PRENATAL AND POSTNATAL DEVELOPMENT OF THYROID FUNCTION IN THE BOVINE

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Marco V. Hernandez

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ABSTRACT

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By

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Thyroid hormones play a very important role in regulating the metabolic processes of farm animals, especially growth, reproduction and lactation. Normal thyroid function during fetal life must have a great influence on fetal development and possibly on the future perfomance of the animal. This study contributes to a better understanding of bovine thyroid physiology during intrauterine and early postnatal life.

Caesarean sections were performed in a total of 40 pregnant cows at either 90, 180 or 266 days of gestation. Maternal blood samples were taken from the jugular vein, uterine artery and uterine vein. Mixed arterial and venous fetal blood samples were taken at 90 days. At 180 and 266 days separate samples were taken from the umbilical artery and umbilical vein. Uterine and umbilical samples were drawn through a polyethylene catheter attached to a 19 or 16 gauge needle. Fetal body weights and trimmed thyroid weights were recorded. In addition, jugular vein samples

were taken from 19 newborn calves at days 1 - 7 after birth.

Blood serum was analyzed for thyroxine (T_4) by a competitive binding method (Tetrasorb I^{125} - Abbott Laboratories). Protein bound iodine (PBI) determinations were also run on the same samples. In the 90 day fetal samples only T_4 was determined.

When the fetal thyroid weights (Y) and the fetal body weights (X) were plotted on log-log graph paper, these parameters were shown to be related by the equation $\log Y = - 0.4582 + 0.9439 \log X$, with a correlation coefficient of 0.9962. The regression coefficient of the equation (0.9439) is not significantly different from 1.0. This demonstrates that from 90 to 266 days of gestation the bovine fetal thyroid gland grows in direct proportion to body growth.

Fetal serum T_4 values in $\mu g/100$ ml averaged 2.18 ± 0.201, 11.66 ± 0.499 and 17.16 ± 0.702 at the first, second and third trimesters. The second trimester T_4 value is more than 5 times ($P < 0.001$) that of the first trimester, and the third trimester value is about one-half higher ($P < 0.001$) than the second. Maternal T_4 values at the same stages averaged 6.67 ± 0.205, 6.42 ± 0.162 and 7.97 ± 0.305 $\mu g/100$ ml. The maternal serum T_4 showed a significant increase ($P < 0.01$) during the third trimester. No significant arteriovenous differences were found in either the fetal or maternal samples at any trimester of gestation.

At the second trimester of gestation 6 female fetuses had a significantly higher absolute thyroid weight of 2.268 ± 0.103 gm than the value for 8 males of 1.752 ± 0.111 gm ($P < 0.001$). At the same stage the fetal female serum T_4 value of 13.28 ± 0.371 µg/100 ml was also significantly higher ($P < 0.01$) than the male serum T_4 value of 10.45 ± 0.693 µg/100 ml.

Mean serum T_4 of neonatal calves was 16.92 µg/100 ml at day 1 and declined exponentially from day 2 - 5 according to the equation, $\log Y = 1.3959 - 0.09014\ X$ ($r = -0.9997$). It appears that T_4 secretion was shut off during this period and the equation represents the degradation of extrathyroidal T_4 released earlier. The fractional degradation rate of 0.1872 daily is about 50% the value reported by Post and Mixner (1961) for 18-day-old calves. At the 7th postnatal day the serum T_4 had a value of 7.29 µg/100 ml.

T_4 values closely paralleled the PBI values throughout this experiment.

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INTRODUCTION

The thyroid gland is a primitive organ that begins to function early in fetal life. Thyroid hormones are essential for embryonogenesis in all vertebrates and continue to play an important role in regulating body functions throughout life. Considerable work has been reported on the development of thyroid function in mammals during the fetal and perinatal periods. Thyroid hormone formation in the fetus begins at about the end of the first one-third of pregnancy in most species. However, there are many differences between species in the time at which thyroid hormone formation begins and also its level in the fetal as compared to the maternal circulation. There is a great deal of evidence that the fetus can supply its own thyroid hormone for some time before birth, but little or no direct proof as to when this hormone begins to have an essential biological function.

Referring specifically to the bovine, no reports have been found on the levels of circulating thyroxine in either the developing fetus or the newborn calf. It was reported by Lewis and Ralston (1953), that calves less than 48 hours old had higher plasma protein-bound iodine (PBI) levels than older animals.

In the research to be reported in this thesis both serum thyroxine (Serum T_4) and PBI were determined on blood serum samples, obtained during Caesarian section, from the uterine and umbilical cord vessels in cows at the first, second and third trimester of pregnancy. Similar determinations were done in serum obtained daily from calves on the first to seventh day after birth.

The data will be discussed in terms of fetal thyroid growth and development, probable maternal and fetal production of thyroid hormones, maternal and fetal utilization, possible placental transfer, and adaptation of thyroid function during the first seven postnatal days.

REVIEW OF LITERATURE

Anatomical Development of the Thyroid Gland.

The thyroid gland is derived from entoderm in the anterior portion of the embryonic alimentary tract. The thyroid anlage appears as a medial unpaired evagination from the floor of the pharynx. The wall of the distal portion of this diverticulum increases in size and by active mitotic division of its cells becomes multi-layered and gradually expands until a bilobal form is reached; in the meantime, the stalked attachment narrows to form the thyroglossal duct. As the diverticulum becomes lobulated it migrates caudally and assumes a position on the anterior ventral surface of the trachea. The thyroglossal duct at the base of the tongue is later obliterated (Boyd, 1964; Turner p. 197, 1967).

In the human, the thyroid anlage can be identified as early as three weeks after conception. By the seventh week of gestation the thyroid has reached the final paratracheal position, and between the seventh and twelfth week the thyroid follicles begin to appear. Colloid appears only in fully organized follicles. (Boyd, 1964; French and Van Wyk, 1964).

Hormonogenesis by the Fetal Thyroid Gland.

Many workers have shown that when radioiodine is injected into pregnant females, it rapidly becomes available to the fetal thyroid. Using this technique, it has been possible to demonstrate that the stage of pregnancy at which thyroid tissue starts to accumulate iodide and synthesize its iodine compounds differs between species (Myant, 1964). In the mouse, (20 day gestation period) the fetal thyroid starts to accumulate iodide from the circulation around the 15th day. The ability to bind this iodide organically and the formation of colloid appears between the 15th and 16th days, whereas the formation of follicles and the ability to produce thyroxine appears between the 16th and 17th days of age (Van Heynigen, 1961).

In the rat, (21-22 day gestation period) the ability of the fetal thyroid to store iodine begins around day 18 to 19 of gestation, which may be correlated with the first differentiation of follicles complete with lumen (Gorbman and Herbert, 1943). At day 21 of gestation the fetal rat is able to synthesize monoiodotyrosine, diiodotyrosine, 3,5,3' triiodothyronine and thyroxine (Geloso, 1956).

In the rabbit, (30 day gestation period) the thyroid anlage appears around the 9th to 10th day of gestation; primitive follicles appear around day 17; at day 19 the fetal thyroid starts to accumulate iodine and between days 20 and 22 the colloid appears in the follicles. At this time the

thyroid starts to synthetize thyroxine (Jost et al., 1949; Waterman and Gorbman, 1956; Myant, 1958a).

In the beagle dog, (63 day gestation period) the fetal thyroid does not secrete thyroxine before the 42nd day of gestation (Beirwaltes, 1967).

The human fetal thyroid has the ability to accumulate demonstrable amounts of iodine by the 80th day and thyroxine has been identified by direct analysis of the gland between the twelfth and fourteenth weeks of gestation (Palmer et al., 1938; Chapman et al., 1948; Hodges et al., 1955; Costa et al., 1965).

In the sheep (average gestation period 150 days) the fetal thyroid gland starts to accumulate iodine by the 50th day of gestation and the formation of thyroid follicles is observed histologically on the 52nd day (Barnes et al., 1957).

In the bovine (282 day gestation period), iodinated organic compounds have been detected in the fetal thyroid between day 53 and 70 of gestation, even before the formation of follicles and the histological appearance of intracellular colloid. Between days 75 to 118 the developing thyroid acquires a follicular structure; this gradual development is accompanied by a continuous increase in its organic iodine content (Koneff et al., 1949).

It is clear that the fetal thyroid gland starts to accumulate iodine and to synthesize thyroxine early in pregnancy, but there are only a few studies where the amount

of thyroxine actually present in the fetal circulation at different stages of pregnancy has been measured.

Pickering and Kontaxis (1961), working with Macaque monkeys found fetal serum butanol-extractable iodine values of 1.2 µg per 100 ml of serum at 75 days of gestation (total gestational period in the monkey 150 days), after that, iodine values increased gradually approaching those of the maternal serum near term.

Costa et al. (1965), have done an extensive study of thyroid function in the human fetus, and have found PBI values of 2-3 µg/100 ml of serum at the second trimester of gestation. At term the concentration of PBI was almost the same as in the maternal serum.

Pituitary Regulation of Fetal Thyroid Function.

Many workers proved that secretion of thyroxine by the fetal thyroid gland is regulated by the fetal hypophysis and that the pituitary and thyroid of the fetus interact with each other in the same way as they do during postnatal life (Jost, 1959-60). It also appears that the differentiation of the thyroid gland from the early undifferentiated anlage does not depend upon the pituitary, but thyroid growth and secretion is definitely stimulated by the pituitary in the later stages of gestation (Jost, 1959-60; Sobel et al., 1960).

The thyroid gland of rabbits decapitated *in utero* fixes less iodine than those with intact pituitaries (Jost, *et al.*, 1949). Pituitary control of the thyroid gland begins in the fetal rabbit at about 22 to 23 days of gestation (Jost, 1959-60).

Removal of the fetal pituitary gland by decapitation in near term rats, produces a decrease in size and number of thyroid follicles; injections of thyrotrophin in these fetuses not only prevented thyroid atrophy, but caused accelerated thyroid growth including an increase in height of the follicular epithelium. (Sethre, 1950; Sethre and Wells, 1951).

Under normal conditions the placenta, is impermeable to maternal thyrotrophin. This has been demonstrated in the guinea pig by Peterson and Young (1952), and in rats by Sobel *et al.* (1960).

In the human, a clinical case of a child born with congenital hyperthyroidism from a hypothyroid mother, has been interpreted as a fetal thyroid response to excess maternal thyrotrophic hormone, which might have crossed the placenta (Koerner, 1954).

More evidence that fetal thyroid function is controlled by the fetal pituitary has been obtained by the administration of goitrogenic drugs to pregnant females. Goitrogenic agents easily cross the placenta and block hormone production in the maternal and fetal thyroid (French and Van Wyk, 1964). Intact and hypophysectomized

pregnant rats were given a mixture of 5% $KClO_4$ and 0.05% propylthiouracil in the drinking water. This treatment caused enlargement and follicular disorganization in the thyroid of all the fetuses, indicating development of a fetal goiter as a consequence of an increased secretion of fetal TSH in response to decreased production of thyroid hormone by the fetal gland. Administration of thyroxine to the same rats prevented goiter development (Sobel et al., 1960).

The same kind of thyroid enlargement was induced in fetuses of pregnant guinea pigs administered propylthiouracil (Peterson and Young, 1952). Myant (1964) reported that essentially the same effect has been observed in hamsters, rabbits, goats, humans and mice.

Placental Transfer of Thyroid Hormones.

It has been demonstrated that inorganic iodide easily crosses the mammalian placenta (Chapman et al., 1948; Koneff et al., 1949; Jost et al., 1949; Hodges et al., 1955; Barnes et al., 1957; Van Heynigen, 1961; Costa et al., 1965). Also, there is an active iodide transport mechanism across the placenta, which is inhibited by sodium thiocyanate (NaCNS) (Longothetopoulus and Scott, 1956).

The problem of placental permeability to thyroxine has attracted the attention of many workers and with the availability of radioactive thyroxine a great deal of progress

has been made in this area.

A clear demonstration that thyroxine crosses the placenta and that the pituitary-thyroid axis is already working in the fetus, was made by the administration of propylthiouracil to guinea pigs during the last three weeks of gestation. This treatment produced a hyperplasia of the fetal thyroid and pituitary that could be counteracted by thyroxine administration. It was also shown that injections of thyroxine alone into pregnant animals suppressed the development of both the fetal thyroid and pituitary (Peterson and Young, 1952). Another study in the guinea pig showed that thyroxine crosses from maternal to fetal circulation within five minutes after intravenous injections of thyroxine-I^{131} (Contopoulos et al., 1964)

The rabbit placenta is almost completely impermeable to thyroxine before the 19th day of gestation (Hall and Myant, 1956). The fetal/maternal ratio of butanol-soluble I^{131} 24 hours after the injection of radioactive thyroxine, in 20-days-pregnant rabbits, was about 0.05, this value increased to about 0.3 at the end of pregnancy (Myant, 1958a).

