thus the magnitude and rate of increase in the concentration of plasma  $T_3$  in response to feeding may be accounted for (3.3).

### 3.8.3 Summary

Thyroidectomy was found to depress the rate of heat production in sexually immature birds during both the light and dark periods. Fasting depressed the levels of the rhythm in heat production in intact hens and depressed it further in the thyroidectomized hens. Additionally fasting reduced the amplitude of the daily rhythm in heat production in intact and thyroidectomized birds. Thus conditions which depress the levels of plasma  $T_3$  are associated with a decrease in the rate of heat production and so supports the view that in the bird concentrations of plasma  $T_3$  and energy metabolism are closely linked.

Low levels of thyroid hormone were detectable in the blood of thyroidectomized birds. These concentrations were not affected by the stress of repeated handling or by an injection of TRH and so suggests that they are of non thyroidal origin.

Studies on the effect of feeding patterns on daily changes in plasma levels of  $T_3$  and  $T_4$  suggested that the concentration of  $T_3$  was regulated by the conversion of  $T_4$  into  $T_3$  in the peripheral tissues. In order to demonstrate this possibility broiler cockerels were surgically thyroidectomized when they were 4 weeks old and 2 weeks later were given a standard dose (10 ug) of thyroxine.  $T_3$  appeared in the blood after the injection of thyroxine; the levels of plasma  $T_4$  were maximal 30 minutes after injection while the levels of  $T_3$  reached their maximum 2.5 hrs later. Thus in birds with an available supply of  $T_4$  in the blood, the magnitude and rate of increase in the concentration of plasma  $T_3$  after feeding may be accounted for.

### 3.9 Role of binding to plasma components

The importance of the binding of thyroid hormones to plasma components has been reviewed in section 1.7. The concentration of the binding proteins has been shown to be affected by age, photoperiod, food intake and disease (Heaf et al., 1980; El-Sayed et al., 1980). In addition such factors as whether a bird is in lay can markedly affect certain plasma components. Hawkins and Heald (1966) have shown that the in vitro synthesis of triglycerides in the livers of laying hens is much greater than what is produced in the liver of the immature pullet. Lipoproteins have been suggested to be the main carriers of thyroid hormone in the chicken and the duck (Castay et al., 1978) and so changes in the concentrations of plasma lipoproteins in a bird might be expected to result in a concomitant change in the levels of plasma thyroid hormones.

Triglycerides are transported to the oocyte of a laying bird as  $\beta$ - lipoproteins in the plasma (Schjeide et al., 1963) and eventually within the fluid yolk compartment of the egg as lipid globules. Although lipoproteins occur in the plasma of immature as well mature birds there is a pronounced lipemia at the onset of vitellogenesis (storage of large quantities of yolk in the maturing oocyte) (Lofts and Murton, 1973; Griffin et al., 1982a) including the production of a specialised type of VLDL which ensures the transfer of lipoproteins to the yolk rather than other tissues (Griffin et al., 1982b). Thus triglycerides

and the component lipoprotein classes might serve as a carrier of thyroid hormones to the yolk and serve as a reservoir for thyroid hormones and iodine in the developing embryo. Indeed Hilfer and Searls (1980) have suggested that most of the thyroxine in the blood of the embryo originates from supplies of maternal hormone deposited in the yolk. The levels of thyroxine in the egg yolk are equivalent to those measured in a laying hen, thus the amount of hormone which becomes available to the blood of a developing embryo is dependent on the amount deposited in the yolk, the amount synthesised by the embryonic thyroid gland and the rate at which the hormone is inactivated (Hilfer and Searls, 1980; Borges et al., 1980).

The purpose of the first study was to determine if there is a relationship between the levels of plasma  $T_3$  and  $T_4$  in immature birds selected for high and low concentrations of plasma triglyceride. In this manner methodological problems encountered in previous studies (1.8) could be eliminated and the results of the present investigation reflective of the in vivo environment in the birds.

In a second study the separate classes of lipoproteins were isolated and the relative affinity of each to  $T_4$  and  $T_3$  was determined. This may lead to a clearer understanding of how thyroid hormones (and iodine) may enter the yolk and play a role in embryonic thyroid function.

### 3.9.1 Correlation of plasma $T_3$ and $T_4$ to plasma triglyceride

Broiler cockerels (Ross Breeder Ltd., Newbridge, Scotland, n = 70) were housed from day-old in floor pens (3.72 m<sup>2</sup>) containing a deep litter of wood shavings (35 birds/pen). The birds were fed a commercial type broiler diet and provided free access to food and water throughout the experiment. They were maintained on a photoperiod of 23 h light/day (lights off between 00.00 and 01.00). Blood samples were obtained between 09.00 and 13.00 h during which period food consumption was unlikely to vary significantly (Cherry and Barwick, 1962).

Blood samples were mixed with sufficient 0.1% EDTA in saline (pH 7.4) to give a concentration of approximately 2 mg EDTA/ml and stored in ice. The concentration of very low density (VLDL) and low density (LDL) lipoproteins were determined by Dr. H. D. Griffin using a semi-automated fluorimetric method (Kessler and Lederer, 1965, Noble and

