FAT DEPOSITION IN RELATION TO SEXUAL MATURATION

OF JAPANESE QUAIL

by

Boma Magnus Oruwari

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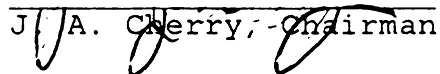

J. A. Cherry, Chairman

W. L. Beane

R. A. Teekell

H. P. Van Krey

K. E. Webb

J. H. Wolford

April, 1984

Blacksburg, Virginia

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INTRODUCTION

Growth, fat deposition and sexual maturation are related developmental processes having important implications in the production of animal protein. Reduced growth and the accompanying reduction in body fat are associated with a delay in the onset of sexual maturity, but the underlying mechanisms involved are not known. Examination of body composition data obtained at sexual maturity led Frisch *et al*. (1977) to propose that a minimum percentage or amount of body fat is required for the onset of sexual maturity. Inherent in their hypothesis is that hyperplastic and/or hypertrophic fat deposition accelerates as an animal approaches maturity.

Although controversial, there is evidence suggesting that hyperplastic lipid deposition usually ceases at some point prior to sexual maturity and subsequent fat deposition proceeds solely through adipocyte hypertrophy. It is conceivable, therefore, that the cessation of adipocyte hyperplasia and the subsequent increase in adipocyte hypertrophy are associated with the onset of sexual maturity in a mechanistic context.

The experiments reported in this dissertation were designed to clarify the relationships among body weight, adipose cellularity, body composition, and the onset of sexual maturity in Japanese quail (*Coturnix coturnix*

japonica). Particular emphasis was placed on examining adipose cellularity in relation to sexual maturation.

REVIEW OF LITERATURE

Interrelated maturational processes under neuroendocrine control are responsible for the initiation of sexual maturity. Puberty may be regarded as the expression of a young female's capability for ovulation and fertile mating, and is conspicuously marked by first estrus in the rat, the onset of menstruation in the human, and the first oviposition in birds. Because of these easily observable events, studies involving sexual maturation have usually utilized female subjects. This review will concentrate on concepts derived from such studies and on their applicability to the domestic fowl.

Body Weight, Body Composition, and Sexual Maturity

Permanent impairments of growth rate in rats subjected to preweaning undernutrition because of large litter size led to observations that a reduced growth rate was associated with a delay in sexual maturation. As a result of such studies, several groups of investigators concluded that sexual maturation was more closely related to the attainment of a minimum threshold body size than to a particular chronological age (Kennedy, 1957; Widdowson and McCance, 1960; Kennedy and Mitra, 1963). Although subsequent studies showed that preweaning undernutrition resulted in permanent reductions in body weight without

delaying either age at vaginal opening or age at first estrus (Bakke *et al*., 1975; Ronnekleiv *et al*., 1978), it appears reasonable to assume that sufficiently severe preweaning undernutrition will effectively delay sexual maturation; the restriction of food intake in postweaning periods is one of the more powerful means of delaying puberty (see review by Barrows and Roeder, 1977).

Widdowson and McCance (1960) reported that different planes of nutrition resulted in sexual maturity occurring at widely different ages but at fairly constant body weights. Monteiro and Falconer (1966) also concluded that the attainment of sexual maturity was weight rather than age-dependent. This relationship was supported statistically by observations that the variance for weight at sexual maturity was less than the variance for weight at a specific chronological age. More recent studies supporting the concept of a minimum threshold body weight for the onset of sexual maturity were reported by Wilen and Naftolin (1978) and by Merry and Holehan (1979). Kennedy (1969) regarded the weight-dependency of sexual maturity as an example of the general tendency for growth to be self-regulatory.

Although a reduced growth rate can delay sexual maturity, a constant body weight at sexual maturity does not occur invariably. Glass *et al*. (1976) observed an inverse relationship between pubertal delay and growth rate, and

concluded that pubertal timing was more closely related to growth rate than to the attainment of a fixed body weight. Wilen and Naftolin (1978) reported a significant correlation between pubertal age and pubertal weight among individual animals; later maturing animals were heavier than earlier maturing ones at puberty. Similar results were reported by Frisch et al. (1975), rats fed high-fat diets entered estrus at an earlier age and smaller body weight than did rats fed low-fat diets. Glass and Swerdloff (1980) concluded that, because body weight at puberty can be altered by diet, puberty is not triggered by the attainment of a critical body weight. Similarly, Johnston et al. (1975) emphasized that within a particular species or population, a constant body weight at puberty is a central tendency rather than a critical threshold factor.

It is obvious that the nutritional status of an individual is important for the onset and maintenance of reproductive function in humans. Undernutrition delays the adolescent growth spurt and menarche in girls while chronic undernourishment and rapid weight loss disrupts established menstrual cycles in otherwise normal older women (Frisch, 1972). Dietary treatment of underweight amenorrheic patients is usually more effective in inducing ovulation than gonadotropin therapy (Knuth et al., 1977). Puberty is also associated with marked changes in somatic development,

a process that comprises alterations in body composition. In rats, the onset of puberty has long been associated with appetite or a lipostat (Kennedy and Mitra, 1963; Kennedy, 1969), and Frisch and McArthur (1974) hypothesized that a particular body composition may be an important determinant of reproductive capability in human females. Support for this hypothesis was provided by observations that maturation of leutinizing hormone patterns in women was associated with body weight and that changes in body composition affect the hypothalamic-pituitary regulation of estrogen secretion and metabolism (Boyar _et_ _al_., 1978). Frisch _et_ _al_. (1977) have also reported that too much or too little body fat inhibits sexual function in human females, and it was suggested that adipose tissue serves as an extragonadal source of estrogen (Frisch, 1980). Aromatization of androgens to estrogens occurs in human adipose tissue (Nimrod and Ryan, 1975), but Crawford _et_ _al_. (1975) found that menarche was not necessarily triggered by the achievement of a critical body weight or by reduced metabolism. They also concluded that increasing levels of estrogens in adolescence were not solely responsible for the characteristic increase in body fat, although the close association between menarche and the attainment of a characteristic body composition was not discounted as a contributing factor in menarche.

Gonadotropin secretion, body composition, and diet are

undoubtedly interrelated in their influences on the onset of sexual maturity. Urinary and plasma gonadotropins are low in underweight patients with and without anorexia nervosa. The response to leutinizing hormone releasing hormone (LHRH) is reduced when body weight is low and is restored when body weight returns to a normal range (Palmer et al., 1975). Similarly, a normal response to clomiphene citrate occurs only when body weights are within a normal range (Marshall and Fraser, 1971), suggesting that body weight or body composition plays a permissive role in the central nervous system's control of hypothalamic function and gonadotropin secretion (Frisch, 1980).

Neuroendocrine mechanisms involved in the onset of sexual maturity of avian females appear similar to those for mammals. Initiation of sexual maturity in the chicken has been associated with increased LH secretion which was correlated with the duration of rapid comb growth (Sharp, 1975). A peak in plasma LH appears to occur between 16 and 19 weeks of age (Williams and Sharp, 1977). A decreased sensitivity of the negative feedback system regulating LH release may be involved in this peak since the pituitary does not become more responsive to LHRH at this developmental phase in the hen (Wilson and Sharp, 1975). The highest content of urinary estradiol and estrone also occurs only a few days before egg laying begins (Common et

al., 1965; Mather and Common, 1969) and a decreased estrogen to progesterone ratio occurs in the hen prior to the first oviposition, presumably because of changes in the sensitivity of hypothalamic positive feedback systems (Wilson and Sharp, 1975). Although not entirely clear, these observations suggest that central nervous system-pituitary-gonadal interrelationships play a dominant role in the initiation of sexual maturity of birds. Concomitant with these neuroendocrine changes are metabolic changes associated with nutrient stores. The concentration of plasma protein (Vanstone *et al.*, 1955), total lipids and free fatty acids (Heald and Badman, 1963) and phosphoproteins (McIndoe, 1959; Heald and Badman, 1963) peak several days prior to the onset of lay. Senior (1977) suggested that the determination of metabolic constituents and gonadotropic hormones in the same blood samples might clarify the process of sexual maturation in the chicken.

The influence of body composition on sexual maturation of the domestic fowl is not clear. Chickens do resemble other species in that body weight is more closely associated with age at sexual maturity than is chronological age (Brody *et al.*, 1980), although both critical age and critical weight requirements apparently exist (Leeson and Summers, 1983; Dunnington *et al.*, 1983). Furthermore, Zelenka *et al.* (1984) presented evidence that multiple threshold traits

associated with body composition were required for the onset of lay of Japanese quail. When growth was maximized through the feeding of high-protein diets *ad libitum*, lay commenced when a minimal chronological age or minimal body fat requirement was reached. When age at sexual maturity was delayed nutritionally, linear and lean body growth appeared to be critical factors for the onset of lay.

In summary, body weight is more closely associated with the onset of sexual maturity than is chronological age. It is likely that body composition plays a permissive role in this phenomenon, although it is not known which specific body component is involved. Interpretation of the available data are undoubtedly complicated by interaction among critical body weight, critical age, and critical body composition requirements.

Adipose Tissue Cellularity and Sexual Maturity

Early studies concerning the cellular development of adipose tissue indicated that changes in both cell number and cell size accompanied changes in body fat of mature rats (Enesco and Leblond, 1962; Peckham *et al.*, 1962). Subsequently, the theory that the number of fat cells present in adult animals is constant and changes in adiposity of adult animals are related to differences in cell size became widely accepted (Knittle and Hirsch, 1968; Hirsch and Gallian, 1968; Hirsch, 1972; Stern and Greenwood,

1974; Hirsch, 1976; Hirsch and Batchelor, 1976; Cleary et al., 1977; Hood, 1977; Kirtland and Gurr, 1979). According to this "fixed adipose cell" hypothesis, adipocyte hyperplasia provides the primary impetus to lipid deposition in early stages of growth followed by a transient period when lipid accumulation is both hyperplastic and hypertrophic in nature. Finally, lipid deposition becomes solely a hypertrophic phenomenon in adult animals (James, 1976; Bulfur and Allen, 1979).

Although hyperplastic and hypertrophic contributions to fat deposition undoubtedly vary at different critical periods, a significant body of evidence has accumulated indicating that adipose cell number is not always constant in adult animals. Increases in both adipose cell number and cell size apparently occur in mature animals of several genetically obese strains (Johnson et al., 1971; Johnson and Hirsch, 1972; Eisen et al., 1978; Trayhurn et al., 1979). A high caloric intake has been shown to increase fat cell number in adult laboratory rodents (Lemonnier, 1972; Faust et al., 1978). The number of fat cells in the perirenal depots of male Fischer 344 rats, a non-obese strain, was shown to double between 6 and 18 months of age (Bertrand et al., 1978; 1980). Plasticity of fat cell number in adult animals was also reported by Stiles et al. (1976) and recently by Bertrand et al. (1984).

Conflicting data concerning adipocyte hyperplasia appears to have resulted because of the procedures utilized. Early studies suggesting that hyperplastic fat deposition occurred in mature animals used DNA content as an index of adipose cell number (Bjurulf, 1959; Enesco and Leblond, 1962; Peckham et al., 1962). These studies, however, were generally considered inconclusive because it was not established if new cells formed were adipocytes or supporting tissue cells; a substantial proportion of adipose tissue DNA is contained in non-adipocyte fractions (Cleary et al., 1977). Conversely, the majority of evidence supportive of the "fixed adipose cell" concept has been provided by studies in which adipocyte number and size were measured electronically using methods originally developed by Hirsch and Gallian (1968). Although such techniques are rapid and provide relatively accurate cell diameter measurements, it is impossible to distinguish between the formation of new adipocytes and the lipid filling of small existing fat cells because a minimum cell size is required for detection. Consequently, interpretation of the available data requires careful attention to the methodology employed.

The incorporation of radioactive thymidine into adipose tissue has been used to study adipocyte differentiation. Hollenberg and Vost (1968) administered tritiated thymidine

to rats and found that within two days of administration, only 10% of the total radioactivity in adipose tissue was found in the adipocyte fraction. The specific activity in the adipocyte fraction rose from two to five days post-injection, and more slowly thereafter. Frohlich *et al*. (1972) also observed a slight rise in DNA specific activity of the adipocyte fraction of adipose tissue after three days in an *in vitro* study using cultured adipose tissue from weanling rats. These two studies were interpreted as evidence that primordial adipose cells within the stromal fraction of adipose tissue differentiate into new adipocytes during prepubertal growth. Similarly, Pilgrim (1971) studied the development of subcutaneous adipose tissue in pre- and post-natal rats and found that the proliferative index, as measured by tritiated thymidine, was highest in preadipocyte type cells. This study also suggested a functional participation of cell replication in the control of the differentiation process, and indicated that DNA synthesis does not cease in differentiating fat cells. Faust *et al*. (1978) also concluded that hyperplastic growth of adipose tissue was inducible after the attainment of adult body size. Conversely, Greenwood and Hirsch (1974) used tritiated thymidine to provide evidence indicating that adipocyte synthesis in the epididymal fat pad of rats occurred at a rapid rate from birth but ceased before sexual

maturity.

