PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 106-109 (1978)

Antenatal Diagnosis of Cretin Lambs by Measurement of Amniotic Fluid Thyrotropin[1] (40001)

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Documentation of fetal hyper- or hypothyroidism before birth by measurement of amniotic fluid thyroxine (T_4) iodine by column or triiodothyronine (T_3) by radioimmunoassay (1, 2) has been difficult. Persistent, long-term postnatal effects of insufficient (3, 4) or excessive (5) levels of thyroxine *in utero* have been described in children. Since these developmental problems may not be fully reversed by treatment after birth, the search continues for feasible diagnostic measures to detect thyroid disorders *in utero*. These experiments were undertaken in sheep to investigate the possibility that amniotic fluid thyrotropin (AFTSH) and 3,3′,5′-triiodothyronine (reverse T_3; rT_3) levels might reflect fetal hypothyroidism.

Materials and methods. Nine crossbred Hampshire, Suffolk, and Rambouillet ewes with dated matings were obtained from the University of Kentucky Ovine Research Farm and maintained at environmental temperatures of 22–24° with free access to water and Purina Omolene grain containing iodized salt. *In utero* fetal thyroidectomy was performed in six pregnant ewes at 50–93 days of gestation by the microsurgical technique previously described (2). Completeness of thyroidectomy was assessed in all experimental animals by later postmortem dissection and histological examination of serial sections of the neck. Eleven normal lambs were studied as controls. These included four twin pregnancies in which only one fetus was subjected to thyroidectomy, with the nonoperated twin in the contralateral noncommunicating uterine horn serving as a control. In the three remaining pregnancies in which no fetal thyroidectomies were performed, there were two sets of normal twin lambs and one set of normal triplets.

At the time of surgery amniotic fluid was aspirated preoperatively from each noncommunicating horn of all animals for TSH, T_4, and T_3 determinations. At Caesarean section amniotic fluid was aspirated with an 18-gauge needle by amniocentesis from each uterine horn before rupture of membranes and delivery. In two pregnancies with cretin lambs (one twin pair with an unoperated control) and three normal pregnancies (seven animals including two sets of twins and one set of triplets), rT_3 determinations were also performed.

Delivery was accomplished by Caesarean section at term (143–147 days in this flock) under general anesthesia. Neonatal jugular blood was drawn after cord cutting within 10–60 min of delivery for TSH, rT_3, T_3, and T_4 determinations. Timing of newborn hormone measurements in twins was within 5 min in control and experimental animals.

T_4 was measured by the method of Kessler and Pileggi (6), whereas T_3 was determined by the method of Larsen (7). TSH measurements were done by the method of Borger and Davis (8). The concentration of rT_3 was measured in ethanol extract of amniotic fluid by a previously described radioimmunoassay (9, 10). Cortisol levels in amniotic fluid were determined by the method of Mattingly (11) in five normal and two cretin lambs. *P* values for statistical analysis of the data were obtained by Student's *t* test.

Results. Completely thyroidectomized lambs showed the cretinoid habitus previ-

[1] Supported in part by Grant CRBS-299 from the National Foundation March of Dimes and USPHS Grants AM16155 and HD07830. Presented in part at the Southern Society for Pediatric Research, New Orleans, Louisiana, January 31, 1976.

0037-9727/78/1571-0106$01.00/0

ously described (2). When thyroidectomy was less than total, partial cretins had milder stigmata of congenital hypothyroidism (2).

Table I summarizes the thyroid hormone and thyrotropin findings in amniotic fluid aspirated at term, and in neonatal lamb serum immediately after delivery.

Amniotic fluid studies. Amniotic fluid obtained at surgery during the middle third of pregnancy did not contain measurable amounts of TSH, rT_3, T_3, or T_4.

At term, very low levels of T_4 were found in normal animals (range, 0.8–2.8 μg/dl) and cretins (range, 0.1–1.0 μg/dl). The difference was significant ($P < 0.05$), but overlap in the two groups prevented diagnostic accuracy.

T_3 was not detectable in our assay system at term in any pregnancy. Reverse T_3 in term pregnancies in two cretin animals and five normal controls was <10 ng/dl. In one triplet pregnancy with three normal animals a low level of 13 ng/dl of rT_3 was measured in amniotic fluid from one uterine horn and 28 ng/dl from fluid in the contralateral uterine horn containing two fetuses.

AFTSH was present in low levels (mean, 3.7 ng/ml) in 10 normal animals at term. In contrast, four cretin animals had a mean AFTSH value of 54 ng/dl.

At postmortem examination two additional cretin lambs (28B and 234A) had tiny thyroid remnants (ca. one-quarter normal size). In both instances AFTSH was higher in these partial cretins than in their normal twins (9.3 vs 2.9 ng/ml in 28B and 28A, 4.1 vs 0.7 ng/ml in 234A and 234B; see Table I).

Amniotic fluid cortisol measurements were obtained at term in five normal and two cretin pregnancies. The mean values of 1.6 μg/dl in normals and 1.3 μg/dl in cretins were similar.

Thyroid hormones and thyrotropin in neonatal lamb serum. Normal newborn lambs within 60 min of Caesarean section had a mean T_4 level of 5.4 μg/dl as compared with only 1.2 μg/dl in cretin animals. Aside from one partial cretin (234A) with a level of 4.1 μg/dl (small thyroid remnant was found at autopsy in this case), four athyrotic cretins and one partial cretin had T_4 measurements of <1.0 μg/dl at birth.

