Iodine metabolism and the effect of TSH in thyroid glands of early bovine embryos

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Abstract. The ontogeny of the thyrotrophin-thyroid axis during the first trimester was studied in 104 bovine embryonic thyroids taken from foetuses of crown-rump length 1.4 to 19.2 cm (25–120 days). The uptake of labelled iodine in vitro in the absence or presence of TSH was measured. The per cent incorporation of radioiodine into iodotyrosines and iodothyronines in the presence and absence of TSH was also studied. It was found that the foetal tissue displayed radioiodine uptake by 25 days of foetal life and the uptake increased with age. TSH caused a further increase in radioiodine uptake in foetuses of 40 days or older. Incorporation of radioiodine into MIT and DIT was apparent at 25 days and into T3 and T4 by 40 days of foetal life. Addition of TSH increased the proportion of total radioiodine found as MIT and DIT in foetuses of 40 days older. This TSH stimulation of radioiodine incorporation increased with age. However, the proportion of radioiodine found as MIT and T3 was not affected before 120 days of foetal life. This was in marked contrast to the adult thyroid where the proportion of radioiodine found as T3 was increased by the addition of TSH. It is concluded that the foetal thyroid can respond to TSH by at least 40 days of foetal life and that this response differs from that seen in the adult.

Considerable data on the ontogeny of the pituitary-thyroid axis is available for rat, sheep, and human (Shepard 1967; Fisher et al. 1977). It has been found that the gland develops in distinct histological stages progressing from pre-colloid to follicular. It is not clear if the growth of the foetal gland or the formation of thyroglobulin require TSH although it has been reported that hormone synthesis and iodine uptake are TSH dependent (Jost 1966). However, the ontogeny of the TSH-thyroid axis during the first trimester has not been fully described in any species nor has the foetal bovine thyroid been studied in any depth.

In a previous study (Avivi et al., in press) on the thyroid function of the early bovine thyroid, it was found that embryos as small as 1.2 cm (25 days) crown-rump length (CRL) contained radioimmunoassayable amounts of both T4 and T3 (4 and 6 ng/lobe, respectively). The amount of these hormones increased steadily with gestation and by 120 days, 45 ng/lobe of T4 and 2.2 ng/lobe of T3 were measured. The thyroid hormone content in these experiments was measured directly in tissue homogenates without pronase digestion. Addition of TSH to in vitro incubations of thyroid slices, induced a 2–3-fold increase in secretion of T4, but not of T3, by glands from foetuses of 3.0 to 25.0 cm (40–130 days) CRL.

In the present study we investigated the effect of TSH on labelled iodine uptake by the bovine foetal thyroid during the first trimester (25–120 days). The incorporation of labelled iodine into iodotyrosines and iodothyronines was also studied.
Materials and Methods

Animals

One hundred and four bovine embryos from the 1st trimester of pregnancy ranging in size from 1.4 to 19.2 cm CRL (25 to 120 days of gestation) were obtained from Holstein-Friesian cows with a gestational period of 275 days. Embryos were collected about 20 min after slaughter and placed immediately in chilled medium. Embryos collected more than 60 min after slaughter were not used as it was found that there was a 50% decrease in the ability of their thyroids to concentrate iodine in vitro. Chronological age was estimated using curves for CRL from Evans & Sack (1973). When embryos of CRL < 8.0 were used, the thyroid gland was cultured with the surrounding tissue as it was impossible to detach it from its cartilage. In the present report the terms embryo and fetus are used interchangeably.

Organ culture

Tissue fragments (5—15 mg) were placed in 1 ml of medium in glass tubes (70 x 100 mm) and shaken for 3½ h in a water bath at 37°C. A control piece of tissue of comparable size, usually neck muscle or tongue, was obtained from the same foetus and similarly incubated. Each test tube was supplemented with 1—2 μCi of Na[I^125I] (Amersham, Buckinghamshire). TSH (NIH-TSH-S8), ovine, National Pituitary Agency, Maryland) was added in a concentration of 0.5 mU/ml unless otherwise noted. This was the concentration previously observed to give optimal secretion of endogenous T4 (Avivi et al., in press). The medium used was medium 199 with Eagle's salts and 25 mM Hepes buffer (Gibco, Grand Island NY) pH 7.4, with 5% calf serum and gentamycin (50 μg/ml).

