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From: The Department of Dairy Husbandry, College of Agriculture
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of Missouri - Columbia, Columbia, Missouri, 65201

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Contract No. AT(11-1)-1758-Anderson during the Period
January, 1968, thru December, 1971.

Project Title: Factors Involving Secretion Rates and Utilization
Rates of Thyroid Hormones Using Iodine-131 as
a Tracer

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Technical Progress Report

During the past 4 years of work on the Atomic Energy Commission Contract No. AT (11-1)COO-1758, a total of 8 journal articles and 13 abstracts of oral presentations at scientific meetings have been published. An additional 8 journal articles have been sent to journals and 11 journal articles are being prepared. Four graduate students completed the Ph.D. during the tenure of this Contract, while 3 other graduate students have been trained in part during this Contract's execution and will complete the requirements for the Ph.D. within 6 months to 18 months of this Contract's expiration.

The graduate students trained, their thesis titles, dates graduated and present positions are as follows:

Dr. Kapilldea N. Sinha, Ph.D., Thyroid activity of the rat and fowl, June, 1968, research associate at University of Cincinnati.

Dr. Thomas R. Bauman, Ph.D., Comparative thyroid secretion rates and thyroxine metabolism in mammals, June, 1969, assistant professor of biology at the University of Alabama, Tuscaloosa, Alabama.

Dr. Ved Parkash, Ph.D., Oxytocic activity in the posterior pituitary glands of experimental animals and blood of dairy cattle, October, 1970, fellow in laboratory animal medicine, Johns Hopkins University, Baltimore, Maryland.

Dr. Samarendra N. Baksi, Ph.D., Thyrocalcitonin relationships with thyroid hormone secretion rate and lactation in rats, June, 1971, fellow at Worchester Foundation for Experimental Biology, Shrewsbury, Massachusetts.

Mr. George K. Littleton, M.S., A procedure for measuring the disappearance and concentration of 17 β -estradiol in plasma and red cells of rat blood, June, 1969.

Those now in training toward the Ph.D. degree include:

Mr. Ming-Hsiung Lu, Thyroxine secretion rates and induced hyperthyroidism during pregnancy and reproduction in the rat, expected graduation date June, 1972.

Mr. George K. Littleton, Blood levels of thyrotropin in dairy cattle measured by radioimmunoassay, expected graduation date August, 1972.

Mr. F. Patrick Wynne, Thyroid gland function in obese rats and mice, expected graduation date January, 1973.

Full length journal articles which have been published as a direct result of Contract AT (11-1)-1758 include the following:

1. Dryden, G. L., T. R. Bauman, C. H. Conaway and R. R. Anderson. 1969. Thyroid secretion rate and biological half-life ($t_{1/2}$) of L-thyroxine- ^{131}I in the musk shrew (*Suncus murinus*). Gen. and Comp. Endocrinol. 12:536-540. (AT (11-1)COO-1758-5).

A mean thyroid hormone secretion rate (TSR) of 1.61 ug/100g body wt/day was determined for 26 Asian musk shrews (*Suncus murinus*) born and raised in captivity. Young females had statistically higher TSR's (1.90 ± 0.17 ug) than old males but the biological significance of this is unknown. The mean TSR of the entire group is not excessive for mammals in this weight class (mean 32.2g) and is actually less than that of the Missouri Valley mole.

The mean $t_{1/2}$ of L- T_4 in young males and females (15.19 ± 0.98 hr) is significantly shorter than in old males (20.38 ± 0.46 hr) when Tapazole is administered to prevent ^{131}I recycling. An overall mean $t_{1/2}$ of 18.08 ± 0.28 hr for 17 shrews does not indicate inordinate thyroid activity when compared with the $t_{1/2}$ of laboratory rodents.

These data indicate that the thyroid activity, and presumably, the overall metabolic rate of this tropically distributed insectivore species does not justify placing *Suncus murinus* in a metabolically peculiar category.

2. Bauman, T. R., R. R. Anderson and C. W. Turner. 1969. The effects of induced, milk hyperthyroidism on serum PBI, thyroxine distribution volume and biological half-life of thyroxine- ^{131}I in dairy cattle. J. Dairy Science, 52:245-249. (AT(11-1)COO-1758-7).

Lactating and dry dairy cattle of several breeds were injected with exogenous L-thyroxine at levels 25 and 50% in excess of the normal thyroid hormone secretion rate. The biological half-life of L-thyroxine ^{131}I became shorter as the degree of induced hyperthyroidism increased. Serum protein-bound iodine levels increased markedly above control values as the level of injected L-thyroxine increased. Thyroxine distribution volume showed an apparent increase over control values at 125 and 150% of normal thyroid secretion rate, but values

at the 150% level declined below those at the 125% level. Thyroid secretion rates of control animals as determined by replacement technique and isotope dilution technique were in fair agreement with each other. The calculated utilization rates of L-thyroxine at the 125 and 150% of normal thyroid secretion rate level were two to three times higher than the known amounts of L-thyroxine injected daily. Excess thyroxine, up to 50% above normal secretion rate, seemed to have been eliminated from the body by rapid removal from the blood after the thyroxine-binding proteins were saturated.

3. Anderson, R. R. 1969. Thyroid secretion rate and lactation. Proc. Fifth Midwest Conf. on the Thyroid, Columbia, Mo., pp. 1-16. (AT(11-1)COO-1758-10).

The TSR was compared in the nonlactating and lactating states in laboratory rats. An increase in TSR of 100% was observed in lactating rats. However, the low correlation coefficient of 0.2 between TSR and milk production served to indicate that the six other hormones known to influence milk synthesis or removal must also play a significant role in controlling the level of milk production. The correlation coefficient of 0.29 between TSR and milk production obtained from 32 observations in Holstein and Jersey cows served to reinforce this concept.

The increase in TSR of 54% by the lactating cow would seem too low to satisfy the increased demand for thyroid hormones during lactation, but it was suggested that changes in metabolism of thyroid hormones by the liver might account for the seemingly small increase in thyroid secretion rate in the lactating cow.

4. Singh, D. V. and C. W. Turner. 1969. Effect of graded levels of parathyroid extract and calciferol upon thyroid hormone secretion rate (TSR) in normal female rats. Proc. Soc. Exptl. Biol. Med. 132:142-145. (AT(11-1)COO-1758-16).

The effect of graded doses of parathyroid extract and calciferol upon thyroxine secretion rate (TSR) of normal female rats was studied with the aid of radioactive iodine ^{131}I . Administration of 20, 30, and 40 USP units of PTE/100 g of body wt/day for 14 days induced a significant ($P < .025$) increase in TSR of 25.4, 35.6, and 54.1%, respectively, over their own controls associated with slight increase in body weight. Administration of calciferol (Vit. D₂) in doses of 0.1, 0.15, and 0.2 mg/100 g of body wt induced a nonsignificant reduction in TSR of the first two groups (groups IV and V) but a significant 26.7% ($P < .05$) increase in TSR above their own control in group

VI associated with loss of body weight of 14.2% when 0.2 mg calciferol/100 g of body wt was given daily for the same period. A nonsignificant increase in weight of ovaries, uteri, and a decrease of thymus glands with significant increase in thyroid and adrenal weights was noticed in the group treated with 0.2 mg of calciferol/100 g of body wt as compared to 0.1 mg of calciferol/100 g of body wt.

5. Sinha, K. N., R. R. Anderson and C. W. Turner. 1970. Low dietary iodine intake and the functional activity of the rat thyroid. *Proc. Soc. Exptl. Biol. Med.* 134: 571-576. (AT(11-1)COO-1758-17).