The rat placenta is also permeable to thyroxine (Hoskins et al., 1958). They recovered butanol-extractable radioiodine from homogenates of fetuses from mothers injected with thyroxine-I^{131}. A decrease in weight of the fetal thyroids was demonstrated after hypophysectomized pregnant

rats were injected with 100 µg of thyroxine daily beginning at the 17th day of gestation (Sobel et al., 1960). Radioactive thyroxine was detected in the fetal circulation five minutes after the intravenous injection of I^{131} labeled thyroxine into the mother (Contopoulos et al., 1964).

The placentae of sows, ewes and ferrets are also permeable to thyroxine. In the sheep and pig radioactive thyroxine could not be detected in fetuses of injected mothers before the 80th day of gestation. It was possible to detect thyroxine-I^{131} in the fetal ferret as early as 60 minutes after intravenous injections to the mother (Contopoulos, 1964).

In the human the transport of thyroxine across the placenta was studied by administering I^{131}-labelled thyroxine to pregnant women at various times before parturition and the concentration of PBI^{131} was measured in the fetal and maternal circulation at birth. It was found that a period of 1 to 3 days is required for an equilibrium to be reached between the mother and the fetus and that the concentration of radioactive thyroxine was 3 times greater in the mother than in the cord blood (Grumbach and Werner, 1956). In another experiment, using the same procedures, it was found that the concentration of organic I^{131} in the fetus was less than 10% that in the mother 14 to 53 hours following injection (Hirvanen and Lybeck, 1956).

In some of the same experiments described above the permeability of the placenta to triiodothyronine was studied.

It was found that triiodothyronine crosses the placenta more readily than thyroxine in rabbits (Myant, 1958a) and in humans (Grumbach and Werner, 1956; Myant, 1958b).

In all the species studied it seems that the passage of thyroid hormones from mother to fetus follows the same pattern. There is almost no thyroxine transport in the early stages of pregnancy and the placental permeability to this hormone increases as pregnancy advances. But even in late stages of pregnancy the rate of transport is slow and limited (Hall and Myant, 1956; Hirvonen and Lybech, 1956; Myant, 1958b; Osorio and Myant, 1960; French and Van Wyk, 1964; Glass et al., 1964).

Up to now we have only considered the passage of thyroid hormones from mother to fetus; we already know that the fetal thyroid starts to secrete thyroxine in the last quarter of gestation in common laboratory animals and at mid-gestation in other species. If it is supposed that the placenta does not behave only as a one sided permeable membrane to thyroxine, then it is possible that thyroxine secreted by the fetus will cross the placenta towards the maternal circulation. The fact, that thyroxine crosses from fetus to mother has been demonstrated by some interesting studies.

There is a clinical report about a myxedematous woman that became pregnant after she had been under thyroidin (desiccated thyroid) treatment for two years. At the beginning of pregnancy no myxedematous syptoms were present.

The thyroidin treatment was discontinued after the first three months of gestation, and surprisingly, no symptoms reappeared up to the time of delivery. Relief of myxedema symptoms was certainly due to the presence in the mother's circulation of thyroid hormone produced by the fetus (Zondek, 1940). Myxedema recurred in the mother after parturition. A similar result was reported in a goat that was thyroidectomized and subsequently became pregnant (Reineke and Turner, 1941).

Body growth of heifers was retarded following thyroidectomy but body growth was resumed during the last stages of pregnancy. Resunption of growth was attributed to the thyroid hormones that crossed the placenta from the fetus (Spielman et al., 1945).

In the rabbit (Hall and Myant, 1956), radioactive thyroxine was detected in the maternal circulation at 1 to 4 hours after the injection of thyroxine I^{131} into the fetus, but the amount did not exceed 7.1% of the dose.

In rats, thyroxine I^{131} was injected into the peritoneal cavity of three fetuses in one uterine horn and within five minutes radioactive thyroxine was shown in the maternal circulation. Radioactivity was also detected in the blood of the uninjected fetuses of the contralateral uterine horn. Between 30 to 40 per cent of this radioactivity was in the form of thyroxine and the remainder as inorganic I^{131} (Contopoulus et al., 1964).

Distribution of Thyroid Hormone Between Mother and Fetus. Fetal Thyroid Hormone Concentration.

It was established in the foregoing section that the passage of thyroid hormones across the placenta increases as pregnancy advances. This increase could accur in many ways: an increase in the permeability of the placenta, a decrease in the thickness of the membranes that separate the fetal from the maternal circulation, and an increase in the placental blood flow (Osorio and Myant, 1960).

The thyroid hormones in the circulation are reversibly bound to thyroxine-binding proteins (TBP), and under physiological circumstances an equilibrium is maintained between bound and unbound thyroxine. The amount of free thyroxine is relatively low because of its high affinity for the serum proteins, especially the thyroxine-binding globulins (TBG) (Robbins and Rall, 1960). In the human, for instance, the concentration of free thyroxine is less than 0.001 times the concentration of bound thyroxine (Robbins and Rall, 1957).

French and Van Wyk (1964), suggested that protein-bound thyroxine probably is not available for placental transport and that only free thyroxine would cross the placenta. Based on this fact, they proposed that the transfer of thyroxine between mother and fetus would be determined primarily by the relative levels of free thyroxine on each side of the placenta; these levels in turn, would be controlled by the relative binding capacity of the two sera,

the thyroid hormone output and the thyroxine turnover rate of mother and fetus. The most important of these factors seems to be the difference in composition, affinity and binding capacity of the serum thyroxine binding proteins between mother and fetus (Osorio and Myant, 1960; Osorio and Myant, 1962).

Myant and Osorio (1959), using electrophoresis and radioactive thyroxine, found that thyroxine binding proteins of the fetal rabbit (fetal TBP) are different from those of the adult rabbit (adult TBP). The fetal TBP's make their initial appearance at about the 19th day of gestation, then gradually increase to levels characteristic of the adult at two weeks of post-natal life. The fetal TBP exhibits a displacement between the α-2 and β-globulin, and the adult TBP between albumin and α 1-globulin. Myant and Osorio (1959) also demonstrated that the binding capacity of the serum TBP of the fetus at 25 days of age is about 10% that of the adult and the affinity of the fetal TBP for thyroxine is five times greater than that of the adult TBP.

The same investigators mixed together equal parts of adult and fetal TBP with radioactive thyroxine, and using electrophoresis, they found that at 25 days of age the fetal TBP binds about one third of the thyroxine, but it has a binding affinity 5 times greater than the adult. At 28 days of age the fetal TBP binds as much thyroxine as the adult TBP (Osorio and Myant, 1960).

During pregnancy in women, the augmented blood levels of estrogen are an important factor in stimulating increased thyroxine binding capacity of the serum proteins, which reaches a level of about twice the nonpregnant values (Dowling et al., 1956; Robbins and Nelson, 1958; Russell et al., 1960; Russell et al., 1964).

Serum of human fetuses, between 12 and 35 weeks of gestation, contains a specific thyroxine-binding protein (TBP), that exhibits the same electrophoretic mobility as adult TBP. By the 24th week the maximal binding capacity of the fetal TBP is much less than the maternal TBP; however, the binding capacity rises towards the end of pregnancy (Osorio and Myant, 1962). At term, this capacity is one and one-half times greater than in the nonpregnant adult, but still about 50% lower than in the mother (Dowling et al., 1956; Robbins and Nelson, 1958).

It has been found in the human that at term the amount of fetal TBG is 29.1 µg per cent with a range from 20.2 to 38.7 µg, as compared with the maternal TBG that ranges from 31.1 to 47.8 µg with a mean of 42.1 µg; the last value is approximately twice the levels for nonpregnant females (Russell et al., 1964).

The PBI of human mothers at term is greater than in nonpregnant adults, but it is approximately the same as in the fetus. The concentration of free thyroxine is significantly higher in the fetus than in the mother (Robbins and

Nelson, 1958). It has also been shown that the fetal binding proteins are slightly but significantly more saturated than their respective maternal proteins, resulting in a higher concentration of free thyroxine (Robin et al., 1969).

The current thinking is that thyroxine crosses the placenta in its free rather than in its protein-bound form (Myant, 1964; Robin et al., 1969), which supports the theory proposed by French and Van Wyk (1964) that the higher concentration of free thyroxine in fetal blood results in a positive net transport from fetus to mother. The existence of a free thyroxine transplacental gradient has been proposed by Robbins and Nelson (1958), but denied by De Mayer et al. (1966).

All the functional changes that the thyroid undergoes during pregnancy contribute to increasing the concentration of endogenous hormone in the fetal blood towards the end of pregnancy (Myant, 1964). In the fetal rabbit the concentration of serum protein-bound iodine at 18 days of age is so low that it is difficult to measure; afterwards it increases, equaling that of the mother's serum at 27 days of gestation (Myant, 1958b).

In the Macaque monkey, the butanol-extractable iodine of the fetal serum, has a value of 1.2 µg/100 ml at 75 days of age; it increases with pregnancy, reaching a value of 4.5 µg at 150 days (Pickering and Kontaxis, 1961) and reaches the same value as in the mother at the time of parturition (Pickering, 1964).

In the serum of human fetuses removed by Caesarean section between 12 to 24 weeks of age, the concentration of protein-bound iodine is less than in serum of myxedematous patients (Osorio and Myant, 1962). The concentration of PBI at the second trimester was found to be 2-3 µg/100 ml of serum (Costa et al., 1965); and at parturition, the mean PBI was 6.2 µg with a range from 3.8 to 9.1 µg (Russell et al., 1964).

Measurable amounts of iodine have been detected in fetal bovine thyroid at 60 days of gestation. Total and thyroxine-like iodine content increased steadily as the fetus grew. The rate of increase of these two iodine fractions was related exponentially to fetal body weight, body length and calculated age. The amounts of iodine found in the bovine fetal thyroid with increasing age were greater than could be accounted for by mere increase in thyroid mass (Wolff et al., 1949).

Thyroid Function in the Perinatal Period.

In the human, the PBI concentration in blood samples taken from the mother during labor and from the umbilical cord soon after delivery have been found not to be significantly different (Danowski et al., 1951; Man et al., 1952; Pickering et al., 1958; Russell et al., 1960; Russell et al., 1964). The thyroxine binding capacity was significantly lower in the newborn (26.9 µg%) than in the mother

(39.6 μg%), but both were greater than in the nonpregnant state (20.3 μg%), (Russell et al., 1960).

Monkey fetal and maternal serum BEI also reaches the same level at the time of parturition (Pickering, 1964).

Tracer quantities of radioiodine were injected into pregnant cows 1 week prior to parturition; at birth, the calf plasma showed radioactivity 10 times higher than that of the dam. The radioiodine in the calf plasma was in the protein-bound form, mostly as thyroxine (Monroe et al., 1951). Two pregnant cows were sacrified 24 hours after radioiodine injection and it was found that the fetal thyroids contained twice as much I^{131} as the maternal thyroids and the concentration of I^{131} was six to seven times greater in the fetal thyroids. The fetal thyroglobulin was 27 times more radioactive than the mother's and analysis of the hydrolyzed thyroglobulins revealed that more than one-fifth of the radioactivity was due to newly formed thyroxine. The blood sera of the fetuses contained only slightly more radioactivity than those of the cows (Gorbman et al., 1952). In another experiment it was found that the fetal thyroids contained as much radioiodine as the maternal thyroids and were higher in concentration per gram. The concentration of I^{131} in the calf plasma was over four times that in maternal plasma (Miller et al., 1967).

It has been reported that the newborn human baby experiences a sharp increase in serum thyroid hormone

concentration during the first two to three days of life. This is followed by a gradual decrease, approaching adult values by the 18th to 20th day of age (Danowski, 1951; Man, et al., 1952; Pickering et al., 1958). These high PBI values together with the finding of an increased thyroid iodine uptake, led to the suggestion that the newborn infant may experience a period of physiological hyperthyroidism (Van Middlesworth, 1954).

Ponchon et al. (1966) found that increased iodine uptake by the thyroid of the newborn is accompanied by a very active thyroxine clearance; the daily accumulation of iodine is up to ten times higher than in adulthood, and the total disposal rate is up to three times faster in infants than in adults.

