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In utero treatment of fetal goiter with a non-halogenated thyroid hormone analog

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IN UTERO TREATMENT OF FETAL GOITER WITH A NON-HALOGENATED THYROID HORMONE ANALOG

Florence Comite
B.S. Brooklyn College of C.U.N.Y.

Presented in partial fulfillment of the requirements
for the degree of Doctor of Medicine,
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TABLE OF CONTENTS

"The stunted stature, the semi-bestial aspect, the blubber lips, retroussé nose, sunken at the root, the wide-open mouth, the lolling tongue, the small eyes, half closed with swollen lids, the solid, expressionless face, the squat figure, the muddy, dry skin, combine to make the picture of what has been well-termed the 'pariah of nature'.

Not the magic wand of Prospero or the brave kiss of the daughter of Hippocrates ever effected such a change as that which we are now enabled to make in these unfortunate victims..."

William Osler, M.D.: Internal Secretions; Considered in their Physiological, Pathological and Clinical Aspects. Sporadic Cretinism in America. Transactions of the Congress of American Physicians and Surgeons. 1867

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Dedicated to Henry.

INTRODUCTION

The recent suggestion that congenital hypothyroidism may occur as often as 1 in 6,000 deliveries has reawakened interest in the diagnosis and treatment of this disease (2,3,5). Even though congenital hypothyroidism may be identified during newborn screening programs, it is not clear whether thyroid hormone therapy, even when begun shortly after birth, will result in full attainment of intellectual potential (6,72). Consequently, there has been interest in the early diagnosis and treatment of hypothyroidism in utero (11-14,94). Chopra and Crandall (13) have suggested that 3,3',5'-triiodothyronine (reverse T_3) levels in amniotic fluid may be a good measure of fetal thyroid function. Van Herle et al (16) have treated a fetus exposed to radioactive iodide with injections of thyroxine (T_4) in utero. However, despite in utero thyroid hormone therapy, the patient was hypothyroid at birth.

If maternal thyroid hormone were available to the fetus in adequate amounts, hypothyroidism in utero would not be a matter of concern. However, available evidence suggests that placental transfer of iodothyronines is minimal (26,27, 56,64-68). The ability of compounds to cross the placenta depends on a number of factors including molecular weight, lipid solubility, and protein binding (41,42). Recently, Jorgensen and his coworkers (69,70) have synthesized

biologically active thyroid hormone analogues which are smaller molecules with increased lipid solubility and decreased binding to thyroxine-binding-globulin (TBG) in comparison to the iodothyronines.

The current study was undertaken in an attempt to assess placental transmission of the thyroid hormone analogues and their thyromimetic activity in the hypothyroid rat fetus.

LITERATURE REVIEW

Hypothyroidism can be detected at birth by measuring serum thyroxine (T_4) and thyroid-stimulating hormone (TSH). Early clinical diagnosis was difficult because hypothyroid infants typically appear euthyroid at birth (1). Recent pilot screening programs, using the radioimmunoassay for T_4 or TSH, have suggested that congenital hypothyroidism may occur in as often as 1 in 6,000 deliveries (2,3). This incidence surpasses that of phenylketonuria (PKU), which occurs with a national frequency of 1 in 14,300 (4), and is the subject of a national screening program.

The various methods of detection of hypothyroidism in the newborn include umbilical cord and capillary blood determinations of T_4 and TSH either directly or with eluates of filter paper blood spots prepared from capillary blood (2,3). Determination of TSH level is the most discriminating test in the diagnosis of primary hypothyroidism. However, determining serum TSH is more expensive as a screening technique and, in addition, would fail to detect TBG abnormalities (2). A more satisfactory method might be to obtain cord blood for T_4; if serum T_4 is low, then TSH levels could be determined immediately. Follow-up time would also be shortened in obtaining serum for TSH that would be necessary using the filter paper technique (5).

Whether the early diagnosis and postnatal treatment of the athyreotic newborn prevents residual brain damage is not clear. Klein, Meltzer, and Kenny (6) observed that thyroid hormone replacement in the human infant with congenital hypothyroidism improved mental retardation, if treatment was begun before three months of age. However, if the critical period of brain development begins prenatally in humans (7), early postnatal detection and treatment of congenital hypothyroidism may not completely prevent central nervous system (CNS) damage (72). More than half of fifty patients with congenital hypothyroidism had signs of cerebellar dysfunction such as ataxic gait, dysdiadochokinesis and clumsiness for variable periods of their life, despite being diagnosed and treated within the first three months of life (8). Smith, Blizzard and Wilkins (72) observed that the greater the extent of intrauterine hypothyroidism the worse the prognosis in severe cretins even though adequately treated by four months of age. In utero diagnosis and treatment of congenital hypothyroidism might allow for an improved prognosis of the disease.

Previous studies have indicated that the fetal thyroid is capable of synthesizing iodothyronines by ten weeks of gestation (9) with detectable fetal serum T_4 levels by the eleventh week (10). Thyroid hormone has also been found in amniotic fluid (11). But measurements of amniotic

fluid thyroxine (T_4) has not proven helpful in the diagnosis of fetal thyroid status (11-13). No correlation was found between amniotic fluid total free T_4 levels and either maternal or fetal serum TSH concentrations; amniotic fluid T_4 levels probably reflects both maternal and fetal hormone (11,12). TSH and T_3 were undetectable in human amniotic fluid, although T_3 was easily measured in both maternal and fetal blood (11).

Recently, Chopra and Crandall (13) have shown that it may be possible to diagnose fetal hypothyroidism early in pregnancy by measuring 3,3',5'-triiodothyronine (reverse T_3) level in amniotic fluid. Reverse T_3 is a normal component of human serum and thyroglobulin. Peripheral metabolism of T_4, by the action of an α-ring deiodinase, is an important source of this iodothyronine (14,101). This hormone has little calorigenic activity (95), although it has been shown capable of exerting an anti-thyroxine effect (96). At 15 weeks of pregnancy, reverse T_3 concentration in amniotic fluid were found to be high and thought to be primarily of fetal origin as levels exceeded those in maternal serum (13). Although after the 30th week of gestation amniotic fluid reverse T_3 decreased, levels always exceeded maternal serum reverse T_3 concentration which remained essentially unchanged throughout pregnancy (13). Amniotic levels of T_3 and reverse T_3 parallel serum concentrations in the newborn and are distinct from maternal serum levels. In the fetus at term, serum T_3

was found to be markedly subnormal while serum reverse T_3 was clearly elevated (14,15). Chopra (14) has also demonstrated decreased serum reverse T_3 concentrations in hypothyroidism while increased concentrations were found in hyperthyroidism. Presumably, the hypothyroid fetus would have low levels of amniotic fluid reverse T_3 in comparison to the euthyroid fetus. The measurement of reverse T_3 by amniocentesis, as early as 15 weeks of gestation, may prove to be helpful in the diagnosis of congenital hypothyroidism in utero (13).

The majority of cases of congenital hypothyroidism occur sporadically. However, clinical situations exist in which the liklihood of fetal hypothyroidism is high. Of particular note is the destruction of the fetal thyroid in the pregnant mother inadvertently exposed to radioiodine during the latter two-thirds of gestation (16,17,97). The developing fetal thyroid is capable of concentrating radioactive iodine by the third month of gestation (17). ^{131}I given to a mother near term reaches a tenfold greater concentration in the fetal thyroid than in the mother's gland (18). Asthmatic pregnant women treated with inorganic iodide can give birth to infants with large goiters (19,20). Most of these infants do well providing they escape death by asphyxiation due to tracheal obstruction (19). However, signs of hypothyroidism at birth may cause permanent damage in some of these infants, even if they become euthyroid rapidly (21).

