THE ROLE OF MYOFIBRILLAR ATPase AND THYROID HORMONES IN THE DEVELOPMENT OF THERMOREGULATION IN THE DOMESTIC FOWL, (*Gallus domesticus*)

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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ABSTRACT

The development of homeothermy was studied in the domestic fowl by measuring changes in oxygen consumption ($\dot{V}O_2$) and body temperature (T_{re}; chicks only) with decreasing ambient temperatures (T_a) during the last week of incubation for embryos and first week of life for chicks. During the same period, myofibrillar ATPase (m-ATPase) activity and thyroid hormones (Thyroxine, T_4; and triiodothyronine, T_3) were also measured.

$\dot{V}O_2$ for 15, 16, and 17 day old embryos diminished with decreasing T_a. Embryos at 18, 19, 20 and 21 days, initially maintained their $\dot{V}O_2$ for decrements of 3, 3, 4, and 5°C in T_a respectively, but their $\dot{V}O_2$ fell with decreasing T_a thereafter. For chicks, $\dot{V}O_2$ increased with decreasing T_a upto a peak and then fell with further decrements in T_a. The difference between the initial and final T_{re} taken before and after the experiments decreased as the chicks became older.

Myofibrillar ATPase activity increased with incubation to reach a critical value in 18 day old embryos and thereafter undulated about the same level. Both T_3 and T_4 levels increased exponentially during the last week of incubation and peaked at hatching. T_4 fell below prehatching levels to a nadir 2 days post-hatching and thereafter rose slowly, while T_3 never fell below prehatching levels. T_3/T_4 ratio increased exponentially from day 19 of incubation and peaked 2 days post-hatching.

It is concluded that up to day 17 of incubation, chicken embryos are poikilothermic and incipient endothermic homeothermy starts on day 18. At hatching, the qualitative adult homeothermic response is present and it approximates that of adults 7 days post-hatching. The strong correlation between $\dot{V}O_2$ and m-ATPase during the embryonic stages and the critical value of m-ATPase activity attained on day 18 suggests a critical role for m-ATPase activity in the development of thermoregulation. Similarly, a stronger correlation between $\dot{V}O_2$ and T_3 than T_4 within the period of study suggests a greater role for T_3 than T_4 in the development and maintenance of homeothermy. T_3 and T_4 also play an important role in the hatching process as indicated by their peak values then. The increasing T_3/T_4 ratio suggests increased conversion of T_4 to T_3 around the perinatal period.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

In the adult stages, birds like mammals are homeotherms. They can maintain a relatively constant deep body temperature. This thermoregulatory ability requires that heat production by the body and heat gain from the environment equal heat losses from the body. The domestic fowl (*Gallus domesticus*) is a precocial species - meaning that its thermoregulatory mechanism is developed at or before the time of hatching. The ability to thermoregulate is linked to the ability to increase metabolic heat production in the face of cold exposure. The increase in heat production during cold exposure is in turn reflected in an increase in oxygen consumption ($\dot{V}O_2$). The development of the ability to thermoregulate is however dependent upon fundamental tissue and organ changes i.e a well developed mature and functional thermoregulatory system. Shivering thermogenesis which involves involuntary muscle contraction is very important in heat production during thermoregulation in cold environments, and so plays an important role in the development of endothermy.

Central to the transition from poikilothermy to homeothermy is the acquisition of sufficient thermogenic

capacity to balance the heat losses to the environment. Metabolic compensation during cold exposure has been used as an indicator of homeothermy for many years, (Romanoff, 1941; Freeman, 1964; Vleck and Hoyt, 1979; Booth, 1984 and Mathiu, 1988). They have found that maintenance of a constant deep body temperature during cold exposure corresponds to an increase in oxygen consumption ($\dot{V}O_2$), thus increased heat production.

The thermogenic increase in $\dot{V}O_2$ during cold exposure when homeothermy is achieved demonstrates that the thermoregulatory apparatus is functional in the developing embryos. The thermoregulatory apparatus (thermosensors, controllers and effectors) in turn depends on functional neurotransmission, adequate functional enzyme systems and hormonal profiles. These fundamental and basic components, which are a pre-requisite to thermoregulation have not been studied much in relation to development of thermoregulation. During the contractile process, myofibrillar ATPase (m-ATPase) breaks down Adenosine triphosphate (ATP) to Adenosine diphosphate (ADP) and inorganic phosphate (Pi). Therefore, m-ATPase is an important enzyme in the mechanism of heat production during thermoregulation.

Thyroid hormones are also known to be important in enhancing thermoregulation by increasing metabolic rate, thus heat production by increasing the ATPase activity of the

sodium potassium cellular pumps (Ismail-Beigi and Edelman, 1970) and also by enhancing gluconeogenesis during exposure to cold, (Nakagawa and Nagai, 1971).

The question then arises; could low levels of thyroid hormones or myofibrillar ATPase activity be a limiting factor in the ability of the developing animal (homeotherm) to thermoregulate? In other words, could it be that during development, an increase in the levels of m-ATPase and/or thyroid hormones above a certain threshold corresponds to the attainment of the ability to thermoregulate? These are the questions that formed the basis for this study.

In this study, the thyroid hormone levels in circulation and the m-ATPase activity in the pectoral muscles of late incubation stage chicken embryos, hatchlings and chicks were measured. The above measurements were then related/compared to the development of thermoregulation evidenced by the ability of the embryos/hatchlings/chicks to either maintain or increase their $\dot{V}O_2$ when exposed to cold.

1.2 LITERATURE REVIEW

1.2.1 **Ontogeny of thermoregulation in birds**

(a) <u>General</u>

The establishment of thermoregulatory capacity at low and moderate ambient temperatures in developing birds is linked to a rise in metabolism and with the acquisition of

abilities to augment heat production beyond this level (Dawson and Hudson, 1970). The timing of these changes is linked to the mode of development, altricial or precocial characterising the particular species. Birds are classified as precocial or altricial depending on their themoregulatory ability at the time of hatching (Vleck and Hoyt, 1979; Vleck *et al.*,1979a). Nice (1962) classified developmental modes of birds into precocial, semi-precocial, semi-altricial and altricial.

At hatching, precocial chicks are mobile, can feed themselves, have open eyes and are covered by down. Hatchlings in the galliforme family megapodidae which have flight feathers and need no post hatching parental care are the precocial extreme. Altricial hatchlings such as those of the passerine birds are naked, have closed eyes and are completely dependent on their parents for nourishment and thermoregulation. Species whose hatchlings lie in between these extremes are classified as either semi-altricial or semi-precocial (Vleck and Vleck, 1987).

The emergence of homeothermy in birds depends on the development of several physiological and physical features including; metabolic rate, body weight and surface area, insulation, evaporative water loss via panting, the development of endocrine glands like the thyroids and behavioral thermoregulation. Thermoregulatory abilities of newly hatched precocial birds vary considerably among orders, generally being greatest in Anseriformes, intermediate in

Galliformes and least developed in Charadriiformes (Ricklefs, 1974). Adult body temperatures vary between 39°C to 42°C. The thermoregulatory abilities of precocial birds are largely a function of their capacity for heat production (measured as BMR and thermogenic capacity) and their ability to restrict heat loss (insulation and vasomotor control of extremities). Body size through its allometric relation to both metabolic rate and insulation also contributes to thermoregulatory abilities.

Chicks vary greatly in their thermoregulatory capacities immediately after hatching. In precocial species, immediately after hatching, T_b may drop to below 30°C because down feathers are wet and a lot of heat is lost due to evaporative cooling (Pembrey *et al.*, 1895; Freeman, 1963). But T_b increases to reach adult levels in about 3 weeks (Freeman, 1963). The increase results in part from an increase in the chicks metabolically active mass and metabolic rate without a commensurate increase in surface area and partly due to an increase in set point temperature and insulation (Ringer, 1976). The highly precocial hatchling of the Mallee fowl (*Leipoa ocellata*) has been found to increase its heat production 3 times during cold exposure and to thermoregulate over a wide range of temperatures, 3° to 46°C (Booth, 1984). Features such as evaporative water loss, excellent insulation and its large body size also helped in this thermoregulatory ability.

Thermoregulatory ability of the Xantus murrelet (*Synthliboramphus hypoleucus*) has been investigated (Eppley, 1984) from hatching up to 2 weeks. Hatchlings were found to increase their metabolism 3.5 times at cooler temperatures. Basal metabolic rate (BMR) increased by a factor of 2.7 during the next 2 weeks. Thermal conductance of hatchlings approached predicted values for passerines within 4 days. Xantus murrelet chicks used partial hypothermia at low temperatures to reduce energetic cost. They showed tolerance to severe hypothermia and could sustain T_b as low as 26°C without motor impairment.

The Slender billed shearwater (*Puffinus tenuirostris*) chicks were found to have sufficiently adequate thermoregulation to maintain essentially adult T_b of 38°-41.5°C in their burrow environment of 22°C (Farner and Serventy, 1959). The thermoregulatory abilities of *Puffinus tenuirostris* was found to be comparable to many other precocial species. They were comparable to those of the Common Eider (*Somateria mollissima*) in which thermoregulation is well established 2-7 hours after hatching (Rol'nik, 1948). Thermoregulation ability appears to develop earlier in *Puffinus tenuirostris* than in such galliformes as *Gallus domesticus* (Randall, 1943), the quail, (*Cortunix cortunix)* and the Ring-necked pheasant, (*Phasianus colchicus*; Ryser *et al.*, 1954).

In the Manx shearwater (*Puffinus puffinus*), chicks below 80g, oxygen consumption increased 2.65 times when ambient temperature was lowered from 32°C (thermoneutral) to 5°C. This increase decreased with age and was only 53% for adults subjected to the same temperature change (Bech *et al.*, 1982). This improved thermoregulatory ability was attributed to increased thermal insulation. In the young of Pelicans (*Pelicanus occidentalis californicus*), the Great Blue heron (*Ardea herodias treganzai)* and the Western Gull (*Larus occidentalis*), homeothermy was found to increase with the development of down (Bartholomew and Dawson, 1954).

Bartholomew and Dawson (1952; 1954) observed that chicks of the Western gull (*Larus occidentalis*) develop fairly good thermoregulatory ability by the end of the first day and after 3 days can maintain near adult thermoregulation except under severe conditions. Dunn (1976) has shown that in the Herring gull chicks (*Larus argentatus*) the T_b of the chicks can be effectively controlled after 3 to 4 days, but the pattern of $\dot{V}O_2$ continues to change as the chicks grow, with decreasing metabolic effort being required at low T_a. This also fits the pattern shown for growing chicks of all developmental modes (Ricklefs, 1974).

The newly hatched Western gull chick can shiver and may also augment its heat production by non-shivering thermogenesis (Dawson and Bennett, 1980). The ability of

hatchling Western gulls to remain homeothermic when unbrooded by a parent in a moderate to cool environment depends primarily upon capacities for chemical thermoregulation (Dawson and Bennett, 1980). Thermogenesis in gulls appears primarily through shivering. Evaporative water loss and panting are also important mechanisms in thermoregulation in the hatchling Western gulls (Dawson and Bennett, 1980). However the mechanism of non shivering thermogenesis is not fully understood and may in some respects differ from that of mammals (Freeman, 1977). Chicks do not have brown fat, an important site for non-shivering thermogenesis in mammals. The role of thyroid hormones, norepinephrine and glucagon in non shivering thermogenesis has been postulated for different species (Dawson, 1984; Freeman, 1966; 1969; 1971b). Thyroid hormone is also known to be calorigenic in the neonatal Domestic fowl (Freeman 1970; 1971a).

In the Japanese quail, another precocial species, the rate of cooling of the chick during exposure to an air temperature of 30°C for 30 minutes has been shown to decrease from the 3rd to the 11th day post hatching. This indicated an increase in thermoregulatory ability during this period that was correlated with a diminution of heat seeking behavior and increased thyroid gland activity (Spiers *et al.*, 1974). In the Painted quail the attainment of homeothermy represents decreasing rates of evaporation especially from the skin and

increasing rates of metabolic heat production (Bernstein,1971). An increase in heat production rather than increasing insulation is known to be the key to the improved ability of the growing chick of the Carpercaillie (*Tetrao urogallus*) to respond to cold (Hissa *et al.*,1983)

Pectoral muscle development was shown to correspond to an increase in heat production by shivering in the Willow ptarmigan, (Aulie, 1976b). Fourteen day old embryos of the Willow ptarmigan showed no signs of thermoregulation (Aulie and Per Moen, 1975) while in the fully developed embryo a 10°C drop in ambient temperature decreased the metabolic rate by only 26% indicating a thermoregulatory response. Newly hatched (wet) chicks were unable to increase their metabolism when exposed to 29°C. Half a day old chicks increased their metabolism by 45% at this temperature. No sign of shivering was observed and thermogenesis is thought to be due to increased muscular activity. At one week old the Willow ptarmigan had a lower critical temperature of 30°C (Aulie and Per Moen, 1975). This was 4°C lower than that of the domestic fowl of 34°C at the same age (Freeman, 1963), suggesting that the Willow ptarmigan has a better insulation and/or that their behavioral temperature regulation is better developed than in the domestic fowl at this stage.

In the semi-precocial gulls, an elevation of heat production occurred in the newly hatched chick during exposure to cold. The increase varied from 50-100% of the

basal metabolic rate (Palokangas and Hissa, 1971; Dawson *et al.*, 1976; Dawson and Bennett,1980). In the semi-precocial California gull (*Larus californicus)* chicks were found to respond to cold temperatures of 6-10°C by increasing $\dot{V}O_2$ 160-200 % while high temperatures beyond 39-40°C caused increased rates of evaporative water loss via panting, (Chappell *et al.*,1984). Metabolic rate of precocial species increases rapidly in the first 80% of incubation and then increases slowly, stabilises or actually declines in the remainder of the period before hatching (Vleck *et al.*, 1979a; 1979b; 1980). Species in which metabolic rate declines in the last 20% of development include, Rhea (*Rhea americanus)* and Emu (*Dromecius novaehollandiae* ; Vleck and Hoyt, 1979; Vleck *et al.*,1980), and the Ostrich, (*Struthio camellus)* Hoyt *et al.*, 1978).

In altricial birds, embryonic metabolic rate (per embryo) increases at an accelerating rate throughout incubation (Vleck, 1978; Vleck *et al.*,1979b) and the acquisition of thermoregulatory ability appears to be related to heat producing tissues notably skeletal muscle (Dunn,1975). Growth is accompanied by the development of plumage and reduction in surface area relative to body mass. These factors help to curtail heat loss and so facilitate the regulation of body temperature. In the nestling Bank swallow (*Riparia riparia*) an effective thermoregulatory response to cold is more closely related to increase in body mass than to the

development of plumage. Initially the increased resistance to cooling is due to increase in body mass rather than an improvement in metabolic regulation (Marsh, 1979). Dunn (1975) has shown that the greater the rate of growth the earlier the age at which altricial chicks are able to maintain their body temperatures during exposure to cold. Growth rate, she concluded was the best predictor of endothermy in altricial nestlings.

Some altricial species like the Cattle egret *(Bubulcus ibis)* have evaporative cooling mechanisms operative on the first day of hatching (Hudson *et al.*,1974). Nestling Cattle egrets were able to lose by evaporative cooling, heat equivalent to more than double their metabolic heat production (Hudson *et al.*, 1974). In general altricial nestlings develop the ability to respond to heat stress before that of cold (O'Conor,1975). Morton and Carey (1971) have shown that in the mountain White Crowned sparrow, nestlings were not able to respond to cooling before day 4, but were able to pant on exposure to heat on day one. Dawson and Evans (1957) have shown that the Chipping sparrows develop effective temperature control at moderate environmental temperatures (20-25°C) at approximately 7 days post hatching. Before this they are poikilothermic.

Generally, studying thermoregulatory abilities of the eggs/embryos and hatchlings also involves defining the thermal limits of growth and development at this stage, and

the identification of the acute effects of variations in egg/hatchling temperature and any compensatory mechanisms that help to dampen these effects. The incubating adult bird serves as a heat source for the eggs under cooler circumstances, but may also serve as heat sink in very hot environments (Russel, 1969; Grant, 1982). Despite thermal protection from the parents for the incubating egg, sometimes fluctuations in egg temperature do arise. Birds have therefore developed thermal tolerance to varying extents. In the week old embryos of the Heermans gulls (*Larus heermanni*) for instance, incubation temperatures approximate 37°C. When warmed to 41.1°C blockage of heart action occurs (Dawson, 1984). The blockage is reversible even after an hours exposure to 42-43°C, but temperatures above 43°C are lethal. Embryos of the domestic fowl cannot survive continous exposure to temperatures lying outside the range 35-43°C (Lundy, 1969)

The general thermal sensitivity of metabolism or heart rate of ectothermic embryos can be assessed via consideration of the relevant temperature coefficients (Q_{10}) for various intervals. Q_{10} seems to decline with increasing temperatures (Scholander *et al.*, 1953; Dawson, 1967). The coefficient ranges from 3.6 to 15 in embryos between 10 and 15°C, but only from 1.1 to 2.0 between 35 and 40°C in which the incubation temperatures of birds lie. Embryos of only two species come close to complete independence in their

metabolic and heart rates, the domestic fowl and the Heermans gull (*Larus heermanni*). Below 30°C these parameters are strongly temperature dependent (Bennett and Dawson, 1979). In the domestic fowl Q_{10} for heart rates between 30 and 40°C and oxygen consumption between 34 and 40°C are 1.2 and 1.0 respectively (Romanoff, 1960; Grieff, 1952). In the Heermans gulls Q_{10} for heart rate between 35° and 40°C and oxygen consumption between 30 and 40°C is 1.1 and 1.0 respectively. The relatively high Q_{10}'s for metabolism and heart rates of avian embryos below 25°C shows the sensitivity of these processes to moderate and cool temperatures and may contribute to the cessation of development that occurs under prolonged cooling.

(b) Domestic fowl (*Gallus domesticus*)

During the incubation period, eggs may be exposed to excessive heat or cold depending on the care that they receive from the parent birds. During the first 10 days, the evaporative heat loss from the artificially incubated egg of the domestic fowl exceeds the heat production, so that the temperature of the egg may be slightly lower than that of the environment (incubator). Towards the end of incubation the major avenue of heat loss from the egg is non-evaporative. After about the mid-point of incubation in chicken eggs, the heat production by metabolism overtakes the heat loss and thereby raising the egg temperature above that of the surroundings (Romanoff, 1941; Romijn and Lokhorst, 1955;

1956; 1960; Tazawa and Nakagawa, 1985; Tazawa and Rahn, 1986).

The domestic fowl's embryo cannot regulate its body temperature until the latter stages of development. Freeman (1964) reported the first indication of a homeothermic metabolic response on the 19th day of incubation. If the reduction in ambient temperature was small (5°C), there was a transient rise in metabolic rate. This is supported by an increase in carbohydrate catabolism in the 19 day old embryo during cooling (Freeman, 1967) and the RQ of late embryos was found to increase in response to cold exposure (Romijn and Lokhorst, 1955).

Before day 18 of incubation the Chicken embryo was found to be essentially a poikilotherm (Tazawa *et al.*, 1988). Thereafter the embryo could respond to a small reduction in ambient temperature by a transient rise in metabolic rate. After external pipping the compensatory metabolic response to gradual cooling was stronger (Tazawa *et al.*, 1988). The metabolic response to cold also appears to be associated with an increase in thyroid activity. Tazawa *et al.*, (1989) have also demonstrated an incipient homeothermic metabolic response before prenatal life in intact or saline treated embryos, but the response was found to be lacking in thiourea treated embryos.

Chicks of the Domestic fowl also respond to cold exposure by increasing heat production (Misson, 1977). The ability to increase oxygen uptake on cold exposure has been demonstrated by several workers (Pembrey *et al.*, 1895; Romijn,1954 ; Romijn and Lokhorst, 1955; Freeman, 1964). Freeman (1966) showed that the neonate Domestic fowl responds to cold by greatly increasing its metabolic rate. Little or no shivering thermogenesis was detected and he concluded that the chick was able to increase its heat production by non-shivering means. In day old chicks there was an increase in oxygen consumption of 150% when ambient temperature was changed from 35°C to 25°C, before reaching a constant level. At 35°C oxygen uptake was normal and this was found to be the thermoneutral temperature for *Gallus domesticus* (Freeman, 1963).

Randall (1943) showed that the neonate fowl relies on non-shivering thermogenesis in a cold environment. The lower critical temperature of the fowl chick decreases with age and the rate of increase in heat production below the critical temperature diminishes (Ringer, 1976). The upper critical temperature is higher in the chick than in the adult, conforming with the relatively greater surface area of the chick and its higher thermal conductance, both features promoting heat loss to the environment. Newly hatched chicks respond to heat exposure with an identifiable thermal

polypnoea which commences at a lower body temperature in chicks than in the adults (Ringer, 1976).

Dawes (1979) found that when the temperature of incubation of the pipped egg was increased from 39.4°C to 46.5°C the respiratory frequency of the embryos doubled within a period of 30 minutes and then fell to zero. There was a progressive increase in the frequency of slow rhythmic movements together with a gradual decline in their amplitude. This compares with the response of the adult Domestic fowl to prolonged heat stress which is in two phases. First there is an increase in frequency of respiratory movements and a reduction in their amplitude, then there is decrease in frequency and an increase in amplitude (Randall and Hiestand, 1939; Frankel *et al.*, 1962; Weiss *et al.*, 1963; Linsley and Burger, 1964). Randall (1943) noted similar effects in immature birds.

Romijn (1954) found that acquisition of the temperature regulation mechanisms and of the maximum T_b was at 6-7 days after hatching. Beattie and Freeman (1961) found a decrease in acceleration of oxygen uptake related to this period. King (1956) reported that light breeds achieved maximal T_b at about 7 days and heavy breeds 10 days after hatching. Hutt and Crawford (1960) showed that T_b reaches a normal level about 4 days after hatching. The development of brain temperature regulation from hatching up to day 21 has also been studied (Arad, 1991). It was shown that body and

brain temperature at hatching were both low compared to adult levels. However both were effectively regulated at T_a's of 30 and 35°C, with only minor differences between the two temperatures. The results also indicated the existence of different patterns of post hatching development of brain temperature regulation in relation to the degree of precocity of the species.

1.2.2 **Myofibrillar ATPase: role in thermogenesis**

Skeletal muscle is the primary thermogenic tissue (Hartmann, 1961; West, 1965). Studying the changes taking place during skeletal muscle development can shed some light on the transition from poikilothermy to endothermy. The pioneering study in this field was done by Marsh and Wickler (1982) on the altricial Bank Swallow (*Riparia riparia*). The study indicated that mass specific aerobic capacity as measured by citrate synthase increases throughout the transition period, but myofibrillar ATPase abruptly doubled when endothermy was achieved. This suggests presence of a mature and functional aerobic pathway providing ATP plus adequate contractile elements but inadequate m-ATPase - limiting effective thermogenesis. In the semi-precocial Wedge-tailed shearwater (*Puffinus pacificus*), no significant difference in protein specific activity of Ca-activated myofibrillar ATPase was found between embryos in pip-holed eggs and hatchlings (Mathiu *et al.*, 1992; in press); the period when an endothermic response appears. This observation

appears to provide tentative support for the view that the appearance of endothermy in the Wedge-tailed shearwater at hatching is linked more with the elimination of the physiological consequences of physical confinement than with abrupt biochemical maturation. Nevertheless a Wedge-tailed shearwater parafetus was observed to shiver during the hatching process.

Shivering (involuntary muscle contraction) is an emergency mechanism for homeotherms that functions in severe cold (Hemmingway,1963). It can be started, stopped or adjusted by the central nervous system when the need arises. It's an involuntary response of skeletal muscles which are usually under voluntary control and can be determined by both qualitative and quantitative means including metabolic, electromyographic, mechanical, palpation and visual means. Shivering is the first line of defence against cold and non-shivering thermogenesis only goes into action as a result of prolonged exposure to cold.

Regulatory non-shivering thermogenesis has not been conclusively demonstrated in birds, including nestlings, (Dawson and Hudson, 1970). This suggests that the development of shivering heat production may underlie the rapid maturation of the thermogenic response in passerine nestlings, (Odum, 1942; Morton and Carey, 1971; Marsh, 1979). The development of shivering will in turn depend on central and peripheral neural development and the development of the

skeletal muscles. Given an adequate oxygen supply, the capacity to generate heat aerobically will be a function of the total aerobic capacity of the muscle tissue as determined by the muscle mass and activity of aerobic enzymes per unit mass. Shivering is also dependent on the maturity of the contractile apparatus which serves to break down the ATP generated by the aerobic pathways, (Marsh and Wickler, 1982).

Contractile function of muscle can be assessed by measuring myofibrillar ATPase activity, which correlates with contractile speed in both developing and adult muscle, (Barany, 1967; Drachmann and Johnston, 1973). In birds, pectoral muscles represent an increasing fraction of total body mass during development. It is the main heat producing mass making up about 15-20% of total body weight (Hartmann,1961). The Electromyographic activity of this muscle (EMG) has been found to increase with decreasing ambient temperature (Hart, 1962) and there is a linear correlation between EMG activity and oxygen consumption. Oxygen consumption also follows the same pattern as shivering (Aulie, 1976b). In the Willow ptarmigan, the growth of pectoral muscles has been shown to accompany the development of thermoregulation in the chicks (Aulie, 1976a).

Aerobic muscles like the thigh and leg muscles of the domestic fowl appear to be responsible for shivering thermogenesis between critical temperature (24°C) and the shivering threshold, (T_{sh}; Aulie and Toien, 1988). Anaerobic

muscles like pectoralis on the other hand appear to be recruited at lower T_a's or when the heat loss during incubation becomes severe. In other birds pectoral muscles are aerobic and so are the most important thermogenic sites (Steen and Enger, 1957; Hart, 1962). In the Willow ptarmigan increase in heat production by shivering was found to increase with the development of the pectoral muscles. The pectoral muscles of the pigeon are also aerobic (George and Berger, 1966) and so shiver at T_a's as high as 30°C (Hart, 1962). Muscle fibres containing high oxidative enzyme activities (type I) are recruited during smaller contraction tensions, whereas those containing greater myosin ATPase activities (type II) are involved when a greater degree of tension is required (Fedde *et al.*, 1969).

The protein content of muscle increases steadily during development (Ricklefs, 1979). It does not show the sharp transition observed in whole animal thermoregulatory response. Thermogenic ability and myofibrillar ATPase clearly indicate a distinct shift in muscle function which cannot be deduced from the increase in protein content or decrease in water content. For Bank Swallows, the development of maximum heat production is mirrored by the activity of m-ATPase in the pectoralis muscle (Marsh and Wickler, 1982). An increase in m-ATPase activity is a common phenomenon during development of fast-twitch skeletal muscle in birds and mammals, and this enzymatic

change underlies an increase in contractile speed. The transition occurs after birth in altricial mammals like rat and cat, but occurs largely before birth, or hatching in the precocial guinea pig and domestic fowl (Trayer and Perry, 1966). In the fowl, Le Peuch *et al.*, (1979) have shown that the synthesis of a number of proteins related to the regulation of contraction is activated at the same time as a shift in myofibrillar ATPase. Barnes and Hasson (1983) have traced the rise in cytochrome oxidase and succinoxidase activity in the leg and breast muscles of embryonic domestic fowl from 11 to 19 days of age. The results indicate a partial differentiation of skeletal muscles over this period.

Acclimation of newly hatched chicks of *Gallus domesticus* to cold has been found to result in higher oxygen consumption during exposure to cold than in non acclimated controls (Aulie and Grav, 1979). The cytochrome oxidase activity of skeletal muscles and liver was elevated in the cold acclimated, indicating a role for this enzyme in the increased oxidative metabolism during cold acclimation. The leg muscles were considered to be a significant source of heat production in the cold acclimated chicks. The complex process of migration and winter acclimation in passerine birds has also been shown to be closely linked with the metabolic properties of the highly aerobic skeletal muscles contained within the flight apparatus (Dawson *et al.*, 1983).

The changes in enzyme profiles (of semi-precocial and precocial birds) of skeletal muscles appear potentially promising in accounting for the increased metabolic capacities of the late incubation stage embryos.

1.2.3 **Thyroid hormones: role in thermogenesis**

As far as the hormone profiles are concerned, Scanes *et al.*, (1987) contend that the full endocrine system is developed during the embryonic stages of the domestic fowl and possibly in all avian species. The thyroid gland appears on the second day of incubation as a midline growth from the ventral pharyngeal wall at the level of the first and second pharyngeal pouches (Romanoff, 1960). The patterns of change of circulating fetal T_3 (fT_3) and fT_4 with incubation age are similar to those of circulating total T_3 and T_4 respectively, (McNabb and Hughes, 1983). In the avian embryo, thyroid hormones are thought to be involved in;

-control of growth and development of a number of organs including brain, liver, skeleton and nervous tissue.

-thyroid hormones are also involved in hatching. If peripheral conversion of T_4 to T_3 is inhibited in ovo the length of incubation of a chick embryo is increased, (Decuypere, 1982). Administration of T_3 or T_4 has been found to enhance percentage hatchability of turkey embryos (Christensen, 1985; Christensen and Biellier, 1982; Christensen *et al.*, 1982). Apart from growth hormone, metabolic hormones like thyroid

hormones and glucocorticoids are more relevant to thermoregulation due to their effects on metabolism and the maturation process. Glucocorticoids are more involved in the maturation process (e.g. synthesis of lung surfactant), while thyroid hormones have been associated more closely with the development of endothermy.

McNabb (1987) compared thyroid development in the precocial Japanese Quail (*Cortunix japonica*) and the altricial Ring Dove (*Streptopelia risoria*), and observed two phases differing in timing and the nature of transition. Phase one is characterized by increasing functional capacity of the thyroid gland but low circulating concentrations of hormones while the second phase is marked by increased gland and hormone activity in the periphery with hormone concentrations approaching or exceeding adult levels. In both patterns of development, a good correlation exists between the initiation of endothermic response to cooling and the transition into phase two of thyroid development. In the Japanese quail the first phase occurs during the later half of embryonic life and there is an abrupt transition to phase two beginning with a perinatal hormone peak. In the ring doves the first phase continues into the first 6-8 days of life.

McNabb (1987) also contends that in birds T_3 is the metabolically active hormone and T_4 is of significance as a substrate for production of T_3. Peripheral tissue 5-monodeiodinase is known to convert T_4 into the more active

T_3. Mcnabb *et al.*,(1981) has shown that in quail, at the time of hatching (day 16 of incubation) and 24 hours post hatching, thyroid activity is dramatically increased and so does the serum T_3/T_4 ratio, coinciding with the time of internal pipping. In the first few days after hatching, the serum T_4 concentration decreases, but this decrease is somewhat dampened by the effect of binding proteins, hence fT_4 decreases more slowly than total T_4. The same pattern of increase in the serum T_3/T_4 ratio has been shown to occur in the chicken, (Decuypere *et al.*, 1979a; 1979b; Borges *et al.*,1980) suggesting an important role for T_3 in the process of pipping and hatching. It has also been shown that in quail chicks, thyroid activity is correlated with the pattern of thermoregulatory development, (McNabb *et al.*, 1984 and Spiers *et al.*, 1974). McNabb and McNabb (1977) and Rol'nik, (1948) have also shown that the thyroid gland of altricial species differentiates into follicles and becomes functional later than in precocial species.

Thommes, (1987) has shown that in the domestic fowl's embryo the thyroid gland can synthesise the thyroid hormones quite early, before day 5.5 of incubation. But it is not until day 10.5-11.5 of incubation that the feed forward and feed backward components of the hypothalmo-adenohypophyseal-thyroid (HAT) axis mature. Trace amounts of T_4 could easily be detected between days 9.0 and 10.0. It had earlier been shown by Thommes and Tonnetta,(1979) that chicken embryos

when treated with thiourea on day 5.5 showed significant decreases in total T_4 concentrations on days 7.9, 9.5, 10.5 and 11.5 of incubation. On day 10.5 the pituitary and hypothalamus of the chicken embryo first responded to decreases in circulating T_4 levels. Thus between days 10 and 13 of development, there is maturation of the HAT axis that may be similar to that found in sheep and humans during mid-gestation. The adenohypophyseal-thyroid (AT) axis of chicks is also known to be functional on or about day 11.5 of incubation, Thommes and Hylka (1977).