Recent studies have demonstrated that the serum free thyroxine values are higher in neonatal life (Marks, 1965; Siersbaek-Nielsen and Molholmhansen, 1967), and that the plasma tyrosine and thyroxine are significantly higher than in euthyroid adults (Siersbaek-Nielsen and Molholmhansen, 1967). This confirms the impression of a physiological hyperactivity of the thyroid gland during the neonatal period.

Fisher et al. (1962), confirmed the presence of an elevated I^{131} uptake rate very early in the newborn infant (12 to 24 hours after birth). They suggested that this phenomenon together with the higher PBI values at the same

age may be associated with an increased secretion of thyrotrophin (TSH) by the pituitary gland. They considered, among other factors, that the cooler temperatures to which the newborn is exposed may be a powerful factor that, by way of the hypothalamus, stimulates TSH release. More recent studies conducted by the same authors (Fisher and Oddie, 1964; Fisher et al., 1966; Fisher and Odell, 1969), confirm that the cold exposure that the neonate experiences is the initial stimulus responsible for thyroid hyperactivity of the newborn.

The mechanism of thyroid hyperactivity and hyperthyroxinemia of the newborn infant have been further understood by the use of a sensitive and specific TSH radioimmunoassay and the demonstration of a marked acute peak in serum TSH concentration soon after birth; the mean values increased from 9.4 μU/ml in cord blood to a peak at 30 minutes of 93 μU/ml, there was a rapid fall between 30 and 90 minutes, and values had dropped to 14 μU/ml at 24 hours (Fisher and Odell, 1969).

In the goat, local cooling of the preoptic anterior hypothalamic region, the "heat loss center" caused a marked increase in the release of protein-bound iodine (PBI^{131}) from the thyroid gland with the development of an extreme hyperthermia (Andersson et al., 1963).

It has been reported that in the bovine the PBI concentration tends to decrease with age. Calves less than

48 hours old showed a PBI of 13.7 µg/100 ml of plasma, while those between 2 days and 12 months averaged 7.2 µg per cent (Lewis and Ralston, 1953).

When the in vitro erythrocyte uptake (EU) of I^{131} labelled triiodothyronine was used as an indicator of thyroid function in cattle, it was found that the EU values declined with age. Mean EU in calves under one month old was 13.5 and in heifers of 12-18 months old 6.2 (Thorell, 1965).

Kossila (1967), published an excellent review, together with very important data of her own, on the development of bovine thyroid function from early fetal life to old age. Kossila's study is mostly based on the weight and basic structural components of the thyroid gland.

The total weight of the fetal gland increased throughout gestation, but the rate of increase slowed considerably near term. Colloid was first detected in the follicular lumen at the 84th day of intrauterine life. The amount of colloid increased from this stage until the end of gestation, being most marked from the 140th day to term. The percentage of glandular epithelial tissue was very high before the 100th day and showed a tendency to decrease as gestation progressed. Between 160 and 195 days, and sometimes earlier, the absolute amount of epithelial tissue had already reached the level observed in newborn calves.

The mean thyroid weight of 135 newborn calves was

7.52gm, with a percentage of epithelial tissue of 18.84. Histologically, the activity of the thyroid appeared to be rather low at birth.

Thyroid glands obtained from 55 calves over one month old, showed an absolute thyroid weight increase, whereas the relative weight rapidly decreased during the first 12 months of age. Mean percentage of epithelial tissue was lower in calves under 6 months old than in those over 6 months old.

MATERIALS AND METHODS

For the study on fetal and maternal thyroid relationships, 40 pregnant Holstein Friesian heifers of known breeding dates were purchased from Michigan dairymen by Wayne D. Oxender DVM for use in his project on the development of the endocrine and reproductive systems in the bovine fetus. After being delivered to the Campus, the heifers were kept at the loose housing barn of the Michigan State University Dairy Department under the usual conditions of feeding and management until scheduled for an operation. Dr. Oxender generously supplied serum samples for the T_4 and PBI analyses and fetal thyroids were collected by Dr. E. P. Reineke. The serum samples for 19 newborn calves were kindly supplied by Winston G. Ingalls of the Michigan State University Dairy Department. They were taken daily at ages 1 to 7 days from calves born in the Michigan State University Dairy Herd.

Blood Sample Collection.

The blood samples were collected from pregnant cows divided into three groups according to the stage of gestation. Sampling dates were timed to coincide with the end of the first, second and third trimester of gestation.

Caesarean sections were performed at the Veterinary Clinic of Michigan State University by Senior Veterinary students under the direction of Dr. Wayne D. Oxender.

Depending on the side of pregnancy, the animals were first clipped and surgically scrubbed with a germicide on the respective paralumbar fossae. Paravertebral or nerve blocking anesthesia was done by infiltration of 2.5% procaine hydrochloride solution with epinephine 1/50,000[1]. The first blood sample was withdrawn from the dam's jugular vein before initiation of surgery.

Once the laparotomy opening was made, the median uterine artery and uterine vein were cannulated with a 30 inch long polyethylene catheter attached to a 19 or 16 gauge needle (Minicath-16, infusion set)[2] and blood samples were withdrawn from each vessel. Afterwards, the uterus was brought up and hysterotomy was performed. In the 180-and 260-day-old fetuses, blood samples were taken from the umbilical artery and umbilical vein using the same kind of polyethylene catheters, but with a 16 gauge needle. In the case of the 90-day-old fetuses the blood was taken from the fetal aorta and the heart.

After removal of the fetus, the uterus was closed with a Cushing suture pattern, using #1 chromic catgut. The peritoneum and muscle layers were sutured with #2 chromic

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[1]Bio-Ceutic Laboratories, Inc. St. Joseph, Missouri

[2]Desert Pharmaceutical Co., Inc. Sandy, Utah.

catgut. The skin closure was made with Vetafil* 0.6 mm.

Fetal thyroids were removed, trimmed and weighed. One part of each thyroid was sectioned and fixed in Dietrich solution for histological studies to be reported elsewhere. The remainder was frozen and stored for subsequent analyses also to be reported elsewhere.

In each case blood samples of about 40 cc were collected into oxalated polypropylene tubes and placed in ice. After 10 to 30 minutes they were centrifuged in a refrigerated centrifuge and the plasma was transfered into the same kind of polypropylene tubes with $CaCl_2$ added in amount sufficient to cause clotting. The samples were stored under refrigeration and within 24 to 48 hours the serum was separated from the fibrin clot. Serum samples for T_4 and PBI determinations were kept frozen until the respective analyses were carried out.

Blood samples from the newborn calves were withdrawn from the jugular vein into polypropylene tubes and were submitted to the same treatment as described above.

Serum Thyroxine (T_4) Analysis

a) Principle of the Test.

The serum thyroxine (serum T_4) was measured by the Tetrasorb-125 method (Radio-Pharmacentical Division, Abbott

*Vetafil Beugen, Haver-Lockhart, Kansas City, Mo.

Laboratories, North Chicago, Illinois). This method uses the principle of "competitive protein binding" that was first described by Ekins (1960), and further developed and simplified by Murphy and Pattee (1964). A resin-sponge to separate bound from unbound thyroxine was introduced by Nakajima et al. (1966) and by Kennedy and Abelson (1967). Kaplan (1966) showed that ^{125}I-thyroxine could be bound in advance to the standard thyroxine-binding globulin solution, and that this treated solution is a satisfactory test reagent for thyroxine estimation.

The principle of the test is described briefly as follows:

There is only a small amount of thyroxine-binding globulin (TBG) in serum. The T_4 binding sites can be readily saturated by the addition of small amounts of thyroxine either labeled or unlabeled. If a small amount of ^{125}I-thyroxine is added, the fraction which is protein-bound can be determined. As more unlabeled T_4 is added, the amount of ^{125}I-labeled bound thyroxine decreases, since both the labeled and unlabeled thyroxine compete for the same binding sites. If instead of pure T_4 a sample of deproteinized plasma is added, the T_4 which it contains competes for the thyroxine binding sites. When equilibrium is reached, the resin-sponge separates the TBG-bound thyroxine from the unbound thyroxine, and the amount of T_4 contained in the serum sample can be measured according to the fall in

bound isotope which it causes.

b) Procedure.

In the present study some minor modifications to the Tetrasorb-125 method were introduced. Serum samples were thawed and brought to room temperature. One ml was used for each determination. In some cases duplicates of the same sample were run. Two ml of 95% ethanol were added and the solution was mixed for 30 seconds by means of a Vortex mixer. The tubes were tightly stoppered. To facilitate maximal T_4 extraction, the solution was allowed to stand for ten minutes and then centrifuged at 1000 g. for 20 minutes. One 0.3 ml aliquot of the supernatant liquid was transferred into polypropylene test tubes and evaporated to dryness by means of a mild stream of clean air, while the tubes were immersed in a warm-water bath (37° C).

After complete drying, 1 ml of ^{125}I-T_4-TBG was added to each sample by using a "syringe pipette" devised by Dr. E. P. Reineke. The tubes were gently shaken for a few moments and placed in a warm-water bath at 22° C for ten minutes to allow a better and constant equilibration between the labeled and unlabeled thyroxine with the TBG molecule. The tubes were then placed in an ice bath at 1.5-4° C. At the end of five minutes in the ice bath, one Tetrasorb resin-sponge was placed in successive tubes at 20 second intervals and the air was expressed by using the Abbott plastic plunger.

After 30 minutes of incubation one initial count (I) of

the total radioactivity contained in each tube was obtained. At the end of exactly 1 hour of incubation, the reaction was stopped in successive tubes at 20 second intervals by adding about 10 ml of cool (5-10°C) glass-distilled water. Then, the washings were withrawn as soon as possible by depressing the sponge 5 times with the Abbott aspirator. Each tube was washed three additional times with cool glass-distilled water. Each tube remained in the ice bath until it was removed for the washing steps. The final radioactivity count (F) was taken using a well-type scintillation counter (Nuclear Chicago, Model Ds-5), and analyzer-scaler (Nuclear Chicago, Model 8725). Finally, the percentage ^{125}I-T_4 uptake by the resin-sponge was calculated by using the following formula:

$$\text{Resin Sponge Uptake } \% = \frac{F\ (CPM) - \text{background } (CPM)}{I\ (CPM) - \text{background } (CPM)} \times 100 \quad (1)$$

c) Standard Curve and Calculations

A standard T_4 curve was run with the determinations of each day. The standard stock solution was prepared from crystalline free thyroxine purified by Dr. E.P. Reineke from monosodium thyroxine pentahydrate*. Ten mg of free T_4 was dissolved in 95% ethanol with the aid of a few drops of a 0.5N NaOH solution and diluted to a concentration of

*Baxter Laboratories, Morton Grove, Ill.

5 µg/ml. The final concentration of the working standard was 0.05 µg/ml. The working standard was prepared aproximately every 3 weeks and kept at 5° C in tightly closed siliconized glass containers.

Based on the results obtained from previous studies in this laboratory (Wan, 1969; Lorscheider, 1970), it was decided that 3 concentrations of the working standard solution would be run for each standard curve, at 1, 4 and 8 µg/100 ml of solution. The three tubes were carried through the same procedure as the alcoholic serum extracts already described. The standard curve was obtained by plotting on linear coordinate graph paper, the T_4 concentration being expressed as µg/100 ml on the X axis and the % resin-sponge uptake on the Y axis.

In the same work mentioned above, it was found that in all the species studied the serum T_4 concentration did not exceed 12 µg/100 ml, and it was established that in the range of 0-12 µg T_4/100 ml a perfectly linear relationship exists between T_4 concentration and % resin-sponge uptake. Thus, instead of reading values from the standard curve, the data were fitted by the method of "least squares" (Li, 1964) and the T_4 values were calculated by use of the "linear regression equation" (Li, 1964). The calculations were made by using the following equation:

$$X_u = \frac{Y - a}{b} \qquad (2)$$

X_u = Serum T_4 (μg/100 ml) uncorrected for recovery.

Y = % resin-sponge uptake

a = intercept of the Y-axis

b = slope of the standard curve.

The ethanol extracts only 77.3% the T_4 from the serum proteins. The results were corrected for extraction losses as follows:

$$X_c = X_u \left(\frac{100}{77.3} \right) \qquad (3)$$

X_c = serum T_4 (μg/100 ml) corrected for extraction recovery.

As a routine control of the determinations, two duplicates of the same pool of steer serum were run each time. The mean T_4 value for 24 determinations on this serum was 6.41 μg/100 ml. In some cases the day's value for the steer serum was used as a standard to correct the experimental T_4 values in order to get more consistent results.

All equations and calculations were programmed on an Olivetti-Underwood Programma 101 desk computer.

Protein-Bound Iodine (PBI) Analysis.