The risk of fetal hypothyroidism or goiter is also present in fetuses of hyperthyroid pregnant women treated with anti-thyroid medication (28). It is thought that goitrogenesis results from the increased secretion of fetal pituitary TSH in response to the decreased production of fetal thyroid hormone induced by the anti-thyroid drug which has crossed the placenta (28,29,39). The occurrence of fetal complications has not been found to be directly dose related. It is postulated that the effects of fetal exposure to an anti-thyroid drug may be genetically determined (22,23,27). Most of the children exposed to an anti-thyroid drug in utero with subsequent goiter formation have no lasting effects, although there have been isolated case reports of mental retardation occurring in such children (22). In a retrospective study of 30 women treated with propylthiouracil (PTU) during 41 pregnancies, five children were born with goiters. There was a question of mental retardation in a follow-up exam of one of the five goitrous newborns (22). Selenkow (24,25) advocates that thyroid hormone supplementation is important for pregnant women on anti-thyroid medication to prevent maternal hypothyroidism and protect the fetus from goiter. However, it is not at all clear that fetal hypothyroidism would be avoided; evidence suggests that transplacental passage of iodothyronines is minimal (22,26). It is not known why treatment of thyrotoxic mothers with anti-thyroid drugs

only infrequently results in the birth of hypothyroid or goitrous infants. There are two case reports of only one of each set of dizygotic twins born with a goiter as a result of maternal treatment with anti-thyroid medication during gestation (27). Serum TSH was elevated in both affected infants; diminished serum T_4 was found in one twin. This selective effect of anti-thyroid drugs may be due to genetic differences in metabolic clearance rates of the anti-thyroid compounds similar to that observed with other drugs, or perhaps, differences in the thyroidal uptake by the fetal gland of the anti-thyroid drugs may be responsible (27).

Sporadic congenital hypothyroidism can be due to athyreosis secondary to an embryologic developmental defect of the thyroid gland (31,32). Rarely, neonatal athyreosis may have resulted from destruction of the thyroid gland by autoimmune diseases such as Hashimoto's thyroiditis (33-35). Inborn errors of thyroid hormonogenesis (36), or peripheral tissue resistance to the action of thyroid hormones (37,38) are other very uncommon causes of sporadic hypothyroidism.

If amniotic fluid concentration of reverse T_3 proves to correlate well with fetal thyroid status, amniocentesis would be of great diagnostic value. Initially, cases with a known increased risk of fetal thyroid dysfunction would probably be evaluated as amniocentesis does engender some risks (40). Pregnancies in which the fetus had been exposed

to radioiodine or in which the mother had been treated with anti-thyroid therapy or potentially goitrogenic drugs would also be included. Amniocentesis might also prove to be very beneficial for the mother with a positive family history of inborn defects of thyroid hormone metabolism (40).

Conflicting data exists about the extent to which the iodothyronines cross the placenta. Although some placental transfer of maternal thyroid hormone probably occurs during the last third of pregnancy in the rat (43-45), guinea pig (46-49,57), rabbit (50), sheep (51-53), monkey (54,55), and man (26,56), available evidence suggests that it is limited (30,54,57).

In general, it is thought that placental transmission of drugs is based upon lipid solubility, ionic charge and concentration gradient of undissociated molecules, serum protein binding, and molecular size (41). Compounds with molecular weights of less than 500 tend to cross more easily, while those with a higher molecular weight cross with increasing difficulty. A high lipid/water solubility enhances placental transfer, and the non-ionized molecule is also more lipophilic. In addition, protein binding is very important as only unbound drugs are available for transfer (41,42). It is not clear which of the above factors or combination of factors dominates in placental transfer of iodothyronines.

The administration of a large dose of T_4 (200 µg/day) to pregnant rats will prevent the development of propylthiouracil (PTU) goiter in the fetus (58). These same observations have been made in the guinea pig and rabbit (46,50). In all of these species, the placental transfer of labelled T_4 and T_3 is found to be limited (30,47-49). In the sheep, Fisher et al (53) shows marked base line fetal-maternal serum concentration gradients of T_4, free T_4, T_3, and free T_3. After fetal ovine thyroidectomy, there is rapid disappearance of T_4 and T_3, and an increase in serum TSH (61-63). Reduction in fetal T_4 and T_3 production rates to 5-10% of normal values occurs (63). When the pregnant rhesus monkey is given T_4 in quantities increasing serum levels to 300%, fetal serum T_4 does not change and T_4 synthesis by the fetal thyroid gland is not inhibited (54).

Human data is similar. Limited placental transfer of iodothyronines is observed at term. When the pregnant woman is given large quantities of T_4 (1500 -8000 µg) or T_3 (300 µg) at term or during labor, maternal-fetal hormone transfer is noted (26,64). Only about 1% of the 8000 µg T_4 load reaches the fetus and the chronic 300 µg/day of T_3 reduces the fetal serum T_4 levels minimally and inconsistently (65,66). In all species investigated, little or no hormone transverses the placenta at physiological serum concentrations, although at very high blood levels some maternal-fetal transfer may occur. However, Keynes (67) reports that administration of

500 μg T_4 daily to a pregnant woman on PTU did not prevent PTU goiter development in the infant. Carr at al (68), after giving approximately 1400 mg dessicated thyroid daily to a pregnant woman observed cord blood BEI of only 4.5 μg% in the fetus, who was shown to have a small residual of hyperfunctioning thyroid tissue; 2-hour RAI uptake was 7% and 24-hour uptake was 3%. In studies of the rat (43,60), guinea pig (46) and sheep (51) placental transfer of TSH is found to be insignificant. Dussault et al (51) have shown that in preliminary studies in which ^{131}I and ^{125}I-labeled TSH is injected into fetal and maternal sheep, respectively, placental TSH transfer does not occur. In humans, fetal pituitary and serum TSH concentrations increase markedly between 18 and 22 weeks of gestation from very low to relatively high levels, while maternal TSH concentration is fairly stable and show no correlation with fetal values (102). The data suggests that the fetal pituitary-thyroid axis seems to function nearly independently of the maternal system.

Recently, Jorgensen and his coworkers (69) have synthesized biologically active thyroid hormone analogues (Figure 1), which are smaller molecules having increased lipid solubility and decreased thyroxine-protein binding in comparison to the iodothyronines (70). The synthesis of these compounds raises the possibility that alteration of molecular structure of thyroid hormone may facilitate placental transfer.

Of particular interest is a non-halogenated thyroid hormone analog, 3,5-dimethyl-3'-isopropyl-L-thyronine known as DIMIT. DIMIT is approximately 20% as active as T_4 in the adult rat antigoiter assay (69,71). In addition, two other thyroid hormone analogues were evaluated; 3,5-diiodo-3'-isopropyl-L-thyronine abbreviated DIIIT and 3,5-diiodo-3'-secondary butyl-L-thyronine or DISBT. Both compounds have similar properties compared to DIMIT, except they are 15 times more active in the adult rat antigoiter assay and are slightly heavier molecules (70).

It is known that early diagnosis and treatment are important in minimizing central nervous system damage in newborn infants with hypothyroidism (6,72). When hypothyroid children are treated prior to three months of age, they attain significantly higher IQ's in comparison to infants who are administered thyroid hormones at four months or older (6). It is not clear to what extent brain growth and development are dependent on thyroid hormone in utero. A critical period certainly exists, during which normal central nervous system development is dependent on the presence of thyroid hormone (6, 7,72-24). This period has been shown to occur postnatally in the rat (7,74), but in man and other species in which the central nervous system is more mature at birth, this critical time presumably begins in utero (7). Whether subsequent administration of thyroid hormones can correct abnormalities due to intrauterine lack of thyroid hormones is not known. In the rat, hypothyroidism induces skeletal and cerebellar

abnormalities which are only prevented if T_4 is given during the first two postnatal weeks (74,75). In species with a more differentiated central nervous system at birth, thyroxine administered within three months after birth may not correct prenatal abnormalities created by the lack of thyroid hormone during gestation (8,72,73). In the ovine fetus thyroidectomized in utero, clinical manisfestations of hypothyroidism are most apparent in the skin, bone, and nervous system. Findings include a low body weight, shortened limbs, and retarded maturation and growth of wool follicles and osseous tissue (76,77). Athyreotic lambs exhibit no inclination to suck despite assistance, perhaps indicative of retarded brain development (76). In addition, thyroid deprivation is found to cause myelin fragmentation and a decrease in cerebellar intra-cytoplasmic vesicles and synapses in neonatal cretin lambs (77). There are some recent observations in fetal sheep suggesting that intrauterine hypothyroidism does not impair brain growth and only minimally delays myelination (78).

