Effect of hypophysectomy by partial decapitation and treatment with thiourea on iodine metabolism in the developing chick embryo

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SUMMARY

The hypothesis of an action of the pituitary gland of the developing chick embryo in the transfer of iodide from the yolk of the egg to the circulation of the embryo, through the yolk sac, was tested. Plasma iodide levels and thyroidal iodine contents were determined in hypophysectomized (by partial decapitation), thiourea-injected and control embryos. From day 11 of incubation these parameters were always lower in the 'hypophysectomized' embryos than in controls, and plasma iodide levels of the thiourea-treated embryos were higher than those of controls. These results indicate a reduced iodide transfer from the yolk to the 'hypophysectomized' embryo, and an increased iodide transfer to the thiourea-treated embryo. This occurred in spite of a reduced thyroid hormonal secretion in both series. The pituitary gland could therefore have a direct action (not through the thyroid gland) at the yolk sac level, to augment the transfer of iodide from the yolk in intact embryos from day 11 to the end of incubation. Thyroid-stimulating hormone (TSH) could be the pituitary hormone acting at the yolk sac level, the increased iodide transfer observed in the thiourea-injected embryos being due to a raised TSH secretion responding to the decreased plasma thyroxine levels.

INTRODUCTION

Iodine is present, in low concentrations, in the thyroid gland of the chick embryo from day 7 of incubation. From day 10 the iodine content of the thyroid increases until the end of incubation on day 20 (Daugéras & Lachiver, 1972; Daugéras, Brisson, Lapointe-Boulu & Lachiver, 1976). Iodine comes from the yolk where it is accumulated during oogenesis essentially in the form of iodide (Daugéras-Bernard & Lachiver, 1980a). During the incubation of the egg, this iodide passes into the circulation of the embryo after traversing the yolk sac, the endomesoblastic extraembryonic membrane enclosing the yolk. The plasma iodide concentration of the embryo also increases from day 10 to 20 of incubation, suggesting that increasing amounts of iodide are transferred to the embryo from the yolk through the yolk sac (Daugéras-Bernard & Lachiver, 1980a).

The beginning of the increase in thyroid gland iodine and in plasma iodide levels coincides on day 10 with the establishment of the pituitary-thyroid axis in the chick embryo (Tixier-Vidal, 1958). Such data suggest that the embryonic pituitary gland could act on the thyroid gland and on the yolk sac increasing the iodide supply from yolk to embryo from day 10 on.

To validate this hypothesis the effects of hypophysectomy of the embryo on plasma iodide levels and on total iodine accumulation in the thyroid gland throughout incubation were studied. Studies on the role of the pituitary gland in iodine metabolism in the chick embryo have previously been limited to the uptake of radioiodide by the thyroid gland (Maraud, Stoll & Blanquet, 1957; Hámori, Mess & Székely, 1959; Mess & Straznicky, 1964; Maraud, Audine & Stoll, 1975). Although Thommes & Pall (1973) reported a fall in plasma...
iodide concentrations in the chick embryo after hypophysectomy, no quantitative data were given. The pattern of plasma iodide concentrations in embryos hypophysectomized by partial decapitation was also compared with that in embryos in which the activity of the thyroid gland was blocked with thiourea.

Preliminary reports of this work have been reported (Lachiver, Daugèras & Boulu, 1976; Daugéras-Bernard & Lachiver, 1980b).

MATERIALS AND METHODS
Eggs from white Leghorn hens were incubated at 37.8 ± 0.3 °C.

Hypophysectomy by partial decapitation
Hypophysectomy was carried out by partial decapitation of embryos at 36 h of incubation according to the method of Fugo (1940). This method is the most effective for removal of the influence of the adenohypophysis in the chicken embryo (Betz, 1975). Sham-operated embryos (controls) underwent the same experimental manipulation but were not actually decapitated.

Injection of thiourea
A sterile solution of 0.07 m-thiourea (0.5 ml) in 0.05 m-NaCl was injected into the yolk sac on day 6 of incubation. Control embryos received 0.5 ml of a sterile solution of 0.12 m-NaCl.