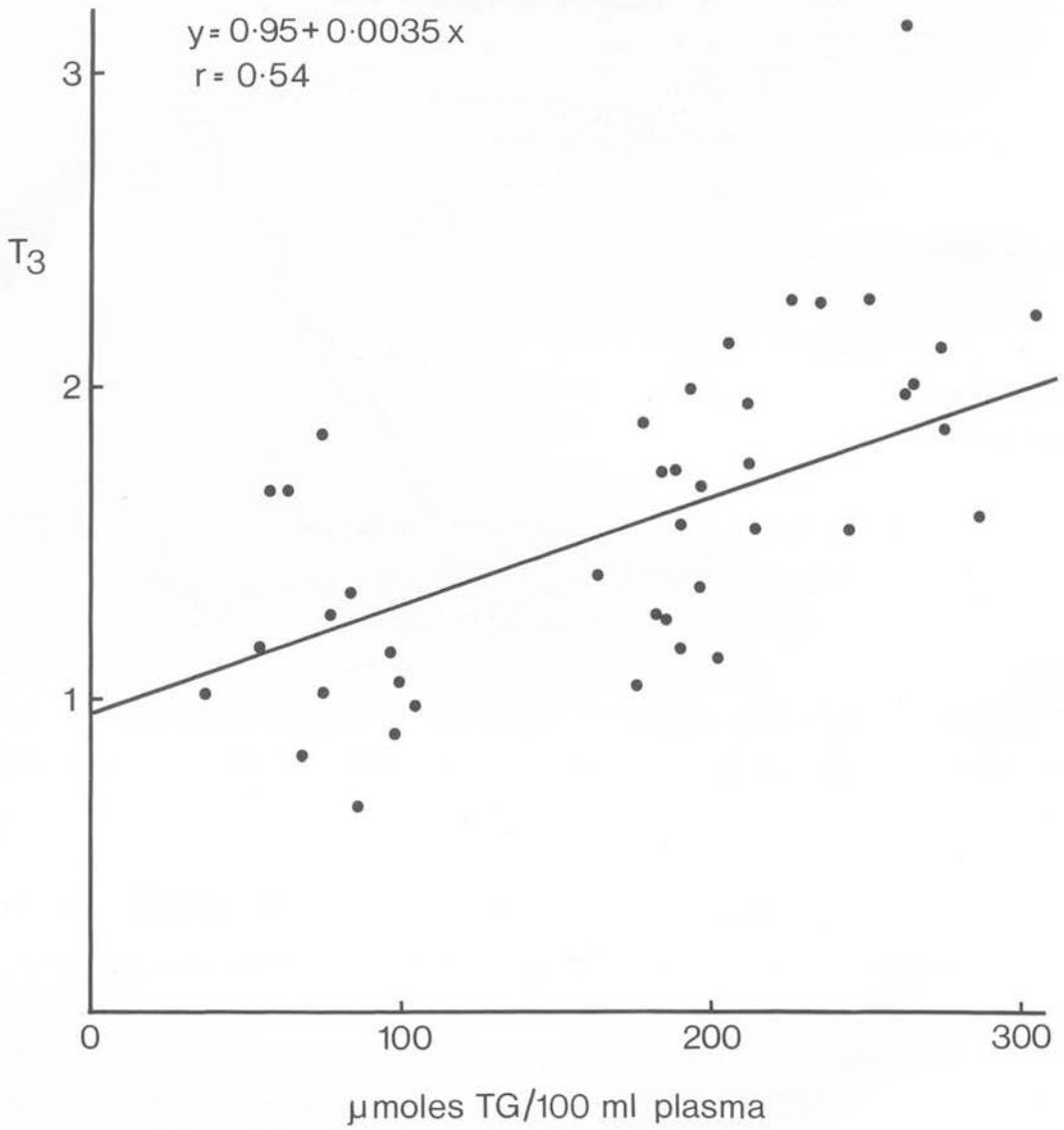


FIGURE 39 The relationship between the concentration of plasma  $T_3$  and the concentration of plasma triglyceride in 8-week-old broiler cockerels.

Campbell, 1970). Samples selected for high and low concentrations of plasma triglyceride were randomly assorted before assay of plasma  $T_3$  and  $T_4$ .

Concentrations of plasma triglyceride varied from 47 to 246  $\mu$  moles/100 ml. No correlation between the levels of plasma  $T_4$  and triglyceride were found (regression line  $y = 12.53 \pm 0.11x$ ;  $r = 0.07$ ) but there was a significant relationship between the concentration of plasma  $T_3$  and triglyceride ( $y = 0.95 + 0.0035x$ ;  $r = 0.54$ ) (Fig. 39).

The results of this study suggest a role for triglycerides in the transport of plasma  $T_3$  in the blood of a bird. This view is supported by the finding that in birds fasting reduces the concentration of plasma  $T_3$  (3.3.1; 3.7.2) as well as the concentration of plasma triglycerides (H. D. Griffin, unpublished observations) while the concentration of plasma  $T_4$  is either unaffected or is seen to increase. In addition thyroid hormone bound to triglyceride could be taken up in tissues such as heart muscle (high lipoprotein lipase activity) (Kaciuba-Uscillao *et al.*, 1980). Thus tissues with an elevated energy metabolism and which require a constant supply of metabolic fuel would have an increase in the available supply of the calorigenically active  $T_3$ . The increase in intracellular  $T_3$  could then accelerate lipid turnover and metabolism (1.6).

### 3.9.2 Binding of $T_4$ and $T_3$ to plasma components

Very low density ( $d < 1.006$ ), low density ( $1.006 < d < 1.063$ ) and high density lipoproteins ( $1.063 < d < 1.225$ ) were isolated by Dr. H. D. Griffin using sequential ultra centrifugation of plasma from immature (10 weeks old) and laying hens (30 weeks old) of the Poultry Research Centre's S-Line (derived from a Shaver White Leghorn-type egg laying strain). Birds were fed a standard commercial diet for laying hens. The birds were kept on a photoperiod of 23L : 1D with the lights off between 00.00 and 01.00 hrs.

Dialysis tubing was rinsed and soaked overnight in distilled water. Each fraction (1.0 ml) was run in quadruplicate with either 0.02 $\mu$ Ci  $^{125}$ I  $T_3$  or 0.025 $\mu$ Ci  $^{125}$ I  $T_4$  added. The tubing was tied with string and inverted 3 times to assure complete mixing before placement in 4.0 ml Tris-EDTA buffer. The tubes were periodically vortexed and allowed to stand for

36 h at room temperature. At this time 0.5 ml of the liquid in the tubing and 0.5 ml of the dialysis buffer were counted for 220 sec. A ratio of the counts remaining in the dialysis tubing versus counts in the buffer was then determined and a relative affinity thus determined. The following components were assayed for binding activity:-

1. Water blank
2. Very low density lipoprotein (VLDL) from laying Thornber's at 1 x plasma concentration
3. VLDL from immature Thornber's at 2 x plasma concentration
4. Low density lipoprotein (LDL) from immature Thornber's at 2 x plasma concentration
5. High density lipoprotein (HDL) from immature Thornber's at 2 x plasma concentration
6. Residue at  $\frac{1}{2}$  x plasma concentration

In the absence of binding to plasma components labelled hormone was found to freely diffuse through the dialysis tubing into the buffer (Table 14) such that the amount of activity left in the tubing was almost equivalent to that in the surrounding dialysis buffer. VLDL in laying and immature birds was found to bind  $T_3$  about 2-fold greater than  $T_4$ . Similarly HDL and the protein residue were found to bind more  $T_3$  than  $T_4$  but when the figures are adjusted to plasma concentration the protein residue contains the principal carriers of thyroid hormone. In addition the levels of  $T_4$  in the plasma are about 10-fold higher than the levels of  $T_3$  (Astier, 1980) and so more  $T_4$  would be available for transport to the yolk. Furthermore the observation that plasma triglyceride concentrations are correlated with the levels of  $T_3$  (3.9.1) and in view of the finding that lipoproteins are transported and concentrated in the yolk of the developing oocyte (Schjeide *et al.*, 1963) these data suggest that HDL and VLDL are responsible in part for the transfer of thyroid hormone to the developing embryo. Egg yolk consists of approximately 30% VLDL; metabolism of yolk during development would release the bound thyroid hormone from the lipoprotein and make it available to the circulation of the embryo. Thus  $T_3$  would be capable of

TABLE 14 - Relative affinity of  $T_4$  and  $T_3$  to plasma components

	Water Blank	VLDL, laying	VLDL, imm	LDL, imm	HDL, imm	Residue, imm
$T_3$	0.90 ±	56.7 ±	6.1 ±	4.1 ±	42.5 ±	48.9 ±
n = 4	0.01	5.1	0.4	0.1	1.6	0.8
$T_4$	0.92 ±	24.8 ±	3.6 ±	2.9 ±	29.7 ±	35.0 ±
n = 4	0.02	1.1	0.2	0.1	0.6	3.4

exerting an effect on development and metabolism and if in sufficient supply would obviate the need for any de novo synthesis of  $T_3$  in the embryo. In support of the view Borges *et al.* (1980) has shown that the embryo preferentially degrades  $T_4$  into  $rT_3$  until the time of pipping and this may be due, in part, to sufficient endogenous levels of  $T_3$ . Thus at the time the requirement for  $T_3$  exceeds the available supply there would be a shift in the metabolism of  $T_4$  from  $rT_3$  to  $T_3$ .