Clinically, combined maternal-fetal hypothyroidism in the rhesus monkey results in marked retardation of skeletal growth and maturation and the recognizable clinical syndrome of cretinism (80). Radioiodine-treated rhesus monkeys have a 20% decrease in CNS protein and RNA synthesis and ganglioside deposition (79). The data indicates that in the absence of thyroid hormone both neuronal and neuroglial cell populations

are functionally affected in the cerebrum and cerebellum (79). Rhesus monkeys are shown to be comparable to humans in terms of placental structure, morphological thyroid development, serum protein species transporting thyroid hormone, and the thyroidal and blood hormone iodine levels (81,82).

Early clinical recognition of human cretins is difficult. Some athyreotic infants do manifest signs and symptoms of hypothyroidism in the newborn period (83). Characteristics of congenital hypothyroidism include prolonged gestation, large birth weights (>4 kg), protracted icterus, edema, abdominal distension, vomiting, and poor feeding. Other, less common findings, include hypothermia (<95^{o} F), lag in stooling, large posterior fontanel, respiratory distress, peripheral cyanosis, and hypoactivity or lethargy. Radiologically, osseous retardation and epiphyseal dysgenesis are diagnostic (84-86) Smith et al (72) studied 128 cases of hypothyroidism and found that in eight of 15 patients diagnosed as severe cretins, and adequately treated by four months of age, the mean IQ was <90. In five of these patients, osseous development is noted to be less than an eight month fetal level, IQ attained in this group ranges from 28 to 68 with a mean of 52. In an additional six cases with osseous development of eight month fetal level or greater, the IQ ranges from 80 to 111 with a mean of 93. This data suggests that "...intrauterine hypothyroidism can exist, and the greater the extent of the intrautering hypothyroidism, as

evidenced by osseous retardation and epiphysial dysgenesis, the poorer is the mental prognosis in severe cretins even though adequately treated by four months of age (72)." Klein et al (6), shows that when hypothyroid infants are treated before the age of three months, 85% have an IQ above 85, whereas when treatment is delayed three to seven months, 85% have definitive mental deficiency. However, even when replacement therapy is initiated shortly after birth, the congenital cretin may remain clumsy, poorly coordinated and handicapped by speech problems (77).

Should hypothyroidism in utero be diagnosed, the most appropriate mode of therapy has not been resolved, assuming maternal thyroid hormone is not readily accessible to the fetus. As evidence indicates that placental transfer of T_4 or T_3 is limited and erratic, the administration of massive doses of T_4 or T_3 to the mother would be relatively ineffective, with the additional risk of maternal thyrotoxicity. Van Herle et al (16) have attempted to treat a fetus exposed to radioactive iodide with four biweekly intramuscular injections of L-thyroxine (120 µg T_4) in utero, beginning at 32 weeks of gestation. During the 24th week, the mother was begun on oral doses of L-triiodothyronine (100 µg T_3) daily. However, despite therapy, the patient was hypothyroid at birth with cord blood levels of T_4 and T_3 undetectable and TSH markedly elevated. In contrast, the maternal serum T_3

concentration was normal and a marked fetal to maternal TSH gradient was present, implying that the placenta is impermeable to T_3 and TSH. It was noted that an adequate T_4 dose would be difficult to supply via intramuscular in utero injections, as the fetus would require approximately 350 µg/week of T_4 based upon daily iodothyronine turnover in the newborn and equal to 18 µg/kg/day (93). Intra-amniotic injections of T_4 have been suggested (40). Thyroid hormone injected into amniotic fluid in the sheep is quantitatively absorbed by the fetus within 24 hours (87). However, the proper dose of thyroid hormone would be difficult to determine. This is crucial as acceleration of events in the brain due to hyperthyroidism can be just as disrupting as deceleration due to hypothyroidism (88). Even a temporary state of hyperthyroidism or hypothyroidism may interfere with normal brain development. A number of events occurring in the brain are known to be dependent on thyroid hormone. In fatty acid metabolism, chain elongation of fatty acids is thyroid-dependent, while thyroid hormone is not necessary for the development of polyunsaturates (88). If a delay caused by the lack of thyroid hormone or acceleration due to an excess of thyroid hormone occurs, normal interrelationships are disrupted. This leads to disordered timing of maturation, causing asynchronous development (98). Nicholson and Altman (99) show that disturbed timing of cellular proliferation in the brain

of hypothyroid or hyperthyroid rats is associated with alterations in the number and type of cells present in the cerebellar cortex. In hypothyroidism, smaller, more tightly packed and less differentiated cells are, in part, responsible for a decreased weight of adult rat cerebrum and cerebellum. While in hyperthyroidism, the cerebral and cerebellar weight deficits are attributed to a permanent decrease in total cell number due to early differentiation and associated termination of cell proliferation (99). The number of arborations between neurons in the brain may also be reduced in hypothyroidism (88). Eayrs (100) shows that hypothyroid rats demonstrate perseveration, and feels this may be due to the decreased arboration and neuron-neuron interaction. Mongoloid children, with decreased arboration in the brain, show a decreased variability in the amplitude of electrical potentials in EEG tracings, which can also be shown in hpothyroid children (88). In the cerebellum of hyperthyroid rats, the external granular layer disappears earlier and DNA synthesis stops earlier (99). These changes in humans early in life are associated with an accelerated functional development, but, in adulthood are associated with decreased functional ability (89). Changes in fetal thyroid hormone status may cause alterations of the developing central nervous system in utero.

The current study was undertaken to investigate the possible role of thyroid hormone analogues in the treatment of in utero hypothyroidism.

MATERIAL AND METHODS

Pregnant Sprague-Dawley rats were obtained on day ten post-coitus from Charles Dawson Laboratories. The animals were kept in a controlled environment with a constant temperature of 75-80° F. and with lighting from 7:00 a.m. until 7:00 p.m. The animals were given access to food and water ad libitum for five days prior to beginning the experiment. The pregnant rats were then divided randomly into groups. The control group was given ground Purina Laboratory Chow (containing 1.7 ppm iodine); while the experimental groups were given 0.3% propylthiouracil (PTU) in ground Purina Laboratory Chow from day 16 of gestation to induce fetal goitrogenesis (43,45,58). (Figure 2) At the same time, 0.5 ml. daily intramuscular injections of either T_4, T_3 or the thyroid hormone analogues dissolved in ethanol were begun. The control group and the PTU-diet control group were injected with 0.5 ml. of pure ethanol.

Food intake was measured daily. Since the groups on the 0.3% PTU-diet did not consume as much as the control group, food intake of the former groups was averaged and this mean intake was given to the group on a normal diet.

The dose of thyroxine was based on data suggesting that a euthyroid replacement dose of T_4 in thyroidectomized rats is 1.3 µg per 100 gram body weight per day (43,44,90-92).

Doses of thyroid hormones and analogues were calculated as μg per 100 gram maternal body weight in pregnant rats. Weights ranged from 200 to 300 grams by day 16 of gestation. Daily IM injections were alternated in both thighs for five days prior to sacrifice (Figure 2). Solutions of the thyroid hormones and analogues were kept refrigerated and used over a two week period.

Rats were sacrificed by exsanguination under ether anesthesia on day 21 of gestation. Maternal blood was obtained from the inferior vena cava. Following hysterotomy, the fetuses were sacrificed and exsanguinated by decapitation; blood from fetuses of a single litter were pooled. With the use of a dissecting microscope, fetal thyroids were removed intact with the trachea. The thyroids were then separated from the trachea. Each thyroid was weighed on a torsion scale (45), and all thyroids of a single litter were pooled in ten percent formalin for histological staining. Several studies were done in a double blind fashion to judge the reliability of the weighing procedure.

Maternal and fetal serum TSH concentrations were measured by radioimmune assay using ^{131}I. On the final day of each experiment, the maternal and fetal blood obtained was centrifuged and the serum placed in the freezer for subsequent assay. Fetal sera from one or two experiments were pooled to obtain a sufficient quantity for assay; separate

determinations of corresponding maternal serum TSH concentrations were done at the same time. TSH values of control and experimental groups injected with the same compound (T_4, T_3, DIMIT, DIIIT or DISBT) were then averaged.

The Student T-test was utilized for determinations of the significance of differences between means. All values were reported as ± standard error of the mean.

