

THYROID DEVELOPMENT IN PRECOICIAL AND ALTRICIAL AVIAN EMBRYOS

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ABSTRACT.—Histological criteria indicate that thyroid glands are organized into follicles at about the same time of incubation in Japanese Quail (precocial) and Ringed Turtle Dove (altricial) embryos, but dove thyroids develop more slowly, are much smaller and are much less active than quail thyroids by the time of hatching. These results indicate thyroid readiness for participation in early endothermic responses of precocial hatchlings, and relative thyroid inactivity in young ectothermic altricial nestlings.—*Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061. Accepted 12 April 1976.*

THYROID hormones are known to be important determinants of metabolic rate and heat production in adult birds. In addition, the time course of developing thyroid function closely parallels developing thermoregulatory ability in precocial young that hatch at a relatively advanced stage of development (see Freeman 1971, for the data on domestic chickens; McNabb et al. 1972 and Spiers et al. 1974 for the data on Japanese Quail [*Coturnix coturnix japonica*]). These precocial hatchlings exhibit some thermoregulatory ability shortly after hatching although they do not become truly homeothermic for several weeks; the actual time course of homeothermic development depends on the species. In contrast, altricial birds hatch at a less developed stage and are dependent on parental care and feeding for some time. Altricial hatchlings are ectothermic for the first few days after hatching, but acquire thermoregulatory ability during the nestling period, and are homeothermic by the time of fledging (usually at about 2–3 weeks of age, Dawson and Hudson 1970). Using the single criterion of epithelial cell height, Dawson and Allen (1960) concluded that thyroid function in nestling Vesper Sparrows (*Pooecetes gramineus*) matches or exceeds that of adults and, therefore, anticipates the development of homeothermy. Rol'nik (1968) stated that the thyroid of altricial birds differentiates into follicles and becomes physiologically active relatively later than in precocial birds, but this comparison is based on birds that differ markedly in body size and length of their incubation periods.

The objective of this paper is to compare thyroid gland development during the embryonic period in a precocial species (Japanese Quail), and in an altricial species (Ringed Turtle Doves, *Streptopelia risoria*). Although these species both have an incubation period of about 16+ days duration, quail exhibit endothermic responses to cold shortly after hatching (Spiers et al. 1974) while doves are presumably ectothermic for at least the first few days after hatching (Breitenbach and Baskett 1967 have shown that nestling Mourning Doves are ectothermic for at least the first 3 days). Our choice of these species was determined by the equal length of their incubation periods plus patterns of development typical of those considered precocial and altricial.

METHODS

Fertile eggs from a white race of Ringed Turtle Doves were obtained from a game bird breeder. Japanese Quail eggs were obtained from the Department of Poultry Science at VPI & SU. Eggs of both species were incubated at 38–39°C. Embryos between 6 and 16 days of incubation age (N = 2–6 for each species for each age sampled) were removed from the shell, fixed and stored in Bouin-Hollande-sublime

fixative. For all of the doves and the early quail embryos, the neck and upper thoracic region was used. After day 9, quail thyroids were dissected out of the embryo. Tissues were embedded in wax by routine histological methods, and serial sections were stained with Heidenhain's Azan. This trichrome stain selectively stains chromophilic colloid blue (basophilia) in an active gland and chromophilic colloid red (acidophilia) in an inactive gland. Unstained colloid is referred to as chromophobic (see Romanoff 1960 for acidophilic, basophilic, and chromophobic staining patterns in chicken embryonic thyroids; use of Heidenhain's Azan for thyroid tissue is cited by Pickford and Atz 1957).

Development of the thyroid was evaluated by counting the numbers of cell clumps (which could be either prefollicular units or the walls of organized follicles), follicles with colloid of each staining reaction, noting the degree of vacuolization of colloid, and by measuring follicle diameter, colloid diameter, epithelial cell height, and cross sectional area in the largest cross section of each gland. The dimensions of 20 follicles were measured in the largest cross section of each gland. Our comparisons of tissue dimensions assume that tissue shrinkage during histological preparation was equal in both species. In a few thyroids, cell membranes did not stain intensely, so we could not measure follicle and cell dimensions accurately. Approximate measurements indicated the dimensions of these glands were typical of other thyroids at the same developmental age. Differences in N values between figures reflect this lack of cell and follicle dimensions for some thyroids.

Statistical comparisons of pairs of means were made using Student's *t*-test. Values of *t* with $P \leq 0.05$ were accepted as indicative of statistically significant differences and are designated as such in the text.

