SELECTED STUDIES OF THYROID HORMONES IN DOGS, HORSES, AND CATTLE

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TABLE OF CONTENTS

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EFFECT ON CHRONIC DIETARY NITRATES ON CANINE THYROID FUNCTION[1]

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Effect of Nitrates on the Canine Thyroid

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Effect of Chronic Dietary Nitrate on Canine thyroid Function.[1] Kelley, S. T., Oehme, F. W., And Hoffman, S. B. (1973). Toxicol. Appl. Pharmacol. . An investigation was conducted to gain information concerning the effects of dietary nitrate on thyroid function in Beagle dogs and their offspring. The dogs received 0 ppm, 300 ppm, 600 ppm, and 1000 ppm sodium nitrate in drinking water under normal management and feeding conditions. Continuous consumption of sub-lethal levels of nitrate by dogs did not produce a syndrome of chronic nitrate toxicosis characterized by thyroid dysfunction.

The existence of potentially toxic levels of nitrate in the water of many rural areas is a recognized occurrence (Olson et al., 1972). Subacute and chronic nitrate toxicity has been observed in food animals to produce signs of anorexia, dyspnea, grinding of teeth, restlessness, vasodilation, reduced blood pressure, abortion, decreased milk secretion, possible avitaminosis A, and possible thyroid malfunction (Blood and Henderson, 1968; Dolahite, 1970).

The potential influence of nitrate toxicity on thyroid function has been studied in several animal species. In rats a definite correlation exists between thyroid hypofunction and nitrate poisoning (Bloomfield et al., 1961; Lee et al., 1970; Wyngaarden et al., 1953), and it appears that nitrate is an antithyroid substance in that species. This effect is probably a result of nitrate interference with the thyroidal iodide-concentrating mechanism (Lee et al., 1970). When nitrate is present, higher levels of iodine are needed for the maintenance of normal thyroid glands. Thyroid abnormalities in sheep were associated with the level of nitrogen fertilization (Reid et al., 1969). However, nitrate was fed at relatively high rates to pregnant heifers without any change in several measures of thyroid function and suggests that ruminants may not respond to nitrate in the same way as rats (Jainudeen et al., 1965).

The relationship between nitrate consumption and thyroid function is thus unclear. This study was undertaken to determine if there was any correlation between chronic dietary nitrate and the occurrence of thyroid malfunction in dogs.

METHODS

A total of 28 mature, breeding Beagle dogs, 4 males and 24 females in good health were used. They were randomly divided into 4 groups of 1 male and 6 females each. Each group received 0 ppm, 300 ppm, 600 ppm, or 1000 ppm sodium nitrate in their drinking water. All the dogs were housed in identical conditions and fed a commercial dry dog food ad lib.[2] The males and females were bred within their respective groups and the resulting puppies maintained on respective feed and water.

After 1 year, blood samples were collected monthly for 4 months from the adults in each group. Samples were drawn at the same hour of the day each time with heparin used as the anticoagulent. The plasma was separated and frozen. Samples were also collected from all puppies in each group at 9 weeks of age during the test period and were handled in the same manner as blood from the adults.

Plasma samples were thawed to room temperature at the end of the test period and evaluated for thyroid function using two _in vitro_ tests. One procedure used was total serum thyroxine by competitive protein binding of labeled thyroxine (^{125}T-4)[3]; this test was recently designated thyroxine (displacement) or T_4(D) (Soloman _et al._, 1972). Determination of T_4(D) was according to manufacturer's instructions except that 0.9 ml of supernatant rather than 0.3 ml was removed after plasma centrifugation. This in turn necessitated division of results by 3 to obtain the thyroxine (T_4) values reported here. The modification was necessary due to the low T_4 concentration found in canine plasma (Furth _et al._, 1968). The second procedure was resin sponge uptake of radioactive triiodothyronine (^{125}T-3 test)[4], designated resin triiodothyronine uptake or RT_3U (Soloman _et al._, 1972). The same deep well counter[5] was used. After determination of T_4(D) and RT_3U values for each adult and puppy sample, the thyroxine-resin T_3 index (T_4-RT_3 index) was calculated. The T_4-RT_3 index is also known as the thyroid activity index, corrected thyroid concentration, T_7, and free thyroxine index (Solomon et al., 1972) and is calculated as the T_4(D):RT_3U ratio or (T_4(D)) X (RT_3U) = (T_4-RT_3).

Mean and standard error of the mean (SEM) were computed for each experimental group of adult and puppy Beagles, and Student's t test of significance was applied to evaluate differences between groups of adults or puppies using a programmed electronic calculator.[6]

RESULTS

Table 1 shows results obtained for the thyroid function studies performed on the adult dogs in each group. No significant differences were observed between groups receiving 0 ppm, 300 ppm, 600 ppm, or 1000 ppm sodium nitrate.

Table 2 shows the results obtained for the thyroid function studies performed on the puppies in each group. No significant differences in the thyroid function evaluations were observed among the puppies from dams receiving 0 ppm, 300 ppm, 600 ppm, or 1000 ppm sodium nitrate.

No clinical manifestation of hypothyroidism was observed in any of the groups of adult or puppy Beagles.

DISCUSSION

Various investigators of canine thyroid function have observed that T_4(D) and RT_3U are valuable indicators of thyroid function (Anderson and Dorner, 1971; Hightower and Miller, 1969; Hightower et al., 1969; Kallfelz, 1968; Kallfelz, 1969a; Kallfelz, 1969b; Kyazr et al., 1972). In humans the T_4-RT_3 index has proved a valuable measure of total thyroid function since it overcomes possible inherent errors when either T_4(D) or RT_3U is used alone (Howorth and Ward, 1972; Stein and Price, 1972). This value is also of use in animals since it gives an index of the free circulating T_4, which is the active form (Siegal, E. T. personal communication, 1972).

In performing the T_4(D) test in this study plasma was used instead of serum as is called for in the manufacturer's directions; however, this modification does not alter the results obtained (Kelley and Oehme, 1973).

Chronic nitrate toxicity in rats mimics the effect of iodide deficiency by causing an increased dietary requirement for iodide (Lee et al., 1970). This observation questions whether long-term restricted dietary iodide consumption would effect the thyroid status in the dog. To answer this question Beagle dogs were maintained on low dietary iodide for up to two years (Norris et al., 1970). During the first year the dogs manifested abnormal thyroid function as measured by uptake and release of ^{131}I by the thyroid and gross and microscopic examination of the thyroid, but the only thyroid function test remaining abnormal after two years was ^{131}I thyroid uptake. Since the adult dogs used in our study were consuming sodium nitrate for one year before thyroid status was measured, a parallel between the experiments may be attempted. If chronic nitrate consumption in dogs mimics iodide deficiency, the dogs in the present study may have accommodated to the condition at the time samples were collected. At no time, however, did the dogs in this study exhibit clinical signs of hypothyroidism.

The reported incidence of hypothyroidism in cattle due to chronic nitrate intake may have been due to other causes mimicking hypothyroid clinical signs (Jainudeen et al., 1965). From the results of this study there is no evidence that dietary nitrate in the doses and for period of time administered has any effect on canine thyroid function.

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2. Golden Choice Dog Meal, Coors Company, Denver, Colo.
3. Tetrasorb-125 (T-4 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.
4. Triosorb-125 (T-3 Diagnostic Kit). Abbott Laboratories, North Chicago, Ill.
5. Logic Series Model 101 Well Counter, Abbott Laboratories, North Chicago, Ill.
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TABLE I

Thyroid Function Studies in Adult Dogs Receiving Sodium Nitrate in Drinking Water

Dose level (ppm)	N	No. of samples	Thyroid function test (± SEM) T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
0	7	21	1.20±0.57	50.56±0.68	0.60.±0.06
300	7	20	1.19±0.64	50.45±0.96	0.59±0.96
600	7	22	0.87±0.17	49.68±0.81	0.44±0.08
1000	7	20	0.94±0.15	50.36±1.02	0.47±0.07
All dogs	28	85	1.08±0.08	50.14±0.08	0.53±0.04

TABLE II

Thyroid Function Studies in Puppies from Dogs Receiving Sodium Nitrate in Drinking Water

Dose level (ppm)	N	No. of Samples	Thyroid function test ($\pm$ SEM)		
			T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
0	41	41	2.27±0.09	47.01±0.09	1.08±0.04
300	11	11	2.16±0.13	48.10±1.00	1.04±0.07
600	4	4	2.47±0.24	43.80±0.74	1.09±0.07
1000	48	48	2.30±0.05	46.83±0.40	1.08±0.03
All puppies	104	104	2.30±0.05	46.83±0.40	1.08±0.03

INFLUENCE OF ESTRUS CYCLE IN NORMAL MARES ON SERUM THYROID LEVELS

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SUMMARY

Serum thyroxine (T_4), resin triiodothyronine uptake (RT_3U), and thyroxine resin T_3 index (T_4-RT_3 index) were followed in 9 normal mares during their estrus cycles. A statistically nonsignificant decrease in serum T_4 was observed after ovulation. However, there were no significant differences in the thyroid function test results during various stages of the estrus cycle. It appears that the stage of estrus cycle does not significantly influence the serum thyroid hormone levels in normal mares.

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The influence of the thyroid upon reporduction in many animal species and man has been extensively investigated and reviewed.[1-6,13,15,16,18,20,23] However, there appears to be considerable confusion concerning the relationship between thyroid function and reproduction. Some investigators contend that female reproductive performance can proceed normally only in euthyroid individuals.[18] Others disagree, saying that reproductive capacity is frequently maintained even in the complete absence of normal thyroid function.[23] The confusion may lie in difference of experimental design or means of evaluation or may be due to species variation.

Much of the research conducted on the thyroid-reproduction relationship has been done on thyroidectomized animals, animals fed antithyroid compounds, or animals receiving exogenous thyroid hormones. Reports can be found in a large number of different species that thyroidectomy or antithyroid compounds appear to adversely affect reproduction in some way.[6,13,18,23] Conflicting reports maintain that, at least in some species, thyroidectomy or antithyroid compounds do not adversely affect reproduction.[5,6,13,23] It has been noticed clinically and experimentally that exogenous thyroid hormones enhance reproductive function.[5,16] This has lead to the theory that thyroid replacement may aid infertility problems in both man and animals.[6,12,16]

It has been observed that problems of infertility in mares, particularly anestrus or irregular estrus, will respond to thyroid replacement therapy.[12] This study was undertaken to determine if the estrus cycle in normal mares influenced serum thyroid hormone levels.

Materials and Methods

Nine mature (over 5 years) quarter-type mares were used. All were fed a ration consisting of brome and alfalfa hay mixture and oats. The mares were obtained at different times during the experimental period: mares 1 and 2 were procured in March; mares 3, 4, and 5 in May; mares 6, 7, and 8 in June; and mare 9 in July. Each mare was given a complete physical examination, including genital examination before being placed on experiment. The genital examination included rectal palpation, vaginal examination, and cervical and uterine culture. If available, a reproductive history was obtained.

For purposes of relating the stage of the estrus cycle to serum thyroid hormone levels, the mares' estrus cycles were divided into 5 periods determined by daily rectal palpation of each mare: 1) Proestrus 1 (P_1), the first day a developing follicle could be palpated on one of the ovaries; 2) Proestrus 2 (P_2), the day the follicle was large and soft, approximately 24 to 48 hours prior to ovulation; 3) Estrus (E), the day of ovulation; 4) Metaestrus 1 (M_1), 6 days after ovulation; 5) Metaestrus 2 (M_2), 11 days after ovulation. Blood samples were collected from the jugular vein of each mare at the periods outlined at the same hour of the day. The serum was separated and frozen.

Serum samples were thawed to room temperature at the end of the test period and evaluated for thyroid function using two <u>in vitro</u> tests. One procedure was total serum thyroxine by competitive protein binding of labeled thyroxine (^{125}T-4)[a]; this test was recently designated thyroxine (displacement) or T_4(D).[21] Determination of T_4(D) was as described previously.[8] The second procedure was resin sponge uptake of radioactive triiodothyronine (^{125}T-3 test)[b], designated resin triiodothyronine uptake or RT_3U.[21] This test was performed according to manufacturer's instructions using a deep well counter.[c] After determination of $T_4(D)$ and RT_3U values for each sample, the thyroxine-resin T_3 index (T_4-RT_3 index) was calculated. The T_4-RT_3 index is also known as the thyroid activity index, corrected thyroid concentration, T_7, and free thyroxine index[21] and is calculated as the $T_4(D):RT_3U$ ratio or $(T_4(D) \times (RT_3U) = (T_4\text{-}RT_3)$.

Mean and standard error of the mean (SEM) were determined for each stage of the estrus cycle in all mares, and Student's t test of significance was applied to evaluate differences between the various estrus cycle stages using a programmed electronic calculator.[d]

During the period of this study, a co-study measured luteinizing hormone (LH) and estradiol in peripheral blood of mares no. 1, 2, 4, 5, and 9 during one or more of their estrus cycles.[19]

Results

A total of 35 estrus cycles were followed in the 9 mares during the test period. However, the mares varied considerably as to number and regularity of their estrus cycles (Table 1).

Table 2 provides the mean results of thyroid function studies in the 9 mares. Significant differences were noted when comparing individual mares to the total group and when comparing individual mares to each other.

Table 3 indicates the results of thyroid function studies at different stages of the estrus cycle in all nine mares. No significant differences in thyroid function evaluations were observed when comparing the results of each stage of the estrus cycle to the total or when comparing each stage of the estrus cycle to the other stages.

To examine the data obtained for the thyroid function tests during the estrus cycle visually, graphs were made of each thyroid function test value for each mare's cycle. Graphs of the mean and SEM values for each of the 3 tests performed, T_4(D), RT_3U, and T_4-RT_3 index, for all mares are shown in Fig. 1, 2, and 3.

Fig. 1 is the mean and SEM of the total serum thyroxine (T_4) for all mares during each stage of their estrus cycle. It should be noted that there was considerable variance in the level of serum T_4 between mares, but there was a consistant decrease in serum T_4 after ovulation. This decrease was seen in 73.6% (25 or 34) of the cycles observed. Further, in 56% (19 of 34) of the cycles observed, the curve of the serum T_4 followed the shape demonstrated in Fig. 1. Fig. 2 provides the mean and SEM of RT_3U for all mares during each stage of their estrus cycle. The shape of the curve is not consistant between mares or between estrus cycles in the same mare. Fig. 3 demonstrates the SEM of T_4-RT_3 for all mares during each stage of their estrus cycle. As with Fig. 2, the shape of the curve in Fig. 3 is not consistant between mares or between estrus cycles in the same mare.