It has been observed, (Scanes *et al.*, 1987), that the circulating concentration of thyroxine (T_4) rises exponentially during the last two thirds of the developing chick embryos. On the other hand, circulating concentrations of triiodothyronine (T_3) in the chick embryo appears to remain low, and relatively constant, during the mid incubation period but increases dramatically in a linear manner between day 18 of incubation and hatching (Scanes *et al.*, 1987). Most T_3 and T_4 in the circulation is bound to plasma proteins. The ability of T_3 and/or T_4 to exert a negative feed back effect is also established relatively early in embryonic development (Thommes and Tonetta, 1979).

Between day 8 and 20, the plasma T_4 concentration appears to double every 3.1 days (Scanes *et al.*, 1987). In later incubation, it would appear that T_4 is being converted to T_3. The importance of thyroid hormones in embryonic development

is not fully characterised. In early and mid embryonic development, thyroid hormones are involved in development of many organs including brain, liver and skeleton (Scanes *et al.*,1987). Thyroid hormones have also been shown to play a role in triggering endothermic responses to cooling in avian embryos prior to the perinatal period, (McNabb *et al.*, 1972). Thommes *et al.*,(1977) have also demonstrated that plasma concentration of reverse T_3 (rT_3) shows a steady rise from day 9.5 to 11.5. Values for T_3 showed a decrease from day 9.5 through day 11.5 followed by a gradual rise to day 18 and then a sharp rise to day 21.5

Tata and Shellabarger, (1959) conducted a study seeking to explain the differences between the responses of mammals and birds to T_3 and T_4. In most mammals the higher potency of T_3 is thought to be due to the faster rate of diffusion from blood to tissues for T_3 than T_4. This concept is upheld by the low binding affinity of T_3 for serum T_4 binding proteins and the two to four times shorter biological half life of T_3. In chicken both T_3 and T_4 are known to have similar $t_{1/2}$. There is lack of the specific T_4 binding protein in avians so the only protein available binds the two hormones with the same avidity. Shellabarger *et al.*, (1955) and Newcomer, (1957), have shown that in chicken both T_3 and T_4 had the same potency whatever the parameter used for comparison.

The development of thermoregulatory ability, heat seeking activity and thyroid function has been studied in the

Japanese quail (*Cortunix cortunix japonica*, Spiers *et al.*, 1974). Histological studies (thyroid gland cell height, colloidal diameter, colloidal staining properties) and stable iodine analyses indicated that the time course of developing thyroid function parallels the time course of developing thermoregulatory ability. Between days 13 and 15 of age, chicks appear homeothermic and some thyroid parameters approach adult levels. This suggests that activity of the thyroid gland is an important factor in the development of thermoregulation in the Japanese quail.

It has been observed (Decuypere,*et al.*,1979a) that there is a sharp increase in heat production after pipping. Thyroid hormone levels are also high at this stage. After hatching both hormones decrease until the adult concentration is reached. Thyroid hormone levels have been measured by several workers. Using a competitive binding assay, Refetoff *et al.*, (1970) reported plasma T_4 levels for the chicken as 1.4-1.6 µg/100ml. For a 10 day incubation embryo, Daugeras *et al.*, (1976) found 0.75 ng/ml for plasma T_4 levels, while Thommes and Hylka (1978) found 1.4 ng/ml (T_3) and 3.36 ng/ml (T_4) in a 10.5 day old chicken embryo. In the 21.5 day (post-hatch) chick, T_3 and T_4 levels were found to be 4.99 ng/ml and 10.78 ng/ml respectively (Thommes and Hylka, 1978)

A circadian rhythm of thyroid hormones has been demonstrated due to the short $t_{1/2}$ of these hormones in birds

The iodohormones show two peaks of plasma concentrations at 0800 hours and 1600 hours (Sadovsky and Bensadoun, 1971). At 1600 hrs the concentration is twice as high as at 0400 hours. Newcomer (1974), and Klandorf *et al.*, (1978) reported that in chicken the concentrations of plasma T_4 increased during the dark period and decreased during the light period where as T_3 increased during the day and decreased at night.

The pattern of food intake has also been found to influence the daily rhythm in serum T_3/T_4 concentrations (Sharp and Klandorf, 1985). Energy intake appears to be the main factor regulating the plasma T_3 concentrations, and presumably the conversion of T_4 to T_3. Cogburn and Freeman (1984) reported that plasma T_3 concentrations were consistently depressed in cockerels held at 38°C but were slightly elevated in cockerels exposed to 10°C. Hendrick and Turner (1967) measured the $t_{1/2}$of T_4 and reported no change on short term exposure to cold. For the chicken the biological $t_{1/2}$ of thyroxine in plasma has been found to be 8.3 hours (Henninger and Newcomer, 1964), and 3.3 hours (Singh *et al.*,1967).

Thyroid hormones play a major role in regulating oxidative metabolism of birds. Triiodothyronine the metabolically active hormone plays an important role in energy metabolism and metabolic processes (Klandorf *et al.*, 1978; 1981). Hyperthyroidism and hypothyroidism is reflected in an altered metabolic rate. Bilezikian *et al.*, (1980) observed

an increase in body temperature (41°C) in mature turkeys and reduced metabolism (39.8°C) in those made hypothyroid.

Age related changes in oxygen consumption and plasma thyroid hormone concentration in the young chicken have been studied by Bobek *et al.*, (1977). They measured oxygen consumption and plasma concentration of circulating T_3 and T_4 . Maximum oxygen consumption was observed in the first and second weeks after hatching. T_3 levels attained maxima in the second week when it was about 67.7% higher than for the days immediately after hatching. Plasma T_4 levels rose slowly by an average of 29.8% between day 2 and the fourth week after hatching and then showed great fluctuations. The coefficient of correlation between oxygen consumption and T_3 or T_4 concentrations in the different age groups of chicken was found to oscillate from 0.78 to 0.98 and 0.19 to -0.63 respectively. In contrast to mammals in which prolonged metabolic rate (MR) stimulation follows T_4 injection, the chicken exhibits only a transitory rise. A small increase in MR following administration of T_4, T_3 or a combination of both T_3 and T_4 lasts only 2-3 hours. Singh *et al.*, (1968) found that the maximum effect produced by T_4 occurred 2.0 hours after its administration.

In the neonate T_3 and T_4 injected intra-peritoneally has been found to be thermogenic (Freeman, 1970). Rectal temperature was elevated within 30 minutes after T_3 or T_4 injection. Thiouracil administration impairs thermoregulation

in the neonate chicken (Freeman, 1971a). The thyroid gland can also influence thermoregulatory ability indirectly by affecting growth and thereby influence the surface to volume ratio at any age.

It has been postulated (Davison, 1973) that gluconeogenesis plays a role in non-shivering thermogenesis. Thyroid hormones also have a calorigenic and thermogenic effect in adult birds and mammals, Chaffee and Roberts (1971) and in the neonate Mallee fowl (Freeman, 1970; 1971b). Williamson *et al.*, (1971) proposed that in the rat the liver is a major site for heat production in non-shivering thermogenesis through increased gluconeogenesis. Both thyroxine and glucagon increase gluconeogenesis in the rat liver (Exton and Park, 1966; Bottger *et al.*, 1970). Thyroid hormones are known to increase gluconeogenesis and mobilise glycogen in the mammalian liver (Bogtter *et al.*, 1970, Heitzman *et al.*, 1971). Increased fat oxidation probably makes a major contribution to increased heat production since Freeman (1967) has shown that oxygen consumption was increased by more than 200% with a concomitant fall in RQ to below 0.7 in cold exposed day old chicks.

Nakagawa and Nagai (1971) have found increased protein catabolism and gluconeogenesis in rats exposed to cold. They suggested that gluconeogenesis may play a special role in heat production by the specific dynamic action (SDA) of protein. Davison (1973) reported increased gluconeogenesis and protein

catabolism in cold exposed day old chicks. They suggested that thyroxine and glucagon may be active in directing metabolic changes in the chick during cold exposure.

Several methods of heat production at cellular level have been proposed. Uncoupling of oxidative phosphorylation seems to be of major importance in non-shivering thermogenesis (NST) in mammalian brown adipose tissue. There is little evidence that uncoupling takes place in other tissues (Hittelman and Lindberg, 1970). In producing more thermal energy , all cells involved must dispose of ATP produced in order to maintain the increased level of oxidative phosphorylation. In shivering this is brought about by the actinomyosin ATPases of the myofibrils (Hemmingway, 1963) and in thyroid calorigenesis by increased ATPase activity of the sodium potassium cellular pumps (Ismail-Beigi and Edelman, 1970).

1.3 OBJECTIVES

The study aims to:-

1. Quantify myofibrillar ATPase activity in pectoral muscles of the domestic fowl and correlate this with the development of endothermy.
2. Measure thyroid hormones (T_3 and T_4) in the peripheral circulation of the Domestic fowl and correlate them with the emergence of endothermy.

The transition from poikilothermy to homeothermy in the developing embryos and chicks of the Domestic fowl (*Gallus domesticus*) is fairly well documented (Freeman, 1964; Tazawa and Rahn, 1987) but the enzyme and hormone profiles have not been studied with a view to relating them specifically to the transition from poikilothermy to homeothermy. This study was designed to investigate whether or not critical changes in m-ATPase and thyroid hormones are a common denominator in the development of endothermy in the domestic fowl.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 GENERAL SUMMARY

Newly laid fertile eggs of the Isa Brown line (*Gallus domesticus*) were obtained from a commercial hatchery belonging to Kenchic (k) limited at Athi River, located seventeen kilometers east of Nairobi city at a latitude of 1°25'S and longitude 37°00'E. The eggs were incubated in the laboratory at 37.5°C in a Curfew incubator, model 300. Relative humidity (60-100%) was maintained by placing a tray of water in the incubator. The eggs were turned automatically every one hour. Air circulation was maintained by an in-built fan. After hatching, the chicks were placed in an open box with a 100 watt bulb as a source of heat. Water and chick marsh were provided *ad libitum*. Oxygen consumption measurements for embryos were done from day 15 to day 21 of the incubation period using a closed system (Mathiu, 1988).

For hatchlings and chicks oxygen consumption was measured using a flow-through respirometry system. After oxygen consumption measurements, the embryos, hatchlings and chicks were sacrificed to obtain blood and muscle samples. Blood samples were obtained from the allantoic artery in embryos and from the jugular veins in hatchlings and chicks. Blood plasma levels of triiodothyronine (T_3) and

thyroxine (T_4) were measured by radioimmunoassay (RIA). The muscle samples were obtained by dissection of the pectoral muscles. Some muscle samples were processed immediately in a cold room but others were deep frozen (for less than a month) until the determination of myofibrillar ATPase activity could be performed. The myofibrillar ATPase activity was determined as described by Marsh and Wickler, (1982).

2.2 OXYGEN CONSUMPTION IN EGGS

A closed system (waterbath) was used to measure oxygen consumption of the eggs (Mathiu, 1988). It consists of a water-bath with a thermostated heating element to regulate the water temperature at a desired level (±0.1°C). Two plexiglass (perspex) egg chambers, cylindrical in shape, with internal dimensions (height vs. diameter) of 10 vs. 6.4 cm and 16 vs. 8.8 cm respectively were used. These chambers, having water/air tight lids were held together by a plexiglass slab with O-ringed protrusions to fit and seal holes on the lids. A perspex rod attached to the middle of the slab and held by a metal bar served to hold the egg chambers while immersed in the water-bath. The egg chambers opened to the outside through holes in the plexiglass slab.

Three-way stopcocks closed the holes from the top of the slab. Perpendicular holes through the plexiglass slab and extended by plexiglass tubes connected to a U-shaped water manometer via tygon tubing. The two egg chambers were

linked by the water manometer. One chamber contained a dead (boiled) egg that did not consume oxygen and served as a pressure compensating chamber. In both chambers, there was CO_2 absorbent (Ascarite II) above which the egg stand was placed.

The egg chambers were immersed in the water-baths and left for one hour for the temperatures to equilibrate. Then the egg chambers were removed, wiped dry and opened. A pre-selected egg at the desired developmental stage was removed from the incubator, weighed and placed into the egg chamber. The egg chamber was closed and re-immersed in the water-bath. Oxygen from an external source, and stored in a 10 ml syringe connected to the three-way stopcock was used to flush the egg chamber. With the water manometer reconnected and the 3-way stop-cocks closed, the syringe was refilled with oxygen. The viable egg used up O_2 and the water column in the manometer rose. Injecting oxygen from the syringe at intervals restored the water meniscus to the initial level and supplied the egg with the necessary oxygen (see plate I for the experimental apparatus).

The experiment ran for at least 1.5 hours at 38 °C with the last 15 minutes being used to measure $\dot{V}O_2$. Starting with the water menisci at the same level, five minute intervals were timed and the rise in water level corrected by addition of more oxygen. The amount of O_2 in mls added per 5

minutes was recorded. Three 5 minute readings were taken for each experiment, averaged and multiplied by 12 to get $\dot{V}O_2$ in ml/hr. The $\dot{V}O_2$ readings at 38°C were added together and averaged to get values for the control $\dot{V}O_2$ for any developmental stage.

Eggs were then cooled using the "gradual cooling" method, (Tazawa and Rahn, 1987). After measuring $\dot{V}O_2$ at 38°C the water-bath heater was turned off. The water-bath, test chamber and egg were allowed to cool gradually to room temperature. The water-bath took on average six (6.0) hours to cool to room temperature. The experiments proceeded for the same length of time. While the egg cooled, its oxygen consumption was continuously monitored for every 1.0°C decrease in ambient temperature (T_a).

$\dot{V}O_2$ values in ml/hr.were then corrected to STPD as follows. Gas in the syringe was assumed to be saturated with water vapour at that temperature. Barometric pressure and the room temperature near the syringe were determined.

$\dot{V}O_2$ values are corrected to STPD by the equation:

$$\dot{V}O_2\,(\text{ml/hr})_{(\text{STPD})} = \frac{P_B - P_{H_2O}}{760} \cdot \frac{273.1}{273.1+T} \cdot \dot{V}O_2$$

P_B = Barometric pressure.

Measured P_B was 623mmHg in the laboratory.

P_{H_2O} = Saturated water vapour pressure at the syringe temperature obtained from vapour pressure tables.

T = Syringe temperature in ^{o}C. Assumed to be the prevailing room temperature.

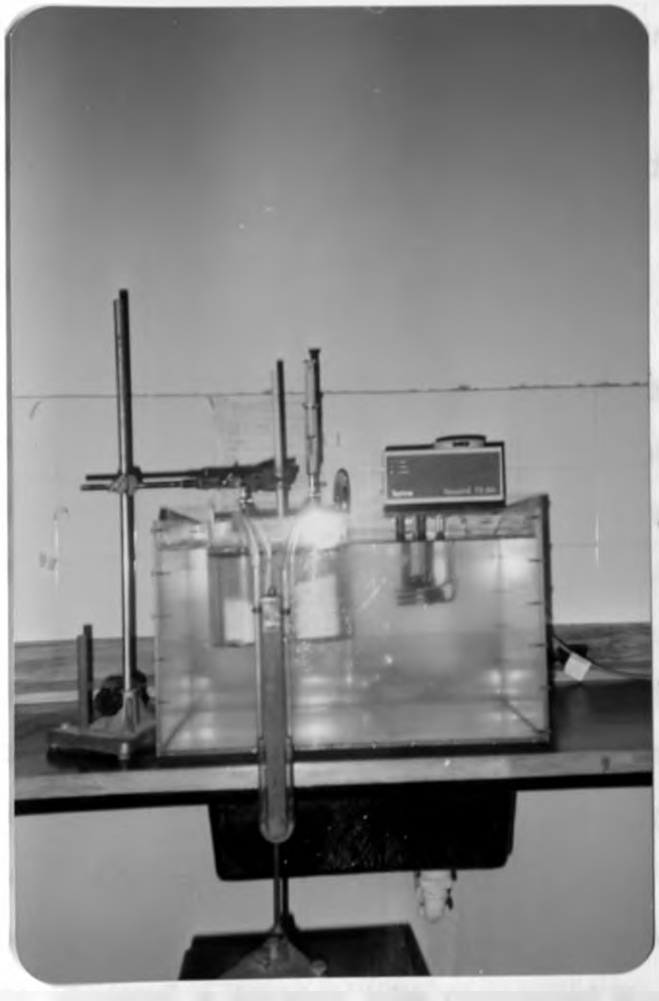

Plate I. Apparatus for the measurement of oxygen consumption in eggs using the closed system.

2.3 OXYGEN CONSUMPTION IN CHICKS

The procedure described here was adopted from the method of Taylor *et al.*, (1982). A flow-through system was used to measure oxygen consumption for hatchlings (zero days) and chicks (one to seven days). Measurements of $\dot{V}O_2$ in hatchlings were carried out after the hatchling down had dried. The experimental arrangement as shown in diagram 1 was used.

It consisted of a cylindrical plexiglass (perspex) chamber with internal dimensions (height vs diameter) of 12 cm vs. 8.5 cm respectively, and a chamber volume of approximately 700 cm^3. A plexiglass lid with an O-ring fitted into the chamber tightly. The chamber had an inlet port at the bottom and the outlet one at the top. A perspex tube of internal diameter 1.0 cm and 15 cm long was connected to the inlet port. The outlet port was fitted with a rubber stop cork through which was inserted a thermocouple probe and a glass tube through which air was drawn out. Another outlet port to which was attached a perspex tube connected the hatchling/chick chamber to a water manometer used to monitor pressures at atmospheric level. At the bottom of the chamber was a wire mesh on which the hatchling stood. The chamber was placed in a water-bath with a Masterline (model 2095) thermostated heating element and cooling coil. This

could regulate water temperature to any desired level between 12°C and 40°C.

Room air was pumped through the metabolic chamber at known flow rates. A small Brooks flowmeter metered the air before it entered the animal chamber as shown in diagram 1. Air flow rates of 1.0 to 1.3 liters per minute were used for these experiments. Pure Nitrogen could also be metered through this system at known rates via a different Brooks flowmeter. The flow of air through the hatchling chamber was determined by bleeding nitrogen into the chamber using a small Brooks flowmeter previously calibrated by use of a Brooks Vol-U-meter and noting the fractional change in oxygen concentration at different nitrogen flow rates. Leakage of exhaled air from the hatchling chamber was checked by increasing the rate of nitrogen flow through the system by specific fractions (while keeping air flow constant), which led to proportionate decreases in oxygen tension in the air reaching the analyzer.

Tygon tubing carried exhaled air from the chamber outlet via a tube containing drierite (anhydrous calcium sulphate) to remove water vapour and then via another tube containing ascarite II (sodium hydroxide) to remove carbon dioxide. Hydrated drierite (pink) was regenerated everyday to the anhydrous form (blue) in a hot air oven. The dry CO_2 free air was then sampled and the fractional concentration of oxygen in the exhaled air determined by a Beckman F3 paramagnetic

oxygen analyzer set at full scale deflection of 1.0 percent (20-21%), with a response of 90% in 40 seconds. The analyzer was in turn connected to an Omniscribe recorder.

Oxygen concentration values were then simultaneously displayed and recorded by the oxygen analyzer panel and recorder respectively. The principle of the oxygen analyzer is as follows: Oxygen exhibits a positive susceptibility in a magnetic field, much higher than other common gases so that the field of force tends to concentrate where oxygen is present. The F3 model measures the total magnetic susceptibility of the sample which is the summation of the susceptibilities of the individual gases in the sample. This total is almost entirely due to oxygen, so the measurement is an accurate indication of oxygen content.

Procedure:

To measure $\dot{V}O_2$ the hatchling was removed from the incubator or brooder and its mass determined using a sartorius balance, model 2255 (GMBH). Rectal/body temperature was taken using a thermistor probe and a YSI tele-thermometer, model 401. The hatchling was then placed in the chamber through which air was circulating at a known flow rate. The chamber was quickly returned to the water bath which was at a pre-set temperature. The water bath and chamber (ambient) temperatures were allowed to equilibrate over the next 45

minutes. Ambient temperatures inside the hatchling chamber were monitored by use of a thermocouple probe. Successive determinations of oxygen uptake were made over periods of 10 minutes after the bird had settled down and the air in the apparatus had come in thermal equilibrium with the water bath. Three consecutive readings were made that varied by less than 5% from the mean value before observations were terminated .

The bird was then removed and rectal/body temperature (assumed equal to core temperature) taken at the end of the experiment. The water bath temperature was then adjusted to a new value. Each bird was used for the measurement of $\dot{V}O_2$ at only one temperature setting except those used at 36°C (brooding temperature) for which another temperature setting was used before they were sacrificed for collection of blood and muscle samples. These measurements were carried out on hatchlings and chicks during days one, two, three, five and seven after hatching.

In these experiments CO_2 and water were both removed from expired air before oxygen analysis was done on the samples. The parameters that were recorded for each experiment include:

$\dot{V}_{mask}$ = the air flow rate through the hatchling chamber in ml/hr (STP).

F_I = the mole fraction of oxygen entering the hatchling chamber.

F_E = the mole fraction of oxygen leaving the hatchling chamber.

An R value of 0.8 has been assumed for these experiments ,(Romjin and Lokhorst, 1955; Rahn *et al.*, 1974).

$\dot{V}_N$ = rate of nitrogen flow into the hatchling chamber through a precision flowmeter.

0.9581= a constant assuming an R value of 0.8 (Taylor *et al.*, 1982)

0.2094 = fractional concentration of oxygen in room air drawn into the hatchling chamber (normal for the laboratory conditions for this experiment).

Oxygen consumption under these conditions was calculated as follows:

$$\dot{V}O_2 = \frac{\dot{V}_{mask}(F_I - F_E)}{0.9581} \quad (1)$$

$$\dot{V}_{mask} = \frac{0.2094\ \dot{V}_N}{0.2094 - F_E} \quad (2)$$

Equations (1)and (2) correspond to equations (3)and (5) of Taylor *et al.*, (1982).

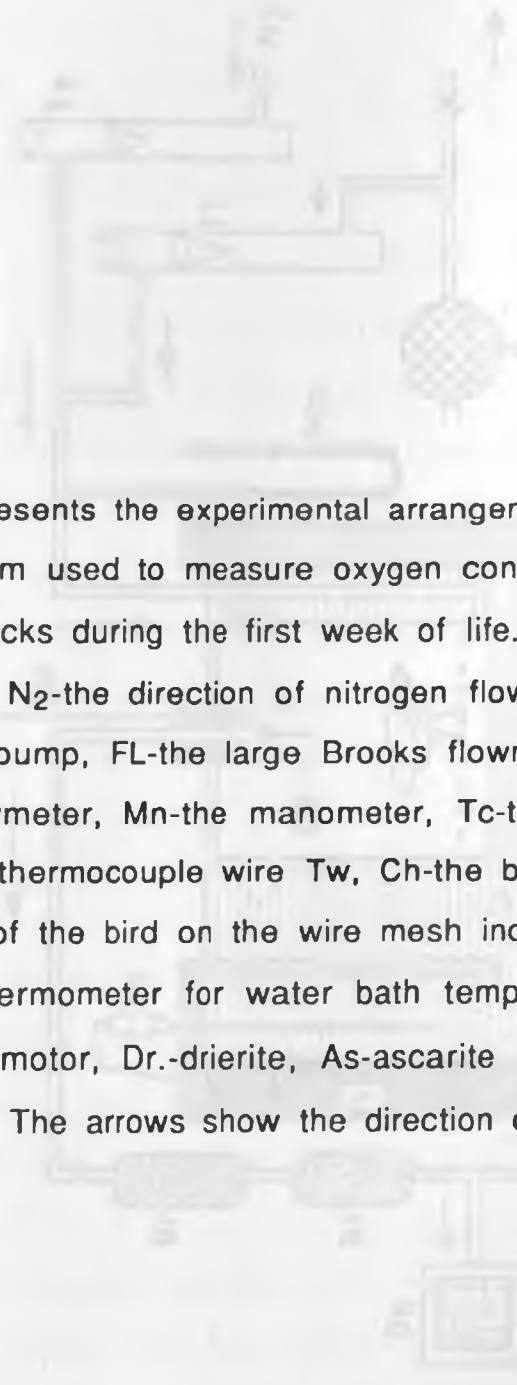

Diagram 1. Represents the experimental arrangement for the flow-through system used to measure oxygen consumption in hatchlings and chicks during the first week of life. Shown in the diagram are: N_2-the direction of nitrogen flow through the apparatus, P-the pump, FL-the large Brooks flowmeter, Fs-the small Brooks flowmeter, Mn-the manometer, Tc-the thermometer and thermocouple wire Tw, Ch-the bird chamber with the position of the bird on the wire mesh indicated, W-water bath, Th-thermometer for water bath temperature regulation, S-the motor, Dr.-drierite, As-ascarite and Oa-the oxygen analyzer. The arrows show the direction of air flow.

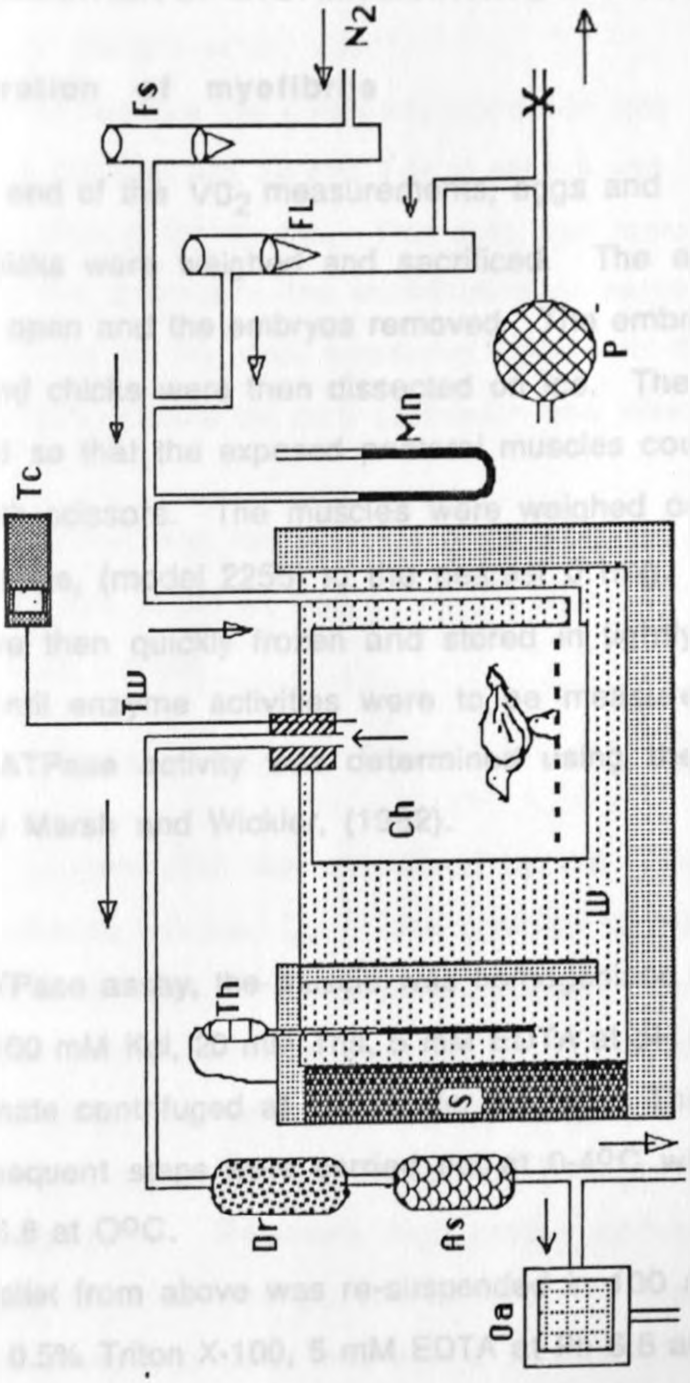

Diagram 1

2.4 DETERMINATION OF MYOFIBRILLAR ATPase ACTIVITY

2.4.1 Preparation of myofibrils

At the end of the $\dot{V}O_2$ measurements, eggs and hatchlings/chicks were weighed and sacrificed. The eggs were broken open and the embryos removed. The embryos, hatchlings and chicks were then dissected on ice. The skin was removed so that the exposed pectoral muscles could be dissected with scissors. The muscles were weighed on a sartorius balance, (model 2255) to the nearest 0.1mg. The samples were then quickly frozen and stored in tightly sealed containers until enzyme activities were to be measured. Myofibrillar ATPase activity was determined using the method described by Marsh and Wickler, (1982).

Procedure

(i) For ATPase assay, the muscle was homogenized in 10 volumes of 100 mM Kcl, 20 mM Tris, 5 mM EDTA at pH 6.8 and the homogenate centrifuged at 1000 g for 10 min. This step and all subsequent steps were carried out at 0-4°C with pH adjusted to 6.8 at O°C.

(ii) The pellet from above was re-suspended in 100 mM Kcl, 20 mM Tris, 0.5% Triton X-100, 5 mM EDTA at Ph 6.8 and centrifuged at 1000 g for 10 minutes. This step was repeated once. Treatment with TritonX-100 solubilises the sarcoplasmic reticulum and reduces possible contamination

with membranous ATPases without affecting the myofibrillar ATPase activity (Solaro *et al.*, 1971).

(iii) In order to remove the detergent the pellet was resuspended in 100 mM Kcl, 20 mM Tris at pH 6.8 and centrifuged at 1000 g for 10 min. This step was repeated twice. Using this procedure the contribution of sarcoplasmic reticulum ATPases to the total measured activity in the original preparation should be zero (Johnston and Welsby, 1977).

(iv) The final pellet was resuspended in 20 volumes of 100 mM Kcl, 20 mM Tris at pH 6.8 and an aliquot taken for determination of protein.

2.4.2 **Protein determination**

Protein concentration was measured by the Biurette method. In alkaline solution, Cu II ions complex with the peptide bonds of proteins to form a purple colour. The intensity of the purple colour is proportional to the protein concentration. Since the number of peptide bonds per unit mass is about the same for all proteins, the Biurette method is generally applicable. Relatively high protein concentrations are readily determined by the Biurette method. In this method, the assay procedure was used first with a solution of known protein concentration over a suitable range in order to establish a standard curve. Bovine serum albumin (BSA) was used for this procedure in order to plot a standard curve (see appendix I for protein standard curve). In the other

experiments, the protein concentrations were determined by comparing the colour intensity with the standard curve.

Procedure:

(i) Dilutions of the standard protein solution were prepared as follows:

Vol. of protein solution (ml)	0	0.2	0.4	0.6	0.8	1.0
Vol. of distilled water (ml)	1.0	0.8	0.6	0.4	0.2	0

Duplicates were prepared at each dilution.

(ii) 4.0 mls of Biurette reagent were added to each tube and allowed to stand for 30 minutes.

(iii) Absorbency was measured at a wavelength of 540 nm on a Perkin Elmer spectrophotometer, model 550s. The graph of absorbency at 540 nm against the quantity of protein (in mg) in the sample was plotted.

(iv) A dilution factor of two (2) for the protein (in the muscle sample) was based on the plot of the standard curve.

Steps i to iii were repeated for the samples using the above dilution factor of 2.

By referring to the standard curve and muscle sample (supernatant) absorbency the protein concentration of the muscle samples was determined.

2.4.3 Enzyme (m-ATPase) assay

This was done in stirred tubes at 37°C with a total volume of 1.5 ml containing 100 mM Kcl, 20 mM Tris, 2.0 mM Mg-ATP, 2 mM $Mgcl_2$, 0.25 mM $Cacl_2$ and ~ 0.4 -0.5 mg

myofibrillar protein at pH 7.4 (adjusted at 37°C). The assay was started by addition of ATP and stopped after 30 sec. by addition of 0.1 ml of 30% trichloroacetic acid. Zero time controls were run by adding the chloroacetic acid first. Tubes were put on ice and finally analyzed for free phosphate by the method of LeBel *et al.*, (1978). Duplicates were run for all assays.

In this text the designation Ca^{2+} stimulated myofibrillar ATPase has been adopted as used by Johnston and Welsby, (1977) because Ca^{2+} in low concentrations have been found to reduce the activation enthalpy of MgATPase at temperatures greater than 15-20°C. The measured ATPase activity therefore closely approximates the in vivo physiological myofibrillar ATPase activity, Fuchs *et al.*, (1975).