RESULTS

Thyroid glands, histologically distinguishable by staining patterns and the beginning of organization into prefollicular cell clumps, were present in doves by day 7 and in quail by day 8 of incubation. Gland size, as indicated by the largest cross sectional area, was similar in both species on day 10, but increased more rapidly in quail than in doves during the incubation period (Fig. 1). Cross sectional area of quail thyroids was 3× greater than that of doves on day 16 (significantly different). This difference in cross sectional area represents a five-fold increase in volume of a spherical gland.

The total number of follicle units (cell clumps and follicles with colloid) in the largest cross section of each thyroid gland increased about three-fold in each species between 10 and 16 days, and was significantly greater in quail than in doves at both 10 and 16 days (Fig. 2). Colloid was first observed on day 10 in both species but was extremely rare in dove thyroids (1% of the follicle units with colloid) and common in quail thyroids (59% with colloid) at that time. In quail thyroids the proportion of follicles with colloid reached 80% by day 11 and remained at that level for the rest of the incubation period. After day 11 the remaining cell clumps are probably follicle walls rather than prefollicular cell clumps. All colloid observed in quail thyroids was chromophilic and blue-stained (active) throughout the incubation period.

In dove thyroids the proportion of follicles with colloid reached 54% by day 14 and did not change further. This suggests that organization into follicles is complete by day 14, with a higher percentage of follicle walls cut than in quail thyroids because of smaller follicle size in dove thyroids (see follicle size data, below). The proportion of follicles with chromophilic blue colloid increased to 40% by day 14, then decreased to 16% by day 16 in doves (Fig. 2). On the expected day of hatching (day 16) dove thyroids had equal numbers of chromophobic, chromophilic blue (active), and chromophilic red (inactive) follicles. Follicle dimensions (follicle diameter-FD, colloid diameter-CD, and epithelial cell height-ECH) did not differ significantly between follicles with different staining reactions in the colloid.

Vacuolization, which usually is considered to be indicative of hormone mobilization, was apparent to some extent in all blue-stained colloid. In quail embryos the degree of colloid vacuolization was more extensive from days 11 to 15 than on day 16

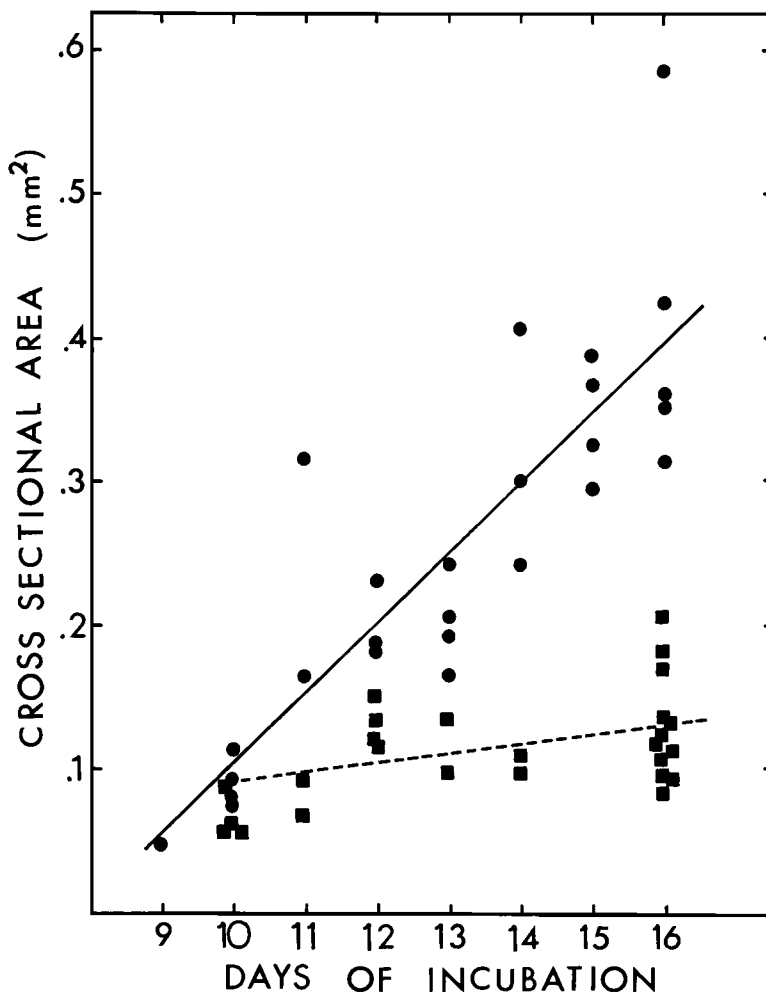


Fig. 1. Thyroid gland cross-sectional area through the largest portion of the gland in relation to incubation time; solid line/circles (quail) and dashed line/squares (doves), are regression lines fitted by the least squares method.

of incubation. In dove embryos, similar degrees of colloid vacuolization were seen in both red and blue stained colloid; vacuolization was lacking in chromophobic colloid.