The PBI technique used in this laboratory is an adaptation of the alkaline ashing method of Barker and Humphrey (1950). Before analysing, the serum samples were

thawed and brought to room temperature. One ml of serum was pipetted into incineration tubes.

Serum proteins were precipitated by adding 1 ml of 10% $ZnSO_4$ and 1 ml of 0.5N NaOH. The precipitate was washed three times with glass-distilled water. One ml of 4N Na_2CO_3 was added to the precipitate, mixed well and dried overnight at 94-100° C. The sample was then incinerated in a muffle furnace for 2.5 hours at 615-625° C. After ashing and cooling, the iodide from the ash was dissolved with 2 ml 2N HCl and 2 ml 7N H_2SO_4. Then the ash digest was diluted to a volume of 11 ml by adding 7 ml of glass-distilled water.

Two duplicate 5 ml aliquots of the dissolved ash solution were pipetted into colorimetric cuvettes and 0.5 ml of arsenious acid was added. The timed reaction with ceric ammonium sulfate was run for exactly 15 minutes at 37° C and then stopped with brucine sulfate (Faulkner et al., 1961). The final readings were taken by means of a Coleman Spectophotometer (Model 6/35) set at a wave lenghth of 480 mu and adjusted to 100% transmittance through a glass-distilled water blank.

A reagent blank and two standards containing 1 ml of Iodotrol*, or 1 ml of standard lamb serum were run each time and submitted to the same treatment as the serum samples. Iodine content was calculated by means of a standard curve

*Dade Reagents, Inc. Miami, Florida (Distributed by Scientific Products).

(intermediate range) prepared under identical conditions and using Hycel iodine standard stock solution* (1 μg/ml) with glass-distilled water to a final concentration of 0.02 μg/ml. In this standard curve, the net % transmittance (standard minus reagent blank) was plotted on the ordinate of linear graph paper, against iodine concentration in micrograms on the abscissa.

Computations.

When the fetal thyroid weights were plotted against body weight on log-log paper they formed a straight line. Thus they would be expected to fit the equation for a parabolic function.

$$Y = a X^b \qquad (4)$$

In logarithmic form this equation may be written

$$\log Y = \log a + b \log X, \qquad (5)$$

where log Y = log of thyroid weight expressed in gm

X = body weight expressed in kg

log a = log of the Y-axis intercept

b = slope of the line (regression coefficient).

As described by Brody (1945 p. 398) two normal equations are needed to fit the data by the method of least squares:

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*Hycel, Inc. Houston, Texas (Distributed by Scientific Products).

$$I \quad \Sigma(\log Y) = N\Sigma(\log a) + b\Sigma(\log X). \qquad (6)$$

$$II \quad (\text{Log } X.\log Y) = \log a \; (\log X) + b \; (\log^2 X) \qquad (7)$$

The data were fitted and the line of best fit was drawn as shown in Figure No. 1.

The mean serum T_4 and PBI of the newborn calves were plotted on semilog paper. The values from neonatal days 2 to 5 formed a straight line, and thus could be fitted by the equation,

$$\log Y = a + b X, \qquad (8)$$

where log Y = log of T_4 or PBI values (μg/100 ml serum)

X = days of age

a = Y-axis intercept

b = slope of the line.

The line of best fit for each slope was determined by the method of least squares (Li, 1964).

Thyroxine biological half-life ($t_{\frac{1}{2}}$) was calculated according to the expression:

$$t_{\frac{1}{2}} = \frac{0.301}{b} \qquad (9)$$

where,

$0.301 = \log_{10}^{0.5}$

and,

b = $\log_{10}$ slope of the line.

The thyroxine fractional turnover (TFTR) per day was calculated using the equation,

$$1 - e^{-X}, \qquad (10)$$

where $X = 1_n$ slope of the line,

or b x 2.302, or $\frac{0.693}{t_{\frac{1}{2}}}$

The thyroxine volume of distribution (TVD) was calculated using the value 22.0 (TVD per cent of body weight) reported by Post and Mixner (1961) in 18-day-old bull calves. The extrathyroidal thyroxine (ETT), T_4 degraded daily and T_4 degraded daily per 45.4 kg of body weight (Table 4) were also calculated.

Significance of the differences between means were obtained by the Student (t) test (Li, 1964).

RESULTS

Primiparous cows 1½ to 2 years of age were used for the experiment on thyroid development in the bovine fetus. The animals were in good heath and nutritional condition at the time of the operation. The fetuses all showed body markings of the Holstein breed, except for fetus No. 561 and fetus No. 564 that had breed characteristics of Hereford and Angus, respectively.

After the fetus was removed from the uterus, it was weighed and transported to the necropsy building. There, it was decapitated and the thyroid gland dissected. Thyroids were weighed to the nearest 0.1g. (on a Gram-atic balance in our laboratory).

The means and standard errors of the body weight expressed in kg and thyroid gland weight expressed in gm are shown in Table 1. No significant difference in body weight between male and female fetuses was found at any trimester of gestation. The same was true for the thyroid gland weight at the first and third trimester. But, at the second trimester of gestation the mean female thyroid gland weight (2.268 gm) was significantly higher ($P < 0.001$) than the mean male thyroid gland weight (1.752 gm). In the third trimester group the thyroid gland from fetus No. 539

TABLE 1

FETAL CALF BODY AND THYROID WEIGHT*

Trimester of Gestation	No.	Body Weight (Kg)	Thyroid Weight (gm)	Relative Thyroid Weight gm/Kg
First	15	0.439±0.056	0.162±0.020	0.374±0.010[a]
Second	14	6.257±0.249	1.973±0.103	0.317±0.010[b]
Third	11	26.845±2.380	7.777±0.873	0.295±0.017[b]

*Mean ± S.E.

[a,b]As determined by the t test, there is a significant difference ($P < 0.001$) between values with different superscripts.

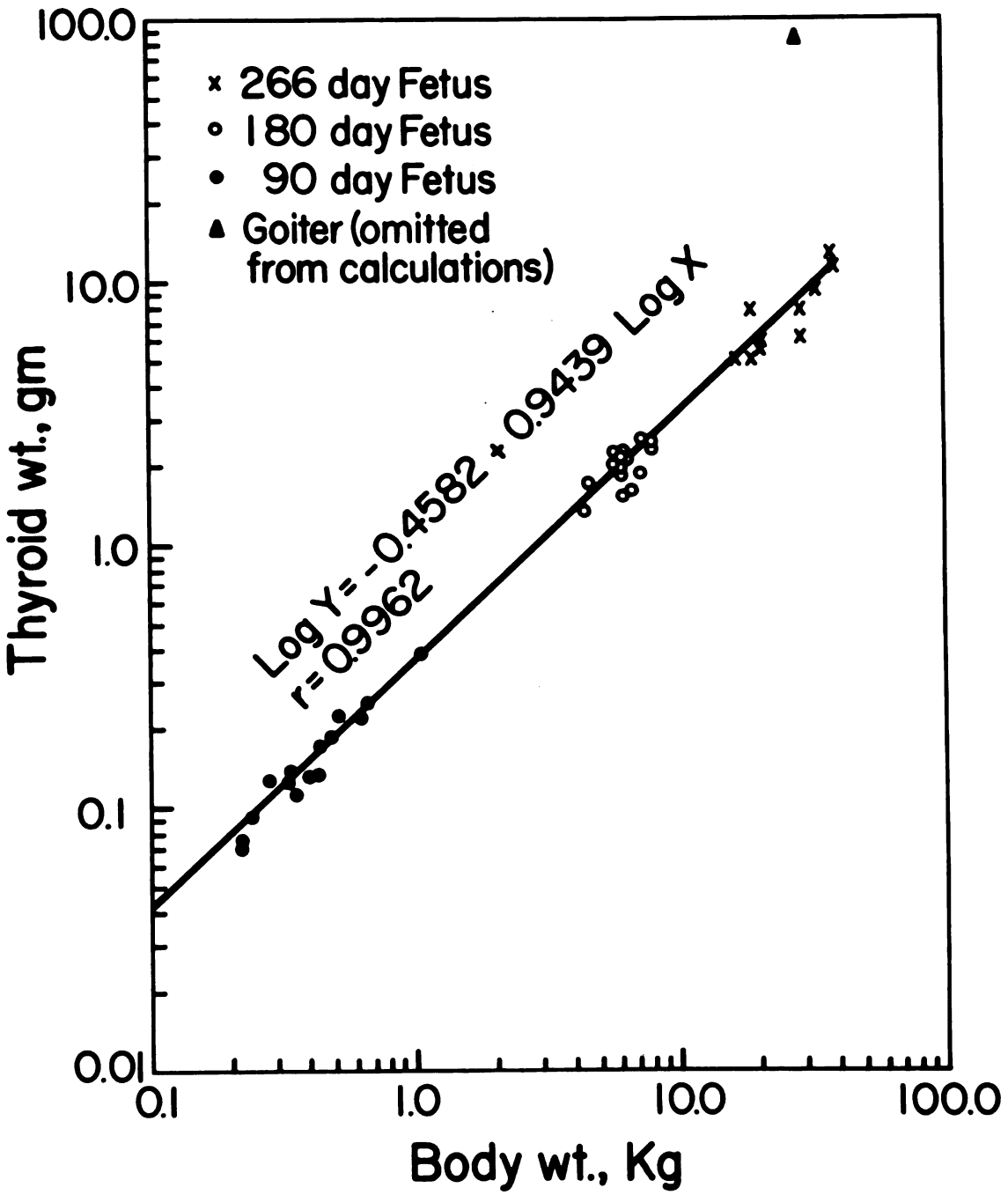

Figure 1. Exponential relationship between fetal thyroid and body weights.

weighed 80.50 gm. Because of this it was excluded from the calculations and classified as a goiter.

When the thyroid gland weight is divided by the body weight, the relative thyroid weight (RTW) in gm/Kg is obtained. The means and standard errors of RTW are also included in Table 1. Attention is called to the fact that the first trimester fetuses have a significantly higher ($P < 0.001$) RTW than the fetuses from the second and third trimester. No difference was found in RTW between the second and third trimester fetuses.

Looking at Table 1 it is easy to appreciate that body and thyroid weights increase as pregnancy advances. Furthermore, when individual values (Appendix I,IV,VII) are plotted on log-log paper (Figure 1) and the line of best fit calculated, there is a very close relationship ($r = 0.9962$) between the increase in fetal body and thyroid gland weight throughout pregnancy. This relationship is described by the equation $\log Y = -0.4582 + 0.9439 \log X$. The regression coefficient of this equation (0.9439) is the relative growth constant of the fetal thyroid with respect to body weight, which is not significantly different ($P > 0.05$) from 1.0. At the second trimester of gestation 6 female fetuses had a significantly higher absolute thyroid weight of 2.268 ± 0.103 gm than the value for 8 males of 1.752 ± 0.111 gm ($P < 0.01$).