T_3 in normal lambs ranged from <25 to 293 ng/dl 10–60 min after birth, with a mean of 114 ng/dl. In contrast, in all cretin lambs values were <70 ng/dl, with a mean value of 37 ng/dl. There was overlap in the

TABLE I. AMNIOTIC FLUID AND NEWBORN SERUM STUDIES OF THYROID FUNCTION IN LAMBS AFTER TOTAL OR INCOMPLETE THYROIDECTOMY *in Utero*.

	Amniotic Fluid				Serum			
	TSH (ng/ml)	T_4 (μg/dl)	rT_3 (ng/dl)	Cortisol (μg/dl)	TSH (ng/ml)	T_4 (μg/dl)	rT_3 (ng/dl)	T_3 (ng/dl)
Normal								
Mean	3.7 (10)[a]	1.6 (9)	<10 (7)	1.6 (6)	1.9 (11)	5.4 (11)	511 (8)	114 (11)
SEM	0.73	0.32	2.5	0.2	0.29	0.51	43	28
Total thyroidectomy								
4B	117	0.8	–	–	210	0.9	–	25
941A	13	0.7	<10	1.3	472	0.8	<10	27
810A	63	0.2	<10	1.3	443	0.4	<10	30
240A	21	0.6	–	–	592	0.6	–	45
Incomplete thyroidectomy								
28B	9.3	1.0	–	–	136	0.7	–	29
28A	2.9	0.9	–	–	2.4	4.9	–	161
Normal twin								
234A	4.1	0.1	–	–	16	4.1	–	69
234B	0.7	0.1	–	–	2.1	4.9	–	58
Normal twin								
P	<0.01[b]	<0.05	NS	NS	<0.005	<0.005	<0.005	<0.1

[a] Numbers of determinations are in parentheses.

[b] *P* values compare normal animals with those confirmed to have total thyroidectomy.

two groups, and the difference between them was not significant ($P < 0.1$).

Serum rT_3 levels were measured in two cretin lambs and found to be <10 ng/dl. Eight normal lambs had very high levels of serum rT_3 at birth, ranging from 400 to 740 ng/dl, with a mean of 511 ng/dl ($P < 0.005$). The high rT_3 levels in normal newborn lambs were in sharp contrast to the mean ewe rT_3 level of 50 ng/dl (range, 24–85 ng/dl) in simultaneously drawn samples from the maternal jugular vein.

TSH levels were low in normal lambs immediately following Caesarean section (mean, 1.9 ng/ml). This was in marked contrast to the mean value of 312 ng/ml in cretin lambs ($P < 0.005$).

All athyrotic lambs and one animal with incomplete thyroidectomy (28B) died of pulmonary failure on the day of birth in spite of intensive neonatal care. Lamb 234A (partial thyroidectomy) died on Day 1 during cardiac function studies.

Discussion. Previous studies in sheep have shown minimal maternal-to-fetal transfer of T_4 and T_3 (12–14). Chopra *et al.* have reported significant levels of rT_3 in human cord blood and in serum of normal newborn lambs (9, 15). It has not been previously possible to document congenital hypothyroidism antenatally in human infants or lambs by measurement of decreased T_3 or T_4 in amniotic fluid (1, 2). Measurements of T_4 in amniotic fluid have not been clearly diagnostic because of the low levels normally present, and T_3 is not detectable in human or ovine amniotic fluid with present assay methods.

In newborn lambs the higher serum T_3 values after cord cutting may reflect the alteration in T_4 to T_3 conversion and the TSH surge which occur within the first hour of life (16).

These studies were undertaken in our well-established model for cretin lambs with the hope that AFTSH and rT_3 might provide a useful diagnostic tool for antenatal thyroid diagnosis. Unexpectedly, rT_3 was not found in ovine amniotic fluid. Ovine amniotic fluid was clearly very different from human amniotic fluid with respect to rT_3 concentration. Very high levels of rT_3 were found, however, in the serum of normal newborns, as observed previously (15). Similarly, as expected, serum rT_3 levels were found to be very low (undetectable) in two newborn cretin lambs. One of these animals had a normal twin in the contralateral horn. This newborn lamb had a serum rT_3 level of 470 ng/dl at birth. The reasons for differences in rT_3 concentration in ovine amniotic fluid compared to human amniotic fluid are not clear. There is a significant difference, however, in placentation between sheep and man. The sheep placenta consists of a group of separate cotyledons instead of a single mass of placental tissue. The information so far available does not suggest a major difference in T_4 monodeiodination between man and sheep (15).

In contrast, significantly elevated levels of AFTSH clearly predicted the birth of athyrotic lambs. The findings in twins were particularly impressive, and in four instances AFTSH clearly distinguished the severely hypothyroid fetus. In two lambs with incomplete thyroidectomy, AFTSH levels were higher than those in their normal twins. When sufficient residual thyroid tissue was present to provide a normal neonatal serum T_4 level (lamb 234A), amniotic fluid values were less helpful diagnostically than neonatal serum TSH levels. Amniotic fluid TSH levels correlated with the severity of hypothyroidism.

Summary. Elevated TSH levels are highly reliable in predicting neonatal lamb hypothyroidism. However, the method is not as sensitive as serum TSH measurement, and mild hypothyroidism can exist with normal amniotic fluid thyrotropin and elevated serum TSH.

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Received September 1, 1977. P.S.E.B.M. 1978, Vol. 157.