The effects of low dietary iodine intake on the functional activity of the thyroid were studied in young female albino rats. The low iodine diet contained 0.05 ppm iodine, with casein as the primary protein source. Feeding this diet to 30-day-old rats for periods of up to 3 months increased the thyroid hormone secretion rate (TSR). The PBI was within the normal range but thyroid size was slightly increased as compared to the corresponding control rats of the same age fed Purina Lab Chow. On the low iodine diet, the percentage uptake of thyroidal- ^{131}I was greatly increased, and the biological half-life of thyroidal- ^{131}I was significantly reduced, indicating a faster turnover of thyroidal-iodine (hormones). The DNA content of the thyroids indicated hyperplasia and hypertrophy suggesting stimulation of the gland. It was concluded that under these experimental conditions the rat thyroid has the capacity to increase its activity to compensate for any moderate low dietary iodine for at least 3 months by augmenting recycling mechanisms and thyroidal turnover of hormones.

6. Bauman, T. R. and R. R. Anderson. 1970. Thyroid activity of the ground squirrel (*Citellus tridecemlineatus*) using a cannula technique. *Gen. and Comp. Endocrinol.* 15:369-373. (AT(11-1)COO-1758-19).

Chronically implanted aortic and jugular cannulae were used to study thyroid status in the ground squirrel by a body pool turnover technique. Thyroid secretion rates (TSR) increased after cold exposure, and then began to decline with time in the cold until hibernation. Protein-bound iodine values were unchanged from controls through cold exposure and into hibernation. Binding of L-T_3 - ^{125}I by serum thyroxine-binding proteins increased after cold exposure and was twice the control values during hibernation. It is believed that alterations in thyroxine metabolism begin before hibernation is entered, hence reduction of body temperature is not the only factor involved in reduced thyroid activity.

7. Turner, C. W. 1969. Method of estimating thyroid hormone secretion rate of rats and factors affecting it. Mo. Agri. Expt. Sta. Research Bul. No. 969. (AT(11-1)COO-1758-28).

With the aid of radioactive iodine (I^{131}), a method has been developed in this laboratory for the estimation of the thyroid hormone secretion rate (TSR). Using this method, an extensive study was initiated to determine the factors influencing the TSR of the rat.

Starting in the 1950's, the results of these studies have been published in various endocrine journals. The object of the present publication is to summarize these observations and point the way for future research.

Of the physiological factors influencing TSR, it was noted that sex had little or no effect. However, there was observed a significant decline in TSR with age from birth to maturity. While there is believed to be a further decline in old age, further research is required. There is no clear evidence for a change in TSR during pregnancy. There was observed, however, a marked increase during lactation, which returns to normal during involution.

It has been suggested for a long time that the functional activity of the thyroid gland is altered by changes in environmental temperature. In this study it was shown that low temperature stimulates a marked increase in TSR, which is maintained or increased further by continued cold. On the other hand, the change from cold to a warm or hot environment quickly reduces the TSR.

A reduction in food intake has been shown to cause a reduction in TSR which is increased as the restriction in food intake is increased to 50 percent. That this is caused primarily by a reduction in calories rather than in protein was shown by the feeding of a protein-free diet and graded levels of protein in the diet. While a protein-free diet reduced TSR about 28 percent, 5, 10, and 15 percent protein reduced the TSR only about 10 percent.

To our surprise, it was observed in the rat that a ration very low in iodine was without effect upon the TSR. The study indicated that the rat has mechanisms by which the iodine is conserved. There is great efficiency in the uptake of iodine by the thyroid gland and the same system recovers and conserves the iodine produced in the metabolism of the hormone. There is evidence that under these conditions, more $L-T_3$ is secreted, which has over twice the biological activity of $L-T_4$.

Increased vitamin A in the diet had no effect on TSR. On the other hand, it was observed that calciferol (vitamin D_2) at a level of 0.2 mg/100 g body weight increased TSR 26.7 percent.

Nitrate at a level of 2.5 percent KNO_3 caused a reduction in TSR but this could be corrected when the iodine content of the diet was increased.

The influence of other hormones on TSR has been studied by the administration of these hormones to normal animals, by the removal of the endocrine gland and by replacement therapy of the various hormones. These studies indicate that the sex hormones (estrogen and testosterone), insulin, and parathyroid hormones increase the TSR of normal animals. Melatonin, from the pineal gland, however, depressed TSR.

When the ovary, testis, adrenal glands, and pancreas (destruction of the B-cells) were removed, the TSR was depressed. The pineal gland removal, however, stimulated an increase. By replacement therapy, the TSR was returned to normal or above.

These observations clearly indicate that the TSR is influenced by the level of secretion of other hormones. If the secretion rate of a hormone is low, the TSR of the animal would be low. If optimal levels of several hormones are being secreted, this should tend to increase the TSR. If this is true, then rats with higher normal TSR might also have optimal secretions of the other hormones. This could be demonstrated by the comparison of the secretion rates of various hormones in groups of rats which differ markedly in TSR.

The question of the effect of a high TSR or by maintaining a high level of L-T_4 in the blood by replacement therapy upon the secretion rate of other endocrine glands should be explored.

The method of estimating the TSR of animals is also useful in determining the biological potency of analogues of L-T_4 . By this method, D-T_4 has been observed to be only 1/10 as active as L-T_4 . Using L-T_3 , it has been observed to be more than two times as active as L-T_4 .

Comparisons of the subcutaneous and oral administration of thyroidal materials may also be made. In the rat, L-T_4 , L-T_3 and thyroprotein (containing 1 percent L-T_4) were found to be about 37 percent as effective orally as by injection. In female fowls, the oral effectiveness has been shown to be about 55 percent, whereas in cattle the oral effectiveness is only about 10 percent.

The marked variation in the oral effectiveness of thyroidal substances in mammals and birds requires further study.

The use of a method for estimating the TSR of the rat has contributed greatly to our understanding of the functional

activity of the thyroid gland. However, much further research must be conducted to determine the mode of inheritance of TSR and the development of strains of animals with optimal secretion of L-T₃ and L-T₄.

8. Anderson, R. R. 1971. Secretion rates of thyroxine and triiodothyronine in dairy cattle. J. Dairy Science. 54: 1195-1199. (AT(11-1)COO-1758-29).

Thyroid hormone secretion rates (TSR) were determined in 7 lactating dairy cows by the substitution method (suppression of thyroidal ¹³¹I release) and averaged .21 mg L-thyroxine (L-T₄) equivalents per 45.4 kg body weight per day. In the same animals, TSR for thyroxine (T₄SR) and for triiodothyronine (T₃SR) were determined separately by the radioiodine labelled hormone pool turnover method. Pool sizes in the 7 cows were 36.9 liters (8.27% of body size) for L-T₄ and 107.1 liters (22.1% of body size) for L-triiodothyronine (L-T₃) when no goitrogen was given but were increased to 56.0 liters (12.2% of body size) for L-T₄ and 201.0 liters (41.5% of body size) for L-T₃ when the goitrogen methimazole (tapazole) was given orally at 4 g per 454 kg body weight per day (P < .05). Turnover rates as half-lives in days were 1.29 and .79 for L-T₄ and L-T₃ in the absence of goitrogen and 1.59 and 1.02 with goitrogen. Concentration of L-T₄ in the plasma pool was 7.49 ug per 100 ml without the goitrogen and 6.18 ug per 100 ml with it while the concentration of L-T₃ in the plasma pool, measured in the absence of goitrogen treatment only, was .149 ug per 100 ml. Mean T₄SR in the 7 cows was .143 mg per 45.4 kg body weight per day without a goitrogen and .148 mg in the presence of the goitrogen; T₃SR was .0139 mg without methimazole and .0193 with it. If L-T₃ is 2.1 times as effective as L-T₄ in suppressing ¹³¹I release by the thyroid, the combined TSR of L-T₃ and L-T₄ without a goitrogen would be .172 mg of L-T₄ equivalents per 45.4 kg body weight per day and .189 mg with the goitrogen. On this basis the substitution method overestimates TSR in cattle by 12 to 22%. Correlation coefficients for TSR with milk production were positive but low and nonsignificant, varying from .64 to zero and being .30 in 39 comparisons.