2.4.4 **Free phosphate determination**

Determination of inorganic phosphate released from the ATPase assay was done using the method of Lebel *et al.*, (1978); itself a modification of the method of Fiske and Subbarow (1925). It is sensitive and reproducible and there is no interference by ATP in colour development. The method is based on the reduction of a phosphomolybdate complex by Elon (P-methyl aminophenol sulphate) in a copper acetate buffer. The reagents used are:

Reagent A: Copper acetate at pH 4.0;

0.25% Copper sulfate pentahydrate and 4.6% sodium acetate trihydrate in 2 N acetic acid.

Reagent B: Ammonium molybdate, 5%.

Reagent C: Elon (P methyl aminophenol sulphate), 2% in 5% sodium sulphite.

Reagent D: Phosphorous standard: 1.0 mg Pi/ml. Prepared by dissolving anhydrous KH_2PO_4 (0.4387 g/100 ml) in distilled water.

All the above reagents are known to be stable for months at 4°C including reagent C which has to be protected from light.

Procedure:

After incubation with a phosphorylated substrate (ATP), proteins were precipitated with trichloroacetic acid (as described above) and centrifuged at 3500 revolutions per minute (RPM), at 4°C for 10 min. The supernatant fluid was used for the determination of inorganic phosphate.

To 1.0 ml of supernatant fluid, 3.0 ml of reagent A and 0.5 ml of reagent B were added and mixed with a vortex mixer. Then 0.5 ml of reagent C was added and mixed. After seven minutes, the absorbency was read at 700 nm by a Perkin Elmer spectrophotometer model 550S. The optical densities so obtained were compared with the standard curve obtained as follows:

Phosphate standard curve:-

The phosphate standard solution was prepared as mentioned above (reagent D). 2.0 ml of this solution was picked, diluted a 100-fold and the new solution used in the drawing of the standard curve (see appendix II for experimental protocol). Optical densities at the various concentrations were determined on a Perkin Elmer spectrophotometer, model 550S at a wavelength of 700nm. A standard curve was then drawn (see appendix III). This procedure was done in quadriplicate. Phosphate concentrations in the samples above were determined by comparing with the known standard on the curve.

2.5 THYROID HORMONES

2.5.1 **Collection of blood samples**

(a) Embryos:

Incubated eggs from day fifteen to twenty-one of incubation were used. The eggs were candled to ascertain viability and also visualize the major blood vessels. The egg shell and the shell membranes were removed gently by cracking the shell and peeling it off together with the membranes, using hand forceps. This exposed the major allantoic blood vessels. The allantoic artery was identified by its dark red blood and gently lifted while being freed from the adjoining connective tissue. The direction of blood flow was determined. Blood flow was then obstructed by use of a

forceps and the artery cut downstream in relation to the forceps. The severed artery was then directed into a 1.0 ml plastic centrifuge tube that had previously been sprinkled with EDTA. The forceps was then released and all blood from the allantoic artery allowed to spurt into the tube. Between 0.25 ml to 0.5 ml of blood could be collected from each egg. In order to get enough plasma for analysis in duplicates blood samples from eggs at the same developmental stage were pooled. The pooled blood samples were then centrifuged at 3,500 revolutions per minute (RPM) at 4°C for 20 minutes. The plasma so obtained was kept in labelled tightly sealed tubes and frozen at -20°C until plasma T_3 and T_4 was measured two to three weeks later.

(b) Chicks:

Blood samples were collected from the jugular veins. The neck region was plucked of all down. The chick was stunned by a light blow on the head. The jugular veins were severed and blood collected into a 1.0 ml centrifuge tube that had been sprinkled with EDTA. As with embryo blood, it was centrifuged at 3,500 RPM at 4°C for 20 minutes and plasma stored in plastic tubes at -20°C until assaying of T_3 and T_4 could be done. Before determination the assay samples were allowed to thaw to room temperature and then mixed gently by swirling or inverting the tube.

2.5.2 Thyroxine (T_4) Assay

(a) Principle and theory

The Coat-A-count technique was used. This method was designed by Diagnostic Products Corporation (DPC). It is a solid-phase ^{125}I radioimmunoassay for the quantitative measurement of total circulating thyroxine (T_4) in serum and plasma. It is used as an aid in the clinical assessment of thyroid status. It is based on antibody coated tubes and human serum calibrators. ^{125}I-labelled T_4 competes for a fixed time with T_4 in the plasma sample for antibody sites, in the presence of blocking agents for thyroid hormone binding proteins. After the tubes are decanted and counted, the T_4 concentration is read from a standard curve.

The DPC assay is optimised for linearity in a logit-log representation throughout the range of its calibrators. The computation can be simplified by omitting the correction for non-specific binding (NSB), without compromising accuracy or quality control. The kit used was equiped with human serum based standards having T_4 values ranging from 1.0 to 24 µg/dl (12.9 to 309 nmol/liter). The technique is very sensitive and can detect T_4 levels as low as 0.7 µg/dl. The antiserum is highly specific for T_4 with very low cross-reactivity to other compounds that might be present in plasma samples. Neither protein, lipemia, bilirubin nor haemolysis has any significant effect on the assay. Heparinised and EDTA plasma yield essentially the same results as serum.

(b) Materials

Total T_4 antibody-coated tubes. 100 polypropylene tubes coated with antibodies (ab) to T_4 were provided in the assay kit. ^{125}I total T_4 (tracer). One vial of iodinated T_4, ready to use, with blocking agents to thyroid hormone binding protein. The vial contained 105 ml of tracer. Total T_4 calibrators were provided. One set of vials labelled A through F of T_4 calibrators in processed human serum. All were provided in liquid form ready for use.

-Gama counter - compatible with standard 12x75 mm tubes,

-Vortex mixer

-Plain 12x75 mm propylene tubes for total counts (TC) and non-specific binding (NSB) tubes

-Micropipettes

-Water-bath

-Foam decanting rack

(c) Procedure

(i). The assay was started at room temperature.

-Four plain (uncoated) 12x75 mm polypropylene tubes were labelled TC (total counts) and NSB (nonspecific binding) in duplicate.

-Twelve total T_4 ab-coated tubes were labelled A (maximum binding) and B through F in duplicate.

Table 1. Concentration of the calibrators in the T_4 assay kit

Calibrations	µg/dl	nmol/L
A	0	0
B	1	12.9
C	4	51.5
D	10	129
E	16	206
F	24	309

(ii). 25 ml of the zero calibrator A was pipetted into the NSB and A tubes, and 25 ml of each remaining calibrator, control and unknown plasma samples into each of the appropriate tubes prepared.

(iii). 1.0 ml of (^{125}I) total T_4 was added to every tube and vortex mixed.

(iv). The tubes were incubated in a water bath at 37°C for 60 minutes.

(v). After incubation the tubes were decanted thoroughly. To enhance precision all visible moisture was removed and the tubes allowed to drain for 2 to 3 minutes in a decanting rack. The tubes were then struck sharply on absorbant paper to shake off all residual droplets.

(vi). Counting was done for 1 minute in a gamma counter.

(vii). A calibration curve was drawn on a logit-log representation (see appendix IV). T_4 concentrations were then calculated from the calibration curve.

2.5.3 **Triiodothyronine (T_3) assay**

(a) Principle and theory

The principle of the assay for T_3 is similar to that for T_4 assay. The (DPC) **coat-A-count** technique was used. It is also a solid phase RIA, where in ^{125}I labelled T_3 competes for a fixed time with T_3 in the unknown plasma sample for antibody sites. This reaction also takes place in the presence of blocking agents which serve to liberate bound triiodothyronine from carrier proteins; hence the assay measures total T_3, since both free and protein bound T_3 from the unknown plasma sample are able to compete with radio labelled T_3 for antibody sites. The antibody being immobilized to the wall of the polypropylene tube, decanting the supernatant suffices to terminate the competition and to isolate the antibody bound fraction of the radio labelled T_3. Counting the tubes in a gamma counter then yields a number, which when converted by way of a calibration curve gives a measure of the T_3 present in the unknown plasma sample.

For this **coat-A-count** method, the DPC kit provided was equiped with human serum-based standards having T_3 values ranging from 20-600 ng/dl (0.31-9.22 nmol/L). The antiserum is highly specific and sensitive, detecting amounts of T_3 as little as 7 ng/dl with no observable drift.

(b) Materials and procedure

It was the same as for T_4 assay with only slight differences as indicated below:

(i). The coated tubes had T_3 antibody.

Table 2. Concentration of the calibrators in the T_3 assay kit

Calibrators	ng/dl	nmol/L
A (mB)	0	0
B	20	.31
C	50	.77
D	100	1.54
E	200	3.07
F	600	9.22

(ii). Aliquotes of 100 ml were used for both the calibrators and the unknown plasma samples.

(iii). 1.0 ml of (^{125}I) total T_3 was added to every tube and vortex mixed briefly.

(iv). Incubation was in a water bath at 37°C for 120 minutes.

(v). The tubes were similarly decanted, drained and counted for 1 minute in a gamma counter as for T_4.

(vi). A calibration curve was similarly drawn on a logit-log representation (See appendix V) and the amounts of T_3 in the samples calculated.

2.6 DERIVED VALUES

2.6.1 T_3/T_4 ratio

The ratio of triidothyronine to thyroxine levels in plasma was computed from day fourteen incubation embryos, through hatching up to seven day old chicks. T_4 is the precursor for T_3, the active hormone. A high plasma T_3/T_4 ratio can therefore result from increased conversion of T_4 to T_3 with the resultant increase in metabolic rate and heat production

2.6.2 Q_{10} Effect

It is a measure of the change in metabolism with increasing or decreasing body temperature. In biological systems, metabolic rate appears to approximately double for every 10°C change in body temperature, corresponding to a Q_{10} of 2.

$\dot{V}O_2$ at 38°C was taken as the standard for the incubating eggs and so $\dot{V}O_2$ for eggs at 28°C was calculated using the formula:

$$Q_{10} = \left[\frac{\dot{V}O_2(38^{\circ}C)}{\dot{V}O_2(Ta)}\right]^{(10/38-Ta)}$$

Where Ta = Ambient temperature.

Since T_a's of 28°C only were used, this equation simplified to

$$Q_{10} = \left[\frac{\dot{V}O_2(38^oC)}{\dot{V}O_2(28^oC)}\right]^{1.0}$$

The Q_{10} and $\dot{V}O_2$ so obtained for the eggs was then compared to $Q_{10} = 2$ and $\dot{V}O_2$ observed at 28°C respectively. If the egg is thermoregulating, then metabolism should be uncoupled from this temperature effect.

2.7 STATISTICS

Masses of eggs, hatchlings and chicks are given as means ± Standard deviation (SD). Oxygen consumption and myofibrillar ATPase activities are both given as raw data points and as means ± SD. In the case of the eggs, regression lines were fitted through the raw data points and regression equations given. Rectal temperatures of the hatchlings, chicks and plasma thyroid hormones are also presented as means ± SD. Standard error bars are also shown for mean oxygen consumption on day fifteen, rectal temperatures, myofibrillar ATPase activity and plasma thyroid hormones. All values in the text are given as means ± Standard deviations.

Correlation coefficients (r) and their statistical levels of significance were obtained by the analysis of variance from Statview (Abacus concepts, Inc., Berkely California U.S.A.), a statistical programme in the Apple Macintosh

computer (Apple computers, Inc. Cupertino, U.S.A). Throughout the text, r=R and r^2 is the same as R^2.

Oxygen consumption and rectal temperatures for hatchlings/chicks at the thermoneutral zone (TNZ; used as a baseline) and their corresponding values at lower T_a's were tested for any statistically significant differences using the Students unpaired t-test (two tailed). The t-test was used to determine at what ambient temperature a significant increase in $\dot{V}O_2$ in chicks occurred compared to $\dot{V}O_2$ at the thermoneutral zone (TNZ). The t-values having probabilities (P) of less than 0.05 are considered significant. The t-test was also used to determine at what point a significant fall in $\dot{V}O_2$ of embryos occurred with decreasing ambient temperature. The Students unpaired t-test (two tailed) was used to compare the m-ATPase activity on day 18 of incubation (used as a baseline) with that for the subsequent days up to day seven post-hatching, in order to determine any statistically significant increase.

ANOVA was used to compare differences between initial and final rectal temperatures for both hatchlings and chicks in order to determine at what ambient temperature a statistically significant fall in rectal temperature occurred. F-value probabilities of less than 0.05 were considered significant. ANOVA, t-tests, F and t-value probabilities were all obtained from Statview.

2.8 PRESENTATION OF RESULTS

The results for the embryos are presented before those for hatchlings and chicks. Oxygen consumption results for both embryos and chicks are presented in order of increasing age. Oxygen consumption expressed as ml/hr or $ml(g.hr)^{-1}$, rectal temperatures, myofibrillar ATPase activity and plasma thyroid hormone levels are the dependent variables(Y axis). The ages in days of the embryos and chicks together with ambient temperatures are the independent variables (X axis). Mean oxygen consumption is shown plotted against age in days for both the embryos and chicks.

From 15 day old embryos up to seven day old chicks raw data points of oxygen consumption vs ambient temperatures are shown. Summaries of mean oxygen consumption in $ml(g.hr)^{-1}$ against T_a are also presented. For embryos, the Q_{10} values corresponding to the change in $\dot{V}O_2$ between 38° and 28°C are calculated and given in the text. All ages of embryos have first order linear regression lines fitted to describe their $\dot{V}O_2$, for uniformity in the presentation of results and to view the degree of association between $\dot{V}O_2$ and T_a.

Myofibrillar ATPase activity, plasma T_3 and T_4 are also plotted against age in days for both the embryos and chicks. Both raw data points and means are shown. The computed plasma T_3/T_4 ratio was also plotted against age in days.

CHAPTER THREE

3.0 RESULTS

3.1 OXYGEN CONSUMPTION

3.1.1 Embryos

Oxygen consumption ($\dot{V}O_2$) at 38°C increased with age from day fifteen to day twenty one of incubation, (Figure 1). The $\dot{V}O_2$ rose from 17.88 ± 1.00 ml/hr (n=6) on day fifteen to 24.73 ± 0.99 ml/hr (n=5) on day seventeen of incubation and then stayed fairly constant (plateau phase) upto day nineteen when it was 25.16 ± 0.86 ml/hr (n=5). $\dot{V}O_2$ then rose to 28.65 ± 2.2 ml/hr. on day twenty one. $\dot{V}O_2$ in ml $(g\ hr)^{-1}$ at 38°C also followed the same trend (Figure 2), rising from 0.37 ± 0.02 $ml(g.hr)^{-1}$ (n=6) on day fifteen upto 0.49 ± 0.02 $ml(g.hr)^{-1}$ on day seventeen of incubation. Between day seventeen and nineteen of incubation, there was no significant increase in $\dot{V}O_2$ ($P > 0.05$). The $\dot{V}O_2$ then rose upto 0.65 ±0.04 $ml(g.hr)^{-1}$ on day twenty before falling to 0.58 ±0.02 $ml(g.hr)^{-1}$ on day twenty one of incubation, (Figure 2).

From day fifteen to twenty one of the incubation $\dot{V}O_2$ for the embryos was measured with decreasing ambient temperatures. Day fifteen mean egg weight was 49.15 ± 2.6 g (n=14). On day fifteen, (Figure 3a) $\dot{V}O_2$ at 38°C was 0.37 ±

0.02 ml(g.hr)$^{-1}$ and decreased to 0.19 ± 0.02 ml(g.hr)$^{-1}$ at Ta of 28°C. The Q_{10} (Vant Hoff factor) corresponding to this 10°C fall in Ta is 1.94. The calculated value of $\dot{V}O_2$ at a T_a of 28°C , assuming a Q_{10} of 2, is 0.185 ml(g.hr)$^{-1}$. This is slightly lower than the observed value, but not significantly different. The $\dot{V}O_2$ at 28°C was 48.6% of that at the control temperature of 38°C (T_a), (Figure 3a). All subsequent results of $\dot{V}O_2$ vs ambient temperatures (T_a) for the embryo are represented as scatter diagrams in Figures 3b to 9. On day fifteen of the incubation, (Figure 3b) $\dot{V}O_2$ decreases with decreasing T_a. The fitted regression line has the equation y = 0.018x - 0.319; R^2 (r^2)= 0.867. There is a statistically significant correlation between $\dot{V}O_2$ and T_a (r = 0.931; P < 0.001).

On day sixteen of incubation mean egg mass was 51.74 ± 2.5g (n=16). The embryo $\dot{V}O_2$ decreased from 0.42 ± 0.01 ml(g.hr)$^{-1}$ (n=5) at 38°C to 0.22 ± 0.02 ml(g.hr)$^{-1}$ at 28°C representing a decrement of 47.6% for the 10°C change in ambient temperature, (Figure 4). The Q_{10} corresponding to this change in metabolism is 1.9. The calculated $\dot{V}O_2$ at T_a of 28°C assuming Q_{10} = 2, is 0.21 ml(g.hr)$^{-1}$ which is just below the observed $\dot{V}O_2$, but is not significantly different. The equation for the regression line through this scatter-gram is y = 0.022 x - 0.41; r^2= 0.918. There is a statistically significant correlation between T_a and $\dot{V}O_2$ (r = 0.958; P < 0.001).

On day seventeen, the mean egg mass was 50.78 ± 3.2 g. (n=15). The embryo $\dot{V}O_2$ changed from 0.49 ± 0.02 ml(g.hr)$^{-1}$ at 38°C, to 0.2 ±0.01 at 28°C corresponding to a 59% fall in $\dot{V}O_2$ for a 10°C change in ambient temperature, (Figure 5). The equation for the fitted regression line is y=0.026x - 0.55; r^2 = 0.906. The correlation of Ta and $\dot{V}O_2$ is statistically significant (r=0.95; P<0.001). The Q_{10} for this reaction is 2.05.

On day eighteen of incubation, the mean egg mass was 48.1 ± 2.8 g.(n=16). The embryo $\dot{V}O_2$ at 38°C was 0.49 ± 0.02 ml(g.hr)$^{-1}$ and fell by 40.8% to 0.29 ± 0.01 ml(g.hr)$^{-1}$ at 28°C, (Figure 6). This change in metabolism corresponds to a Q_{10} of 1.69 from 38°C to 28°C. The equation for the fitted regression line is y = 0.023x - 0.378; r^2=0.86. The correlation coefficient between Ta and $\dot{V}O_2$ is statistically significant (r=0.927, P<0.001). Cooling the eggs from 38°C to 36°C caused no significant difference in $\dot{V}O_2$ (P >0.5). $\dot{V}O_2$ fell with T_a's below 36°C, (Figures 6 and 10).

On day nineteen, mean egg mass was 46.46 ± 2.5 g. (n=14). Embryo $\dot{V}O_2$ fell by 40% from 0.55 ± 0.01 ml(g.hr)$^{-1}$ at 38°C to 0.33 ± 0.01 ml(g.hr)$^{-1}$ at 28°C, (Figure 7). This change in metabolism corresponds to a Q_{10} of 1.66. The fitted regression line is described by the equation y = 0.026x - 0.44; r^2=0.884. The correlation coefficient for Ta vs $\dot{V}O_2$ for this equation is statistically significant (r=0.94; P<0.001), (Figure

7). From 38°C to 36°C there was no significant change in $\dot{V}O_2$ ($P > 0.5$), (Figures 7 and 10).

On day twenty, the mean egg mass was 46 ± 0.5g.(n=12). The embryo $\dot{V}O_2$decreased by 38% from 0.65 ± 0.04 ml $(g.hr)^{-1}$ at 38°C to 0.4 ± 0.01 ml$(g.hr)^{-1}$ at 28°C, (Figure 8). This change corresponds to a Q_{10} of 1.62. The equation for the fitted regression line is $y=0.029x - 0.395$; $r^2 = 0.734$. The correlation coefficient for Ta and $\dot{V}O_2$ is statistically significant, ($r=0.857$; $P< 0.001$). There was no significant change in $\dot{V}O_2$ from 38°C upto 33°C ($P>0.2$), (Figures 8 and 10). In fact there is a general but slight increase in $\dot{V}O_2$ with decreasing Ta from 38°C to 33°C.

On day twenty one, the mean egg mass was 45.3 ± 2.2 g. (n=14). The embryo $\dot{V}O_2$ decreased by 31% from 0.58 ± 0.02 ml $(g.hr)^{-1}$ at 38°C to 0.4 ± 0.02 ml $(g.hr)^{-1}$ at 28°C, (Figure 9). This corresponds to a Q_{10} of 1.45. The fitted regression line has the equation; $y = 0.017x - 0.036$; $r^2=0.692$. The correlation between temperature and oxygen consumption is statistically significant ($r= 0.831$; $P< 0.001$). From 38°C to 33°C there was no statistically significant change in $\dot{V}O_2$ with decreasing Ta; ($P > 0.2$), (Figures 9 and 10)

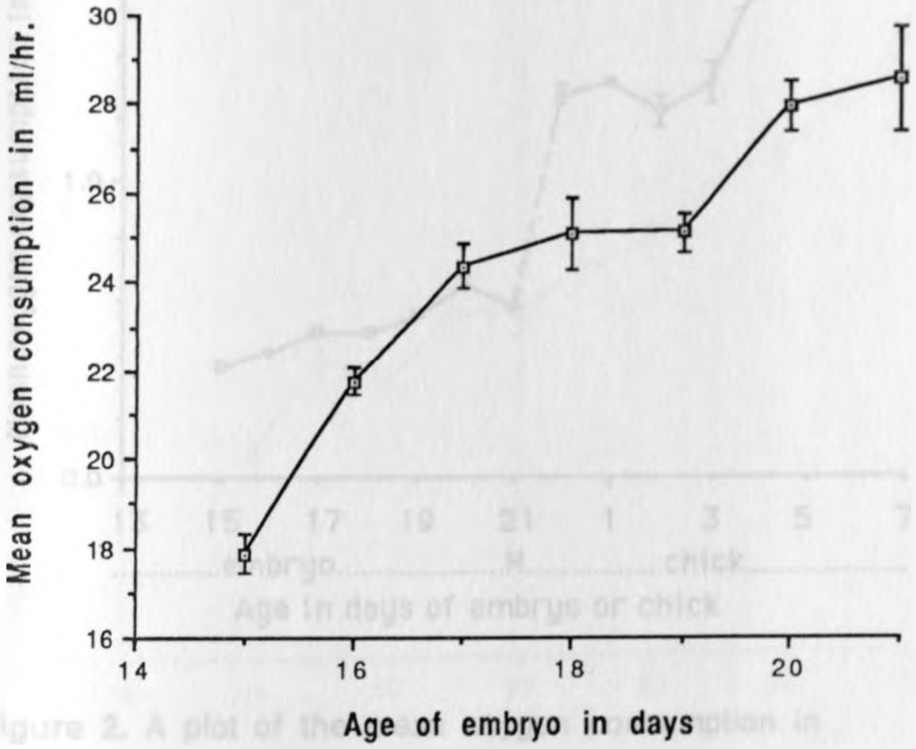

Figure 1. A plot of the mean oxygen consumption in ml/hr. at 38°C. against age in days for the developing embryo of the domestic fowl, *Gallus domesticus*, between day fifteen and twenty one of incubation.

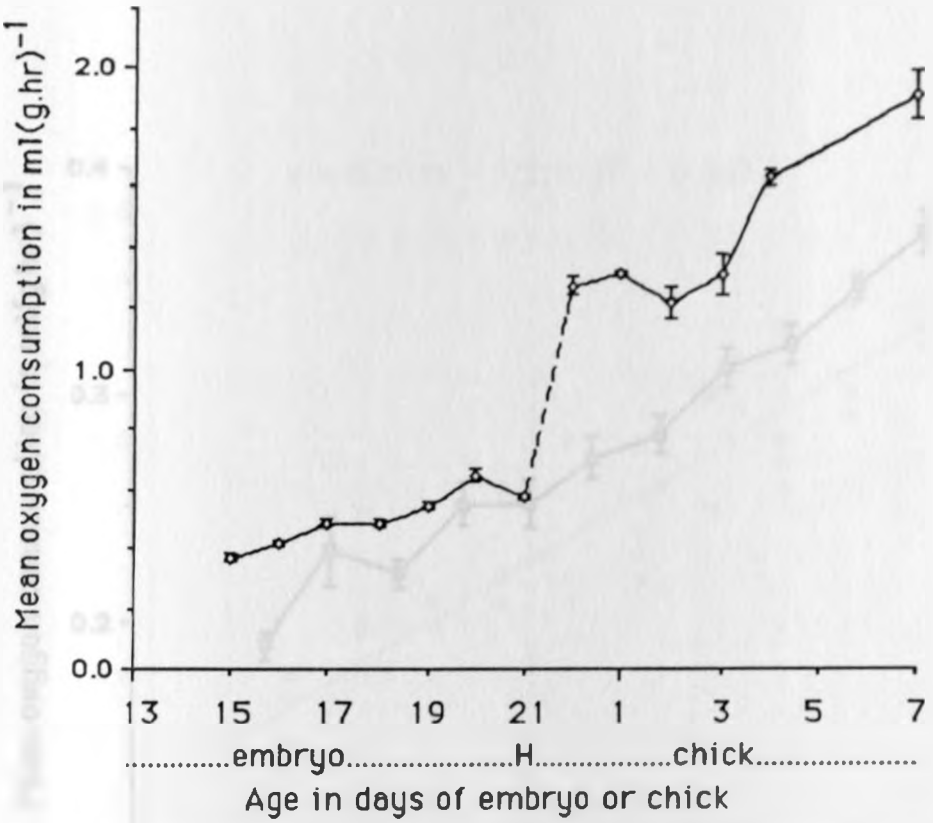

Figure 2. A plot of the mean oxygen consumption in ml(g.hr)$^{-1}$. (respiratory intensity) against age in days for the embryo and chick of the domestic fowl, *Gallus domesticus*, from day fifteen of incubation through the first week after hatching. H denotes the time of hatching at day twenty one. The five hours period between hatching and day zero when the down dries is shown by the broken line .

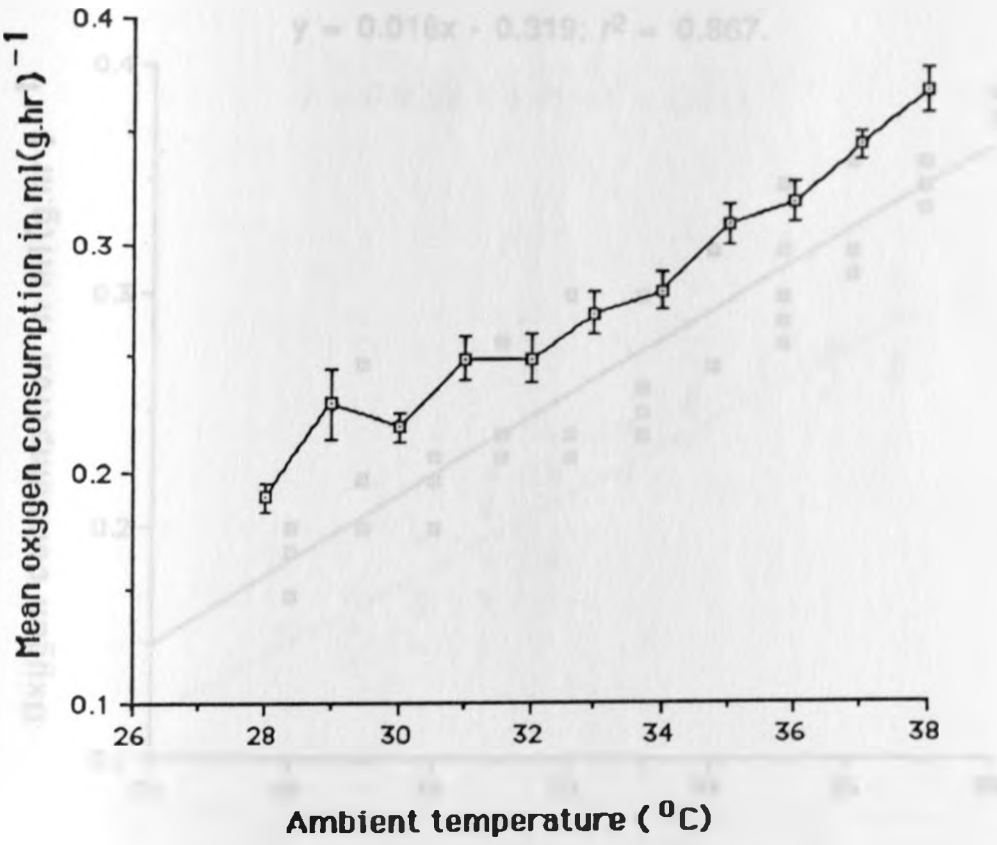

Figure 3a. A plot of the mean oxygen consumption in ml(g.hr)$^{-1}$ (respiratory intensity) against ambient temperature in degrees centigrade for the fifteen day old embryo of the domestic fowl, *Gallus domesticus*. The bars represent standard error of the means.

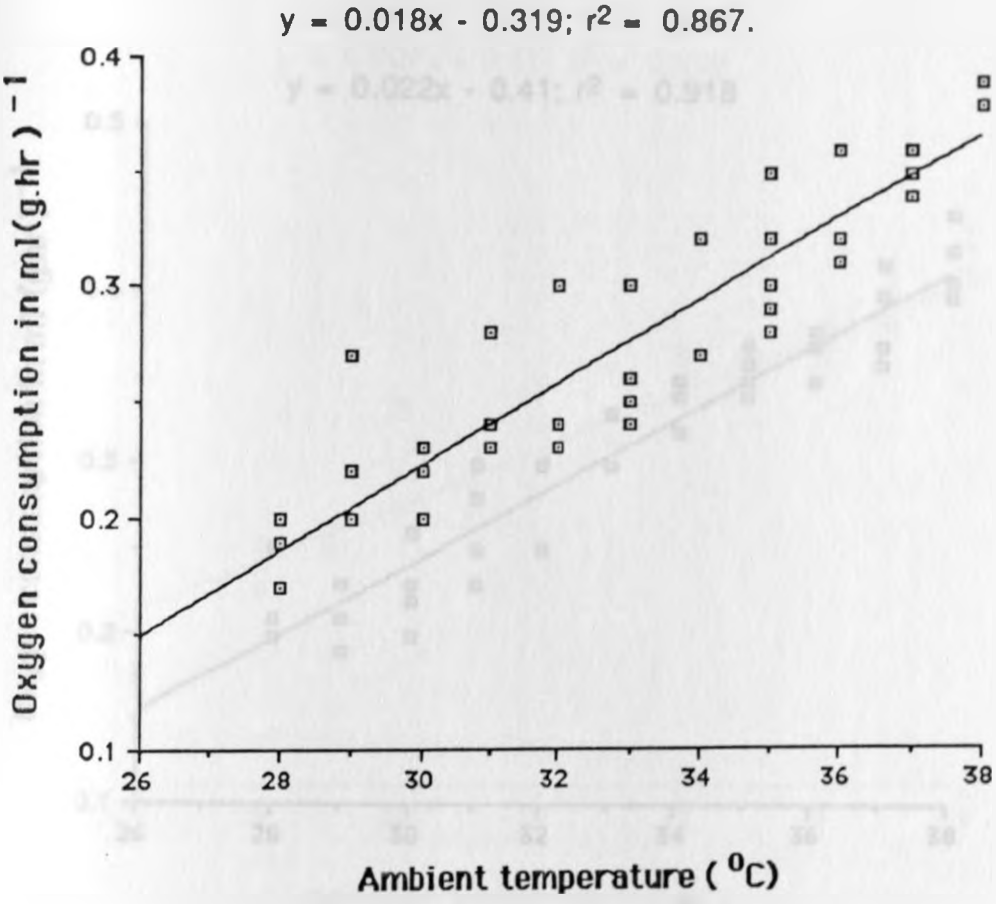

Figure 3b. A scatter plot of oxygen consumption in $ml(g.hr)^{-1}$ (respiratory intensity) against ambient temperature in degrees centigrade for the fifteen day old embryo of the domestic fowl, *Gallus domesticus*. Regression line for best fit is shown.