Proteolytic digestion

At the end of the incubation period, the thyroid tissue was transferred to saline to free the tissue fragments from unbound I^125I in the medium. Tissue was mildly agitated in 1 ml of saline for 15 min, removed with forceps and blotted on filter paper. Three such washings were found to remove 95—99% of unincorporated I^125I that might contaminate the thyroid fragments. The tissue was then homogenized and digested with pronase (50 μl of a 3.5% solution) in the presence of 0.05 M 1-methyl-2-mercaptoimidazole which served as a deiodination inhibitor. The digestion was carried out anaerobically at 37°C for 18 h in a manner similar to that described in detail by Inoue & Taurog (1967).

Biochemical studies

The digestion mixture was centrifuged for 15 min at 3000 r.p.m. at 4°C. The resulting supernatant was lyophilized and re-dissolved in ethanol:NH_4OH (1:1 v/v). Separation of iodine containing compounds namely T_4, T_3, monoiodotyrosine (MIT) and diiodotyrosine (DIT) was performed by thin layer chromatography (TLC) on silica gel. The plate was run in a developing system of tertiary-ethyl alcohol, acetone and 2 N NH_4OH (25:8:7 v/v) containing 0.1% sodium thiosulphate as described by Shapiro & Gordon (1966).

Radioactive spots on the chromatographs were identified by the addition of cold carriers of MIT, DIT, T_3 and T_4. They were located by spraying the plate with a mixture of 0.2% ninhydrin in a solvent consisting of 5% acetic acid in n-butanol. Nearly all the radioactivity (Table 2) was present either as I^- (R_f = 0.78) or as MIT (R_f = 0.28), DIT (R_f = 0.22), T_3 (R_f = 0.53) and T_4 (R_f = 0.42). About 2% was present as unidentifed material in the origin. Na[I^125I] spotted in a separate lane was used to locate I^-.

Since there was no significant radioactivity after the third wash (< 5%) following incubation, it was assumed that the I^- present on the plate represents intrathyroidal iodine and not contamination from the media. The plate was then divided into 0.5 cm width bands scraped into tubes and counted in a gamma counter (Packard, Model 5320).

Fig. 1.

Time course of in vitro I^125I uptake by thyroid gland slices of bovine embryos (15—18 cm CRL) in the presence and absence of TSH (0.5 mU/ml). Time zero corresponds to the time of TSH addition (1 h after I^125I was added). The values of I^125I uptake are expressed a log of the T/M ratio. Each point represents the mean ± SE of 5 different embryos.
Results

Fig. 1 illustrates the time course of labelled iodine uptake by bovine embryonic thyroid (90—120 days) in the presence or absence of 0.5 mU/ml TSH. Following a lag period of 2 h, the uptake of labelled iodine was significantly elevated. In the presence of TSH, there was a significant decrease in radioiodine uptake during the lag period, but iodine uptake was significantly raised above the control by 3 h.

Fig. 2 illustrated the effect of the concentration of TSH on radioiodine uptake over the range of 0.1 to 2 mU/ml. Radioiodine uptake into the tissue required concentrations of 0.3 mU/ml, or higher. Table 1 shows the radioiodine uptake by bovine embryonic tissue following a 3½ h incubation. It increased 5-fold as gestation progressed from 40 to 120 days (3.0—19.2 cm) and in the presence of TSH it was significantly elevated even when only the primordium could be seen histologically. Table 2 shows the per cent distribution of the 125I-labelled iodothyronines and iodothyronines in embryonic thyroid from 25 to 120 days of gestation (1.4—19.1 cm CRL). Tissues were incubated for 3½ h in the presence or absence of 0.5 mU/ml TSH. The earliest sign of thyroid activity was the incorporation of labelled iodine into MIT and DIT which occurred even before the histological primordium appeared. Incorporation into T3 and T4 was low throughout the period studied, but traces (0.5%) were apparent at the time of primordium formation. Although the per cent of incorporation into T3 and T4 increased slightly with age, the ratio of the compounds was constant. Similarly, there was no major change in the ratio of DIT to MIT during follicular maturation. However, the total percentage of radioactivity recovered as iodoamino acids increased from 8.2% in undifferentiated neck tissue at 30 days to 60.3% by 120 days. Control tissue contained non-detectable amounts (<0.1%) of labelled compounds and the T/M ratio was consistently lower than 1 (0.6—0.8).