The measurement of LH and estradiol in peripheral blood of mares during their estrus cycle revealed no observeable correlation between the thyroid test values and the LH and estradiol levels when all parameters were measured simultaneously.

Discussion

Investigators of equine thyroid function have observed that T_4(D) and RT_3U are valuable indicators of thyroid function.[7-9,11,14] In humans the T_4-RT_3 index has proved an important measure of total thyroid function since it overcomes possible inherent errors when either T_4(D) or RT_3 are used alone.[10,22]

The total mean values reported in this study for T_4(D) and RT_3U in all mares agree favorably with values reported by other investigators.[2-9, 11,14] The results indicate that there is a significant variation between the thyroid function test results in different mares. However, this wide variation in values in different individuals has been observed in both man and animals, including horses.[7-9,11-,17]

In other species it has been reported that there is a definite relationship between the thyroid status and the estrus cycle.[1,2,5,6,18] In women it has been observed that there is a decrease in free serum T_4 levels in the luteal phase of the menstral cycle.[1] In rats, hamsters, and guinea pigs increased thyroid activity has been observed after ovulation.[2] Female mice have increased thyroid activity during proestrus.[18]

The fact that there was a mean decrease in serum T_4 after ovulation in most cycles is an interesting trend. This suggests a possible species variation since, as noted earlier, rats, hamsters, and guinea pigs show increased thyroid activity after ovulation. It is difficult to postulate a reason for the observed decrease in serum T_4 after ovulation in mares. It is known that in the mare there is a peak in serum estrogen shortly before ovulation.[19] In women it has been observed that during period of increased serum estrogen, such as pregnancy or in women receiving estrogen containing contraceptive drugs, there is an increase in serum thyroid-binding globulin (TBG) which causes an increase in serum T_4.[23] If there is an increase in the mare's serum proteins that bind T_4 during the increase in estrogen prior to ovulation, there would also be a slight increase in T_4. After ovulation there may be a decrease in T_4-binding proteins due to the decrease in serum estrogen. This could cause a feedback inhibition of T_4 from the thyroid gland and a decrease in serum T_4 after ovulation. Since the RT_3U did not change significantly during the estrus cycles, this theory to explain the drop in T_4 following ovulation is questionable.

Another theory for the consistant decrease in serum T_4 following ovulation is that thyroid changes are a consequence of ovulation and not related to estrogen concentration. This theory is supported by one investigator who maintains that thyroid activity appears to be associated with ovulation.[2] However, since no correlation appeared between LH and estradiol levels and thyroid levels, it could be suggested that neither LH nor estradiol affect thyroid activity directly. Further investigation needs to be conducted to determine the consistency and cause of the decrease in serum T_4 after ovulation.

When examining Table 1, which shows the number and regularity of estrus cycles of the mares, and Table 2, which shows the results of thyroid function studies in the mares, an interesting trend is observed. The mare with the highest mean serum T_4 level (mare no. 2) had very irregular estrus cycles. Mare no. 8, which had the lowest serum T_4 level, also had few and irregular estrus cycles. However, when examining LH and estradiol levels during estrus cycles,[19] both mares no. 2 and 8 were entirely normal and exhibited curves similar to the other mares studied. Mares no. 3

and 7 exhibited only one estrus cycle during the test period, but the results for T_4(D), RT_3U, and T_4-RT_3 index were similar to the means for all the samples. There was no significant difference for the thyroid function tests between the various stages of the estrus cycles measured (Table 3).

Another group of investigators has studied reproductive function in thyroidectomized mares and stallions.[13] The thyroidectomized animals subsequently showed clinical signs of hypothyroidism, but the thyroidectomized mares had estrus cycles and teasing patterns similar to those of controls. Together with results of the present study, this suggests that the estrus cycle does not significantly influence serum thyroid hormone levels in normal mares.

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Footnotes

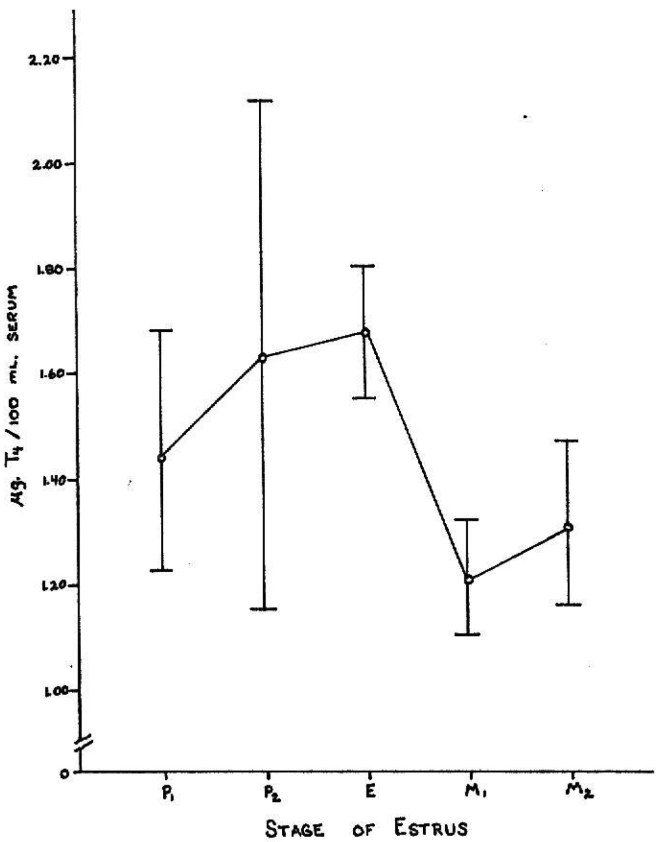
a. Tetrasorb-125 (T-4 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.

b. Triosorb-125 (T-3 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.

c. Logic Series Model 101 Well Counter, Abbott Laboratories, North Chicago, Ill.

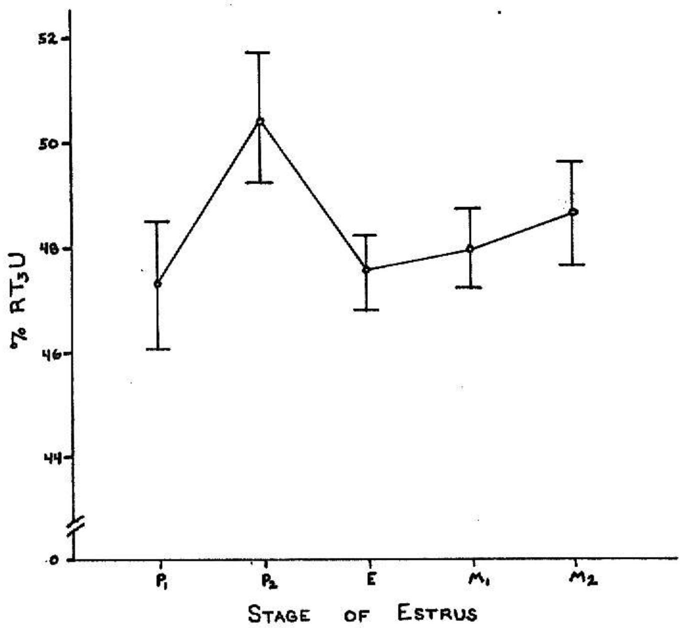
d. Wang 360K, Tewksbury, Mass.

Fig. 1--Mean and SEM of Serum thyroxine (T_4) during different stages of the estrus cycl of 9 normal mares. P_1 is proestrus 1, the first day a developing follicle could be palpated on one of the ovaries; P_2 is Proestrus 2, the day the follicle was large and soft, and approximately 24-48 hours prior to ovulation; E is Estrus, the day of ovulation; M_1 is Metaestrus 1, 6 days after ovulation; and M_2 is Metaestrus 2, 11 days after ovulation.



2.20
2.00
1.80
1.60
1.40
1.20
1.00
0
μg. T4 / 100 ml. SERUM
P1
P2
E
M1
M2
STAGE OF ESTRUS

Fig. 2--Mean and SEM of resin triiodothyronine uptake (RT_3U) values during different stages of the estrus cycle of 9 normal mares. P_1 is Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries; P_2 is Proestrus 2, the day the follicle was large and soft, and approximately 24-48 hours prior to ovulation; E is Estrus, the day of ovulation; M_1 is Metaestrus 1, 6 days after ovulation; and M_2 is Metaestrus 2, 11 days after ovulation.



52
50
48
46
44
0
% RT3U
P1
P2
E
M1
M2
Stage of Estrus

Fig. 3--Mean and SEM of thyroxine-resin T_3 index (T_4-RT_3 index) during different stages of the estrus cycle of 9 normal mares. P_1 is Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries; P_2 is proestrus 2, the day the follicle was large and soft, and approximately 24-48 hours prior to ovulation; E is Estrus, the day of ovulation; M_1 is Metaestrus 1, 6 days after ovulation; and M_2 is Metaestrus 2, 11 days after ovulation.

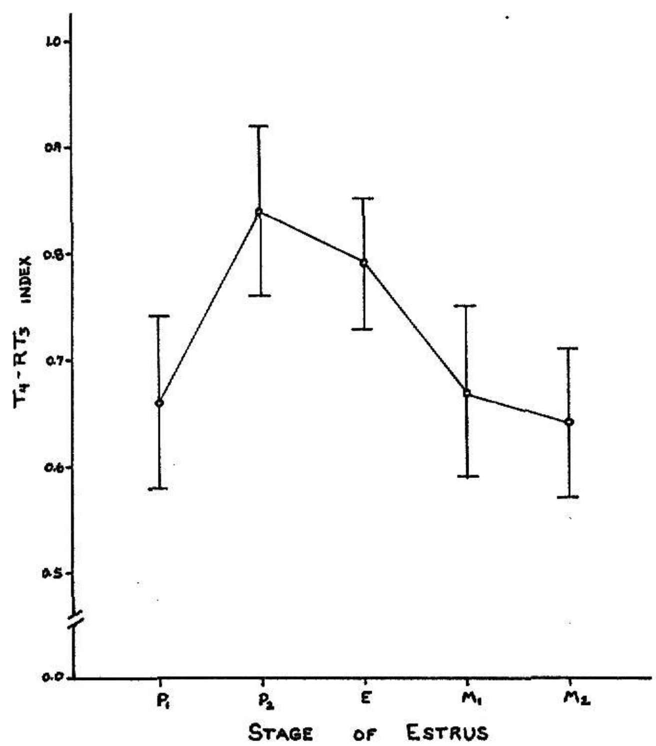

1.0
0.9
0.8
0.7
0.6
0.5
0.0
T4-RT3 INDEX
P1
P2
E
M1
M2
STAGE OF ESTRUS

TABLE 1-Number and Regularity of Estrus Cycles of 9 Normal Mares

Mare	No. of estrus cycles	Days between estrus cycles
1	7	24 23 24 25 27 25
2	5	51 23 56 18
3	1	..
4	6	19 20 20 22 23
5	5	15 21 24 19 26
6	2	47
7	1	..
8	3	23 46
9	4	24 22 23

TABLE 2-Thyroid Function Studies in 9 Normal Mares

Mare	No. of samples	Thyroid function test (± SEM) T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	28	1.22±0.15	46.70±0.83	0.55±0.06
2	25	2.13±0.17	51.74±1.18	1.08±0.08
3	5	1.32±0.17	50.83±1.37	0.66±0.09
4	25	1.67±0.16	47.97±1.43	0.81±0.08
5	24	1.55±0.12	46.42±0.45	0.72±0.06
6	12	1.30±0.23	47.24±0.89	0.61±0.11
7	8	1.91±0.17	43.05±0.33	0.82±0.07
8	15	0.51±0.12	55.00±1.36	0.28±0.05
9	16	1.32±0.98	48.16±0.98	0.63±0.09
All mares	158	1.47±0.07	48.57±0.45	0.70±0.03

TABLE 3-Thyroid Function Studies at Different Stages of the Estrus Cycle in 9 Normal Mares

Stage of cycle	No. of samples	Thyroid function test (± SEM) T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
P_1^*	32	1.44±0.16	48.30±1.13	0.69±0.08
P_2^{**}	28	1.61±0.13	50.76±1.25	0.82±0.07
E^+	34	1.68±0.13	47.75±0.72	0.79±0.06
$M_1^‡$	33	1.20±0.14	47.91±0.77	0.68±0.07
$M_2^§$	32	1.33±0.16	48.57±1.02	0.63±0.07
Total	158	1.47±0.07	48.57±0.45	0.70±0.03

*Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries

**Proestrus 2, the day the follicle was large and soft, approximately 24-48 hours prior to ovulation

+Estrus, the day of ovulation

‡Metaestrus 1, 6 days after ovulation

§Metaestrus 2, 11 days after ovulation

A COMPARATIVE STUDY OF
CIRCULATING THYROID LEVELS

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Circulating Thyroid Levels

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A Comparative Study of Circulating Thyroid Levels. Kelley, S. T. and Oehme, F. W. (1973). Toxicol. Appl. Pharmacol. Three thyroid function tests: thyroxine (displacement), resin triiodothyronine uptake, and thyroxine-resin T_3 index, were used to measure thyroid function in dogs, horses, and cattle. A comparison was also made of the use of serum or plasma. The choice of serum or plasma did not interfere with or alter the test results. The thyroid function tests gave similar results in dogs and horses, but there were significant differences in cattle. This variation may be due to basic species differences in thyroid physiology.

Thyroid malfunction has been incriminated in several toxicological and clinical disorders in dogs, horses, and cattle (Blood and Henderson, 1968; Bloom, 1968; McDonald, 1969; Nodine and Siegler, 1964). Until recently it has been difficult to evaluate thyroid function in domestic animals, but with the advent of simpler and more accurate methods for laboratory evaluation, it is now possible to better relate clinical and subclinical thyroid disorders with laboratory data. In order to determine the normal values for these laboratory methods, this study was undertaken to measure circulating serum thyroxine (displacement) (T_4(D)), resin triiodothyroxine uptake (RT_3U), and thyroxine-resin T_3 index (T_4-RT_3 index) in a group of normal dogs, horses, and cattle.