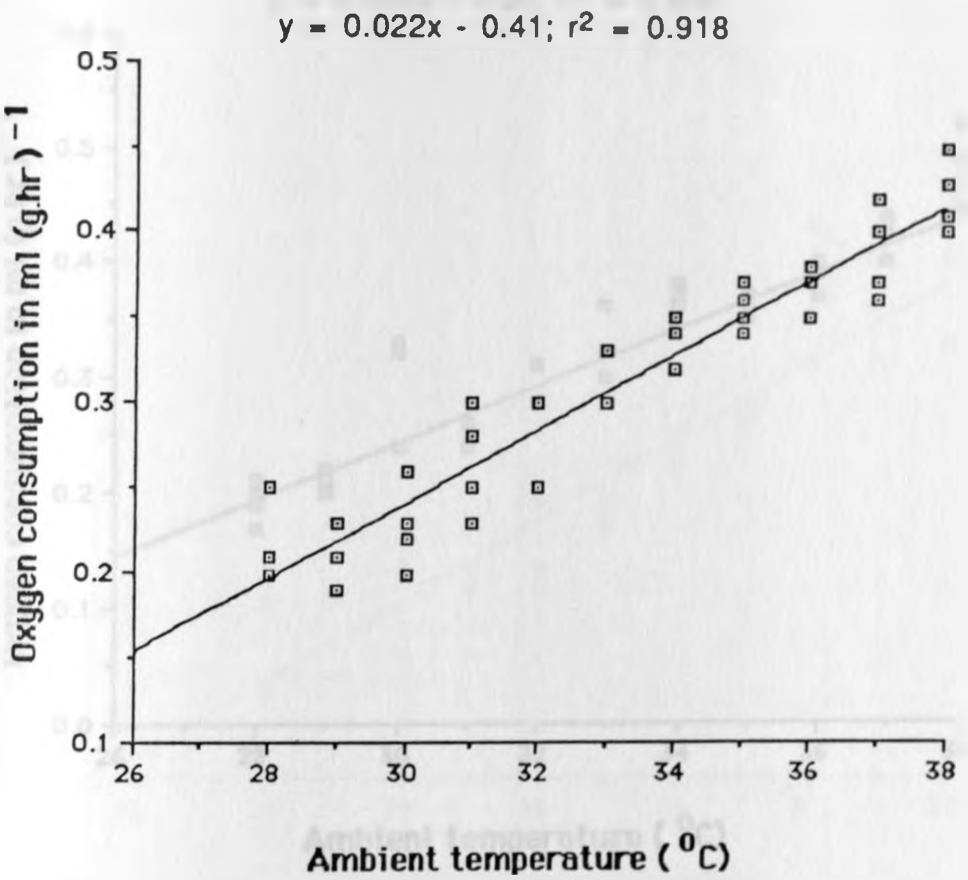

Figure 4. A scatter plot of oxygen consumption in ml(g.hr)$^{-1}$ (respiratory intensity) against ambient temperature in degrees centigrade for the sixteen day old embryo of the domestic fowl, *Gallus domesticus*. Regression line for best fit is shown.

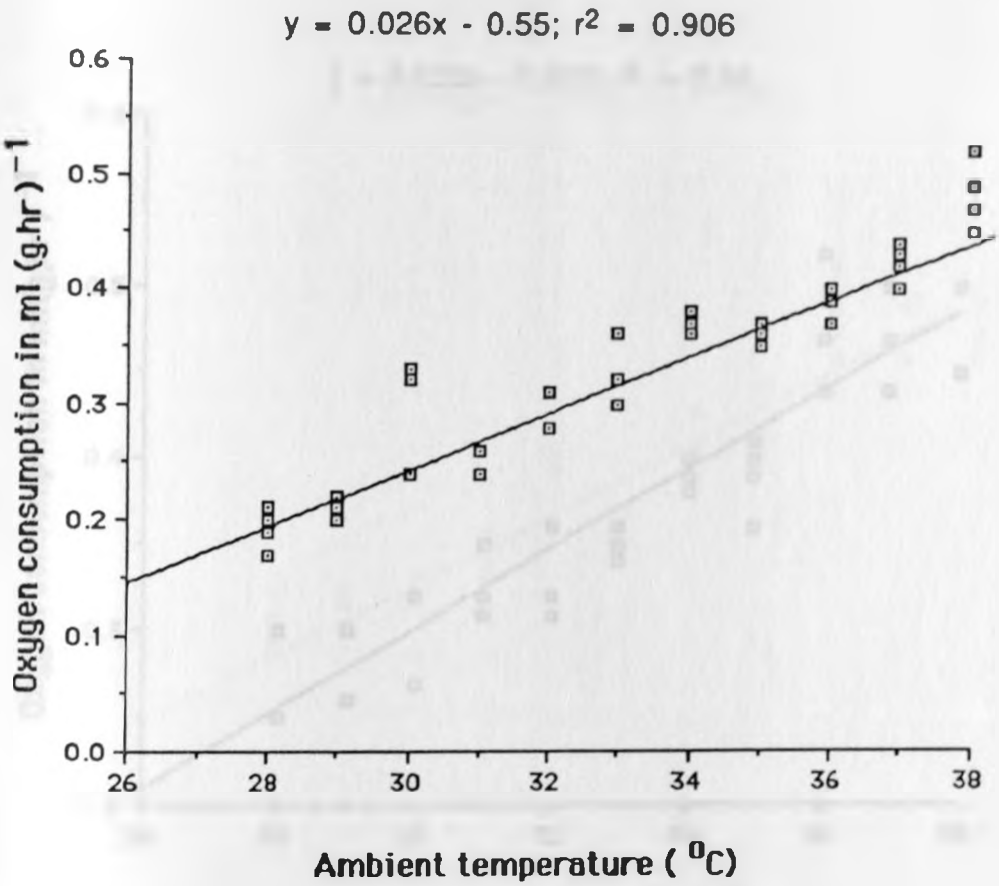

Figure 5. A scatter plot of oxygen consumption in ml(g.hr)$^{-1}$ (respiratory intensity) against ambient temperature in degrees centigrade for the seventeen day old embryo of the domestic fowl, *Gallus domesticus*. Regression line for best fit is shown.

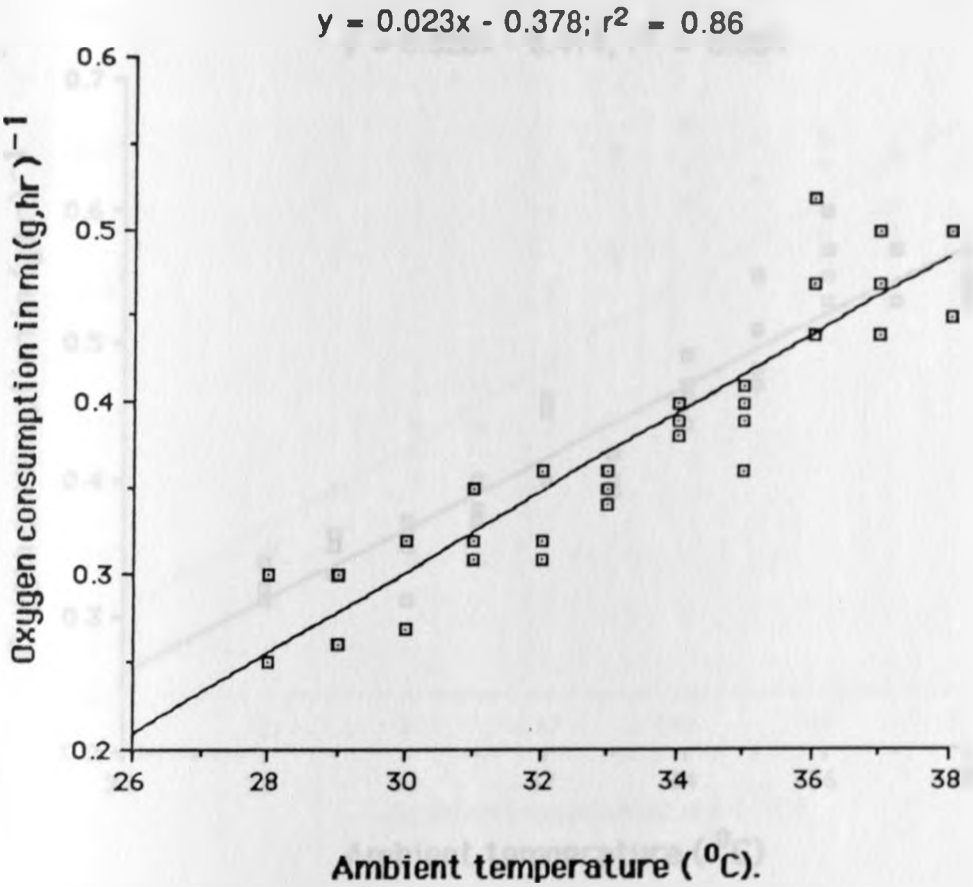

Figure 6. A scatter plot of oxygen consumption in $ml(g.hr)^{-1}$ (respiratory intensity) against ambient temperature in degrees centigrade for the eighteen day old embryo of the domestic fowl, *Gallus domesticus*. Regression line for best fit is shown.

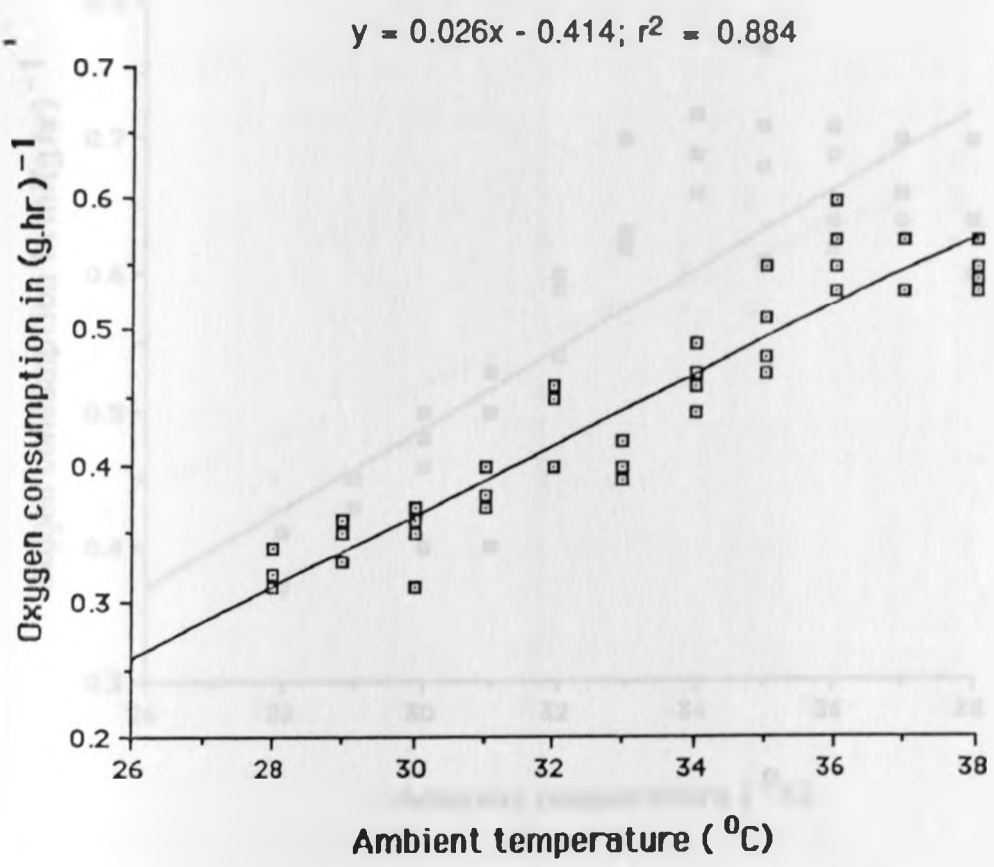

Figure 7. A scatter plot of oxygen consumption in ml(g.hr)$^{-1}$ (respiratory intensity) against ambient temperature in degrees centigrade for the nineteen day old embryo of the domestic fowl, *Gallus domesticus*. Regression line for best fit is shown.

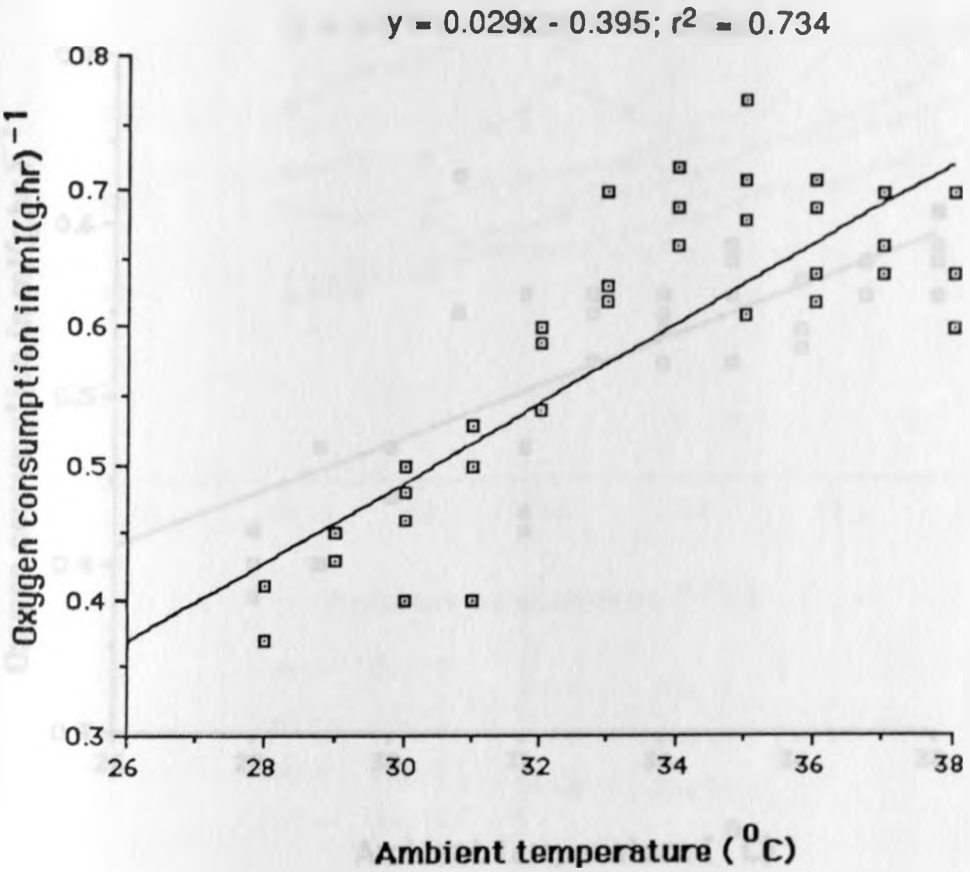

Figure 8. A scatter plot of oxygen consumption in $ml(g.hr)^{-1}$ (respiratory intensity) against ambient temperature in degrees centigrade for the twenty day old embryo of the domestic fowl, *Gallus domesticus.* Regression line for best fit is shown.

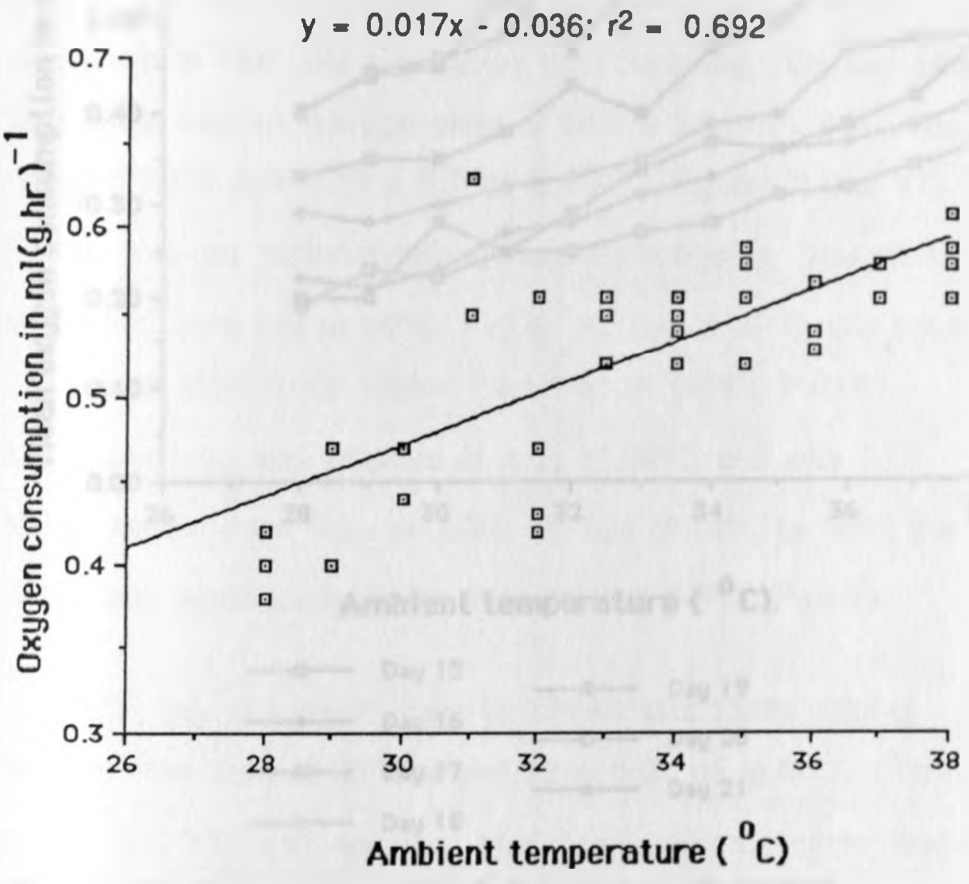

Figure 9. A scatter plot of oxygen consumption in $ml(g.hr)^{-1}$ (respiratory intensity) against ambient temperature in degrees centigrade for the twenty one day old embryo of the domestic fowl, *Gallus domesticus*. Regression line for best fit is shown.

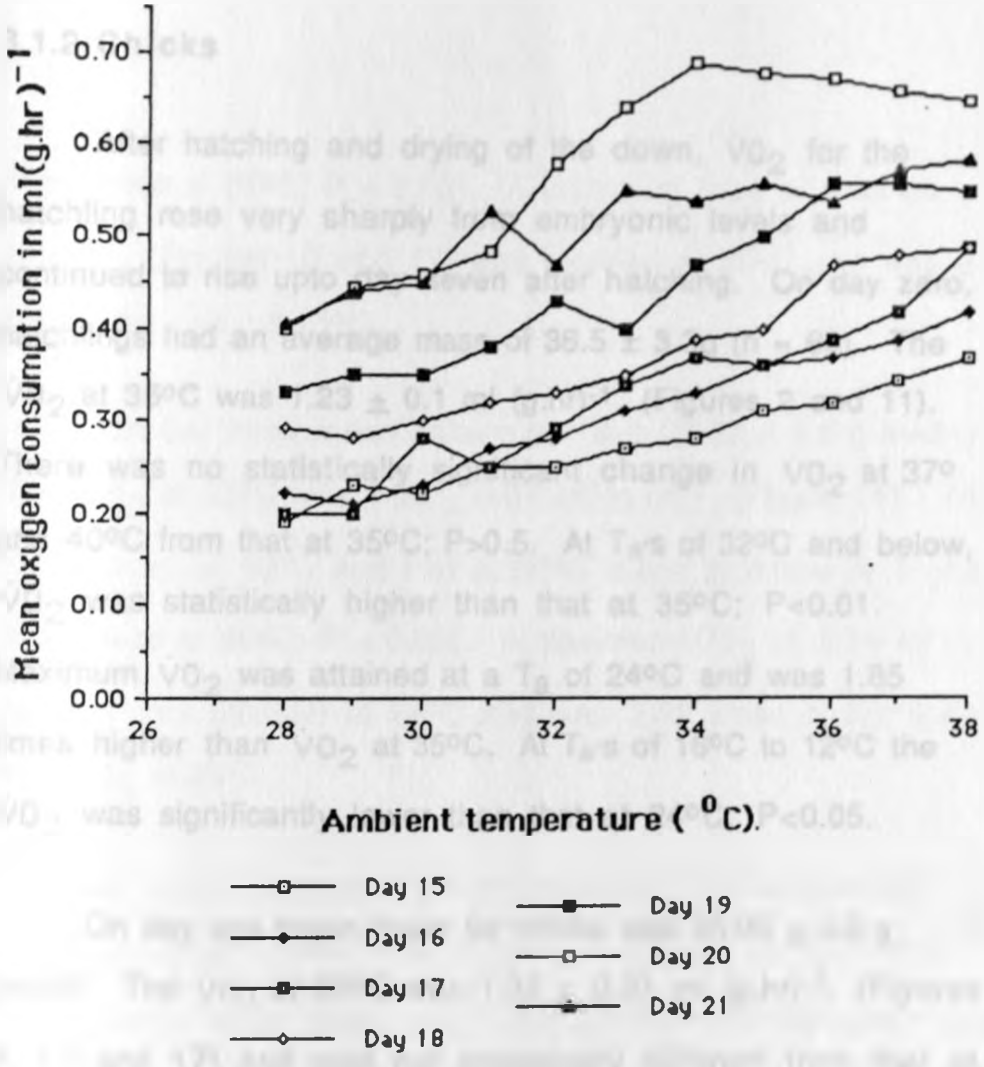

Figure 10. A summary plot of the means of oxygen consumption in $ml(g.hr)^{-1}$ (respiratory intensity) against ambient temperatures in °C during the last seven days (15-21) of incubation for the embryos of the domestic fowl, *Gallus domesticus*. The key to the graph is also shown above.

3.1.2 **Chicks**

After hatching and drying of the down, $\dot{V}O_2$ for the hatchling rose very sharply from embryonic levels and continued to rise upto day seven after hatching. On day zero, hatchlings had an average mass of 36.5 ± 3.3g (n = 60). The $\dot{V}O_2$ at 35°C was 1.23 ± 0.1 ml $(g.hr)^{-1}$, (Figures 2 and 11). There was no statistically significant change in $\dot{V}O_2$ at 37° and 40°C from that at 35°C; $P>0.5$. At T_a's of 32°C and below, $\dot{V}O_2$ was statistically higher than that at 35°C; $P<0.01$. Maximum $\dot{V}O_2$ was attained at a T_a of 24°C and was 1.85 times higher than $\dot{V}O_2$ at 35°C. At T_a's of 16°C to 12°C the $\dot{V}O_2$ was significantly lower than that at 24°C; $P<0.05$.

On day one mean mass for chicks was 35.05 ± 2.3 g (n=58). The $\dot{V}O_2$ at 35°C was 1.32 ± 0.01 ml $(g.hr)^{-1}$, (Figures 2, 12 and 17) and was not statistically different from that at 40°C; $P>0.05$. At T_a's of 30°C and below, $\dot{V}O_2$ was statistically higher than at 35°C; $P<0.01$. Maximum $\dot{V}O_2$ was attained at 24°C and was 2.27 times higher than the $\dot{V}O_2$ at 35°C. There was no significant difference between $\dot{V}O_2$ at 24°C and that of temperatures as low as 12°C; $P> 0.2$.

On day two, the mean chick mass was 35.45 ± 25 g (n=56). The $\dot{V}O_2$ at 35°C was 1.23 ± 0.5 ml $(g.hr)^{-1}$, (Figure 13) The $\dot{V}O_2$ values at 40°C and 28°C were significantly higher

than those at 35°C; $P < 0.001$. A maximum $\dot{V}O_2$ of 3.35 ml $(g.hr)^{-1}$, (Figures 13 and 17) was attained at 20°C and was 2.7 times the $\dot{V}O_2$ at 35°C.

On day three mean chick mass was 37.23 ± 3.8 g (n=51) The $\dot{V}O_2$ at 35°C was 1.32 ± 0.07 ml $(g.hr)^{-1}$, (Figure 14). The $\dot{V}O_2$values at 40°C and that at 30°C were significantly higher than those at 35°C; $P < 0.001$. A maximum $\dot{V}O_2$ of 3.24 ml $(g.hr)^{-1}$ was attained at 18°C and was 2.45 times higher than the $\dot{V}O_2$ at 35°C.

On day five, mean chick mass was 40.7 ± 3.4g (n=50). The $\dot{V}O_2$ at 35°C was 1.65 ml $(g.hr)^{-1}$, (Figure 15) and was significantly lower than $\dot{V}O_2$ at 26°C; $P<0.05$. A maximum $\dot{V}O_2$ of 2.92 ml$(g.hr)^{-1}$ was attained at 16°C and was 1.77 times higher than the $\dot{V}O_2$ at 35°C, (Figures 15 and 17).

On day seven mean chick mass was 47.92 ± 5.4 (n=51) The $\dot{V}O_2$ at 35°C was 1.93 ± 0.08 ml$(g.hr)^{-1}$, (Figure 16) and was significantly lower than that at 28°C; $P<0.05$. A maximum $\dot{V}O_2$ of 3.2ml$(g.hr)^{-1}$ was attained at at T_a of 16°C and was 1.7 times the $\dot{V}O_2$ at 35°C, (Figures 16 and 17).

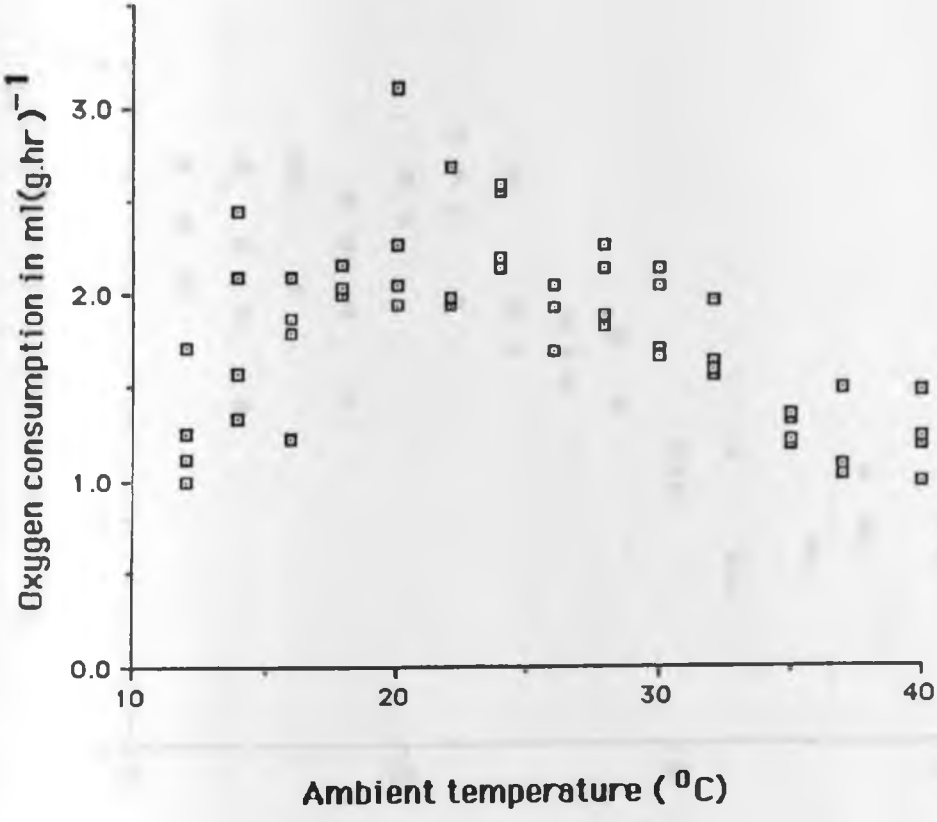

Figure 11. A scatter plot of oxygen consumption in ml(g.hr)$^{-1}$ (respiratory intensity) against ambient temperatures in ^{o}C for the zero day old chick of the domestic fowl, *Gallus domesticus*.

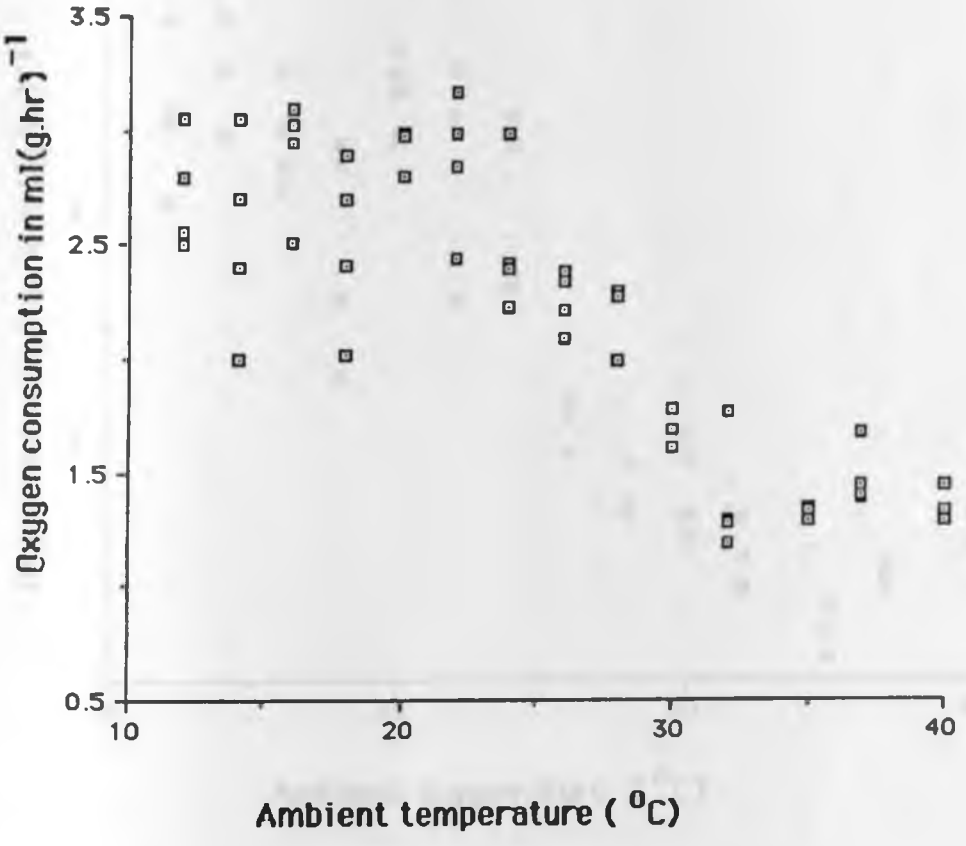

Figure 12. A scatter plot of oxygen consumption in ml(g.hr)$^{-1}$ (respiratory intensity) against ambient temperatures in °C for the day old chick of the domestic fowl, *Gallus domesticus*.