Time of death and determination of the concentration of iodine in the plasma and thyroid gland
‘Hypophysectomized’ embryos and controls were killed between days 9 and 18 of incubation. Thiourea-treated and control embryos were killed between days 8 and 15 by which latter time histological changes in the thyroid gland indicated that the effect of thiourea treatment was no longer apparent (Tixier-Vidal, 1958). The age of each embryo was expressed in days of incubation in agreement with the table of development in Hamburger & Hamilton (1951).

As the eyes, upper beak and the other derivatives of the prosencephalon are missing in partially decapitated embryos, each embryo of the operated and control series was completely decapitated to make the weight of normal and operated embryos comparable.

Thyroid glands were removed and blood collected from the vitelline artery using heparin as an anticoagulant (heparin was a gift of Laboratories Fournier, Paris, and was iodine-free). The plasmas were separated by centrifugation and, with the thyroid glands, were stored at −25 °C before analysis.

Thyroid glands from control and experimental embryos of 8, 9 and 10 days of age were pooled and the iodine contents measured directly. From day 11 of incubation thyroid glands were dissolved in 0.2 ml 2M-NaOH, evaporated to dryness at 100 °C and then diluted in 0.5 ml twice-distilled water in the case of partially decapitated or thiourea-injected embryos, or in 2–5 ml water in the case of control embryos. Measurements were then carried out on 20, 50 or 100 μl samples.

Plasma iodine, which occurs essentially in the form of iodide (Daugéras-Bernard & Lachiver, 1980a), was measured in 8–10-day-old embryos in 100–300 μl samples of a pool of plasma. From day 11 onwards, iodine was measured in 20–50 μl of individual plasma samples.

Iodine was measured by a semi-automatic colorimetric method in a Technicon-Analyser after mineralization by a mixture of sulphuric, nitric and perchloric acids. Details of the method have been described previously (Daugéras & Lachiver, 1972; Daugéras et al. 1976).
Measurement of plasma levels of thyroid hormones

Thyroxine (T₄) and tri-iodothyronine (T₃) concentrations were measured each day, from day 10 to day 15, in the pooled plasmas of thiourea-injected embryos and controls by radioimmunoassay (Daugères-Bernard, Leloup & Lachiver, 1976). The detection limits of these assays were determined according to Ekins (1974) and the index of precision according to Midgley, Niswender & Rebar (1969). They were, respectively, 2 pg and 0·05 pg for T₃ and 4 pg and 0·02 pg for T₄.

RESULTS

Partially decapitated embryos

The mean weight of hypophysectomized embryos was approximately 88% of controls but the difference between the two groups was not statistically significant at any age. In the two groups growth followed a power function of the form \( W = Kt^a \) (where \( W \) = body weight in g, \( K \) = a constant, \( t \) = number of days of incubation and \( a \) = the exponent). For controls, the growth curve was expressed by \( W = 8 \times 10^{-5} t^{1·16} \) (\( r = 0·97, P < 0·01 \)) and for hypophysectomized embryos by \( W = 13 \times 10^{-5} t^{3·96} \) (\( r = 0·96, P < 0·01 \)); the difference between the two exponents was not significant.

Mean values for the weight and iodine content of the thyroid glands and for plasma iodide concentrations in hypophysectomized and sham-operated embryos are shown in Table 1. Thyroid weight increased from 0·45 mg on day 9 to 3·74 mg on day 18 in controls whereas in operated embryos it reached a weight of 0·91 mg on day 13 and did not change significantly thereafter. There was no difference between the thyroid weights of the two groups on days 9 and 10. From day 11 of incubation, however, the differences were highly significant (\( P < 0·01 \)).

Table 1. Iodine content of thyroid glands and plasma iodide concentrations of control (C) and hypophysectomized (HPX) chick embryos. From day 11 values are means ± S.E.M.; the numbers of embryos in each group are given in parentheses