In addition, a manuscript was recently published based on work supported by the AEC Contract No. AT(11-1)301-Turner.

1. Singh, D. V., R. R. Anderson and C. W. Turner. 1971. Effect of decreased dietary protein on the rate of thyroid hormone secretion and food consumption in rats. J. Endocrinol. 50:445-450. (AT(11-1)COO-148).

One hundred and twenty albino female rats (Sprague-Dawley-Rolfsmeier) were divided into five equal groups. Rates of thyroxine secretion (TSR) and food consumption

were determined during the control period, and 10 and 25 days after initiation of dietary treatment. Animals in each group served as their own controls for the following modifications of their diet: (1) protein-free diet, (2) 5% protein (casein) diet, (3) 10% protein diet, (4) 15% protein diet, and (5) 20% protein diet. Purina lab chow (23.4% protein) and the 20% casein diet served as control diets. The TSR, the body weight and amount of food consumed were depressed significantly in the group fed on a protein-free diet for 10 and 25 days. The group fed 5% protein diet had a non-significant decrease in TSR as compared with the controls. Similarly, TSR was not reduced by 10, 15 or 20% protein diets. Food consumption decreased significantly in the groups fed a 5, 10 and 15% protein diet, but not in the group on 20% casein. Body weight decreased significantly in the groups on a protein-free diet and on a 5% protein diet.

It would appear from these results that protein content of the diet does not become a limiting factor for TSR until it is lower than 5%. It is suggested that the calorie intake plays a more important role in regard to TSR than a low protein content of the food.

Abstracts of papers presented at annual meetings include the following:

1. Bauman, T. R., R. R. Anderson and C. W. Turner. 1968. Biological half-life ($t_{1/2}$) of thyroxine in dairy cattle during increasing degrees of induced hyperthyroidism. Proc. Fourth Midwest Conf. on the Thyroid, Columbia, Mo., p. 70 (abstract). (AT(11-1)COO-1758-1).

The biological half-life ($t_{1/2}$) of thyroxine ^{131}I was determined on 19 "lactating" dairy cattle and 6 "dry" dairy cattle under normal conditions and during administration of exogenous thyroxine (T_4) at levels 25 and 50% above their estimated thyroxine secretion rate (TSR). The mean $t_{1/2}$ of T_4 in the "lactating" cows has been observed to be 2.39 days, with a turnover rate of 29.0% per day (Pipes, *et al.*, Dairy Sci. 42:1606, 1959; Premachandra *et al.*, Proc. Soc. Expt. Biol. & Med. 106:818, 1961). After daily administration of T_4 for 7 weeks at 25% above normal TSR the $t_{1/2}$ T_4 was reduced to 1.41 days with a turnover rate of 51.4% per day. When the daily dose of administered T_4 was raised to 50% above TSR for 14 weeks, the mean $t_{1/2}$ was reduced to 1.16 days, with a turnover rate of 61.8% per day. The mean $t_{1/2}$ of the "dry" cows was observed to be 2.67 days, with a turnover rate of 26.0% per day. After daily administration of T_4 for 4 weeks at 25% above TSR, the $t_{1/2}$ was reduced to 2.11 days, with a turnover rate of 32.8% per day. The mean $t_{1/2}$ after 4 weeks of daily administration of T_4 at 50% above TSR, was reduced to 1.65 days with a turnover rate of 42.0% per day. Data on the changes in thyroxine pool and serum PBI will be presented in an attempt to explain the decreased $t_{1/2}$ of T_4 .

2. Anderson, R. R. and T. R. Bauman. 1968. Thyroid hormone secretion rates of cows in the dry period and during lactation. J. Dairy Science. 51:955. (abstract) (AT(11-1)COO-1758-2).

Seven Jersey cows were used to measure thyroid hormone secretion rates (TSR) by blocking release of thyroidal ^{131}I with increasing increments of 110 ug L-thyroxine/100 kg body wt/day for 2 days. Dry cows (6 pregnant) were tested for TSR during May or September and the mean TSR was 267 ± 33 ug L-thyroxine/100 kg body wt/day. The cows averaged 386 kg body wt. They were again tested in October or January when lactating for periods of 4 to 13 weeks. Peak milk production varied from 9 to 20 kg/day on 2X milking of 8- and 16-hr intervals. Mean TSR was 410 ± 66 ug L-thyroxine/100 kg body wt/day during lactation. Mean body wt was 404 kg. This increase of 53% was not significant ($P > .05$). It compares to a 50 to 100% increase in TSR of lactating versus normal rats.

3. Anderson, R. R., S. N. Bakshi and M. H. Lu. 1968. DNA and potassium (K) in udders of dairy cows. J. Animal Science 27:1187. (abstract) (AT(11-1)COO-1758-3).

Udders were removed from six lactating and five dry cows immediately after slaughter. The mammary glands were weighed, halved, trimmed of excessive adipose tissue, and K^{40} was counted in a low level radiation counter. The glands were then sampled for DNA determinations. The tissue was weighed and extracted with lipid solvents. The dried fat-free tissue of the six lactating glands averaged 13.0% and of five dry cows 18.3% of wet weight. The DNA ranged from 20.6 to 52.4 gm. with a mean of 34.5 gm. in the lactating glands and from 4.7 to 15.2 gm. with a mean of 10.3 gm in the dry cows. In the six lactators, the correlation coefficients were 0.90 between DNA and wet weight, 0.98 between DNA and dry weight, 0.94 between DNA and K, 0.97 between K and wet weight, and 0.96 between DNA and milk production which ranged from 2.7 to 5.9 kg. per day prior to slaughter. In the five dry cows, the correlation coefficients were 0.94 between DNA and wet weight, 0.92 between DNA and dry weight, 0.65 between DNA and K, and 0.82 between K and wet weight. Because of the small size of the udders from dry cows the K contents could not be determined very accurately. The DNA of wet mammary tissue was 0.46% and The K was 0.20% in the eleven cows.

4. Bakshi, S. N. and R. R. Anderson. 1969. Thyroid secretion rates and thyrocalcitonin contents of thyroids in mature female rats. Proc. Fifth Midwest Conf. on the Thyroid, Columbia, Mo., p. 90 (abstract). (AT(11-1)COO-1758-11).

Thyrocalcitonin (TCT), a hypocalcemic hormone secreted by the parafollicular cells of the mammalian thyroid, plays

an important role in calcium homeostasis. Thyroxine, the hormone secreted by the follicular cells, has an important role in regulating metabolic rate. A study was undertaken to determine any possible relationship between these two hormones secreted by the thyroid gland. Thyroid hormone secretion rate (TSR) was estimated by thyroxine substitution in eleven mature female rats using I^{131} and tapazole. The rats were given sufficient time to recover their normal thyroid gland function. TCT was estimated in thyroid homogenates of individual rats by bioassay in young male Holtzman rats. Parallel line techniques were employed using MRC standard 'B' to establish TCT potency in the glands. Average TCT in the thyroid was found to be 466 mU/gland (range 248 to 1047 mU). The mean TSR was 1.29 ug thyroxine/100 g body wt (range 0.5 to 2.0 ug). It was found that TSR and TCT content have an inverse relationship; the regression (b) was -207.3 and the correlation coefficient (r) was -0.38 (not significant).