When serum proteins were precipitated with ethanol in the T_4 analyses, the fetal serum precipitate showed a light brown color, while the dam's serum precipitate was usually

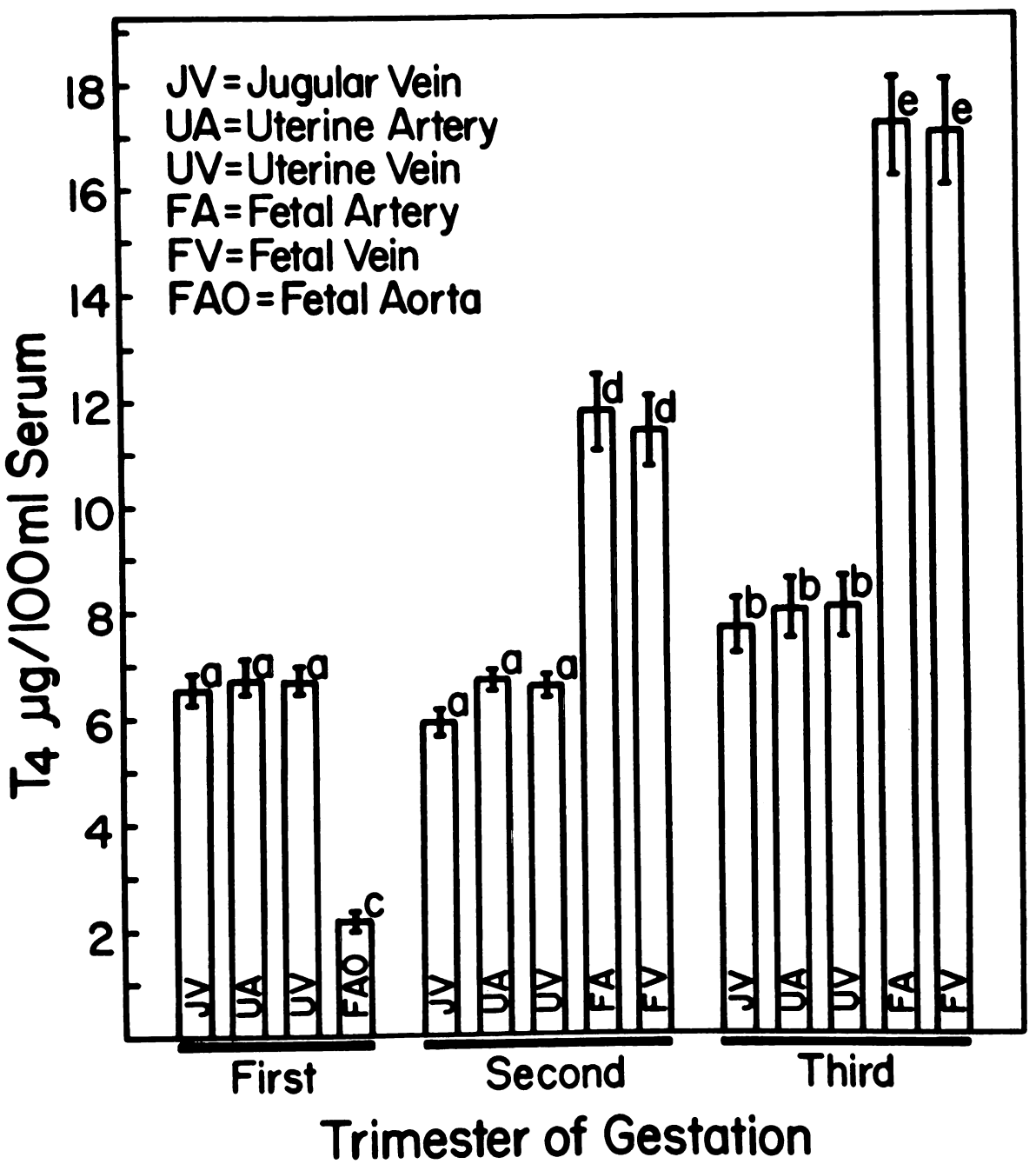

Figure 2. Maternal and fetal serum T_4 during pregnancy.
As determined by the t test, there is a significant difference between values with different superscripts. a,b,c,d,e, ($P < 0.001$).

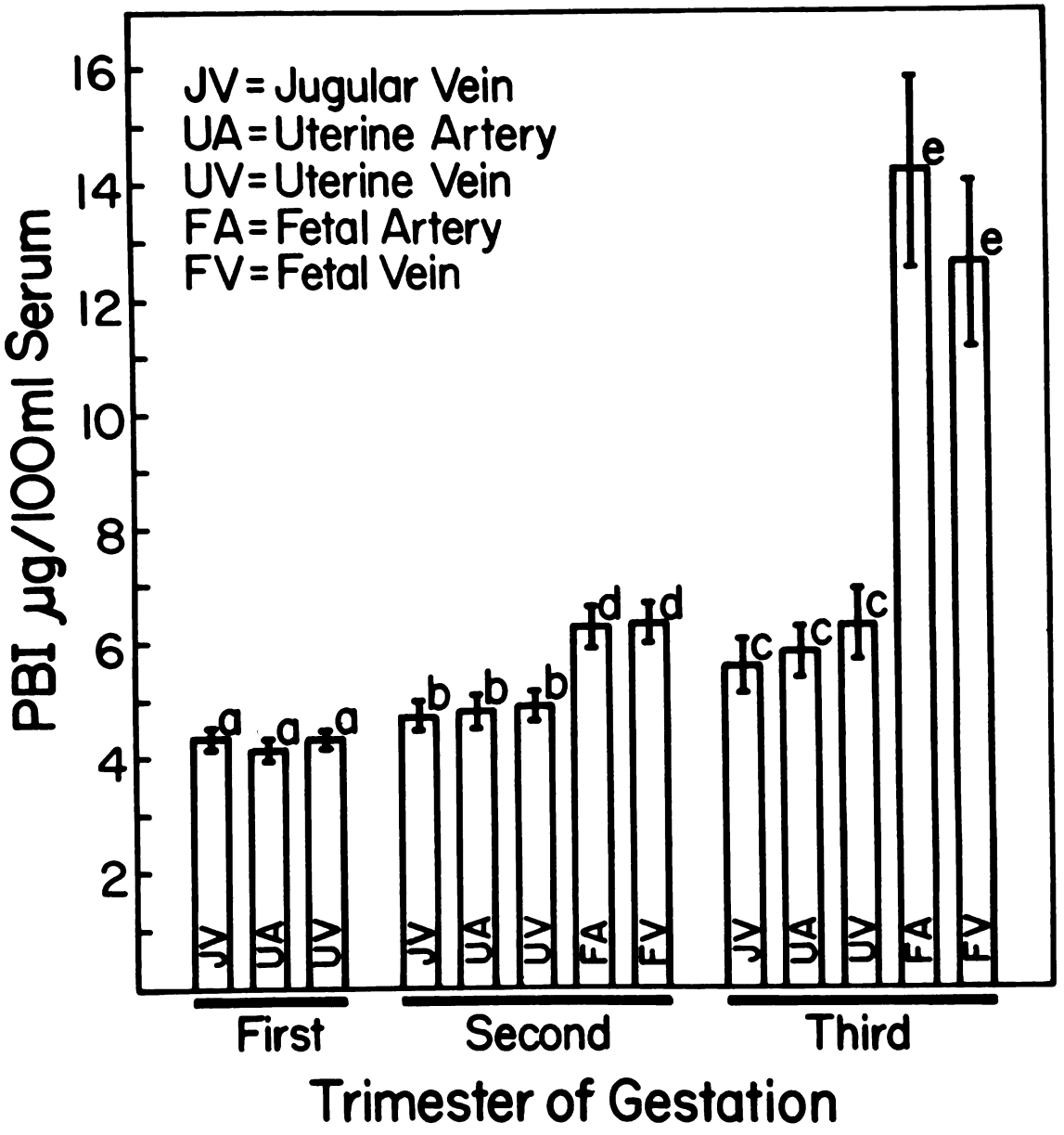

Figure 3. Maternal and fetal serum PBI during pregnancy.

As determined by the t test, there is a significant difference between values with different superscripts. a,b, ($P < 0.02$); b,c, ($P < 0.01$); d.e. ($P < 0.001$).

clear. The total amount of serum obtained from the first trimester fetuses was only a few mililiters and enough serum was available to run T_4 tests only.

The dam and fetal serum T_4 and PBI values (Mean±S.E.) are represented graphically in Figure 2 and 3, respectively. The complete data are given in Appendices II,III,V,VI,VIII and IX. Thyroxine was detected in the serum of first trimester (90 days) fetuses. The mean value was 2.18 µg of T_4/100 ml of serum.

Comparing serum T_4 values from the fetal artery and fetal vein, no significant difference was found at the second (180 days) and third (266 days) trimesters of gestation. The same was true for the PBI values. In general, the mean fetal artery values tended to be slightly higher than those for the fetal vein (Figures 2 and 3).

At the second trimester of gestation the fetal serum T_4 level had a mean value of 11.66 µg/100 ml, which is more than 5 times that of the first trimester (2.18 µg). In turn the third trimester value (17.16 µg of T_4/100 ml) is significantly higher ($P < 0.001$) than that for the second trimester. The fetal PBI at the third trimester (13.42 µg/100 ml serum) is also significantly higher ($P < 0.00$) than that of the second trimester (6.24 µg/100 ml serum).

The levels of maternal T_4 and PBI found in the jugular, uterine artery and uterine vein, are not significantly different from each other at any of the three trimesters.

TABLE 2

MEAN FETAL AND MATERNAL SERUM T_4 IN $\mu g/100$ ml*

Trimester of Gestation	Fetal	Maternal
First	2.18 ± 0.201^{a} (10)	6.67 ± 0.205^{d} (15)
Second	11.66 ± 0.499^{b} (14)	6.42 ± 0.162^{d} (14)
Third	17.16 ± 0.702^{c} (11)	7.97 ± 0.305^{e} (11)

*Mean $\pm$ S.E. One serum T_4 analysis was done for each fetus at the first trimester. At the second and third trimesters, values for umbilical artery and vein were included in each average. On the maternal side T_4 values for jugular, uterine vein and uterine artery are included in each average.

The number of animals represented in the average for each group is shown in parenthesis.

As determined by the t test, there is a significant difference between values with different superscripts. a,b,c,d,e, ($P < 0.001$); d,e, ($P < 0.01$).

TABLE 3

MEAN FETAL AND MATERNAL SERUM PBI IN μg/100 ml*

Trimester of Gestation	Fetal	Maternal
First	- - - - - - - - -	4.29 ± 0.110^{c} (15)
Second	6.24 ± 0.272^{a} (14)	4.81 ± 0.184^{d} (14)
Third	13.42 ± 1.140^{b} (11)	5.94 ± 0.316^{e} (11)

*Mean ± S.E. On the maternal side PBI values for jugular vein, uterine vein and uterine artery are included in each average. For each fetus the values for umbilical vein and artery are included in each average.

The number of animals represented in the average for each group is shown in parenthesis.

As determined by the t test, there is a significant difference between values with different superscripts. a,b;c,e, ($P < 0.001$); c,d, ($P < 0.05$); d,e, ($P < 0.01$).

Usually, the uterine vein levels have the tendency to be higher than the jugular and uterine artery values (Figures 2 and 3). In neither the fetal nor maternal samples were the differences large enough to establish an arteriovenous difference.

The maternal T_4 at the first trimester of pregnancy had a value of 6.67 μg/100 ml serum. This value is not significantly different from that of the second trimester (6.42 μg/100 ml serum); but the value for the third trimester (7.97 μg/100 ml serum) is significantly higher ($P < 0.01$) than that of the second. The maternal PBI at the second trimester of pregnancy is significantly higher ($P < 0.05$) than that of the first. In turn, the third trimester PBI level is significantly higher ($P < 0.01$) than that of the second trimester. The maternal and fetal T_4 and PBI values are summarized in Tables 2 and 3.

The fetal T_4 and PBI level from the second and third trimester are significantly higher ($P < 0.001$) than the respective maternal levels during those trimesters.

At the first trimester of gestation the maternal T_4 level is three times larger than the respective fetal level. At the second trimester the fetal T_4 level is almost twice the maternal level, while at the third trimester the fetal level is more than 2 times the maternal level (Table 2).

When fetal female serum T_4 values are compared with the fetal male values, no difference is found at the third trimester. But, at the second trimester the female T_4