<table>
<thead>
<tr>
<th>Age of embryos (days of incubation)</th>
<th>Thyroid weight (mg)</th>
<th>Thyroid total iodine (as nmol I)</th>
<th>Plasma iodide (nmol I/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>HPX</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0·45(11)</td>
<td>0·45(8)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0·59(6)</td>
<td>0·62(7)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0·87±0·08</td>
<td>0·61±0·03**</td>
<td>0·16±0·028</td>
</tr>
<tr>
<td>12</td>
<td>1·46±0·15</td>
<td>0·82±0·08**</td>
<td>0·62±0·08</td>
</tr>
<tr>
<td>13</td>
<td>1·28±0·11</td>
<td>0·91±0·13**</td>
<td>1·04±0·23</td>
</tr>
<tr>
<td>14</td>
<td>2·76±0·44</td>
<td>0·95±0·08**</td>
<td>3·13±0·92</td>
</tr>
<tr>
<td>15</td>
<td>2·94±0·37</td>
<td>0·96±0·19**</td>
<td>10·01±2·28</td>
</tr>
<tr>
<td>18</td>
<td>3·74±0·68</td>
<td>1·17±0·14**</td>
<td>17·4±4·13</td>
</tr>
</tbody>
</table>

\* \( P < 0·05 \), \** \( P < 0·01 \) compared with controls on the same day of incubation (Student's \( t \)-test).

From day 11, the iodine content of the thyroid of intact embryos increased to 17·4 nmol (2·2 µg)/gland by day 18. The iodine content of the thyroid of operated embryos was always significantly lower (\( P < 0·01 \)) than that of controls and reached a maximum of only 0·39 nmol (50 ng)/gland. By day 18, the concentration was only 0·34 mmol/kg (43 ng/mg) of tissue in decapitated embryos whereas it was 4·6 mmol/kg (591 ng/mg) in controls.
Plasma iodide levels also increased much more rapidly after day 10 in intact than in hypophysectomized embryos and, by day 18, the concentration was twice as high in controls, 3717 nmol/l (47·2 µg/dl) as in operated animals, 1583 nmol/l (20·1 µg/dl), P < 0·01.

**Thiourea-injected embryos**

Mean values for the iodine content of the thyroid gland and plasma iodide levels are given in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Iodine content of thyroid glands and plasma iodide concentrations of thiourea-injected chick embryos. On days 8 and 10 of incubation the data are from a pool of similarly aged individuals. From day 11 values are means ± S.E.M.; the numbers of samples are given in parentheses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age of embryos (days of incubation)</strong></td>
</tr>
<tr>
<td>Controls</td>
</tr>
<tr>
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</tr>
<tr>
<td>8</td>
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<tr>
<td>10</td>
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<td>11</td>
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<td>12</td>
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<td>14</td>
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<tr>
<td>15</td>
</tr>
</tbody>
</table>

*P < 0·05, **P < 0·01 compared with controls on the same day of incubation (Student's t-test).

The quantity of iodine in the thyroid was the same in controls and treated embryos on days 8 and 10 but, from day 11 onwards, it was significantly (P < 0·01 or P < 0·05) greater (except on day 12) in controls than in the experimental group.

Plasma iodide levels were similar in the two groups on days 8 and 10. They increased in control and treated embryos from day 11, but were already higher in the treated embryos by day 11 and remained significantly raised until day 15.

Plasma T₄ concentrations did not differ on day 10 in control and treated embryos (0·19 nmol/l). Levels in controls rose from 0·5 nmol/l on day 11 to 1·8 nmol/l on day 15. In thiourea-injected embryos, T₄ levels, which were always lower than in controls (0·17 nmol/l on day 11), remained constant from day 12 to day 15 (0·5 nmol/l). Tri-iodothyronine was never detected.

**DISCUSSION**

A reduction in the weight of hypophysectomized embryos has been reported previously (Fugo, 1940; Case, 1952; Vogel, 1957, 1965; Love & Königsberg, 1958; Betz, 1967, 1968). Our results can only be compared with those of Case (1952) and Love & Königsberg (1958) who weighed the headless bodies of normal and hypophysectomized embryos during the second half of incubation. Case (1952) reported that on day 18 the body weight of the ‘hypophysectomized’ embryos was 77% of that of controls whereas in the present experiments it was 88%o. A greater retardation of growth was reported by Love & Königsberg (1958): on day 18, body weight of hypophysectomized embryos was 63% of that of controls (minus head). The difference between the results could be explained by the use of eggs from a different strain of fowls by Love & Königsberg (1958).