RESULTS

Initially, a series of experiments were done to determine doses of thyroid hormones and analogues which prevented PTU-induced fetal goiter. When pregnant rats were fed a 0.3% PTU-diet from day 16 to day 20 of gestation (Figure 2), mean fetal thyroid weight was found to be significantly increased compared to control thyroid weight. (Table 1, Figure 3) Control fetal thyroids weighed 1.10 ± 0.05 mg, while the mean fetal thyroid weight of PTU-fed rats was 1.92 ± 0.11 mg ($p < .0001$). 5.2 µg of T_4 (sodium L-thyroxine pentahydrate) per 100 gram maternal body weight from day 16 to day 20 were required to prevent the occurrence of fetal goiters in rats fed PTU. This dosage of T_4 produced a mean fetal thyroid weight of 1.18 ± 0.07 mg. There was no suppression of fetal goiter with 2.56 µg T_4: mean thyroid weight was 1.68 ± 0.14 mg. However, 2.56 µg of T_3 (sodium salt of triiodo-L-thyronine) per 100 gm maternal B.W. was sufficient to prevent goiter formation and resulted in a mean fetal thyroid weight of 1.27 ± 0.07 mg. When 1.28 µg of T_3 was injected into rats fed PTU, fetal goiters were not inhibited; mean fetal thyroid weight was 1.77 ± 0.08 mg. In contrast, 0.45 µg of DIMIT (3,5-dimethyl-3'-isopropyl-L-thyronine) per 100 gm maternal B.W. prevented fetal goiter formation. Mean fetal thyroid weight was 1.05 ± 0.05 mg. A dose of 0.23 µg of DIMIT did not

prevent fetal goiter with a mean fetal thyroid weight of 1.76 ± 0.09 mg.

The thyroid hormone analogues, 3,5-diiodo-3'-isopropyl-L-thyronine (DIIIT) and 3,5-diiodo-3'-secondary butyl-L-thyronine (DISBT), were also evaluated. Although both of these compounds have 15 times more thyromimetic activity when compared to DIMIT in the adult rat antigoiter assay (70), they are larger molecules with increased protein binding to TBG (70). Both these characteristics may be associated with decreased placenta transfer.

Control fetal thyroid weight was 1.15 ± 0.06 mg and the mean fetal thyroid weight of PTU-fed animals was 1.78 ± 0.13 mg ($p < .001$). (Table 2, Figure 4) 0.45 µg of DIMIT per 100 gm maternal body weight daily administered to PTU-fed rats again prevented the development of fetal thyroid goiter; mean thyroid weight was 0.90 ± 0.07 mg, slightly less than mean control fetal thyroid weight. Fetal goiters were not prevented when 0.45 µg of DIIIT or 0.45 µg of DISBT was injected into rats fed PTU with mean fetal thyroid weights of 1.78 ± 0.08 mg and 1.68 ± 0.09 mg, respectively. However, 0.90 µg of these compounds did prevent fetal goitrogenesis with mean fetal thyroid weights of 1.05 ± 0.06 mg in PTU-fed rats injected with DIIIT and 1.01 ± 0.06 mg in rats given DISBT. There was no significant difference between DIMIT and these analogues in the prevention of goitrogenesis when calculated by molecular weight.

Histological examination of the fetal thyroids did not reveal objective differences between the various groups. Ingestion of PTU by the mother resulted in enlargement and some minimal disorganization of the follicular structure of the fetal thyroid glands.

Mean pooled fetal TSH levels paralleled mean fetal thyroid weights. (Figure 5) Control fetal serum TSH was 88 ng/ml (19 μU/ml) compared to 135 ng TSH/ml (30 μU TSH/ml) in fetuses exposed to PTU. When 0.45 or 0.90 μg of DIMIT per 100 gm maternal body weight was given with PTU, fetal serum TSH was not detectable. In animals injected with 5.20 μg T_4, mean fetal serum TSH was about 1/3 control fetal TSH values (25 ng TSH/ml; 6 μU TSH/ml). Fetal serum TSH approximated control fetal TSH concentration (85 ng TSH/ml; 19 μU TSH/ml) when 2.56 μg T_3 was administered. When the thyroid hormone analogues, DIIIT and DISBT were injected in doses of 0.90 μg per 100 gram maternal body weight daily, no TSH was detectable in fetal serum.

Maternal serum TSH was 24 ng/ml (5 μU/ml) in control rats; a PTU-diet resulted in serum TSH increased to 89 ng/ml (20 μU/ml). (Figure 6) Although 5.20 μg T_4 or 2.56 μg T_3 per 100 gram maternal body weight administered along with PTU suppressed maternal serum TSH to less than control maternal TSH concentration, 0.45 μg DIMIT failed to completely counteract the increase in maternal serum TSH due to the PTU. Rats treated with T_4 or T_3 has serum TSH levels of 15 ng/ml

(3 μU TSH/ml) and 4.5 ng/ml (1 μU TSH/ml), respectively. Maternal serum TSH in rats fed PTU and injected with 0.45 μg DIMIT was 46 ng/ml (10 μU TSH/ml). Serum TSH concentration in rats exposed to twice the goiter preventing dose of DIMIT, or 0.09 μg, was 5 ng/ml (1 μU TSH/ml). When 0.09 μg DIIIT per 100 gm maternal B.W. was administered to rats fed PTU, serum TSH was undetectable. Rats injected with 0.09 μg DISBT had 7.5 ng TSH/ml (2 μU TSH/ml).

Since the thyroid hormone analog DIMIT is not a natural hormone, several experiments were done to determine possible teratogenic effects. Injections of 3.6 μg DIMIT per 100 gm maternal body weight, or approximately eight times the dose of DIMIT which suppresses goiter formation were begun on the eighth day of gestation, after implantation of the embryo had occurred. Subsequent injections were given on alternate days. Control rats and experimental rats were sacrificed on day 12, day 16, and day 19 of gestation. No gross abnormalities could be found in the fetuses exposed to DIMIT in utero. However, two pregnant rats injected with the same amount of DIMIT and permitted to undergo natural delivery, ate the newborn rats, which might indicate that the fetuses were abnormal. Earlier experiments with very high doses of DIMIT, more than 30 times the dose of DIMIT inhibiting fetal goitrogenesis, administered to PTU-fed rats daily from day 16 through day 20 of gestation, did produce fetuses that appeared

slightly erythematous and edematous in comparison to control fetuses. Weights of these fetuses did not consistently differ significantly compared to control fetal weight.

DISCUSSION

In the present study, pregnant rats received propylthiouracil (PTU) in an attempt to produce fetal goiters. When these rats were given an 0.3% PTU-diet for the last five days of gestation, the mean absolute fetal thyroid weight approximately doubled compared to control fetal thyroid weight which is consistent with previous observations. Hamburgh et al (43) and Knobil and Josimovich (45) found that a maternal diet of 0.1%-0.2% PTU throughout the last week or two weeks of gestation in pregnant rats, caused fetal goiters which were twice as large as thyroids of control fetuses. Hoskins, Van Arsdel and Williams (58) reported that newborn offspring of mothers who had received 1% PTU from the beginning of gestation (22-23 days), had thyroids that weighed approximately five times control newborn thyroid weight. Since Maternal thyroidectomy or hypophysectomy did not affect fetal thyroid weight, they calculated that goiter formation in the fetus following a PTU-diet in pregnant rats was mediated by the fetal-pituitary axis (45).

Pharmocologic doses of thyroxine (T_4) and triiodothyronine (T_3) had to be administered to the pregnant rat on a PTU-diet in order to prevent fetal goitrogenesis. 5.20 µg T_4 or 2.56 µg T_3 per 100 gram maternal body weight daily were necessary. The

physiologic replacement dose of T_4 in rats has been shown to be 1.3 µg per 100 gram body weight (90,91). Cullen, Doherty and Ingbar (92) showed that 6.7 µg of T_4 or 2.2 µg of T_3 per 100 gram body weight caused thyrotoxicosis in rats. The previous data suggested that the large doses of iodothyronines required to cross the placenta and prevent fetal goiter formation due to a maternal diet of PTU would cause thyrotoxicity in the pregnant mother.

