The effect of thyroid function on hemoglobin differentiation in the chick

By ROBERT W. ATHERTON

Department of Physiology, University of California, Berkeley

The switching off of the synthesis of embryonic hemoglobin (EHb) and the turning on of adult hemoglobin (AHb) synthesis has served as one of several major examples of biochemical differentiation. However, the control mechanisms underlying this transformation remained elusive, except in the case of *Rana catesbeiana* (Moss & Ingram, 1965, 1968a, b) where the classical thyroxine-induced metamorphosis effected a precocious shift from EHb to AHb. In the chick embryo such a dramatic metamorphosis does not occur, but a change from EHb to AHb can be detected electrophoretically on the sixth day of incubation (Hashimoto & Wilt, 1966; Manwell, Baker & Betz, 1966).

Normal thyroid development can be seen on the second day in the chick embryo, appearing as a medial outgrowth from the pharyngeal floor. By the sixth day, when AHb is first recognized electrophoretically, the thyroid concentrates $^{131}\text{I}$ 500 times more than does muscle (Olonoski, Lengeman & Comar, 1960). At 7 days, but not before, TSH has been detected *in vitro* (Tixier-Vidal, 1956). On the eighth day, yolk-sac absorption can be inhibited by injection of 100 $\mu$C of $^{131}\text{I}$, and 50 $\mu$C of $^{131}\text{I}$ will delay hatching and significantly reduce body and thyroid uptake of isotope (Maraz, 1966). By 8 days, anterior pituitary tissue placed on the chorioallantoic membrane causes an accelerated thyroid morphogenesis (Studitskii, 1938), indicating the presence of biochemical interrelationships. Thus, in the chick, there is evidence that the thyroid-pituitary axis is anatomically developed and biochemically active during and shortly following the switch from EHb to AHb. Experimentally it has been shown that the chick embryo, hypophysectomized at 33–36 h of development, exhibited a normal switch from embryonic to adult hemoglobin (Manwell & Betz, 1966). It would appear that the lack of embryonic TSH does not affect the differentiation of hemoglobin. However, evidence is available that egg yolk has a thyroid stimulating effect that increases the weight, function and histological structure of the gland (Tsuji, 1922).

In the present study, electrophoretic patterns of hemoglobin and erythrocyte morphology were investigated in the chick rendered hypo- or hyper-thyroid by

---

1 Author's address: Department of Physiology–Anatomy, University of California, Berkeley, California 94720, U.S.A.
the administration of thyroxine, $^{131}$I, thiourea, and tapazole (1-methyl-2-mercaptoimidazole). Because of the close functional relationship between thyroid activity and growth hormone, the effect of growth hormone on hemoglobin differentiation was also investigated.

**MATERIALS AND METHODS**

Incubation was started within 3 days after receipt of eggs of the species, *G. gallus*, from Kimber Farms in Niles, California. Montgomery Ward incubators were maintained at 37.7 °C and 80% relative humidity. Thyroxine and growth hormone were injected into the air sac according to the method of Romijn, Fung & Lokhorst (1952). This method was chosen for several reasons. First, by 8 days in development, this route will allow both thyroxine and thiourea to reach the embryo and affect embryonic respiration (Romijn *et al.* 1952). By this time, vascularization is sufficiently established to allow diffusion and absorption to facilitate transport. That compounds may reach the embryo before vascularization is complete is shown by the finding that propylene glycol (0.05 ml), injected from day zero to day 7 of incubation, results in a high incidence of mortality. Up to the fourth day when vascularization is incomplete, mortality increases rapidly (> 90%) and falls by day 7 (~ 30%) (Gebhardt, 1968). Thyroxine and thiourea, when injected into the air sac prior to incubation, have significant effects on the thyroid of newly hatched chicks (McCartney & Shaffner, 1949). In the present research, pilot studies indicated that doses of thyroxine (30 /µg/0.05 ml of saline) increased mortality (40%) over control figures. The minimal dose at which thyroxine was effective was determined to be 2.5 /µg/0.05 ml.

To induce hypothyroidism, $^{131}$I, thiourea, and tapazole were injected by the same route as the two hormones; the doses injected are indicated in Table 3. Single injections were administered at 24, 48, 72, and 96 h. incubation and multiple injections were given from day 3 through day 8. A minimum of twelve embryos was used in each control and experimental group.

Blood smears were stained with Wright’s Stain and also phosphate buffer (pH = 6.4). Five hundred cells from each embryo were catalogued according to the number of erythroblasts, mitotic figures, and the distribution (percentage) of polychromatic erythrocyte cell types (Lucas & Jamroz, 1961). Electrophoresis of the cyanomethemoglobin derivative was carried out on starch gel (Manwell, 1963).