Atrophy of the thyroid gland after removal of the pituitary gland by partial decapitation has also been reported by Fugo (1940), Betz (1967), Mess & Straznicky (1970) and Maraud.
et al. (1975). The weak accumulation of iodide that we observed in the thyroid glands of embryos without pituitary glands is also in agreement with the low fixation of radioiodide by the gland found in other studies (Maraud et al. 1957; Hámori et al. 1959; Mess & Straznicky, 1964; Maraud et al. 1975).

The increase in plasma iodide concentration of the intact embryos from day 11 and thus of total body iodide, as well as the increase in the iodine content of their thyroids, indicate an increase in the iodide flux from the yolk to the embryo during the second half of incubation. Early hypophysectomy by partial decapitation greatly reduced this transfer of iodide since the plasma iodide level and the thyroidal iodine content of the hypophysectomized embryos were lower than those of controls. These results suggest an action of the pituitary gland, which would stimulate the iodide transfer from the yolk to the embryo by acting at the level of the yolk sac from days 10–11.

This action may be either direct or mediated by the thyroid because the gland shows a very decreased secretory activity after partial decapitation (Daugéras-Bernard et al. 1976; Thommes, Vieth & Levasseur, 1977; Hilfer & Sears, 1980). The embryonic plasma iodide level was not decreased when the secretion of thyroid hormones was blocked by thiourea; on the contrary, it increased. The increased plasma level could be due to the decreased iodine fixation that was seen in the thyroid gland after the treatment with thiourea. A theoretical value for this increase can be calculated from the volume of distribution of iodide in the chick embryo body (N. Daugéras-Bernard & F. Lachiver, unpublished data); such values are always less than those actually observed, indicating that thiourea treatment increases the net transfer of iodide from the yolk to the embryo. This increase occurs in spite of the decrease in \(T_4\) and absence of \(T_3\) in the plasma of these embryos. It is thus unlikely that the pituitary gland could act by means of thyroid hormones, at the level of the yolk sac, to augment iodide transfer from the yolk to the embryo. This would not be in agreement with the results of Thommes & Pall (1973), which indicated an augmentation of serum iodide levels in both hypophysectomized and intact embryos injected with \(T_4\), and suggested a role for the thyroid hormones in the regulation of serum iodide levels. However deiodination of the injected \(T_4\) could account for the increased serum iodide levels.

The marked and simultaneous increases in iodine content of the thyroid gland and plasma iodide levels of the intact chick embryos on day 11 and their modifications by either hypophysectomy or thiourea treatment suggest that thyroid-stimulating hormone (TSH) has an action both on the thyroid gland and on the yolk sac; thus, the increased plasma iodide levels measured after thiourea injection could be due to raised TSH concentrations provoked by a fall in thyroid hormone levels. Such an increased TSH secretion responding to decreased plasma \(T_4\) levels in 10-5- and 11-5-day-old thiourea-treated embryos is reported by Thommes & Tonetta (1979) and morphological and histological changes in the chick embryo thyroid gland after thiourea treatment are usually interpreted as being the results of a raised TSH secretion (see references in Daugéras, 1971). Thyroid-stimulating hormone could therefore act on the transfer of iodide between the yolk and the embryo, at the level of the yolk sac, this transfer being reduced in hypophysectomized and increased in thiourea-treated embryos.

In the model of iodide bidirectional exchanges between the two compartments, yolk and embryo, which we have already proposed (Daugéras-Bernard & Lachiver, 1980a, b), we have suggested a passive transport from the yolk sac to the embryo and an active transport from the embryo to the yolk sac, as early as days 7 or 8 of incubation. In intact embryos the pituitary gland, probably through the secretion of TSH, could modify, from days 10–11, the bidirectional exchanges of iodide across the yolk sac by increasing the passive transport to the embryo and/or by decreasing active transport back to the yolk. In hypophysectomized embryos these transport mechanisms persist even after days 10–11 since the partial decapitation reduces but does not eliminate the transfer of iodide from yolk sac to embryo. The mechanisms involved in this change are currently under study.

In conclusion, the pituitary gland, probably through TSH, may be involved in the iodine metabolism of the developing chick embryo by regulating the iodide supply to the thyroid.
gland from days 10–11 of incubation, at the time when the pituitary-thyroid axis is established.

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REFERENCES