5. Lu, M. H., M. H. Mayan and R. R. Anderson. 1969. Induced hyperthyroidism on reproduction in the rat. Proc. Fifth Midwest Conf. on the Thyroid, Columbia, Mo., p. 89 (abstract). AT (11-1)COO-1758-12).

Groups of female Sprague-Dawley-Rolfsmeyer rats were treated as follows: normal, thyroidectomized, 3 ug thyroxine ($L-T_4$)/100 g body wt/day, 6 ug $L-T_4$, 12 ug $L-T_4$, 24 ug $L-T_4$, 48 ug $L-T_4$, and 96 ug $L-T_4$. Animals were smeared daily for estrous cycling and sperm detection. The normal control group had 6 of 6 pregnant, 6 of 6 to parturition, and 5 of 6 to twenty days lactation, while other groups in the above order had 0, 0 and 0; 5, 4 and 2; 4, 4 and 3; 5, 5 and 2; 3, 3 and 0; 6, 5 and 0; and 5, 5 and 0. All levels of $L-T_4$, which are from 3 to 96 times the normal TSR in the rat, allowed estrous cycling and pregnancy maintenance. Levels from 3 to 12 ug $L-T_4$ permitted lactation to proceed. However, all levels above 12 ug $L-T_4$ /100 g bw/day suppressed lactation and observations indicated that the mothers lost their instinct to prepare a bed, to care for the young or to nurse the young. Mammary gland wet weights and dry weights were lower than controls in all experimental groups. Nucleic acid contents showed trends similar to dry weight changes. Since it is believed that a relative hypothyroidism occurs at parturition in normal animals, the results suggest that such a state parallels maternal behavior of the mother in caring for the young. This theory is supported by the evidence that maternal behavior is lost in the presence of an excessively hyperthyroid state.

6. Anderson, R. R. and T. R. Bauman. 1969. Blood and whole-body half-lives ($t_{1/2}$) of L-thyroxine ($L-T_4$) and L-triiodothyronine ($L-T_3$) in experimental animals. J. Dairy Science 52:913. (abstract). (AT(11-1)COO-1758-13).

After intravenous or intracardiac injection of tracer doses of either 1-thyroxine- ^{131}I or 1-triiodothyronine- ^{131}I , blood counts were made semidaily or daily to 72 hr and whole body counts daily to 12 days in mice, rats and rabbits. In all cases, whole body half-lives were greatly extended. 1-Thyroxine- ^{131}I and 1-triiodothyronine- ^{131}I half lives in blood were: mice, 11.3 and 9.9 hr; rats, 21.2 and 16.5; and rabbits, 18.5 and 17.0. Whole body half-lives after four days or eight days of counting 1-triiodothyronine- ^{131}I were: mice, 34 and 65 hr; rats, 21 and 50; and rabbits, 27 and 84. To determine the reason for deviation of whole body counts from a first-order regression, rats were sacrificed at two-day intervals after 1-thyroxine- ^{131}I injection. Radioactivity was eliminated at a decreasing rate with time in all organs and tissues measured, especially in the skin and hair.

7. Bauman, T. R. and R. R. Anderson. 1969. Thyroid activity in the thirteen-lined ground squirrel (*Citellus tridecemlineatus*) during hibernation and induced hypothermia. Trans. Mo. Acad. Science. 3:99-100 (abstract). (AT(11-1)COO-1758-14).

Parameters of thyroid activity, serum PBI, $t_{1/2}$ of thyroxine- ^{131}I , thyroxine secretion rate, T_3 uptake and free thyroxine index were used to compare thyroid hormone status at 25.5°C, and at 5.0°C during hibernation or cold exposure. A technique was developed for placement of an aortic cannula via the right carotid artery. The cannula, approximately 10 inches long, was made of PE-10 polyethylene tubing with a tip of equivalent size Silastic tubing. The cannula was chronically implanted in *Citellus* prior to hibernation or cold exposure. The $t_{1/2}$ of thyroxine- ^{131}I (T_4 - ^{131}I) in the control animal at 25.5°C, was about 28-30 hours. In the cold exposed animals, at 5.0°C, the $t_{1/2}$ of T_4 - ^{131}I was decreased to about 18-20 hours. In the hibernating animal, the $t_{1/2}$ of T_4 - ^{131}I was increased to about 65-70 hours.

8. Wynne, F. P. and R. R. Anderson. 1969. Thyroxine secretion rate in rabbits. Trans. Mo. Acad. Science 3:99 (abstract). (AT(11-1)COO-1758-15).

Thyroxine secretion rates (TSR) were determined in New Zealand whites by the substitution method. Five males and nine females were injected with 20 μC ^{131}I and allowed 48 hr for maximum thyroidal iodine uptake which was measured by external counting over the thyroid with a NaI crystal scintillation detector. Release rates were observed for 10 days after injecting Tapazole, a goitrogen, at 1 mg/100g b.w./24 hr to prevent iodine recycling. On day 10, in addition to Tapazole, the first of a series of graded doses of L-thyroxine (L- T_4) was initiated. Each dose was continued for two days and the animals counted on day 3, which was also

day 1 for the second graded dose. L-T₄ dosages were increased from 0.2 ug/100 g b.w. in increments of 0.2 ug until counts were obtained which equaled or were greater than 95% of counts made 48 hrs. previously. Mean pooled TSR for males and females was 0.68 ug L-T₄/100 g b.w./24 hr. (0.2-1.2 range) in animals weighing 870 to 1820 g. Male mean TSR was 0.74 ug L-T₄/100 g b.w./24 hr. (0.6-1.0 range) with a body weight range of 915 to 1500 g and mean female TSR was 0.64 ug/100 g b.w./24 hr. (0.4-1.2 range) with a weight range of 870 to 1820 g.

9. Anderson, R. R., J. Clark and T. R. Bauman. 1970. Potassium (⁴⁰K) measurements of dairy cattle. J. Animal Science 31: 158 (abstract). (AT(11-1)COO-1758-24).

Measurement of whole body potassium by the K⁴⁰ counting technique was carried out in a number of dairy animals of the Jersey breed to develop average values for growth curves. These values will then be used as a base for endocrine experiments relating to K changes in the body. Two Jersey bulls were counted once a month from 2 weeks of age to 29 months, when they were slaughtered and the carcasses counted. Total K in the whole body ranged from 75 g at 2 weeks of age to 1168 g at 29 months. The concentration of K ranged from 1.5 g to 2.0 g/kg body weight in the whole body. Parts of the body had the following K concentrations: gut, 0.96 g/kg; pluck, 2.15; head and hide, 1.09; rumen contents, 1.25; and remainder (muscle and skeleton), 2.36. Recovery of K in the parts was 1163 g in relation to 1168 g K in the whole animal count of one bull, while the parts totaled 1025 g K and the whole animal count was 1069 g K in the second bull. The difference was due to the loss of blood at the time of slaughter. Total K in females ranged from 28 g at 1 day of age to 975 g in a 4-year-old cow. The concentration of K varied from 1.3 to 2.4 g/kg. Mean K concentration in 132 observations from 15 females was 1.63 while in 103 observations from 8 males it was 1.76 g/kg (P < .01).

10. Friedlander, R. C. and R. R. Anderson. 1970. Clearance of ¹³¹I into milk of dairy cows. J. Dairy Science 53:663 (abstract). (AT(11-1)COO-1758-25).