Further evidence which suggests that the yolk is a source of thyroid hormone comes from studies on hypophysectomised embryos. In the absence of TSH normal levels of  $T_4$  were measured in the plasma of these chicks (Hilfer and Searls, 1980; Thommes and Hylka, 1977b; Daugeras-Bernard and Lachiver, 1980). The yolk contains sufficient hormone supply for the continued development and growth of the embryo; thus the plasma lipoproteins are suggested to be one of the factors responsible for the transfer of thyroid hormone into the yolk.

### 3.9.3 Summary

The concentration of thyroid hormone binding proteins regulates both the concentration and the metabolism of plasma thyroid hormones; factors which affect the levels of these proteins are responsible for fluctuations in the total levels of thyroid hormones and thus the amount of hormone available to the target tissues. The levels of plasma triglycerides,

which are important in laying hens, were found to be positively correlated with the levels of plasma  $T_3$  ( $r = 0.54$ ). Plasma lipoproteins (principally VLDL and HDL) were found to bind both  $T_3$  and  $T_4$ . It is suggested that the uptake of lipoproteins in tissues with an increased rate of heat production would thus have increase availability of both thyroid hormone and metabolic fuel. Storage of these lipoproteins in the yolk of oocytes would provide a reservoir of thyroid hormone and iodine for the developing embryo.

### 3.10 Role of genotype

Evidence for a role of the genotype in affecting the levels of circulating thyroid hormones is dramatically illustrated in families with inherited X-linked thyroid binding-globulin (TBG) abnormalities (review Refetoff, 1979). Although even small alterations in TBG concentration result in significant changes in serum  $T_4$  and  $T_3$  concentrations the abnormality is not associated with any metabolic consequences due to the levels of serum free (unbound) thyroid hormone remaining within normal levels. The intact hypothalamo-pituitary axis is suggested to be capable of maintaining normal physiologic function (Hansen *et al.*, 1975) and so, comparably, lack of a specific TBG in man represents the situation found in the plasma of birds. Thus thyroid hormone binding proteins are of secondary importance in the maintenance of normal thyroid function.

In birds there has been extensive work in genetic selection in order to improve the rate of growth relative to food intake, the quality of the egg shell and the rate of lay. Recently, interest has arisen over possible predictive parameters which may be used to select birds for these features in order to breed them into successive generations. In particular the concentration of plasma LH before the onset of lay has been observed to be related to subsequent egg production (Wilson, 1978). Thus it may also be possible to predict egg production in a flock of hens by measuring the concentration of  $T_4$  before the onset of lay. In support of this view the secretion of thyroxine has been shown to be related to the rate of lay and to be involved in the regulation of ovarian development. White Leghorn hens laying four-egg sequences were found to have a higher thyroid secretion rate than hens laying two-egg

sequences (Booker and Sturkie, 1950). Furthermore Turner et al. (1945) showed that the seasonal decline in egg production in the summer was due to a reduction in the secretion of  $T_4$ . Thus the purpose of the first study was to investigate the possibility that a single measurement of plasma  $T_4$  in birds of a pedigreed flock could be a suitable criterion in the selection of laying strains for improved production.

In addition to playing a role in egg production the concentration of thyroid hormone is also known to be related to the quality of an egg shell (1.12). Thus eggs with thin shells are laid by thyroidectomized hens (Taylor and Burmester, 1940), by hens fed a diet containing drugs which depress the secretion of thyroid hormones (Berg and Bearse, 1951; Gabuten and Shaffner, 1954) and by hens exposed to high temperatures (Premovich and Chiasson, 1976). It has also been shown that hens with poor shell quality early in the laying year continue to lay eggs of poor shell quality even after a forced moult (Britton and Washburn, 1978) and so remain a problem for the egg industry. Thus it was proposed to investigate the relationship between shell quality and the levels of plasma thyroid hormone in two pedigreed strains of birds.

The yolk sac of the chick embryo is withdrawn into the abdominal cavity during the last few days of incubation with retraction being complete approximately 14 h before hatching (El-Ibian et al., 1966). Incomplete withdrawal results in chicks with unhealed navels which represents a problem of financial concern to the poultry industry.

Yolk sac retraction occurs both by shrinkage of the extra-embryonic membranes, especially the inner allantoic membranes around the yolk sac, and by constriction which results in its being squeezed through the navel into the abdominal cavity (Wishart et al., 1977). Thyroid hormones are suggested to initiate the process of yolk sac retraction; thiourea retards retraction (Grossowicz, 1946; Adams and Ball, 1949; Balaban and Hill, 1971; Ockleford and Vince, 1980) whereas administration of thyroid hormone accelerates yolk sac retraction without exerting a general effect on the maturation of the embryo (Vidal, 1953; Moog, 1961; Wishart et al., 1977; Ockleford and Vince, 1980).

An increase in the incidence of incomplete retraction of the yolk sac is found in a pedigreed egg laying strain of Ross Poultry Ltd. The

incidence of unhealed navels occurred with greater frequency in the female parent line and not in the male parent line. The purpose of this study was to determine the relationship between the levels of thyroid hormone and the problem of incomplete yolk sac retraction in a pedigreed line of birds. In addition 3 control lines with a low incidence of the unhealed navel problem were also sampled.

### 3.10.1 Prediction of subsequent egg production

The birds used in this study were of two homogeneous parent strains derived from White Leghorn stock and used to produce a commercial hybrid. The male and female strains were designated 030 and 035 respectively. Approximately 200 birds of each strain were transferred to a photoperiod of 17L : 7D (lights on 02.00 to 19.00) when they were 16 weeks of age. Individual records of egg production were kept for the first 270 d of lay (Dr. J. H. Van Middelkoop). A blood sample was taken between 08.00 and 12.00 h from each bird when they were 9 weeks of age.

The mean ( $\pm$  SEM) concentrations of plasma  $T_4$  at 9 weeks of age for all birds in strain 030 and all birds in strain 035 were respectively  $12.4 \pm 0.6$  ng/ml ( $n = 186$ ) and  $12.0 \pm 0.4$  ng/ml ( $n = 202$ ). No significant differences were found between the two strains.

The birds were divided into groups of poor, intermediate and good egg producers at 90, 180 and 270 d of lay (Table 15). On the basis of this classification there was no relationship between the levels of  $T_4$  and egg production in either strain (Table 15). Although  $T_4$  is known to be involved in the regulation of ovarian function in the hen, this study suggests that the levels of  $T_4$  in the immature pullet are not suitable as a genetic marker for increased egg production. This observation is disappointing in view of the current interest in the use of hormone measurements as a marker of fecundity in domestic mammals (Land and Carr, 1978). Possibly the timing of the sample relative to the photoperiod may have influenced the results of the study. Levels of plasma  $T_4$  would be minimal in birds bled in the morning (3.2; 3.3) due to its conversion into  $T_3$  in response to feeding. The study may have been better carried out during the dark period when the levels of  $T_4$  are elevated or, alternatively after the birds had been fasted for 24 h in order to eliminate the diurnal feeding effects on the levels of plasma thyroid hormones.