Hamburgh et al (43) found that at least 50 µg T_4 (free acid base of L-thyroxine) per day from day 15 of geatation was necessary to inhibit fetal goiter due to maternal ingestion of 0.2% PTU. 25 µg T_4 daily did not appreciably inhibit fetal goitrogenesis. In the study by Knobil and Josimovich (45), 15 µg T_4 (sodium salt of L-thyroxine) or the same amount of T_3 daily was sufficient to prevent fetal goiter due to a maternal diet of 0.1% PTU from day 11 of gestation. Hoskins, Van Arsdel and Williams (58) noted that newborn rats of animals on 1% PTU that were injected with 100 µg T_4 (sodium L-thyroxine pentahydrate) every 12 hours throughout gestation had thyroid weights equal to control thyroid weight.

Results of maternal and fetal serum TSH concentrations correlated well with the activity of the thyroid hormones and analogues as determined by fetal thyroid weight. As

expected, a PTU diet significantly increased both maternal and fetal serum TSH. When ovine fetuses were thyroidectomized in utero, serum TSH was significantly elevated at birth (63). It should also be noted that significant thyroid enlargement in fetuses of thyroidectomized or hypophysectomized rats and guinea pigs treated with PTU, occurred only when the fetal hypophysis was intact (30,45). In humans, treatment with PTU, during gestation, caused elevation of serum TSH in affected offspring (27). The hypothyroid newborn of a mother exposed to radioiodine during gestation, had significantly elevated levels of cord blood TSH compared to the TSH concentrations in cord blood of euthyroid newborns (16). It is clear that, in man as in animals, PTU can produce fetal goiter and that the athyreotic fetus manifested low T_4 and T_3 concentrations and increased serum TSH in cord blood (16,27,63,77).

In the present study, the mean serum TSH values in mothers who had received goiter preventing doses of the iodothyronines were equal to the mean serum TSH in the control group. In the fetuses of the T_4 and T_3 injected mothers, serum TSH levels were less than or equal to control fetal TSH. When 0.45 µg of DIMIT was administered to pregnant rats fed PTU, maternal TSH concentration fell between control maternal serum TSH level and maternal serum TSH of rats fed PTU; yet, in the fetuses of DIMIT-injected rats no TSH was detected. DIIIT completely suppressed maternal TSH; while animals treated

with DISBT and fed PTU had a serum TSH concentration that was less than 1/3 control maternal serum TSH. Fetal serum TSH levels of animals injected with DIIIT or DISBT were undetectable. The data implied that while DIMIT crossed the placenta and prevented fetal goitrogenesis, the concentration in the mother was not high enough to suppress TSH.

Which of these properties - molecular weight, lipid solubility, or protein binding is most important in increasing placental transfer remains to be determined. (Figure 7) DIMIT is the smallest of the three analogues and therefore should cross the placenta more easily. All three compounds have increased lipid solubility in comparison to T_4 (70), which should enhance placental permeability. The addition of iodide to DIIIT and DISBT should increase lipid solubility because iodide is two and a half times as lipophilic as a methyl group on DIMIT. However, this increased lipid solubility is offset by heightened protein binding secondary to increased ionization of the phenolic hydroxy (OH) group on the β-ring caused by the addition of iodide. In addition, DIMIT does not bind to TBG as well as the other analogues (70). This would allow an increased amount of unbound hormone to be available for placental transfer.

Another factor that must be considered is the possibility that the iodinated thyroid hormone analogues, DIIIT an DISBT, may be acted upon by the fetal α-ring

deiodinase, which forms the inactive reverse T_3 (101), whereas DIMIT lacks the iodide necessary for such a reaction.

Finally, if the dose of thyroid hormone which prevents fetal goiter is so large that it causes maternal thyrotoxicosis, clinical benefit would be marginal. Therefore, the relationship between the effect of thyroid hormone on the fetus and the effect of thyroid hormone on the mother is very significant. In Figure 8, the numbers are all relative to the activity of thyroxine (T_4). The numbers in the first column compare the activity of T_3 and the thyroid hormone analogues to the activity of T_4 in the mother in terms of moles (70). The numbers in the second column compare the molar activity of T_3 and the thyroid hormone analogues to the dose of T_4 in moles required to block fetal goiter. As is shown, DIMIT is only 20% as active as T_4 (69); yet, it has four and a half times the activity of T_4 in the fetus, presumably secondary to its increased placental permeability. The other two analogues, DIIIT and DISBT, are three times as active as T_4 in the mother; and are a little more than three and a half times as active as T_4 in the fetus. Therefore, DIMIT has an effect in the fetus compared to an effect on the mother that is more than 20 times that of T_4. In comparison, DIIIT and DISBT have an effect that is less than one and a half times that of T_4. The data indicates that the amount of non-halogenated thyroid

hormone analog, DIMIT, preventing fetal goiter would not increase maternal thyroid hormone activity significantly.

Thyroid hormone analogues like DIMIT may have a role in the management of fetal hypothyroidism in utero.

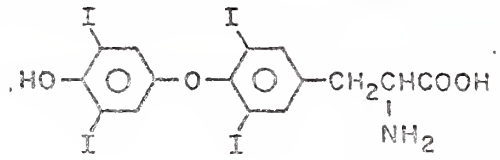
SUMMARY

1. Doses of thyroxine (T_4) and triiodothyronine (T_3) which prevented fetal goiter would cause maternal thyrotoxicosis.

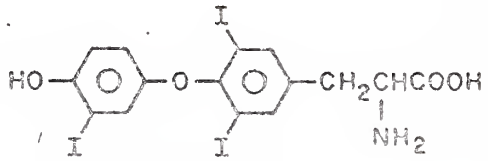
2. The non-halogenated thyroid hormone analog, 3,5-dimethyl-3'-isopropyl-L-thyronine (DIMIT), prevented fetal goiter at a maternal dose that had little thyromimetic activity.

3. The data indicated that thyroid hormone analogues with smaller molecular weight, increased lipid solubility, and decreased binding to TBG crossed the placenta more easily than the parent compounds.

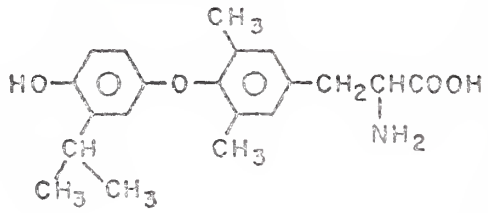
L-THYROXINE (T_4)



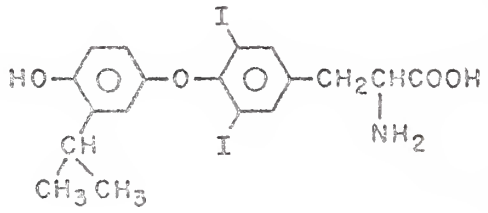
L-TRIIODOTHYRONINE (T_3)



L-3, 5-DIMETHYL-3'-ISOPROPYLTHYRONINE (DIMIT)



L-3, 5-DIIODO-3'-ISOPROPYLTHYRONINE (DIIIT)



L-3, 5-DIIODO-3'-SECONDARY BUTYLTHYRONINE (DISBT)