METHODS

To test thyroid function in dogs, three groups were used: 8 mature breeding Beagles; 39 nine-week old puppies; and 12 randomly-selected male and female mixed-breed dogs. The horses used were a group of 9 mares on an estrus cycle study (Kelley et al., 1973a) and a group of four mares admitted to Dykstra Veterinary Hospital, Kansas State University, for infertility problems. Three of the latter mares were aged Thoroughbreds showing signs of anestrus, but with no signs of hypothyroidism. The fourth mare was a Quarter horse showing signs of anestrus and also exhibiting clinical signs associated with hypothyroidism. To evaluate thyroid function in cattle, 19 mature Holstein cows, pregnant and in mid-lactation, and 4 Hereford steers were used.

At least 5 ml. of blood was collected from each animal. Heparin was used as an anticoagulant for half the sample and the remainder was allowed to clot. The plasma or serum was separated and frozen. Samples were later thawed to room temperature and evaluated for thyroid function.

Two *in vitro* tests were used. Total serum thyroxine (T_4) was determined by competitive protein-binding of labeled T_4 (^{125}T-4)[1]; this test was recently designated thyroxine (displacement) or T_4(D) (Soloman *et al.*, 1972). Resin sponge uptake of radioactive triiodothyronine uptake or RT_3U (Soloman *et al.*, 1972) was also measured. Determinations of T_4(D) and RT_3U were as outlined previously (Kelley *et al.*, 1973$_{a,b}$). The thyroxine-resin T_3 index (T_4-RT_3 index), also known as the thyroid activity index, corrected thyroid concentration, T_7, and free thyroxine index (Soloman *et al.*, 1972), was then calculated as the T_4(D):RT_3U ratio or (T_4(D)) X (RT_3U) = (T_4-RT_3). Extraction effeciency of T_4 from serum or plasma of each species being studied was determined as previously described (Kallfelz, 1969$_a$).

Mean, standard deviation (S.D.), and standard error of the mean (SEM) were calculated for each experimental group, and Student's *t* test of significance ($P < 0.05$) was applied to evaluate differences between groups using a programmed electronic calculator.[3]

RESULTS

The results of the thyroid function studies and the serum-plasma comparison from dogs are shown in Tables 1, 2, and 3. There was a significant difference between the mean T_4(D) and T_4-RT_3 index of the adult and puppy Beagles, with the puppies showing significantly higher values. There was no significant difference between adult and puppy values for RT_3U. There was no significant difference in the test results when serum or plasma was used (Table 3). The mean values for T_4(D) and T_4-RT_3 index were lower for the mixed-breed dogs than for the adult

Beagles; but dog no. 5 had a T_4(D) and T_4-RT_3 index value of 0, there-by materially lowering the means of these tests for that group. If this dog's results are omitted, the mean values are similar.

The results of the thyroid function tests performed on the horses are summarized in Tables 4 and 5.

The thyroid function test results for cattle are shown in Tables 6 and 7. There was no significant difference when serum or plasma was used. Significant differences were found in the T_4(D) and T_4-RT_3 index when comparing the dairy cattle to the beef cattle, but not when the RT_3U results were compared.

The extraction efficiencies of T_4 from serum and plasma of dogs, horses, and cattle are given in Table 8.

DISCUSSION

Investigators of thyroid function have observed that T_4(D) and RT_3U are valuable indicators of thyroid function in dogs (Anderson and Dorner, 1971; Hightower and Miller, 1969; Hightower et al., 1969; Kallfelz, 1968; Kallfelz, 1969a; Kallfelz, 1969b; Kyzar et al., 1972), horses (Hightower and Miller, 1969; Hightower et al., 1969; Hightower et al., 1971, Kallfelz and Lowe, 1970; Lowe and Kallfelz, 1970; Motley, 1972), and cattle (Hightower et al., 1969; Lorscheider, 1970). In humans the T_4-RT_3 index has proved a more valuable measure of total thyroid function, since it overcomes possible inherent errors when either T_4(D) or RT_3U are used alone (Howorth and Ward, 1972; Stein and Price, 1972). T_4-RT_3 index is also of use in animals since it gives an index of the free circulating T_4, which is the active form (Siegal, E. T., personal communication, 1972).

Our studies and those elsewhere indicate that the use of either serum or plasma in testing dogs, cattle, or horses (Hightower et al., 1971) should not interfere with test results. This is important because now a single blood sample can be reliably used for these thyroid function tests as well as for other clinical laboratory procedures utilizing plasma.

The observation of significantly higher serum T_4 concentrations in young than in adult dogs (Tables 1 and 2) has also been made in other species (Lowe and Kallfelz, 1970; Lorscheider, 1970; Murray et al., 1971).

The mean values for T_4(D) in dogs are lower than those reported by other investigators (Anderson and Dorner, 1971; Hightower et al., 1969; Kallfelz, 1969a,b; Kyzar et al., 1972), but the method for performing this procedure was slightly modified (Kelley et al., 1973a) from those previously reported. The results for RT_3U in the adult dogs agree favorably with those previously reported (Hightower and Miller, 1969; Kallfelz, 1969).

The results for T_4(D) and RT_3U in horses also agree favorably with results published by other investigators (Hightower and Miller, 1969; Hightower et al., 1969; Hightower et al., 1971). However, the T_4(D) values were below those others reported in horses (Kallfelz and Lowe 1970; Lowe and Kallfelz, 1970). This difference is believed due to the modifications in the T_4(D) test.

The T_4(D) test results in cattle correlate favorably with the reports of other investigators (Hightower et al., 1969; Lorscheider, 1970). While the dairy cattle had significantly higher values than beef cattle, there was also nonsignificantly higher RT_3U values in the beef cattle as compared to the dairy cattle.

The thyroid function tests measured had very similar results in dogs and horses; however, cattle had significantly higher mean values for T_4(D) and T_4-RT_3 index and lower values for RT_3U. This observation may be due to basic species differences in thyroid physiology (Refetoff et al., 1970).

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Footnotes

1. Tetrasorb-125 (T-4 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.
2. Triosorb-125 (T-3 Diagnostic Kit). Abbott Laboratories, North Chicago, Ill.
3. Wang 360K, Tewsbury, Mass.

TABLE 1

Thyroid Function Studies in Adult Beagle Dogs

Dog no.	Sample	Thyroid function test: T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	1	0.89	52.74	0.47
	2	1.21	55.50	0.67
	3	0.18	47.08	0.37
2	4	0.89	52.45	0.47
	5	0.93	54.25	0.50
	6	0.67	51.68	0.35
	7	0.26	48.37	0.13
3	8	2.33	51.77	1.21
	9	1.68	52.13	0.88
	10	1.51	53.09	0.80
4	11	0.47	54.58	0.26
	12	1.77	52.79	0.93
5	13	2.51	51.93	1.30
	14	0.47	50.86	0.24
	15	0.90	46.34	0.42
6	16	1.15	45.99	0.56
	17	1.84	45.99	0.85

TABLE 1 (cont.)

Dog no.	Sample	T_4(D) (μg/100 ml)	RT_3U (%)	T_4-RT_3 index
6	18	2.40	44.54	1.07
7	19	0.99	49.41	0.49
	20	2.08	39.53	0.47
	21	1.58	45.77	0.72
8	22	1.21	52.57	0.64
	23	1.21	50.66	0.61
Mean		1.29	50.18	0.64
S.D.		0.63	3.69	0.30
SEM		0.13	0.77	0.06

TABLE 2

Thyroid Function Studies in Beagle Puppies

Puppy no.	Thyroid Function test: T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	2.11	43.18	0.91
2	1.41	45.14	0.64
3	1.41	44.66	0.77
4	1.38	42.25	0.59
5	2.11	45.08	0.95
6	2.36	47.87	1.13
7	2.91	45.49	1.32
8	1.38	47.69	0.66
9	2.20	48.06	1.06
10	2.59	48.50	1.26
11	2.91	47.89	1.39
12	3.37	46.50	1.57
13	2.59	45.42	1.18
14	1.80	39.57	0.71
15	1.65	43.76	0.72
16	2.83	48.27	1.37
17	1.88	49.14	0.92
18	3.14	46.33	1.45
19	2.11	43.69	1.40
20	2.59	47.87	1.24

TABLE 2 (cont.)

Puppy no.	T_4(D) (μg/100 ml)	RT_3U (%)	T_4-RT_3 index
21	3.53	46.81	1.65
22	2.74	42.98	1.31
23	2.36	44.45	1.05
24	2.11	39.73	0.84
25	2.71	50.80	1.38
26	2.36	47.24	1.11
27	2.51	48.89	1.23
28	2.81	49.97	1.41
29	2.59	48.31	1.25
30	2.11	52.82	1.11
31	2.98	50.84	1.51
32	1.73	47.66	0.82
33	1.48	48.47	0.72
34	2.03	45.64	0.93
35	1.80	47.38	0.85
36	1.73	48.74	0.84
37	0.95	47.05	0.45
38	2.11	51.79	1.09
39	1.93	54.28	1.05
Mean	2.27	47.01	1.08

TABLE 2 (cont.)

Puppy no.	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
S.D.	0.59	3.03	0.29
SEM	0.09	0.47	0.04

TABLE 3

Thyroid Function Studies in 12 Mixed-Breed Dogs Using Serum and Plasma

Dog no.	Serum: Thyroid function test T_4(D) (µg/100 ml)	Serum: RT_3U (%)	Serum: T_4-RT_3 index	Plasma: Thyroid function test T_4(D) (µg/100 ml)	Plasma: RT_3U (%)	Plasma: T_4-RT_3 index
1	0.63	47.53	0.30	0.58	55.01	0.32
2	0.73	49.52	0.36	1.05	47.35	0.50
3	0.63	49.52	0.31	0.63	48.17	0.30
4	0.83	54.06	0.45	1.36	56.05	0.76
5	0	38.69	0	0	39.13	0
6	0.79	51.49	0.41	0.67	51.23	0.34
7	0.67	53.99	0.24	0.78	57.02	0.44
8	1.93	51.40	0.99	1.30	52.98	0.69
9	1.15	49.93	0.52	1.06	51.16	0.54
10	1.21	47.63	0.58	1.96	47.15	0.92
11	1.73	45.17	0.78	1.88	50.09	0.94

TABLE 3 (cont.)

Dog no.	Serum: Thyroid function test $T_4(D)$ (µg/100 ml)	RT_3U (%)	T_4-RT_3 index	Plasma: Thyroid function test $T_4(D)$ (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
12	0.93	51.94	0.48	0.93	57.46	0.25
Mean	0.94	49.24	0.46	0.97	51.12	0.50
S.D.	0.49	4.05	0.25	0.56	4.97	0.27
SEM	0.14	1.17	0.07	0.16	1.44	0.09

TABLE 4

Thyroid Function Studies in 9 Normal Mares

		Thyroid function test (±SEM)		
Mare no.	No. of samples	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	28	3.22±0.15	46.70±0.83	0.55±0.06
2	25	2.13±0.17	51.74±1.18	1.08±0.08
3	5	1.32±0.17	50.83±1.27	0.66±0.09
4	25	1.67±0.16	47.97±1.43	0.81±0.08
5	24	1.55±0.12	46.42±0.45	0.72±0.06
6	12	1.30±0.23	47.24±0.89	0.61±0.11
7	8	1.91±0.17	43.05±0.33	0.82±0.07
8	15	0.51±0.12	55.00±1.36	0.28±0.05
9	16	1.32±0.10	48.16± .98	0.63±0.09
Mean		1.47±0.07	48.57±0.45	0.70±0.03
S.D.		0.84	5.65	0.40

TABLE 5

Thyroid Function Studies in 4 Mares Admitted for Clinical Evaluation

Horse no.	Breed	Thyroid function test: T_4(D) (μg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	Thoroughbred	1.52	53.35	0.81
2	Thoroughbred	3.09	47.06	1.51
3	Thoroughbred	4.30	47.48	7.04
4	Quarter horse	1.41	47.44	0.67

TABLE 6

Thyroid Function Studies in 19 Holstein Cows Using Serum and Plasma

	Serum			Plasma		
	Thyroid function test			Thyroid function test		
Cow no.	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	3.61	29.92	1.08	3.89	29.92	1.35
2	—	27.19	—	—	—	—
3	2.85	33.74	0.96	2.99	35.40	1.06
4	4.93	30.26	1.49	5.17	67.77	1.38
5	3.61	26.79	0.97	3.88	27.62	1.07
6	4.39	26.83	1.39	4.77	26.78	1.28
7	3.44	24.87	0.85	5.17	26.28	1.36
8	4.04	31.17	1.26	4.23	31.34	1.32
9	3.50	29.01	0.88	3.88	30.79	1.19
10	4.33	27.18	1.18	4.48	29.34	1.31
11	3.86	33.20	1.28	3.58	33.67	1.20

TABLE 6 (cont.)

Cow no.	Serum: Thyroid function test T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index	Plasma: Thyroid function test T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
12	5.57	31.76	1.77	5.35	32.04	1.71
13	4.39	33.43	1.47	4.28	35.11	1.50
14	4.63	28.79	1.33	5.06	28.09	1.42
15	5.11	25.50	1.30	5.35	28.83	1.54
16	4.86	30.89	1.50	5.17	31.31	1.62
17	—	30.34	—	—	—	—
18	4.80	34.64	1.66	4.42	34.35	1.51
19	4.86	28.20	1.37	4.82	28.29	1.36
Mean	4.28	29.67	1.28	4.50	30.35	1.36
S.D.	0.70	2.80	0.26	0.67	2.90	0.17
SEM	0.17	0.64	0.06	0.16	0.70	0.04

TABLE 7

Thyroid Function Studies in 4 Hereford Cattle

Cattle no.	Thyroid function test T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	2.46	35.17	0.86
2	3.95	35.76	1.41
3	2.20	33.44	0.73
4	3.82	33.29	1.27
Mean	3.11	34.41	1.07
S.D.	0.78	1.08	0.18
SEM	0.39	0.54	0.14

TABLE 8

Extraction Efficiency of Thyroxine from Serum and Plasma of Dogs, Horses, and Cattle

Species	Media	Extraction efficiency (%)
Dog	Plasma	97.50
Dog	Serum	97.40
Horse	Serum	81.82
Cattle (beef)	Serum	85.20
Cattle (dairy)	Plasma	85.57
Cattle (dairy)	Serum	85.93

AN EVALUATION OF SELECTED COMMERCIAL THYROID FUNCTION TESTS IN DOGS

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SUMMARY

Five different commercial test kits for studying thyroid function were evaluated using normal dogs. Two of the tests were found unsatisfactory for use without considerable modification and two tests were of value. One test appeared promising for evaluating thyroid function, but further investigation is needed.