Regardless of the contribution of hyperplastic growth to adipose tissue accumulation in adult animals, enlargement of individual fat cells can account for a majority of lipid accumulation, because the diameter of a single fat cell is capable of a seven- to ten-fold increase (Wasserman, 1965). In all probability, however, cell size alone cannot account for the enormous masses of body fat that an animal is capable of accumulating. The limited amount of cytoplasm covering the intracellular fat droplet must ultimately limit the flexibility of a fat cell (Wasserman and McDonald, 1960; Faust et al., 1978). As a result, it has been suggested that new fat cells are added to fat depots only after a "saturation level" or a critical maximum size of existing adipocytes are reached (Liebelt, 1963; Faust et al., 1978). Thus, body fat may accumulate by hypertrophic growth until a critical limit is reached, after which additional cells are recruited for lipid storage. Additional evidence for this hypothesis has been provided by studies in which a bimodal size distribution for adipocytes was observed (March et al., 1982).

Differentiated fat cells display a remarkable tendency to persist during prolonged periods of weight reduction (Hausberger, 1965). Napolitano and Gagne (1963) used electron microscopy to examine adipose tissue from animals

maintained in good health but gradually depleted of fat stores through the feeding of a low-calorie diet. Morphological changes including a reduced cell volume, alterations in cell metabolism, and a diminution of tissue protein were observed; however, the cells did not become dedifferentiated. The differentiated fat cell, therefore, maintains its high degree of specialization (Napolitano, 1965) with the nucleus, mitochondria, endoplasmic reticulum and golgi material preserving the same characteristics in fat depleted cells as in lipid-laden cells.

As presently understood, perivascular reticular cells in mesenchymal lobules represent a source from which new fat cells proliferate (Simon, 1965; Hausberger, 1965; Napolitano, 1965). Prior to proliferation into the adipocyte pool, these reticular cells, or primordial fat cells, are virtually indistinguishable from other stromal elements such as histiocytes and fibroblasts, although these latter cells do not accumulate lipid (Simon, 1965). When lipid first appears in a primordial fat cell, the cell loses its capacity to divide and is called an adipogenic reticular cell which transits to an adipoblast. With further lipid inclusion, a preadipocyte (Moruloid cell) is formed. The preadipocyte is an obligatory step in adipogenesis since the preadipocyte develops into a mature adipocyte (Signet-ring cell). In the mature adipocyte, the nucleus is forced to

the periphery by a centralized confluence of multiple lipid droplets. The adipocyte has the ability to grow to its maximum capacity by adding to the central lipid droplets in its cytoplasm.

Several groups of investigators have used collagenase digestion (Rodbell, 1964) to separate fat cells from stromal elements for studying the turnover of adipocytes and their progenitors. These procedures generally involved an initial injection of tritiated thymidine which was followed by a "chaser" of unlabeled thymidine to create a brief pulse of exposure to the isotope. The animals were then sampled over a period of several days to trace the movement of label from the stromal to adipocyte fractions. From such experiments, Hollenberg and Vost (1968) determined that radioactivity initially accumulated in the stromal (primordial fat cell) fraction, and then required two to five days to migrate into the adipocyte fraction. Their observations were consistent with previous histological evidence that mature adipocytes do not display mitotic activity (Simon, 1965). Greenwood and Hirsch (1974) used similar procedures and concluded that adipocyte proliferation in the rat ceased prior to sexual maturity. In subsequent studies in that laboratory (Klyde and Hirsch, 1979a; 1979b), radioactivity was found in the adipocyte fraction of collagenase digests of adipose tissue from rats fed both a normal stock diet and a high-fat diet,

but they concluded that adipocyte progenitors reside both in the stromal and adipocyte fractions. More recently, Bertrand *et al*. (1984) estimated fat cell number in collagenase extracts of adipose tissue and concluded that fat cell number was not fixed in adult animals.

Adipose cellularity studies with the domestic fowl are limited. In both broilers and Leghorns under *ad libitum* feeding, hyperplastic growth of adipose tissue appears to cease by 12 to 15 weeks of age (Pfaff and Austic, 1976; Hood, 1982). Although this age can apparently be altered by nutrient restriction (Pfaff and Austic, 1976; March and Hansen, 1977; Ballam and March, 1978), there is no evidence that adipocyte hyperplasia persists in chickens after sexual maturity is reached (March *et al*., 1982).

EXPERIMENT I

SEXUAL MATURITY AND BODY COMPOSITION OF JAPANESE QUAIL IN RESPONSE TO PHOTOPERIODIC AND HORMONAL MANIPULATIONS

Introduction

A clear understanding of the role of body weight, growth rate and chronological age in the neuroendocrine initiation of sexual maturity is lacking. It has been postulated that sexual maturity is triggered by the attainment of a minimum threshold body weight rather than a minimum chronological age (Frisch and Revelle, 1970; Frisch, 1972; Frisch et al., 1977; Brody et al., 1980). An interplay between minimum chronological age and minimum body weight requirements for sexual maturation, however, has been demonstrated (Leeson and Summers, 1983; Dunnington et al., 1983). Moreover, considerable evidence suggests that body composition is either actively or permissively involved in the initiation of sexual maturity. Some workers have emphasized the importance of attaining minimal amounts of body protein (Wilen and Naftolin, 1976; 1977; 1978), while others concluded that a minimum amount or percentage of body fat is required for the onset of sexual maturity (Widdowson and McCance, 1960; Frisch et al., 1973; Frisch, 1980; 1982).

It was proposed recently that multiple threshold traits associated with growth and body composition are critical for the onset of lay in female Japanese quail (Zelenka et al., 1984). Based on experiments in which the body composition of quail fed restricted amounts of diets differing in crude protein was examined, they suggested that early maturing

quail tended to enter lay when a minimum chronological age or a critical body lipid level was reached. Late maturing quail, on the other hand, entered lay when a critical lean body mass or skeletal size was reached.

In the experiment reported here, body composition and sexual maturity were examined in response to hormonal and photoperiodic manipulations. Male and/or female Japanese quail were treated with phenylthiouracil (PTU), turkey growth hormone (GH), anti-sera to turkey growth hormone (AGH), estrogens (E), testosterone (T), and an inhibitory photoperiod (IP). At 63 days of age, when approximately 50% of the females had entered lay, each quail was sacrificed to determine if differences in sexual maturation could be related to differences in body composition.

Materials and Methods

Japanese quail were hatched and caged in modified chick battery brooders. Feed and water were provided *ad libitum* throughout the study. A daily photoperiod of four hours light and 20 hours darkness was maintained until the quail reached 42 days of age and, unless specified otherwise, 14 hours of light and 10 hours of darkness, thereafter.

At 14 days of age, a control group of 24 unsexed quail were randomly selected and maintained under the conditions described above. Three treatments were provided by feeding diets containing 0.1% (20 quail), 0.01% (15 quail), and 0.001% (12 quail) PTU (4-phenyl-2-thiouracil; Sigma Chemical Co., St. Louis, MO) to unsexed quail beginning at 14 days of age and continuing to 63 days of age. In addition, GH and AGH (Avian Physiology Laboratory, USDA/SEA, Beltsville, MD) were administered intramuscularly (0.1 ml/bird/day) for 12 consecutive days beginning at 14 days of age. GH and AGH were administered to 13 and 14 unsexed quail, respectively. Silastic capsules (2 x 1.6 cm) containing 10 mg of T (Androstene-17β-ol-3-one, Sigma Chemical Company, St. Louis, MO) or E (17β-estradiol, Sigma Chemical Co., St. Louis, MO) were implanted subcutaneously into 12 male and 12 female quail, respectively, at 28 days of age. The amounts of T and E actually absorbed, as measured by recovery at sacrifice, were about one and 0.5 mg/bird, respectively.

Finally, 24 unsexed quail were maintained under an IP consisting of 4 hours of light and 20 hours of darkness until the experiment was terminated.

At 63 days of age, the quail were weighed and killed by cervical dislocation. Each individual was classified as either mature or immature; this classification was based on cloacal gland secretions in males and on ovary-oviduct development in females. Shank length, breast weight, abdominal fat weight, and ovary-oviduct or testes weights were determined. Defeathered carcasses were then subjected to solvent extraction for carcass lipids (Folch *et al.*, 1957).

The data were analyzed, within sexes, by analysis of variance. When significance occurred, treatment means were separated by the multiple range test of Duncan (1955).

Results and Discussion

Quail subjected to the IP treatment failed to reach sexual maturity by 63 days of age. This lack of maturation was evidenced by reduced testes weights (Table 1) and ovary-oviduct weights (Table 2), as well as by an absence of cloacal gland secretions in males and oviposition in females. The failure of the quail subjected to this treatment to mature was associated with a significant reduction in body weight of females (Table 2) but not males (Table 1). Regardless of sex, shank length did not differ between those subjected to the IP treatment and the controls (Tables 1 and 2). Breast weight, a measurement of lean body mass (Plotkin, 1982), tended to be higher for the quail receiving the IP treatment than for the controls. When expressed as a percentage of body weight, these differences were significant for both sexes (Tables 1 and 2). On a total weight basis, the differences were significant only for females (Table 2). In females, abdominal fat weights were less for the IP treatment than for the controls (Table 2), but abdominal fat weights of males did not differ between these treatment groups (Table 1). No significant differences in total carcass fat between the quail subjected to the IP treatment and the controls were obtained for either sex (Tables 1 and 2).

Minimum chronological age, body weight, linear body

growth, lean body weight, and body lipid stores have all been implicated as critical traits for the onset of sexual maturity. Because all quail were subjected to a daily photoperiod of four hours until 42 days of age, chronological age requirements should have been satisfied for all groups. The failure of the females exposed to the IP treatment to reach sexual maturity could have been associated with critical body weight requirements *per se* (Frisch and Revelle, 1970; Brody *et al*., 1980; Summers and Leeson, 1983; Dunnington *et al*., 1983), but this was clearly not true for males. Lean body mass requirements (Wilen and Naftolin, 1976; 1977; 1978; Zelenka *et al*., 1984) were apparently met or exceeded by the quail receiving the IP treatment. The results were, however, suggestive of a critical lipostatic requirement (Widdowson and McCance, 1960; Frisch *et al*., 1973; Frisch, 1980; 1982; Zelenka *et al*., 1984). Both males and females exposed to the IP treatment had significantly less abdominal fat than the controls. Total body fat also tended to be reduced by the IP treatment, but the differences were not significant for either sex.

An inverse relationship between gonadal activity and triiodothyronine (T_3) concentrations has been suggested (Oishi and Konishi, 1978). In the present experiment, PTU was administered in an effort to stimulate gonadal

development by inhibiting the conversion of thyroxine (T_4) to T_3. This effect did not occur. The males treated with .001% PTU actually had smaller testes than the controls (Table 1), while ovary-oviduct weights of females were significantly reduced by 0.1% PTU (Table 2). In no instance was there evidence that PTU stimulated gonadal development. Neither were shank length, breast weight nor carcass fat significantly altered by PTU in either sex.

Neither GH nor AGH significantly influenced body weight (Tables 1 and 2). This failure of GH to stimulate growth was consistent with several reports that circulating GH is negatively associated with growth rate in chickens (Harvey et al., 1977; Jones et al., 1980; Scanes et al., 1980; Burke and Marks, 1982). Proudman and Wentworth (1978), however, demonstrated parallel dose-response curves between turkey GH and pituitary extracts, as well as between sera samples from turkeys, chickens, and quail. AGH, nevertheless, may have inhibited gonadal development. Ovary-oviduct weights of the females were smaller for the AGH treatment than for the controls (Table 2). The slight reduction in testes weight induced by AGH in males was not significant (Table 1). GH and AGH had little effect on body composition.

Administration of E to females resulted in a significant reduction in body weight and total carcass fat (Table 2), but had little effect on sexual maturity. In

males, the administration of T resulted in a significant reduction in testes weight (Table 1). This inhibition of gonadal development, however, did not appear to be associated with differences in body weight or body composition.

The principal objective of this experiment was to determine if photoperiodic and hormonal effects on sexual maturation were associated with differences in body weight and/or body composition. Unfortunately, with the exception of the IP treatment, none of the treatments used completely prevented sexual maturity. In males, T significantly reduced testes weights in comparison to the controls (Table 1). These results were consistent with those of Desjardins and Turek (1977) who reported that about an 18-fold increase in plasma T above normal levels through the administration of exogenous T resulted in a reduction of about 60% in testes weights and in the relative number of germ cells. Nevertheless, gonadal development in the T-treated quail was appreciably greater than for the IP treatment. The males treated with T also exhibited cloacal gland secretions by 63 days of age. Therefore, these quail may have been sexually mature although the cloacal gland secretions induced by exogenous T were not necessarily indicative of testicular maturation. In females, both the AGH and the 0.1% PTU treatments resulted in a significant reduction in ovary-

oviduct weights in comparison to the controls, but the weights of these reproductive organs were substantially larger than those obtained with the IP treatment (Table 2).

To further examine the relationship between body composition and sexual maturity, individual females within each treatment were separated into mature and immature groups for further analyses. Because of small sample sizes, the PTU treatments were omitted. Similar procedures were not feasible for the males because only a few individuals were immature at 63 days of age.

Regardless of treatment, no significant differences in body weight between mature and immature females were observed (Table 3). There was no indication, therefore, that the onset of sexual maturity was associated with the achievement of a minimal threshold body weight. There was also no indication that the lack of sexual maturity by some of the quail in this experiment was associated with minimal body composition requirements. Shank length differences between mature and immature females were significant only within the GH treatment, and the immature quail had longer shanks than the mature ones. When treated with E, the immature quail had larger breasts regardless of whether expressed on a total weight basis or as a percentage of body weight. Percentage breast weight was also higher for the immature quail than for the mature ones within the GH

treatment. Similar results were obtained for body fat. In every instance in which significance was obtained, the immature quail possessed more abdominal fat and/or more total carcass fat than did the mature females.