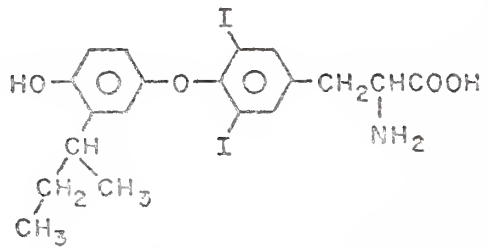


Figure 1: Molecular structures of thyroid hormones and analogues

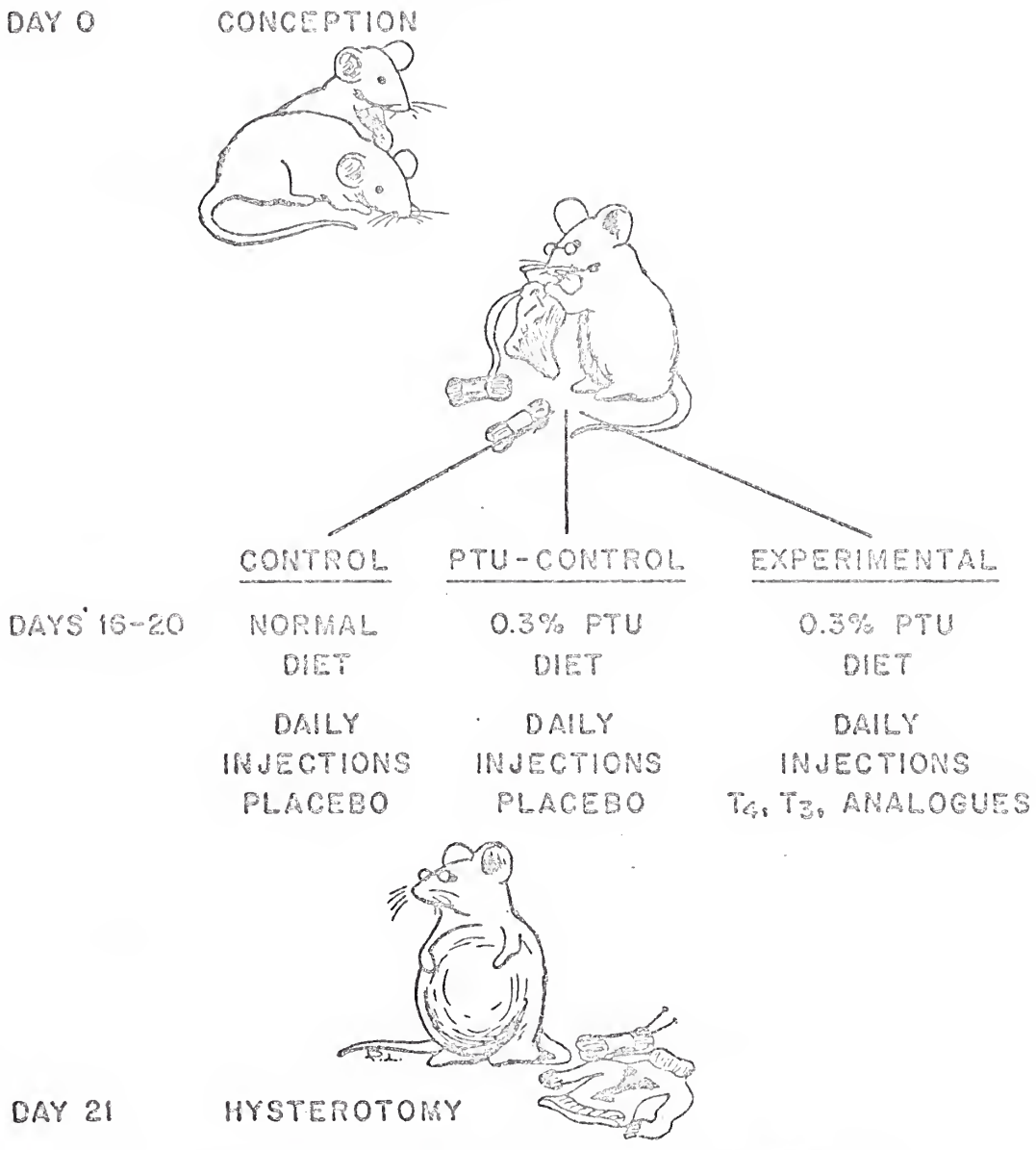


Figure 2: Experimental design

TABLE 1

GROUP	DIET	DOSE 100gm/B.W./day	MEAN THYROID WT.* (mg)	T-TEST** Gp.I	T-TEST** Gp.II
I	NORMAL	-	1.10 ± 0.05	-	$p<.0001$
II	PTU	-	1.92 ± 0.12	$p<.0001$	-
III	PTU	5.20 µg T_4	1.18 ± 0.07	N.S.	$p<.001$
IV	PTU	2.60 µg T_4	1.68 ± 0.14	$p<.001$	N.S.
V	PTU	2.56 µg T_3	1.27 ± 0.07	N.S.	$p<.001$
VI	PTU	1.28 µg T_3	1.77 ± 0.08	$p<.0001$	N.S.
VII	PTU	0.45 µg DIMIT	1.05 ± 0.05	N.S.	$p<.0001$
VIII	PTU	0.23 µg DIMIT	1.76 ± 0.09	$p<.0001$	N.S.

ALL VALUES ARE ±S.E.M.

*ONE OR TWO LITTERS (5-13 FETUSES/LITTER)

**REPRESENTS COMPARISON BETWEEN EXPERIMENTAL ANIMALS GIVEN T_4, T_3 OR ANALOGUES AND CONTROL GROUP (GP. I) OR PTU GROUP (GP. II).

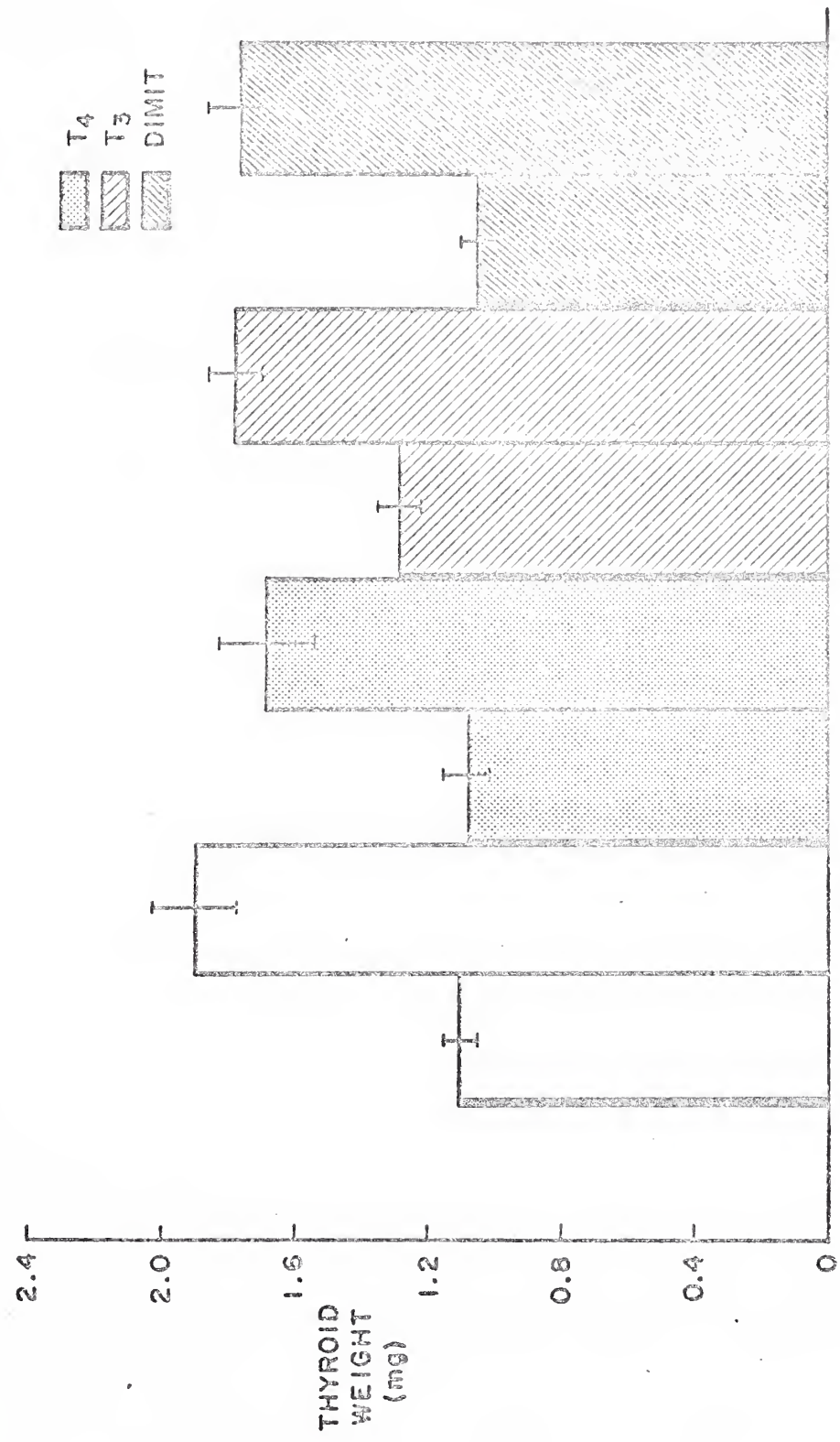


Figure 3: Effect of thyroxine (T_4), triiodothyronine (T_3) and thyroid hormone analog (DIMIT) on fetal thyroid weight. Each bar represents the mean fetal thyroid weight of one or two litters. Each litter contains 5 to 13 fetuses.