Lactating Jersey and Guernsey cows were injected subcutaneously with 150 uCi of ¹³¹I, or L-thyroxine (T₄)-¹³¹I, or L-triiodothyronine-(T₃)-¹³¹I. Milk samples were taken 8 hours after injection for 12 consecutive milkings. Thyroid counts were also taken. The ¹³¹I disappeared exponentially from the milk in the six days. Three to 8% of the injected ¹³¹I was recovered in milk. The rate of passage of ¹³¹I was directly proportional to the milk yield. A delay was observed in the L-T₄-¹³¹I (60 hr) and L-T₃-¹³¹I (40 hr) experiments in the passage of ¹³¹I into the milk. The per cent dose of ¹³¹I uptake by the thyroid was 1.6 to 4.7% and 2.2 to 5.3%

with T_4 - ^{131}I and T_3 - ^{131}I , while the per cent uptake by the thyroid of ^{131}I alone was 12%. Seemingly the ^{131}I was separated from the T_4 and T_3 molecules and only the ^{131}I was trapped by the mammary gland.

11. Wynne, F. P. and R. R. Anderson. 1971. Survival, obesity, rate of weight gain, and L-thyroxine turnover in goldthio-glucose treated mice. Trans. Mo. Acad. Science 5: (abstract). (AT(11-1)COO-1758-31).

Thirteen and forty female Swiss Webster mice were given, respectively, single i.p. injections of isotonic saline (C) and gold thioglucose (GTG, 0.8 mg/g body weight). Individual body weights were recorded on day 3 post injection and weekly for weeks one through six, at which time all animals were injected with 4.2 to 5.0 uCi of L-thyroxine- ^{131}I via a lateral tail vein. Blood samples were collected from the opthalmic venous plexus at 12, 24.5, 36.7, and 50.7 hours post injection and the plasma counted in the Packard scintillation spectrometer. All control and 25 (62.5%) of ATG injected animals survived, deaths due to ATG toxicity occurring within three days. Control animals increased their body weight an average of $12.2 \pm 8.2\%$ and ATG-survivors (ATG-S) $43.5 \pm 20.9\%$. Obesity (ATG-O), defined as a weight gain exceeding final control mean percent body weight increase plus three standard deviations ie. 36.72% (herrero, 1969, AJP 17, p. 403), occurred in twelve (48%) of the ATG-S group with a mean increase of $60.5 \pm 17.3\%$. C-animals increased their body weight at an average rate of 1.9%/week ($r = .97$) and ATG-O animals 11.4%/week ($r = .99$). Mean thyroxine biological half lives ($t_{1/2}$) and slopes (b) were as follows: C; $t_{1/2} = 15.20 \pm 2.07 \text{ hrs}^2$ ($r = .98$), $b = .0464 \pm .0065\%$ dose/hr. ATG-O; $t_{1/2} = 14.63 \pm 3.34$ ($r = .98$), $b = .0494 \pm .0098\%$ dose/hr.

12. Wynne, F. P. and R. R. Anderson. 1971. A simplified technique for quantitating plasma and plasma radioactivity in radiothyroxine turnover studies. Proc. Seventh Midwest Conf. on Thyroid and Endocrinology, Columbia, Mo. (AT(11-1)1758-34).

Plasma half-times ($t_{1/2}$), fractional turnover rates (k) and time-zero per cent-dose values (a) of injected (i.v.) L-thyroxine- ^{131}I were determined for 5 female Sprague-Dawley-Rolfsmeier rats with 0.1 ml whole plasma samples and the dissolved washed trichloroacetic acid precipitates (TCAP) of the same samples. These same parameters were compared to values determined from whole-plasma samples obtained from blood collected concurrently in microhematocrit pipets. Plasma volumes were quantitated by calculations based on packed-cell length (PCL) and mean pipet volumes (75.08 ul)

and by calculations based on plasma column lengths (PL) and a pipet length/volume ratio (1.0 mm = 1.0725 ul). Mean volume and mm-ul values were determined by gravimetry on a representative sample of microhematocrit pipets. No statistically significant differences (AOV, $P > .05$) were observed among $t_{1/2}$, k , or a values.

Whole Plasma	% Activity	$t_{1/2}$, hrs.	k	a
0.1 ml	100	12.67 $\pm$.49	.0550 $\pm$.0022	4.26 $\pm$.31
PCL	100	13.15 $\pm$.44	.0530 $\pm$.0019	4.25 $\pm$.42
PL	100	12.86 $\pm$.37	.0540 $\pm$.0016	4.47 $\pm$.43
TCAP	93 $\pm$.87	12.61 $\pm$.44	.0552 $\pm$.0019	4.09 $\pm$.34

13. Wynne, F. P. and R. R. Anderson. 1971. Selection of control animals for studies on the effect of goldthioglucose-obesity on thyroid function. Proc. Seventh Midwest Conf. on Thyroid and Endocrinology, Columbia, Missouri. (AT(11-1) COO-1758-35).

Goldthioglucose (GTG) is frequently employed to induce obesity in mice and the various observed parameters related to saline injected control values. Preliminary to observing the relationship between GTG-obesity and thyroid function, it was desirable to justify this convention by determining the extra-CNS effects due to gold per se. Ten CBA female mice were injected i.p. with 0.1 ml per 10 grams body weight of an 8% aqueous solution of goldthiomalate (GTM), a compound that does not induce obesity, so that each animal received 0.8 mg of GTM per gram of body weight. Ten animals similarly injected with normal saline served as controls. Six weeks later all animals were injected i.v. with 5.0 uCi of L-thyroxine- ^{131}I per animal. Whole plasma samples obtained from blood collected 12, 24, 36 and 48 hours post injection were used to calculate plasma radiothyroxine half-time ($t_{1/2}$) and clearance (C), the product of the volume of distribution and fractional turnover rate. All animals were sacrificed and body, spleen, liver and kidney weights obtained. No statistically significant differences ($P > .05$) among variables were observed by unpaired variate "t" tests.

	<u>Body Weight, g</u>	
	Initial	Final
Saline	22.8 $\pm$.43	26.5 $\pm$.68
GTM	22.5 $\pm$.36	27.6 $\pm$.38

Organ Weights, g

	Spleen	Kidneys	Liver
Saline	.167 $\pm$.01	.344 $\pm$.01	1.136 $\pm$.03
GTM	.176 $\pm$.01	.342 $\pm$.01	1.267 $\pm$.04

Plasma T₄ Values

	t _{1/2} , hrs.	C, ml/hr
Saline	12.19 $\pm$.41	0.20 $\pm$.02
GTM	10.85 $\pm$.74	0.18 $\pm$.01

All values mean $\pm$ SEM

Journal articles which have been prepared and submitted to various journals for consideration for publication include the following:

1. Sinha, K. N., R. R. Anderson and C. W. Turner. Relation of thyroid activity to wild and tame behavior in cockerels. Submitted to Proc. Soc. Exptl. Biol. Med. (AT(11-1)COO-1758-4).

The TSR was estimated in different lines of "wild" and "tame" cockerels which were grouped on the basis of their temperament. These birds were crossbred for broiler production and their mean age ranged from 32 to 53 weeks. The mean TSR in different groups ranged from 0.33 to 0.50 ug L-T₄/100 g bw/day. No significant difference was observed in TSR between a "wild" and the corresponding "tame" controls. In some groups a significant difference was observed in 48 hour thyroidal-¹³¹I uptakes between a "wild" or "intermediate" group and their respective "tame" controls, but the overall uptake values were low. It was observed that nervous temperament of the cockerels was not related to hyperthyroidism.