It is clear that sexual maturity is more closely correlated with body weight than with chronological age in numerous species (Mandl and Zuckerman, 1952; Crichton et al., 1959; Joubert, 1963; Kennedy and Mitra, 1963; Dickerson et al., 1964; Wiltbank et al., 1966; Wilen and Naftolin, 1976; Sohn and Crows, 1977; Brody et al., 1980; Frisch et al., 1980). It also appears that minimum chronological age and minimum body weight requirements must both be exceeded for sexual maturity to occur (Leeson and Summers, 1983; Dunnington et al., 1983). The achievement of age and weight requirements, however, does not necessarily result in sexual maturation (Grindeland et al., 1974; Bakke et al., 1975; Crawford and Osler, 1975; Frisch et al., 1975; Ronnekleiv et al., 1978; Kirtley and Maher, 1979; Glass and Swerdloff, 1980; Ramaley, 1981; Wilen et al., 1981; Zelenka et al., 1984), and it has been suggested that minimal body composition requirements must be achieved for sexual maturity to occur. In the present experiment, the apparent achievement of minimal body weight, lean body mass, and body fat requirements did not necessarily result in the onset of sexual maturity.

Summary

Body composition and sexual maturation were examined in response to photoperiodic and hormonal manipulations. Attempts were made to modify body composition, and thereby the age at sexual maturity, by feeding diets containing 0.1, 0.01, and 0.001% PTU (4-phenyl-2 thiouracil) from 14 to 63 days of age, by subjecting birds from hatch to 63 days of age to a daily photoperiod (IP) of four hours, by the subcutaneous implantation of E (10 mg of 17β-estradiol) to females or T (10 mg of Androsten-17β-01-3 one) to males, and by the intramuscular injection of GH (0.1 ml of turkey growth hormone/bird/day) or AGH (0.1 ml of anti-sera to turkey growth hormone/bird/day) for 12 consecutive days beginning at 14 days of age. All surviving quail were sacrificed at 63 days of age. Traits measured included body weight, breast weight, abdominal fat weight, shank length, total carcass fat, and ovary-oviduct or testes weight.

Quail reared under the IP treatment failed to reach sexual maturity by 63 days of age. This failure to mature was not clearly attributable to a failure to achieve threshold body weight requirements; a reduction in body weight in response to the IP treatment was observed for females but not for males. Lean body mass or linear body growth requirements were apparently met or exceeded by the quail receiving the IP treatment; regardless of sex, shank

lengths did not differ between the quail receiving the IP treatment and the controls, and breast weights tended to be higher for the IP treatment than for the controls. The results, however, did not preclude a critical lipostatic requirement; both males and females exposed to the IP treatment had significantly less abdominal fat than the controls, although total carcass fat did not differ significantly.

Attempts to alter body composition and sexual maturity hormonally met with only limited success. Neither gonadal development, shank length, breast weight nor carcass fat were consistently influenced by PTU. GH and AGH had little effect on body weight or body composition, although AGH may have inhibited gonadal development. The administration of E to females reduced body weight and total carcass fat but had little effect on sexual maturity. The administration of T to males, in contrast, reduced testes weights but this inhibition of gonadal development did not appear to be associated with differences in body weight or body composition.

Finally, individual females within each treatment group were further examined by separating them into mature and immature groups. Similar procedures were not feasible for males because only a few males were immature at 63 days of age. When examined in this manner, there was no indication

that a lack of sexual maturity corresponded with a failure to meet minimal body weight or body composition requirements.

Table 1. Means for body weight, testis weight, shank length, abdominal fat weight, breast weight, and carcass fat for male Japanese quail treated with an inhibitory photoperiod (IP), phenylthiouracil (PTU), growth hormone (GH), anti-growth hormone (AGH), and testosterone (T)

Treatment	No.	Body wt.	Testis wt.	Shank length	Abdominal fat wt.		Breast wt.		Carcass fat
		g	g	cm	g	% body wt.	g	% body wt.	% body wt.
Control	14	100 ± 2a	2.8 ± 0.2d	2.7 ± 0.1a	6.9 ± 1.7a	6.9 ± 1.6a	23.6 ± 0.6a	23.6 ± 0.3b	27 ± 2a
IP	12	104 ± 2a	0.3 ± 0.1a	2.6 ± 0.1a	4.2 ± 0.8a	4.0 ± 0.8a	28.0 ± 0.8b	26.9 ± 0.6a	23 ± 1a
PTU									
(.001%)	6	97 ± 2a	1.8 ± 0.3bc	2.6 ± 0.1a	5.2 ± 1.9a	5.4 ± 1.2a	22.7 ± 0.9a	23.4 ± 1.5b	28 ± 3a
(.01%)	8	99 ± 4a	2.6 ± 0.3cd	2.7 ± 0.1a	8.3 ± 2.8a	8.4 ± 1.1a	24.3 ± 1.2a	24.6 ± 1.8b	26 ± 3a
(.1%)	5	94 ± 6a	2.3 ± 0.4d	2.6 ± 0.1a	2.9 ± 1.4a	3.0 ± 1.5a	22.8 ± 1.9a	24.3 ± 2.5b	24 ± 4a
GH	5	103 ± 4a	3.0 ± 0.4d	2.4 ± 0.1a	7.4 ± 3.3a	7.2 ± 2.8a	25.5 ± 0.9ab	24.8 ± 0.8b	29 ± 4a
AGH	8	96 ± 4a	2.3 ± 0.3cd	2.6 ± 0.1a	4.4 ± 1.6a	4.6 ± 1.3a	22.3 ± 1.2a	23.2 ± 0.7b	23 ± 2a
T	12	103 ± 3a	1.2 ± 0.2b	2.5 ± 0.1a	8.4 ± 2.1a	8.2 ± 1.9a	25.3 ± 0.7ab	24.6 ± 0.4b	29 ± 1a

[a,b,c,d] Means within a column having the same letter were not significantly different ($P > .05$).

Table 2. Means for body weight, ovary-oviduct weight, shank length, abdominal fat weight, breast weight, and carcass fat for female Japanese quail treated with an inhibitory photoperiod (IP), phenylthiouracil (PTU), growth hormone (GH), anti-growth hormone (AGH), and estrogen (E)

Treat-ment	No.	Body wt.	Ovary-Oviduct wt.	Shank length	Abdominal fat wt.		Breast wt.		Carcass fat
		g	g	cm	g	% body wt.	g	% body wt.	% body wt.
Control	12	129 ± 3cd	7.4 ± 1.4cd	3.4 ± .02a	2.7 ± 0.7bc	2.1 ± 0.5ab	29.2 ± 1.0ab	22.6 ± 0.6b	29 ± 2bc
IP	8	114 ± 4a	0.1 ± 0.0a	3.5 ± 0.0ab	1.0 ± 0.3a	0.9 ± 0.2a	29.9 ± 1.2b	26.2 ± 1.0a	23 ± 4ab
PTU									
(.001%)	3	122 ± 3abc	8.3 ± 0.6d	3.4 ± 0.1a	1.4 ± 0.2ab	1.2 ± 0.2ab	26.4 ± 0.4a	21.6 ± 0.5b	32 ± 2c
(.01%)	5	139 ± 5cd	6.4 ± 2.1bcd	3.6 ± 0.1b	3.0 ± 1.6d	2.2 ± 0.8ab	29.6 ± 0.8ab	21.3 ± 0.6b	31 ± 6c
(.1%)	7	130 ± 4cd	4.6 ± 1.4b	3.5 ± 0.0ab	2.6 ± 0.8bc	2.0 ± 0.6ab	28.1 ± 0.8ab	21.6 ± 0.7b	26 ± 1abc
GH	8	126 ± 4cd	5.8 ± 2.2bc	3.4 ± 0.0a	2.4 ± 0.6abc	1.9 ± 0.4ab	28.9 ± 0.6ab	22.9 ± 0.5b	32 ± 3c
AGH	6	122 ± 5abc	4.8 ± 1.8b	3.4 ± 0.1a	1.4 ± 0.3ab	1.2 ± 0.2ab	28.6 ± 0.6ab	23.4 ± 1.1b	30 ± 2c
E	11	117 ± 2ab	5.3 ± 1.3bc	3.4 ± 0.0a	1.3 ± 0.3ab	1.1 ± 0.2ab	26.7 ± 0.8ab	22.8 ± 0.6b	22 ± 2a

[a,b,c,d]Means within a column having the same letter were not significantly different ($P < .05$).

Table 3. Means for body weight, ovary-oviduct weight, shank length, abdominal fat weight, breast weight, and carcass fat for mature and immature female Japanese quail treated with IP, GH, AGH and E

Trait	Treatment				
	Control	IP	GH	AGH	T
No. of Individuals					
Mature	8	0	3	3	6
Immature	4	8	5	3	5
Body weight (g)					
Mature	129	-	134	128	119
Immature	130	114	122	117	116
Ovary-oviduct wt. (g)					
Mature	10.3B	-	13.2B	8.4B	8.4B
Immature	1.7A	0.1	1.3A	1.2A	1.5A
Shank length (cm)					
Mature	3.4	-	3.3A	3.4	3.5
Immature	3.4	3.4	3.4B	3.4	3.4
Abdominal fat wt. (g)					
Mature	1.4A	-	1.0A	0.8A	0.9
Immature	5.3B	1.0	3.3B	1.9B	1.7
Abdominal fat wt. (% body wt.)					
Mature	1.1A	-	0.8A	0.6A	0.8
Immature	4.1B	0.9	2.7B	1.7B	1.5
Breast wt. (g)					
Mature	26	-	29	29	25A
Immature	30	30	29	28	29B
Breast wt. (% body wt.)					
Mature	20	-	21A	23	21A
Immature	23	26	24B	24	25B
Carcass fat (% body wt.)					
Mature	25A	-	24A	28	19
Immature	36B	23	36B	33	26

A,B Different letters denote a significant ($P \leq .05$) maturity effect. An absence of letters indicates a lack of significance. The number of individuals were not subjected to statistical analyses.

EXPERIMENT II

ADIPOCYTE HYPERPLASIA IN RELATION TO SEXUAL MATURITY IN JAPANESE QUAIL

Introduction

When fed *ad libitum*, hyperplastic growth of adipose tissue appears to cease by 12 to 15 weeks of age in both White Leghorn and broiler-type chickens (Pfaff and Austic, 1976; Hood, 1982). Although this age can be altered by nutrient restriction (Pfaff and Austic, 1976; March and Hansen, 1977; Ballam and March, 1978), there is no evidence for hyperplastic modification of adipose tissue in adult fowl (March *et al*., 1982). Consequently, sexual maturity of chickens apparently occurs during periods of hypertrophic fat deposition. It is also conceivable that adipocyte size is associated mechanistically with sexual maturation. Adipose tissue serves as an extragonadal source of estrogens in mammals (Nimrod and Ryan, 1975; Frisch, 1980), and the rate at which the estrogens are synthesized and released by adipose tissue may be related to adipocyte size.

The principal objective of the present study was to determine whether a cessation of adipocyte hyperplasia coincided with the onset of sexual maturity in Japanese quail. Total DNA concentrations in the abdominal (retroperitoneal) fat depot were determined on a chronological age basis. Because a substantial proportion of the DNA found in fat depots is contained in non-adipocyte fractions (Cleary *et al*., 1977), collagenase digestion (Rodbell, 1964) was used to separate abdominal fat into

lipid and non-lipid fractions. The results obtained were then compared to similar values collected at sexual maturity. Because of evidence that adult animals are sometimes capable of hyperplastic lipid deposition (Lemonnier, 1972; Faust *et al.*, 1978; Bertrand *et al.*, 1978; 1984), the study was also extended to ages well beyond the onset of sexual maturity.

Materials and Methods

Japanese quail were hatched and caged in a modified chick battery brooder under a continuous photoperiod. Feed and water were provided *ad libitum*. At 21 days of age, the quail were sexed, wingbanded and, with the exception of 20 females assigned to individual cages, caged in groups of three birds of the same sex. Beginning at 21 days of age, a daily photoperiod of 14 hr was provided.

At intervals of seven days, beginning at 28 days of age and continuing through 91 days of age, ten male and ten female quail were weighed and sacrificed for the determination of DNA concentrations in the abdominal fat pad. Prior to sacrifice, plasma was collected via cardiac puncture from each female for the subsequent determination of plasma lipid (Zollner and Kirsch, 1962) and calcium (atomic absorption spectrophotometry). When 10 of the 20 individually caged females had entered lay (52 days of age), they were killed to estimate the DNA content of adipose tissue at sexual maturity. An additional ten females were sacrificed at 240 days of age. Plasma calcium and lipid were not determined on the latter quail.

Immediately after sacrifice, the abdominal fat pad was removed, weighed, rinsed with saline, blotted and subjected to collagenase digestion. The method used was the modification (Free, 1972) of the method originally described

by Rodbell (1964), except that adipose tissue was not minced prior to digestion, because preliminary studies indicated that the separation of nonadipose tissue (stroma) was more complete when the intact depot was digested. Incubation was accomplished in a shaking water bath (37C) with Krebs-Ringer phosphate-albumin buffer and 3-5 mg collagenase (depending on the activity of the specific lot) per g fat. Collagenase was obtained from Sigma Chemical Company (St. Louis, MO).