TABLE 1

GROUP	DIET	DOSE 100gm/B.W./day	MEAN THYROID WT.* (mg)	T-TEST** Gp.I	T-TEST** Gp.II
I	NORMAL	-	1.15 ± 0.06	-	p<.001
II	PTU	-	1.78 ± 0.13	p<.001	-
III	PTU	0.45 µg DIMIT	0.90 ± 0.07	p<.05	p<.001
IV	PTU	0.45 µg DIIIT	1.78 ± 0.08	p<.0001	N.S.
V	PTU	0.90 µg DIIIT	1.05 ± 0.06	N.S.	p<.0001
VI	PTU	0.45 µg DISBT	1.68 ± 0.09	p<.0001	N.S.
VII	PTU	0.90 µg DISBT	1.01 ± 0.06	N.S.	p<.0001

ALL VALUES ARE ±S.E.M.

*ONE OR TWO LITTERS (5-13 FETUSES/LITTER)

**REPRESENTS COMPARISON BETWEEN EXPERIMENTAL ANIMALS GIVEN DIMIT, DIIIT OR DISBT AND CONTROL GROUP (GP. I) OR PTU GROUP (GP. II).

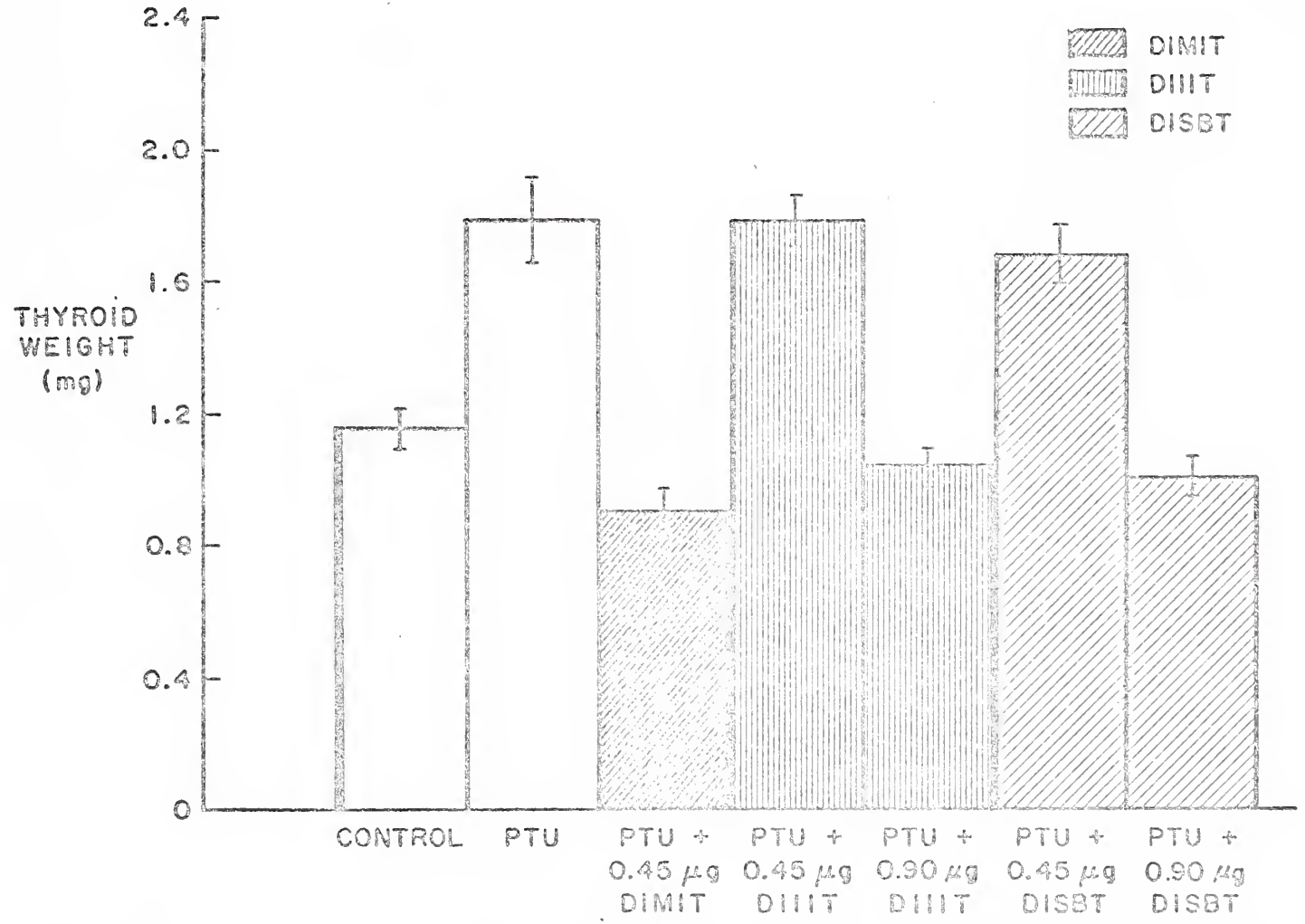


Figure 4: Effect of thyroid hormone analogues, 3,5-dimethyl-3'-isopropyl-L-thyronine (DIMIT), 3,5-diiodo-3'-isopropyl-L-thyronine (DIIIT) and 3,5-diiodo-3'-secondary butyl-L-thyronine (DISBT) on fetal thyroid weight. Each bar represents the mean fetal thyroid weight of one or two litters. Each litter contains 5 to 13 fetuses.