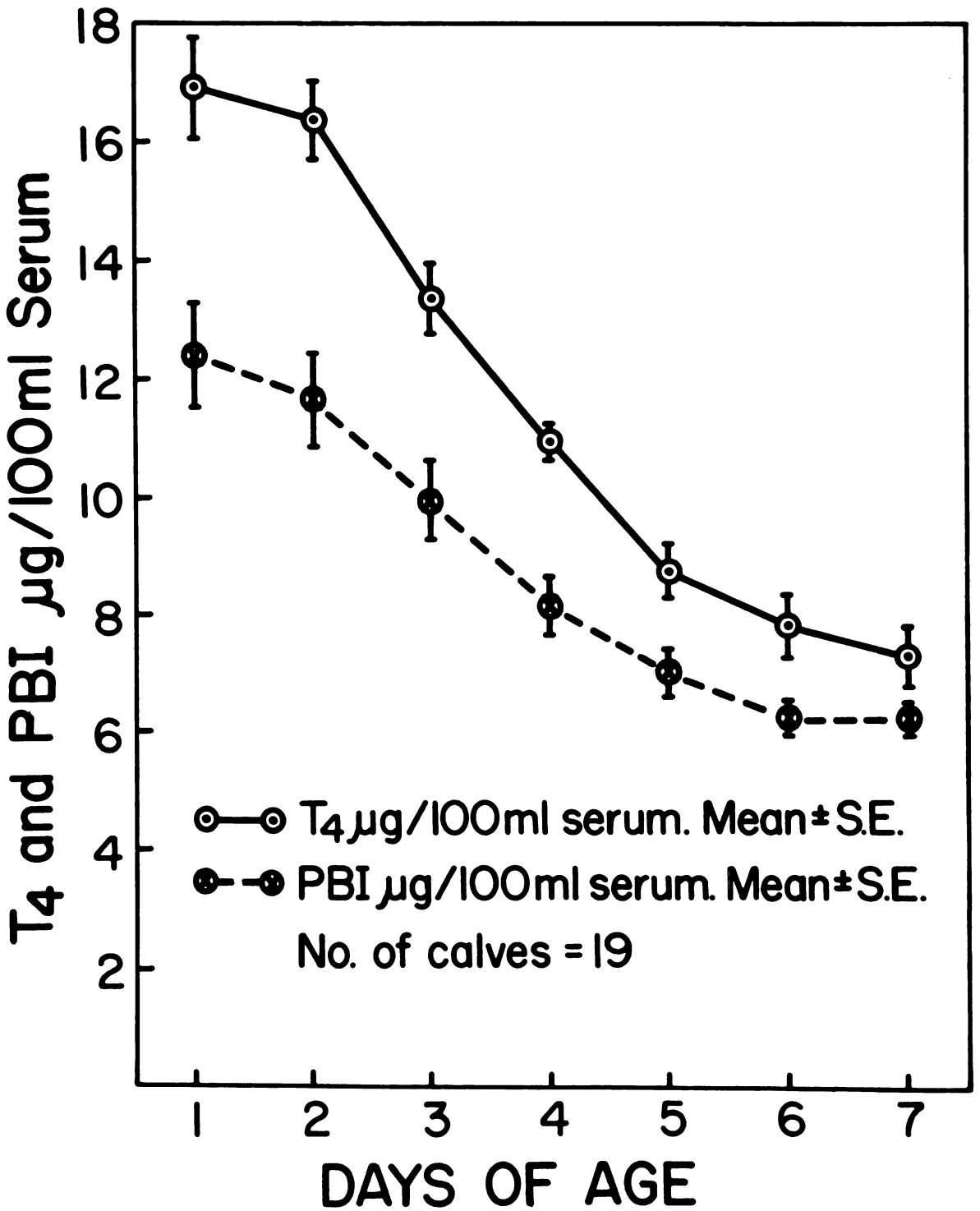

Figure 4. Newborn calf serum T_4 and PBI from days 1 to 7 after birth.

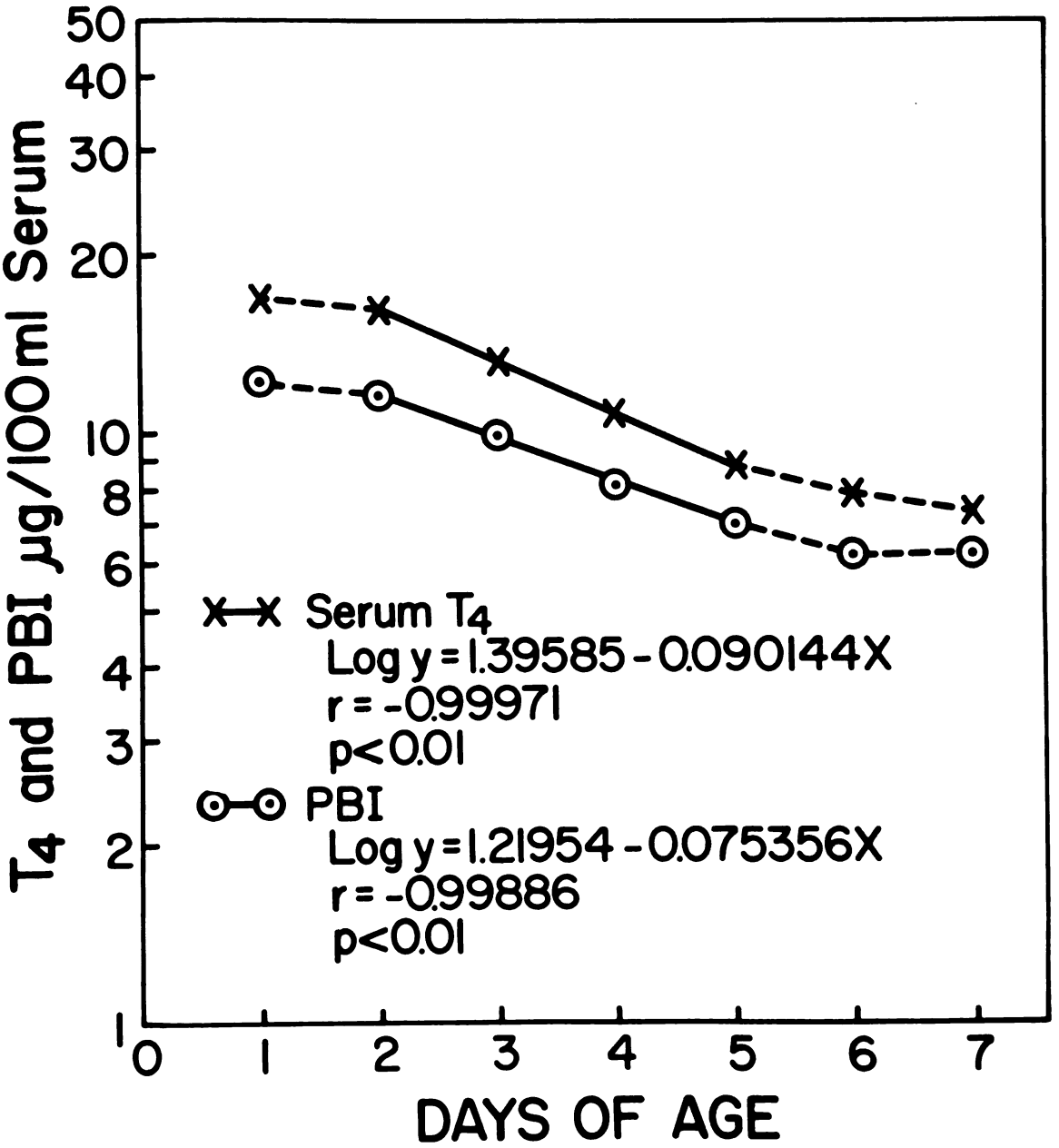

Figure 5. Semilogarithmic plot of serum T_4 and PBI of neonatal calves.

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value of 13.28 ± 0.371 µg/100 ml is significantly higher ($P < 0.01$) than the male value of 10.45 ± 0.693 µg/100 ml. The PBI values do not show a sex difference at the third trimester either. At the second trimester PBI only in the female fetal artery was significantly higher ($P < 0.05$) than in the male fetal artery. This may be a chance difference.

The mean body weight of 17 newborn calves (including a pair of twins) was 37.92 kg, with a range of 26.31 - 43.55 kg. No sex difference in birth body weight was found (Appendix X).

The mean serum T_4 levels of nineteen newborn calves showed a steady decrease from the first (16.92 µg/100 ml serum) to the seventh postnatal day (7.29 µg/100 ml serum). The same constant decrease was observed in the PBI values from 12.39 µg/100 ml serum at the first day to 6.24 µg/100 ml serum at the seventh day (Appendices XI - XII). The shape of this decrease can be better appreciated in Figure 4, where we can observe that the T_4 and PBI curves follow the same trends.

The mean values were plotted on semilog paper, and the line of best fit was calculated for days 2 through 5 by the method of least squares. A straight line was obtained for both T_4 and PBI, with a correlation coefficient of 0.9997 for the T_4 curve and $r = 0.9989$ for the PBI curve (Figure 5).

The biological half-life ($t_{\frac{1}{2}}$) of the serum T_4 from days 2 to 5 was 3.34 days, with a daily thyroxine fractional turnover rate of 0.18739 mg. The daily values for

TABLE 4

MEAN THYROXINE DEGRADATION IN NEWBORN CALVES FROM DAYS 2 TO 5*

Days of Age	T_4 mg%	ETT, mg**	T_4 degraded daily (mg)	T_4 degraded daily mg/45.4 Kg
2	0.01637	1.3656	0.256	0.304
3	0.01336	1.1145	0.209	0.248
4	0.01093	0.9118	0.171	0.202
5	0.00875	0.7299	0.137	0.162

*Calculated by the method of Post and Mixner (1961). The mean $t_{\frac{1}{2}}$ was 3.34 days.

**Extra Thyroidal Thyroxine (ETT) = Serum T_4 (mg%) X T_4 Distribution Volume (8.34 l).

extrathyroideal thyroxine (ETT), T_4 degraded daily and T_4 degraded daily per 45.4 kg of body weight are given on Table 4.

DISCUSSION

The thyroid gland is one of the first endocrines to appear in the developing mammal. The thyroid anlage can be identified as early as three weeks after conception. In the present study it was found that from 90 to 266 days of gestation the bovine fetal thyroid gland grows in direct proportion to body weight. Thyroid weight increases with body weight according to the equation $\log Y = - 0.4582 + 0.9439 \log X$. The coefficient 0.9439 is not significantly different from 1.0. This means that during fetal life the growth of the thyroid is in direct proportion to body growth. Abeloos (1942), and Nichols *et al.* (1949) report coefficients of 0.95 and 1.0, respectively, for relative thyroid growth in the fetal calf. Costa *et al.* (1965) reported a 100-fold increase in total thyroid weight between the third month and the end of gestation in the human, whereas the relative growth did not change. Brody and Kibler (1941) found a value of 0.924 for the relation between thyroid weight and body weight in many species of mature mammals.

The rate of growth of the fetal thyroid seems not to be affected by the stage of pregnancy. But, having found a higher relative thyroid weight at 90 days of gestation, it might be that around this stage of development the growth

of the thyroid gland is faster than in later stages. A considerably faster increase of the absolute thyroid weight between the 75th and the 195th day than during the last 1 - 1.5 month of gestation was observed by Kossila (1967). Nichols et al. (1949), showed that the percentage growth rate of the fetal thyroid decreases steadily with advancing pregnancy between the 50th and the 250th day of gestation. Fenger (1913) reported that thyroid glands from fetuses 6 to 9 months of gestation are relatively larger than those from full term calves.

It appears that active proliferation of the epithelial tissue is mainly responsible for the increase in size end weight of the thyroid in earlier stages of pregnancy, while colloid accumulation may be responsible at later stages (Kossila, 1967). A very active proliferation of epithelial tissue in the fetal calf thyroid at 53 to 70 days of gestation was found by Koneff and associates (1949). Kossila (1967) reported a close relationship between the amount of epithelial tissue in the fetal thyroid and body weight.

A significantly higher thyroid weight found in females at the second trimester of gestation will be discussed later together with serum T_4 differences. Kossila (1967) reports that the sex of the fetus does not appear to influence the development of the thyroid gland.

According to present knowledge, it appears that thyroid hormones are not necessary for conception,

differentiation and early embryonic growth. Furthermore, it is possible that embryonic fetal development might take place in the absence of thyroid hormone, at least until the fetus can suply its own. Good evidence for this supposition is the fact that thyroidectomized heifers were able to conceive and bear normal calves. (Spielman et al., 1945). There are wide differences between species in regard to the stage of pregnancy at which the fetal thyroid begins to function; it appears that these differences are correlated with the stage of development and maturity reached by the fetus at the time of birth (Myant, 1964).

In the present study it was possible to detect measurable amounts of circulating thyroxine (2.18 µg/100 ml of serum) in the blood of 90-day-old fetuses. This might be an indication that at this stage of development the thyroid gland has reached the functional capacity to synthesize and release hormone into the circulation. Thyroxine-like and other organically bound iodine compounds have been demonstrated in the fetal calf thyroid gland between the 53rd and 60th day of age, even before intracellular colloid of follicle formation was histologically evident (Koneff et al., 1949; Wolff et al., 1949). Intrafollicular colloid was clearly detected at the 84th day of gestation (Kossila, 1967). These observations contribute to the belief that the 90-day-old bovine fetus already has a functional thyroid gland and that hormonal synthesis has begun before the complete differentiation of the gland. But, it is possible

that at least some of this thyroxine is of maternal origin. There is evidence that the placenta is hardly permeable to thyroxine at this stage of bovine pregnancy. Contopoulos et al. (1964) found that the sheep placenta is not permeable to radioactive thyroxine before the 80th of pregnancy.

It has been demonstrated that fetal thyroid growth and hormone synthesis is entirely under fetal pituitary control (Jost et al., 1949; Jost, 1959-60; Sobel et al., 1960). Then, it becomes obvious that in the 90-day-old calf fetus the pituitary-thyroid gland axis is already working, a relationship that will be maintained in later stages of pregnancy and throughout postnatal life.

The concentration of serum thyroxine increases in the fetus as pregnancy advances. At the second and third trimesters of gestation it is 5 and 8 times greater, respectively, than at the first trimester. Furthermore, the fetal serum thyroxine level is about twice that of the maternal level during the same stages of pregnancy. This means that the bovine fetus builds up a very high level of circulating thyroxine during its intrauterine life.