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Appreciation is expressed to Abbott Laboratories, North Chicago, Ill., Ames Co., Elkhart, Ind., and Mallinckrodt Chemical Works, St. Louis, Mo. for supplying the respective tests and instrumentation, and to Mrs. D. C. Kelley and Dr. George Cardinet for their assistance in this project.

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Two new methods for measuring thyroid function have recently been developed. One method is thyroxine (displacement) (T_4(D)), which measures the serum concentration of thyroxine (T_4) and is often called thyroxine (Murphy-Pattee), displacement analysis, isotope displacement assay, saturation analysis, competitive protein binding, and radioligand binding.[22] The second method is resin triiodothyroxine uptake (RT_3U), which measures uptake of radioactive iodine-labeled triiodothyronine (T_3) on a resin.[22] Several commercial laboratories have developed kits for conducting T_4(D) and RT_3U studies, and clinical laboratory is faced with the decision of which commercial procedure to employ.

This experiment was undertaken to study the results of simultaneously using the thyroid function testing kits available from three commercial sources[a,b,c,d,e] on normal dogs. All the kits had been extensively tested in humans,[2,3,5,16,17,19,24] but little investigation had been conducted in animals. Only two kits, one for testing T_4(D)[c] and one for testing RT_3U,[a] had been previously used in animals; the results have been reviewed.[1,6,7,9-11,13]

Materials and Methods

Five thyroid function testing kits were studied. Two kits evaluated RT_3U;[a,b] two other kits tested T_4(D);[c,d] and one test combined the evaluation of RT_3U and T_4(D) so as to determine and express serum T_4 concentration as a percent.[e]

The first two kits, measuring RT_3U[a] and T_4(D),[c] were studied using 8 adult Beagles, 39 Beagle puppies, and 12 mixed-breed dogs, all from the Laboratory colony. The other three kits[b,d,e] used mixed-breed dogs varying in age from 3 months to 5 years, and German Shepards, German

Shepard-Greyhound crosses, and Greyhounds, from 5 months to 4 years of age, which had been raised and maintained in a campus colony.[f] All dogs were healthy and had no clinical signs of thyroid disorder.

At least 10 ml. of blood was collected from the cephalic vein of all the dogs and divided into two portions. Heparin was placed in one portion and the other was allowed to clot. The serum or plasma was separated and frozen. The samples were later thawed to room temperature and used in the thyroid function test procedures in duplicate.

The methods used in the first two tests (for RT_3U[a] and $T_4(D)$[c]) have been previously described,[11,12] as has that for the third thyroid function test studied (RT_3U[b]).[14] Several modifications of the fourth thyroid function test ($T_4(D)$[d]) were attempted before a method was determined that gave satisfactory results. The final procedure was a modification of a method originally reported in the human literature.[2] In the original human method, the serum was applied directly to a Sephadex G-25 column; the modification required for dogs necessitated that 1 ml. of serum or plasma be first extracted with 2 ml. of 95% ethanol. Following mixing for 15 sec. and allowing to stand for 5 min., the samples were centrifuged at 2000 rpm for 5 min. The supernatant was separated and dried with air in a water bath at 55°C. The dried product was reconstituted with 1.0 ml. deionized water and mixed gently for 5-10 min. After the phosphate buffer in the Sephadex columns was discarded and 1 ml. of the $^{125}I\text{-}T_4$ reagent applied to each column, 0.8 ml. of the reconstituted sample was introduced to each column and mixed gently. The columns were allowed to drain completely. Four ml. of pH 8.6 barbitol buffer were placed on the column and drained through. They were then placed in a well counter[g] and the activity determined. After counting, 1 ml. of eluting reagent was placed on each column and allowed to drain. Four ml. of the barbitol buffer was also added to each column and drained. The columns were again placed in the well counter and the final activity determined. Although the well counter used in this study gave a direct reading of percent retention, this value could also be calculated by the following formula:

$$\% \text{ Retention} = \frac{\text{final counts obtained from column}}{\text{initial counts obtained from column}} \times 100.$$

A corrected percent retention was calculated as follows:

$$\text{Corrected } \% \text{ Retention} = \frac{\% \text{ Retention}}{\% \text{ Extraction Efficiency}}$$

The percent extraction efficiency for canine serum was determined by pipetting 0.2 ml. ^{125}I-T_4 reagent into several test tubes. To each tube was added 1.0 ml. of canine serum or plasma. The tubes were incubated for 10 min. at room temperature while being shaken gently. After incubation the tubes were counted in a well counter for 1 min. Two ml. of 95% ethanol was added to each tube and the contents mixed for 15 sec. The tubes were allowed to stand for at least 5 min. and were then centrifuged for 5 min. at 2000 rpm. One ml. of the supernatant was counted in a well counter for 5 min. The percent extraction efficiency was calculated as follows:

$$\% \text{ Extraction Efficiency} = \frac{\text{cpm final count} \times 3.2 \times 100}{\text{cpm initial count}}.$$

T_4I (µg./100 ml.) was calculated from a standard curve prepared using the corrected % retention. T_4I (µg./100 ml.) was converted to T_4 (µg./100 ml.) by dividing by 0.653, since there is 65.3% I in T_4. The actual concentration of T_4 was then calculated as follows:

$$\text{Actual } \mu g.T_4/100 \text{ ml.} = \frac{\mu g.T_4/100 \text{ ml.}}{8}$$

The division by 8 was required because 0.8 ml. of the reconstituted serum extract was used in the modification instead of the recommended 0.1 ml. This was necessitated by the low circulating T_4 level in dogs.[4]

A standard curve was prepared using human serum provided with each kit. For each curve 0.05 ml., 0.10 ml., and 0.20 ml. of standard serum were used, which is equivalent to 2.5 ug.T_4/100 ml., 5.0 ug.T_4I/100 ml., and 10.0 ug.T_4/100 ml. respectively.

The final thyroid function test studied was an estimation of serum or plasma T_4 concentration (T_4 ratio[e]) in test serum, expressed as percent of the serum T_4 concentration in similarly determined normal serum. The methodology has been previously described.[24]

After both RT_3U[b] and T_4(D)[d] were determined, the thyroxine resin T_3 index (T_4-RT_3 index) was calculated. The T_4-RT_3 index is also known as the thyroid activity index, corrected thyroid concentration, T_7, or free thyroxine index and is calculated as the $T_4(D):RT_3U$ ratio or $(T_4(D)) \times (RT_3U) = (T_4\text{-}RT_3)$.[22]

Mean, standard deviation (S.D.), and standard error of the mean (SEM) were computed for each experimental group, and Student's t test of significance ($< P$ 0.05) was applied to evaluate differences between groups using a programmed electronic calculator.[h]

Results

The extraction efficiency for both canine serum and plasma was 86.9%. Extraction efficiency for human control serum used to establish the standard curve was 87.8%. The results of using serum and plasma in the thyroid function tests studied are outlined in Table 1. No significant differences could be noted.

The results using the first two thyroid function test kits (RT_3U^a and $T_4(D)^c$) are summarized in Table 2. No significant sex differences were noted in any of the groups.

The results of the third test kit evaluated (RT_3U^b) are summarized in Table 3. Significant differences were noted between males and females, Greyhounds and mixed-breeds, German Shepard-Greyhound crosses and mixed-breeds, and 0-6 mo.-old pups and the total population.

Results for the fourth thyroid function test kit studied (modified $T_4(D)^d$) are shown in Table 4. Significant differences were found for Greyhounds compared to mixed-breed dogs and 0-6 mo.-old dogs compared to the total population.

Results for T_4-RT_3, established by multiplying RT_3U^b by $T_4(D)^d$, are summarized in Table 5. The 0-6 mo.-old dogs and significantly higher values and German shepard-Greyhound crosses had significantly lower values when compared to the mixed-breed dogs and the total population.

The results for the final thyroid function test studied (T_4 ratio^c) are given in Table 6. The only significant difference noted was in comparing Greyhounds to mixed-breed dogs.

Discussion

The finding that either serum or plasma can be used to perform any of the thyroid function tests studied allows a single blood sample to be employed to perform thyroid function and other clinical laboratory procedures.

[illegible] [illegible] interesting trends were observed by examining the data from each thyroid function test. The measurements of T_4(D) and T_4-RT_3 using four different methods[a,b,c,d], had high thyroid function values in the young dogs (up to 0.6 mo.). This observation has also been made in other species.[15,18] When the dogs of different breeds were compared, Greyhounds and German Shepard-Greyhound crosses had significant differences from the mixed-breed dogs. Breed differences in thyroid function test values have been noted previously using different testing methods.[20] Although additional investigations are needed, this suggests that true breed differences in thyroid function exist. In one test (RT_3U[b]) a sex difference was observed; females had higher values than males. The opposite observation has been made in humans, with males having higher RT_3U values using this procedure.[25]

The T_4-RT_3 index has proved a valuable measure of total thyroid function in humans since it overcomes possible inherent errors when either T_4(D) or RT_3U are used alone.[8,23]

The mechanism by which the T_4(D) test[c] determines serum T_4 concentration has been previously discussed.[10] The column method of measuring serum T_4 (T_4(D)[d]) was designed on a different principle. The first method[c] used ethanol to extract T_4 from the serum or plasma proteins, and the patient's T_4 then competed with ^{125}I-T_4 for binding sites on thyroid-binding globulin (TBG). The column method (T_4(D)[d]) used a Sephadex G-25 column (pH 5.3) in place of the ethanol to extract the T_4, which than competed with ^{125}I-T_4 for binding sites on TBG. When canine serum was applied directly to the column without prior extraction, the canine T_4 was not retained in the Sephadex as expected and necessitated the modification described. It is postulated that the canine T_4 did not remain on the column because of inherent differences in the binding of canine T_4 by serum proteins as compared to humans.[4,21] With the modification of the column method ($T_4(D)^d$), results comparing favorably with those of the first method ($T_4(D)^c$) were found. However, the modification required additional time, making the entire test longer to perform than the first[c], and thereby negated the advantage this method has in human medicine. The column method also required the calculation of a new standard curve for each group of tests performed, thus, further increasing the time and cost.

When examining the data for the two methods measuring RT_3U, the values for the first test[a] are seen to be considerably lower than the values for the column test[b]. This same phenomenon was observed to occur in humans,[25,26] although good correlation was found when comparing the two methods in humans.[14]

The mechanism of the T_4 ratio thyroid function test[e] and the results of its use in humans has previously been described.[24] The results of this test in dogs were consistant, with little variance between individuals, and suggest that further evaluation should be conducted with this method on dogs with abnormal thyroid function. It would appear that this method could be of importance in veterinary medicine, since it combines two tests ($T_4(D)$ and RT_3U) into one quick and relatively simple technique.

Of the thyroid function tests studied, two methods[a,c] were shown of value. Because of the modifications required, two tests[b,d] were too involved and time-consuming for practical use. With further investigation, the T_4 ratio test[e] may be of value in measuring thyroid function in the dog.

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25. Trilute ^{125}I Column T-3 Test for Thyroid Function (Instruction Manual) Ames Co. Elkhart, Ind.

26. Triosorb-125 T-3 Diagnostic Kit (Instruction Manual) Abbott Laboratories, North Chicago, Ill.

Footnotes

a. Triosorb-125 (T-3 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.

b. Trilute (^{125}I Column T-3 Test for Thyroid Function), Ames Co., Ilkhart, Ind.

c. Tetrasorb-125 (T-4 Diagnostic Kit), Abbott Laboratories. North Chicago, Ill.

d. Tetralute (^{125}I Column T-4 Test for Thyroid Function), Ames Co. Elkhart, Ind.

e. Res-O-Mat ETR Diagnostic Test, Mallinckrodt Chemical Works, St. Louis, Mo.

f. Neuro-Muscular Disease Laboratory, Dept. of Physiological Science, Kansas State University, Manhattan, Kansas.

g. Thyrimeter (Direct Ratio Reading Gamma Counter), Ames Co., Elkhard, Ind.

h. Wang 360 K, Tewksbury, Mass.

TABLE 1-Comparison of Serum and Plasma for Selected Thyroid Function Tests in Dogs

Thyroid function test	N	Serum ($\pm$SEM)	Plasma ($\pm$SEM)
RT_3U* (%)	24	49.24$\pm$1.17	51.12$\pm$1.44
RT_3U** (%)	32	82.39$\pm$1.09	84.36$\pm$1.22
$T_4(D)^+$ (ug./100 ml.)	24	0.94$\pm$0.14	0.97$\pm$0.16
$T_4(D)^{++}$ (ug./100 ml.)	30	0.96$\pm$0.10	1.10$\pm$0.11
T_4-RT_3 index‡	24	0.46$\pm$0.07	0.50$\pm$0.09
T_4-RT_3 index‡‡	29	0.75$\pm$0.08	0.92$\pm$0.09
T_4 ratio§ (%)	40	87.57$\pm$0.58	86.78$\pm$0.58

* Triosorb-125 (T-3 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.

** Trilute (^{125}I column T-3 Test for Thyroid Function), Ames Co. Elkhart, Ind.

\+ Tetrasorb-125 (T-4 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.

++ Tetralute (^{125}I Column T-4 Test for Thyroid Function), Ames Co. Elkhart, Ind.

‡ T_4-RT_3 index determined by multiplying RT_3U* by $T_4(D)^+$.

‡‡ T_4-RT_3 index determined by multiplying RT_3U* by $T_4(D)^{++}$.

§ Res-O-Mat ETR Diagnostic Test, Mallinckrodt Chemical Works, St. Louis, Mo.

TABLE 2-Values of RT_3U*, $T_4(D)$**, and T_4-RT_3 Index+ in Dogs Using Two Commercial Thyroid Function Test Kits

Group	N	Test	Mean	S.D.	SEM
Adult Beagles	8	$T_4(D)$(ug./100 ml.)	1.20	0.57	0.12
	8	RT_3U (%)	50.56	3.10	0.68
	8	T_4-RT_3 index	0.60	0.27	0.06
Puppy Beagles	39	$T_4(D)$(ug./100 ml.)	2.27	0.59	0.09
	39	RT_3U (%)	47.01	3.03	0.47
	39	T_4-RT_3 index	1.08	0.29	0.04
Mixed-Breeds	12	$T_4(D)$(ug./100 ml.)	0.97	0.58	0.16
	12	RT_3U (%)	51.12	4.97	0.16
	12	T_4-RT_3 index	0.50	0.27	0.09

* Triosorb-125 (T-3 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.

** Tetrasorb-125 (T-4 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.

\+ T_4-RT_3 index determined by multiplying RT_3U* by $T_4(D)$**.