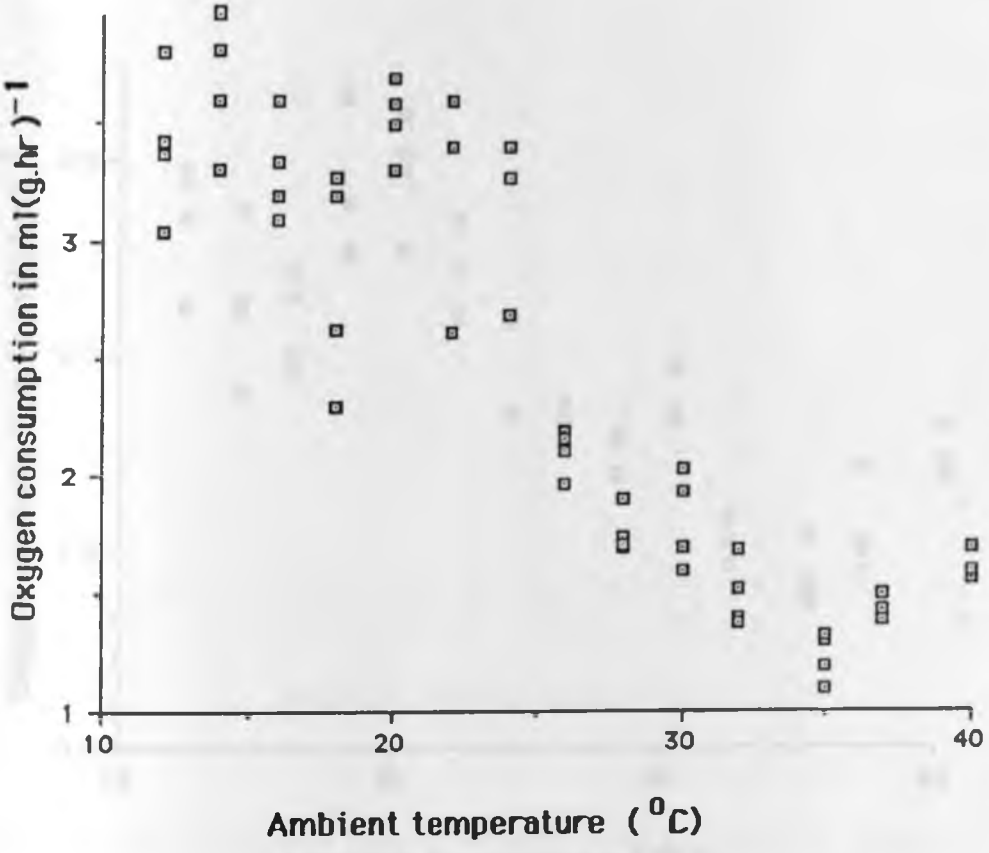

Figure 13. A scatter plot of oxygen consumption in ml(g.hr)$^{-1}$(respiratory intensity) against ambient temperatures in °C for the two day old chick of the domestic fowl, *Gallus domesticus.*

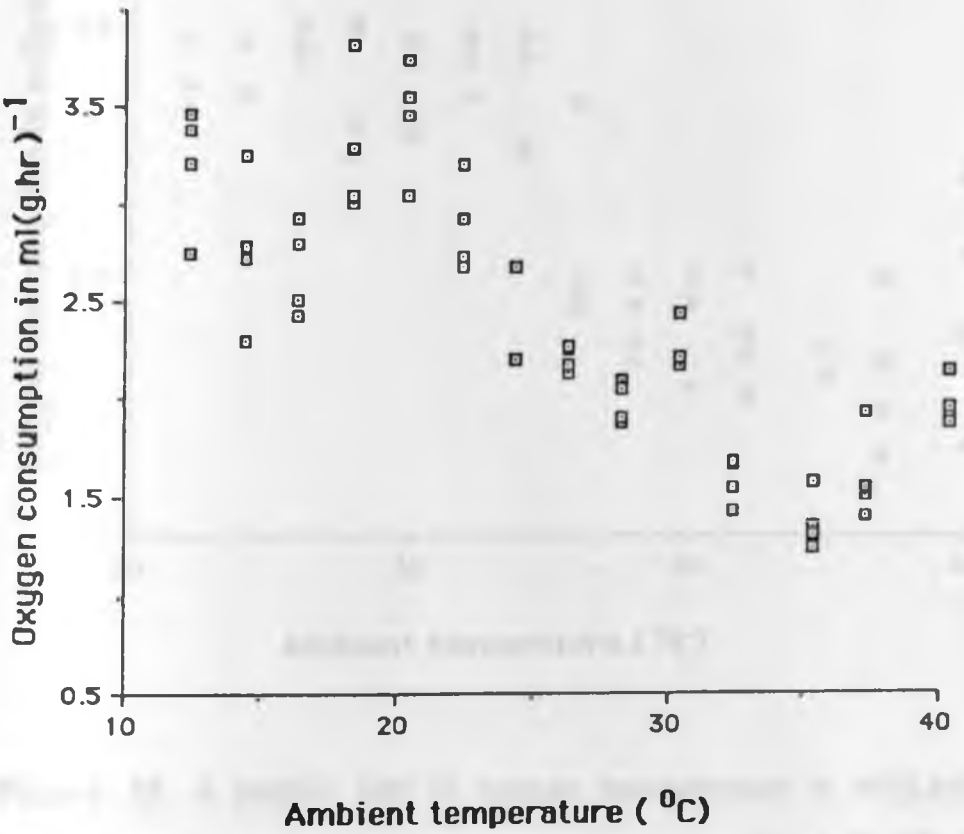

Figure 14. A scatter plot of oxygen consumption in ml(g.hr)$^{-1}$ (respiratory intensity) against ambient temperatures in ^{o}C for the three day old chick of the domestic fowl, *Gallus domesticus*.

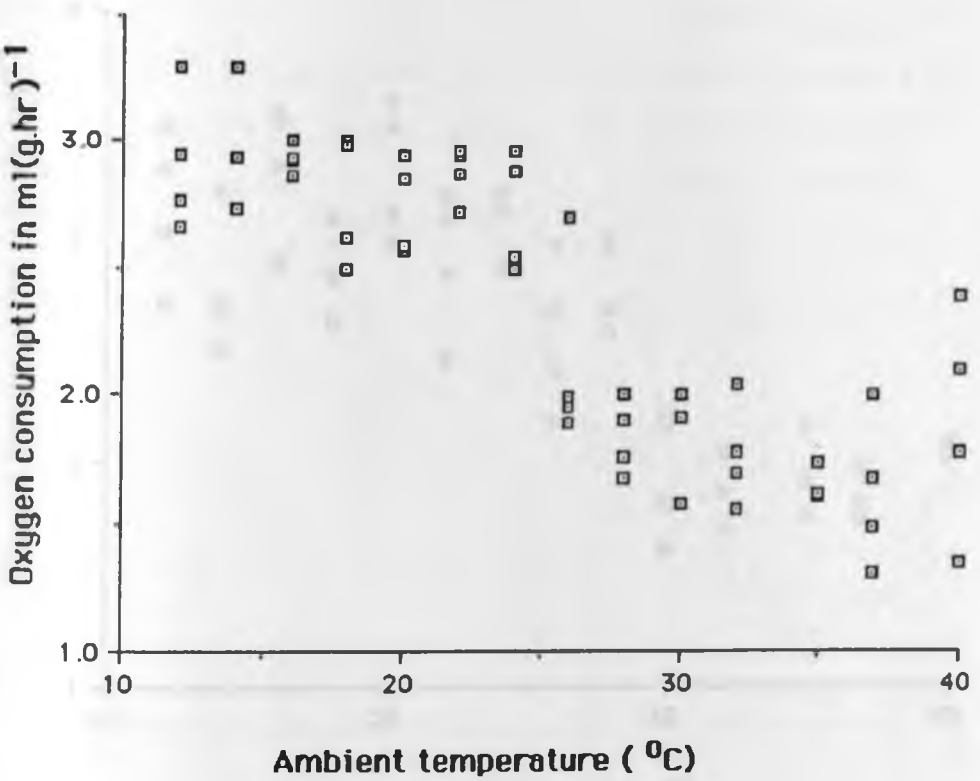

Figure 15. A scatter plot of oxygen consumption in $ml(g.hr)^{-1}$ (respiratory intensity) against ambient temperatures in °C for the five day old chick of the domestic fowl, *Gallus domesticus*.

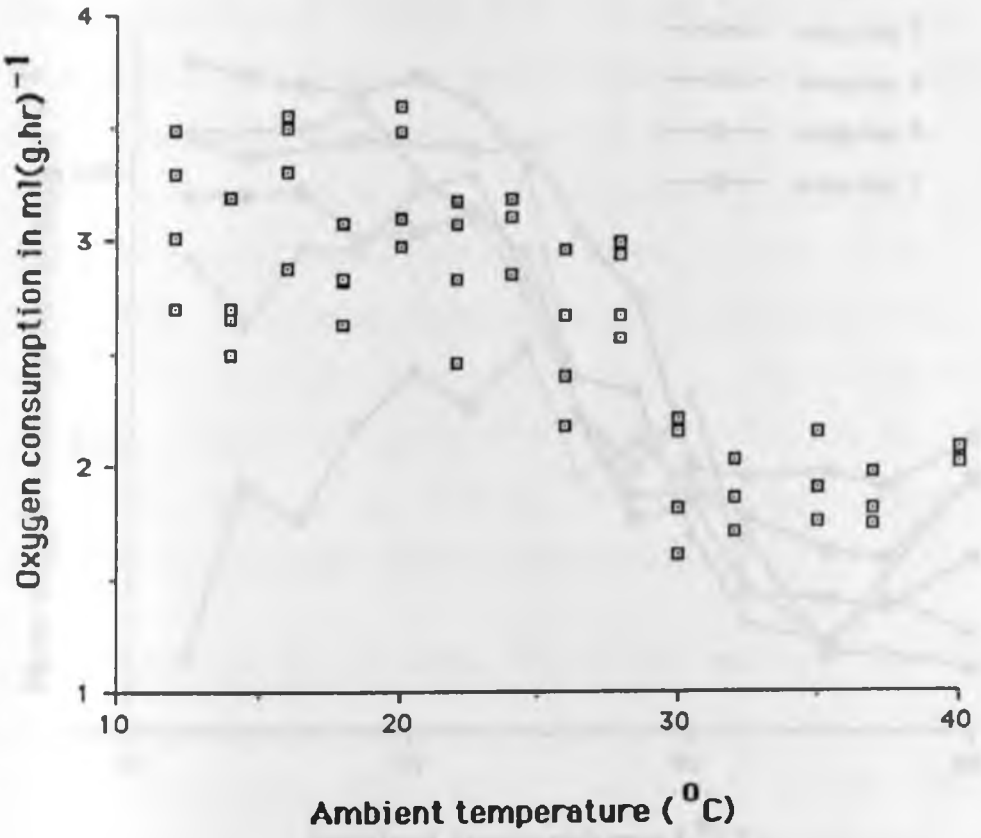

Figure 16. A scatter plot of oxygen consumption in ml(g.hr)$^{-1}$ (respiratory intensity) against ambient temperatures in ^{o}C for the seven day old chick of the domestic fowl, *Gallus domesticus*.

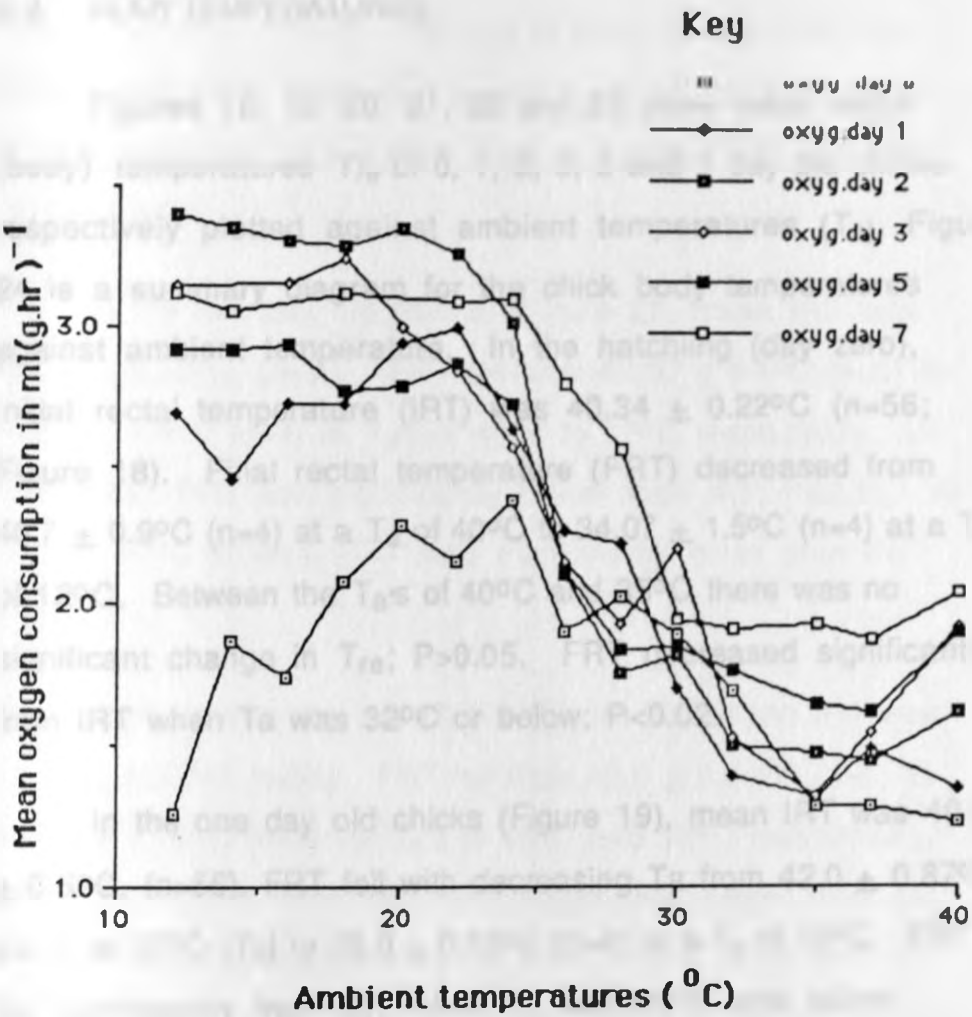

Figure 17. A summary plot of the means of oxygen consumption in ml(g.hr)$^{-1}$(respiratory intensity) against ambient temperatures in ^{o}C during the first seven days of life for the chicks of the domestic fowl, *Gallus domesticus*.

3.2 BODY TEMPERATURES

Figures 18, 19, 20, 21, 22 and 23 show mean rectal (body) temperatures T_{re} of 0, 1, 2, 3, 5 and 7 day old chicks respectively plotted against ambient temperatures (T_a). Figure 24 is a summary diagram for the chick body temperatures against ambient temperature. In the hatchling (day zero), initial rectal temperature (IRT) was 40.34 ± 0.22°C (n=56; Figure 18). Final rectal temperature (FRT) decreased from 40.7 ± 0.9°C (n=4) at a T_a of 40°C to 34.07 ± 1.5°C (n=4) at a T_a of 12°C. Between the T_a's of 40°C and 35°C there was no significant change in T_{re}; $P>0.05$. FRT decreased significantly from IRT when Ta was 32°C or below; $P<0.02$.

In the one day old chicks (Figure 19), mean IRT was 40.5 ± 0.5°C, (n=56). FRT fell with decreasing Ta from 42.0 ± 0.87°C (n=4) at 37°C (Ta) to 36.9 ± 0.19°C (n=4) at a T_a of 12°C. FRT fell significantly from IRT when Ta was 30°C and below; $P<0.01$.

For the two day old chicks (Figure 20) mean IRT was 40.47 ± 0.35°C, (n=56). FRT fell from 42.5 ± 0.63°C at a T_a of 40°C to 37.25 ± 0.22°C at a T_a of 12°C. At 40°C (T_a) FRT was significantly higher than IRT ($P<0.001$). At T_a's of 26°C and below, FRT was significantly lower than IRT; $P<0.05$.

In the three day old chicks, (Figure 21) the mean IRT was 40.68 ± 0.31°C,(n=56). At 40°C (T_a) FRT was significantly

higher than IRT; $P<0.01$. For T_a's of 30°C or less, FRT was significantly lower than IRT; $P<0.001$. FRT fell from 41.35 ± 0.15°C (n=4) to 37.5 ± 0.49°C (n=4) at T_a's of 40°C and 12°C respectively.

In the five day old chicks, (Figure 22) mean IRT was 40.77 ± 0.32°C, (n=56). FRT fell from 42.07 ± 0.4°C (n=4) to 39.62 ± 0.4°C (n=4) at T_a's of 40°C to 12°C respectively. At 40°C (Ta), FRT was significantly higher than IRT; $P<0.05$. At T_a's of 30°C or less, FRT was significantly lower than IRT ($P<0.001$).

In the seven day old chicks (Figure 23), mean IRT was 41.17 ± 0.30°C (n=56). FRT fell from 42.8 ± 0.25°C (n=4) to 39.45 ± 0.16°C (n=4) for T_a's of 40°C and 12°C respectively. At T_a's of 37°C and 40°C FRT was significantly higher than IRT; $P<0.01$, but significantly lower than IRT for T_a's of 24°C or less; $P<0.05$. For all ages of chicks studied (day 0 to day 7) FRT was always much higher than the prevailing ambient temperatures (See broken lines in figures 18 to 24). The fall in FRT with decreasing Ta became smaller with increasing age from hatchlings to day seven chicks, (Figure 24).

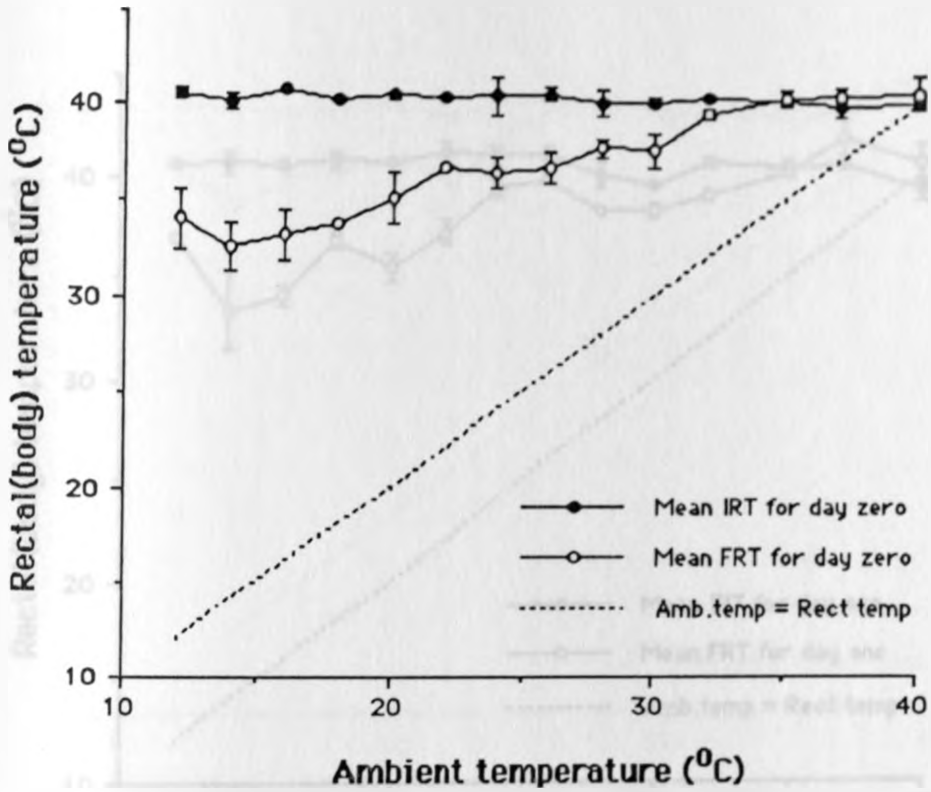

Figure 18. A plot of mean body temperatures against ambient temperatures for the zero day old chick of the domestic fowl, *Gallus domesticus*. Initial rectal temperatures(IRT/before experiments) and final rectal temperature (FRT/after experiments) are shown.Bars denote standard error of the means. The dashed line denotes where rectal temperature would be equal to ambient temperature.

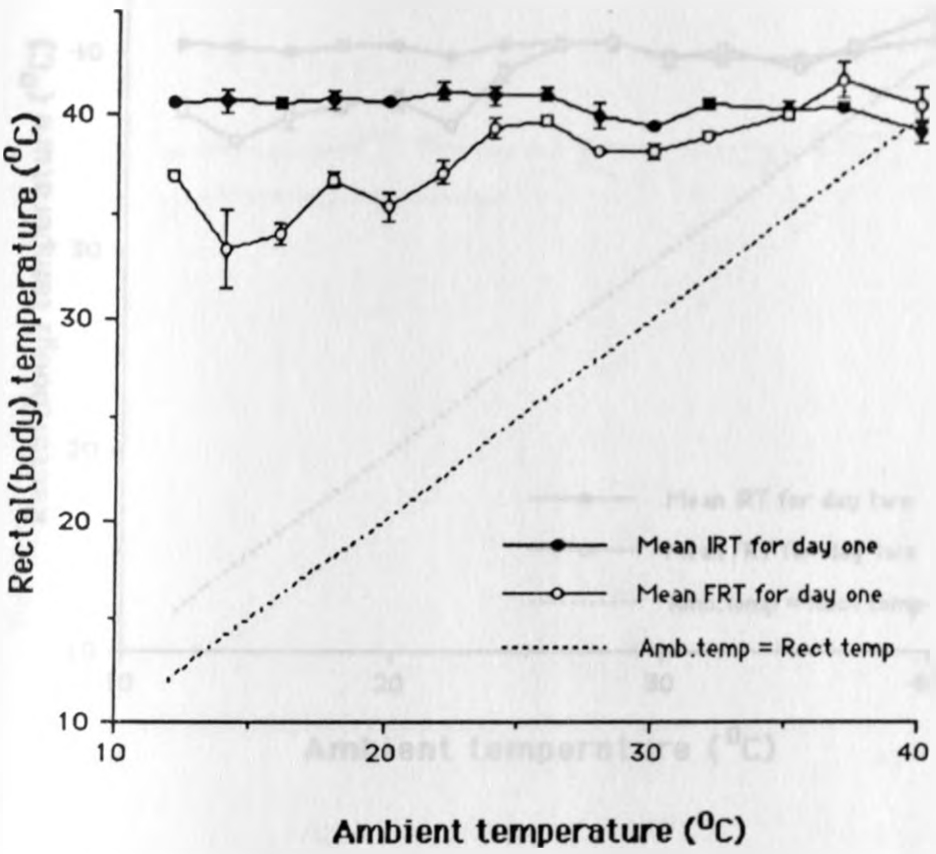

Figure 19. A plot of mean body temperatures against ambient temperatures for the one day old chick of the domestic fowl, *Gallus domesticus.* Initial rectal temperatures(IRT/before experiments) and final rectal temperature (FRT/after experiments) are shown. Bars denote standard error of the means. The dashed line denotes where rectal temperature would be equal to ambient temperature.

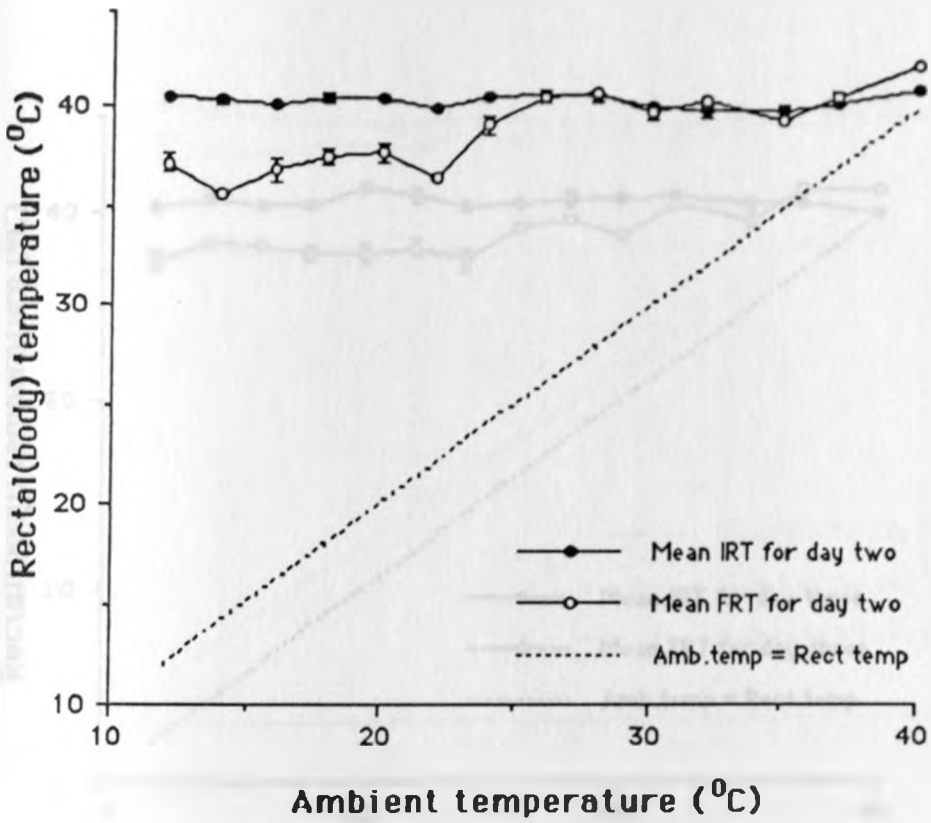

Figure 20. A plot of mean body temperatures against ambient temperatures for the two day old chick of the domestic fowl, *Gallus domesticus*. Initial rectal temperatures(IRT/before experiments) and final rectal temperature (FRT/after experiments) are shown. Bars denote standard error of the means. The dashed line denotes where rectal temperature would be equal to ambient temperature.

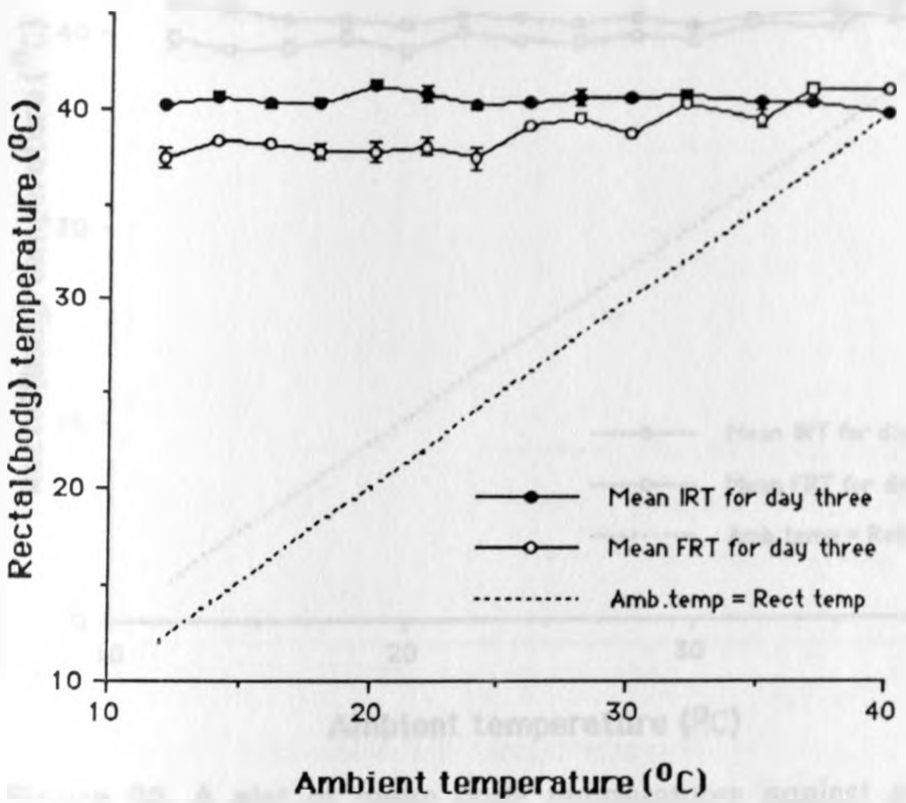

Figure 21. A plot of mean body temperatures against ambient temperatures for the three day old chick of the domestic fowl, *Gallus domesticus.* Initial rectal temperatures(IRT/before experiments) and final rectal temperature (FRT/after experiments) are shown.Bars denote standard error of the means. The dashed line denotes where rectal temperature would be equal to ambient temperature.

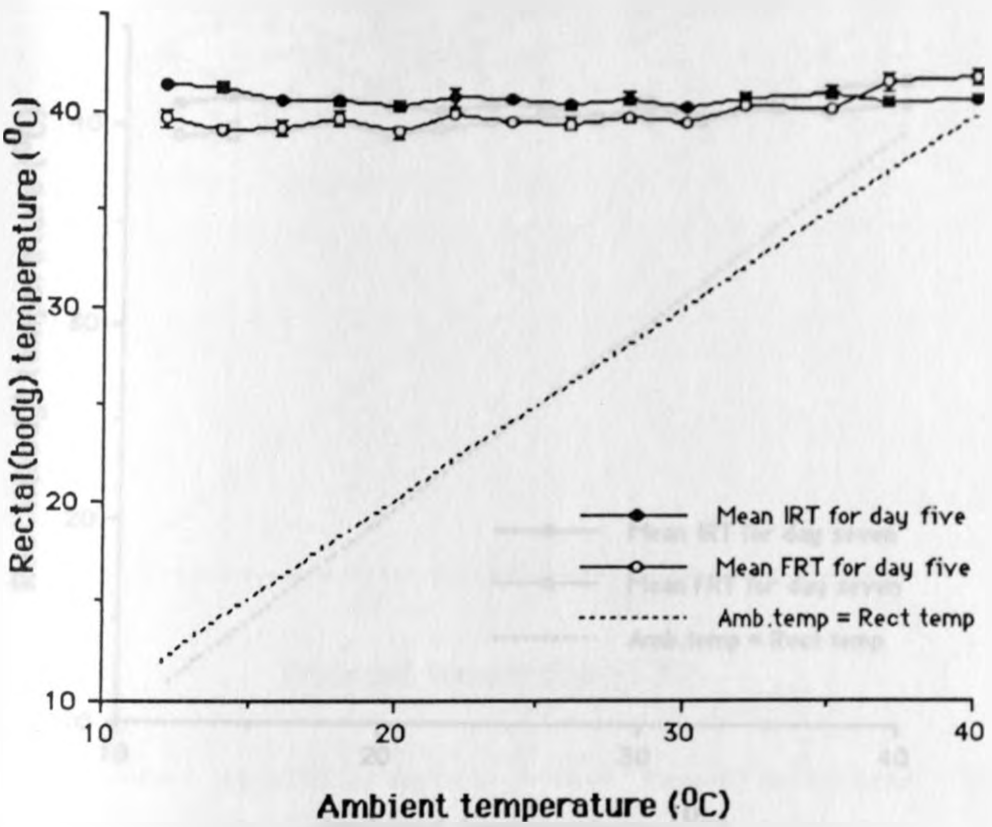

Figure 22. A plot of mean body temperatures against ambient temperatures for the five day old chick of the domestic fowl, *Gallus domesticus.* Initial rectal temperatures(IRT/before experiments) and final rectal temperature (FRT/after experiments) are shown. Bars denote standard error of the means. The dashed line denotes where rectal temperature would be equal to ambient temperature.

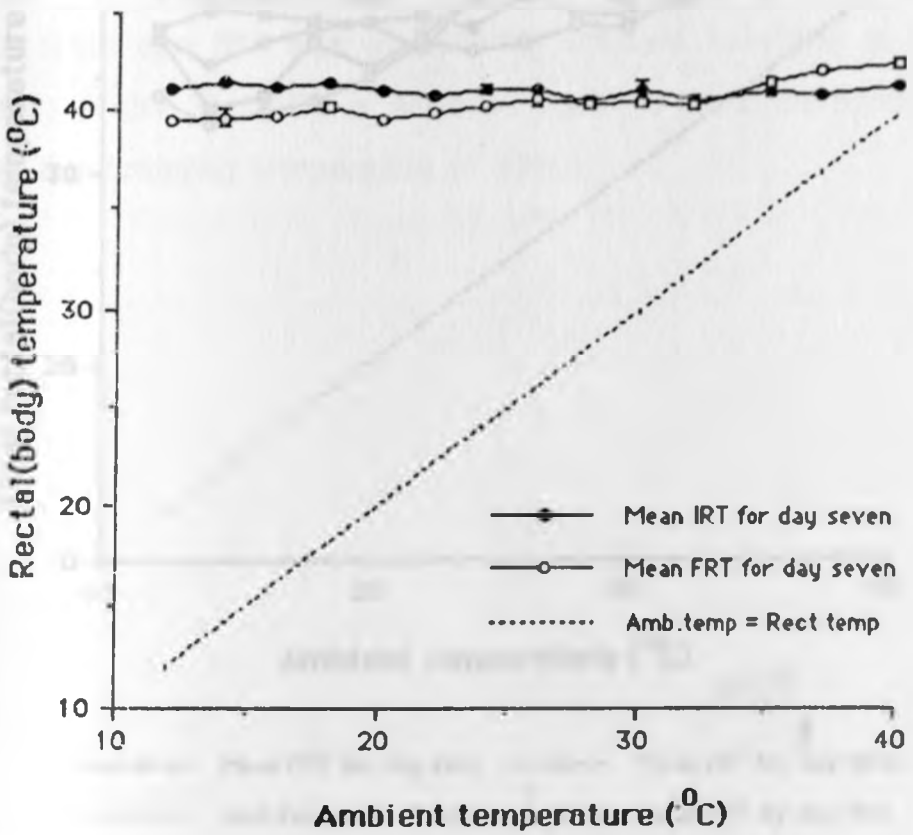

Figure 23. A plot of mean body temperatures against ambient temperatures for the seven day old chick of the domestic fowl, *Gallus domesticus*. Initial rectal temperatures(IRT/before experiments) and final rectal temperature (FRT/after experiments) are shown. Bars denote standard error of the means. The dashed line denotes where rectal temperature would be equal to ambient temperature.