The serum thyroxine level of the dam also increases between the second and third trimester of gestation, but it is much less than in the fetus.

It was stated in the literature review that the placenta is almost impermeable to thyroxine in early stages of pregnancy, becoming increasingly permeable with the progress of gestation. Even in later stages the permeability

is slow and limited. Not having found significant arteriovenous differences at any stage of pregnancy, it becomes evident that maternal and fetal circulations are functioning to some extent as two separate pools for thyroxine.

A number of possible explanations may be considered with regard to the increasingly higher fetal levels of serum thyroxine during pregnancy. The fetus is surrounded by an ideal environment, and it probably does not need to metabolize much thyroxine to fulfil such demanding functions as maintenance of body temperature, respiration or digestion. Consequently the fetal thyroxine turnover must be minimal. In fact that seems to be true even in the first days of postnatal life, as will be discussed later.

The recycling of iodine seems to be more favorable in the fetus than the mother. Although exchange of some iodine across the placenta probably occurs, the fetus does not lose any iodine through the urinary and digestive systems. This together with a higher fetal iodine uptake and faster thyroxine synthesis (Gorbman et al., 1952), might be an indication of a relatively greater activity of the fetal thyroid as compared with the maternal gland.

It is well established that thyroxine in the circulation is bound to at least three serum proteins, one of the α- globulins, one of the prealbumins and one albumin. The globulin (TBG) is the most important thyroxine binding protein, and no thyroxine binding prealbumin (TBPA) has been detected in the cow (Robbins and Rall, 1960). The

serum of the fetal rabbit shows a high concentration of $\alpha 2$ - globulin in early pregnancy that becomes relatively and absolutely higher than in the maternal serum during the last week of pregnancy. Furthermore, the binding affinity of the fetal TBP is about 5 times that of the maternal TBP (Myant and Osorio, 1959).

If the same occurs in the bovine, this might be an important factor in the progressive increase of circulating thyroxine level in the growing fetus. On the other hand, an increase in the thyroxine binding capacity of the fetal serum proteins, seems to be very feasible at least during the last stages of gestation. Robin et al. (1969) found in the human fetus at term a slight but significantly higher saturation of the thyroxine binding proteins than in the mother.

The significantly higher serum thyroxine levels found in the cow during the third trimester of gestation can perhaps be interpreted as a consequence of an increase in the thyroxine binding capacity of the serum proteins. On the other hand, they may be due to transfer of thyroxine from the fetus to the mother. The increase in thyroxine binding capacity has been shown to be true in the human (Robin et al., 1969), but not in the sheep (Annison and Lewis, 1958). Dowling et al. (1960), among other workers, have demonstrated the influence of estrogen in increasing the binding power of thyroxine binding globulins (TBG), together with a diminution in the peripheral fractional turnover rate of thyroxine. It is possible that the rapid rise in estrogen

levels that the cow experiences during the third trimester of gestation (Nalbandov p. 266, 1964) may be the stimulus that increases the binding capacity of the serum proteins and consequently the circulating thyroxine levels.

The sex difference in absolute thyroid weight and serum thyroxine found in the calf fetus at the second trimester of gestation, might be evidence of a more active pituitary function in the female than in the male. It is possible that at this stage the fetal female pituitary gland produces more TSH, which in turn intensifies thyroid gland growth and secretion. At the end of the third trimester there is no sex difference in thyroid weight or serum thyroxine.

Serum thyroxine levels of the bovine fetus begin to rise during the 2nd trimester of gestation and reach maximal values before birth. This is in marked contrast to the human infant where thyroid hormone levels are reported to be the same as in the mother at birth, but to rise sharply immediately after birth.

When radioiodine was injected into term cows, the calf's serum at birth showed 4 to 10 times more radioactivity, mostly as thyroxine, than the dam's serum (Monroe et al., 1951; Miller et al., 1967).

Studies in the monkey (Pickering, 1964) and in the human (Danowski et al., 1951; Man et al., 1952; Pickering et al., 1958; Russell et al., 1960; Russell et al., 1964) have not shown differences in serum PBI or BEI levels

between the human mother and the fetus at the time of parturition. But, it has been found that the newborn infant experiences a sharp increase in serum thyroxine during the first two to three days after birth, followed by a gradual decrease (Danowski et al., 1951; Van Middlesworth, 1954; Ponchon et al., 1966; Marks, 1965; Siersbaek-Nielsen and Molholmhansen, 1967). Fisher and associates (1964 - 1969) confirmed that the "physiological hyperthyroidism" of the newborn infant is a consequence of an increase in TSH secretion by the pituitary gland during the first 30 minutes after birth. These authors believe that the initial stimulus for this high TSH secretion is the cooler temperature that the infant experiences soon after birth.

Studies in the rat (Woods and Carlson, 1956), in the guinea pig (Del Conte and Stux, 1954), in the dairy goat (Flamboe and Reineke, 1959), in the sheep (Henneman et al., 1955; Hoersch et al., 1961) and in the bovine (Pipes et al., 1963; Turner, 1968) demonstrated that decreases in ambient temperature stimulate thyroid gland hormone release. Even more, Andersson and coworkers (1963) showed that cold stimulation of the anterior preoptic region of the hypothalamus in goats, produces an increase in hormone secretion by the thyroid gland.

The thyroid functional state in the newborn calf seems to be somewhat different than in the newborn human infant. It is possible that the cooler temperatures to which the calf

is exposed immediately after birth and the stress of birth by itself might stimulate the pituitary and thyroid gland by way of the hypothalamus. But, the calf having built up a high level of circulating thyroxine during its intrauterine life, it may be that these high levels of hormone oppose up to some point the effects of such cooler temperatures in stimulating the hypothalamus, pituitary and thyroid gland, avoiding in that manner a further increase in serum thyroxine.

It also appears that at the end of the gestation period the calf is morphologically and maybe physiologically more mature than the human baby. Probably the homeostatic mechanisms are also more developed to face the challenges of the new postnatal life.

In the present study it was found that the calf experiences a gradual decrease in serum thyroxine levels from the first to the seventh day after birth. A postnatal decrease in PBI values was reported by Lewis and Ralston (1953). The decrease between the second and fifth day follows a straight line ($r = -0.9997$), according to the exponential equation $\log Y = 1.39585 - 0.09014\ X$ used to calculate the slope of the curve. It appears that hormone secretion by the thyroid gland has been shut off during those days. One interpretation is that the high concentration of circulating thyroxine, by way of the feed-back mechanism, has inhibited the pituitary TSH secretion and consequently the activity of the thyroid gland is blocked.

At the sixth day of age, when serum thyroxine levels are approaching normal values, the pituitary and thyroid are in normal balance and thyroxine secretion is resumed.

The thyroxine degradation rate during the same period (0.187) is about one-half the value (0.379) reported by Post and Mixner (1961) in 18-day-old bull calves. Another indication of the slower thyroxine turnover in the newborn calf, is the longer biological half-life ($t_{\frac{1}{2}}$), which value is 3.34 days in comparison with a $t_{\frac{1}{2}}$ of 1.9 days found in 18-day-old calves.

Roy et al. (1957) studying the basal metabolism of the newborn calf, found an increase in heat production during the first 3 days after birth, followed by an exponential decline from days 3 to 8. This finding seems to correlate with the metabolism of the excess of circulating T_4, that occurs during the same period in the newborn calf.

The T_4 values determined by the Tetrasorb I^{125} method follow the same trends as the PBI values. A similar correlation was found by Godwin and Swoope (1968) working with human samples.

SUMMARY AND CONCLUSIONS

1. Development of thyroid function during intrauterine and early postnatal life was studied in the bovine.

2. Caesarean sections were performed in 40 primiparous Holstein cows, 1½ to 2 years of age, at either 90, 180 and 266 days of pregnancy. Maternal blood samples were taken from the jugular vein, uterine artery and uterine vein. Mixed arterial and venous fetal blood was taken at 90 days. At 180 and 266 days the umbilical vein and umbilical artery were sampled separately. After the fetus was removed from the uterus, body weights and trimmed thyroid weights were recorded. Jugular vein samples were also taken from 19 newborn calves at days 1 to 7 after birth.

3. Blood serum was analyzed for thyroxine (T_4) by a competive protein binding method, utilizing the Tetrasorb-I^{125} kit provided by Abbott Radiopharmaceutical Laboratories. Protein bound iodine (PBI) was also determined, except that for the 90 day fetal samples only T_4 was analyzed.

4. When fetal thyroid weights (Y) and fetal body weights (X) were plotted on log-log graph paper and the line of best fit calculated, a very close relationship between the

increase in fetal body weight and thyroid weight was found. This relationship is described by the equation, $\log Y = -0.4582 \pm 0.9439 \log X$ ($r = -0.9962$). The coefficient of 0.9439 is not significantly different from 1.0. This means that from 90 to 266 days of gestation the fetal thyroid gland grows in direct proportion to body growth. Relative thyroid weight was significantly higher ($P < 0.001$) in the female than in the male fetus at the second trimester of gestation.

5. Pooled fetal serum T_4 values in μg/100 ml averaged 2.18 ± 0.201, 11.66 ± 0.499 and 17.16 ± 0.702 at the first, second and third trimesters. It is believed that at 90 days of gestation the fetal thyroid gland is already functioning. Afterwards the fetus builds up a high level of endogenous thyroxine. The second trimester T_4 value is more than 5 times ($P < 0.001$) that of the first. In turn, the third trimester value is about 50 percent higher ($P < 0.001$) than the second. At the second trimester the female serum T_4 is significantly higher ($P < 0.01$) than the male. This finding together with a higher absolute thyroid weight ($P < 0.001$), may be a consequence of a more active pituitary function in the female fetus at this stage of gestation.

6. Pooled maternal T_4 values at the same stages of pregnancy averaged 6.67 ± 0.205, 6.42 ± 0.162 and 7.97 ± 0.305

μg/100 ml. There is a significant increase ($P < 0.001$) in maternal serum T_4 at the third trimester of gestation. This can perhaps be interpreted as a consequence of an increase in the thyroxine binding capacity of the serum proteins. It may also be due to transfer of thyroxine from the fetus to the mother.

7. No significant arteriovenous differences in serum T_4 were found in either fetal or maternal samples at any trimester of gestation. At the second and third trimesters the fetal serum thyroxine concentration is about twice the maternal value.

8. Mean serum T_4 of neonatal calves was 16.92 ± 0.872 μg/100 ml at day 1 and declined exponentially from day 2-5 according to the equation, $\log Y = 1.3959 - 0.09014 X$ ($r = -0.9997$). It appears that T_4 secretion was shut off during this period and the equation represents the degradation of extrathyroidal T_4 released earlier. The fractional degradation rate of 0.1872 mg per day is about ½ the value reported for 18 day-old calves by Post and Mixner (1961). At the 7th postnatal day the serum T_4 had a value of 7.28 ± 0.509 μg/100 ml.

9. It appears that no major changes in thyroid function occur in the bovine during the perinatal period, since the serum T_4 value at the third trimester is not significantly different from the value on the first postnatal day.