After 60 minutes of digestion, stromal material was removed and the remaining suspension (lipocyte fraction) passed through a nylon mesh with 250 µm openings (Fisher Scientific Company, Raleigh, NC). The lipocytes were then washed three times by suspending them in fresh buffer, centrifuging for one minute at 400 g in a tabletop centrifuge and aspirating the infranatant buffer to the stromal fraction of the sample. The stromal fraction with buffer was then centrifuged, the buffer decanted, and the precipitate washed one final time. This procedure yielded a stromal fraction considered to be essentially devoid of lipid droplets and a lipocyte fraction considered to be essentially free of nonlipid contaminants. The term lipocyte was used to designate all adipose cells in the digested fraction regardless of cell size.

The DNA content of both the lipocyte and stromal fractions was determined colorimetrically by the procedure

of Curtis-Prior et al. (1975). The procedure was modified to allow analysis of the entire volume of each lipocyte sample instead of the recommended one ml of fat cell suspension. The lipocyte fraction was treated with 35 to 40 ml of a cold ethanol-diethyl ether (3:1) extraction mixture containing 10% (w/v) trichloroacetic acid (TCA). After centrifugation at 4000 g for 10 minutes, the lipid and acid soluble nucleotide fraction was discarded. The remaining "nucleotide pellet" was then washed with 35-40 ml of an ethanol-diethyl ether (3:1) mixture without TCA, and the stromal nucleoproteins were washed with 10 ml of a diethyl ether-ethanol (3:1) solution.

Isolation of DNA from both the lipocyte and stromal pools was accomplished by adding three ml of a 5% aqueous solution of TCA and incubating the mixture at 90C in a shaking water bath for 20 min. The nucleoprotein pellets were scattered by frequent agitation with a glass rod during incubation. The samples were then cooled and centrifuged for 20 min at 4000 g.

Samples (one ml) of the lipocyte and stromal DNA solutions were diluted with one ml of a 5% aqueous TCA solution, and then treated with 40 µl of freshly prepared p-nitrophenylhydrazine (20 mg dissolved in 2 ml ethanol) color reagent. This mixture was incubated by refluxing in a boiling water bath for 20 min. DNA standard solutions,

prepared by adding 50 ml of a 5% (m/v) TCA solution to 25 mg of salmon-sperm DNA (Sigma Chemical Company, St. Louis, MO) were treated in an identical manner. Excesses of p-nitrophenylhydrazine were removed by washing the samples and standard solutions three times with seven ml of n-butyl acetate. Each washing was accompanied by vigorous shaking followed by brief low-speed centrifugation; the upper phase was then aspirated and discarded.

One-ml samples of the washed lower phase was transferred into one-ml centrifuge tubes and spun briefly at low speeds. Any visible n-butyl acetate and the uppermost layer of the aqueous solution were then aspirated and discarded. Color was developed by transferring 500 µl of the remaining solution to a microcuvette and adding 200 µl of 4N NaOH. These solutions were mixed by repeated inversion, and exactly three minutes after the addition of NaOH, absorbance at 560 nm was determined using a Beckman Model 35 spectrophotometer equipped with a Model 39 printer. The data were expressed as total DNA in the stromal and lipocyte fractions of the abdominal fat pad for statistical analyses.

The data were analyzed, within sex, by analyses of variance with age considered a fixed effect. The statistical model was:

$$Y_{ij} = \mu + A_i + e_{ij}$$

where i = 28, 35, 42 . . . 240 days of age and j = 1, 2 . . . 10 individuals. When significance was obtained, the means were separated by Duncan's (1955) multiple range test.

Results and Discussion

The development of the abdominal fat depot as the male quail aged did not parallel changes in body weight (Table 4). No significant differences in body weight occurred beyond 49 days of age, while a substantial increase in abdominal fat was observed at older ages. Significant increases in the DNA content of both the stromal and lipocyte fractions accompanied the increased accumulation of abdominal fat at these older ages.

The relationships among DNA concentrations, fat deposition, and body weight are graphically depicted in Figure 1. Lipocyte DNA increased from 28 to 35 days of age, remained fairly constant from 35 to 70 days of age, then increased again between 70 and 77 days of age. Because 70 days is well beyond the expected age at sexual maturity of *ad libitum*-fed quail (Zelenka *et al*., 1984), the data suggested that new adipocytes were being formed for lipid storage after sexual maturation.

Although stromal DNA concentrations were fairly constant (Figure 1), some increases appeared to occur at older ages. It was not known if this increase represented a synthesis and turnover of new fat cells or an increase in supporting connective tissue; stroma weights exhibited some variability but were only moderately correlated (r = .39) with abdominal fat weights. The results, however, do

emphasize the substantial contribution of non-adipose cells to the total DNA content of a fat sample (Rodbell, 1964; Cleary et al., 1977). At 28 days of age, stromal DNA concentrations were equal to the DNA content of adipose cells. At later ages, the DNA content of stromal fraction was 20-50% of that contained in the lipocyte fraction.

The results obtained with females were similar to those for the males in that a significant increase in the DNA content of the lipocyte fraction of abdominal fat occurred at ages well beyond sexual maturity (Table 5). For females, the median age at the onset of lay was estimated to be 52 days, and a significant increase in lipocyte DNA occurred between 70 and 77 days of age.

Growth of the female quail in this experiment appeared to be generally typical of this species (Figure 2), although an inexplicably high value was obtained at 84 days of age. Similar to the results reported by Zelenka et al. (1984), there was no evidence of a "growth spurt" accompanying the onset of sexual maturity similar to that often described in mammals (Frisch and Revelle, 1970; Frisch et al., 1973). It appeared, however, that the neuroendocrine stimulation of sexual maturity was initiated somewhere between 35 and 42 days of age. Both plasma calcium (Figure 3) and plasma lipids (Figure 4) had increased dramatically by 42 days of age. These values tended to remain elevated to 91 days of

age.

Developmental changes in abdominal fat of the females are shown in Figure 5. Abdominal fat appeared to accumulate rapidly up to an age near sexual maturity. This accumulation was probably both hypertrophic and hyperplastic in nature (Pfaff and Austic, 1976). Abdominal fat weights at sexual maturity (52 days) and at 56 days of age, however, were significantly smaller than those at 49 days of age (Table 5). This reduction in abdominal fat was not associated with a reduction in lipocyte DNA. The onset of lay, therefore, appeared to be associated with a hypotrophic reduction in the amount of abdominal fat. The results obtained in Experiment I were consistent with this conclusion; at the same chronological age, females that had entered lay tended to have less body fat than nonlaying females (Table 3). This apparent decrease in abdominal fat associated with the onset of lay may represent a mobilization of stored lipid for deposition in egg yolk.

Subsequent to sexual maturity, abdominal fat tended to remain constant to 77 days of age, after which a significant increase occurred (Figure 5). The quail sampled at 240 days of age had substantially less abdominal fat than those at 84 or 91 days of age. These changes were not reflected entirely by changes in lipocyte DNA concentrations (Figure 6). As mentioned previously, substantial mitotic activity

appeared to occur between 70 and 77 days of age. An associated increase in abdominal fat was not observed until 84 days of age (Figure 5) suggesting that an interval of time was required for the newly recruited fat cells to accumulate sufficient lipid to result in a net increase in depot fat. Furthermore, after this period of mitotic activity, lipocyte DNA appeared to remain constant (Figure 6), although a significant reduction in abdominal fat was observed at 240 days of age (Figure 5). This observation was consistent with reports that increases in differentiated adipocytes persist after stored lipid is mobilized (Hausberger, 1965; Haugebak et al., 1974, March et al., 1982).

It may be of importance to note that the results of this experiment up to 70 days of age would have been consistent with evidence obtained with rats (Hirsch and Gallian, 1968; Harris, 1970), pigs (Hood and Allen, 1977), sheep (Hood and Thornton, 1979), humans (Hirsch and Knittle, 1970), and chickens (Pfaff and Austic, 1976; March and Hansen, 1977; Ballam and March, 1979; Hood, 1982; March et al., 1982) that adipocyte cell number is fixed in adult animals. In females, lipocyte DNA tended to be constant from 42 to 70 days of age (Figure 6), while in males lipocyte DNA was generally constant from 35 to 70 days of age (Figure 1). In both sexes, however, an increase in

abdominal fat accompanied by an increase in the DNA content of the lipocyte fraction of abdominal fat was observed at ages well beyond the age at sexual maturity. It is not known if these increases are typical of Japanese quail or whether an environmental influence was responsible. It is conceivable that the increased body fat observed was due to normal fattening since Japanese quail is a migratory species (Yokoyama, 1977). Nonetheless, the data strongly support previous evidence that adult animals are capable of hyperplastic lipid deposition (Lemonnier, 1972; Faust *et al.*, 1978; Bertrand *et al.*, 1978; 1984). In addition, the onset of sexual maturity did not appear to be associated with a cessation of adipocyte hyperplasia or an increase in adipocyte hypertrophy.

Summary

Adipocyte hyperplasia in relation to sexual maturation was examined in Japanese quail. The DNA content of the abdominal fat depot was used as a measurement of the hyperplastic development of adipose tissue. Data collected on a chronological age basis were compared to similar data collected at sexual maturity to determine whether sexual maturation was associated with a cessation of adipocyte hyperplasia.

All abdominal fat samples were initially subjected to collagenase digestion to separate non-lipid (stroma) from lipocyte fractions; the term lipocyte was used to designate all adipose cells harvested regardless of size. The DNA content in both the lipocyte and stromal fractions was then determined colorimetrically using salmon-sperm DNA as the reference standard.

At intervals of seven days, beginning at 28 days of age and continuing to 91 days of age, ten male and ten female quail were weighed and sacrificed for the determination of DNA concentration in the abdominal fat depot. Prior to sacrifice, plasma samples were collected from each female for the subsequent determination of plasma calcium and total plasma lipids. Ten females were also slaughtered on the day of first oviposition to estimate abdominal fat DNA at sexual maturity. An additional ten females were examined when 240

days of age.

In males, the development of the abdominal fat depot with age did not parallel changes in body weight. No significant differences in body weight were observed subsequent to 49 days of age, while a substantial increase in abdominal fat was observed at older ages. The DNA content of the lipocyte fraction increased from 28 to 35 days of age, remained rather constant from 35 to 70 days of age, then increased again between 70 and 77 days of age. Thus, the increase in abdominal fat observed at the older ages appeared to be associated with the recruitment of new adipocytes for lipid storage.

Based on dramatic rises in plasma lipids and plasma calcium, it appeared that the neuroendocrine stimulation of sexual maturity in females commenced between 35 and 42 days of age. The median age at the onset of lay in turn was 52 days. Up to an age near the onset of lay, abdominal fat accumulated rapidly and was associated with increased DNA concentrations. The onset of lay, however, was accompanied by a a reduction in abdominal fat weights, probably representing a mobilization of stored lipid for deposition in egg yolk. This reduction in abdominal fat was apparently hypotrophic in nature; a corresponding decrease in abdominal fat DNA did not occur. Subsequent to sexual maturity, however, significant increases in abdominal fat weights were

associated with increases in the DNA content of abdominal fat. It was concluded that the onset of sexual maturity was not associated with a permanent cessation of adipocyte hyperplasia and an increase in adipocyte hypertrophy.

Table 4. Accumulation of DNA in stromal and lipocyte fractions of the abdominal fat pad of male Japanese quail from 28 to 91 days of age

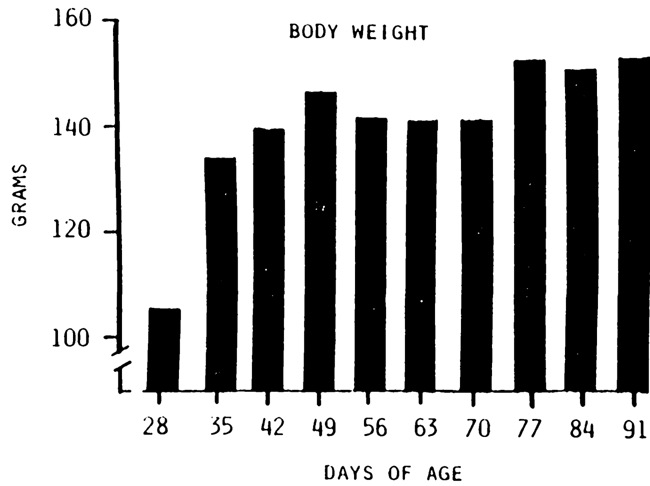
Age	Body wt.	Abdominal fat wt.	Stroma wt.	Total DNA	
				Stroma	Lipocyte
(days)	-g-	-g-	-g-	-µg-	
28	115±3a*	0.19±.04a	.10±.01abc	33± 5a	31± 5a
35	134±3b	0.74±.10ab	.08±.01a	49± 6b	95± 9b
42	140±2bc	1.34±.15b	.11±.01bcd	52± 6b	100± 8b
49	147±2bcd	1.70±.22b	.08±.01ab	42± 4b	100±10b
56	142±3bcd	1.44±.30b	.14±.01d	51± 2b	106± 9b
63	142±2bcd	1.62±.17b	.08±.01ab	46± 6b	117± 8b
70	142±5bcd	1.45±.20b	.07±.01a	32± 3b	122± 9b
77	153±3d	3.67±.45cd	.12±.01cd	83± 5c	253±29c
84	151±3d	3.14±.41c	.12±.01cd	60± 6b	260±17c
91	153±3d	4.41±.54d	.13±.01cd	100± 7c	262±21c

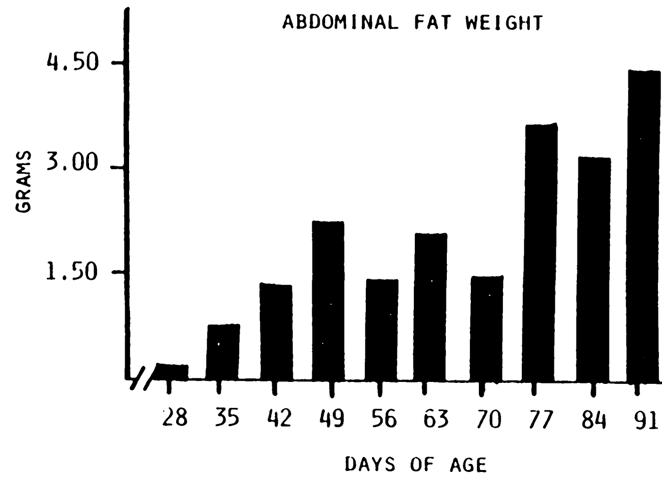
a,b,c,d Means for each trait having the same letter were not significantly different ($P \geq .05$).
*Means ± standard errors.