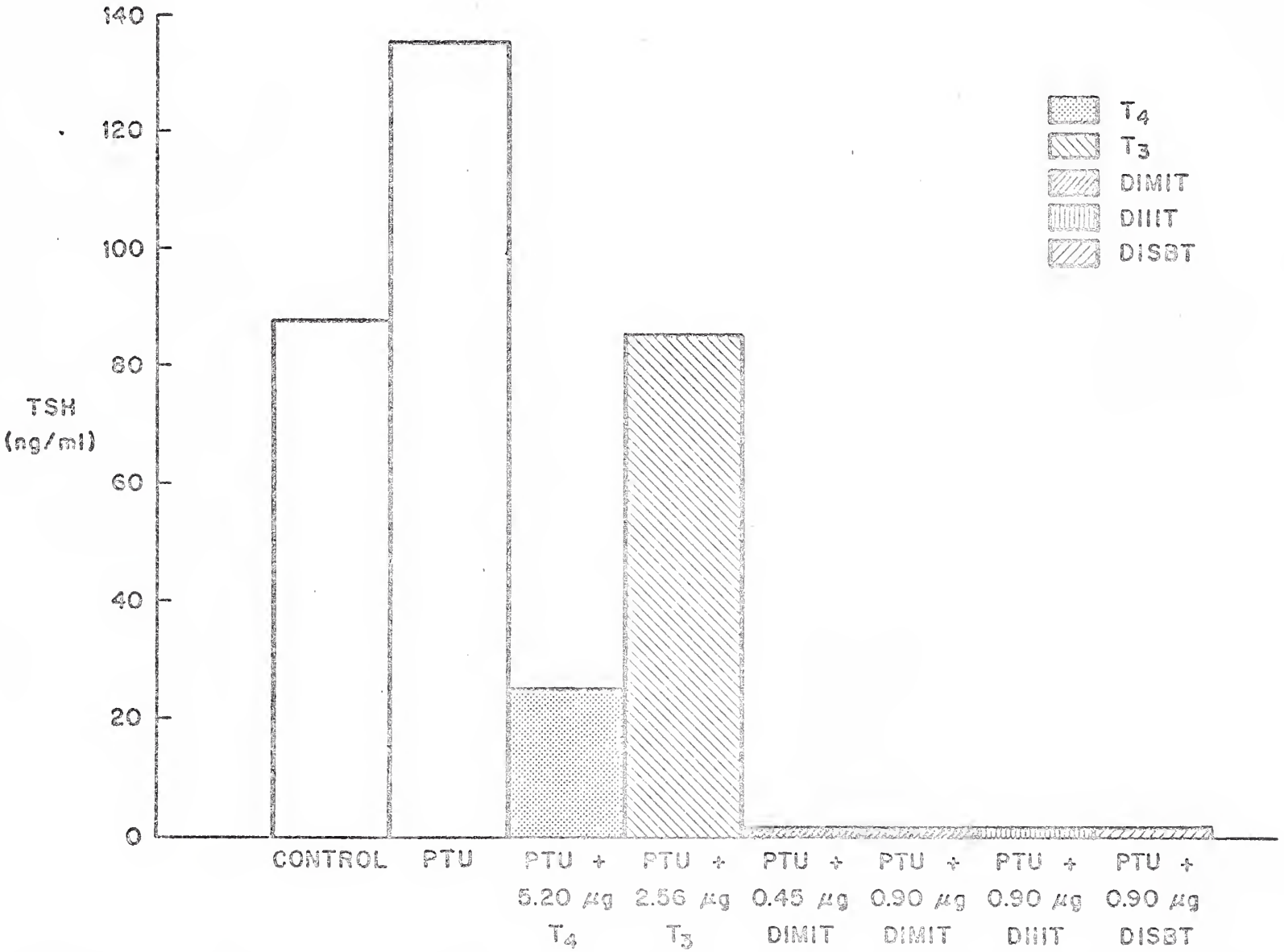


Figure 5: Effect of thyroxine (T_4), triiodothyronine (T_3) and thyroid hormone analogues (DIMIT, DIIIT, DISBT) on fetal serum TSH concentration. Control group is the mean of 5 litters (5-13 fetuses/litter): PTU group - 6 litters; PTU + 5.20 µg T_4 - 1 litter; PTU + 2.56 µg T_3 - 4 litters; PTU + 0.45 µg DIMIT - 3 litters; PTU + 0.90 µg DIMIT, PTU + 0.90 µg DIIIT and PTU + 0.90 µg DISBT - 2 litters in each group.

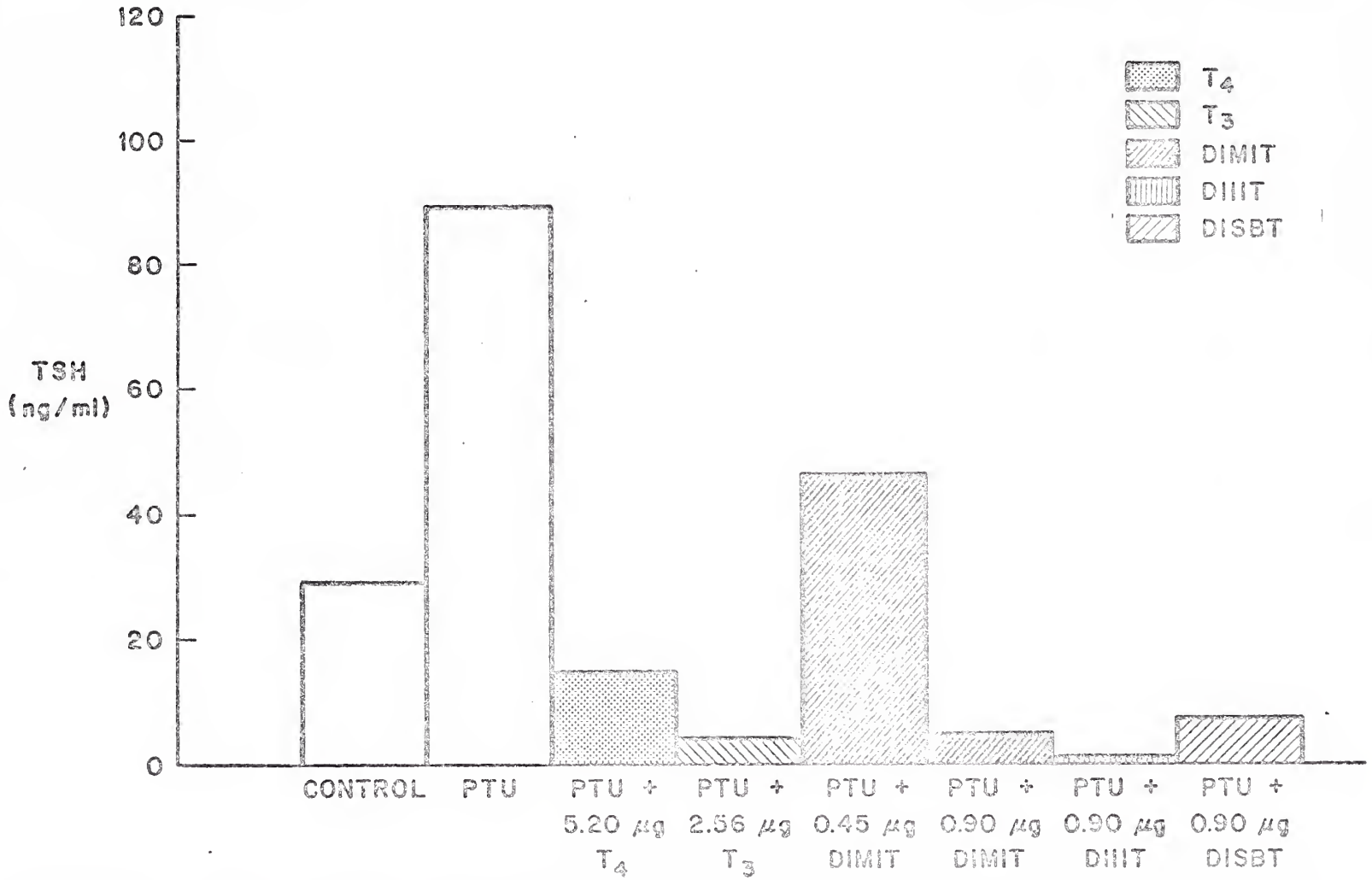


Figure 6: Effect of thyroxine (T_4), triiodothyronine (T_3) and thyroid hormone analogues (DIMIT, DIIIT, DISBT) on maternal serum TSH concentration. Control group is the mean of 5 rats; PTU group - 6 rats; PTU + 5.20 µg T_4 - 1 rat; PTU + 2.56 µg T_3 - 4 rats; PTU + 0.45 µg DIMIT - 3 rats; PTU + 0.90 µg DIMIT, PTU + 0.90 µg DIIIT and PTU + 0.90 µg DISBT - 2 rats.

	Molecular Weight	Lipid Solubility	Protein Binding to TBG (%)	Dose which Inhibits Fetal Goiter	
	gm/mole	(Relative to T_4)		gm$\times 10^{-6}$	mole$\times 10^{-9}$
T_4	888.96	—	100	5.20	5.85
T_3	673.04	—	10	2.56	3.93
DIMIT	343.20	↑	0.8	0.45	1.31
DIIIT	567.18	↑	2	0.90	1.59
DISBT	581.19	↑	2	0.90	1.55

Figure 7: Properties of thyroxine (T_4), triiodothyronine (T_3) and the thyroid hormone analogues, 3,5-dimethyl-3'-isopropyl-L-thyronine (DIMIT), 3,5-diiodo-3'-isopropyl-L-thyronine (DIIIT) and 3,5-diiodo-3'-secondary butyl-L-thyronine (DISBT).

	Maternal	Fetal	$\frac{\text{Fetal}}{\text{Maternal}}$
T_4	1*	1**	1
T_3	3	1.5	0.5
DIMIT	0.2	4.5	22.5
DIIIT	3	3.8	1.3
DISBT	3	3.7	1.2

*1 = T_4 activity in maternal serum

**1 = amount of T_4 in moles required to block fetal goiter (5.85×10^{-9} mole T_4/100 g maternal body weight)

Figure 8: Relative molar activity of thyroxine (T_4), triiodothyronine (T_3) and thyroid hormone analogues (DIMIT, DIIIT, DISBT) in maternal serum and fetal serum as defined by the adult and fetal rat antigoiter assay.

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