**TABLE 15** - Concentration (means  $\pm$  SEM) of plasma thyroxine in birds at nine weeks of age in relation to subsequent egg production

Strain	Period of laying (d)	Egg production (per 100 bird d)	Number of hens	Plasma T <sub>4</sub> ng/ml
030	90	$\leq 86.5$	39	12.4 $\pm$ 1.2
		86.6 - 94.5	111	12.8 $\pm$ 0.8
		$\geq 94.6$	34	12.0 $\pm$ 1.0
	180	$\leq 78.5$	20	13.0 $\pm$ 1.6
		78.6 - 88.5	65	11.2 $\pm$ 0.8
		$\geq 88.6$	97	13.2 $\pm$ 0.8
270	$\leq 70.5$	12	11.4 $\pm$ 2.0	
	70.6 - 82.5	57	12.8 $\pm$ 1.0	
	$\geq 82.6$	109	12.6 $\pm$ 0.8	
035	90	$\leq 86.5$	95	12.4 $\pm$ 0.6
		86.6 - 94.5	83	12.0 $\pm$ 0.8
		$\geq 94.6$	20	11.2 $\pm$ 1.6
	180	$\leq 78.5$	43	12.2 $\pm$ 1.0
		78.6 - 88.5	105	12.0 $\pm$ 0.6
		$\geq 88.6$	45	12.6 $\pm$ 1.0
270	$\leq 70.5$	39	13.0 $\pm$ 1.0	
	70.6 - 82.5	79	11.2 $\pm$ 0.8	
	$\geq 82.6$	71	13.0 $\pm$ 1.0	

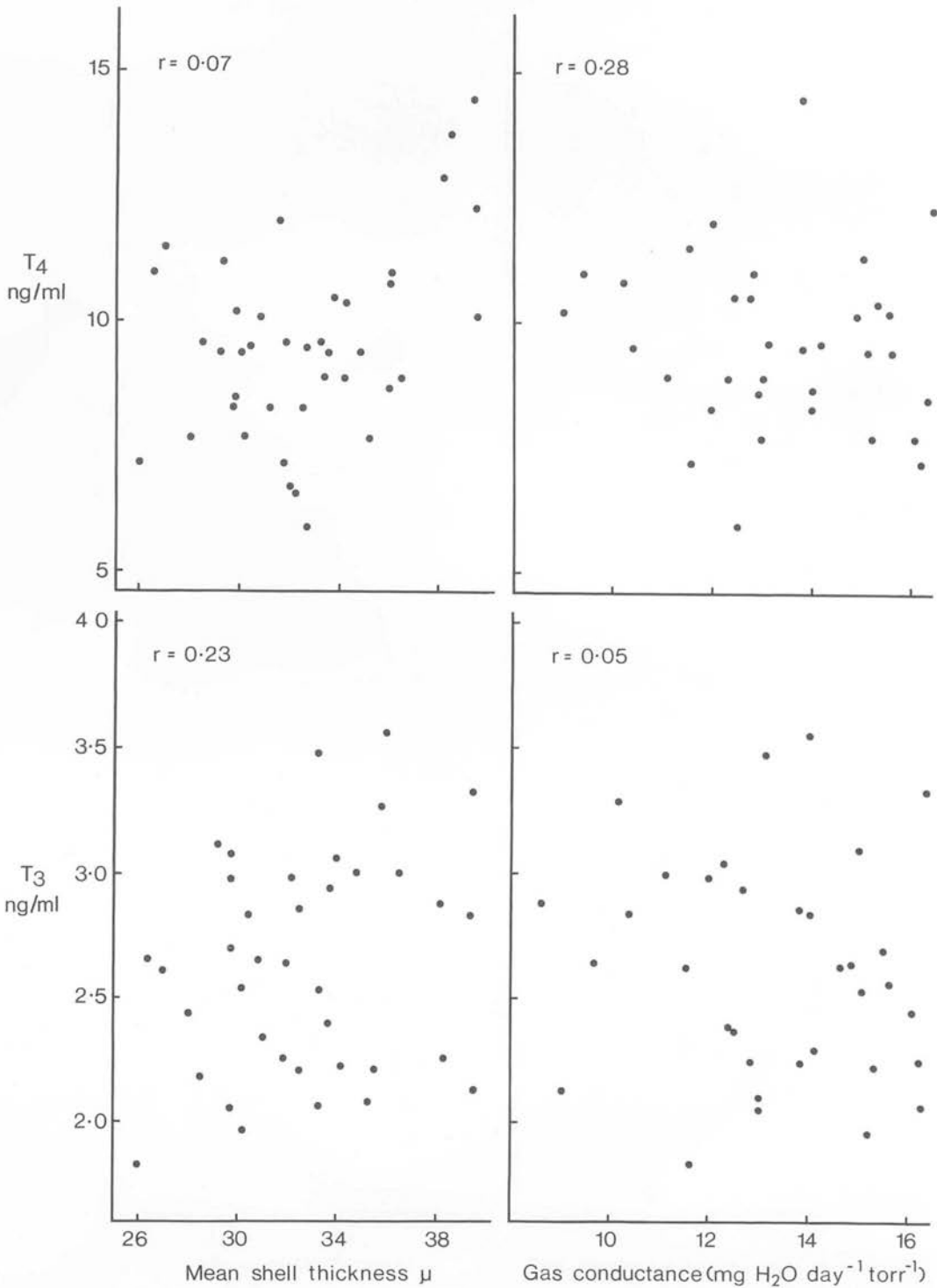
### 3.10.2 Strain differences and egg shell quality

Blood samples were taken from 2 pedigreed strains of hens ( $n = 200$ ) (Ross Poultry Breeders, Gretna main lines 73 and 921) during the time of lay. Birds were selected for sampling on the basis of shell quality. A measurement of specific gravity was obtained on 3 eggs from each bird in the flock and all the results were provided on computer printouts 2 days after the last measurement. Birds with the highest and lowest value of specific gravity were randomly bled and the samples stored for assay of plasma  $T_3$  and  $T_4$ . Additionally one egg from each of these birds was collected for analysis of shell thickness and gas conductance at the Poultry Research Centre. Measurements of gas conductance were carried out by Dr. K. F. Laughlin according to the technique of Rahn and Ar (1974).

The mean ( $\pm$  SEM) levels of thyroid hormone in birds selected on the basis of shell quality are listed in Table 16. Using this parameter there was no relationship between the concentration of plasma thyroid hormones and eggs of good and poor quality, even though there was a highly significant difference between shell quality within each strain of birds ( $p < 0.001$ ) (Table 16). There was no significant difference in the measurement of gas conductance between the two groups. The data were also subjected to linear regression analyses and an example is shown for line 73 (Figs. 40 and 41). There was no significant relationship between the concentration of  $T_3$  or  $T_4$  and shell quality or shell thickness in either strain.

The lack of a relationship between thyroid hormones and egg shell quality is disappointing in view of the numerous published studies which have reported an effect of thyroid status on shell quality (1.12). Furthermore egg shell breakage, which amounts to about 10% of all eggs laid, is due in part to thinner shells which are more easily damaged (Britton, 1980) and correlation of this parameter with thyroid function could have been of benefit to the egg industry. This data suggests that the decline in levels of plasma thyroid hormone with age (3.6.1) is not responsible for the decline in shell quality. It is thus suggested that thyroid hormone may be exerting a permissive rather than an active role in shell synthesis.

There was a significant difference between the concentration of



**FIGURE 40**

The lack of a relationship between the concentrations of plasma  $T_4$  or  $T_3$  and shell thickness or gas conductance in laying hens. Blood samples were obtained from hens selected for laying eggs with a high measurement of specific gravity.