2. Sinha, K. N., R. R. Anderson and C. W. Turner. Relation of thyroid hormone secretion rate (TSR) in rats to hormone content of their thyroid glands. Submitted to Proc. Soc. Exptl. Biol. Med. (AT(11-1)COO-1758-6).

By the determination of the thyroid hormone secretion rate (TSR), the amount of hormone secreted each 24 hours has been estimated. An attempt has been made in this study to determine the relation of the daily TSR to the amount of stored hormone (thyroglobulin) in the thyroid of the rat in terms of L-thyroxine (L-T₄) equivalents. Graded amounts of

thyroid homogenate from donor rats of known TSR were injected intraperitoneally to the recipient rats with known TSR to block the secretion of the thyroid stimulating hormone (TSH) as used in the TSR technique. The intraperitoneal route of administration of the homogenate was based on the kinetic studies of radioactive L-T₄ and thyroglobulin to match the rate of absorption and disappearance. The biological assay of the homogenate indicated that the hormones stored in the thyroids of the donor rats in terms of L-T₄ equivalent contained a mean of 3.87 times the daily TSR with a range from 2.09 to 6.22 days. The biological half-life of thyroidal-I¹³¹ with tapazole treatment was also studied in the rat to supplement the observation.

3. Singh, D. V. and C. W. Turner. Effect of melatonin on thyroid hormone secretion rate (TSR) in female hamsters. Submitted to J. Endocrinol. (AT(11-1)COO-1758-21).

Thyroid hormone secretion rate (TSR) of 43 female hamsters (25 days of age) was determined by the substitution method as 0.75 ug L-thyroxine equivalent/100 g body wt/day. At 55 days of age the animals were divided into 3 groups: a control group of 15 receiving physiological saline, a group of 13 receiving 50 ug melatonin/100 g body wt/day for 7 days prior to and during TSR determination, and a group of 15 receiving 100 ug melatonin/100 g body wt/day for 7 days prior to and during TSR determination. TSR in the control saline group was 0.60 ug, in the group on 50 ug melatonin 0.48 ug, and in the group on 100 ug melatonin 0.48 ug L-thyroxine equivalent/100 g body wt/day. The reduction of 20% in TSR due to melatonin treatment was significant ($P < .05$). Food consumption was measured in individual hamsters for a 13-day period of the treatment. It was reduced by 8% in hamsters receiving 50 ug melatonin and by 16% ($P < .05$) in hamsters receiving 100 ug melatonin.

4. Singh, D. V. and C. W. Turner. Endocrine effects of melatonin in female rats at increasing ages. Submitted to Acta Endocrinologica (AT(11-1)COO-1758-22).

Eighty female Sprague-Dawley-Rolfsmeyer rats were divided equally into four groups at 25 days of age. One half of each group, serving as controls, received saline, the other half received 100 ug melatonin/100 gram body weight at 25, 35, 45, and 55 days of age, for 10 days. They were killed 24 hours after the last injection. Pituitaries, ovaries, uteri, adrenals, and thyroids were collected and weighed. DNA determination by the method described by Webb and Levy (20) was used for ovaries, uteri, and adrenals. In general, pituitaries, ovaries, and uteri weights decreased; thyroid weights increased at increasing age by melatonin treatments.

Ovarian total DNA and DNA/100 gram body weight increased significantly at 35 days of age, but the total DNA and DNA/100 gram body weight were significantly decreased at 45 and 55 days of age and a non-significant decrease at 65 days of age in melatonin-treated group as compared to the corresponding controls. There was no significant decrease in adrenals DNA/100 gram body weight at any age. DNA/100 gram body weight of uteri increased significantly at 35 days of age and decreased significantly at 55 and 65 days of age by 33% and 20%, respectively, as compared to control groups. These data also show that the DNA/mg of endocrine glands DFFT decreased with increasing age, suggesting a reduction in functional cells per unit of tissue in older animals.

5. Anderson, R. R. and T. R. Bauman. Thyroid hormone secretion and utilization rates in the guinea pig. Submitted to Proc. Soc. Exptl. Biol. Med. (AT(11-1)COO-1758-23).

Thyroid hormone secretion rates (TSR) of L-thyroxine ($L-T_4$) and L-triiodothyronine ($L-T_3$) were determined by the substitution method in albino guinea pigs of both sexes. Mean TSR of $L-T_4$ in 30 animals was 0.57 ± 0.02 ug $L-T_4$ equivalent/100 g body wt/day. No significant difference between sexes was found; TSR in 13 females was 0.55 ± 0.04 ug $L-T_4$ and in 17 males averaged 0.58 ± 0.02 ug $L-T_4$. Mean TSR of $L-T_3$ determined by the substitution method in 36 guinea pigs of both sexes was 0.18 ± 0.01 ug $L-T_3$ equivalent/100 g body wt/day with no significant difference between sexes. Thus, $L-T_3$ was 3.2 times as effective as $L-T_4$ in suppressing thyroid release of ^{131}I via suppression of thyrotropin secretion. TSR's were also determined for $L-T_4$ and $L-T_3$ using the thyroid hormone pool-turnover method. In 14 animals the TSR of $L-T_4$ was 0.39 ± 0.06 ug/100 g body wt/day. Parameters used to measure TSR by pool-turnover were concentration in pool, volume of distribution, and half-life in the plasma and were 3.23 ug%, 18.2% of body weight, and 26.3 hrs for $L-T_4$. For $L-T_3$, the volume of distribution was 36.1% and the half-life was 14.5 hr.

6. Bakshi, S. N. and R. R. Anderson. Thyroid secretion rate and thyrocalcitonin content of the thyroid in female rats. Submitted to J. Endocrinology (AT(11-1)COO-1758-30).

The thyroid hormone secretion rates (TSR) of forty Sprague-Dawley-Rolfsmeyer rats, average body weight 233 g (150-317 g), were estimated using radioactive ^{131}I and increasing increments of exogenous L-thyroxine ($L-T_4$). The mean daily TSR was found to be 1.10 ug $L-T_4$ /100 g bw (range 0.50-2.0 ug). The thyroidal content of thyrocalcitonin (TCT) of these rats was estimated by bioassay using young rats. The average TCT content was found to be 395 MRC mU/gland (range 248-772 mU). It was found that thyroidal TCT content and

TSR have an inverse correlationship ($r = -0.384$, $p < 0.05$ with 38 d.f.). It is concluded that higher TSR depletes thyroidal TCT.

7. Littleton, G. K. and R. R. Anderson. Characterization of 17B-estradiol- ^3H single-injection disappearance curves in rat plasma and red cells. Submitted to Proc. Soc. Exptl. Biol. Med. (AT(11-1)COO-1758-33).

17B-estradiol- ^3H was injected intravenously into female rats and disappearance rates from whole blood, plasma and red cells determined. Disappearance curves were resolved into three components from which three pools were described. The first pool (P_1), assumed to comprise the blood, had very rapid halflives in the blood, plasma and red cell compartments of 5.53, 6.48, and 4.56 minutes, respectively. The second pool (P_2) had halflives of 30.93, 30.55, and 34.68 minutes in the three respective compartments.

Two models are proposed to describe 17B-estradiol disappearance. Model I, representing a series of three pools, yielded estimated for P_2 of 16.27, 17.13 and 18.80 percent of body weight for the three compartments, respectively, while the estimates for the size of P_3 were 60.06, 41.18 and 53.22 percent. Estimates of pool sizes and turnover constants for Models I and II, which represents three pools not in series are presented.

It is concluded that estrogen utilization is best represented by a three pool system.