The role of thyroid hormones in differentiation of other systems was determined indirectly by administering 50 /µc of $^{131}$I at 9 days and measuring body and brain weights at 19 days, since total body growth and brain development have been considered useful parameters of thyroid function in the developing rat (Geel & Timiras, 1967).
RESULTS

Body and brain weights were reduced by early injections of \(^{131}\)I (Table 1), indicating that a state of hypothyroidism was effectively induced. The electrophoretic diagram of the hemoglobin bands presented in Table 2 was adapted from the literature. For comparative purposes, these results are incorporated in Fig. 1, together with the results of the present experiments. The various hormonal treatments—thyroxine (1, 2, 3 and 4 days), growth hormone (3 and 4 days), and thyroxine plus growth hormone (3 and 4 days)—did not alter the electrophoretic patterns, indicating that apparently endocrine influences have not induced a precocious shift to AHb. Further, single injections of \(^{131}\)I and thiourea did not prolong the presence of EHb beyond 5 days of development. Because it is conceivable that the number and type of cells might change before any differences in electrophoretic patterns could be detected, it was decided to employ the more

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry brain weight (mg)*</td>
<td>0.086 ± 0.005</td>
<td>0.072 ± 0.006†</td>
</tr>
<tr>
<td>Wet body weight (g)</td>
<td>21.1 ± 2.4</td>
<td>17.7 ± 1.7†</td>
</tr>
</tbody>
</table>

* Dried at 105 °C, 4 days.
† t test: \( P = 0.05 \)

Table 2. Diagrammatic distribution of chick (embryo and adult) hemoglobins on starch-gel electrophoresis*

<table>
<thead>
<tr>
<th>Embryo</th>
<th>Adult</th>
<th>Embryo</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_1 )</td>
<td>+</td>
<td>( \times )</td>
<td>Minor Adult</td>
</tr>
</tbody>
</table>
| \( A_2 \)       | \( \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \time...
sensitive method of establishing the mitotic index of dividing blood cells, the number of erythroblasts, and the differential counts of the polychromatic erythrocyte series (Table 3). Endocrine treatment did not alter significantly (by the \( t \) test) the mitotic index; some increase of mitotic activity was noted after growth hormone and thyroxine, but not after the combined treatment. When the mean number of erythroblasts decreased the mitotic index increased—an inverse ratio that is not expected. Because of the slight changes observed in

![Electrophoretic patterns of adult chickens, hormone-injected embryos, and controls.](image)

\[
\begin{align*}
T_4 \times T_4 & = T_4 \text{ injected on day 1–3; sampled day 5.} \\
5D & = T_4 \text{ or growth hormone injected on day 3; sampled day 5.}
\end{align*}
\]

mitotic indices and the number of erythroblasts at 5 days, a more detailed differential count of primary, middle and late polychromatic erythrocytes was done at 9 days, when the erythrocytes are more distinguishable. It was confirmed that there is a distinct distribution (percentage) of the cell types as previously reported (Lucas & Jamroz, 1961), but no significant effect of stimulation or inhibition of erythropoiesis by thyroid agents was observed (Table 4).

In another series of experiments, multiple injections of thyroxine and tapazole were administered (daily doses of 0.09 \( \mu g/0.05 \) ml) covering the entire critical
Chick hemoglobin differentiation period of hemoglobin differentiation, that is, between 3 and 8 days. As in the case of single injections, the multiple injections did not modify the date of appearance of AHb differentiation.

Table 3. Hormone and anti-thyroid treatment in chick embryos and embryonic blood response

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose* (μl/0.05 ml)</th>
<th>Time sampled</th>
<th>Hb type</th>
<th>Mitotic index</th>
<th>Mean no. of erythroblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>5 days</td>
<td>EHb</td>
<td>7.2</td>
<td>19.1</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>1000</td>
<td>5 days</td>
<td>EHb</td>
<td>24.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>2.5</td>
<td>5 days</td>
<td>EHb</td>
<td>19.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Thyroxine and growth hormone</td>
<td>2.5 + 1000</td>
<td>5 days</td>
<td>EHb</td>
<td>5.2</td>
<td>21.3</td>
</tr>
<tr>
<td>Thiourea</td>
<td>3000</td>
<td>5 days</td>
<td>EHb</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>5 days</td>
<td>EHb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>¹³¹I</td>
<td>50 μc†</td>
<td>Daily, 5–19 days</td>
<td>AHb</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Given at 24 h of incubation.
† Given at 9 days of incubation.