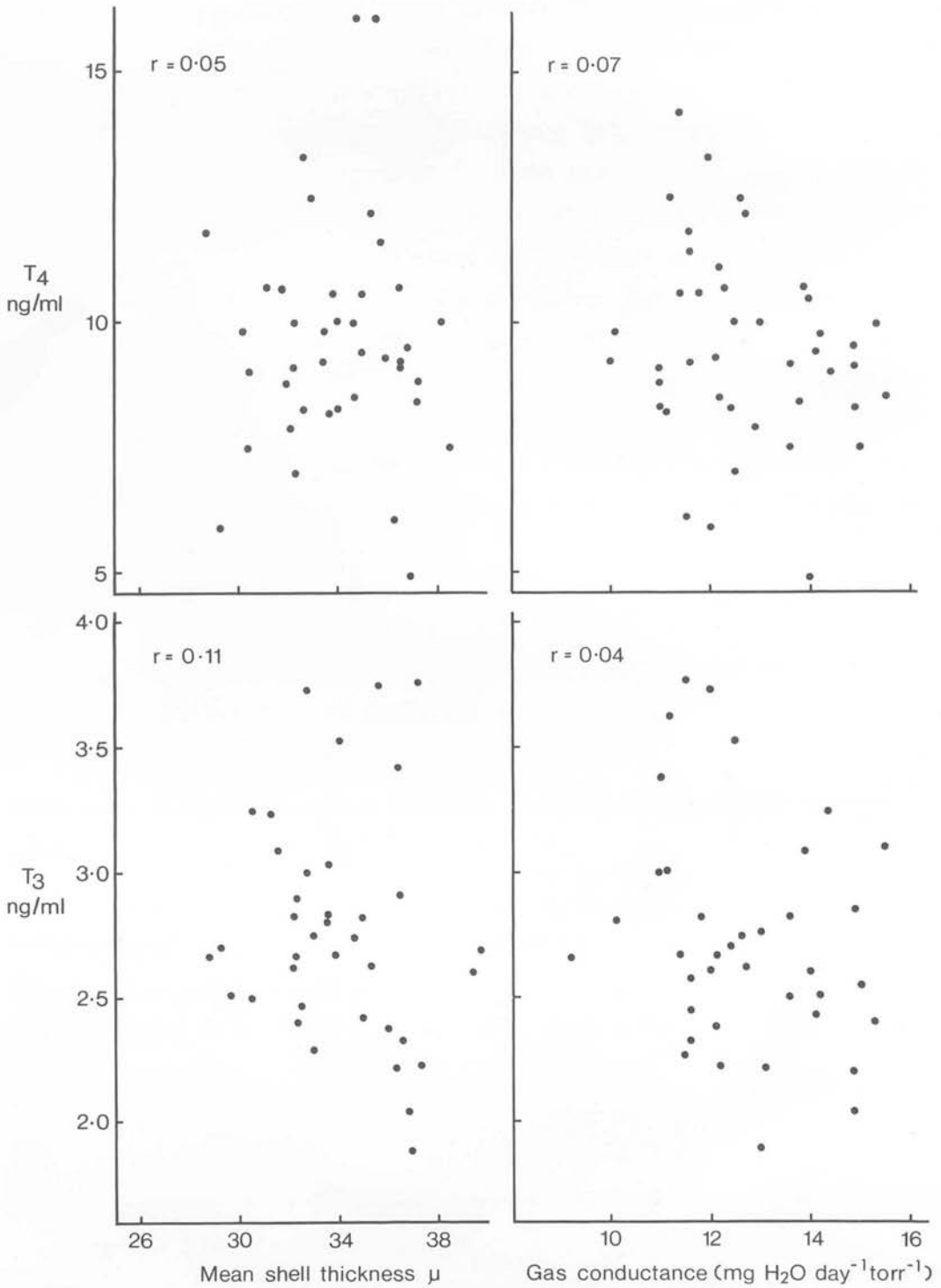


FIGURE 41 The lack of a relationship between the concentrations of plasma T<sub>4</sub> or T<sub>3</sub> shell thickness or gas conductance in laying hens. Blood samples were obtained from hens selected for laying eggs with a low measurement of specific gravity.

plasma  $T_3$  and  $T_4$  and the 2 lines of hens (Table 16;  $p < 0.001$ ). This is the first study to report a difference between the endogenous levels of thyroid hormone within a strain of bird although numerous studies have established a significant difference between the levels of thyroid hormone and the various species of birds (see Astier, 1980). These differences could not have been due to such factors as age, photoperiod, bleeding order or feeding patterns and so suggests a role for the genotype in determining the levels of plasma thyroid hormones within a strain. Furthermore birds in line 73 had higher levels of plasma  $T_3$  than those in line 921 but were found to have lower levels of plasma  $T_4$ . The reciprocal pattern in the levels of plasma  $T_3$  and  $T_4$  supports the general relationship found between these hormones although any significance of these results to the physiology of the bird remains speculative at this time.

TABLE 16 - Relationship between egg shell quality and levels of plasma thyroid hormones

Line	$T_4$ ng/ml	$T_3$ ng/ml	n	Gas Conductance mg/mmHg/d	Shell Quality	Mean Shell Thickness
73	$9.6 \pm 0.3$	$2.83 \pm 0.08$	53	$13.0 \pm 0.3$	$218 \pm 3.9$	$38.3 \pm 0.4$
73	$9.5 \pm 0.3$	$2.59 \pm 0.08$	49	$14.0 \pm 0.4$	$370 \pm 6.7$	$32.6 \pm 0.5$
921	$12.1 \pm 0.05$	$2.24 \pm 0.05$	49	$13.7 \pm 0.3$	$192 \pm 4.5$	$38.0 \pm 0.8$
921	$12.1 \pm 0.4$	$2.27 \pm 0.07$	47	$13.6 \pm 0.4$	$272 \pm 6.4$	$35.8 \pm 0.5$

### 3.10.3 Yolk sac retraction

Plasma was obtained from 2 pedigreed strains of chick embryos (Ross Poultry Ltd.) at the time of hatch. Each of the groups of eggs were incubated together for an identical amount of time at  $38 \pm 0.5^{\circ}\text{C}$  and  $55 \pm 5\%$  relative humidity (Mather and Laughlin, 1976). The female line from the AG strain had been selected for an increased incidence of improper retraction of the yolk sac. Three control lines (no unhealed navel problem) were included in the study, AGM (the male line), ATM and AFT (male and female lines of the AT strain). Blood was obtained by decapitation (2.2.2) and the degree of yolk sac retraction was assessed at this time. The scale ranged from 1 to 5 with a score of 1 implying perfect closure of the navel while a score of 5 indicated a very high degree of protrusion (Laughlin and Mather, 1977).

Incomplete retraction of the yolk sac was recorded in each of the lines of embryos studied but the problem was most severe in the line selected for this trait (Table 17; Fig. 42). In embryos of the male lines AT and AG which had a slight or mild degree of protrusion of the yolk sac there was no significant effect on the levels of plasma  $T_3$  and  $T_4$ . There was an increased incidence and severity of improper yolk sac retraction in the two female lines. This was associated with a decline in the levels of plasma  $T_3$  (Fig. 42) which were markedly depressed in embryo's with the most severe problem (AGF, Group 5,  $p < 0.001$ ). Additionally the embryos in this group also showed a significant ( $p < 0.05$ ) decrease in the concentration of  $T_4$  in comparison to intact embryos of the same line.