8. Wynne, F. P. and R. R. Anderson. A Rapid Method to Quantitate Plasma Volume and Radiothyroxine in Turnover Studies. Submitted to J. Applied Physiology. (AT(11-1)COO-1758-38).

Dimensional uniformity of commercially available micro-hematocrit capillary tubes was evaluated as indicated by volume determinations based on manufacturer's specifications and gravimetry to determine if plasma sample volume could be accurately quantitated by applying a mean volume/length factor (ul/mm) to plasma column lengths (PCL) obtained after centrifugation of blood samples. Plasma radiothyroxine turnover parameters (slope, y intercept) used to derive thyroxine secretion rates were determined and compared in rats utilizing whole plasma quantitated as PCL $\times$ ul/mm and utilizing whole and trichloroacetic acid (TCA) precipitated plasma samples quantitated by lambda pipet. No statistically significant difference ($P > 0.25$) for a given parameter was observed regardless of the method of sample preparation or quantitation. By collecting blood samples from the opthalmic venous plexus in microhematocrit tubes and quantifying both whole plasma sample volume and whole plasma radioactivity directly in the

tubes, as opposed to TCA precipitates, considerable conservation of laboratory resources can be achieved.

Manuscripts now being prepared for submission to scientific journals during the coming year include the following:

1. Lu, M. H. and R. R. Anderson. Effects of induced hyperthyroidism on reproduction in the rat.

Hyperthyroidism was induced by daily administration of thyroid hormones thyroxine (L-T₄) and triiodothyronine (L-T₃). In the study using L-T₄, 48 virgin female Sprague-Dawley-Rolfsmeyer strain rats were smeared daily to determine normal estrous cycling and sperm. Males were added to cages of females and treatments were begun after two normal estrous cycles. Rats were divided into 8 groups of 6 each as follows: normal, thyroidectomized, 3 ug L-T₄/100 g body wt/day, 6 ug L-T₄, 12 ug L-T₄, 24 ug L-T₄, 48 ug L-T₄, and 96 ug L-T₄. In all treatment groups from 3 to 96 ug L-T₄, the animals showed estrous cycles and some maintenance of pregnancy. The levels of L-T₄ represent challenges to the rat of 3 to 96 times its normal thyroid hormone secretion rate. Levels from 3 to 12 ug L-T₄ permitted lactation to proceed. All levels above 12 ug L-T₄ resulted in suppressed lactation. Observations indicated that the mothers lost their instinct to prepare a nest, to care for the young, and to allow the young to suckle. Mammary gland wet weights and fat-free dry weights were lower in all treatment groups than in the controls. When L-T₃ was used on the same strain of rats, 36 animals were allowed to have two normal estrous cycles before being separated into groups and treated. These were normal control, 1 ug L-T₃/100 g body wt/day, 2 ug, 4 ug, 8 ug, and 16 ug. Animals were housed with a male and smeared daily until smear was detected. All animals on 1, 2, or 4 ug L-T₃ had normal estrous cycles and normal pregnancies. All levels above 4 ug L-T₃ resulted in decreased numbers of pregnancies. Only the groups on 1 or 2 ug L-T₃ had parturitions which proceeded normally. Levels of L-T₃ at 4 ug and above resulted in dystocia. Only the group on 1 ug L-T₃ had normal lactation. The gestation length in all experimental groups was increased by one or two days over the normal 22 day gestation. Body weights were significantly less than the normal controls at 8 or 16 ug. Mammary gland wet weights were decreased in all treatment groups except those on 1 ug L-T₃.

2. Lu, M. H. and R. R. Anderson. Thyroxine secretion rates measured by the pool-turnover method at various stages of pregnancy in rats.

Thyroid hormone secretion rates (TSR) were studied at different stages of pregnancy in Sprague-Dawley-Rolfsmeyer rats. The day sperm were detected in the vaginal smear was designated day 1 of pregnancy.

TSR was determined in 7 groups as follows: virgin control and days 5, 10, 15, 20, 22 of pregnancy and immediately prior to parturition. Thyroxine was obtained as L-T₄-¹³¹I and injected into the heart. Blood samples were taken every 12 hr to 48 hr to measure PBI and rate of L-T₄ turnover and volume of pool of L-T₄ in the body. The product of these determined the secretion rate of L-T₄. The results are presented in the following table.

Table - T₄SR measured by pool-turnover at various stages of pregnancy in the rat

Group	n	Body Weight g	PBI ug %	b per day	t _{1/2} hr
Control	20	261	2.42	0.93	19.0
Day 5	22	278	2.11	1.05	18.1
Day 10	17	259	2.81	1.20	14.2
Day 15	12	259	1.66	0.93	21.4
Day 20	17	261	1.34	1.20	13.5
Day 22	10	386	1.83	1.38	14.1
Just before parturition	18	356	1.99	1.44	11.9

Group	Vol. of Dist. ml	Vol. of Dist. % of B.W.	T ₄ Pool ug.	T ₄ SR ug/100g B.W./day
Control	33.9	13.2	1.28	0.46
Day 5	44.7	16.0	1.43	0.51
Day 10	35.7	13.9	1.55	0.75
Day 15	32.6	12.7	0.83	0.28
Day 20	52.9	20.2	1.06	0.46
Day 22	50.0	13.2	1.43	0.49
Just before parturition	106.5	29.9	3.22	1.29

The data show that T_4 SR increased during early pregnancy, dropped at mid-pregnancy and returned to normal in late pregnancy, except just prior to parturition when the secretion rate increased markedly. PBI did not rise during pregnancy as it does in primates. The rate of utilization of $L-T_4$ (b) increased in the later part of pregnancy and especially immediately prepartum. There was also a sudden increase in the size of the body's $L-T_4$ pool immediately before parturition.

3. Anderson, R. R. Recovery of ^{131}I in milk after injection of radiolabelled L-thyroxine or L-triiodothyronine.

L-thyroxine- ^{131}I ($L-T_4$ - ^{131}I) and L-triiodothyronine- ^{131}I ($L-T_3$ - ^{131}I) were administered to lactating dairy cows subcutaneously or intravenously. A goitrogen, methimazole, was administered orally in trials to determine its affect upon removal of ^{131}I into milk after intravenous injection of the radiolabelled thyroid hormones. The percentage of ^{131}I recovered in milk after intravenous injection of $L-T_4$ - ^{131}I was similar whether or not methimazole was given, but the phase relationship for peak concentration of ^{131}I in the milk was delayed 24 hr as a result of methimazole. The percentage of ^{131}I recovered in milk following $L-T_3$ - ^{131}I intravenously was 5.5% over a 6-day period when no goitrogen was given and only 0.4% when methimazole was administered. Whereas peak concentration of ^{131}I in milk occurred 8 hrs after injection of $L-T_3$ - ^{131}I without the goitrogen, it was delayed to 24 hrs after injection when the goitrogen was given. The results suggest that $L-T_3$ is very rapidly utilized by actively secreting mammary epithelial cells and the iodide resulting from deiodination of $L-T_3$ is rapidly transferred to the milk. It appears that methimazole preferentially reduces the movement of $L-T_3$ into mammary gland secretory cells with the result that the iodide from deiodinated $L-T_3$ finds its way into milk at a much slower rate. Competition for iodide by the tyrosine residues in milk protein probably constitutes an important determinant of the amount of iodine in milk.

4. Anderson, R. R. Blood and whole body half-lives of radiolabeled thyroxine and triiodothyronine in experimental animals.