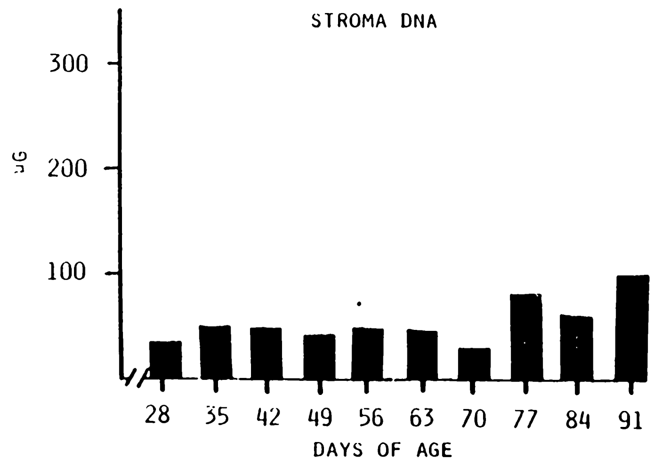
Table 5. Accumulation of DNA in stromal and lipocyte fractions of the abdominal fat pad of female Japanese quail from 28 to 240 days of age

Age	Body wt.	Plasma Ca	Plasma Lipid	Abdominal fat wt.	Stroma wt.	Total DNA	
						Stroma	Lipocyte
days	g	-mg-		-g-		-µg-	
28	115±4a*	39±1a	421± 19a	.27±.05a	.11±.01ab	49± 7abc	46± 6a
35	136±4ab	35±2a	609± 43ab	.55±.07ab	.09±.01ab	49± 4abc	72± 5ab
42	146±2bc	54±4bcd	2056±294ef	1.39±.23bc	.10±.01ab	50± 6abc	104±12bc
49	149±4bc	52±1bc	1541±171cde	1.98±.35cd	.08±.02a	45± 8ab	110± 7bc
52**	156±6bc	55±2bcd	1851±342def	1.06±.11ab	.13±.01b	70± 3cd	115± 9bc
56	155±3bc	59±2d	1912±280def	1.09±.13ab	.11±.01ab	58± 5bcd	111±11bc
63	165±5cd	57±2bcd	2382±198f	1.23±.21bc	.18±.02c	42± 7ab	123±14c
70	167±4cd	54±2bcd	1094± 81bc	1.30±.49bc	.11±.01ab	30± 5a	124±13c
77	170±3cd	57±2bcd	1373±112cd	1.07±.10ab	.11±.01ab	61± 8bcd	220±23a
84	211±3d	58±2d	1479±147cde	2.74±.56d	.10±.01ab	74± 8d	221±23d
91	170±4cd	58±2d	2104±235def	2.07±.33cd	.13±.01b	110±10e	228±16d
240	184±2d			0.91±.14ab	.09±.01a	100± 4e	242±18d

a,b,c,d,e,f Means for each trait having the same letter were not significantly different (P ≥ .05).
*Means and standard errors.
**Mean of ten females killed on day of first egg.







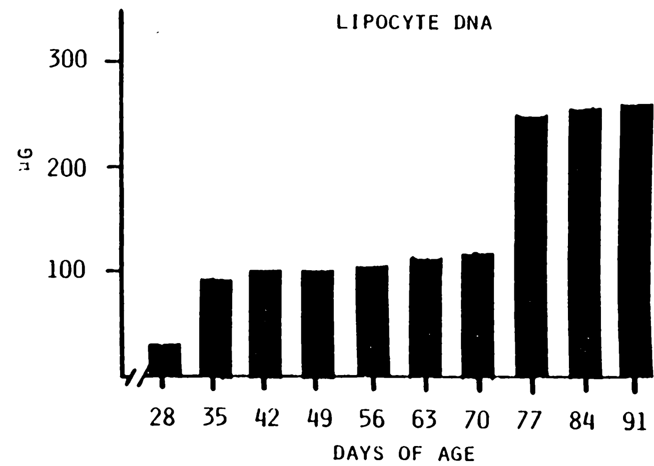


FIGURE 1. CHANGES IN BODY WEIGHT, ABDOMINAL FAT WEIGHT, AND DNA CONTENT OF THE LIPOCYTE FRACTIONS OF ABDOMINAL FAT OF MALE JAPANESE QUAIL FROM 28 TO 91 DAYS OF AGE.

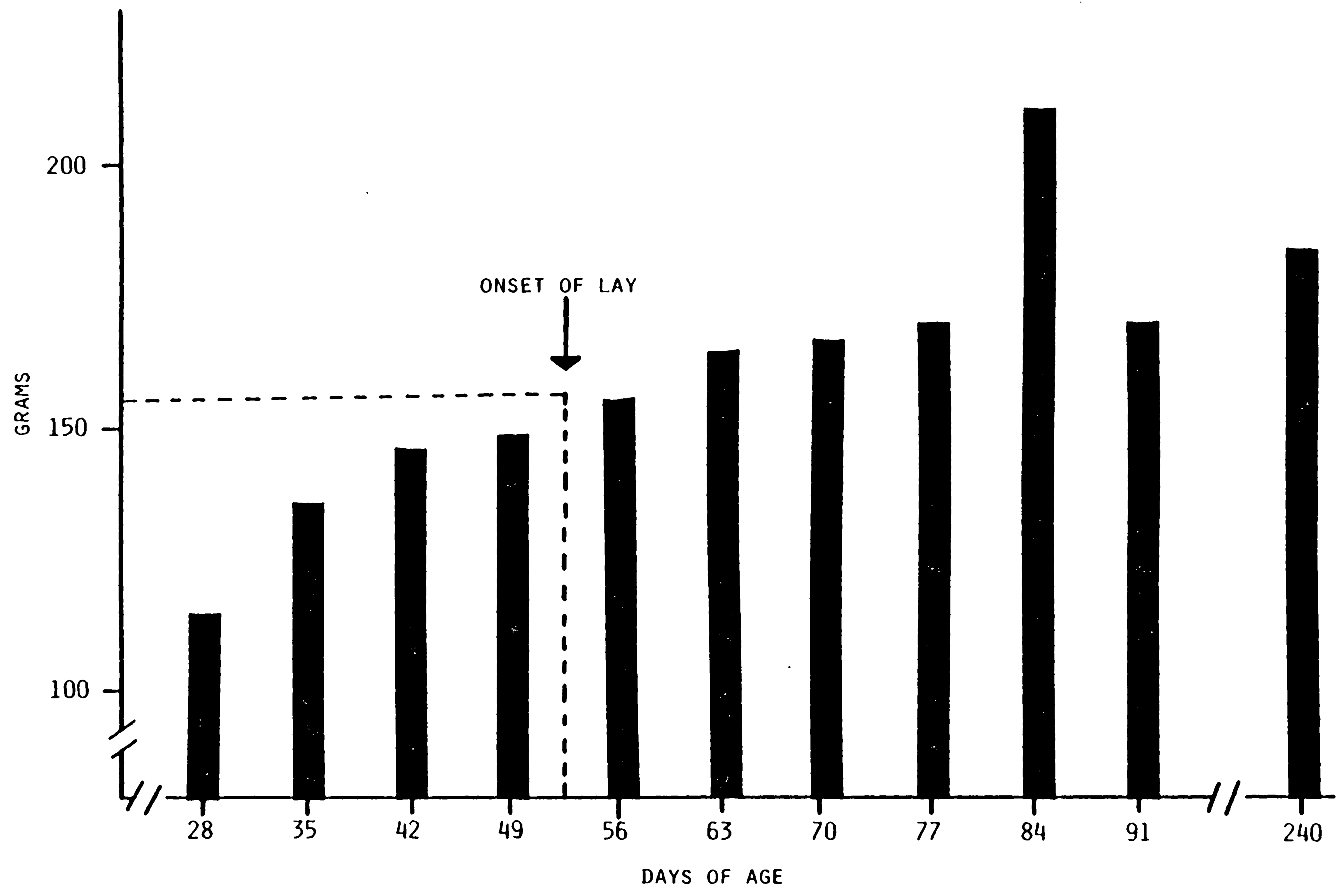


FIGURE 2. CHANGES IN BODY WEIGHT OF FEMALE JAPANESE QUAIL FROM 28 TO 240 DAYS OF AGE IN RELATION TO SEXUAL MATURITY.

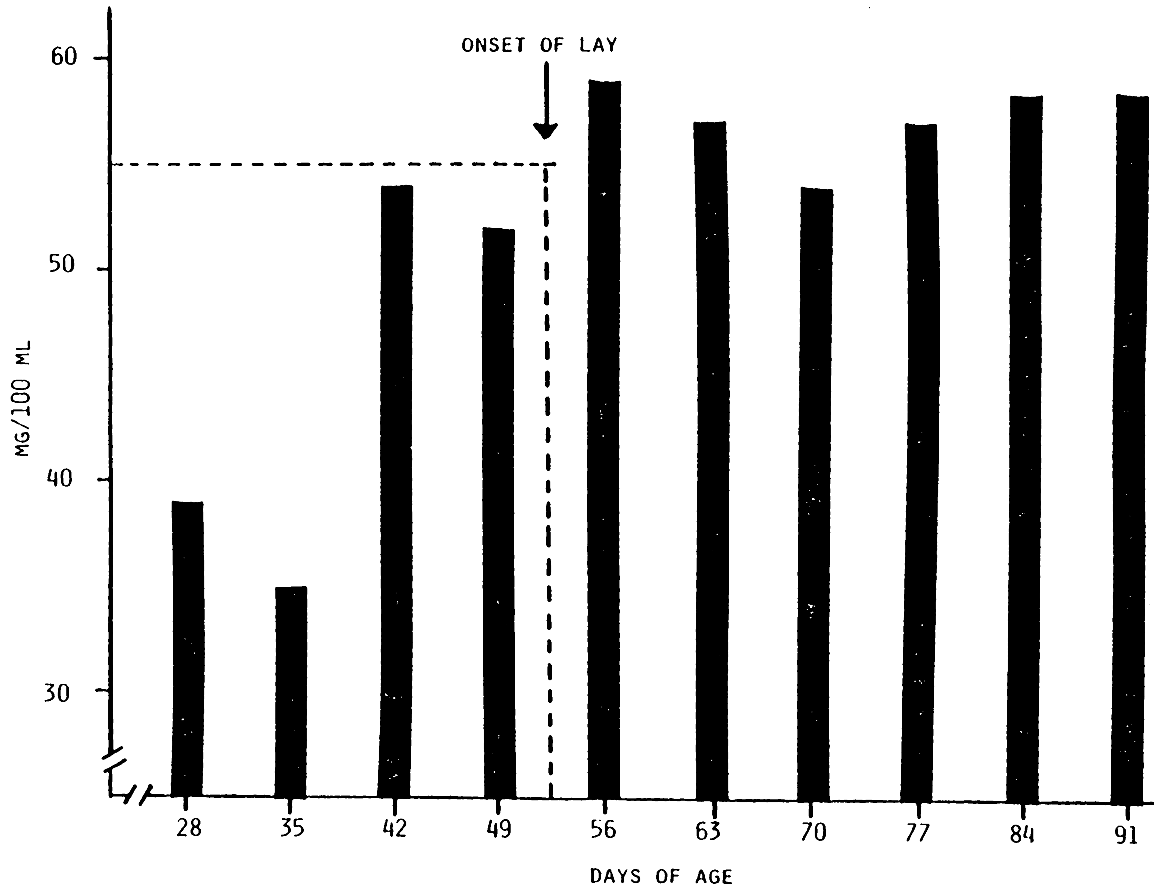


FIGURE 3. CHANGES IN PLASMA CALCIUM OF FEMALE JAPANESE QUAIL FROM 28 TO 91 DAYS OF AGE IN RELATION TO SEXUAL MATURITY.