It can be seen that the levels of plasma  $T_4$  for the affected AG female line were depressed in comparison to the 3 control lines (Table 17; Fig. 42) whereas the levels of plasma  $T_4$  were highest in the control female line (AT) when compared to the male control lines (AT and AG). Thus these results support the findings of the previous section which measured differences in thyroid hormone levels between different lines of pedigreed birds (3.10.2).

The finding that the concentration of  $T_3$  declined with increasing severity of problem further supports a role for  $T_3$  in the retraction of the yolk sac in the developing embryo (Wishart et al., 1977). The

Figure 42 Relationship between the levels of plasma T<sub>3</sub> or T<sub>4</sub> and retraction of the yolk sac in different strains of chick embryos at the time of hatch. The degree of yolk sac retraction was assessed on a scale from 1 to 5 with a score of 1 implying perfect closure of the navel while a score of 5 indicated a very high degree of protrusion. The number of birds for each group are listed in each histogram. Open bars, group 1; hatched bars, group 2; stippled bars, group 3; solid bars, groups 4 and 5.

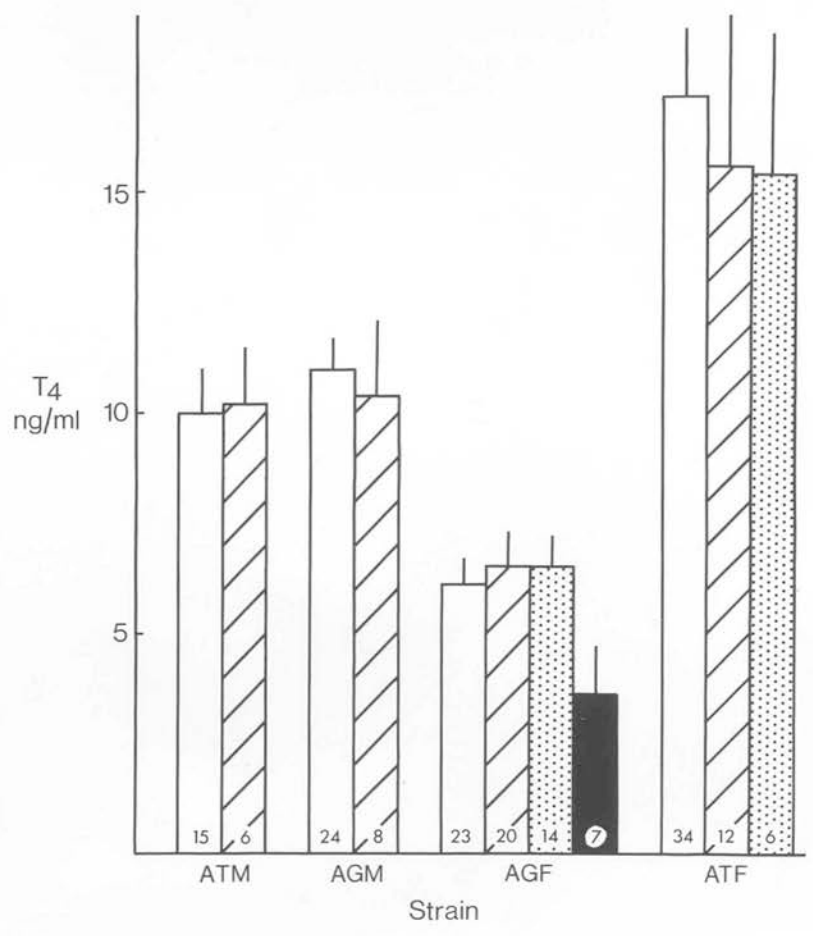
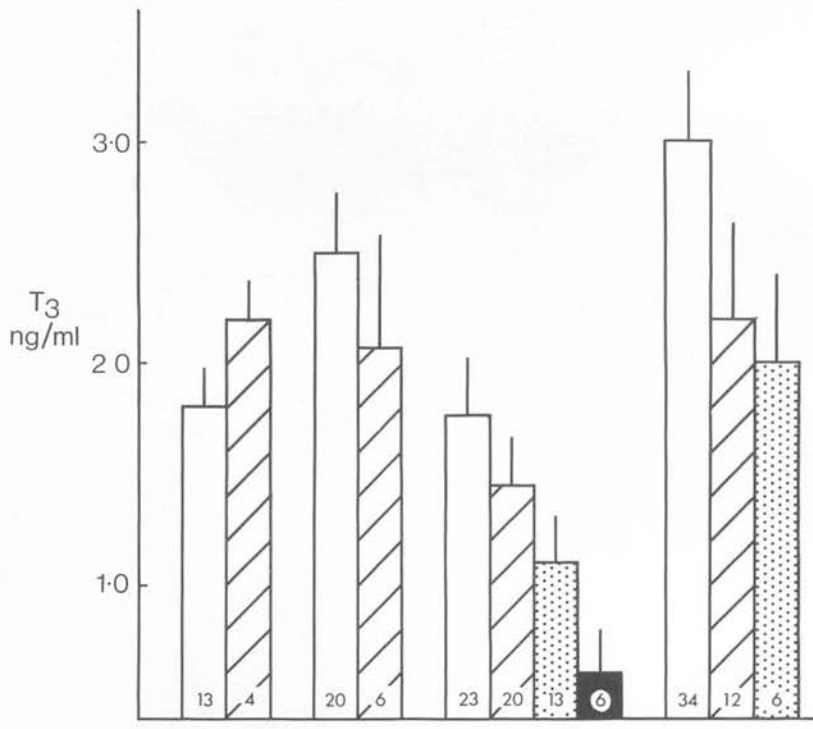


TABLE 17 - Relationship between levels of plasma thyroid hormone and degree of retraction of the yolk sac

Strain	AT male line			AT female line		
	T <sub>4</sub>	T <sub>3</sub>		T <sub>4</sub>	T <sub>3</sub>	
Hormone						
Index of retraction	1	2-3	1	2-3	1	2-3
n	15	6	13	4	34	12
M	10.0	10.2	1.81	2.20	3.01	2.22
S.E.M.	1.0	1.3	0.17	0.17	0.31	0.43

Strain	AG male line			AG female line*		
	T <sub>4</sub>	T <sub>3</sub>		T <sub>4</sub>	T <sub>3</sub>	
Hormone						
Index of retraction	1	2-3	1	2-3	1	2-3
n	24	8	20	6	23	20
M	11.0	10.4	2.51	2.08	1.76	1.45
S.E.M.	0.7	1.7	0.26	0.50	0.22	0.20

\* selected for increased incidence of improper yolk sac retraction

finding that depressed levels of plasma  $T_3$  and  $T_4$  in embryos were associated with a high degree of protrusion of the yolk sac is in agreement with the response of birds and mammals to certain stresses (3.7). The low levels of hormone may in part be due to a reduction in the secretion of TSH in response to an increase in the secretion of adrenal steroids. Alternatively maturational changes which occur in the metabolism of thyroid hormones as the embryos develop may not have transpired. At the time the bird initiates air breathing (internal pipping) the conversion of  $T_4$  is directed to generating  $T_3$  (Borges et al., 1980; Decuyper et al., 1979; McNabb et al., 1981). Normally this process is initiated on the nineteenth day of the 20-21 day incubation period; impairment of this mechanism might have delayed both the retraction of the yolk sac and the hatching process. Studies have shown that if oxygen supply to the developing chick is reduced embryonic heart rate and time of hatch are delayed (Tazawa et al., 1971) presumably due to a reduction in the levels of  $T_3$ .