Addition of TSH (0.5 mU/ml) increased incorporation of 125I into DIT and thyroxine at all stages of development after the primordium was formed. In contrast, little or no effect was seen on the per cent of radioiodine incorporated into MIT or T3.

In order to determine how the incorporation into iodoamino acids was related to the lag period noted in Fig. 1, bovine embryonic thyroid (90—120 days) was incubated for 30 min, and both radio-

Analysis of results

Specific uptake of radioiodine was expressed as the ratio of radioactivity (CPM) between the tissue (T) and the medium (M). T/M was calculated as CPM per 1 g of tissue divided by CPM per 1 ml medium. 125I was considered as specifically incorporated when T/M was found to be greater than 1. The distribution of incorporated radioiodine into the 4 different thyroid iodoamino acids which were studied was expressed as the percentage of the total CPM in the sample applied to the TLC plate. All data were subjected to Student’s t-test.

Histology

Particular stages of histological differentiation corresponding to different CRL ranges of the embryos as shown in the headings in Tables 1 and 2 are based on previously reported histological studies (Avivi et al., in press). These studies are in agreement with the histological observations of Koneff et al. (1949).
iodine uptake and incorporation measured. Table 3 illustrates that the decrease in iodine uptake was unrelated to the incorporation of radioiodine into DIT and thyroxine. Both compounds were significantly elevated after 30 min of incubation in spite of a net decrease in radioiodine uptake.

**Discussion**

The present experiments demonstrate that $^{125}$I uptake and incorporation of radioiodine into MIT and DIT by the bovine foetal thyroid is apparent by at least 25 days of gestation (primordial stage). Furthermore, incorporation of all four iodoamino acids studied were demonstrable prior to the formation of colloid (75-85 days). Both radioiodine uptake and incorporation into iodoamino acids increases steadily with age. This is in agreement with the work of Koneff et al. (1949) who demonstrated the presence of significant amounts of thyroxine and stable iodine in the bovine thyroid at 53-80 days (pre-colloidal) and that the quantity of these substances increased with age. It is in contrast, however, to the work of Shepard (1967) who found no evidence of iodoamino acid formation or

<table>
<thead>
<tr>
<th>Thyroid stage</th>
<th>Neck only</th>
<th>Primordium</th>
<th>Pre-colloid</th>
<th>Colloid beginning</th>
<th>Follicular</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRL cm</td>
<td>1.4-2.9</td>
<td>3.0-7.9</td>
<td>8.0-11.9</td>
<td>12.0-14.9</td>
<td>15.0-19.2</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>16</td>
<td>24</td>
<td>21</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>T/M</td>
<td>1.1 ± 0.1</td>
<td>1.25 ± 0.1</td>
<td>2.4 ± 0.15*</td>
<td>3.7 ± 0.1*</td>
<td>5.6 ± 0.1*</td>
<td>9.1 ± 0.2*</td>
</tr>
<tr>
<td>+ TSH</td>
<td>1.2 ± 0.15</td>
<td>2.0 ± 0.1**</td>
<td>4.1 ± 0.2**</td>
<td>6.8 ± 0.25**</td>
<td>10.9 ± 0.4**</td>
<td>26.7 ± 1.6**</td>
</tr>
</tbody>
</table>

* Significantly greater than preceding stage, at $P < 0.001$.
** Significantly greater than corresponding stage without TSH at $P < 0.05$.

Table 2.

Distribution of labelled iodine metabolites in thyroid glands from bovine embryos (30-120 days) incubated for 3½ h in the presence or absence of 0.5 mU TSH.