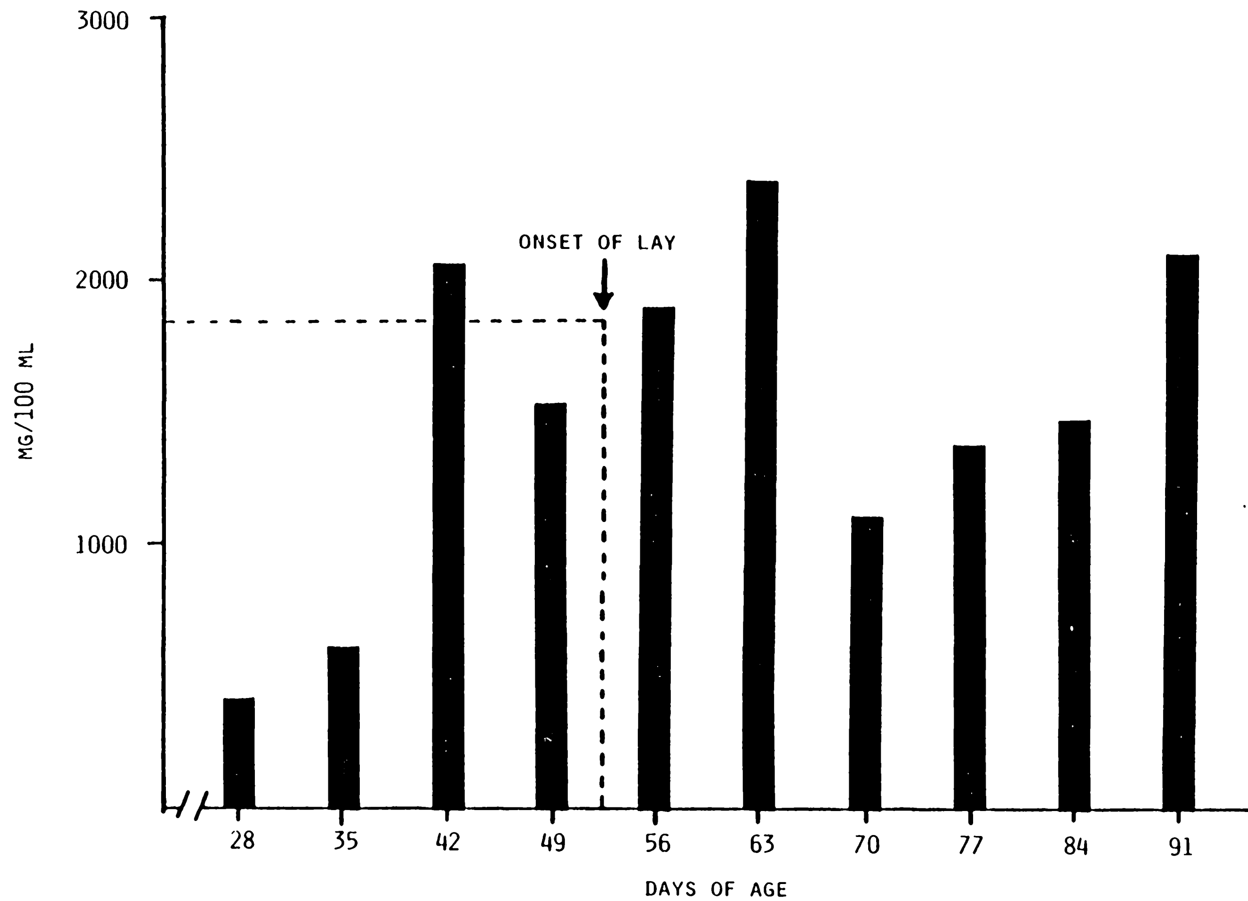


FIGURE 4. CHANGES IN PLASMA LIPID OF FEMALE JAPANESE QUAIL FROM 28 TO 91 DAYS OF AGE IN RELATION TO SEXUAL MATURITY.

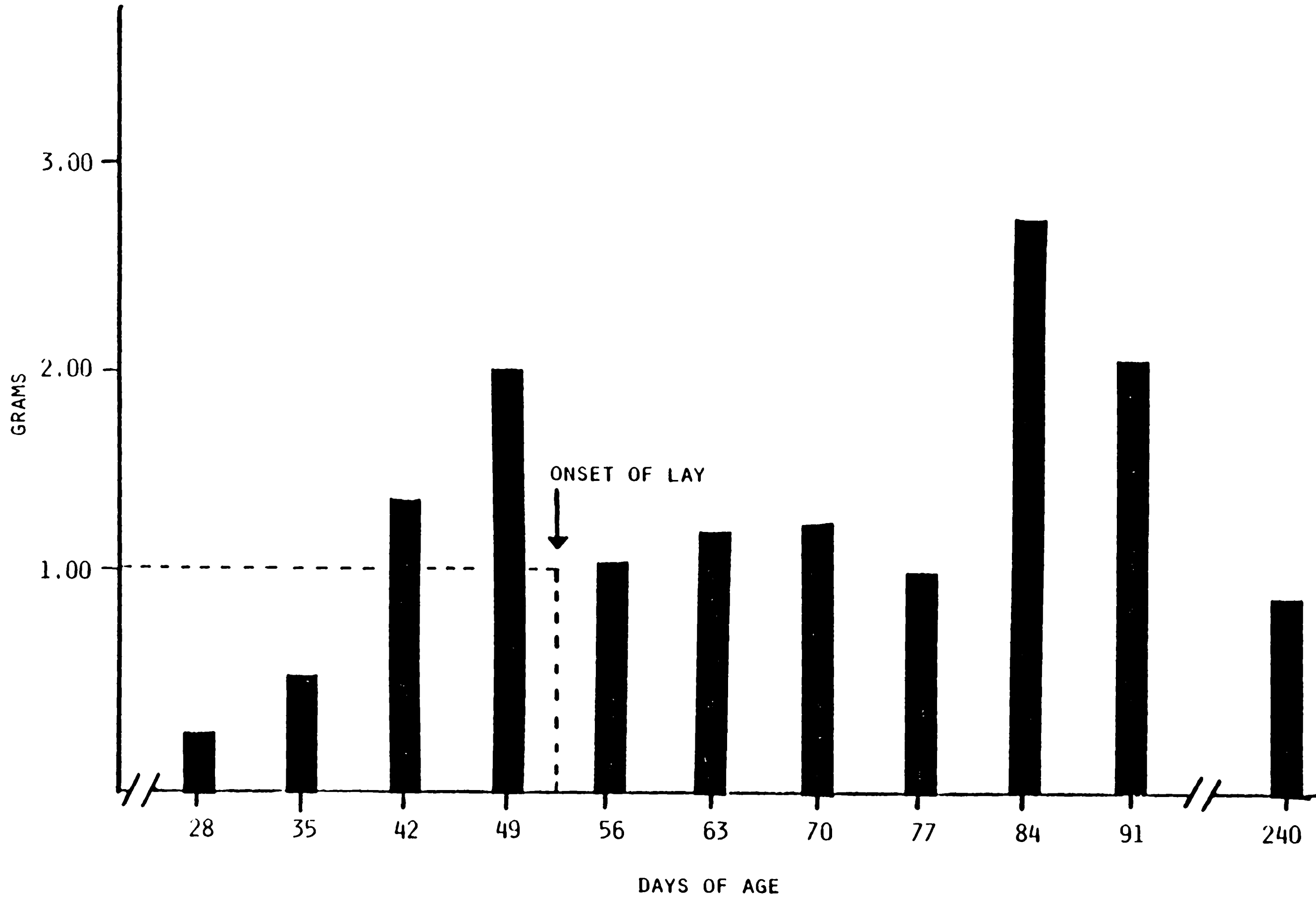


FIGURE 5. CHANGES IN ABDOMINAL FAT WEIGHT OF FEMALE JAPANESE QUAIL FROM 28 TO 240 DAYS OF AGE IN RELATION TO SEXUAL MATURITY.

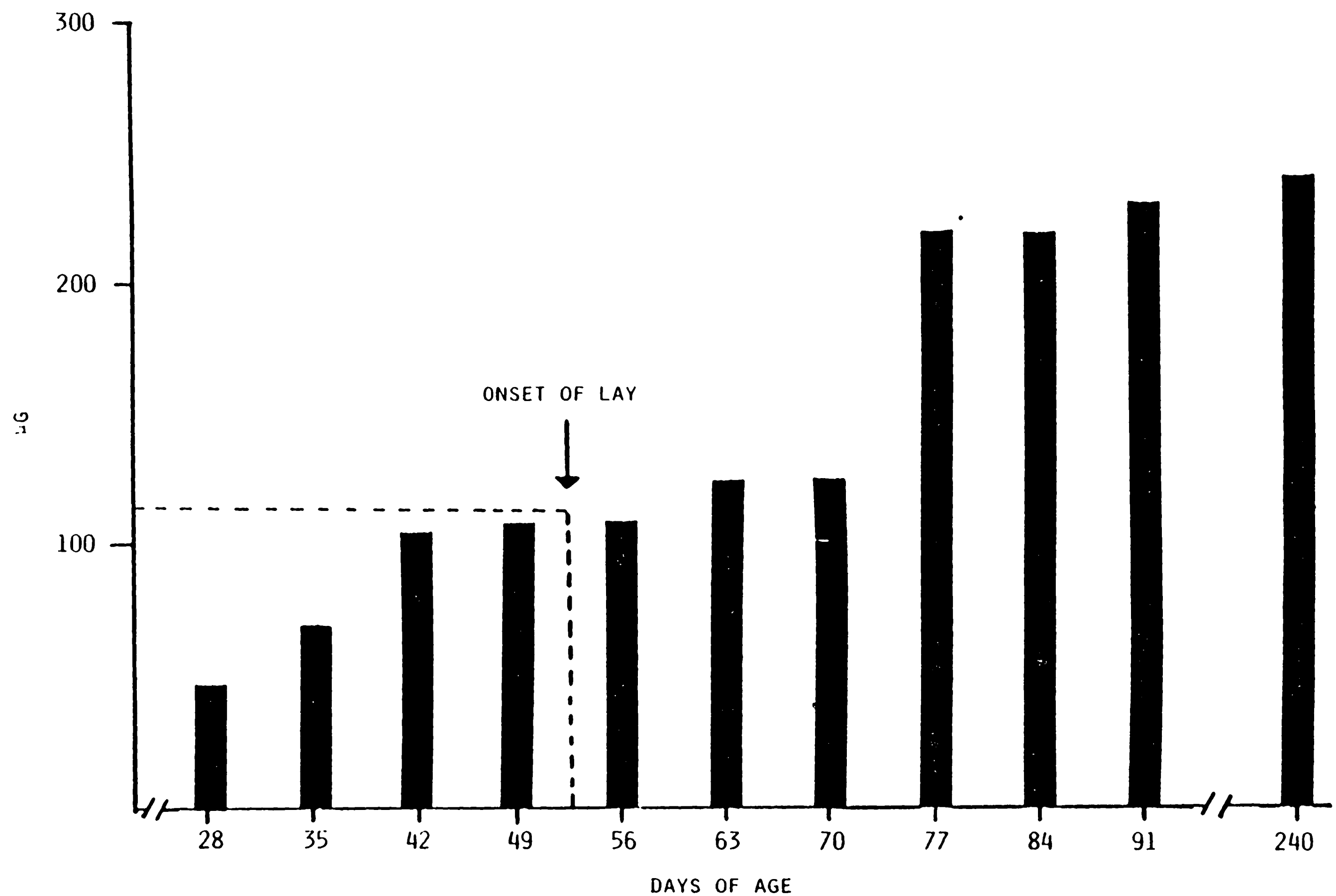


FIGURE 6. CHANGES IN LIPOCYTE DNA CONTENT IN THE ABDOMINAL FAT PAD OF FEMALE JAPANESE QUAIL FROM 28 TO 240 DAYS OF AGE.

EXPERIMENT III

MITOTIC ACTIVITY IN ABDOMINAL FAT OF JAPANESE QUAIL

IN RELATION TO SEXUAL MATURITY

Introduction

Since Hirsch and his colleagues (Knittle and Hirsch, 1969; Hirsch and Han; 1969) first proposed that changes in adipose tissue mass in mature animals are solely dependent upon changes in cell size, a voluminous literature concerning adipose tissue cellularity and fat deposition has been published (see reviews by Kirtland and Gurr, 1979; Bulfur and Allen, 1979). From these data, the "adipose cell hypothesis" has evolved (James, 1976). According to this postulate, fat accretion follows a basic pattern of development consisting of three distinct phases. In early stages of growth, fat deposition proceeds primarily through adipocyte hyperplasia. This hyperplastic period is followed by a transient period in which fat accretion is both hyperplastic and hypertrophic in nature. Finally, either before or at sexual maturity, adipocytes lose their ability to multiply and adipose tissue mass can increase or decrease only through modifications of cell size.

The adipose cell hypothesis has not been without its critics and several reports have contained evidence incompatible with this concept (Lemonnier, 1972; Stiles *et al*., 1975; Faust *et al*., 1978; Bertrand *et al*., 1978; 1984). In the second experiment reported in this dissertation, evidence was presented indicating that adipose tissue in adult Japanese quail undergoes mitotic development. In view

of several reports suggesting that adipose cell number is fixed in mature chickens (Pfaff and Austic, 1976; March and Hansen, 1977; Ballam and March, 1979; Hood, 1982; March *et al*., 1982), this experiment was conducted to verify the previous observations using different methodologies. The incorporation of radioactive thymidine into abdominal fat DNA was used as a measurement of mitotic activity.