The time of hatch is associated with dramatic fluctuations in the secretion of a number of hormones including those of the thyroid. Numerous studies have reported an increase in the plasma concentration of thyroid hormone and PBI at the time of hatch (Davison, 1976b; Thommes et al., 1977a, 1977b; King, 1977; Decuyper et al., 1979a, 1979b; Gaspard et al., 1981; McNabb et al., 1981). High plasma levels of thyroid hormone are associated with the beginning of pulmonary respiration and an increase in energy metabolism. Freeman (1974) has suggested thyroid hormone to be the hatching stimulus while other studies have shown that administration of thyroid hormone can accelerate the hatching process (Balaban and Hill, 1971; Oppenheim, 1973) although Ockelford and Vince (1980) suggested that this effect occurs only when eggs treated with thyroid hormone are incubated in contact with each other (synchronising effect of clicking). Alternatively because administration of thyroid hormone did not shorten the interval between penetration of the air cell membrane and the time of pipping, McNabb et al. (1981) suggested that thyroid hormones stimulate hatching through a generalised stimulation of growth and development.

The results of this study suggest that the levels of plasma thyroid hormone are intimately involved in the process of yolk sac

retraction and that the maturational changes in the metabolism of thyroid hormone have not occurred.

#### 3.10.4 Summary

The possibility of a relationship between certain inherited traits and the levels of plasma thyroid hormones was investigated in pedigreed strains of chickens. Plasma levels of  $T_4$  were found not to be useful in predicting subsequent egg production in individual birds and thus was not a useful criteria for selection of egg laying strains.

A relationship between different lines of birds within a strain and concentrations of plasma  $T_3$  and  $T_4$  were established. The levels of hormone were inversely related to each other; thus the line with an elevated level of plasma  $T_4$  was associated with a lower concentration of plasma  $T_3$ .

The concentrations of plasma  $T_3$  and  $T_4$  were not related to shell quality as determined by measurement of specific gravity or gas conductance. Thus thyroid hormone is likely exerting a permissive effect on shell formation.

A relationship was established between the levels of plasma  $T_3$  and improper healing of the navel in selected lines of newly hatched chicks. With increasing severity of problem the levels of plasma  $T_3$  were seen to decline. The levels of plasma  $T_4$  for the affected line were depressed in comparison to the control lines of embryos and were further depressed in embryos with the most severe unhealed navel problem. These studies further illustrate the importance of thyroid hormones in the hatching process and suggest that the maturational changes that occur in the metabolism of thyroid hormone in the developing embryo have not occurred.

#### 4.1 General conclusions

The following discussion centres on the system depicted in Fig. 43 which is presented as a summary of the factors which are proposed to regulate thyroid function in birds.

From the external environment the organism receives a continuous supply of information which includes changes in daylength and temperature. Various environmental stresses are also encountered and ultimately this information becomes integrated in the hypothalamus. The release of hypothalamic neuropeptides in response to these factors can over-ride the normal functioning between the pituitary-thyroid axis. In birds changes in daylength as well as in temperature are examples of physiological situations which can effect the release of hypothalamic thyrotrophin-releasing hormone. Thus an increase in daylength results in an increase in the levels of plasma thyroid hormone whereas in response to an increase in temperature there is a concomitant decrease in the levels of plasma thyroid hormones. In addition these factors also exert pronounced effects on the level and pattern of food intake which in turn effects both hypothalamic function and peripheral metabolism of thyroid hormones.

In environments with low levels of iodine or with diets containing a high level of goitrogen, thyroid function is reduced and certain metabolic disorders may result, particularly during occasions when the demand for thyroid hormone is great (e.g. low temperature, growth and development). Administration of goitrogens to a bird results in a decrease in the levels of plasma thyroid hormones and an increase in follicular cell activity as well as an increase in the concentration of pituitary TSH. Prolonged exposure to goitrogens results in an increase in the size of the thyroid gland, less efficient digestion and utilisation of ingested nutrients as well as other resultant detrimental peripheral effects.

Thyrotrophin is localised in the rostral lobe of the adenohypophysis of the bird and its concentration is dependent on thyroid status: high levels of thyroid hormone depress the concentration whereas low levels result in an increase in the concentration and release of TSH. The concentration of gonadal steroids does not affect plasma thyroid hormone

Figure 43 Schematic overview of the factors proposed to regulate the production and expression of plasma concentrations of thyroid hormone in birds.

ACTH, Adrenocorticotrophin releasing hormone  
CRF, Corticotrophin releasing hormone  
FSH, Follicle stimulating hormone  
LH, Luteinising hormone  
LHRH, Luteinising hormone releasing hormone  
PIF, Prolactin inhibiting factor  
PRF, Prolactin releasing factor  
PRL, Prolactin  
TRF, Thyrotrophin releasing hormone  
TSH, Thyroid stimulating hormone  
T<sub>3</sub>, Triiodothyronine  
T<sub>4</sub>, Thyroxine

FOOD

SENSORY SYSTEM

PHOTOPERIOD  
TEMPERATURE  
STRESS  
NUTRITION

HYPOTHALAMUS

CRF — TRF — PIF/PRF      LHRH

ANTERIOR PITUITARY GLAND

ACTH

TSH

PRL

LH/FSH

LIVER

ADRENAL

THYROID

?

GONAD

ADRENAL  
STERIODS

T<sub>4</sub>

SEX STEROIDS

T<sub>3</sub>

THYROID HORMONE  
BINDING PROTEINS

TARGET TISSUES

[HEAT PRODUCTION]  
YOLK SAC RETRACTION  
DEVELOPMENT

at the level of the pituitary gland for the concentration of TSH in the gland remains unchanged after gonadectomy or administration of implants of testosterone.

The thyroid gland, having evolved as a structure to trap and store iodine, is now considered a reservoir that provides a substrate  $T_4$  for peripheral deiodination into the calorigenically active thyroid hormone  $T_3$ . It is likely that any metabolic affect of  $T_4$  is contingent upon its peripheral conversion to  $T_3$  either by the liver or by its target tissue.  $T_4$  is still considered in the classic sense of a hormone for its release from the thyroid gland is triggered by a message from the pituitary and it subsequently travels to a target tissue where it is metabolised to  $T_3$ .

Peripheral tissues may now be considered an integral part of the thyroid axis in view of their ability to monodeiodinate  $T_4$  into either  $T_3$  or  $rT_3$ . In addition the liver also synthesises plasma thyroid hormone binding proteins which determines the total levels of thyroid hormone in the blood. Although generation of  $rT_3$  represents an inactivation pathway for thyroid hormone, the mechanisms regulating this conversion have not been established. Conditions which elevate  $rT_3$  are associated with disease, age, drugs, starvation, stress, etc.