In quail thyroids FD increased significantly from 14.5μ on day 10 to 23.3μ on day 16 (Fig. 3). This change in FD in quail due to an increase in CD with no significant change in ECH (mean ECH through days 10–16 was 4.5μ with the greatest cell height of 5.7μ 2 days prior to hatching; [Fig. 3]). In embryonic dove thyroids FD did not increase significantly (19.6μ on day 16), and ECH decreased significantly from 6.5μ on day 11 to 4.9μ on day 16 (Fig. 3). Follicle diameter was significantly greater in quail thyroids than in dove thyroids on day 16; ECH did not differ significantly between the two species (Fig. 3).

DISCUSSION

The beginning of thyroid gland organization into follicles occurs at about the same time of incubation in quail and dove embryos, but the rates of gland growth and

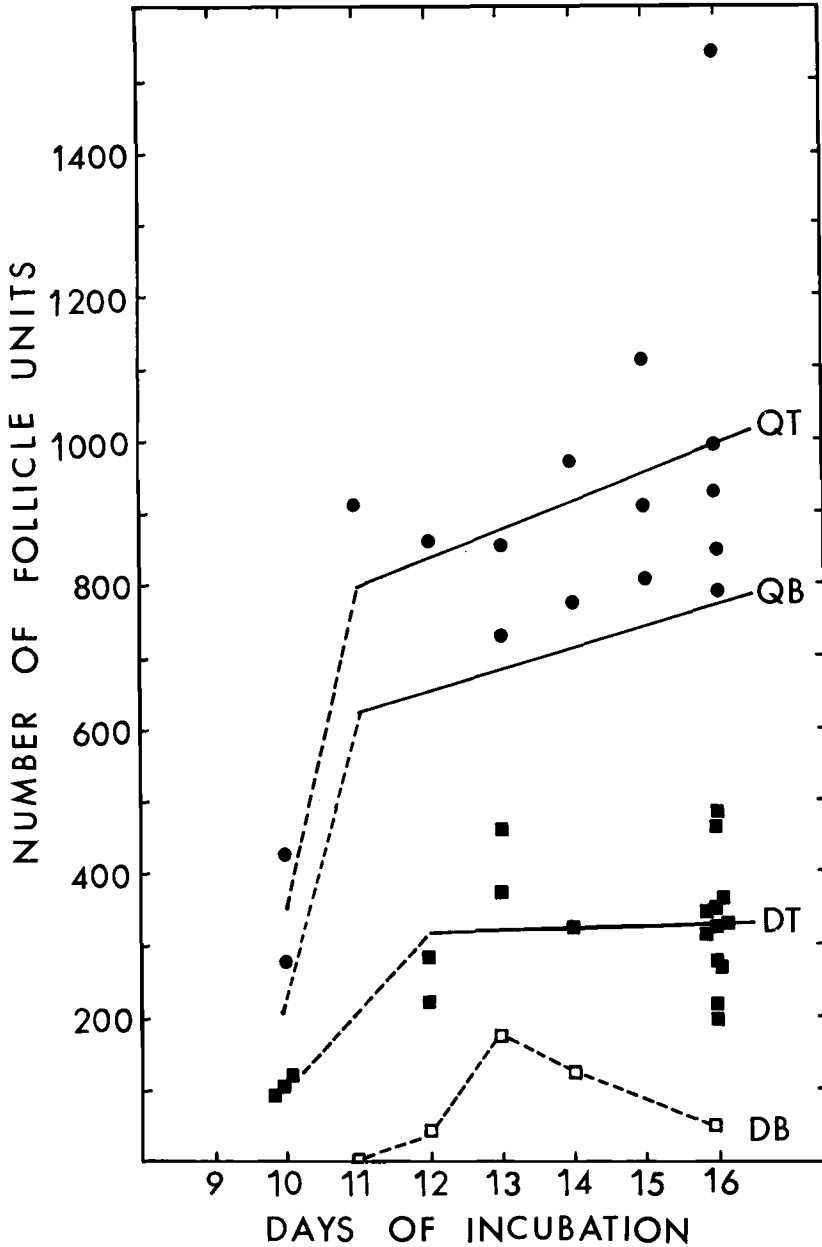


Fig. 2. Total numbers of follicle units, and numbers of follicles with chromophilic blue colloid per largest gland cross section in relation to incubation time. Q = quail, D = doves, T = total number of follicle units, B = number of follicles with chromophilic blue colloid; closed circles = individual data points for the total number of follicles in quail thyroids, closed squares = individual data points for the total number of follicles in dove thyroids, open squares = mean values for the number of dove follicles with blue colloid. Solid lines are regression lines fitted by the least squares