Materials and Methods

The incorporation of methyl-^{3}H-thymidine (specific activity of 30 Ci/mmol; ICN Chemical Company, Irvine, CA) into quail adipose tissue was examined at several different ages. The procedure generally consisted of the intramuscular injection of 25 or 30 µci of the tritiated thymidine per bird, followed by an injection of 25 µmol of unlabeled thymidine (Sigma Chemical Company, St. Louis, MO). The birds were subsequently slaughtered and the abdominal fat pad was removed, weighed and subjected to collagenase-digestion as described for Experiment II. DNA was then isolated from both the stromal and lipocyte fraction using the procedures previously described except the solutions were prepared for liquid scintillation counting rather than for DNA assay by spectrophotometry. For counting, each adipose tissue fraction was dried in a scintillation vial. To the dried sample, 15 ml of a scintillation solution (diotol) was added and each vial was subsequently counted (Buhler, 1962). Activity was corrected for background and the data were expressed as the total disintegrations per minute (DPM) observed in each adipose tissue fraction.

Japanese quail were reared in modified chick battery brooders under a continuous photoperiod. Half were fed *ad libitum* and half were restricted to 70% of *ad libitum*

intake. Adjustments in feeding allotments were made daily. In the initial study, ten female quail fed *ad libitum* and ten subjected to restricted feeding were each injected, when 35 days of age, with 25 µci of tritiated thymidine. Twenty minutes later, 25 µmol of unlabeled thymidine was injected into each bird. After 24 hours, five quail from both the *ad libitum*- and restricted-fed groups were weighed and sacrificed. The abdominal fat pads were then removed, subjected to collagenase digestion, and radioactivity in stromal and lipocyte DNA was measured. The remaining ten quail were slaughtered five days later and treated in a similar manner.

In the second phase of the study, five female quail fed *ad libitum* and ten subjected to restricted feeding were administered, at 35 days of age, 30 µci of tritiated thymidine. Twenty-five µmol of unlabeled thymidine were injected 20 minutes later. Five of the restricted-fed birds were then released to *ad libitum* feeding while the remainder were maintained on their previous feeding regimes. These quail were monitored daily on an individual basis for the beginning of egg production. Unlabeled thymidine, at a dosage of 25 µmol per bird, was administered daily for an additional ten days. This procedure was then discontinued to minimize competition between the labeled and unlabeled thymidine. These birds were slaughtered when all five birds

within each treatment began to lay; the _ad libitum_, released and restricted groups were killed 7, 21 and 28 days, respectively, after the tritiated thymidine was administered. Lipocyte and stromal DNA were then examined for radioactivity.

In the third phase of this experiment, a direct comparison was made between mature and immature females. At 56 days of age, ten _ad libitum_-fed quail, all of which had begun to lay, and five quail under restricted feeding, none of which had entered lay, were administered 30 µci of tritiated thymidine. Twenty-five µmol of unlabeled thymidine were administered 24 hours later. Twenty minutes after administration of the unlabeled thymidine, five of the _ad libitum_-fed mature quail were killed for the determination of radioactivity in abdominal fat tissue. The remaining ten quail were killed five days after administration of the tritiated thymidine; none of the restricted-fed quail entered lay during this period.

Finally, mitotic activity in adipose tissue was examined at ages well past sexual maturity. At 84 days of age, ten quail fed _ad libitum_ and ten under restricted feeding were injected with 30 µci of tritiated thymidine; all quail had entered lay. Five of the restricted-fed birds were then placed on _ad libitum_-feeding. Twenty-four hours later, 25 µmol of unlabeled thymidine were administered.

Five of the *ad libitum*-fed quail and the five remaining on the restricted feeding regimen were slaughtered 20 minutes after the administration of the unlabeled thymidine. The remaining ten, five of which were fed *ad libitum* and five of which had been released to *ad libitum* feeding from restricted feeding, were sampled five days postinjection.

Results and Discussion

Initially, the incorporation of tritiated thymidine into adipose tissue DNA was examined in sexually immature females fed either *ad libitum* or at 70% of *ad libitum* intake. The results are presented in Table 6. One day after the administration of labeled thymidine to the quail fed *ad libitum*, substantial radioactivity was found in both stromal and lipocyte DNA; background radiation was about 20-30 counts per minute. At this sampling time, significantly more radioactivity was located in the stromal fraction than in the lipocyte fraction. Five days after injection of the tritiated thymidine, however, significantly more radioactivity was found in lipocyte DNA than in stromal DNA.

The results obtained with *ad libitum* feeding were consistent with several studies in which thymidine incorporation into adipose tissue DNA was examined in rats (Hollenberg and Vost, 1968; Klyde and Hirsch, 1979a; 1979b). Adipogenesis in Japanese quail, therefore, was apparently associated with the mitotic development of adipocyte progenitors within stromal cells. These primordial cells subsequently proliferated into new adipocytes (Frohlich *et al*., 1977). Hollenberg and Vost (1968), however, estimated that two days were required before substantial radioactivity appeared in lipocyte DNA after rats were treated with

radioactive thymidine. In the present study, about one-third of the total radioactivity recovered from adipose tissue 24 hours after thymidine administration was located in the lipocyte fraction. Thus, it appeared that in quail a substantial migration of cells from the stromal to the lipocyte fraction occurred within 24 hours of thymidine administration. Regardless of the time required, however, the accumulation of radioactivity in lipocyte DNA demonstrated that fat deposition in the *ad libitum*-fed quail was associated with the formation of new fat cells, although a concomitant adipocyte hypertrophy was not excluded as a contributing factor.

Not unexpectedly, fat deposition in the quail subjected to restricted feeding was limited. Both body weights and abdominal fat weights were significantly smaller in the restricted-fed quail than in those fed *ad libitum* (Table 6). This limited fat deposition appeared to be reflected in the estimates of mitotic activity. Although measurable radioactivity was found in stromal DNA from the restricted-fed quail, the accumulation of radioactivity 24 hours after thymidine administration was less than 20% of that observed under *ad libitum* feeding. Moreover, only negligible amounts of lipocyte DNA were harvested after collagenase digestion of adipose tissue from the restricted-fed quail, suggesting that essentially no new lipid-containing cells were

differentiating. This lack of new lipocytes was believed to be attributable to a lack of fat deposition, and not to the absence of the capability for adipocyte hyperplasia.

In the second phase of this study, tritiated thymidine was administered to quail at 35 days of age and the accumulation of radioactivity into adipose tissue DNA was examined when lay had commenced. The quail fed *ad libitum* had all reached lay seven days after the injection of thymidine while those subjected to restricted feeding required 28 days to achieve lay (Table 7). The quail changed from restricted to *ad libitum* feeding at 35 days of age entered lay 21 days later. In all three groups, the accumulation of radioactivity in lipocyte DNA indicated that new adipose cells were being formed. Radioactivity in lipocyte DNA was significantly higher for the quail fed *ad libitum* and for those released to *ad libitum* from restricted feeding when compared to the restricted-fed quail. Again, these differences were probably attributable to differences in net fat deposition. In addition, only negligible radioactivity was found in stromal DNA, regardless of the feeding regime, indicating that the flux of labeled cells from the stromal to the lipocyte fraction was essentially completed by the time these birds were slaughtered.

When a direct comparison of mature and immature females was made, there were no indications that adipocyte

hyperplasia ceased at sexual maturity (Table 8). *Ad libitum*-fed females, all of which had attained lay, were treated with tritiated thymidine and examined one or five days later. The results were remarkably similar to those previously obtained prior to sexual maturity (Table 6). Twenty-four hours after the administration of labeled thymidine, about 70% of the recovered radioactivity was located in the stromal fraction of adipose tissue (Table 8). Five days after treatment, about 70% of the radioactivity was located in the lipocyte fraction. Moreover, significantly more radioactivity was found in lipocyte DNA from the quail fed *ad libitum* and slaughtered five days after thymidine administration than was found in the immature restricted-fed quail killed at the same age. If adipocyte hyperplasia ceased prior to sexual maturity, opposite results would be expected.

In the final phase of the study, radioactivity in lipocyte DNA was examined after the administration of tritiated thymidine to quail at 84 days of age. All of these quail had previously laid eggs. The results differed slightly from those previously obtained, but again there was no evidence that adipocyte hyperplasia had ceased (Table 9). In both *ad libitum*- and restricted-fed quail killed one day after the administration of tritiated thymidine, slightly but nonsignificantly more radioactivity was found in

lipocyte DNA than in stromal DNA. In the previous studies, significantly more radioactivity was found in stromal DNA than in lipocyte DNA when adipose tissue was examined 24 hours after the injection of labeled thymidine (Tables 6 and 8). This discrepancy was not totally surprising, however, because the previous observations suggested that appreciable radioactivity was located in lipocyte DNA within 24 hours of thymidine administration (Tables 6 and 8). Nevertheless, the accumulation of radioactivity in lipocyte DNA should have been negligible in these mature quail if adipocyte hyperplasia was arrested at earlier ages. This was clearly not the case. In both *ad libitum*-fed quail and quail released to *ad libitum* feeding from restricted feeding, a substantial accumulation of radioactivity in lipocyte DNA occurred five days after the administration of tritiated thymidine.

The apparent migration of radioactivity from stromal to lipocyte DNA in this study was very similar to results reported from studies with rats (Hollenberg and Vost, 1968; Klyde and Hirsch, 1979a; 1979b). It might be concluded, therefore, that quail resemble other species in that adipogenesis initially involves mitotic activity of progenitor cells located in non-lipid fractions of adipose tissue, and the subsequent maturation of these progenitor cells into mature adipocytes (Simon, 1965). From similar

data, however, Klyde and Hirsch (1979a; 1979b) concluded that adipocyte progenitors reside in both the stroma and lipocyte fractions of adipose tissue. The results reported were not contradictory to either concept. The consistent appearance of radioactivity in lipocyte DNA 24 hours after the administration of tritiated thymidine could be construed as evidence that mitotic activity was occurring directly in the adipocyte fraction of adipose tissue. The apparent decline in radioactivity in stromal DNA, which was associated with an increase in lipocyte DNA radioactivity, observed subsequent to 24 hours would be representative of the maturation of cells originally derived from stromal progenitors. Conversely, the early appearance of radioactivity in lipocyte DNA may just indicate that the maturation of adipose cells from stromal elements proceeds more rapidly in the quail than in the rat (Hollenberg and Vost, 1968). In retrospect, it would have been valuable to examine these fractions at times less than 24 hours after thymidine administration.

As mentioned previously, adipose tissue cellularity studies with the domestic chicken have failed to provide evidence contradictory to the "fixed adipose cell" hypothesis (Pfaff and Austic, 1976; March and Hansen, 1977; Ballam and March, 1979; Hood, 1982; March *et al.*, 1982; Cherry *et al.*, 1984). In contrast, the results of this

experiment, as well as the results reported in Experiment II of this dissertation, appear to conclusively show that mature Japanese quail are capable of hyperplastic growth of adipose tissue. Either adipogenesis in Japanese quail differs from that in chickens or previous studies with chickens have failed to detect similar adipogenic responses. In addition, the results of this study are supportive of evidence obtained with rats that adipocyte hypertrophy, as well as adipocyte hyperplasia, can contribute to increases in the adipose tissue mass of mature animals (Lemonnier, 1972; Stiles *et al*., 1975; Faust *et al*., 1978; Bertrand *et al*., 1978; 1980; 1984).

Summary

The incorporation of tritiated thymidine into adipose tissue DNA was used to assess the capability of mature Japanese quail to exhibit adipocyte hyperplasia. Twenty-five or 30 μci of methyl-^{3}H-thymidine was injected intramuscularly into both ad libitum- fed and restricted-fed female Japanese quail at several ages. The quail were subsequently slaughtered and the abdominal fat pads were subjected to collagenase digestion. The radioactivity in DNA from both the stromal and lipocyte fractions resulting from collagenase digestion was then determined by liquid scintillation counting.

Regardless of the stage of maturity, substantial radioactivity was recovered from both stromal and lipocyte DNA when quail were sacrificed 24 hours after the administration of tritiated thymidine. When the quail were sacrificed five days after thymidine administration, a substantial migration of radioactivity from stromal to lipocyte DNA had apparently occurred. It was concluded that this pattern represented an initial mitotic activity of adipocyte progenitor cells located in non-lipid fractions of adipose tissue, and the subsequent proliferation of these new cells into maturing lipocytes as they accumulated lipid. The data were not, however, contradictory to the concept that adipocyte progenitor cells reside in both stroma and

lipocyte fractions of adipose tissue. Nonetheless, similar patterns were observed when the quail were examined at ages prior to, at, and subsequent to the onset of lay. Therefore, the results indicated that adipocyte hyperplasia, as well as adipocyte hypertrophy, can contribute to increases in adipose tissue mass of mature Japanese quail.

Table 6. Radioactivity in DNA from lipocyte and stromal fractions of abdominal fat from immature female Japanese quail treated with tritiated thymidine at 35 days of age and sacrificed at 36 or 40 days of age

Feeding Regime	Age	Days after injection	Body wt.	Abdominal fat	Radioactivity Stroma	Radioactivity Lipocyte
			-g-	-mg-	---------DPM---------	
Ad libitum	36	1	137±5b	660±190b	1456±396b	496±177a
Ad libitum	40	5	140±9b	1146±400b	124± 34a	994± 28b
Restricted	36	1	109±5a	110± 36a	211± 54a	---
Restricted	40	5	97±8a	64± 12a	183± 62a	---

a,b Means within a column having the same letter were not significantly different ($P \leq .05$). Under ad libitum feeding, differences in radioactivity between the stromal and lipocyte fractions were significant ($P \leq .05$) at both 36 and 40 days of age. Radioactivity in the lipocyte fraction under restricted feeding was omitted from analysis because only negligible amounts of lipid were harvested after collagenase digestion.