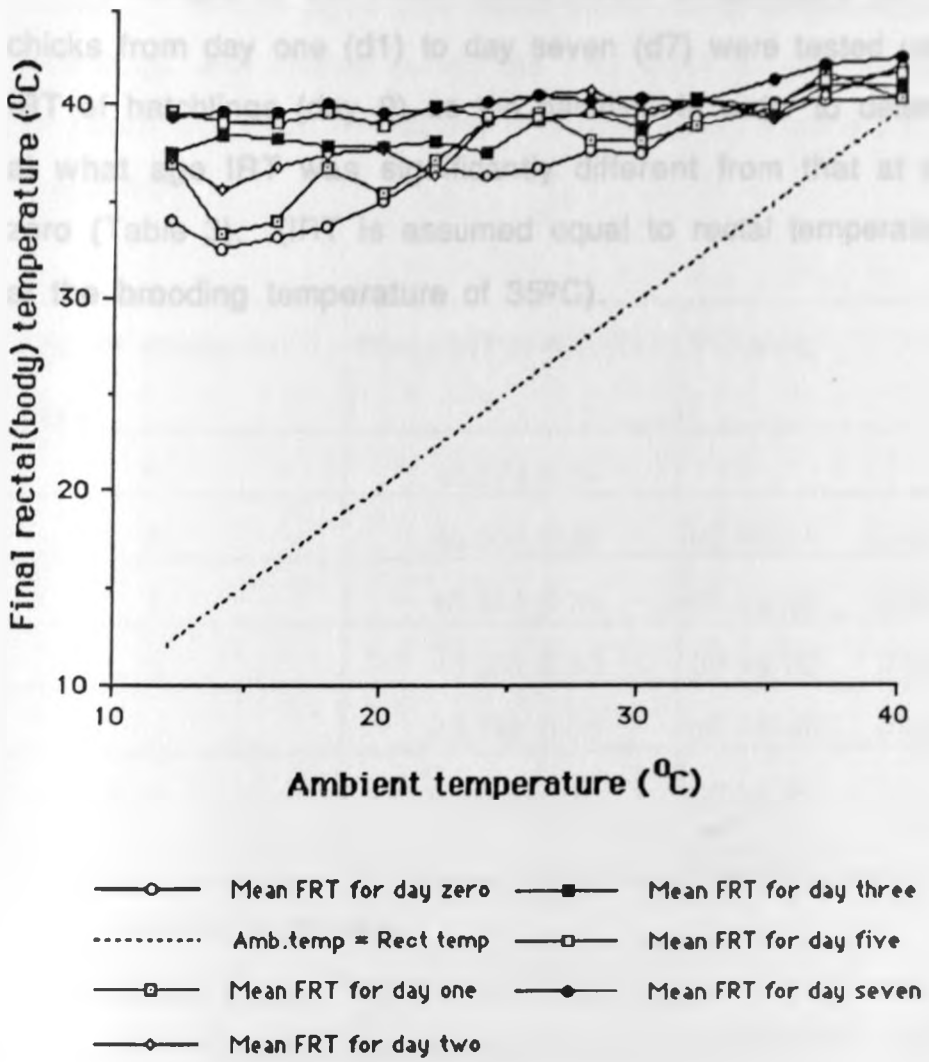

Figure 24. A summary plot of mean final rectal temperatures (FRT/after experiments) against ambient temperature from day zero to seven day old chicks of the domestic fowl, *Gallus domesticus*. The dashed line denotes where a body temperature would be equal to ambient temperature. The key to the graphs is also given above.

Changes in the mean initial rectal temperature for chicks from day one (d1) to day seven (d7) were tested using IRT of hatchlings (day 0) as the baseline in order to determine at what age IRT was significantly different from that at day zero (Table 3). (IRT is assumed equal to rectal temperatures at the brooding temperature of 35°C).

Table 3. Shows ages and mean initial rectal temperatures for hatchlings and chicks during the first week of life. P-values for mean IRT when IRT at day 0 is used as a baseline are indicated.

Age of chicks in days	Mean IRT in °C±SD	P-value
0	40.37±.0.24	
1	40.55± 0.42	d0 vs d1 0.494
2	40.51± 0.34	d0 vs d2 0.323
3	40.69± 0.30	d0 vs d3 0.0468*
5	40.76± 0.08	d0 vs d5 0.0282*
7	41.03±0.10	d0 vs d7 0.0031**

n=6 for all means,

*Significant **Very significant

IRT for day 3 and older chicks was significantly higher than that for day zero ($P<0.05$).

3.3 MYOFIBRILLAR ATPase ACTIVITY

The activity of myofibrillar ATPase in µmoles (min. mg protein)$^{-1}$ rose rapidly from 0.11 ± 0.02 on day eight to 0.64 ± 0.05 on day eighteen of embryonic development, (Figure 25). After day eighteen of incubation, m-ATPase activity for embryos and chicks upto day seven undulated about the same level with no statistically significant increase, ($P>0.05$; with m-ATPase activity for day 18 old embryos used as a baseline). In the embryos, the correlation coefficient between m-ATPase activity and oxygen consumption was statistically significant ($r=+0.789$; $P<0.05$). In chicks (day 0 to day 7) this correlation was not significant ($r=+0.133$; $P>0.5$).

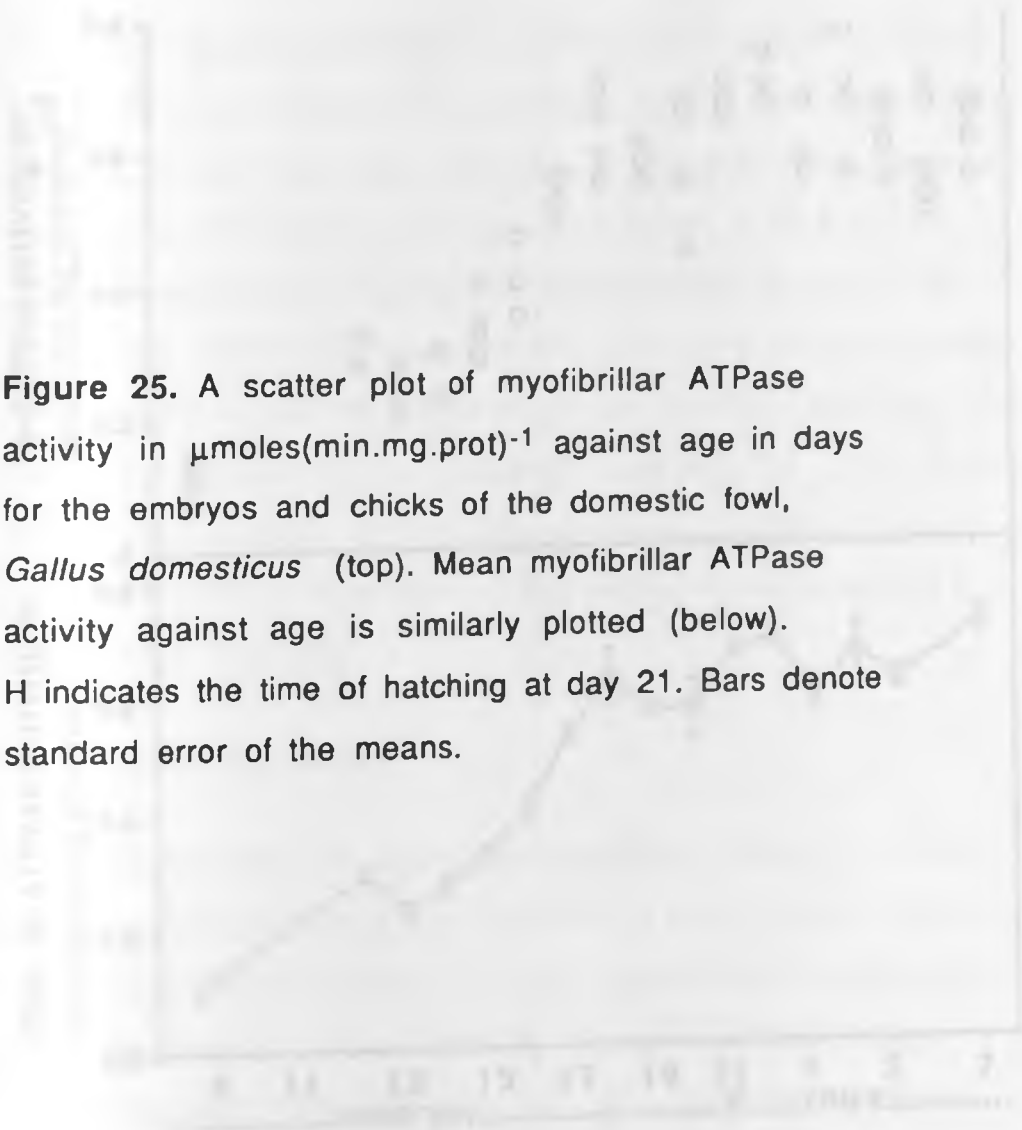

Figure 25. A scatter plot of myofibrillar ATPase activity in μmoles(min.mg.prot)$^{-1}$ against age in days for the embryos and chicks of the domestic fowl, *Gallus domesticus* (top). Mean myofibrillar ATPase activity against age is similarly plotted (below). H indicates the time of hatching at day 21. Bars denote standard error of the means.

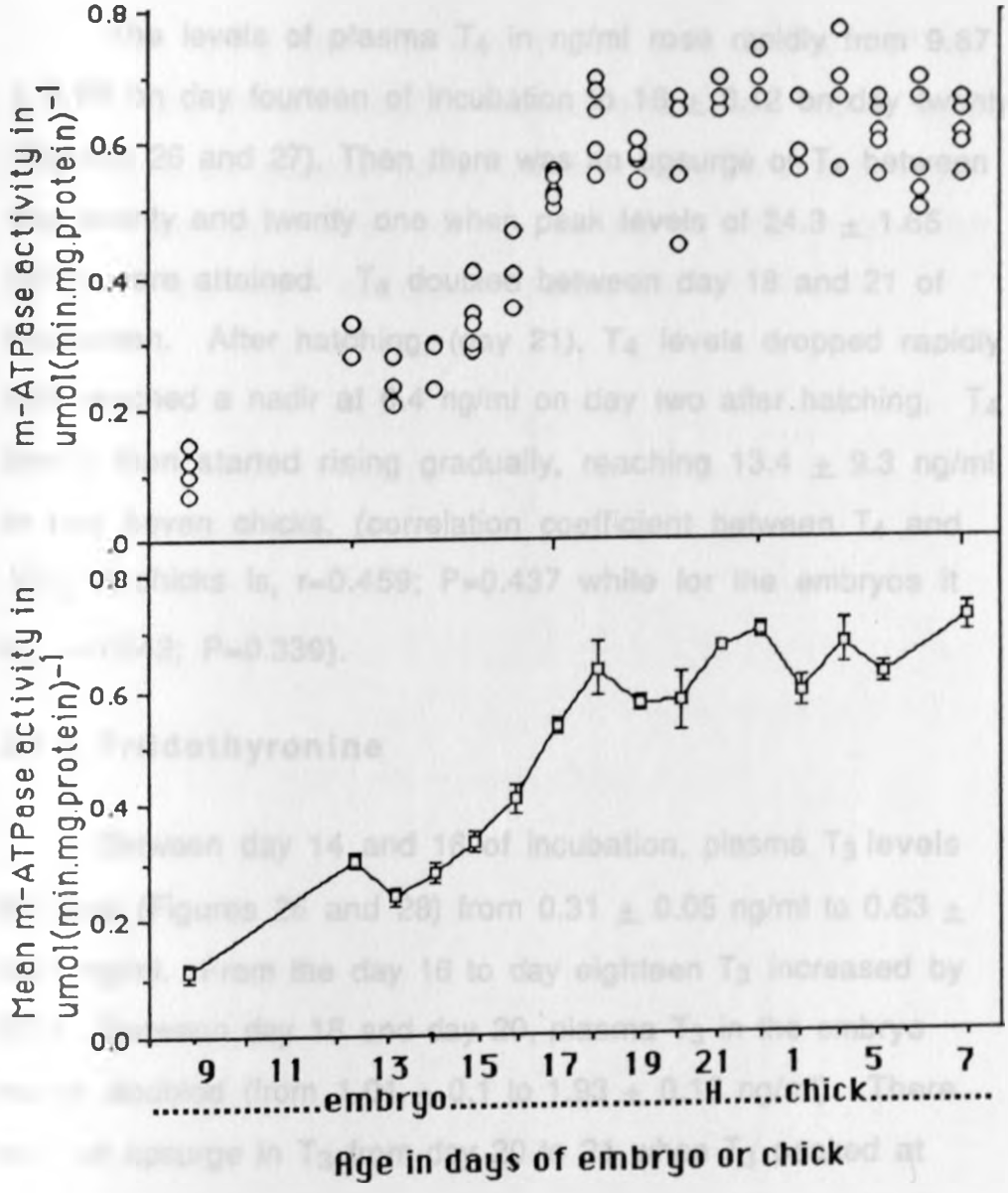
m-ATPase activity in umol(min.mg.protein)$^{-1}$
0.8
0.6
0.4
0.2
0.0
Mean m-ATPase activity in umol(min.mg.protein)$^{-1}$
0.8
0.6
0.4
0.2
0.0
9
11
13
15
17
19
21
1
5
7
.....embryo.....H.....chick.....
Age in days of embryo or chick

Figure 25

3.4 THYROID HORMONES

3.4.1 **Thyroxine**

The levels of plasma T_4 in ng/ml rose rapidly from 9.87 ± 0.65 on day fourteen of incubation to 16 ± 0.12 on day twenty (Figures 26 and 27). Then there was an upsurge of T_4 between day twenty and twenty one when peak levels of 24.3 ± 1.65 ng/ml were attained. T_4 doubled between day 18 and 21 of incubation. After hatching, (day 21), T_4 levels dropped rapidly and reached a nadir at 6.4 ng/ml on day two after hatching. T_4 levels then started rising gradually, reaching 13.4 ± 9.3 ng/ml in day seven chicks. (correlation coefficient between T_4 and $\dot{V}O_2$ in chicks is, r=0.459; P=0.437 while for the embryos it is, r=0.542; P=0.339).

3.4.2 **Triidothyronine**

Between day 14 and 16 of incubation, plasma T_3 levels doubled (Figures 26 and 28) from 0.31 ± 0.05 ng/ml to 0.63 ± 0.05 ng/ml. From the day 16 to day eighteen T_3 increased by 60%. Between day 18 and day 20, plasma T_3 in the embryo nearly doubled (from 1.01 ± 0.1 to 1.93 ± 0.16 ng/ml). There was an upsurge in T_3 from day 20 to 21 when T_3 peaked at 5.16 ng/ml, before falling to 3.9 ng/ml in hatchlings. Unlike T_4 plasma T_3 in chicks remained higher than pre-hatching levels. It showed little increase in day zero to day seven

chicks. Oxygen consumption shows a poor correlation to T_3 in both chicks (r=0.85; P=0.062) and embryos (r=0.497; P=0.394).

3.4.3 The T_3/T_4 ratio

The T_3/T_4 ratio rose very slightly from 0.03 to 0.07 on days 14 and 19 of incubation respectively. There was a rapid increase in the plasma T_3/T_4 ratio from day 19 embryos upto two day old chicks when the ratio peaked at 0.55. It then fell to 0.30 in the day 7 old chicks, (Figure 26).

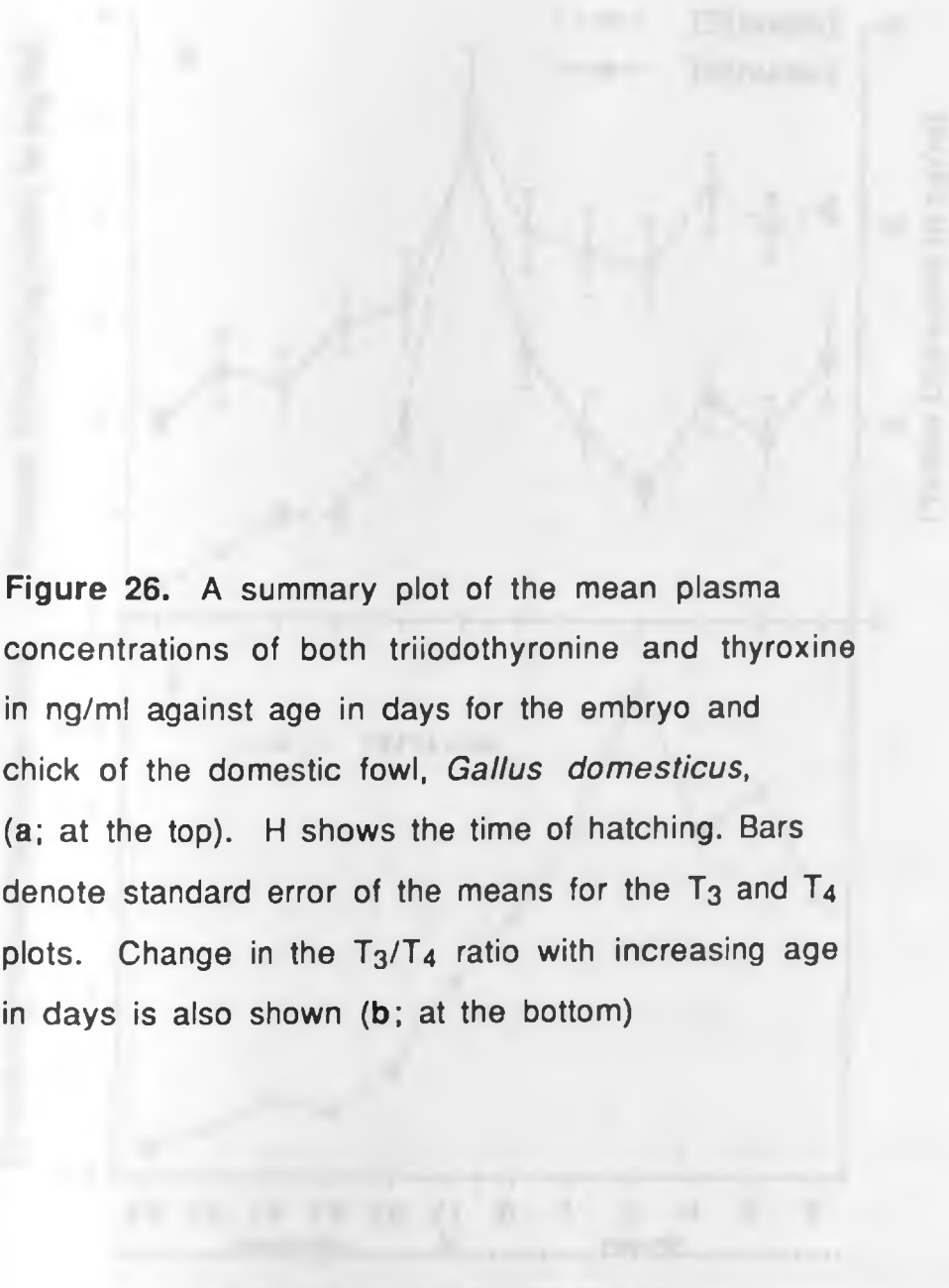

Figure 26. A summary plot of the mean plasma concentrations of both triiodothyronine and thyroxine in ng/ml against age in days for the embryo and chick of the domestic fowl, *Gallus domesticus*, (**a**; at the top). H shows the time of hatching. Bars denote standard error of the means for the T_3 and T_4 plots. Change in the T_3/T_4 ratio with increasing age in days is also shown (**b**; at the bottom)

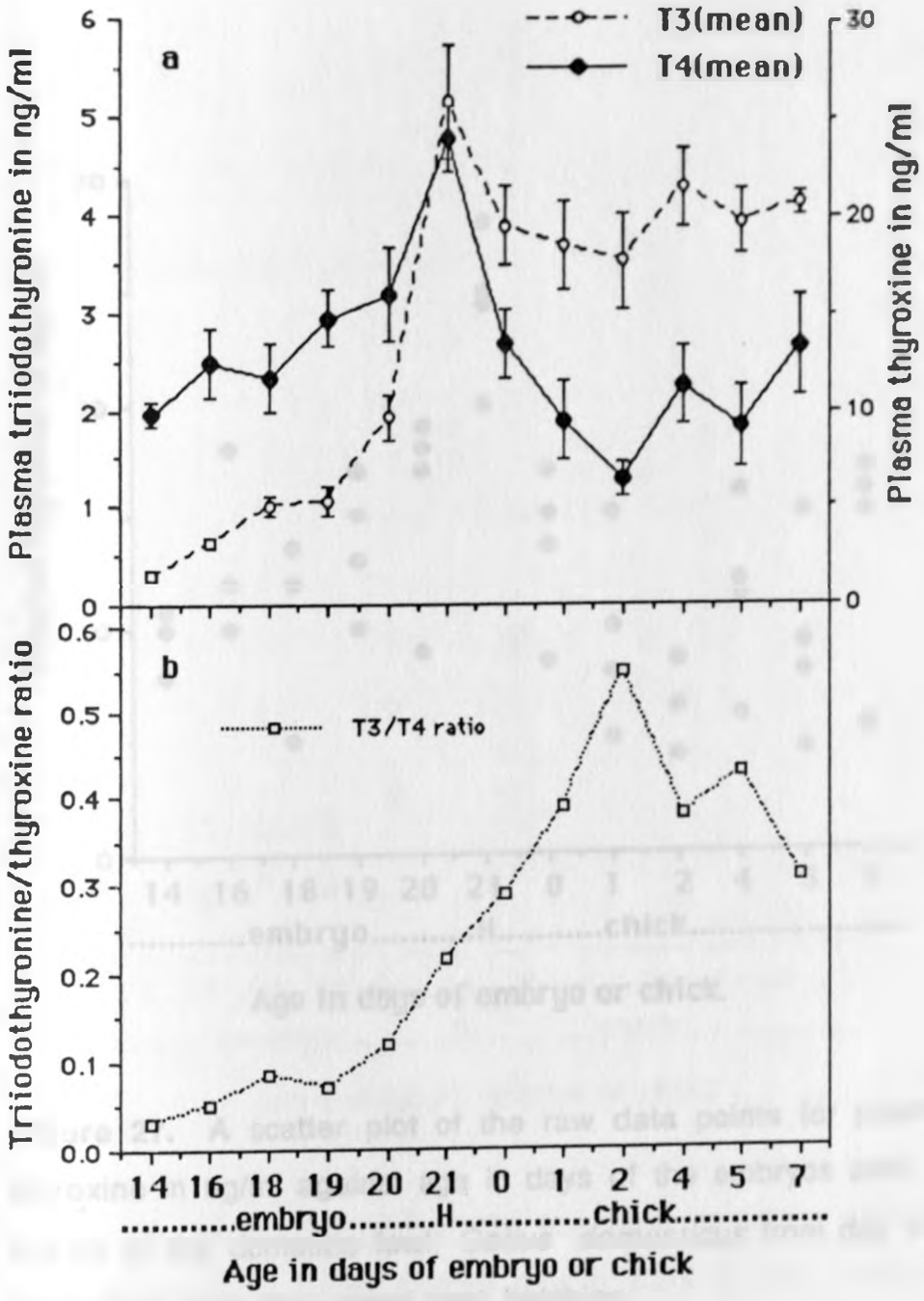
a
T3(mean)
T4(mean)
Plasma triiodothyronine in ng/ml
Plasma thyroxine in ng/ml
b
T3/T4 ratio
Triiodothyronine/thyroxine ratio
14 16 18 19 20 21 0 1 2 4 5 7
.....embryo.....H.....chick.....
Age in days of embryo or chick

Figure 26

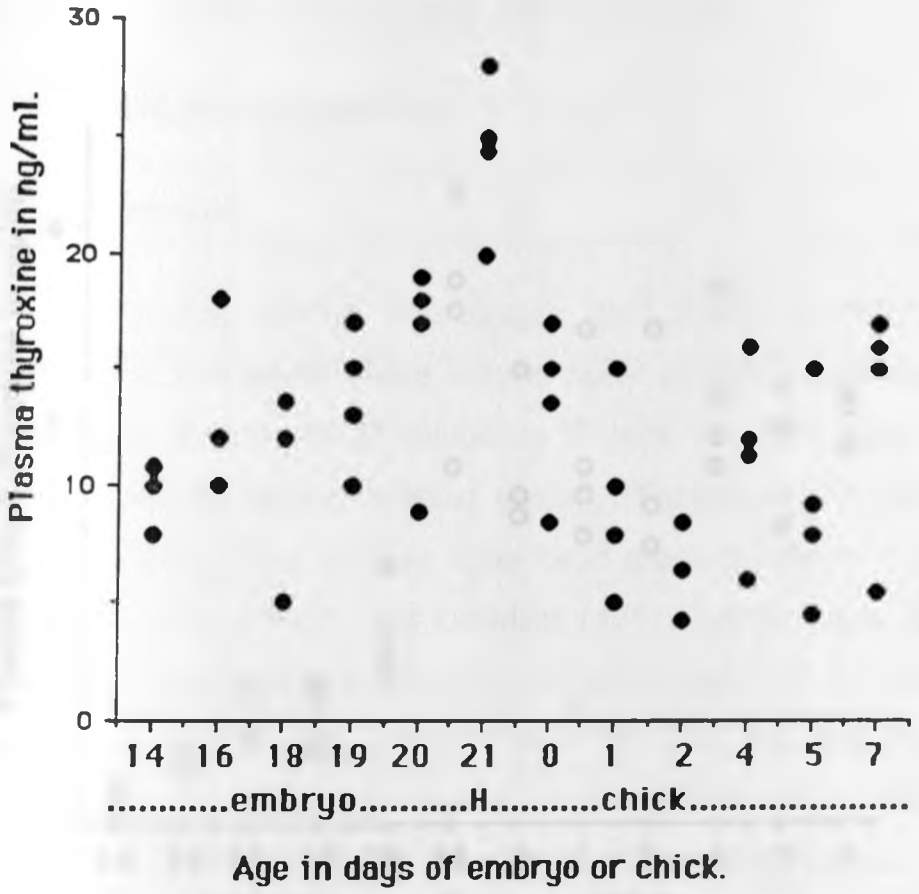

Figure 27. A scatter plot of the raw data points for plasma thyroxine in ng/ml against age in days of the embryos and chicks of the domestic fowl, *Gallus domesticus* from day 14 of incubation upto day seven post hatching.

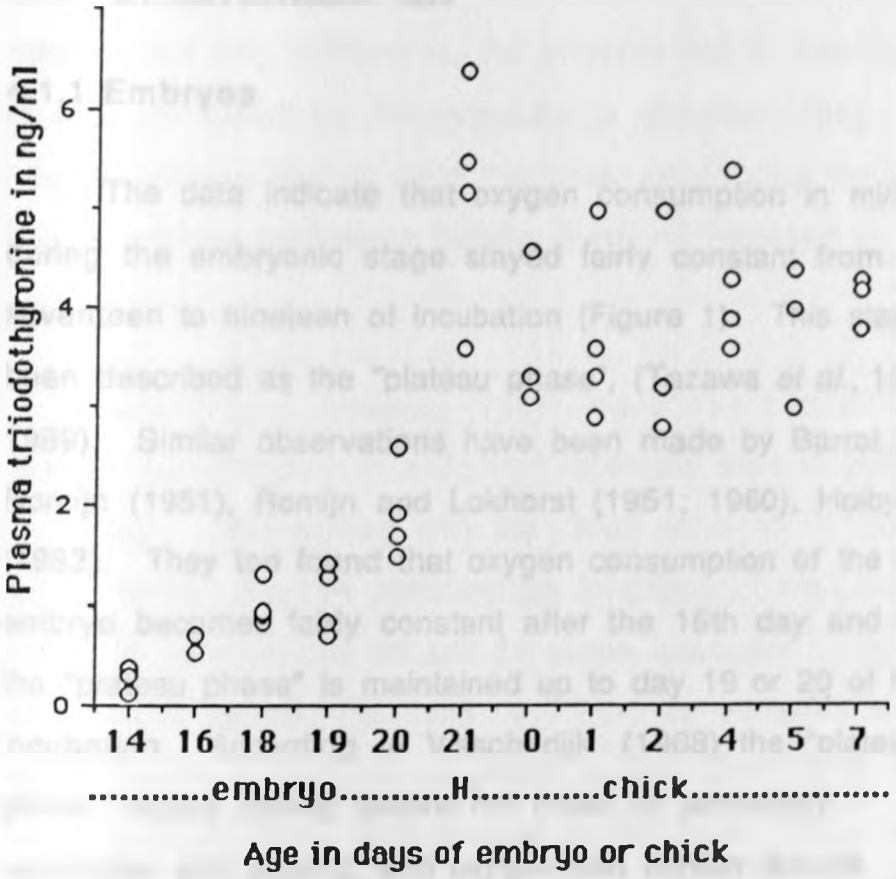

Figure 28. A scatter plot of the raw data points for plasma triiodothyronine in ng/ml against age in days of the embryos and chicks of the domestic fowl, *Gallus domesticus* from day 14 of incubation upto day seven post hatching.

CHAPTER 4

4.0 DISCUSSION AND CONCLUSIONS

4.1 OXYGEN CONSUMPTION

4.1.1 Embryos

The data indicate that oxygen consumption in ml/hr during the embryonic stage stayed fairly constant from day seventeen to nineteen of incubation (Figure 1). This stage has been described as the "plateau phase", (Tazawa *et al.*, 1988, 1989). Similar observations have been made by Barrot (1937), Romijn (1951), Romijn and Lokhorst (1951; 1960), Hoiby *et al.*, (1983). They too found that oxygen consumption of the chick embryo becomes fairly constant after the 16th day and that the "plateau phase" is maintained up to day 19 or 20 of the incubation. According to Visschedijk, (1968) the "plateau phase" occurs shortly before the onset of pulmonary respiration and pipping, and oxygen and carbon dioxide tensions at the "plateau" region represent the maximum values which can be maintained by gaseous diffusion across the egg shell and any increase leads to hypoxia and hypercarbia (hypercapnia) which signals the initiation of pipping of the shell. Freeman and Vince (1974) have similarly argued that the "plateau" in metabolic rate between day 17 of incubation and the onset of pipping is a reflection of the maximum theoretical flux of O_2 and CO_2 through the egg's shell.

After day 19 of incubation (Figure 1) oxygen consumption of the chick embryo increased upto day 21. According to Freeman (1961), this rapid rise in oxygen consumption after day 19 or 20 of incubation is associated with the hatching process. Part of the extra energy from this increased $\dot{V}O_2$ is required for lung ventilation, the physical act of hatching and to a lesser extent for thermoregulation (Dawson,1984). When internal pipping occurs (with the beak penetrating the air cell) and pulmonary respiration begins, then this increase in $\dot{V}O_2$ takes place.

According to Freeman (1961), there was a small fall in oxygen consumption in $ml(g.hr)^{-1}$ after the start of pulmonary ventilation. This agrees with the result obtained in these experiments. Oxygen consumption in $ml(g.hr)^{-1}$ was found to decrease between day 20 and 21 of the incubation, (Figure 2). This weight specific decrease in oxygen consumption is explained by an increase in weight (growth rate) by the embryo during the last stage of incubation (Hoiby *et al.*, 1983) and also by insufficient delivery of oxygen to the tissues. The low level of embryonic mass specific metabolism evident in late incubation appears to be a broadly shared characteristic of precocial and semi-precocial birds (Dawson, 1984).