method. For QB only the regression line and for DB only the means joined by a line are shown to permit clarity on a single graph. N values for the QB and DB lines are equal to those in the T line for each species.

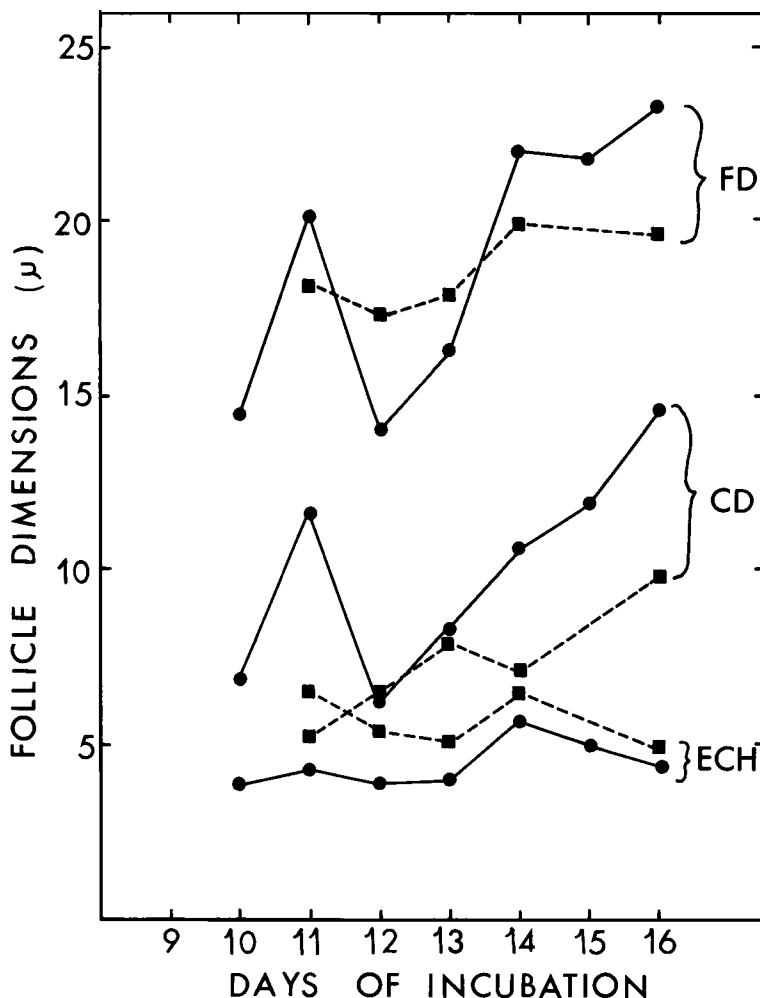


Fig. 3. Follicle dimensions of embryonic thyroids in relation to incubation time. Solid lines = quail, dashed lines = doves, FD = follicle diameter (outside diameter), CD = colloid diameter, ECH = epithelial cell height. Each symbol is the mean of 2-6 measurements except days 11 and 12 for quail and day 14 for doves which are single values.

follicle organization differ markedly between these species. As indicated earlier by McNabb et al. (1972) and confirmed by the more extensive measurements in the present study, the quail thyroid gland is organized into follicles exhibiting histological evidence of activity relatively early in incubation. The blue chromophilia of all colloid in embryonic quail thyroids (this study and McNabb et al. 1972), and the ability to accumulate iodine (McNabb et al. 1972; McNabb, McNabb, and Pinkston, unpublished radioiodine uptake studies), indicate early production of thyroid hormones. In addition, the time course of thermoregulatory development is correlated with post-hatching patterns of thyroid gland activity, and indicates the participation of the thyroid gland in this process in this precocial species (Spiers et al. 1974).