Table 7. Radioactivity in DNA from lipocyte and stromal fractions of abdominal fat from female Japanese quail treated with tritiated thymidine at 35 days of age and sacrificed at the onset of lay

Feeding Regime	Age	Days after injection	Body wt.	Abdominal fat	Radioactivity Stroma	Radioactivity Lipocyte
			-g-	-mg-	------DPM------	
Ad libitum	42	7	171±6a	1722±145a	58±6a	870±33b
Ad libitum	56	21	170±9a	2559±563a	36±4a	828±32b
Restricted	63	28	154±6a	2000±500a	30±2a	302±29a

a,b Means within a column having the same letter were not significantly different ($P \geq .05$). For all three feeding regimes, radioactivity in the lipocyte fraction was significantly ($P \leq .05$) higher than in the stromal fraction

Table 8. Radioactivity in DNA from lipocyte and stromal fractions of abdominal fat from mature and immature female Japanese quail treated with tritiated thymidine at 56 days of age

Feeding Regime	Maturity	Age	Days after injection	Body wt.	Abdominal fat	Radioactivity	
						Stroma	Lipocyte
				-g-	-mg-	-------DPM-------	
Ad libitum	Mature	57	1	176±4c	1987±354b	331±51b	134±38a
Ad libitum	Mature	61	5	156±6b	1428±252b	185±22a	454±21c
Restricted	Immature	61	5	122±8a	635±278a	181±46a	257±34b

a,b,c Means within a column having the same letter were not significantly different ($P \geq .05$). Regardless of treatment, differences in radioactivity between the stromal and lipocyte fractions were significant ($P \leq .05$).

Table 9. Radioactivity in DNA from lipocyte and stromal fractions of abdominal fat from sexually mature female Japanese quail treated with tritiated thymidine at 84 days of age and sacrificed at 85 or 89 days of age

Feeding Regime	Age	Days after injection	Body wt.	Abdominal fat	Radioactivity	
					Stroma	Lipocyte
			-g-	-mg-	-------DPM-------	
Ad libitum	85	1	188±2b	4207±920a	223±37a	549±128a
Restricted	85	1	156±1a	853±267a	157± 9a	223± 29a
Ad libitum	89	5	186±3b	1357±561a	434±98b	1424±145b
Released	89	5	156±1a	794±167a	403±63b	1974±206c

a,b Means within a column having the same letter were not significantly different ($P \geq .05$). Differences in radioactivity between the stromal and lipocyte fractions were significant for the quail at 89 but not at 85 days of age.

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APPENDIX A

Appendix Table 1. Mean squares (M.S.) and degrees of freedom (df) for data presented in Table 1 of text (Experiment I)

Traits	Source of Variation (M.S.) Treatment df = 4	Error df = 46
Testis wt.	13.78*	0.47
Testis wt. (% body wt.)	14.15*	0.43
Shank Length	0.07	0.06
Body wt.	108.64	83.24
Abdominal fat pad wt.	35.94	35.64
Abdominal fat pad wt. (% body wt.)	28.41	27.63
Breast wt.	47.97*	7.09
Breast wt. (% body wt.)	22.95*	2.98
Carcass fat	82.14	41.82

*(P ≤ .0001).

Appendix Table 2. Mean squares (M.S.) and degrees of freedom (df) for data presented in Table 2 of text (Experiment I)

Traits	Source of Variation (M.S.) Treatment df = 4	Error df = 40
Ovary wt.	67.51*	21.21
Ovary wt. (% body wt.)	41.39*	12.55
Shank Length	0.011	0.0080
Body wt.	398.22**	101.13
Abdominal fat pad wt.	5.74	2.36
Abdominal fat pad wt. (% body wt.)	2.83	1.29
Breast wt.	14.44	8.21
Breast wt. (% body wt.)	44.12**	5.14
Carcass fat	157.14*	45.56

*(P ≤ .01).
**(P ≤ .001).

Appendix Table 3. Mean squares (M.S.) and degrees of freedom (df) for data presented in Table 4 in text (Experiment II)

	Source of Variation (M.S.)			
	df	Week	df	Error
Body wt.	9	1277*	90	118
Abd. fat pad wt.	9	18*	90	0.94
Stromal wt.	9	0.006*	90	0.001
Lipocyte DNA	9	67490*	90	2164
Stromal DNA	9	4701*	90	554

*($P \leq .0001$).

Appendix Table 4. Mean squares (M.S.) and degrees of freedom (df) for data presented in Table 5 in text (Experiment II)

	Source of Variation (M.S.)							
	df	Week	df	Error	df	Week	df	Error
Body wt.	11	5785*	108	593	10	--	99	--
Abd. fat pad wt.	11	13*	108	0.812	10	--	99	--
Stromal wt.	11	.007*	108	0.001	10	--	99	--
Lipocyte DNA	11	44336*	108	2165	10	--	99	--
Stromal DNA	11	5641*	108	492	10	--	99	--
Plasma calcium	11	--	108	--	10	502	99	3771603*
Plasma total lipid	11	--	108	--	10	42	99	408795

*(P ≤ .0001).

Appendix Table 5. Mean squares (M.S.) and degrees of freedom (df) for data presented in Table 6 of text (Experiment III)

Source of Variation	df	Mean Squares: Body wt.	Abd. fat pad wt.	Stromal wt.	DPM
Regime	1	12674***	6.65***	0.0001	1366600**
Site DPM	1	--	--	--	2231
Day	1	230	0.49	0.013	175525
Regime x Site DPM	1	--	--	--	21706
Regime x Day	1	436	0.71	0.003	256980
Regime x Site DPM x Day	1	--	--	--	884545*
Site DPM x Day	1	--	--	--	1188450
Error	32	232	0.25	0.001	170376

*($P \leq .0001$).

Appendix Table 6. Mean squares (M.S.) and degrees of freedom (df) for data presented in Table 7 of text (Experiment III)

Source of Variation	df	Mean Squares			
		Body wt.	Abd. fat pad wt.	Stromal wt.	DPM
Regime	2	755	1.81	0.001*	245754*
Site DPM	1	--	--	--	2840007*
Regime x Site DPM	2	--	--	--	437003*
Error	22	267	0.89	0.001	241

*(P ≤ .0001).

Appendix Table 7. Mean squares (M.S.) and degrees of freedom (df) for data presented in Table 8 of text (Experiment III)

Source of Variation	df	Mean Squares				
		Body wt.	Abd. fat pad wt.	Stromal wt.	DPM	
					1 day	5 day
Site DPM (Ad libitum)	1	--	--	--	97338*	
Error	8	--	--	--	10161	
Regime	1	5712**	3.14*	0.01	--	191962
Site DPM	1	--	--	--	--	191179
Regime x Site DPM	1	--	--	--	--	84396
Error	16	242	0.35	0.00	--	23540

*(P ≤ .01).
**(P ≤ .0001).

Appendix Table 8. Mean squares (M.S.) and degrees of freedom (df) for data presented in Table 9 of text (Experiment III)

Source of Variation	df	Mean Squares: Body wt.	Abd. fat pad wt.	Stromal wt.	DPM
Regime	2	4866**	29**	0.001	264760
Site DPM	1	--	--	--	5264501
Day	1	20	41**	0.032	1472996
Regime x Site DPM	2	--	--	--	2897593
Error	33	17	1	0.001	733692

*(P ≤ .01).
**(P ≤ .0001).

APPENDIX B

Bioanalytical Techniques

Collagenase Digestion (Rodbell, 1964)

Incubation in a shaking water bath at 37°C for 1 hr (3-5 mg collagenase/g fat pad/ml Krebs-Ringer-phosphate-albumin buffer). Remove stroma. Wash lipocytes three times and stroma twice. Centrifuge at each washing time for 1 min at 400 g.

DNA Analysis Procedures (Curtis-Prior et al., 1975)

I. Homogenize lipocytes and stroma samples separately. For lipocytes, extract with cold 35-40 ml of 10% w/v TCA in ethanol-diethyl ether (3+1); and for stroma, extract with 10 ml m/v TCA in diethyl-ether-ethanol (3+1). Spin cold at 4000 g for 10 min. Discard lipid and acid soluble nucleotide. This yields nucleotide pellets.

II. Wash nucleotide pellets of lipocytes with 35-40 ml ethanol-diethyl ether (3+1) and cold 10 ml diethyl-ether-ethanol (3+1). Spin cold at 4000 g for 10 min. Discard lipids.

III. DNA isolation: Add 3 ml 5% m/v aqueous solution of TCA to each sample and incubate for 20 min at 90°C in a shaking water bath. Cool and spin at 4000 g for 20 min.

IV. DNA radioactivity: Dry DNA solution samples in scintillation vials and add cocktail for LS counting (modified from Curtis-Prior *et al.*, 1975).

V. Chemical DNA analysis: Take 1 ml DNA solution of each sample and .1 ml of 5% aqueous solution of TCA.

1. Hydrazone formation: Add 40 ul of fresh p-nitrophenyl hydrazine. Incubate by refluxing in boiling water bath for 20 min. This reaction caused formation of hydrazone with deoxyribose.

2. Washing: To remove any excess p-nitrophenyl hydrazine, wash each sample three times with 7 ml n-butyl acetate. Each time shake vigorously for 2 min, spin briefly to separate phases, aspirate, and discard upper phase.

3. Transfer 1 ml of the washed lower phase into 1 ml tube. Centrifuge briefly and aspirate any visible n-butyl acetate, plus the uppermost layer.

4. Transfer 500 ul of the remainder to a microcuvette. Add 200 ul of fresh, aqueous 4N NaOH solution. Mix by repeated inversion.

5. Spectrophotometic measurement of the color developed by alkali. Read the absorbance of the magenta-colored solution at 560 nm, exactly 3 min after the addition of alkaline.

DNA standard solution: Prepare stock solution by adding 50 ml of 5% w/v aqueous solution of TCA to 25 mg of salmon sperm DNA. Standard should be treated as the samples through the steps of Stage V.

Calculations

DNA Concentration DNA = K x OD of sample x 12 - µg

where k = $\frac{\text{Conc. of std.}}{\text{OD of std.}}$

and 12 = constant dilution factor derived from the assay (see protocol)

DNA Radioactivity = $\frac{\text{CPM - BKG}}{\text{Counting efficiency of sample}}$

where counting efficiency = $\frac{\text{Observed CPM-BKG x 100}}{\text{decay corrected DPM of std.}}$ %

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FAT DEPOSITION IN RELATION TO SEXUAL MATURATION

OF JAPANESE QUAIL

by

Boma Magnus Oruwari

(ABSTRACT)

The relationships among body weight, body composition, adipose tissue cellularity and the onset of sexual maturity were studied in Japanese quail (Coturnix coturnix japonica). In an initial experiment, body composition and sexual maturity were examined in response to photoperiodic and hormonal manipulations. Attempts were made to modify body composition, and thereby age at sexual maturity, by feeding diets containing PTU (4-phenyl-2 thiouracil), by imposing a daily photoperiod (IP) of four hours, by the subcutaneous implantation of estrogen to females and testosterone to males, and by the intramuscular injection of turkey growth hormone and anti-sera to turkey growth hormone. The quail were sacrificed at 63 days of age and body composition was examined.

Quail subjected to the IP treatment failed to enter sexual maturity by 63 days of age, while about 50% of the controls receiving a photoperiod of 14 hours were sexually mature at this age. The failure of these quail to mature

was associated with a reduction in body fat. The effects of the other treatments on sexual maturity, however, were not consistently associated with differences in body weight and/or body composition.

In the second phase of this study, adipocyte hyperplasia was examined in relation to sexual maturity. Total DNA concentrations in the stromal and lipocyte fractions of collagenase-digested abdominal fat depots were determined on a chronological age basis from 28 to 240 days of age. In both males and females, significant increases in abdominal fat weights at ages beyond the age at sexual maturity were associated with increased DNA concentrations in abdominal fat adipose tissue. These results suggested that mature Japanese quail were capable of hyperplastic fat deposition.

In the final phase of this study, the incorporation of methyl-^{3}H-thymidine into DNA of lipid and non-lipid fractions of collagenase-digested abdominal fat from both ad libitumand restricted-fed (70% of ad libitum intake) quail was examined. Regardless of the feeding regime and stage of maturity, substantial radioactivity was recovered from both the stromal and lipocyte fractions when the quail were examined 24 hours after the administration of tritiated thymidine. When quail were examined five days after the administration of tritiated thymidine, an apparent migration

of radioactivity from stromal to lipocyte DNA occurred. It was concluded that this pattern represented mitotic activity of adipocyte progenitor cells located in non-lipid fractions of adipose tissue, and the subsequent maturation of these cells into lipocytes as they accumulated lipid. The hypothesis that adipocyte progenitors reside in both the stromal and lipocyte fraction, however, was not disproven. Nevertheless, the similarity of results obtained prior to, at, and subsequent to the onset of lay indicated that adipocyte hyperplasia contributes to increases in adipose tissue mass in mature Japanese quail.