Production of  $T_3$  is associated with an increase in heat production and heart rate and in birds is most important during growth and development. Food intake regardless of the photoperiod increases the rate of generation of  $T_3$  from  $T_4$  in the liver and is responsible for the inverse daily rhythms in the levels of plasma  $T_3$  and  $T_4$  in chickens. Because birds normally eat only when it is light, food intake is restricted to this period as is also an increase in the concentration of  $T_3$  and a decrease in  $T_4$ . If birds do not receive food then the levels of  $T_4$  tend to remain elevated while the concentration of  $T_3$  remains depressed. Whether the daily rhythm in plasma  $T_4$  contains a circadian element must await the development of a radioimmunoassay to measure plasma TSH. In birds maintained under constant lighting conditions the rhythms in the levels are abolished unless food intake is limited to a particular time of day. Withdrawal of food results in a decline in the levels of plasma  $T_3$  and an increase in the levels of  $T_4$ , while refeeding requires a minimum period of time to elapse for these rhythms to be manifested

and the cyclic pattern of  $T_4$  secretion from the thyroid gland to become apparent. Refeeding after a fast results in an increase in  $T_3$  before  $T_4$  levels begin to decline. This suggests that the primary effect of feeding is to alter the production of  $T_3$ . Prior to feeding, the low levels of plasma  $T_3$  are exerting a minimal feedback effect on the secretion of TSH from the pituitary gland and so the release of plasma TSH and  $T_4$  would be maximal. As the levels of  $T_3$  increase, there is a latent period before the release of  $T_4$  is reduced sufficiently to cause a decreased plasma level. Thus the rhythms may persist in ahemeral lighting cycles but not after transfer to constant lighting conditions.

The concentration of thyroid hormone binding proteins is affected by food intake. Fasting decreases the levels of binding proteins in mammals but this effect has not been studied in birds. Production of thyroid hormone binding protein is markedly affected by the levels of gonadal steroids and is suggested to be a factor responsible for the increase in the levels of thyroid hormones at the end of a breeding cycle. A change in the concentration of thyroid hormone binding proteins results in a concomitant change in the levels of plasma thyroid hormone; thus in quail during a photoinduced breeding cycle the increase in the levels of gonadal steroids would account for the depression in both the levels of binding proteins as well as thyroid hormone. In support of this view is the evidence in gonadectomised quail in which the levels of  $T_4$  remain elevated and do not decline after exposure to stimulating daylengths. Similarly during sexual development food intake is initially high as are the levels of binding protein and so the highest levels of thyroid hormone are measured at this time.

An exception to this general pattern seems to occur at the time the hen is incubating eggs. Although food intake and body weight decline in response to a marked reduction in food intake the levels of  $T_3$  remain elevated. An explanation for the raised levels of plasma  $T_3$  may be the increased levels of plasma prolactin found in incubating hens. Alternatively the reduction in the levels of gonadal steroids might have affected the concentration and affinity  $T_3$  possesses for the various classes of plasma binding proteins. An injection of prolactin into laying and broody birds resulted in an increase in the concentration of plasma  $T_3$  and not of  $T_4$ . These results suggest that prolactin may act

peripherally to increase the metabolism of  $T_4$  into  $T_3$ . The maintenance of a higher level of heat production would decrease the amount of time required for incubation but would result in the loss of a substantial amount of body weight and energy reserve. When the hens are brooding the young the levels of  $T_3$  remain elevated but this observation is likely to be associated with the increase in food intake at this time.

Stress is also suggested to play an important role in the regulation of thyroid hormones in birds. Periods of limited food availability and dehydration cause a decrease in the levels of  $T_3$  and an increase in the concentration of plasma  $T_4$ . Fluctuations in temperature also affect thyroid activity, in part, by a change in food intake and consequently are correlated with measurements in heat production. The response of the adrenal gland to stress is also suggested to affect the activity of the thyroid gland. In mammals the release of norepinephrine from adrenergic nerve endings in the thyroid gland increases the release of thyroid hormone into the circulation. In mammals the release of corticosterone from the adrenal glands inhibits the release of TSH from the pituitary gland and the conversion of  $T_4$  into  $T_3$ . Thus the acute response to handling stress in birds is a decrease in the concentration of plasma  $T_4$  which supports the view that the increase in the concentration of plasma corticosterone at this time results in a decrease in the secretion of TSH. The importance of this mechanism in birds remains to be studied.

In hens the conversion of plasma  $T_4$  into the metabolically active  $T_3$  is a rapid process which very likely accounts for the equipotency ascribed to both hormones in the literature. Injection of  $T_4$  into thyroidectomized or intact birds results in an immediate increase in the levels of plasma  $T_3$  which remains elevated for long periods of time. The factors which regulate this monodeiodination of  $T_4$  into  $T_3$  are actively under investigation today. In mammals the carbohydrate content of the diet determines the degree of stimulation of  $T_3$  neogenesis. Diets containing a high fat content lead to a 'fasting-like' response in the levels of plasma thyroid hormones suggesting that food intake per se does not affect the metabolism of thyroid hormones. Secondly, the level of food intake also regulates the extent of the conversion of  $T_4$  to  $T_3$  for, in mammals, overfeeding is associated with an increase

in the levels of  $T_3$  which results in an increase in heat production. Injection of pharmacological doses of  $T_4$  into ducks results in a markedly elevated level of  $T_4$  and a normal level of plasma  $T_3$ . These findings suggest that the peripheral tissues possess a 'homeostatic wisdom' and can regulate the degree of metabolism of  $T_4$  into the active thyroid hormone. The elevated levels of  $T_4$  would be expected to decrease the secretion of TSH by conversion into  $T_3$  in the pituitary cells.

Ultimately the genotype of a particular species regulates the concentrations and actions of thyroid hormone. The differences between species in the types of plasma binding proteins and their respective affinities for thyroid hormones are important in determining the turnover of thyroid hormone in the circulation and correlate with the concentration of unbound (free) hormone in the blood. These differences also account for the different total levels of plasma thyroid hormone measured between the various species thus far investigated. In addition there are differences in the levels of plasma thyroid hormone between different lines of pedigreed hens. The possible role these differences might have on the physiological expressions of thyroid hormone in the bird remains speculative; they are not useful in predicting subsequent egg production and are not related to parameters such as egg shell quality.

The mechanisms regulating the concentrations of thyroid hormone are suggested to change during development. Thus improper expression of the genotype may be responsible for the increase in the incidence of unhealed navels in a selected line of birds. The finding that maturational changes in the levels of plasma  $T_3$  did not occur in the affected line supports the view that thyroid hormone is important in the retraction of the yolk sac at the time of hatch.

The finding that the concentration of plasma triglyceride is correlated with the levels of plasma  $T_3$  and that lipoproteins bind both  $T_3$  and  $T_4$  is important for the developing embryo. These plasma components in the laying hen are taken up into the yolk and so may form an important source of iodine and thyroid hormone for the developing embryo. Similarly, tissues with a high rate of energy metabolism and which utilise triglycerides and lipoproteins as a metabolic fuel would, in addition, have an increase in the amount of uptake of thyroid hormone

in the cell due to the association of thyroid hormone with these components. Thus, once in the cell, thyroid hormone would contribute to the maintenance of the elevated metabolic rate through the biochemical processes outlined in the general introduction.

In conclusion an intact hypothalamo-pituitary-thyroid-peripheral tissue axis is essential for the production and maintenance of plasma levels of thyroid hormones. The total levels of thyroid hormone in the circulation are reflected by their secretion rate, peripheral metabolism, turnover, and concentration and affinity of binding proteins. The ability of this axis to respond to environmental changes is dependent on all of these factors. Furthermore these factors are themselves regulated by the nutritional status, age, sex, other hormones as well as the genotype of the particular species. Thus the magnitude and duration of a response are different and are regulated in part by the physiological state of the bird. Even though the basal levels of thyroid hormone in the blood vary over a wide range of concentrations the mechanisms which regulate the generation of the metabolically active  $T_3$  are dependent on these factors and ultimately are responsible for its ability to adapt and survive in the environment.

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