<table>
<thead>
<tr>
<th>Thyroid stage</th>
<th>Neck only n = 12</th>
<th>Primordium n = 17</th>
<th>Pre-colloid n = 24</th>
<th>Colloid beginning n = 24</th>
<th>Follicular n = 15</th>
<th>Adult n = 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without TSH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIT</td>
<td>6.2 ± 0.4</td>
<td>9.2 ± 0.2</td>
<td>22.2 ± 4.6</td>
<td>27.2 ± 3.2</td>
<td>31.1 ± 3.1</td>
<td>34.1 ± 2.1</td>
</tr>
<tr>
<td>DIT</td>
<td>2.0 ± 0.1</td>
<td>5.1 ± 0.6</td>
<td>12.7 ± 1.6</td>
<td>19.8 ± 1.5</td>
<td>21.1 ± 1.7</td>
<td>24.2 ± 1.6</td>
</tr>
<tr>
<td>T$_3$</td>
<td>-</td>
<td>0.3 ± 0.1</td>
<td>1.2 ± 0.7</td>
<td>3.1 ± 0.4</td>
<td>4.1 ± 0.8</td>
<td>5.0 ± 1.0</td>
</tr>
<tr>
<td>T$_4$</td>
<td>-</td>
<td>0.2 ± 0.05</td>
<td>1.0 ± 0.4</td>
<td>3.3 ± 0.2</td>
<td>4.0 ± 0.6</td>
<td>25.7 ± 0.8</td>
</tr>
<tr>
<td>I$^-$</td>
<td>88.7 ± 0.9</td>
<td>84.1 ± 1.2</td>
<td>58.6 ± 3.4</td>
<td>40.3 ± 2.1</td>
<td>33.9 ± 1.7</td>
<td>10.0 ± 3.4</td>
</tr>
<tr>
<td>Origin</td>
<td>2.3 ± 0.2</td>
<td>1.4 ± 0.4</td>
<td>1.0 ± 0.1</td>
<td>2.2 ± 0.8</td>
<td>0.7 ± 0.1</td>
<td>3.5 ± 1.1</td>
</tr>
<tr>
<td>With TSH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIT</td>
<td>5.9 ± 0.1</td>
<td>9.7 ± 0.3</td>
<td>20.0 ± 3.7</td>
<td>29.1 ± 2.7</td>
<td>30.0 ± 4.2</td>
<td>15.4 ± 1.2*</td>
</tr>
<tr>
<td>DIT</td>
<td>2.9 ± 0.2</td>
<td>12.1 ± 1.2*</td>
<td>21.1 ± 3.4*</td>
<td>39.5 ± 2.0*</td>
<td>42.2 ± 2.7*</td>
<td>28.4 ± 3.4</td>
</tr>
<tr>
<td>T$_3$</td>
<td>-</td>
<td>0.3 ± 0.05</td>
<td>1.4 ± 0.5</td>
<td>3.0 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>10.4 ± 1.3*</td>
</tr>
<tr>
<td>T$_4$</td>
<td>-</td>
<td>0.6 ± 0.1*</td>
<td>3.1 ± 0.7*</td>
<td>11.1 ± 1.1*</td>
<td>17.2 ± 3.1*</td>
<td>43.2 ± 3.2*</td>
</tr>
<tr>
<td>I$^-$</td>
<td>91.0 ± 1.2</td>
<td>76.3 ± 1.9</td>
<td>50.2 ± 2.2</td>
<td>14.4 ± 2.3*</td>
<td>7.2 ± 0.5*</td>
<td>4.7 ± 1.1*</td>
</tr>
<tr>
<td>Origin</td>
<td>1.8 ± 0.6</td>
<td>1.6 ± 0.7</td>
<td>2.1 ± 0.4</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>1.5 ± 0.3</td>
</tr>
</tbody>
</table>

* Per cent of total radioactive iodine recovered on TLC. * Significantly different than without added TSH, at $P < 0.05$.  

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iodine uptake prior to colloid formation (74 days) in human embryonic thyroids.

The effect of TSH on radiiodine uptake by the foetal thyroid is apparent by 40–75 days of gestation. Significant elevation of radiiodine incorporation into DIT and thyroxine following the addition of TSH is also evident prior to colloid formation. Since we could not find any evidence of pituitary thyrotrophs prior to 90 days (Avivi et al., in press), it appears that the thyroid is responsive to TSH before the appearance of foetal TSH.