TABLE 3-Values for a $(RT_3U)^*$ Column Thyroid Function Test in Dogs

Group	N	Mean (%)	S.D.	SEM
Males	25	82.74	4.24	0.85
Females	26	85.06	4.97	0.98
German Shepards	23	82.70	3.26	0.68
Greyhounds	9	87.67	3.87	1.29
German Shepard-Greyhound Crosses	5	86.18	2.27	1.02
Mixed-Breeds	11	81.30	4.89	1.48
Dogs 0-6 mo.	6	81.22	5.55	2.27
Dogs 0-12 mo.	13	83.56	4.77	1.32
Dogs 1-5 yrs.	38	84.05	4.80	0.78
All Dogs	54	83.73	4.72	0.66

* Trilute (^{125}I Column T-3 Test for Thyroid Function), Ames Co., Elkhart, Ind.

TABLE 4-Values for a Modified (T_4(D))* Column Thyroid Function Test in Dogs

Group	N	Mean (μg./100 ml.)	S.D.	SEM
Males	41	1.14	0.61	0.09
Females	29	1.19	0.44	0.08
German Shepards	47	1.29	0.74	0.11
Greyhounds	6	0.72	0.19	0.08
German Shepard-Greyhound Crosses	5	1.00	0.39	0.20
Mixed-Breeds	11	1.05	0.42	0.21
Dogs 0-6 mo.	6	0.52	0.15	0.07
Dogs 0-12 mo.	17	1.20	0.24	0.06
Dogs 1-5 yrs.	53	1.15	0.57	0.08
All dogs	72	1.18	0.41	0.05

* Tetralute (^{125}I Column T-4 Test for Thyroid Function), Ames Co. Elkhart, Ind.

TABLE 5-Column Thyroid Function Test (T_4-RT_3 Index)* Values in Dogs

Groups	N	Mean (units)	S.D.	SEM
Males	24	0.83	0.31	0.06
Females	22	0.93	0.40	0.08
German Shepards	23	1.05	0.32	0.07
Greyhounds	6	0.64	0.17	0.08
German Shepard-Greyhound Crosses	5	0.86	0.36	0.18
Mixed-Breeds	11	0.85	0.35	0.10
Dogs 0-6 mo.	5	1.22	0.19	0.08
Dogs 0-12 mo.	12	0.95	0.38	0.11
Dogs 1-5 yrs.	34	0.88	0.36	0.06
All dogs	49	0.92	0.36	0.05

* T_4-RT_3 index determined by multiplying RT_3U** by $T_4(D)$+

** Trilute (^{125}I Column T-3 Test for Thyroid Function), Ames Co., Elkhart, Ind.

+ Tetralute (^{125}I Column T-4 Test for Thyroid Function), Ames Co., Elkhart, Ind.

TABLE 6-Thyroxine Ratio (T_4 Ratio)* Values in Dogs

Group	N	Mean (%)	S.D.	SEM
Males	56	86.81	2.89	0.39
Females	43	86.95	3.39	0.52
German Shepard	60	87.24	3.21	0.41
Greyhounds	13	85.82	1.98	0.55
German Shepard-Greyhound Crosses	7	85.41	3.06	1.50
Mixed-breeds	15	87.71	2.58	0.66
Dogs 0-6 mo.	9	86.29	2.25	0.75
Dogs 0-12 mo.	24	86.56	3.19	0.65
Dogs 1-5 yrs.	43	86.97	3.36	0.39
All dogs	104	86.89	3.45	0.34

* Res-O-Mat ETR Diagnostic Test, Mallinckrodt Chemical Works, St. Louis, Mo.

APPENDICES

APPENDIX A

INDIVIDUAL ANIMAL DATA

FROM

PREVIOUS STUDIES

TABLE I

Thyroid Function Studies in Adult Beagle Dogs Receiving 0 ppm Sodium Nitrate in Drinking Water

Dog no.	Sample	Thyroid Function Test: T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	1	0.89	52.74	0.47
	2	1.21	55.50	0.67
	3	0.18	47.08	0.37
2	4	0.89	52.45	0.47
	5	0.93	54.25	0.50
	6	0.67	51.68	0.35
	7	0.26	48.37	0.13
3	8	2.33	51.77	1.21
	9	1.68	52.13	0.88
	10	1.51	53.09	0.80
4	11	0.47	54.58	0.26
	12	1.77	52.79	0.93
5	13	2.51	51.93	1.30
	14	0.47	50.86	0.24
	15	0.90	46.34	0.42

TABLE I (cont.)

Dog no.	Sample	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
6	16	1.15	45.99	0.56
	17	1.84	45.99	0.85
	18	2.40	44.54	1.07
7	19	0.99	49.41	0.49
	20	2.08	39.53	0.47
	21	1.58	45.77	0.72
8	22	1.21	52.57	0.64
	23	1.21	50.66	0.61
Mean		1.29	50.18	0.64
S.D.		0.63	3.69	0.30
SEM		0.13	0.77	0.06

TABLE II

Thyroid Function Studies in Adult Beagle Dogs Receiving 300 ppm Sodium Nitrate in Drinking Water

Dog no.	Sample	Thyroid Function Test: T_4(D) (μg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	1	1.68	50.77	0.85
	2	2.31	45.58	1.05
	3	1.58	51.32	0.81
	4	1.62	48.94	0.79
2	5	1.51	52.84	0.80
	6	2.46	45.59	1.12
	7	1.15	48.93	0.56
3	8	0.63	56.28	0.36
	9	0.31	61.96	0.19
	10	0.63	53.48	0.34
4	11	0.47	53.05	0.25
	12	0.31	47.12	0.15
	13	1.45	45.84	0.70
5	14	0.55	56.70	0.31
	15	1.93	50.68	0.98
	16	0.67	44.06	0.29

TABLE II (cont.)

Dog no.	Sample	T_4(D) (μg/100 ml)	RT_3U (%)	T_4-RT_3 index
6	17	1.21	49.88	0.60
	18	1.68	47.94	0.80
	19	1.15	48.10	0.55
7	20	0.47	49.88	0.23
Mean		1.19	50.45	0.59
S.D.		0.64	0.64	0.30
SEM		0.14	0.14	0.07

TABLE III

Thyroid Function Studies in Adult Beagle Dogs Receiving 600 ppm Sodium Nitrate in Drinking Water

Dog no.	Sample	Thyroid Function Test: $T_4(D)$ (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	1	0.08	51.72	0.04
	2	0.05	62.58	0.03
	3	0.89	52.92	0.47
	4	0.93	50.32	0.47
2	5	2.03	51.50	0.79
	6	2.46	49.28	1.21
	7	1.73	51.20	0.88
	8	2.88	45.37	1.31
3	9	0.63	50.14	0.32
	10	1.58	42.85	0.68
	11	0.73	47.37	0.35
4	12	0.31	51.58	0.16
	13	0.47	43.94	0.21
	14	0.89	45.26	0.40
5	15	0	49.85	0
	16	0	49.94	0

TABLE III (cont.)

Dog no.	Sample	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
	17	0.05	46.94	0.02
	18	0.03	47.72	0.01
6	19	0.37	52.48	0.19
	20	1.36	49.00	0.67
7	21	1.10	52.52	0.58
	22	0.63	48.51	0.31
Mean		0.87	49.68	0.44
S.D.		0.81	3.95	0.38
SEM		0.17	0.84	0.08

TABLE IV

Thyroid Function Studies in Adult Beagle Dogs Receiving 1000 ppm Sodium Nitrate in Drinking Water

Dog no.	Sample	Thyroid Function Test: T_4(D) (μg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	1	1.25	44.77	0.56
	2	1.48	45.78	0.68
2	3	0.05	52.24	0.03
	4	1.06	57.40	0.61
3	5	0.67	56.38	0.38
	6	1.28	50.53	0.65
4	7	1.73	47.31	0.82
	8	1.06	50.94	0.54
5	9	0.78	62.80	0.49
	10	2.62	49.75	1.30
	11	0.78	51.20	0.40
	12	1.15	49.85	0.57
6	13	0.58	51.35	0.30
	14	2.08	49.42	1.03
	15	0.89	52.09	0.46

TABLE IV (cont.)

Dog no.	Sample	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
	16	0	49.07	0
7	17	0.47	41.60	0.19
	18	0	45.08	0
8	19	0.63	50.52	0.32
	20	0.37	49.10	0.18
Mean		0.94	50.36	0.47
S.D.		0.66	4.58	0.32
SEM		0.15	1.02	0.07

TABLE V

Thyroid Function Studies in Puppies from Beagle Dogs Receiving 0 ppm Sodium Nitrate in Drinking Water

Puppy no.	Thyroid Function Test		
	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	2.11	43.18	0.91
2	1.41	45.14	0.64
3	1.41	44.66	0.77
4	1.38	42.25	0.59
5	2.11	45.08	0.95
6	2.36	47.87	1.13
7	2.91	45.49	1.32
8	1.38	47.69	0.66
9	2.20	48.06	1.06
10	2.59	48.50	1.26
11	2.91	47.89	1.39
12	3.37	46.50	1.57
13	2.59	45.42	1.18
14	1.80	39.57	0.71
15	1.65	43.76	0.72
16	2.83	48.27	1.37
17	1.88	49.14	0.92
18	3.14	46.33	1.45

TABLE V (cont.)

Puppy no.	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
19	2.11	43.69	1.40
20	2.59	47.87	1.24
21	3.53	46.81	1.65
22	2.74	42.98	1.31
23	2.36	44.45	1.05
24	2.11	39.73	0.84
25	2.71	50.80	1.38
26	2.36	47.24	1.11
27	2.51	48.89	1.23
28	2.81	49.97	1.41
29	2.59	48.31	1.25
30	2.11	52.82	1.11
31	2.98	50.84	1.51
32	1.73	47.66	0.82
33	1.48	48.47	0.72
34	2.03	45.64	0.93
35	1.80	47.38	0.85
36	1.73	48.74	0.84
37	0.95	47.05	0.45
38	2.11	51.79	1.09
39	1.93	54.28	1.05

TABLE V (cont.)

Puppy no.	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
Mean	2.27	47.01	1.08
S.D.	0.59	3.03	0.29
SEM	0.09	0.47	0.04

TABLE VI

Thyroid Function Studies in Puppies from Beagle Dogs Receiving 300 ppm Sodium Nitrate in Drinking Water

	Thyroid Function Test		
Puppy no.	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	1.73	50.51	0.87
2	2.28	48.44	1.10
3	1.77	49.97	0.88
4	1.73	43.63	0.75
5	2.51	50.89	1.28
6	1.48	43.32	0.64
7	2.83	43.54	1.23
8	2.66	50.29	1.33
9	2.59	51.24	1.33
10	2.28	52.23	1.19
11	1.95	45.01	0.88
Mean	2.16	48.10	1.04
S.D.	0.43	3.33	0.24
SEM	0.13	1.00	0.07

TABLE VII

Thyroid Function Studies in Puppies from Beagle Dogs Receiving 600 ppm Sodium Nitrate in Drinking Water

	Thyroid Function Test		
Puppy no.	T_4(D) (µg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	2.71	44.85	1.21
2	2.98	45.06	1.34
3	1.70	41.32	0.70
4	2.51	43.99	1.10
Mean	2.47	43.80	1.09
S.D.	0.48	1.49	0.24
SEM	0.24	0.74	0.12

TABLE VIII

Thyroid Function Studies in Puppies from Beagle Dogs Receiving 1000 ppm Sodium Nitrate in Drinking Water

	Thyroid Function Test		
Puppy no.	T_4(D) (μg/100 ml)	RT_3U (%)	T_4-RT_3 index
1	2.73	45.42	1.24
2	1.41	42.35	0.60
3	2.43	49.04	1.19
4	2.66	47.84	1.27
5	2.11	48.39	1.02
6	2.03	49.07	1.00
7	3.37	44.75	1.51
8	2.36	47.81	1.13
9	2.74	42.11	1.15
10	3.61	44.64	1.41
11	3.98	43.13	1.72
12	1.88	45.25	0.85
13	2.11	48.84	1.03
14	2.20	41.14	0.90
15	1.18	43.08	0.51
16	1.93	47.98	0.93
17	2.98	47.20	1.41
18	3.14	46.31	1.45

TABLE VIII (cont.)

Puppy no.	$T_4(D)$ (μg/100 ml)	RT_3U (%)	T_4-RT_3 index
19	1.58	47.55	0.75
20	2.98	46.51	1.39
21	1.93	49.02	0.97
22	1.88	46.14	0.87
23	1.96	47.75	0.94
24	1.93	46.90	0.90
25	2.36	51.02	1.20
26	2.28	52.38	1.19
27	1.41	52.20	0.74
28	1.25	48.86	0.61
29	3.61	46.50	1.68
30	1.73	48.30	0.84
31	2.66	44.50	1.84
32	1.80	43.12	0.78
33	2.51	49.05	1.23
34	3.46	48.69	1.68
35	2.03	43.17	0.88
36	3.14	47.28	1.48
37	2.73	43.59	1.19
38	2.74	49.31	1.35
39	2.28	45.95	1.05
40	2.71	54.32	1.47

TABLE VIII (cont.)

Puppy no.	T_4(D) (μg/100 ml)	RT_3U (%)	T_4-RT_3 index
41	3.13	47.90	1.50
42	2.03	46.43	0.94
43	1.33	50.72	0.67
44	1.93	46.81	0.90
45	2.03	53.57	1.09
46	1.85	51.60	0.95
47	1.18	46.65	0.55
48	1.58	45.66	0.72
Mean	2.31	47.20	1.10
S.D.	0.68	2.96	0.32
SEM	0.09	0.43	0.05

TABLE IX

Thyroid Function Studies in Normal Mares:

Mare no. 1

T_4(D) (µg/100 ml)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	——	1.67	2.22	3.18
2	3.04	——	2.25	0.83	——
3	2.43	0.92	1.67	——	0.83
4	1.25	0.69	0.98	0.57	0.93
5	0.06	——	1.79	0.61	0.75
6	0.83	——	1.84	0.83	0.20
7	0.49	1.10	0.41	1.10	0.83

RT_3U (%)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	——	47.61	41.80	41.10
2	35.54	——	42.92	56.00	——
3	45.80	51.23	46.74	——	46.45
4	51.51	52.31	54.22	46.63	48.34
5	43.95	——	45.80	42.81	41.43
6	44.72	——	44.50	47.64	47.43

TABLE IX (cont.)