10. T_4 and PBI values correlated well throughout the experiment.

APPENDIX I

FIRST TRIMESTER FETUSES

Cow and Fetus No.	Sampling Date	Days of Gestat.	Fetus' Sex	Body Wt.Kg	Thyroid Wt.	
					Tot.gm	gm/Kg
543	7/21/70	91	F	0.22	0.076	0.345
554	7/21/70	91	M	0.28	0.125	0.446
545	7/21/70	91	F	0.24	0.086	0.358
548	7/24/70	89	M	0.40	0.132	0.330
552	7/28/70	88	M	0.33	0.125	0.383
553	7/28/70	88	F	0.34	0.135	0.400
570	10/13/70	90	F	0.425	0.153	0.360
571	10/13/70	91	F	0.52	0.216	0.416
572	10/13/70	90	F	0.44	0.172	0.390
573	10/15/70	90	F	0.22	0.071	0.323
574	10/15/70	91	F	0.475	0.190	0.400
575	10/20/70	93	M	0.665	0.246	0.435
576	10/20/70	93	F	0.345	0.112	0.325
577	10/27/70	95	M	1.05	0.381	0.363
578	10/27/70	92	F	0.64	0.215	0.336

Total NO. 15

Male fetuses (M) 5

Female fetuses (F) 10

APPENDIX II

DAM AND FETAL T_4 µg/100 ml SERUM FIRST TRIMESTER OF GESTATION

Cow and Fetus No.	Jugular	Uterine Artery	Uterine Vein	Fetal Blood
543	7.84	9.30	8.43	-
544	7.57	8.18	9.13	-
545	7.53	8.93	8.37	-
548	5.65	5.69	6.08	-
552	5.15	5.43	5.95	1.75
553	5.37	6.08	5.37	-
570	7.62	7.53	5.49	1.48
571	6.26	7.22	6.66	2.08
572	6.22	5.92	7.28	3.05
573	5.75	5.57	5.72	2.31
574	6.01	4.02	5.50	1.92
575	10.04	8.65	8.71	1.26
576	6.14	6.20	4.81	3.28
577	6.04	5.73	7.15	2.23
578	5.06	6.55	6.45	2.43
No.	15	15	15	10
Mean ±	6.55 ±	6.73 ±	6.74 ±	2.17 ±
S.E.	0.345	0.390	0.355	0.201

APPENDIX III

DAM PBI µg/100 ml SERUM FIRST TRIMESTER OF GESTATION

Cow and Fetus No.	Jugular	Uterine Artery	Uterine Vein
543	4.80	3.84	3.39
544	5.18	4.34	5.54
545	5.84	3.27	4.31
548	3.50	3.30	3.70
552	4.86	5.29	4.34
553	3.20	3.41	4.62
570	4.49	4.90	4.97
571	4.32	3.87	3.99
572	3.99	4.24	4.41
573	3.33	3.58	3.27
574	3.54	-	3.98
575	4.25	4.22	4.47
576	5.35	5.12	-
577	5.19	5.00	5.50
578	3.88	3.67	4.14
No.	15	14	14
Mean ±	4.37 ±	4.14 ±	4.33 ±
S.E.	0.210	0.187	0.182

APPENDIX IV

SECOND TRIMESTER FETUSES

Cow and Fetus No.	Sampling Date	Days of Gestat.	Fetus' Sex	Body Wt.Kg	Thyroid Wt. Tot.gm	Thyroid Wt. gm/Kg
540	7/14/70	184	M	4.5	1.33	0.296
541	7/14/70	183	F	5.9	2.27	0.385
542	7/14/70	179	M	6.30	1.49	0.237
546	7/21/70	178	F	6.72	2.65	0.394
547	7/21/70	177	F	7.44	2.46	0.330
551	7/24/70	178	F	6.10	2.13	0.349
554	7/28/70	180	M	5.92	1.98	0.334
555	7/28/70	179	M	7.14	1.76	0.246
556	10/6/70	180	M	6.68	1.58	0.237
557	10/6/70	180	F	6.02	1.94	0.322
558	10/6/70	180	M	6.14	1.83	0.298
559	10/6/70	180	M	7.90	2.35	0.297
561	10/8/70	180	M	4.64	1.697	0.366
567	10/15/70	180	F	6.20	2.160	0.348

Total No. 14

Male fetuses (M) 8

Female fetuses (F) 6

APPENDIX V

DAM AND FETAL T_4 µg/100 ml SERUM SECOND TRIMESTER OF GESTATION

Cow and Fetus No.	Jugular	Uterine Artery	Uterine Vein	Fetal Artery	Fetal Vein
540	5.29	4.95	5.46	6.64	7.44
541	5.86	6.12	5.96	12.66	12.38
542	4.22	5.90	5.59	10.47	9.66
546	6.40	8.07	6.26	13.45	14.66
547	8.54	7.87	8.25	14.45	13.01
551	6.22	7.65	6.47	12.63	10.23
554	4.94	5.95	5.73	12.42	13.32
555	4.92	6.34	6.66	15.55	13.26
556	6.92	7.98	7.36	8.94	8.89
557	4.14	5.76	5.69	12.90	13.56
558	6.58	6.82	7.39	10.84	10.26
559	6.07	6.95	7.49	13.14	12.81
561	5.78	7.61	6.83	6.90	6.62
567	7.17	6.61	6.96	14.81	14.58
No.	14	14	14	14	14
Mean ±	5.93 ±	6.65 ±	6.57 ±	11.84 ±	11.47
S.E.	0.320	0.260	0.227	0.738	0.697

APPENDIX VI

DAM AND FETAL PBI µg/100 ml SERUM SECOND TRIMESTER OF GESTATION

Cow and Fetus No.	Jugular	Uterine Artery	Uterine Vein	Fetal Artery	Fetal Vein
540	4.58	4.56	5.37	4.44	4.67
541	5.51	5.73	4.93	8.32	6.62
542	3.40	-	4.00	7.40	7.60
546	4.78	3.80	-	7.13	7.13
547	4.90	5.12	5.89	7.94	7.93
551	3.20	3.40	3.00	6.30	5.40
554	3.11	3.30	3.59	5.05	5.14
555	3.29	4.10	4.38	6.19	6.50
556	7.55	7.43	7.23	5.74	5.66
557	4.70	5.10	5.07	5.75	-
558	3.83	3.79	4.08	2.92	3.29
559	6.44	5.71	5.06	7.52	7.65
561	5.83	5.38	5.45	5.63	5.62
567	5.18	5.16	5.49	7.22	7.61
No.	14	13	13	14	13
Mean ±	4.74 ±	4.81 ±	4.88 ±	6.25 ±	6.29 ±
S.E.	0.352	0.321	0.304	0.396	0.387

APPENDIX VII

THIRD TRIMESTER FETUSES

Fetus No.	Sampling Date	Days of Gestat.	Fetus' Sex	Body Wt.Kg	Thyroid Wt. Tot.gm	Thyroid Wt. gm/Kg
515	6/24/70	270	M	38.8	11.23	0.289
524	6/24/70	270	M	21.3	5.76	0.270
525	6/24/70	270	F	19.3	7.92	0.410
539*	6/22/70	270	M	23.94	80.50	2.690
549	7/24/70	264	M	33.1	9.21	0.278
550	7/24/70	264	F	18.6	5.02	0.270
563	10/6/70	265	F	37.65	13.31	0.354
560	10/8/70	265	F	29.94	6.206	0.207
562	10/8/70	262	M	16.78	5.366	0.320
564	10/8/70	265	F	20.41	5.788	0.284
566	10/13/70	265	M	29.48	7.964	0.270

Total No. 11

Male fetuse (M) 6

Female fetuse (F) 5

*Calf with goiter

APPENDIX VIII

DAM AND FETAL T_4 µg/100 ml SERUM THIRD TRIMESTER OF GESTATION

Cow and Fetus No.	Jugular	Uterine Artery	Uterine Vein	Fetal Artery	Fetal Vein
515	7.00	8.64	8.64	19.65	18.24
524	9.43	10.43	11.00	18.98	18.42
525	9.34	9.92	8.65	19.37	18.19
539	8.87	9.29	9.57	21.72	20.46
549	7.24	6.31	6.60	19.77	18.68
550	7.89	8.28	6.57	15.98	17.39
563	8.10	8.10	8.59	14.81	15.76
560	7.00	7.02	7.14	16.66	16.23
562	5.14	5.15	6.35	10.19	8.72
564	4.66	5.87	5.43	12.70	15.57
566	9.90	10.20	10.90	19.83	20.18
No.	11	11	11	11	11
Mean ±	7.68 ±	8.08 ±	8.13 ±	17.24 ±	17.07±
S.E.	0.514	0.545	0.564	1.065	0.966

APPENDIX IX

DAM AND FETAL PBI μg/100 ml SERUM THIRD TRIMESTER OF GESTATION

Cow and Fetus No.	Jugular	Uterine Artery	Uterine Vein	Fetal Artery	Fetal Vein
515	5.5	6.8	6.7	18.2	17.8
524	6.2	7.3	9.4	24.7	14.6
525	7.1	5.4	5.6	14.5	.4.5
539	6.2	5.9	6.8	20.6	20.1
549	3.00	3.30	3.00	7.70	6.80
550	4.40	4.60	4.70	12.40	9.80
563	4.58	4.70	5.56	10.20	9.33
560	5.05	6.04	7.53	8.09	7.70
562	4.35	3.94	4.69	8.75	6.10
564	6.06	6.06	6.41	11.99	12.46
566	9.14	10.06	9.86	19.40	19.59
No.	11	11	11	11	11
Mean ±	5.59 ±	5.82 ±	6.38 ±	14.23 ±	12.61±
S.E.	0.494	0.556	0.612	1.726	1.532

APPENDIX X

NEWBORN CALVES

Calf No.	Birth Date	Sex	Body wt. Kg	Breed
430	7/20/70	M	-	Holstein
415	7/25/70	F	38.56	"
994	7/26/70	F	27.22	"
407	7/27/70	F	41.73	"
418	7/27/70	M	40.82	"
1031	7/29/70	F	-	"
405	7/30/70	M	39.92	"
431	8/1/70	M	43.55	"
411	8/3/70	F	38.10	"
420	8/4/70	F	32.66	"
408	8/5/70	F	36.29	"
406	8/8/70	F	40.82	"
409	8/11/70	F	39.01	"
424 B	8/13/70	M	31.30	"
424 H	8/13/70	F	26.31	"
417	8/14/70	F	43.55	"
403	8/14/70	F	48.08	"
413	8/14/70	F	39.92	"
427	8/15/70	F	36.74	"

Total No. 19

Males 5

Females 14

APPENDIX XI

NEWBORN CALVES T_4 µg/100 ml SERUM

Calf No.	Days						
	1	2	3	4	5	6	7
430	17.33	18.11	9.70	9.31	8.86	6.60	5.28
415	19.83	15.69	13.17	9.48	7.93	13.03	7.05
405	11.95	12.20	13.73	10.53	11.36	10.77	8.06
1031	19.24	14.89	13.67	11.32	8.79	9.62	10.34
431	15.04	21.34	14.66	11.35	9.74	7.90	7.49
418	18.11	16.25	12.42	9.79	8.02	7.19	6.11
408	22.77	18.14	19.27	12.55	11.62	8.15	7.12
994	27.87	22.51	10.58	10.58	9.18	6.73	6.99
417	16.73	16.09	15.86	13.57	10.91	11.08	10.76
427	17.61	19.38	12.11	10.26	8.42	6.66	8.11
407	13.96	18.58	16.03	13.26	12.79	11.39	11.18
406	17.18	14.54	14.55	11.85	10.58	6.82	4.40
411	14.54	15.21	9.58	10.21	6.41	6.25	-
413	15.82	12.51	10.12	10.07	5.22	3.52	2.19
420	15.71	16.16	14.15	9.55	7.70	6.37	8.60
409	12.36	16.89	16.33	10.24	5.18	7.21	7.37
403	12.78	15.95	15.79	13.52	8.36	5.92	4.84
424B	18.03	15.11	11.21	11.33	6.73	5.62	7.88
424H	14.62	11.54	10.45	9.04	8.60	7.55	6.72
No	19	19	19	19	19	19	18
Mean ±	16.92 ±	16.37 ±	13.36 ±	10.93 ±	8.75 ±	7.80 ±	7.28 ±
S.E.	0.872	0.657	0.613	0.329	0.477	0.541	0.509

APPENDIX XII

NEWBORN CALVES PBI μg/100 ml SERUM

Calf No.	Days						
	1	2	3	4	5	6	7
430	8.75	9.99	5.72	5.58	8.69	5.00	5.74
415	13.83	10.12	8.16	6.43	5.89	5.93	5.94
405	7.05	7.67	6.70	6.09	6.40	8.52	5.35
1031	16.42	12.41	8.43	7.18	6.72	5.86	8.58
431	8.68	13.36	9.85	6.50	6.07	5.20	5.06
418	7.96	7.29	6.93	5.47	5.68	4.23	4.18
408	10.50	11.07	13.34	9.97	7.77	6.98	6.04
994	7.51	5.87	7.88	7.79	5.11	6.14	6.38
417	16.27	17.55	17.22	13.60	11.47	7.76	7.62
427	17.93	18.12	12.52	6.34	6.79	4.92	5.70
407	10.86	17.62	13.86	10.26	9.46	7.71	7.11
406	17.34	12.74	12.25	9.97	9.61	5.17	5.61
411	12.23	10.11	8.11	6.83	7.63	5.60	-
413	18.87	13.47	10.88	8.91	7198	9.62	8.03
420	10.86	10.41	9.90	8.79	5.78	4.88	5.41
409	16.64	12.40	11.35	10.40	3.50	7.19	6.65
403	10.33	13.00	12.26	10.62	6.72	6.57	5.75
424B	14.91	10.37	7.26	7.00	5.46	5.29	6.94
424H	8.54	8.19	6.57	7.52	6.38	6.03	5.65
No.	19	19	19	19	19	19	18
Mean ±	12.39 ±	11.67 ±	9.95 ±	8.17 ±	7.00 ±	6.21 ±	6.24±
S.E.	0.907	0.791	0.703	0.497	0.427	0.317	0.247

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