In the altricial doves, which are presumably completely ectothermic for some period after hatching (see work of Breitenbach and Baskett 1967, on Mourning Doves), embryonic thyroid development is much slower than in quail. In doves,

thyroid size is considerably smaller and organization of the gland into follicles is slower than in quail embryos (Figs. 1 and 2). Staining reactions indicate that only $\frac{1}{3}$ of the thyroid follicles are active in the dove at the time of hatching. In dove thyroids the existence of some chromophobic colloid late in the incubation period parallels the condition in chicken embryos (Romanoff 1960) and is in contrast to the condition in embryonic quail thyroids.

Colloid vacuolization did not appear to be a particularly useful indicator of hormone mobilization in these avian embryos. Vacuolization was present in both active and inactive chromophilic colloid, but the degree of vacuolization was not correlated with either changes in cell height, or times when hormone release is indicated by changes in thyroidal iodine content (McNabb et al. 1972).

Measurements of follicle dimensions indicate that quail thyroid growth, after organization into complete follicles, occurs by increases in both follicle number (Fig. 2) and FD (Fig. 3). As ECH does not increase, but CD does, cell numbers or cell width in the follicle walls must increase. In dove thyroids, FD does not change significantly during this period. The lack of significant differences between species in ECH, although other criteria indicate differences in thyroid activity, suggests this measurement is poor as a single criterion for comparing thyroid activity between different avian species, but the general pattern of ECH changes may be one useful source of information. In this study, ECH in quail thyroids showed the same pattern of changes that McNabb et al. (1972) were able to correlate with the pattern of stable iodine content of the glands. In embryonic doves, ECH measurements are similar to those of hatchling and adult Vesper Sparrows, but less than ECH measurements in 2–8-day nestling sparrows (Dawson and Allen 1960).

The histological criteria for thyroid activity used in this study show good correlation between the time course of changes in thyroid gland activity, and the patterns of developing thermoregulatory ability in these precocial and altricial species. Japanese Quail are precocial, and from the time of hatching use metabolic energy to gain some independence of environmental temperature. These quail chicks have active thyroid glands during most of the latter half of the incubation period. High thyroid hormone secretion occurs just prior to the initiation at hatching of endothermic responses to cold (McNabb et al. 1972, Spiers et al. 1974). The patterns of both thyroid gland development and thermoregulatory ability in Japanese Quail are similar to those described for chickens, the precocial species that has received the most study (see review by Freeman 1971). The time course of developing thermoregulation in Japanese Quail is prolonged, relative to that in chickens, as might be expected because of the high rates of heat loss resulting from the quail's small body size. Japanese Quail attain thermoregulatory ability more rapidly than do the larger Bobwhite Quail (Borchelt and Ringer 1973), a pattern that is reversed from that expected on the basis of body size. Thus while Japanese Quail seem an appropriate choice for studying the role of the thyroid gland in the thermoregulation of precocial birds, their rate of post-hatching development is unusually rapid in comparison to that of other quail.

The altricial pattern of development is characterized by ectothermy in the early hatchling period with high growth efficiency, at least partly because little energy is expended for thermoregulation. The thyroid glands of altricial Ringed Turtle Doves showed little evidence of activity by the time of hatching. These results are consistent with the low metabolic rates in altricial nestlings that remain ectothermic for at least several days after hatching (see Breitenbach and Baskett 1967 for work on Mourning

Doves). The small proportion of active thyroid follicles during the embryonic period in doves indicates at least some production of thyroid hormones. As thyroid hormones appear to be necessary for normal growth and development of avian embryos (see review by Betz 1971), the low level of thyroid hormones may be sufficient for this purpose but insufficient to elevate the metabolic rate to the high levels required for homeothermy. One line of evidence (ECH values in Vesper Sparrow thyroids, Dawson and Allen 1960) indicates that the thyroids of altricial nestlings show histological evidence of activity prior to the time when these nestlings exhibit endothermic responses to cold.

Evidence of thyroid hormone production prior to the initiation of endothermic responses to cold in both quail and doves indicates thyroid participation in these metabolic events, but does not reveal the mechanism involved in their onset. A marked increase in the release of thyroid hormones could be either the trigger for initiating thermoregulatory responses or merely part of the metabolic condition prerequisite to central nervous initiation of thermoregulatory responses. Distinguishing between these two possibilities will require neurophysiological experiments and measurements of circulating thyroid hormone levels during the period of developing thermoregulatory ability.

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