Cycle no.	P_1*	P_2**	E^+	M_1^{++}	M_2‡
7	54.12	46.81	45.90	46.31	48.12

T_4-RT_3 Index

Cycle no.	P_1*	P_2**	E^+	M_1^{++}	M_2‡
1	——	——	0.79	0.93	1.31
2	1.08	——	0.97	0.46	——
3	1.11	0.47	0.78	——	0.38
4	0.64	0.36	0.53	0.27	0.45
5	0.03	——	0.82	0.26	0.31
6	0.37	——	0.82	0.39	0.09
7	0.26	0.51	0.19	0.51	0.40

* Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries

**Proestrus 2, the day the follicle was large and soft, approximately 24-28 hours prior to ovulation

\+ Estrus, the day of ovulation

++Metaestrus 1, 6 days after ovulation

‡ Metaestrus 2, 11 days after ovulation

TABLE X

Thyroid Function Studies in Normal Mares:

Mare no. 2

T_4(D) (µg/100 ml)

Cycle no.	P_1*	P_2**	E+	M_1++	M_2‡
1	1.51	——	——	——	——
2	——	1.50	1.51	——	——
3	——	2.22	——	——	——
4	——	——	——	3.08	3.04
5	2.16	2.84	2.80	2.10	2.87
6	——	2.26	2.94	1.82	0.69
7	2.05	——	——	——	——
8	3.37	1.41	1.68	1.68	1.68
9	——	2.55	0.93	1.07	——

RT_3U (%)

Cycle no.	P_1*	P_2**	E+	M_1++	M_2‡
1	53.82	——	——	——	——
2	——	62.64	54.12	——	——
3	——	——	46.73	——	——

TABLE X (cont.)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
4	——	——	——	52.12	49.60
5	52.44	54.00	41.83	51.12	49.51
6	——	43.21	49.21	49.68	51.08
7	44.11	——	——	——	——
8	43.80	64.16	48.16	52.20	52.42
9	——	58.65	57.10	53.15	——

T_4-RT_3 Index

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	0.81	——	——	——	——
2	——	0.94	1.17	——	——
3	——	1.04	——	——	——
4	——	——	——	1.78	1.50
5	1.13	1.19	1.17	1.07	1.42
6	——	0.98	1.45	0.90	0.35
7	0.90	——	——	——	——
8	1.48	0.90	0.81	0.88	0.88
9	——	1.50	0.53	0.57	——

TABLE X (cont.)

* Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries

** Proestrus 2, the day the follicle was large and soft, approximately 24-48 hours prior to ovulation

+ Estrus, the day of ovulation

++ Metaestrus 1, 6 days after ovulation

‡ Metaestrus 2, 11 days after ovulation

TABLE XI

Thyroid Function Studies in Normal Mares:

Mare no. 3

T_4(D) (μg/100 ml)

Cycle no.	P_1*	P_2**	E+	M_1++	M_2‡
1	1.35	1.34	1.82	0.69	1.82
2	0.93	——	——	——	——

RT_3U (%)

Cycle no.	P_1*	P_2**	E+	M_1++	M_2‡
1	52.61	——	48.29	53.02	46.15
2	54.10	——	——	——	——

T_4-RT_3 Index

Cycle no.	P_1*	P_2**	E+	M_1++	M_2‡
1	0.71	——	0.88	0.37	0.84

TABLE XI (cont.)

Cycle no.	P_1*	P_2**	E+	M_1++	M_2‡
2	0.50	——	——	——	——

* Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries

** Proestrus 2, the day the follicle was large and soft, approximately 24-48 hours prior to ovulation

+ Estrus, the day of ovulation

++ Metaestrus 1, 6 days after ovulation

‡ Metaestrus 2, 11 days after ovulation

TABLE XII

Thyroid Function Studies in Normal Mares:

Mare no. 4

T_4(D) (μg/100 ml)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	——	3.73	1.87	3.12
2	——	1.82	2.00	1.36	1.21
3	1.36	2.11	2.16	0.69	0.81
4	2.49	——	2.49	1.93	1.74
5	1.36	2.29	1.87	0.61	0.49
6	1.00	1.07	1.36	0.84	2.01

RT_3U (%)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	——	50.39	47.83	44.17
2	——	45.89	43.43	38.23	67.65
3	37.56	54.56	38.75	48.61	46.64
4	40.58	——	48.48	47.85	57.39
5	55.26	62.19	51.74	44.01	38.69
6	49.33	46.99	50.52	47.07	45.46

TABLE XII (cont.)

T_4-RT_3 Index

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	——	1.88	0.89	1.38
2	——	0.83	0.87	0.52	0.82
3	0.51	1.15	0.84	0.33	0.38
4	1.01	——	1.21	0.92	1.00
5	0.75	1.42	0.97	0.27	0.19
6	0.49	0.50	0.68	0.39	0.57

* Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries

**Proestrus 2, the day the follicle was large and soft, approximately 24-48 hours prior to ovulation

\+ Estrus, the day of ovulation

++Metaestrus 1, 6 days after ovulation

‡ Metaestrus 2, 11 days after ovulation

TABLE XIII

Thyroid Function Studies in Normal Mares:

Mare no. 5

T_4(D) (µg/100 ml)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	——	1.32	——	——
2	——	2.88	1.68	0.99	1.44
3	1.07	1.30	1.54	0.99	2.00
4	2.29	2.11	1.87	1.50	0.75
5	1.54	2.02	2.95	1.74	0.57
6	——	1.18	1.50	0.69	1.18

RT_3U (%)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	——	45.39	——	——
2	——	48.62	45.32	49.80	48.36
3	48.47	50.80	49.24	45.03	47.94
4	45.99	44.73	46.58	45.74	49.58
5	43.57	44.50	45.27	43.52	43.81
6	——	47.14	46.03	46.04	42.60

TABLE XIII (cont.)

T_4-RT_3 Index

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	——	0.60	——	——
2	——	1.40	0.76	0.49	0.70
3	0.52	0.66	0.76	0.45	0.96
4	1.05	0.94	0.87	0.69	0.37
5	0.67	0.90	1.33	0.76	0.25
6	——	0.55	0.69	0.32	0.50

* Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries

** Proestrus 2, the day the follicle was large and soft, approximately 24-48 hours prior to ovulation

+ Estrus, the day of ovulation

++ Metaestrus 1, 6 days after ovulation

‡ Metaestrus 2, 11 days after ovulation

TABLE XIV

Thyroid Function Studies in Normal Mares:

Mare no. 6

T_4(D) (μg/100 ml)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	1.07	——	——	——	——
2	2.80	0.37	——	——	——
3	0.81	——	0.87	0.25	0.57
4	0.69	——	——	——	——
5	——	2.00	2.10	2.19	1.84

RT_3U (%)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	51.73	——	——	——	——
2	50.47	44.86	——	——	——
3	51.66	——	51.57	44.94	47.69
4	44.68	——	——	——	——
5	——	45.49	43.26	44.97	45.56

TABLE XIV (cont.)

T_4-RT_3 Index

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	0.55	——	——	——	——
2	1.41	0.17	——	——	——
3	0.42	——	0.45	0.11	0.28
4	0.31	——	——	——	——
5	——	0.91	0.91	0.98	0.84

* Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries

** Proestrus 2, the day the follicle was large and soft, approximately 24-48 hours prior to ovulation

+ Estrus, the day of ovulation

++ Metaestrus 1, 6 days after ovulation

‡ Metaestrus 2, 11 days after ovulation

TABLE XV

Thyroid Function Studies in Normal Mares:

Mare no. 7

T_4(D) (µg/100 ml)

Cycle no.	P_1*	P_2**	E+	M_1++	M_2‡
1	1.87	1.41	——	——	——
2	1.93	——	——	——	——
3	2.86	1.83	2.00	1.18	2.18

RT_3U (%)

Cycle no.	P_1*	P_2**	E+	M_1++	M_2‡
1	42.14	43.58	——	——	——
2	42.83	——	——	——	——
3	43.73	43.42	44.70	41.49	42.54

TABLE XV (cont.)

T_4-RT_3 Index

Cycle no.	P_1*	P_2**	E+	M_1++	M_2‡
1	0.79	0.61	——	——	——
2	0.83	——	——	——	——
3	1.25	0.79	0.89	0.49	0.93

* Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries

** Proestrus 2, the day the follicle was large and soft, approximately 24-48 hours prior to ovulation

\+ Estrus, the day of ovulation

++ Metaestrus 1, 6 days after ovulation

‡ Metaestrus 2, 11 days after ovulation

TABLE XVI

Thyroid Function Studies in Normal Mares:

Mare no. 8

T_4(D) (µg/100 ml)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	——	0.75	2.00	0.14
2	0.50	0.75	0.61	0.57	0
3	0.50	0.50	——	——	——
4	0.24	0.61	0.14	0.32	0.09

RT_3U (%)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	——	51.66	46.49	60.54
2	41.05	56.69	58.08	54.40	55.41
3	59.84	59.01	——	——	——
4	59.72	59.24	52.20	57.32	53.18

TABLE XVI (cont.)

T_4-RT_3 Index

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	—	—	0.39	0.93	0.08
2	0.20	0.42	0.35	0.31	0
3	0.30	0.29	—	—	—
4	0.14	0.36	0.07	0.18	0.05

* Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries

**Proestrus 2, the day the follicle was large and soft, approximately 24-48 hours prior to ovulation

+ Estrus, the day of ovulation

++Metaestrus 1, 6 days after ovulation

‡ Metaestrus 2, 11 days after ovulation

TABLE XVII

Thyroid Function Studies in Normal Mares:

Mare no. 9

T_4(D) (µg/100 ml)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	0.50	0.32	0.24	0.87
2	0.03	——	1.09	2.02	2.59
3	——	2.02	1.54	1.51	1.51
4	1.19	——	2.39	1.50	1.87

RT_3U (%)

Cycle no.	P_1*	P_2**	E^+	M_1++	M_2‡
1	——	48.24	46.57	52.64	53.52
2	57.56	——	46.15	52.74	49.79
3	——	45.03	44.47	44.68	43.48
4	44.62	——	47.84	45.08	48.22

TABLE XVII (cont.)

T_4-RT_3 Index

Cycle no.	P_1*	P_2**	E+	M_1++	M_2‡
1	——	0.24	0.15	0.13	0.47
2	0.02	——	0.50	1.06	1.29
3	——	0.91	0.68	0.67	0.66
4	0.53	——	1.14	0.68	0.90

* Proestrus 1, the first day a developing follicle could be palpated on one of the ovaries

** Proestrus 2, the day the follicle was large and soft, approximately 24-48 hours prior to ovulation

\+ Estrus, the day of ovulation

++ Metaestrus 1, 6 days after ovulation

‡ Metaestrus 2, 11 days after ovulation

TABLE XVIII

Individual Data for Dogs Used in Evaluation of Selected Commercial Thyroid Function Tests

Dog no.	Breed*	Sex**	Age (months)	Thyroid Function Test: $T_4(D)^{++}$ (µg/100 ml)	$RT_3U^{‡}$ (%)	T_4-RT_3 ‡‡ index	T_4 Ratio§ (%)
1	GS	F	3	1.72	84.6	1.46	85.3
1+	GS	F	3	1.65	86.6	1.43	86.1
2	Mix	M	24	1.00	74.6	0.80	87.0
2+	Mix	M	24	1.12	80.0	0.90	87.0
3	Mix	F	10	——	——	——	89.5
3+	Mix	F	10	1.23	82.7	1.02	87.8
4	Mix	F	24	——	——	——	91.3
4+	Mix	F	24	1.73	90.7	1.57	93.5
5	Mix	F	3	——	——	——	——
5+	Mix	F	3	1.37	81.1	1.11	82.7
6	Mix	M	2	1.33	74.8	0.99	87.0

TABLE XVIII (cont.)

Dog no.	Breed*	Sex**	Age (months)	$T_4(D)$ ++ (µg/100 ml)	RT_3U ‡ (%)	T_4-RT_3 ‡‡ index	T_4 Ratio § (%)
6+	Mix	M	2	1.52	73.0	1.11	89.1
7	Mix	M	6	—	—	—	89.0
7+	Mix	M	6	—	—	—	85.2
8	Poodle	M	24	0.87	77.9	0.68	82.2
8+	Poodle	M	24	—	—	—	86.0
9	Mix	M	12	0.51	85.8	0.44	83.6
9+	Mix	M	12	0.41	86.8	0.36	85.3
10	GH	F	60	—	—	—	88.6
10+	GH	F	60	0.98	92.4	0.90	84.3
11	Mix	M	24	0.57	78.7	0.45	87.5
11+	Mix	M	24	0.76	81.1	0.62	85.1
12	Pointer	F	36	0.38	92.9	0.35	84.5
12+	Pointer	F	36	0.28	93.3	0.26	84.1
13	GH	F	30	—	79.3	—	86.1

TABLE XVIII (cont.)

Dog no.	Breed*	Sex**	Age (months)	$T_4(D)^{++}$ (µg/100 ml)	$RT_3U^{‡}$ (%)	T_4-RT_3 ‡‡ index	T_4 Ratio§ (%)
13+	GH	F	30	——	88.0	——	87.2
14	GS	M	12	1.03	81.7	0.84	91.4
14+	GS	M	12	1.32	83.1	1.10	87.0
15	GS	M	36	0.76	83.4	0.63	89.0
15+	GS	M	36	0.89	79.9	0.71	84.2
16	GS	M	12	0.74	81.8	0.60	89.4
16+	GS	M	12	0.67	86.1	0.58	92.3
17	GS	F	12	1.22	80.2	0.98	87.1
17+	GS	F	12	——	——	——	——
18	GS	M	12	1.62	——	——	94.0
18+	GS	M	12	1.93	79.5	1.53	87.0
19	GH	F	33	0.78	84.3	0.66	89.0
19+	GH	F	33	0.83	86.2	0.72	88.0
20	GS/GH	M	12	0.97	84.1	0.82	82.4

TABLE XVIII (cont.)

Dog no.	Breed*	Sex**	Age (months)	$T_4(D)^{++}$ (µg/100 ml)	$RT_3U^{‡}$ (%)	T_4-RT_3 ‡‡ index	T_4 Ratio§ (%)
20 +	GS/GH	M	12	0.89	83.6	0.74	88.8
21	GH	F	32	——	86.8	——	82.5
21 +	GH	F	32	——	——	——	——
22	GS/GH	F	5	——	87.2	——	88.8
23	GS/GH	M	8	1.61	89.9	1.45	90.0
24	GS	F	18	0.92	85.0	0.78	84.6
25	GS	F	18	0.91	81.1	0.74	82.1
26	GH	F	32	0.57	90.7	0.52	85.0
26r§§	GH	F	32	0.44	90.0	0.40	84.3
27	GH	M	30	——	91.3	——	85.3
28	GS	F	48	1.07	84.4	0.90	
29	GS	M	13	1.13	85.3	0.96	84.2
30	GS/GH	F	5	——	——	——	83.4
31	GS	M	12	1.17	——	——	86.7

TABLE XVIII (cont.)