Embryos aged fifteen to seventeen days old showed a decrease in oxygen consumption (metabolic rate) with decreasing T_a from 38° to 28°C. This is a poikilothermic

response (Figure 3a, 3b, 4 and 5). No compensatory increase in metabolic rate was therefore evident at this stage when the embryo was gradually cooled. Upto day seventeen of incubation the metabolic rate of the chicken embryo appeared to be "coupled" to ambient temperature. Precocial birds' transition from poikilothermy has been divided into four phases, depending on the limitations to endothermic homeothermy (Tazawa *et al.*, 1988); these are: Arrhenius limited, O_2-conductance limited, power limited and homeothermy phases. According to this classification, the data appear to indicate that chicken embryos upto day seventeen of incubation correspond to the Arrhenius limited phase, where the embryo has neither sufficiently developed controllers nor effectors (Tazawa *et al.*, 1988). Before day 18 of incubation the metabolic rate was low and increased with growth. When the surroundings cooled, the egg cooled with it and the metabolic rate decreased by some proportion, which roughly conforms with the Arrhenius limitation of temperature on chemical kinetics.

On the eighteenth day of incubation, a slight metabolic response was evident when the egg was cooled gradually. Oxygen consumption showed a "plateau phase" when the cooling was less than 3°C (i.e. between 38° and 36°C). This compensatory metabolic response became increasingly stronger on days nineteen, twenty and twenty one of incubation. The chick embryo could resist a 3°, 4° and 5°

decreases in T_a without a decrease in oxygen consumption for days 19, 20 and 21, respectively, of incubation. Comparable results were obtained by Freeman (1964), who reported that 19 day old embryos responded to egg cooling with a transient increase in oxygen consumption that was explained as a compensatory increase of metabolic heat production, (Romijn and Lokhorst, 1955). He however saw no signs of metabolic compensation even in the 20 day old embryo and so concluded rather erroneously that the embryo was a poikilotherm even upto full term. This difference between the present experimental results and those of Romijn and Lokhorst (1955), and Freeman (1964) has been partly attributed to the design of the experiments (Tazawa *et al.*, 1988). In the stepwise ("sudden") cooling method used by Romijn and Lokhorst (1955) and Freeman (1964), the feeble compensatory ability to cooling of the late-term chicken embryo was overwhelmed (swamped) by the much larger losses of heat to the surroundings. In the "gradual" cooling technique developed by Tazawa *et al.*, (1988) and also adopted in these experiments, the imbalance between heat loss and heat production was made as small as possible during egg cooling and so the feeble compensatory ability of the late-term chicken embryo could be more easily detected.

The "plateau phase" in oxygen consumption observed when the chicken embryo was cooled on days 18 to 21 of incubation appears to parallel the O_2-conductance limited

stage of the transition from poikilothermy. During this stage that ends with external pipping, the embryo's thermoregulatory control and effector mechanisms are sufficiently developed to be operative but are throttled by the low conductance of the eggshell to oxygen diffusion (Tazawa *et al.*, 1988).

Similar results as in the present study were also obtained by Tazawa *et al.*, (1988) who showed that prior to day 18 of incubation, chicken embryos were poikilothermic and at about day 18 of incubation upto term, a weak metabolic response to gradual egg cooling appeared and became stronger after external pipping. The data also suggest that transient endothermic homeorthermy in chicken embryos is developed by the eighteenth day of incubation. During the "plateau" phase, metabolic rate appeared uncoupled to ambient temperature (T_a); when the decrement in T_a was small, ($\leq 5^oC$).

The experimental data also appear to indicate that the 20 day old chicken embryo showed a very slight increase in oxygen consumption with decreasing T_a before $\dot{V}O_2$ declined after a (T_a) of 34°C, (Figures 8 and 10). According to Tazawa *et al.*, (1988) if the embryo's resting metabolic rate is still less than the eggshell conductance limit, a slight increase in O_2 consumption can still occur when the egg is cooled, but will not exceed the oxygen conductance limit. If the embryo's resting metabolic rate is already at the conductance limit, a

plateau but not a rise in O_2 consumption will be observed. This also partly explains the different results of Freeman (1964) who observed an increase in metabolism in a cooling egg and of Romijn and Lokhorst (1955), who did not. It also explains the increase in oxygen consumption observed in our experiments for the 20 day old embryo.

The experimental data also indicates that at the point of hatching, (Figure 2) the oxygen consumption of the hatching embryo suddenly rose from 0.58 $ml(g.hr)^{-1}$ to 1.28 $ml\ (g.hr)^{-1}$ in the hatchling. The explanation for this is two-fold; first, the denominator,(egg mass) is reduced by the loss of the egg shell and shell membranes thus mass specific metabolism increases, (Mathiu, 1988) and secondly, at this stage the embryo is no longer throttled/constrained by the egg shell and now uses its lungs to breath in oxygen-rich air. The embryo can then increase its metabolic rate to the maximum possible level. This stage when homeothermy only evades the embryo and hatchling because its capacity to generate heat is not sufficiently great has been referred to as the "power limited" stage of the transition from poikilothermy (Tazawa *et al.*, 1988).

The experimental data also indicates that generally, the Q_{10} for the change in oxygen consumption corresponding to the temperature fall from 38°C to 28°C decreased with successive developmental stages of the embryos. The Q_{10} values for days 15, 16, 17, 18, 19, 20 and 21 of incubation embryos were 1.94,

1.9, 2.05, 1.69, 1.66, 1.62 and 1.45 respectively. For days 15, 16 and 17, the Q_{10} values are seen to approximate 2.0 for this 10°C fall in T_a. For embryos aged day 18 to 21 the Q_{10} value progressively decreases, tending towards unity. Similar observations have been made by Dawson (1984) and Tazawa *et al.*, (1989). This data provides further evidence to support the contention that upto day seventeen of incubation, the embryo of the domestic fowl is a poikilotherm and the transition from poikilothermy is achieved soon after.

The decrease in Q_{10} with increasing age can also be attributed to the rise in the level of metabolism and the acquisition of the abilities to augment heat production when developing embryos are exposed to moderate and cool T_a's. Tazawa *et al.*, (1988; 1989) also contend that the distinctly lowered Q_{10} ($Q_{10} < 2$) for embryo from day 18 to 21 of incubation is further evidence that a weak homeothermic metabolic response to gradual cooling of egg occurs in these late prenatal embryos.

4.1.2 **Chicks**

The neonate chicks were able to increase oxygen consumption in response to a cold challenge for all ages (day 0 to day 7). This ability to increase oxygen consumption in response to cold has also been demonstrated by several workers; Pembrey (1895), Romijn (1954), Romijn and Lokhorst (1955), Freeman (1966). The metabolic rate achieved on

exposure to cold (20°C) was found to increase and the degree of hypothermia to decrease as a function of age. For all ages, chicks showed a partial hypothermia (Figures 18 to 24) as indicated by a fall in rectal temperature with decreasing T_a's The degree of hypothermia was highest in the day 0 old chicks and decreased with increasing age upto day 7. Similar findings have been reported by Wekstein and Zolman (1969). Misson (1977), also had similar findings for chicks of the Domestic fowl aged 2 to 48 hours exposed to 20°C. Comparable results were obtained by Randall (1943) where rectal temperatures of day old chicks fell as low as 31° to 32°C when T_a was 26°C while in the seven day old chicks T_{re} kept at normal levels of 40.5 to 41°C.

In the zero day old chicks, the hypothermia was very severe. There was a decline in oxygen consumption when T_a fell below 20°C. A similar drop in metabolic rate in hatchlings of the Willow ptarmigan (*Lagopus lagopus*) exposed to T_a's below 29°C has been reported, (Aulie and Per Moen, 1975); and also in the Xantus murrelet *(Synthliboramphus hypoleucus*) hatchlings exposed to T_a's below 15°C, (Eppley, 1984). This substantial drop in metabolic rate was attributed to the fact that the down was still wet and a considerable amount of heat was used to vaporise this water. The same is true of the domestic fowl and the effect disappears soon after the down dries (Pembrey *et al.*, 1895; Freeman, 1963). The chick is a homeotherm at hatching but upon exposure to

temperature extremes may revert to the poikilothermic state of its embryological development (Randall, 1943). General depression of all oxidative functions may be involved in the cessation of shivering at temperatures below 20°C (Ta). Partial hypothermia in chicks appears to be a protective mechanism that helps to reduce energetic costs when temperatures drop very low (Eppley, 1984).

The lower critical temperature (T_{cr}; T_a below which T_b cannot be maintained without an increase in heat production) decreased with increasing age. Eppley (1984) obtained similar results in precocial Xantus murrelet chicks. This decrease in T_{cr} is partly attributed to the increasing thermoregulatory ability with age due to the improving insulation as the feathers begin to grow.

According to Randall (1943) T_b was observed to increase from 38°C to 41°C between day zero and day 10 after hatching. Seven day old chicks kept T_b at normal levels of 40.5 to 41°C. The experimental data shows that, T_b increased from 40.3°C on day zero to about 41.04°C on day seven. T_b on day 3 was 40.67 and was significantly different from that on day 0. The slight differences in T_b between these results and those of Randall (1943) could be due to a difference in brooding temperature. A body temperature of 41.03 obtained on day seven approximates that of adult birds as reported by Randall (1943) and Aulie and Toien, (1988).

Body mass in these experiments increased from 36.5 g on day 0 to 47.92 g on day seven. However, for the first 4 days body mass stayed fairly constant. Bernstein (1973), Spiers *et al.*,(1974) and Aulie, (1976b) have obtained similar findings. They attributed this lack of growth in chicks during the first few days of life to the fact that precocial chicks are not fed by the parents. Body size, through its allometric relationship to both metabolic rate and insulation contributes to thermoregulatory abilities of chicks. The body mass of birds increases proportionately faster than does surface area. Thus as more protoplasmic mass is involved in heat production with comparatively little change in surface area for heat dissipation, the normal T_b must rise.

Buchanan and Hill (1947; 1949) have suggested that the development of homeothermy in chicks is related to the degree of myelination of hypothalamal tracts. This mechanism may be linked with the perfection of the homeothermic response which occurs at the end of the first week of post embryonic life (Romanoff, 1941; Romijn, 1954; Romijn and Lokhorst, 1955). However, this theory was not tested for in these experiments.

4.2 MYOFIBRILLAR ATPase ACTIVITY

The data indicates a shift in activity of myofibrillar ATPase in the domestic fowl during the embryonic stages of development. It increased about six times between the

eleventh and eighteenth day of incubation when it appears to have reached a critical value (Figure 25). This finding agrees with that of Trayer and Perry (1966) who found that the shift in m-ATPase in fast twitch skeletal muscles of mammals and birds occurred largely before birth or hatching in the precocial Guinea pig and domestic fowl, but after birth in altricial species like the rat and cat. A similar shift in m-ATPase was observed by Marsh and Wickler (1982) who showed that development of endothermy (measured as peak metabolic response to cold) in the altricial nestling Bank swallows (*Riparia riparia*) coincided with a rapid increase in m-ATPase activity in pectoral muscles. The transition in m-ATPase activity in Bank swallows was as abrupt as the development of the endothermic response to cold.

In the semi-precocial Wedge-tailed shearwater (*Puffinus pacificus*), no significant shift in protein specific activity of Ca-activated myofibrillar ATPase was found between embryos in pip-holed eggs and hatchlings (Mathiu *et al.*,1992; in press), as was observed in nestling Bank swallows (Marsh and Wickler, 1982) or in the embryonic Domestic fowl, (this study). The difference in the results obtained in this study and those for the semi-precocial Wedge-tailed shearwater can partly be explained by differences in methodologies. In the later study, muscle samples were frozen in liquid nitrogen for eight (8) months before analysis for the m-ATPase activity was done. This long period of

storage may have caused significant losses in the activity of m-ATPase.

Myofibrillar ATPase activity is a good measure of contractile function of muscles and so this enzymatic change in m-ATPase activity also underlies an increase in contraction speed of the muscles. After day eighteen of incubation myofibrillar ATPase activity was found to undulate about the same level upto seven day old chicks. The curve for the increase in m-ATPase activity between the eleventh day of incubation and the seventh day after hatching was typically sigmoid in nature.

The increase in oxygen consumption of the embryo between the fifteenth and 20th day was found to closely parallel the increase in myofibrillar ATPase activity over the same period of time. There was a strong correlation between m-ATPase activity and oxygen consumption for the embryo ($r = +0.789$; $P < 0.05$). In chicks, this correlation was very poor ($r = +0.133$; $P>0.5$). It is also observed that on day eighteen of incubation when m-ATPase activity appears to reach a critical value, (in the sigmoid curve before it begins to undulate about the same level) is also the time when a transient homeothermic response to gradual cooling was observed in these experiments and those of Tazawa *et al.*, (1988). This change in the profile of myofibrillar ATPase therefore appears to help in accounting for the increased metabolic capacity observed in late prenatal embryos

It has been suggested that the development of shivering heat production may underly the rapid maturation of the thermogenic response in passerine nestlings (Odum, 1942; Morton and Carey, 1971; Marsh, 1979; Marsh and Wickler, 1982). Development of skeletal muscles is important in development of shivering. Shivering thermogenesis is also dependent on the maturity of the contractile apparatus that serves to break down ATP generated from the aerobic pathways (Marsh and Wickler, 1982). Myofibrillar ATPase is therefore an important enzyme in the development of shivering thermogenesis.

From the results of these experiments, it appears that in the day eighteen incubating embryos of the domestic fowl, m-ATPase activity in pectoralis muscles reaches a critical (threshold) value and so contributing towards the incipient homeothermic response to cold possibly via shivering thermogenesis. The poor correlation between m-ATPase activity and 0_2-consumption in hatchlings and chicks during the first week of life suggests that in this period, endothermic homeothermy is independent of the level of m-ATPase activity.

4.3 THYROID HORMONES

The data show an exponential rise in T_3 and T_4 during the embryonic stages. T_4 levels rose rapidly from day 18 and peaked at day 21 for the embryos. T_3 also rose rapidly from day 19 to 21 and it also peaked on day 21. This period when T_3 and T_4 rose rapidly also parallels the period when transient endothermic homeothermy was observed in the embryo. After hatching, both T_3 and T_4 levels fell but T_3 levels never reached their prenatal levels while T_4 dropped to prenatal levels before beginning to rise again. The increase in T_3 and T_4 upto day 21 when both hormones peaked is similar to the findings of Thommes and Hylka (1977); Decuypere *et al.*, (1979a; 1979b), Ockleford *et al.*, (1983); McNabb, (1987). The drop in T_3 and T_4 levels after hatching is also similar to the findings of Daugeras *et al.*, (1976); Thommes and Hylka, (1977).

The value of 9.5 ng/ml for total T_4 obtained for the 1-day old chick closely approximates that of 10.78 ng/ml reported by Thommes and Hylka (1977), and 11.0 ng/ml reported by Bobek *et al.*, (1977) for the 2-day old chicks, but is much lower than the concentrations reported by Newcomer and Huang (1974) for the 6 week old chickens (14.8 to 31.4 ng/ml) and the values published by Refetoff *et al.*, (1970) for the adult domestic fowl (14-16 ng/ml). The T_3 values obtained in this investigation for the 1-day old chick (3.7 ng/ml) also agree with those found by King *et al.*, (1977) in the

1-day old chicks (2.74 - 3.85 ng/ml) and those of Bobek *et al.*, (1977) for the 2 day old chicks (2.85 ng/ml), but are lower than those of Thommes and Hylka (1977) for the 1-day old chicks (4.99 mg/ml). The different methodologies used for hormone analysis can partly explain the differences.

Tazawa *et al.*, (1989) have shown that thyroid hormones are partly responsible for the feeble metabolic response to cooling observed in late embryos, since this response was blocked in thiourea treated embryos. The present data also indicates that thyroid hormone concentrations are still low during the late prenatal stages as compared with the post-natal period; they might not be high enough to trigger a persistent endothermic response to cooling in prenatal embryos, though thyroid development in late embryos appears responsible for the compensatory metabolic response to cooling (Tazawa *et al.*, 1989).

The plasma ratio of T_3/T_4 rose exponentially and peaked on day 2 after hatching. The ratio was 0.05 and 0.08 on days 16 and 18 respectively of incubation. This ratio increased upto 0.22 on the day (21) of hatching. These results agree with those of Decuypere *et al.*, (1979b) who got a serum T_3/T_4 ratios of 0.06 and 0.27 on days 17 and 21 respectively of incubation of the embryo. One day old chicks had a plasma ratio of T_3/T_4 of 0.29. This compares well with the 0.25 T_3/T_4 ratio that Davison (1976) found in one day old chicks. It

therefore appears likely that there is increased conversion of T_4 to T_3 in the serum towards the onset of hatching.

Similar studies of the serum T_3/T_4 ratio have been conducted on the precocial Japanese Quail (*Cortunix Japonica*) by McNabb (1987) and shown that the T_3/T_4 ratio peaks at the time of hatching just as in the domestic chicken. McNabb (1987) contends that the increase in the serum T_3/T_4 ratio in the perinatal period was due to increased peripheral production of T_3 as shown by a corresponding peak in hepatic 5'Dactivity. (5'D = 5'monodeiodinase). The same pattern occurs in perinatal chickens as shown by in vitro hepatic 5'D assays (Borges *et al.*,1980) and in vivo demonstrations of peripheral deiodination (Decuypere *et al.*, 1982; Decuypere and Scanes, 1983). Comparable findings have also been reported for the rabbit, (Brzezinska and Krysin, 1990). They found a sudden increase of 5'D activity in the liver and kidneys 3 days before birth, falling to a nadir 3 days after birth. They observed that an increase in the conversion of T_4 to T_3 and rT_3 in the liver and kidney correlated with T_3 concentration in the serum from day 3 after birth.

According to Bobek *et al.*, (1977) oxygen consumption in the 1-day old chick was 1.22 ml $(g.hr)^{-1}$ and rose to 1.5 ml $(g.hr)^{-1}$ in the 1 week old chick of the domestic fowl. The coefficient of correlation between oxygen consumption and thyroid hormone concentration in different age groups of chicken ranged from 0.78 to 0.98 and 0.19 to -0.63 for T_3 and

T_4 respectively. These results are similar to the ones obtained in the present experiments in which oxygen consumption was 1.32 ml(ghr)$^{-1}$ for 1-day old chicks and 1.93 ml (g.hr)$^{-1}$ for 7-day old chicks and the coefficient of correlation between oxygen consumption and thyroid hormones was 0.86 and 0.46 for T_3 and T_4 respectively.

These results show a higher degree of association between oxygen consumption and plasma T_3 levels during the first week of life than for T_4. This suggests that T_3 is the main thyroid hormone influencing oxygen consumption and therefore thyroid hormone calorigenesis in young chicks. The higher levels of T_3 after hatching also suggest augmented thyroid stimulating hormone (TSH) secretion (Bobek *et al.*, 1977). TSH stimulation of the thyroid gland is known to increase the serum T_3/T_4 secretion ratio (Taurog and Chaikoff, 1946; Inoue *et al.*,1967). The results obtained in this study suggest that T_3 is more related to the control of metabolic rate of young chickens during cold exposure than T_4. This also agrees with the findings of Bobek *et al.*,(1977) and Freeman (1970).

The high correlation between oxygen consumption and plasma T_3 levels are also indicative of the role of T_3 in increasing thermogenesis and so preventing a drop in body temperature (measured as T_{re}) when chicks are subjected to a cold challenge. Freeman (1970) also showed that T_3 injected chicks caused higher elevations of rectal temperatures and for

longer periods than those treated with T_4 suggesting a greater role for T_3 in thyroid hormone calorigenesis than for T_4.

The possible mechanisms for shivering and thyroid hormone calorigenesis could be as follows: At the cellular level any process that increases coupled mitochondrial oxidative phosphorylation is potentially thermogenic (Prusiner and Poe 1968), for thermal energy is produced as part of the process. Since in intact organs, (except brown adipose tissue), the link between mitochondrial oxidation and phosphorylation is compulsory (Lardy and Wellman, 1952) the rate of oxidation must be controlled by the availability of ADP from energy utilizing processes of the cell. In producing more thermal energy the cells involved must dispose of the ATP produced in order to maintain increased levels of oxidative phosphorylation. In shivering this is brought about by the actinomysin ATPases of the myofibrils (Hemingway, 1963) and in thyroid calorigenesis by increased ATPase activity of the sodium-potassium cellular pumps (Ismail-Beigi and Edelman, 1970; Melikan and Ismail-Beigi, 1991).

Protein catabolism and gluconeogenesis are known to be increased in the rat during cold exposure (Nakagawa & Nagai, 1971) and so it has been suggested that gluconeogenesis may play a special role in heat production by the specific dynamic action of protein. Davison (1973) reported that gluconeogenesis and protein catabolism were increased in one day old chicks exposed to cold; both processes will increase

ATP turnover (Williamson *et al.*, 1971) and consequently increase oxidative phosphorylation. This process is potentially thermogenic (Prusiner & Poe, 1968). Thyroxine is known to increase gluconeogenesis and mobilise glycogen in the mammalian liver (Bottger *et al.*,1970). It has therefore been speculated (Davison, 1973) that thyroid hormones may be active in this way by directing the metabolic changes in the chick during cold stress.

It has also been shown that in the Apodan amphibian (*Gegenophis carnosus)* , in vivo administration of L-thyroxine and triiodothyronine stimulated the activities of cytochrome oxidase, succinate dehydrogenase,and magnesium ATPase (Sutharan *et al.*, 1990). It was therefore suggested that thyroid hormones in vivo increased the oxidative capacity of hepatic mitochondria in *Gegenophis carnosus.* Everts (1990) has also observed that in rat skeletal muscle, T_3 stimulated Ca^{2+} dependent Mg^{2+} ATPase and augmented intracellular Ca^{2+} pools (sarcoplasmic reticulum and mitochondria). The latter results in the enhancement of the passive Ca^{2+} leak, which in turn, may lead to activation of substrate transport systems. It was therefore suggested that increase in intracellular Ca^{2+} cycling after T_3 treatment may at least partly explain the T_3 induced stimulation of energy metabolism.

The data in these experiments also strongly suggests a major role for thyroid hormones, especially T_3 (the active form) in the development and sustenance of thermoregulation

in the domestic fowl. Thyroid hormones also appear to play a crucial role in the hatching process.

4.4 CONCLUSIONS

(i) Up to day seventeen of incubation, the oxygen consumption of the embryo of the domestic fowl was coupled to the ambient temperature. The embryo behaved like a poikilotherm at this stage. In late prenatal embryos (day 18 to 21), a "plateau phase" of oxygen consumption was obtained when the embryo was cooled by less than 5°C. In this stage, metabolic rate is uncoupled to ambient temperature for the small decreases in T_a. This response indicates an incipient homeothermic metabolic response for embryos at this stage.

(ii) The decrease in the Q_{10} values for metabolism with increasing age (day 15 to 21) of embryos suggests improving compensatory ability of the embryos to gradual cooling with increasing age.

(iii) The neonate was found to respond to cold by greatly increasing the metabolic rate. Neonate chicks became hypothermic when exposed to very cold temperatures (<20°C). The degree of hypothermia was found to decrease with increasing age of the chicks. Hypothermia was severest in hatchlings where large drops in T_{re} and oxygen consumption were observed. The lowest values for oxygen consumption were obtained when T_a was 35° C indicating themoneutrality

at this temperature. The lower critical temperature of chicks was also found to decrease with increasing age.

(iv) Rectal temperatures rose from 40.3°C in day zero to 41.03°C in seven day old chicks. Rectal temperatures for day 3 old chicks were significantly higher than for day 0 and by day seven, T_{re} had reached adult levels.

(v) A six fold increase in the levels of myofibrillar ATPase activity was found to occur between days 11 and 18 of embryonic development. In 18 day old embryos, the activity of m-ATPase appeared to have reached a 'critical' value of 0.64 µmoles (min. mg. protein)$^{-1}$. This value appears to be the threshold value at which m-ATPase is no longer limiting to the development of thermoregulation in the developing embryo. The high correlation between m-ATPase activity and oxygen consumption in the embryos and the fact that the enzyme activity appears to reach a threshold value at the same time when an incipient homeothermic response was observed suggests a critical role for the enzyme in the development of temperature regulation in the domestic fowl.

(vi) The rapid rise in the plasma levels of T_3 and T_4 from day 18 to day 21 of incubation suggests that the thyroid hormones may be involved in the development of the incipient homeothermic response observed in embryos at this stage.

(vii) Peak values of T_3 and T_4 on day 21 of incubation also suggest a role for these hormones in the hatching process of the eggs of the domestic fowl.

(viii) After hatching, the levels of T_4 in the neonate fell to their prehatching values while T_3 levels stayed fairly high. This may suggest increased conversion of T_4 to T_3. A crucial role for T_3 in the maintenance of the homeothermic state of the neonate is also suggested by the high correlation between plasma T_3 levels and oxygen consumption at this stage.

(ix) The rapid increase in the plasma T_3/T_4 towards the time of hatching and in the first few days of the neonate suggests a greater role for T_3 than T_4 in the processes of hatching and thermoregulation in this period.

(x) Possible mechanisms explaining thyroid calorigenesis in the pre- and post natal domestic fowl are also suggested. Thyroxine is known to increase gluconeogenesis and mobilise glycogen in the mammalian liver. This process is potentially thermogenic. It is therefore possible that thyroid hormones may be active in this way by directing the metabolic changes in the chick during cold stress. It is also possible that increase in intracellular Ca^{2+} cycling due to increasing T_3 levels may at least partly explain the T_3 induced stimulation of energy metabolism in hatchlings and chicks.

(xi) The results of these experiments therefore suggest a major role for both m-ATPase and thyroid hormones in the development of thermoregulation in the domestic fowl.

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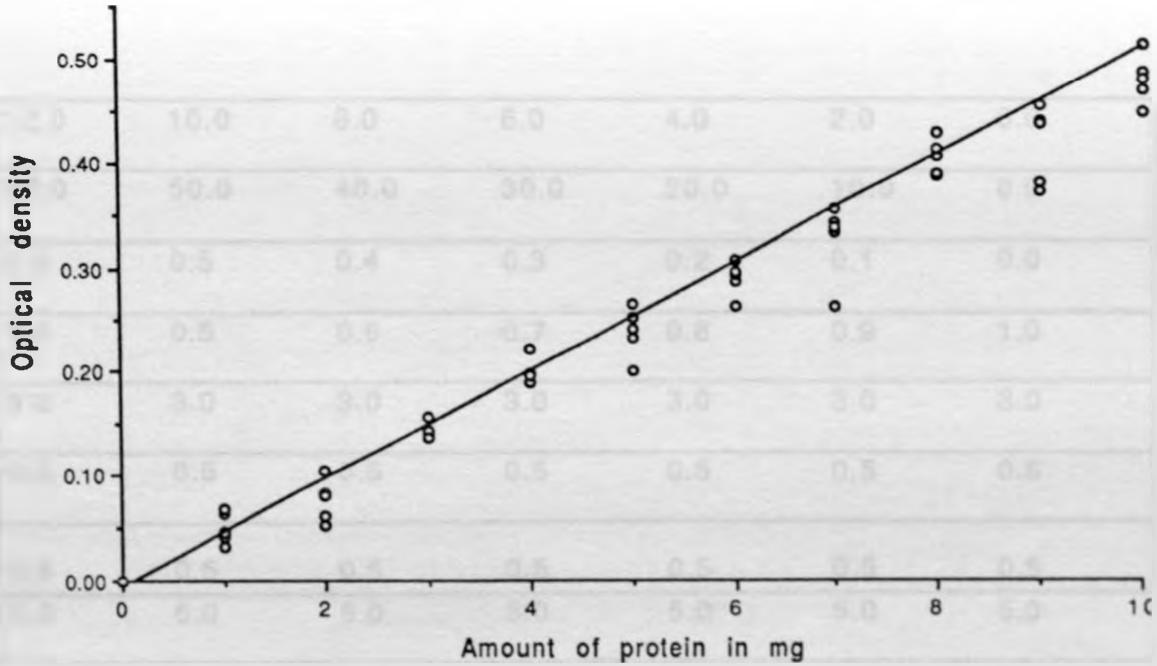
Appendix I

Standard curve for protein Concentration



A plot of optical density against the amount of protein in mg for Bovine serum albumin.

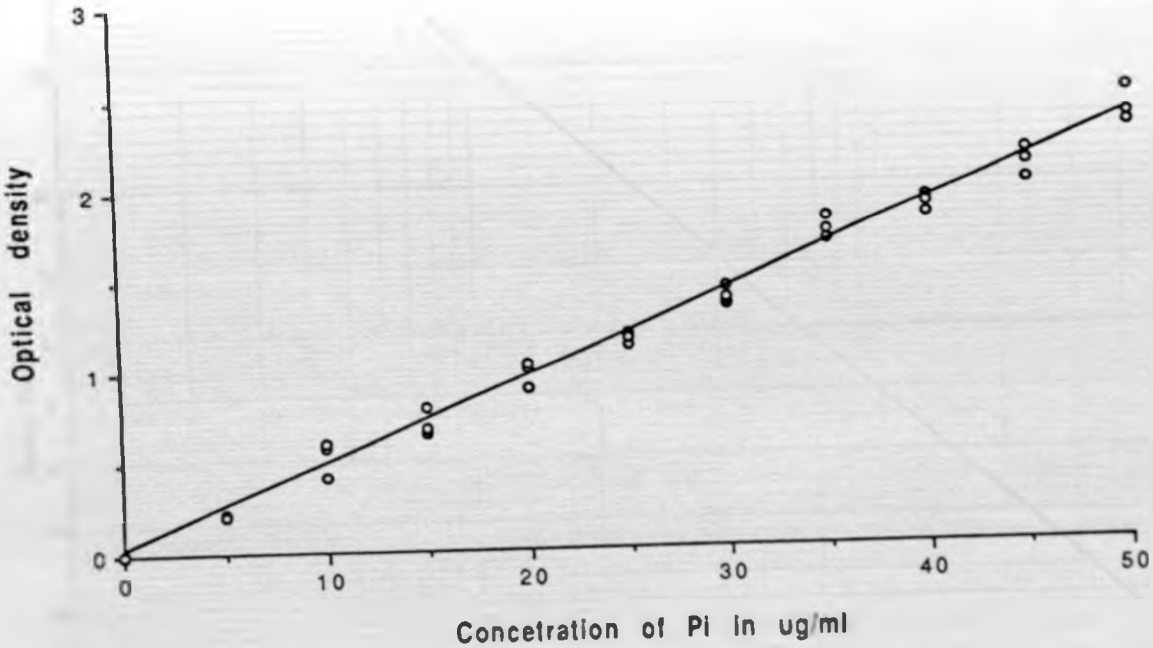
Appendix II

Experimental protocol for for the standard curve of inorganic phosphate showing the different volumes of the reagents A, B, C and standard dispensed.