Table 4. Distribution of blood cell types in the 9-day-old embryo (expressed as percentage)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Primary</th>
<th>Middle</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.6 ± 2.6</td>
<td>19.3 ± 2.2</td>
<td>62.8 ± 2.5</td>
</tr>
<tr>
<td>Thyroxine†</td>
<td>14.5 ± 1.0</td>
<td>17.8 ± 2.0</td>
<td>65.9 ± 1.0</td>
</tr>
<tr>
<td>Tapazole</td>
<td>14.0 ± 1.8</td>
<td>20.0 ± 1.5</td>
<td>65.5 ± 2.0</td>
</tr>
</tbody>
</table>

* Each cell type was significantly different (t test; P = 0.05) from one another, but a given cell type within different treatment groups was not.
† Dose: 0.09 μg/0.05 ml in saline.

DISCUSSION

Contrary to the data reported for R. catesbeiana, thyroxine treatment did not alter the electrophoretic pattern of hemoglobin differentiation in G. gallus. Various morphological parameters of blood differentiation similarly showed no changes consequent to thyroxine and growth-hormone treatment. These findings indicate that erythropoietic tissue is not competent to react to these hormones during thyroid morphogenesis in this species, although the literature and the present research suggest that the thyroid is beginning to function at the time of hemoglobin differentiation. It is suggested that some other mechanism, probably not directly involving the thyroid or other endocrines, is responsible for the signal to alter this particular protein synthesis. It would be of interest to study embryonic blood cells, in vitro without yolk, in order to elucidate further the mechanism by which hemoglobin differentiation occurs.
SUMMARY

Function of the thyroid gland in both the tadpole and the chick has been reported early in development. In the tadpole, early thyroid treatment has been shown to induce precocious differentiation of hemoglobin. The present study was conducted to ascertain the role of the thyroid in the same transformation (from embryonic (EHb) to adult (AHb) hemoglobin) in the chick. At various periods prior and during the time of normal synthesis of AHb (6 days), thyroxine, $^{131}$I, thiourea, or tapazole were injected into the air sac. Several erythropoietic parameters were measured at 5, 6, 7, 8, and 9 days to determine the thyroid effect. Because of the known relation between growth hormone and thyroxine during development, growth hormone was administered both separately and in conjunction with thyroxine, but was found to have no effect on erythropoietic differentiation. The results demonstrate that despite the presence of thyroid function in the chick in early embryonic stages, hemoglobin differentiation, the number of erythroblasts, mitotic figures, and differential counts do not depend upon thyroid function in this species as in $R$. catesbeiana. It is suggested that the mechanism(s) that trigger hemoglobin differentiation in the chick are probably non-endocrine in origin.

RÉSUMÉ

L'effet de la fonction thyroidienne sur la différenciation de l'hémoglobine chez le Poulet

La fonction de la glande thyroïde a été étudiée sur les jeunes stades de développement du Têtard et du Poulet. Chez le Têtard, un traitement précoce de la thyroïde a entrainé une différenciation précoce de l'hémoglobine. Le présent travail a été effectué pour déterminer le rôle de la thyroïde sur la même transformation (depuis l'hémoglobine embryonnaire (EHb) jusqu'à l'hémoglobine adulte (AHb)) chez le Poulet. A différents moments, avant et pendant la période de synthèse normale de l'AHb (6 jours), on a injecté, dans la poche d'air, de la thyroxine, $^{131}$I, thiourée ou du tapazole. Différents paramètres érythropoïétiques ont été mesurés après 5, 6, 7, 8 et 9 jours pour déterminer l'effet de la thyroïde. Etant donné la relation connue qui existe entre l'hormone de croissance et la thyroxine pendant le développement, l'hormone de croissance a été administrée séparément et simultanément avec la thyroxine, mais n'a jamais eu d'effets sur la différenciation érythropoïétique. Les résultats montrent que malgré la présence de fonction thyroidienne dans l'embryon à de jeunes stades embryonnaires, la différenciation de l'hémoglobine, le nombre des érythroblastes, les figures mitotiques et les comptages différentiels ne dépendent pas de la fonction thyroidienne ni chez le poulet ni chez $R$. catesbeiana. Il est suggéré que le (ou les) mécanismes qui font démarrer la différenciation de l'hémoglobine chez le poulet, sont d'origine non-endocriniennes.
Chick hemoglobin differentiation

REFERENCES