Dog no.	Breed*	Sex**	Age (months)	$T_4(D)$ ++ (μg/100 ml)	RT_3U ‡ (%)	T_4-RT_3 ‡‡ index	T_4 Ratio § (%)
32	GS	M	12	——	——	——	88.4
33	GS	M	12	——	——	——	86.3
34	GS	M	12	——	——	——	85.4
35	GS	M	12	1.44	——	——	89.0
36	GS	M	12	1.66	——	——	86.7
37	GS	M	12	——	——	——	81.9
38	GS	M	12	1.28	——	——	86.7
39	GS	M	12	——	——	——	82.6
40	GS	M	12	1.38	——	——	86.7
41	GS	M	12	1.77	——	——	88.8
42	GS	M	12	——	——	——	87.1
43	GS	M	12	1.09	——	——	87.3
44	GS	M	10	2.01	——	——	90.9
45	GS	M	18	——	——	——	89.0
46	GS	M	18	1.29	——	——	85.2

TABLE XVIII (cont.)

Dog no.	Breed*	Sex**	Age (months)	$T_4(D)^{++}$ (µg/100 ml)	$RT_3U^{‡}$ (%)	T_4-RT_3 ‡‡ index	T_4 Ratio§ (%)
47	GS	M	18	0.83	——	——	84.2
48	GS	M	18	1.18	——	——	89.9
49	GS	M	18	1.02	——	——	84.0
50	GS	M	18	0.94	——	——	83.2
51	GS	M	24	1.20	82.2	0.99	90.4
52	GS	M	24	1.20	——	——	84.4
53	GH	M	30	——	——	——	87.3
54	GH	M	30	——	——	——	83.2
55	GS	M	36	——	——	——	86.9
56	GS	M	36	0.86	86.9	0.71	87.0
57	GS	M	48	1.59	86.0	1.37	91.8
58	GS	M	19	1.50	——	——	82.4
59	GS	M	8	1.44	——	——	84.6
60	GS	F	12	1.86	84.9	1.58	93.1

TABLE XVIII (cont.)

Dog no.	Breed*	Sex**	Age (months)	$T_4(D)^{++}$ (µg/100 ml)	$RT_3U^{‡}$ (%)	T_4-RT_3 ‡‡ index	T_4 Ratio§ (%)
61	GS	F	12	1.36	——	——	87.1
62	GS	F	12	1.63	——	——	89.0
63	GS	F	12	——	——	——	83.4
64	GS	F	12	1.44	——	——	77.4
65	GS	F	12	0.98	——	——	86.0
66	GS	F	12	1.50	86.3	1.29	91.3
67	GS	F	12	1.19	——	——	83.2
68	GS	F	10	1.00	——	——	90.9
69	GS	F	18	1.25	79.7	1.00	91.3
70	GS	F	18	1.51	72.6	1.10	88.7
71	GS	F	18	——	——	——	89.9
72	GS	F	18	——	——	——	85.8
73	GS	F	18	1.66	81.0	1.34	89.6
74	GS	F	18	——	——	——	91.3

TABLE XVIII (cont.)

Dog no.	Breed*	Sex**	Age (months)	$T_4(D)^{++}$ (μg/100 ml)	$RT_3U^{‡}$ (%)	T_4-RT_3 ‡‡ index	T_4 Ratio§ (%)
75	GS	F	48	1.03	——	——	88.2

\+ = Plasma was used with heparin as the anticoagulant

* GS = German Shapard, GH = Greyhound, GS/GH = German Shepard;Greyhound Cross

** F = Female, M = Male

++ Tetralute (^{125}I Column T-4 Test for Thyroid Function), Ames Co., Elkhart, Ind.

‡ Trilute (^{125}I Column T-3 Test for Thyroid Function), Ames Co., Elkhart, Ind.

‡‡ T_4-RT_3 index determined by multiplying $T_4(D)^{++}$ by $RT_3U^{‡}$

§ Res-O-Mat ETR Diagnostic Test, Mallinckrodt Chemical Works, St. Louis, Mo.

§§ Repeated determination of the sample

APPENDIX B

THYROXINE RESPONSE TO THYROTROPIN-RELEASING HORMONE IN A DOG

In recent years two in-vitro thyroid function tests have been extensively investigated in the dog.[2,9,10,12-16,18] One procedure is total serum thyroxine by competitive protein binding of labeled thyroxine (T_4); recently designated thyroxine (displacement) or $T_4(D)$.[25] The second procedure is resin sponge uptake of radioactive triiodothyronine, designated resin triiodothyronine uptake or RT_3U.[25]

When compared to humans, the dog has a low level of circulating T_4.[6,20] For this reason it is difficult to diagnose borderline hypothyroidism in the dog using just the previously outlined procedure.[3,23] The thyroid stimulating hormone (TSH) stimulation test has proved valuable in veterinary medicine since it overcomes this problem.[3,23] However, the TSH-stimulation test has one serious disadvantage; the TSH, which is extracted from bovine pituitaries, is antigenic and repeated administration may elicit specific anitbody response.[3]

Recently a new hormone, thyrotropin releasing hormone (TRH) has been identified and synthesized.[7] Present thyroid physiology indicates that TRH, produced in the hypothalamus, is released by proper stimulation into the hypothalamo-hypophoseal portal circulation. There it acts to stimulate the release of TSH which, in turn, stimulates the thyroid gland to release and synthesize thyroid hormones.[21] Considerable investigation has been conducted in humans on the effect of TRH on thyroid physiology and the possible role of TRH in thyroid function testing.[1,4,5,8,11,19,22,24,26,27]

Because of the possible antigenic reaction resulting from repeated use of TSH, thyroid response using $T_4(D)$ and RT_3U may be a measure of thyroid function in the dog. This experiment was designed to measure thyroid response after intravenous administration of TRH to one dog.

MATERIALS AND METHODS

A healthy, 7-year old female Irish Setter was used. Ten min. prior to administration TRH, a 10 ml. blood sample was collected; 120 µg. TRH was then administered intravenously. Ten ml. samples of whole blood were collected at 30 min., 2 hrs., 6 hrs., 12 hrs., 24 hrs., 36 hrs., and 60 hrs. after TRH administration. The samples were allowed to clot, and the serum was separated and frozen. Samples were later thawed to room temperature and evaluated for thyroid function using the two _in vitro_ tests, $T_4(D)$[a] and RT_3U[b], previously described.[17] After determining $T_4(D)$ and RT_3U for each sample, the thyroxine-resin T_3 index (T_4-RT_3 index), known also as the thyroid activity index, corrected thyroid concentration, T_7, and free thyroxine index,[25] was calculated as the $T_4(D)$:RT_3U ratio or $T_4(D)) \times (RT_3U) = (T_4\text{-}RT_3)$.

RESULTS

Fig. 1, 2, and 3 demonstrate the values determined for $T_4(D)$, RT_3U, and T_4-RT_3 index at various times after TRH administration.

DISCUSSION

Most investigators agree that there is a rise in serum TSH after administration of TRH to humans and that this response is dose dependent within limits.[4,5,8,11,19,22] TRH has also been demonstrated to cause increase in serum triiodothyronine (T_3) concentration as measured by radioimmunoassay.[11,22] However, the evidence supporting the TRH-induced rise in T_4(D) and RT_3U values is confusing. One investigator demonstrated that there was no change in T_4(D) or RT_3U values for tests performed before and 24 hours after administration of a single intravenous dose of TRH in humans.[1] Another study found a significant rise in serum T_4, as measured by protein bound iodine (PBI), if TRH was administered orally[19] or infused over a 4-hour period.[27] A significant rise in serum T_4 was measured by T_4(D) after a single intravenous dose of TRH and the collection of samples 10, 20, 40, and 60 min. after the TRH administration.[11]

In the present experiment, the rise in serum T_4 levels after TRH administration was rapid and then quickly dropped to below initial levels. This is in general agreement with similar studies in humans.[11] A possible reason that the human study[26] did not demonstrate an increase in serum T_4 after TRH administration is that blood samples were collected after the serum T_4 peak had occurred. The cause of the increase in serum T_4 36 hours after TRH administration is difficult to postulate; perhaps it was due to a feedback phenomenon on the anterior pituitary gland. There was no significant rise in RT_3U after TRH administration. The decrease in RT_3U one hour after TRH administration may have resulted from an absolute decrease in serum T_3 or from a decrease in binding sites on the serum thyroid binding proteins. The T_4-RT_3 index reflects the free (unbound) T_4 in the serum and may be a more accurate index of the effect of TRH on the thyroid gland.[23]

The results of this experiment are inconclusive in themselves. However, there are indications that TRH administration, followed by measurement of T_4(D), RT_3U, and T_4-RT_3 index, may be a useful diagnostic aid in veterinary medicine. More extensive experiments should be conducted.

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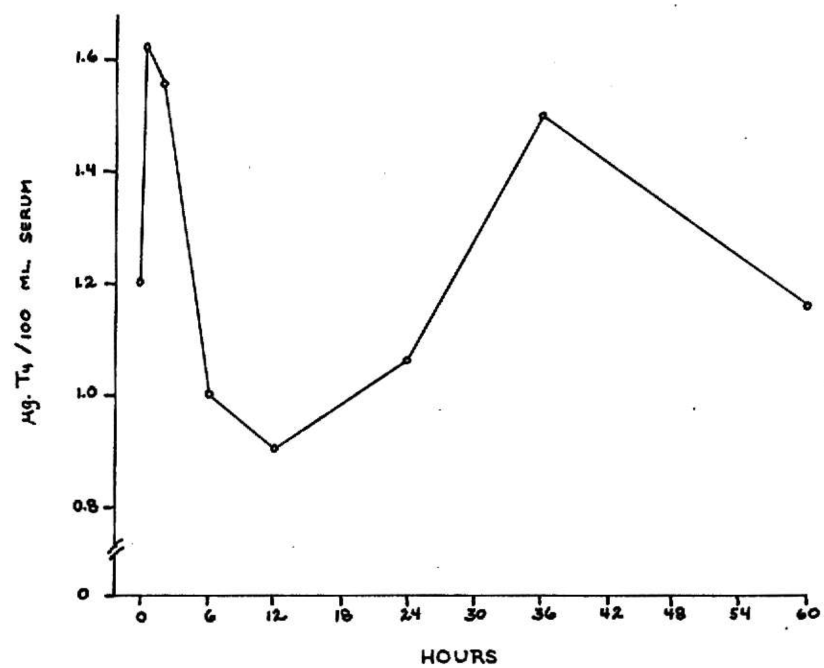
Footnotes

a. Tetrasorb-125 (T-4 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.

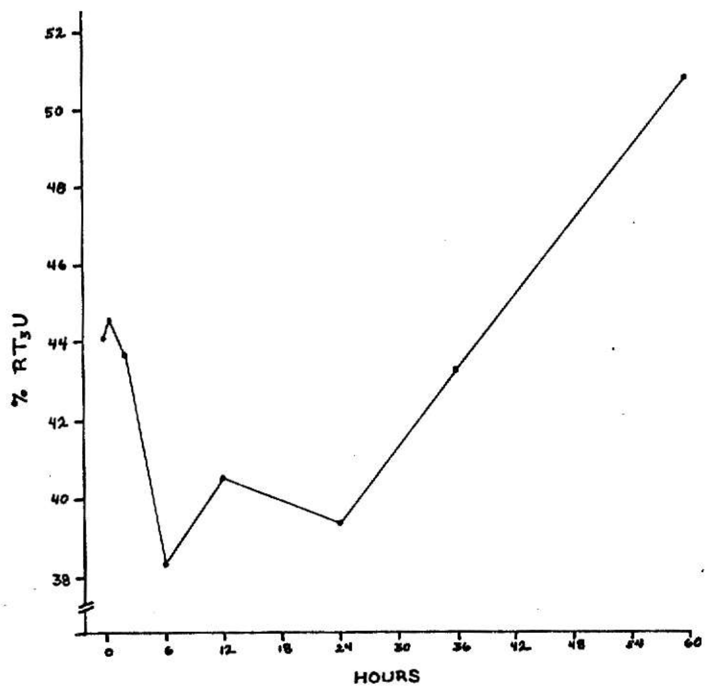
b. Triosorb-125 (T-3 Diagnostic Kit), Abbott Laboratories, North Chicago, Ill.

Fig. 1--Serum thyroxine (T_4) levels before and after administration of thyrotropin releasing hormone (TRH).



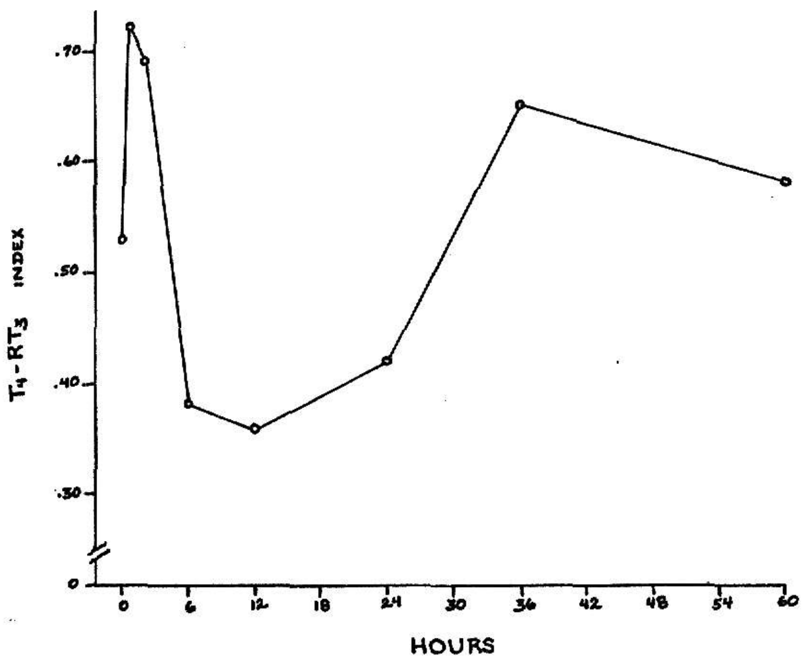
μg. T4 / 100 ML SERUM
1.6
1.4
1.2
1.0
0.8
0
0
6
12
18
24
30
36
42
48
54
60
HOURS

Fig. 2--Serum resin triiodothyronine uptake (RT_3U) before and after administration of thyrotropin releasing hormone (TRH).