Concentration ug/ml	12.0	10.0	8.0	6.0	4.0	2.0	0.0
Total P_i in 5.0 ml in ug	60.0	50.0	40.0	30.0	20.0	10.0	0.0
Vol. of standard diluted 1:10	0.6	0.5	0.4	0.3	0.2	0.1	0.0
Trichloroacetic acid (mls)	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Reagent A (cu-acetate)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Reagent B (molybdate)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Reagent C (Elon)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total volume(mls)	5.0	5.0	5.0	5.0	5.0	5.0	5.0

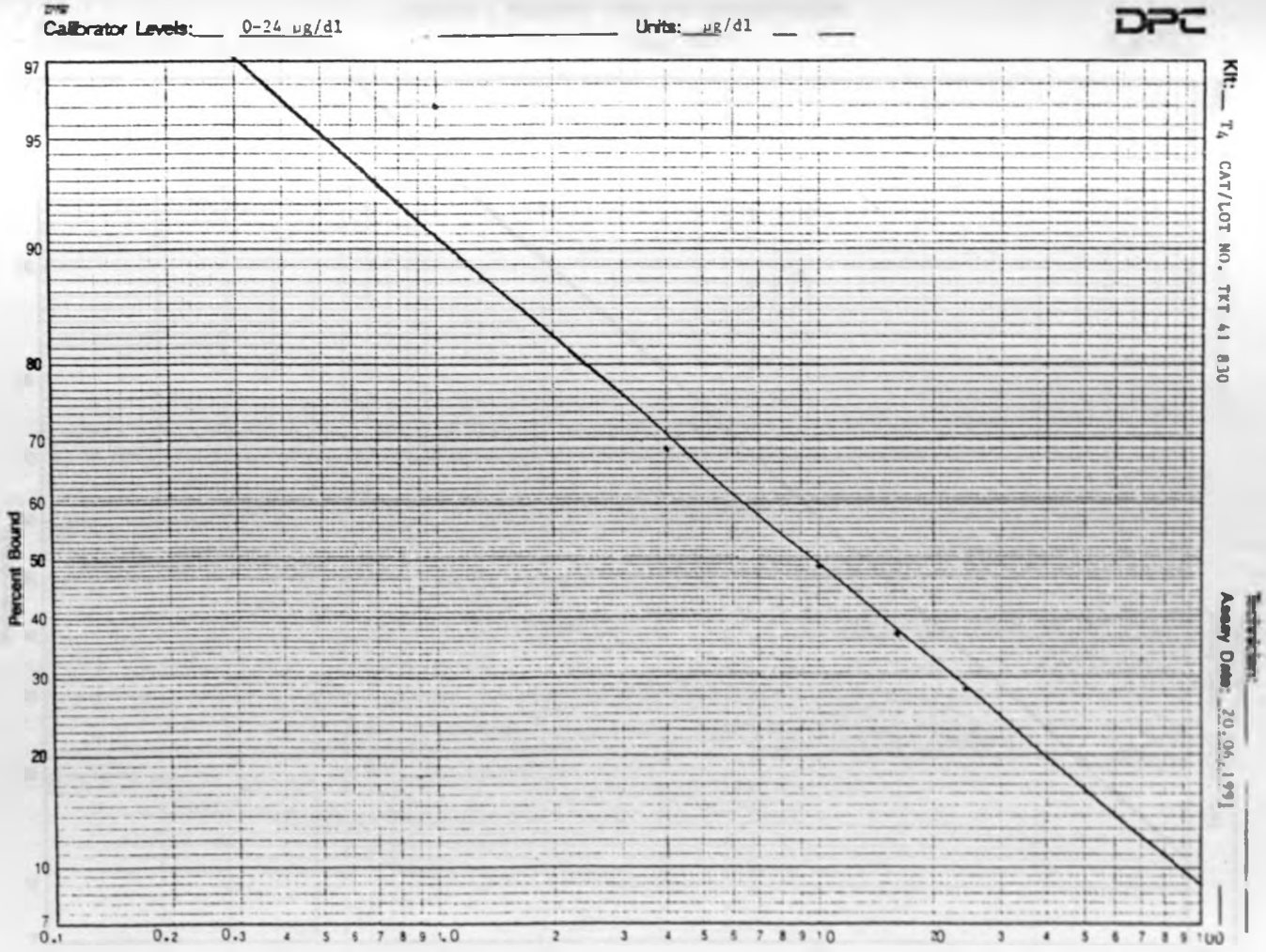
Appendix III

Standard curve for inorganic phosphate.

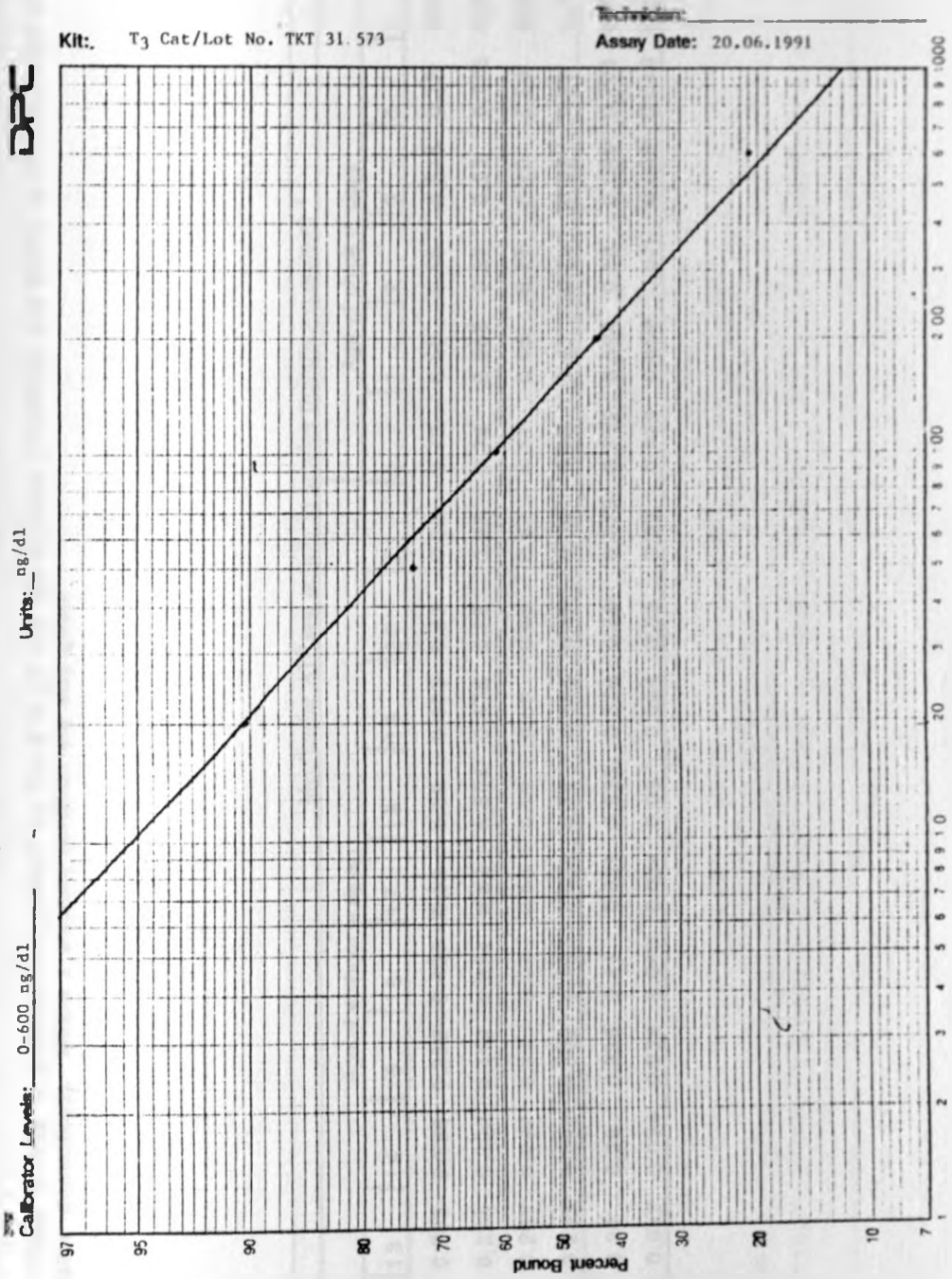


A plot of the optical densities against concentration of inorganic phosphate in ug/ml for the phosphate standard

APPENDIX IV STANDARD CURVE FOR THYROXINE



APPENDIX V STANDARD CURVE FOR TRIIODOTHYRONINE



Appendix VI.

Values of myofibrillar ATPase activity in µmoles(min.mg.prot)$^{-1}$ for the 8 to 21 day old embryos, hatchlings and chicks up to seven days old of the domestic fowl, (*Gallus domesticus*). Means ± standard deviation are also given.

	Myofibrillar ATPase activity in µmoles (minute.mg protein)$^{-1}$																
	Age of embryo in days											Age of chick in days					
	8	12	13	14	15	16	17	18	19	20	21	0	1	2	3	5	7
i	0.07	0.33	0.283	0.296	0.289	0.407	0.53	0.59	0.605	0.67	0.65	0.70	0.56	0.56	0.615	0.72	0.61
ii	0.15	0.33	0.235	0.302	0.410	0.354	0.56	0.594	0.575	0.55	0.67	0.74	0.585	0.67	0.55	0.776	0.65
iii	0.10	0.28	0.208	0.232	0.37	0.47	0.55	0.59	0.58	0.65	0.67	0.67	0.56	0.77	0.60	0.64	0.809
iv	0.12	0.28	0.235	0.296	0.300	0.407	0.505	0.77	0.545	0.45	0.70	0.70	0.67	0.70	0.65	0.72	0.73
Mean	0.11	0.305	0.24	0.28	0.335	0.41	0.536	0.636	0.576	0.58	0.673	0.702	0.594	0.68	0.026	0.721	0.65
± SD	0.03	0.028	0.032	0.034	0.034	0.048	0.024	0.09	0.024	0.05	0.02	0.028	0.052	0.07	0.038	0.046	0.06

Appendix VII

Plasma concentrations of thyroxine and triiodothyronine in ng/ml and their means ± SD. The ratios of T_3/T_4 are also given

		Age of embryo in days						Age of chick in days					
		14	16	18	19	20	21	0	1	2	4	5	7
Plasma T_4 Conc. in in ng/ml	i	10.8	18	5	13	19	20	15	5	6.4	16	4.5	15
	ii	10.8	10	12	10	17	25	13.5	8	4.3	12	9.3	16
	iii	10	10	12	15	19	28	17	15	8.5	6	8	5.5
	iv	8	12	13.5	17	9	24.4	8.5	10	6.4	11.3	15	17
	v			16.0	19.0								
T_4 Mean		9.87	12.5	11.7	14.8	16.0	24.0	13.5	9.5	6.4	11.3	9.2	13.4
±SD		0.65	1.8	1.8	1.5	2.38	1.65	1.8	2.1	0.85	2.0	2.1	2.6
Plasma T_3 Conc. in ng/ml	i	.38	.54	1.32	1.28	1.7	5.5	4.6	5	5	5.4	4.4	4.3
	ii	.13	.54	.84	.72	1.5	6.4	4.6	3.3	2.8	3.6	3	3.8
	iii	.38	.72	.92	1.4	2.6	3.6	3.3	3.6	3.2	3.9	4	4.3
	iv	.32	.72	.96	.84	1.93	5.17	3.1	2.9	3.2	4.3	4.4	4.2
T_3 Mean		0.303	0.63	1.01	1.06	1.93	5.17	3.9	3.7	3.55	4.3	3.95	4.15
±SD		0.05	0.05	0.0.1	0.16	0.23	0 58	0.40	0.45	0.49	0.39	0.33	0.11
Plasma T_3/T_4 ratio		0.0306	0.0504	0.0863	0.0716	0.1206	0.2154	0.289	0.389	0.550	0.380	0.429	0.3097

Appendix VIII

Oxygen consumption in ml(g.hr)$^{-1}$(respiratory intensity) at different ambient temperatures for chicken embryos during days 15, 16, 17 and 18 of incubation. Means ± one standard deviation are given at each temperature.

Oxygen in ml(g.hr)$^{-1}$		Ambient temperatures (°C)										
		38°C	37°C	36°C	35°C	34°C	33°C	32°C	31°C	30°C	29°C	28°C
Oxygen consump. on day 15	i	.38	.34	.32	.29	.27	.26	.23	.23	.23	.22	.2
	ii	.38	.36	.32	.32	.28	.3	.3	.28	.22	.27	.19
	iii	.39	.36	.36	.35	.32	.25	.24	.24	.2	.2	.17
	iv	.39	.35	.31	.3	.29	.24	.24	.23	.22	.2	.19
	v	.35	.34	.31	.3	.26	.27	.23	.27	•	.26	•
	vi	.33	.32	.3	.3	.28	.29	.27	•	•	•	•
Mean±SD		.37±.02	.34±.01	.32±.02	.31±.02	.28±.02	.27±.02	.25±.02	.25±.02	.22±.01	.23±.03	.19±.01
Oxygen consump. on day 16	i	.45	.42	.37	.34	.32	.3	.25	.28	.26	.19	.21
	ii	.41	.36	.38	.35	.34	.3	.25	.25	.23	.21	.2
	iii	.43	.4	.37	.36	.34	.3	.3	.23	.22	.23	.21
	iv	.4	.37	.35	.37	.35	.33	.3	.3	.2	.21	.25
	v	.42	.41	•	•	.29	•	.32	•	•	•	•
Mean±SD		.42±.01	.39±.02	.37±.01	.36±.01	.33±.02	.31±.01	.28±.03	.27±.03	.23±.02	.21±.01	.22±.02
Oxygen consump on day 17	i	.52	.43	.4	.35	.38	.36	.28	.24	.32	.2	.2
	ii	.49	.4	.37	.35	.36	.36	.28	.24	.33	.21	.19
	iii	.47	.44	.4	.37	.37	.32	.28	.26	.24	.2	.17
	iv	.45	.42	.39	.36	•	.3	.31	.26	.24	.22	.21
	v	.5	.43	.38	•	•	•	•	•	•	•	•
Mean±SD		.49±.02	.42±.01	.39±.01	.36±.01	.37±.01	.34±.03	.29±.01	.25±.01	.28± 04	.2±.01	.2±.01
Oxygen consump on day 18	i	.5	.44	.47	.41	.4	.36	.32	.31	.27	.26	.3
	ii	.45	.5	.47	.4	.39	.36	.36	.31	.27	.26	.25
	iii	.5	.5	.47	.39	.39	.35	.32	.35	.32	.3	.3
	iv	.5	.44	.52	.36	.38	.34	.31	.32	.32	.3	.3
Mean±SD		.49±.02	.47±.03	.48±.02	.39±.02	.39±.01	.35±.01	.33±.02	.32±.01	.3±.02	.28±.02	.29±.02

Appendix IX

Oxygen consumption in ml(g.hr)$^{-1}$(respiratory intensity) at different ambient temperatures for chicken embryos during days 19, 20 and 21 of incubation. Means ± one standard deviations are given at each temperature.

Oxygen in ml(g.hr)$^{-1}$		Ambient temperatures(°C)										
		38°C	37°C	36°C	35°C	34°C	33°C	32°C	31°C	30°C	29°C	28°C
Oxygen consump on day 19	i	.54	.53	.6	.55	.44	.4	.46	.38	.37	.35	.34
	ii	.53	.57	.57	.51	.47	.39	.45	.37	.35	.33	.32
	iii	.57	.57	.55	.47	.49	.42	.4	.4	.36	.36	.31
	iv	.57	.57	.53	.48	.46	.4	.4	.37	.31	.35	.34
	v	.55	.55	•	•	•	•	•	•	•	•	•
Mean±SD		.55±.01	.56±.02	.56±.02	.50±.03	.47±.02	.40±.01	.43±.03	.38±.01	.35±.02	.35±.01	.33±.01
Oxygen consump. on day 20	i	.6	.64	.69	.77	.69	.63	.59	.53	.5	.45	.41
	ii	.64	.7	.71	.71	.72	.62	.54	.5	.46	.45	.41
	iii	.7	.64	.62	.68	.58	.62	.54	.4	.48	.45	.37
	iv	.64	.66	.64	.68	.66	.7	.6	.5	.4	.43	.41
Mean±SD		.65±.04	.66±.028	.66±.03	.68±.04	.66±.06	.64±.03	.58±.03	.48±.05	.46±.04	.44±.01	.4± 02
Oxygen consump. on day 21	i	.58	.58	.53	.56	.55	.55	.43	.55	.47	.4	.4
	ii	.61	.56	.53	.59	.56	.56	.42	.55	.47	.47	.42
	iii	.59	.58	.54	.52	.52	.56	.56	.55	.47	.47	.42
	iv	.56	.58	.57	.58	.54	.52	.47	.47	.44	.4	.38
Mean±SD	v	.58±.02	.58±.01	.54±.02	.56±.03	.54±.02	.55±.02	.47±.06	.53±.04	.45±.03	.44±.04	.40±.01

Appendix X

Oxygen consumption im ml(g.hr.)$^{-1}$ (respiratory intensity) for hatchlings, one day and two day old chicks at different ambient temperatures Means ± one standard deviation are given at each temperature.

Oxygen in ml(g.hr)$^{-1}$		Ambient temperatures (°C)													
		40	37	35	32	30	28	26	24	22	20	18	16	14	12
Oxygen consump. on day 0	i	1	1.5	1.33	1.98	2.05	1.82	1.69	2.56	2.7	3.1	2.16	1.22	2.45	1
	ii	1.49	1.5	1.36	1.56	1.7	1.89	1.94	2.21	1.99	1.75	2.16	1.86	1.34	1.7
	iii	1.2	1.03	1.2	1.64	1.66	2.15	2.06	2.15	1.95	2.05	2	1.76	2.1	1.25
	iv	1.24	1.09	1.23	1.6	2.15	2.27	1.94	2.6	1.99	2.27	2.04	2.1	1.57	1.11
Mean±SD		1.23±.2	1.28±..3	1.28±.1	1.67±.1	1.89±.2	2.03±.2	1.90±.2	2.38±.2	2.16±.3	2.29±.5	2.09±.1	1.74±.3	1.87±.5	1.27±.3
Oxygen consump. on day 1	i	1.32	1.4	1.35	1.3	1.7	2	2.1	2.42	2.4	3	2.41	2.52	2.33	2.38
	ii	1.34	1.45	1.34	1.19	1.62	2.28	2.39	3.47	3.18	2.98	2.22	2.22	3.05	2.56
	iii	1.3	1.41	1.3	1.78	1.79	2.3	2.35	2.4	3.23	2.81	2.02	3.03	2.4	3.3
	iv	1.45	1.69	1.3	1.28	1.7	2.28	2.22	2.24	3.2	2.98	2.22	3.1	2	2.5
Mean±SD		1.35±.1	1.49±.1	1.32±.0	1.39±.2	1.7±.06	2.22±.1	2.27±.1	2.63±.5	3.0±.40	2.94±.1	2.22± 2	2.72±.4	2.45±.4	2.69±.4
Oxygen consump. on day 2	i	1.6	1.38	1.2	1.4	1.6	1.7	2.2	2.7	3.6	3.7	3.2	3.2	3.6	3.8
	ii	1.57	1.5	1.1	1.52	1.7	1.74	2.15	2.7	2.62	3.5	2.63	3.34	3.97	3.04
	iii	1.7	1.43	1.3	1.69	2.03	1.9	1.97	3.23	3.4	3.59	2.3	3.1	3.82	3.375
	iv	1.6	1.5	1.33	1.38	1.93	1.71	2.11	3.4	3.4	3.3	3.27	3.6	3.3	3.42
Mean±SD		1.62±.1	1.46±.1	1.23±.1	1.5±.1	1.82±.2	1.76±.1	2.11±.1	3.02±.3	3.26±.4	3.5±.2	2.85±.4	3.31±.2	3.67±.3	3.4±.31

Appendix XI

Oxygen consumption im ml(g.hr.)$^{-1}$ (respiratory intensity) in the 3, 5 and 7 day old chicks at different ambient temperatures

Means ± one standard deviation are given at each temperature.

Oxygen in ml(g.hr)-1		Ambient temperatures (°C)													
		40	37	35	32	30	28	26	24	22	20	18	16	14	12
Oxygen consump on day 3	i	1.86	1.36	1.2	1.39	2.16	2.05	2.2	2.87	3.15	3.4	2.95	2.38	2.24	3.32
	ii	2.1	1.46	1.27	1.5	2.12	2	2.22	2.63	2.68	3	3.24	2.88	2.73	2.68
	iii	1.91	1.89	1.528	1.62	2.39	1.83	2.09	2.62	2.88	3.7	3.78	2.75	2.66	3.4
	iv	1.83	1.5	1.31	1.64	2.16	1.86	2.13	2.15	2.63	3.5	3	2.46	3.19	3.16
Mean±SD		1.92±.1	1.55±.2	1.32±.1	1.53±.1	2.2±.11	1.94±.1	2.16±.1	2.57±.3	2.84±.2	3.4±.3	3.24±.4	2.62±.2	2.7±.38	3.14±.3
Oxygen consump. on day 5	i	1.34	1.3	1.74	1.7	1.91	2.0	1.89	2.88	2.72	2.94	2.62	2.92	2.73	3.3
	ii	2.4	2.0	1.61	2.04	2	1.76	1.99	2.5	2.94	2.57	2.5	2.86	3.3	2.76
	iii	1.78	1.68	1.62	1.56	1.58	1.68	1.95	2.96	2.96	2.85	3	2.93	2.73	2.94
	iv	2.1	1.49	1.62	1.78	1.91	1.90	2.7	2.55	2.87	2.59	2.98	3.0	2.93	2.66
Mean±SD		1.9±.45	1.62±.3	1.65±.1	1.77±.2	1.85±.2	1.83±.1	2.13±.4	2.72±.2	2.87±.1	2.79±.2	2.77±.3	2.93±.1	2.92±.3	2.92±.3
Oxygen consump on day 7	i	2.03	1.97	1.76	2.03	1.61	2.68	2.4	3.2	2.857	3.6	2.63	2.88	2.5	3.02
	ii	2.05	1.82	1.91	2.03	2.21	2.57	2.18	2.86	3.08	2.98	3.08	3.5	2.7	3.49
	iii	2.09	1.75	2.16	1.71	1.82	3	2.68	3.2	3.19	3.1	2.82	3.56	3.2	2.7
	iv	2.02	1.97	1.9	1.86	2.16	2.95	2.97	3.12	2.46	3.49	2.83	3.31	2.65	3.3
Mean±SD		2.05±.1	1.88±.1	1.93±.2	1.91±.2	1.95±.3	2.8±.2	2.56±.3	3.1±.16	2.9±.3	3.29±.3	2.84±.2	3.3±.18	2.74±.3	3.72±.3

Appendix XII

Initial rectal temperatures for hatchlings, one day and two day old chicks. Means ± one standard deviation are given.

Tre in °C		Ambiemt temperatures (°C)													
		40°C	37°C	35°C	32°C	30°C	28°C	26°C	24°C	22°C	20°C	18°C	16°C	14°C	12°C
Initial RT	i	40.5	40.5	40	40.5	40	40.5	41	42	40	40.5	40.4	40.7	40	40.1
on day 0	ii	39.9	40	40	40.5	40	41	40	37.5	40.4	40.2	40.2	41.3	39	40.1
	iii	40.8	38.5	41	40	40.7	40.5	41.4	41	40.4	41	40.2	40.8	40.9	41.1
	iv	39.5	40.9	40	40.5	40.1	41	40.1	41.7	40.6	40.2	40.5	40.4	40.5	40.8
Mean±SD		40.2±.6	39.9±.1	40.3±.5	40.4±.3	40.2±.4	40.1±1.	40.6±.7	40.5±2	40.4±.3	40.5±.4	40.3±.2	40.8±.4	40.1±.8	40.5±.5
	i	40.3	41.2	41	40.6	39.4	39.3	40.8	40.4	40.2	40.7	40.5	40.4	40.8	40.8
Initial RT	ii	39.8	40.5	40.1	40.7	40	42	42.1	42.4	42.5	40.7	42.2	41.3	42	40.5
on day 1	iii	37.5	40.3	41	40.2	39.4	39.3	40.8	40.9	41.3	40.8	40.1	40.5	40.9	40.4
	iv	40	40.5	40	41.4	39.4	40	40.8	40.5	41	40.7	40.5	40	39	40.8
Mean±SD		39.4±1	40.6±.4	40.5±.6	40.7±.7	39.6±.3	40.1±1.	41.1±.7	41±.9	41.2±.9	40.7±.1	40.8±.9	40.6±.5	40.7±1	40.6±.2
	i	41.5	40.5	39.1	39.1	40.2	41.4	41.8	40.8	40.1	41	40.6	40.1	40.3	40.8
Initial RT	ii	41.4	40.4	40	40.5	40	40.5	40.5	40.8	40.1	40.5	41.4	40.5	40.6	40.5
on day 2	iii	40.8	40.3	41	40.2	40.2	40.2	40.8	40.9	40	40.8	40.1	40.5	40.9	40.4
	iv	41.2	40.5	40	40.3	40.5	41.4	40.8	40.5	39.9	40.2	40.5	40	40	40.8
Mean±SD		41.2±.3	40.4±.1	40±.7	40±.6	40.2±.2	40.8±.6	40.9±.5	40.7±.2	40±.1	40.6±.4	40.6±.5	40.2±.3	40.4±.4	40.6±.2

Appendix XIII

Initial rectal temperatures for 3, 5 and 7 day old chicks. Means ± one standard deviation are also given.

Initial RT (oC) for:		Ambient temperature (°C)													
		40°C	37°C	35°C	32°C	30°C	28°C	26°C	24°C	22°C	20°C	18°C	16°C	14°C	12°C
Day 3	i	40.4	40.8	40.9	41	41	40.3	40.3	40.8	40.4	41.1	40.1	40.4	41.3	40.5
	ii	39.9	40.9	40.9	40.6	40.5	40.8	40.3	40.4	42.1	42	40.1	41	40.3	40.3
	iii	39.8	40.9	40.3	41.9	41	41.9	40.8	40.4	40.6	41.5	40.8	40.2	40.7	40.1
	iv	40.5	40.5	41	40.6	41	40.4	40.8	40	41	41.1	41.1	40.3	40.7	40.6
Mean ±SD		40.1	40.7	40.7	41	40.8	40.8	40.3	40.4	41	41.4	40.5	40.4	40.7	40.4
		0.35	0.2	0.3	0.6	0.25	0.7	0.3	0.3	0.7	0.4	0.5	0.4	0.5	0.22
Day 5	i	40.8	40	41.9	40.5	40.4	41.3	40.5	40.4	39.9	39.9	40	40.8	42	41
	ii	41.4	40.6	40.5	41.2	40.5	41.2	40.4	41.1	41.1	40.9	40.8	40.6	40.8	41
	iii	40.7	41.7	41.2	40.6	40.1	40.5	40	40.5	41.8	40.5	40.6	40.6	41	41
	iv	40.8	40.6	41	41.3	40.5	40.1	40.9	40.9	40.5	40.1	40.9	40.3	41	41.4
Mean ±SD		40.9	40.7	41.1	40.9	40.3	40.77	40.45	40.7	40.8	40.4	40.5	40.57	41.2	41.1
		0.3	0.7	0.6	0.4	0.2	0.3	0.4	0.33	0.8	0.44	0.4	0.2	0.05	0.2
Day 7	i	41.8	41.2	40.9	40.3	41.3	41.1	41.1	40.5	41	41.1	41.1	41	40.7	40.5
	ii	41.5	41	40.8	40	41.1	40	41.3	41.5	40.6	41.1	41.7	41.5	40.5	40.9
	iii	41.8	41	41.8	41.2	41.2	40.5	41.2	41.5	40.6	41.2	41.7	41.2	40.7	41.1
	iv	41.5	41.2	41	41.3	41.3	41	41.3	41	41	41.1	41.6	41.4	40.3	39.2
Mean ±SD		41.65	41.1	41.25	40.7	41.22	40.65	41.22	41.12	40.8	41.12	41.5	41.3	41.5	41.12
		0.2	0.11	0.5	0.6	0.1	0.5	0.1	0.4	0.2	0.5	0.3	0.22	0.3	0.1

Appendix XIV

Final rectal temperatures recorded for hatchlings, one day and two day old chicks after a two hour exposure to different ambient temperatures. Means ± one standard deviation are given at each ambient temperature.

Final RT (°C) for:		Ambient temperatures (°C)													
		40°C	37°C	35°C	32°C	30°C	28°C	26°C	24°C	22°C	20°C	18°C	16°C	14°C	12°C
Day 0	i	41	40.5	41.8	39	40.1	37	38	38	36.4	38.6	34	35.2	32.2	29.4
	ii	42	40.5	39.9	39.9	37.7	38.6	34.5	36.5	37	33.2	33.6	29.6	33.3	36
	iii	38	41.4	40	39.9	35.6	38.6	38	34.2	37	32.9	33.5	32.5	29.6	35.6
	iv	42	40	39.9	39.9	37.2	37	37	37.3	36.9	35.9	34	35.6	35.2	35
Mean		40.7	40.6	40.4	39.6	37.65	37.8	36.8	36.5	36.8	35.15	33.8	33.22	32.57	34.00
±SD		1.8	0.5	0.9	0.45	1.86	0.92	1.6	1.6	0.28	2.6	0.26	2.7	2.3	3.0
Day 1	i	41.8	43	40	38.7	37.9	38.2	40	38.6	38.3	34.2	36.5	33.2	37	36.7
	ii	41.1	39.9	40	38.9	39.4	38.7	39.1	41.1	38.3	34.4	38	34	35.2	37.5
	iii	38	41.3	40.8	38.9	37.9	38.2	40.5	38.9	36.1	36.9	36.2	33.8	33.4	36.9
	iv	41.9	43.8	39.9	40	37.9	38	39.9	39	36	36.5	36.8	35.5	28	36.7
Mean		40.7	42	40.2	39.1	38.28	38.28	39.8	39.4	37.2	35.5	36.8	34.1	33.4	36.9
±SD		1.83	1.7	0.4	0.5	0.75	0.29	0.5	1.0	1.3	1.3	0.7	0.9	3.8	0.37
Day 2	i	42.3	41.5	37	40.8	39	41.2	40.2	38.6	36.5	38.1	38.5	36.3	35.5	37
	ii	42.5	41	40.8	40.8	39.9	40.8	41.1	40	36.5	37.4	38	36.5	35.6	38.6
	iii	42.6	40	39.8	40.5	40.9	40.8	40.6	38.4	36.5	39	37	38.8	35.8	37
	iv	42.5	41	40.3	40.3	39.9	41	41	40	36.5	37	36.8	36.3	35.6	36.5
Mean		42.5	40.8	39.48	40.6	39.9	40.95	40.7	39.25	36.5	37.8	37.57	36.9	35.62	37.2
±SD		0.12	0.629	1.7	0.25	0.77	0.191	0.411	0.87	0.0	0.87	0.81	1.32	0.12	0.91

Appendix XV

Final rectal temperatures recorded for the 3, 5 and 7 day old chicks after a two hour exposure to various ambient temperatures. Means ± one standard deviation also given at each ambient temperature.

Final RT (±°C) for:		Ambient temperatures (°C)													
		40°C	37°C	35°C	32±°C	30°C	28°C	26°C	24°C	22°C	20°C	18°C	16°C	14°C	12°C
Day 3	i	41.2	41.3	39	40.7	38.9	40.2	39.3	38.8	38.9	37	37	38.9	38.6	37.5
	ii	41.7	41.1	40.5	40.3	39.4	40	39.4	37.6	39.1	38	38	38	38.6	38.3
	iii	41.5	42.1	39.1	41.2	39	40	39.8	38	38	39.4	38	38.2	38.2	38.1
	iv	41	41.1	40.8	40.3	39	39.3	39.2	36	37	37.2	38.9	38.4	38.2	36.1
Mean		40.15	40.7	40.7	41	40.8	40.85	40.35	40.4	41	41.4	40.5	40.4	40.75	40.37
±SD		0.35	0.18	0.32	0.6	0.25	0.73	0.28	0.32	0.75	0.42	0.50	0.35	0.52	0.22
Day 5	i	41.9	41.9	40.8	40.4	39.5	40	40.2	39.4	39.7	38.3	39	38.4	38.8	40.7
	ii	42.9	41	40.4	40.2	39.8	40	39.7	39.7	40.5	39	40.5	39	39.6	39.8
	iii	42.5	43	40.1	40.4	39.6	40	39.2	39.9	40	38.6	40.2	39	39	39
	iv	41	41	40.1	40.8	39.6	39	38.8	39.2	39.8	40	39	40.2	38.9	39
Mean		42.07	41.7	40.35	40.45	39.6	39.75	39.47	39.55	40	38.95	39.67	39.15	39.05	39.62
±SD		0.8	0.95	0.33	0.25	0.12	0.5	0.6	0.31	0.35	0.74	0.78	0.75	0.35	0.81
day 7	i	42.8	42.5	41.3	40.3	40.8	40.1	41.4	40.5	40	39.5	39.4	39.4	39	39.5
	ii	42.1	42.3	41.5	40.3	41.3	40.9	40.1	40.3	40.1	39.9	40.7	39.9	38.9	39.8
	iii	43.3	42.4	42.5	41.1	40.3	40	41.3	39.9	40	39.6	40.3	39.6	40.7	39
	iv	43	42.5	41.6	41.1	40.2	41	40	40.5	39.8	39.5	40.3	39.7	39.7	39.5
Mean		42.8	42.42	41.7	40.7	40.65	40.5	40.7	40.3	39.98	39.6	40.17	39.65	39.6	39.45
±SD		0.51	0.09	0.53	0.46	0.50	0.52	0.75	0.28	0.12	0.18	0.55	0.20	0.83	0.33