TSH has a negative effect on radiiodine uptake at suboptimal concentrations or incubation times. This biphasic effect has been also reported in the adult by Halmi et al. (1960) and Knopp et al. (1970) who showed an initial negative membrane effect of TSH on iodine uptake mediated through RNA and cAMP. However, the present study indicates that foetal thyroid incorporation of labelled iodine into iodoamino acids is not impaired during the initial apparent depression of iodine uptake. Also Tong (1969) did not report any evidence of a lag time for iodoamino acid formation in the adult bovine thyroid. Halmi et al. (1960) and Grollman et al. (1977) indicated that this pattern of response to TSH involves a rapid hyperpolarization of the thyroid membrane which causes a dramatic increase in its permeability to anions. Hence, there is drastic outward diffusion of iodide together with a slowly developed augmentation of the inwardly directed iodide pump.

The foetal bovine thyroid slices used in our study did however, differ from the adult in one important aspect. Addition of TSH in the adult resulted in an increased incorporation of radiiodine into T₃ while the foetal thyroid showed no such response. Since it is generally agreed that nearly all the T₃ in the foetus is of thyroidal origin (Chopra et al. 1975) it seems possible that the serum T₃ deficiency characteristic of foetal sheep and man (Chopra et al. 1978; Fisher et al. 1977) is related to the low synthetic capacity for T₃ by the foetal thyroid as demonstrated in this study, and not solely due to the lack of extrathyroidal metabolism of T₄ (Chopra et al. 1978). Our observation of enhanced T₄ synthesis evoked by TSH may be directly related to the concomitant increase in DIT:MIT ratio. Similar data were reported by Nataf (1968) in the foetal rat where TSH increased iodine uptake and conversion of MIT to DIT by the foetal thyroid in organ culture. Moreover, when the level of thyroglobulin iodination increases not only total iodotyrosine but also the DI/T ratio increases noticeably (Inoue & Tarog 1968; Pitt-Rivers 1966). Under these conditions T₄ formation is favoured and the coupling efficiency for T₃ decreases. Furthermore, they also demonstrated that when the level of DIT in the thyroglobulin attains some critical value, there is favoured utilization of DIT for T₄ formation rather than for T₃ formation. Hence, we propose that the increased radiiodine uptake and DI/T ratio evoked by TSH in the foetal bovine thyroid plays a role in the increase in formation of T₄ and not T₃. The role of thyroid hormones in foetal development is not known. Nathanielsz (1975) reported that the ovine embryonic gland is necessary for proper development of the lungs and nervous system and also critical for the survival of the newborn. Since the iodothyronines do not cross the placenta (Dussault et al. 1972), T₃ concentrations in the foetal blood is very low and there is no extrathyroidal conversion of T₄ into T₃ during pre-natal life (Chopra et al. 1978), it is possible that thyroxine is the major active thyroid hormone in the foetus as opposed to adult where T₃ is the active hormone (Oppenheimer et al. 1979). Some support for this concept may be derived from the recent report of Galton & Cohen (1980) that T₄ plays a more important role as an active hormone in the pre-metamorphic tadpole than T₃. Alternatively, Chopra et al. (1978) have suggested that the benefit of low T₃ in the foetus is to prevent the catabolic effects of T₃.

The ability of the foetal thyroid to respond to TSH before the differentiation of the pituitary may simply reflect the maturation of the receptors prior to that of the stimulus. Alternatively, these foetal

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**Table 3.**

Incorporation of labelled iodine into iodoamino acids after 30 min of incubation during the time TSH has a negative effect on iodine uptake.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+ TSH</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/M</td>
<td>1.4*</td>
<td>0.35</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>MIT</td>
<td>10.1 ± 0.2**</td>
<td>9.8 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>DIT</td>
<td>4.7 ± 0.3</td>
<td>7.8 ± 0.9</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>T₃</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>T₄</td>
<td>0.9 ± 0.2</td>
<td>5.2 ± 0.7</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

* Tissue to media ratio, n = 11.
** Per cent incorporation.
thyroid TSH receptors may respond to a TSH-like substance present in the bovine placenta (Avivi & Shemesh, unpublished observations). It is interesting to note in this regard, that the bovine ovary and testes can also respond to pituitary hormones prior to the differentiation of the pituitary (Shemesh et al. 1978) and that the placenta does contain an hCG-like substance and a LRF-like substance at this time (see Shemesh 1980 for review).

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References


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