52
50
48
46
44
42
40
38
% RT3U
0
6
12
18
24
30
36
42
48
54
60
HOURS

Fig. 3--Serum thyroxine resin triiodothyronine uptake (T_4RT_3) before and after administration of thyrotropin releasing hormone (TRH).



.70
.60
.50
.40
.30
0
T_4-RT_3 INDEX
0
6
12
18
24
30
36
42
48
54
60
HOURS

APPENDIX C

LITERATURE REVIEW

THYROID FUNCTION TESTING IN DOGS, HORSES, AND CATTLE

Disorders of the thyroid gland are the most common endocrine disorders in man and an extensive accumulation of historical, and scientific literature is available.[13,64,66,79] However, in domestic animals, the thyroid and its disorders are less well known and documented.

Since extensive study has already been done on thyroid function testing in humans most of the tests done in animals are modifications of human methods. Thyroid function tests can be classed into general groupings according to the specific function that is measured. The general groups are as follows: 1) physiologic effects including, basal metabolic rate (BMR), serum cholesterol, and hematology; 2) radioactive tracer studies including, radioactive iodine uptake by the thyroid, urinary excretion of radioactive iodine, protein bound ^{131}I ($BP^{131}I$), conversion ratio, thyroxine ^{131}I plasma clearance half-time, and thyroid scan; 3) serum hormone concentration including, protein bound iodine (PBI), butanol extractable iodine (BEI), thyroxine by column (T_4-COL), free thyroxine determination, radioimmunoassay of thyroid stimulatin hormone (TSH), thyroxine (T_4), and triiodothyronine (T_3), thyroxine (displacement) (T_4(D)), and resin triiodothyronine uptake (RT_3U); 4) combined thyroid function tests including, thyroxine secretion rate (TSR), thyroxine resin T_3 index (T_4-RT_3 index), and effective thyroxine ratio (ETR); 5) stimulation tests including, TSH stimulation test and thyrotropin releasing hormone

(TRH) stimulation test.

The group of thyroid function tests based on the physiologic effects of the thyroid gland were the first group of tests applied to veterinary medicine. Determination of BMR has long been a method of choice for detection of thyroid malfunction in man. However, this test, which measures oxygen consumption under standardized conditions, has found little application in veterinary medicine due to the difficulty of maintaining animals under the conditions required for the test.[10] The level of plasma or serum cholesterol is affected by the status of thyroid activity, and in general, it varies inversely with the degree of activity. However, cholesterol determination is an unreliable indicator of thyroid function because the normal range for serum cholesterol is wide and elevations are frequently seen in a variety of conditions unrelated to thyroid activity. The diagnostic accuracy of serum cholesterol for hypothyroidism in the dog is about 60%. However, when the levels are very high, greater than 500 mg./100 ml. and diabetes mellitus is eliminated, its diagnostic accuracy is increased. Evaluation of serum cholesterol as an indicator of hyperthyroid status is completely ineffective.[36] A moderate normocytic normochromic anemia is sometimes associated with clinical hypothyroidism in the dog. But, because this type of anemia characteristic of a variety of diseases this observation could not be used to diagnose hypothyroidism, just help confirm a suspected case.[36]

All radioactive tracer studies, used in the diagnosis of thyroid gland malfunction, have one thing in common; they require the injection of radioactive iodine (usually ^{131}I) into the subject. This requirement is a distinct disadvantage because of the regulations concerning the <u>in-vitro</u>

use of radioactive isotopes and the elaborate monitoring equipment necessary. This means that these tests can only be conducted at institutions with the proper facilities.

Radioactive iodine uptake by the thyroid is intended to measure the ability of the thyroid gland to concentrate iodine. Radioactive iodine is administered to the subject and at specified times following administration the quantity of radioactive iodine concentrated in the thyroid gland is determined by placing a radiation detector over the gland. A high uptake usually indicates a very active gland and a low uptake a sluggish one.[72] Several investigators have examined uptake of radioactive iodine in dogs with varying results depending on the specific method used.[8,14,16,34,38,59,62,63,65] Data for this test is not as plentiful in other species but investigators have reported on horses[35] and dairy cattle.[73]

Urinary excretion of radioactive iodine is similar to thyroidal uptake of radioactive iodine in that it measures the thyroid gland's ability to concentrate iodine.[68] This procedure is plagued in veterinary medicine with the requirement of total urine collection during the measurement period and also necessitates normal kidney function.[22] In animals this test has been experimentally used only in dogs.[54,62]

Two additional tracer techniques include $PB^{131}I$ and the conversion ratio. These procedures depend upon thyroidal secretion of administered ^{131}I incorporated into the circulation at specified times after administration. In the $PB^{131}I$ method, the hormonal protein-bound moiety is separated from the circulating iodide. The protein-bound fraction is expressed as a percentage of the administered dose. Normal values have been worked out in dogs.[8,62] The conversion ratio represents an alternative calculation that expresses the fraction protein-bound radioactivity as a percent of the total ^{131}I present in plasma.[35] In dogs there are conflicting reports of the normal values for the conversion ratio.[30,39]

Thyroxine ^{131}I plasma clearance half-time is the time required for one-half of and injected amount of tagged thyroxine to disappear from circulation and is a measure of the rate of thyroxine utilization. This procedure has been conducted experimentally in dogs.[54]

Thyroid scan requires a radioisotope scanner. It is a visualization of the shape and size of the thyroid tissue that concentrated radioactive iodine that was previously administered. This diagnostic method has been used successfully to diagnose thyroid neoplasia in dogs.[21]

In-vitro thyroid function tests, which measure circulating levels of thyroid hormones are superior to those requiring injection of radioisotopes because they are simpler for the practioner to make use of. These tests usually require the collection of a serum or plasma sample from the patient and then sending this sample to a laboratory for analysis.

Determination of PBI is a measure of the level of thyroid hormone in circulation. PBI is a rather sensitive test and is easily affected by iodine contamination.[22,36] This method is one of the oldest in use in veterinary medicine and there is considerable data available for dogs,[7,12,32,36,44,53-55,59,61] horses,[28,35,61,76] and cattle.[40,41,61]

The BEI procedure like PBI is a chemical procedure to determine thyroid hormone levels in circulation.[26] It is reported to be more specific for the protein bound iodine containing hormones and less sensitive to other organic iodine compounds which may be present than PBI,[45,60] but it is very complex and for this reason is rarely performed on animals except for research purposes.

T_4-COL is a more specific approach to evaluating thyroid function. This procedure uses an anion exchange resin to remove T_3, T_4 and other iodide containing compounds from the serum.[17] Only the fraction containing T_4 and T_3 are eluted off the column. T_4-COL is reported as µg.I/100 ml. (T_4-COL). Only a limited amount of data for the dog is presently available by this method.[36]

Free (unbound) thyroxine (T_f) is considered to be the metabolically active fraction of total circulating T_4, thus this parameter of thyroid function would be most valuable to have available.[9] However, the concentration of T_f is extremely low in most species.[61] This makes this determination difficult to perform, expensive, and time consuming.

Recently the discovery of radioimmunoassay procedures has opened the way for relatively simple and very accurate procedures for measuring serum levels of T_3, T_4, and TSH in humans.[20,25,57] More investigation needs to be done to adapt these methods to animals.

In 1964 investigators,[49] developed a technique to determine T_4 utilizing the property of protein-binding, recently designated thyroxine (displacement) (T_4(D)), formerly called thyroxine (Murphy-Pattee), displacement analysis, isotope displacement assay, saturation analysis, competitive protein binding, and radioligand binding.[70] The T_4(D) test is based on competitive protein binding and estimates the levels of T_4 in serum or plasma by the amount of ^{125}I-labeled T_4 released from labeled thyroxine-binding-globulin (TBG) as a result of competition with the T_4 present in a test sample.[32] Several commercial test kits have been produced measuring $T_4(D)$ by slightly different modifications and some have been investigated in humans.[50-52] One of the commercial kits utilizing a resin sponge has been extensively investigated in dogs,[2,22,23,31,72,88] horses,[22-24,33,43] and cattle.[22,42]

In 1957, measurement of uptake of radioactive T_3 by RBCs was described as a method of estimating thyroid function in man.[18] This test is based on the fact that T_4 and T_3 are bound by plasma proteins and RBCs, but more firmly bound by the proteins. However, this technique is difficult to perform since it requires the washing of RBCs in a careful and uniform manner to insure repeatable results.[30] In 1961, measurement of resin sponge uptake of liothyronine ^{131}I ($^{131}T_3$) from human serum as a diagnostic test for thyroid dysfunction and pregnancy was reported.[47] This test is an indirect measure of thyroid function. In fact, it is a measure of the relative saturation of thyroid hormone binding sites in circulation.[23] Recently the resin uptake of radioactive triiodothyronine has been designated resin triiodothyronine uptake (RT_3U).[70] Values for RT_3U using ^{125}I has been reported in dogs,[2,23,32] horses,[23,53,48] cattle.[23]

The thyroxine-resin T_3 index (T_4-RT_3 index), also known as the thyroid activity index, corrected thyroid concentration, T_7, and free thyroxine index is calculated as the $T_4(D):RT_3U$ ratio or $(T_4(D)) \times (RT_3U) = (T_4\text{-}RT_3)$.[70] In humans the T_4-RT_3 index has proved a valuable measure of total thyroid function, since it overcomes possible errors when either $T_4(D)$ of RT_3U are used alone.[15,27,71]

TSR is a measure of the rate of secretion by the thyroid gland. The determination requires measuring the plasma clearance half-time of tagged T_4 and PBI. The remainder of the procedure involves mathematical manipulations of the tagged T_4 clearance curve, clearance half-time, and PBI. This assumes that the rate of thyroxine utilization or loss equals the rate of secretion or production.[72] Several investigators maintain that TSR is the best measure of thyroid status in horses[29] and cattle.[3,58] TSR has also been determined in dogs.[62]

Recently a new thyroid function test has been developed that purportes to overcome some of the possible errors using either RT_3U or $T_4(D)$ alone. This test is called the effective thyroxine ratio (ETR). This procedure quantifies only the concentration of T_4 and is expressed as a ratio of T_4 similarly determined in asolute units.[46,75]

A valuable method for indirectly evaluating thyroid function is the thyroid stimulation test. In the TSH- stimulation test, administration of exogenous TSH (extracted from bovine pituitaries) simulates the effect of endogenous TSH by accelerating thyroid gland physiology. This test is used in human medicine principally to differentiate between primary and secondary thyroid disorders.[79] However, it promises to be of far greater importance to veterinary medicine as a primary diagnostic procedure.[4,55]

The test is performed by injecting a quantity of bovine TSH into the patient. A blood sample is collected before injection and 24 hours after administration, $T_4(D)$ or PBI tests are performed on the samples. If the test values rise a significant amount[5], the test is considered normal. The TSH-stimulation test has one serious disadvantage; TSH extracted from bovine pituitaries is antigenic and its continued administration may elicit specific antibody response.[4]

In humans the TRH-stimulation test, based on a principle similar to the TSH-stimulation test, has been investigated as a possible test of the pituitary-thyroid axis.[1,6,11,19,26,56,65,67,69,77,78] No investigations have yet been published concerning the TRH-stimulation test in animals.

Relatively few of the thyroid function tests available to human medicine are practical for routine use to diagnose thyroid dysfunction in dogs, horses, or cattle. Tests most applicable to veterinary medicine are RT_3U, $T_4(D)$, PBI, serum cholesterol, radioactive iodine uptake, conversion ratio, tagged T_4 clearance half-time, TSR, and thyroid scan.[22] Of these tests only RT_3U, $T_4(D)$, PBI, and serum cholesterol are feasible for the practicing veterinarian to use, unless he has access to sophisticated instrumentation.[4]

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SELECTED STUDIES OF THYROID HORMONES
IN DOGS, HORSES, AND CATTLE

by

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Five selected studies of thyroid hormones were conducted in dogs, horses, and cattle. These studies were: 1) effect of chronic dietary nitrate on canine thyroid function; 2) influence of estrus cycle in normal mares on serum thyroid levels; 3) a comparative study of circulating thyroid levels; 4) evaluation of selected commercial thyroid function tests in the dog; 5) thyroxine response to thyrotropin-releasing hormone in a dog.

An investigation was conducted to gain information concerning the effects of dietary nitrates on thyroid function in Beagle dogs and their offspring. The dogs received 0 ppm, 300 ppm, 600 ppm, and 1000 ppm sodium nitrate in drinking water under normal management and feeding conditions. Continuous consumption of sub-lethal levels of nitrate by dogs did not produce a syndrome of chronic nitrate toxicosis characterized by thyroid dysfunction.

Serum thyroxine (T_4), resin triiodothyronine uptake (RT_3U), and thyroxine resin T_3 index (T_4-RT_3 index) were followed in 9 normal mares during theri estrus cycles. A statistically nonsignificant decrease in serum T_4 was observed after ovulation. However, there were no significant differences in the thyroid function test results during various stages of the estrus cycle. It appears that the stage of estrus cycle does not significantly influence the serum thyroid hormone levels in normal mares.

Three thyroid function tests: thyroxine (displacement) (T_4(D)), RT_3U, and T_4RT_3 index, were used to measure thyroid function in dogs, horses, and cattle. A comparison was also made of the use of serum or plasma. The choice of serum or plasma did not interfere with or alter test results. The thyroid function tests showed similar results in dogs and horses, but there were significant differences in cattle. This variation may be due to basic species differences in thyroid physiology.

Five different commercial test kits for studying thyroid function were evaluated using normal dogs. Two of the tests were found unsatisfactory for use without considerable modification, and two tests were of value. One thyroid test appeared promising for evaluating thyroid function, but further investigation is needed.

Thyroxine response to thyrotropin-releasing hormone (TRH) was measured in one dog. Thyroid response was determined by measuring T_4(D), RT_3U, and T_4-RT_3 index. There was a significant change in T_4(D) values after TRH administration. RT_3U and T_4-RT_3 index values did change, but not dramatically. The results from this experiment were inconclusive in themselves. However, there were indications that TRH administration, followed by measurement of T_4(D), RT_3U, and T_4-RT_3 index, may be a useful diagnostic aid in veterinary medicine.