Data have been accumulated to date on whole body disappearance rates on rats, mice, rabbits and guinea pigs. These are then compared to the disappearance rates of $L-T_4$ and $L-T_3$ from the blood. We noted early in the studies that the disappearance rates of $L-T_4$ and $L-T_3$ from the whole body did not show first order kinetics as did the blood over the first 72 hours. As the time of measurement was extended the curves flattened. It was noted by dissecting rats at various times after injection of $L-T_4$ or $L-T_3$ that the ^{131}I accumulated in the skin and hair to the extent that the greatest percentage of ^{131}I left in the animal after 12 days was in the

skin. This lead to trials in mice which had a picture similar to but not as dramatic as that in rats. When we subjected rabbits to a trial for 6 days we found that the ^{131}I did not accumulate in the hair and skin but in the viscera. Guinea pigs were similar to rabbits but the accumulation in the viscera was not as pronounced. We found that short term sampling of blood and whole body for the first 48 hours after injection of L-T_4 gave fairly good agreement. The differences in disappearance rates from blood of L-T_4 and L-T_3 were great in guinea pigs but less marked in rats, rabbits and mice.

Half-lives (hrs) of L-T_4 and L-T_3 in blood

	<u>L-T_4</u>	<u>L-T_3</u>
Guinea Pig	26.3	14.5
Rat	21.2	16.5
Rabbit	18.5	17.0
Mouse	11.3	9.9

5. Anderson, R. R. Thyroid hormone secretion rates in rats following changes in dietary bulk and energy intake.

Thyroid hormone secretion rates (TSR) were determined by the substitution method in mature female rats of the Sprague-Dawley Rolfsmeyer strain after two weeks on (a) control diet (23.4% protein), (b) 50% control-50% cellulose flour diet ad libitum and (c) idem restricted to 15 g per day. The TSR of 24 rats was 1.30 ug L-thyroxine (L-T_4)/100 g bw/day during the period the rats were receiving the control diet. It rose to 1.44 ug L-T_4 after 2 weeks on diet (b) a nonsignificant increase of 11%, and decreased significantly ($P < .01$) to 0.62 ug L-T_4 on diet (c). Food consumption was 15.0 g/day of (a), 25.8 g/day on (b) and 13.3 g/day on (c). Fecal bulk produced per day averaged over a 3-week period was 6.9 g on diet (a), 20.8 g on diet (b), and 11.3 g on diet (c) ($P < .05$). The daily urine volume changed from 10.6 ml on diet (a) to 4.1 ml on diet (b) to 7.0 ml on diet (c) ($P < .05$). Energy intake appears to be more important in TSR control than is dietary bulk.

6. Anderson, R. R. Relation of thyroxine secretion rate to age and body weight in growing Jersey cattle.

The following table includes a compilation of data relating TSR to body weight in a total of 62 individual observations obtained to date.

N	of	Age group	Mean	Mean		T ₄ fractional	T ₄ Vol. of		T ₄ SR/day,
a.	als	mos.	age	B.W.	PBI	turnover	dist.	total	mg/100 kg
			days	kg	ug%	(per day)	l.		body weight
7		0-3	46	45.9	8.6	0.499	5.36	0.341	1.095
5		3-6	144	75.6	6.3	0.467	5.68	0.231	0.306
8		6-9	240	118.9	5.2	0.434	7.11	0.237	0.202
8		9-12	330	169.7	5.8	0.386	9.28	0.308	0.181
9		12-15	410	209.6	5.4	0.388	13.43	0.411	0.196
9		15-18	510	250.1	5.6	0.391	15.04	0.477	0.195
5		18-21	600	265.9	4.7	0.393	19.32	0.540	0.209
5		21-24	660	325.0	6.2	0.364	16.84	0.558	0.169
6		24-36	880	384.1	5.7	0.363	21.15	0.665	0.176

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An analysis of the data was made to determine the exponential rate of change of body weight in relation to changes in TSR. The formula $Y \text{ (TSR, mg)} = a X^b$, where X is the body weight in kg, was solved based on the above data. The regression (b) of the log X on log Y was 0.72, suggesting that TSR increases with the 0.72 power of X or $Y = X^{0.72}$. This is in excellent agreement with data compiled by Sam Brody indicating that body surface increases with body weight to the three-quarters power. Metabolic rate of animals varies with the body surface rather than the body weight, a concept which is completely supported by the TSR data obtained in this study of growing Jersey cattle.

7. Anderson, R. R. Thyroid hormone secretion rates by substitution and turnover methods in gerbils.

Thyroid hormone secretion rates (TSR) were obtained in 44 mature gerbils (*Meriones unguiculatus*) by substitution. The mean TSR at an ambient temperature of 25.5°C was 0.93 ug L-thyroxine equivalents per 100 g body wt per day. Of the 30 males and 14 females included in the study, no difference was found between sexes. In order for the data to be meaningful to us, we plan to determine TSR in a similar group of gerbils by the pool-turnover method. This entails the collection of sufficient blood to measure PBI prior to injection i.v. of a tracer dose of L-thyroxine-¹³¹I and sampling the blood at 12-hr intervals for 3 days to determine the disappearance rate of the ¹³¹I from blood. The data provide a valuable basis for interpretation of other experimental data acquired by either method.

8. Wynne, F. P. and R. R. Anderson. Thyroid hormone secretion rates in obese rats and mice.

Seventy-two CBA/Jax female mice were divided equally into two groups of 36 animals, one group for thyroid hormone secretion rates (TSR) by the substitution method and the other group for TSR by the L-thyroxine (L-T₄) pool-turnover method. Each of these groups was subdivided into two groups of 18, one group serving as the normal controls while the other group received 0.4 mg/g body wt goldthioglucose (GTG) intraperitoneally in a single injection. Sixteen days after injection of GTG, release rates of thyroidal iodine-131 and TSR by substitution were determined in one group of 36 and TSR by pool-turnover in the second group of 36. This period of TSR determinations represents the dynamic phase of weight gain in the obesity-induced mice. Using the same animals, TSR was determined again at 70 days after GTG injection. This represents the static phase of weight gain.

Results determined to date are summarized.

Dynamic phase - TSR by substitution

	Saline Control	GTG-obese	GTG-nonobese
Number	18	10	7
Initial mean body wt, g	25.9	25.7	26.1
Final mean body wt, g	25.0	29.0	26.7
Body wt gain or loss, g	- 0.5	3.3	0.7
Release rate, %/day	12.9	12.5	12.9
TSR, ug L-T ₄ /100 g body wt/day	1.03	1.20	1.28

Dynamic phase - TSR by pool-turnover

	Saline Control	GTG-obese	GTG-nonobese
Number	18	11	7
Initial body wt, g	26.4	26.4	25.8
Final body wt, g	25.9	30.1	26.9
Body wt gain or loss, g	-0.5	3.8	0.4
Turnover rate, %/hr	0.058	0.060	0.061
t _{1/2} , hr	12.3	12.3	11.9
Vol. of dist., ml	3.5	3.8	4.3
Vol. of dist., % of body wt.	13.7	12.5	15.7

Static phase - TSR by substitution

	Saline Control	GTG-obese	GTG-nonobese
Number	16	10	6
Initial mean body wt, g	25.8	25.7	26.1
Final mean body wt, g	29.2	36.8	30.6
Body wt gain or loss, g	3.4	11.1	4.5
Release rate, %/day	15.7	15.6	15.5
TSR, ug L-T ₄ /100 g body wt/day	1.16	1.40	1.33

Static phase - TSR by pool-turnover

	Saline Control	GTG-obese	GTG-nonobese
Number	16	12	4
Initial body wt, g	26.4	26.4	25.8
Final body wt, g	28.3	34.0	28.2
Body wt gain or loss, g	1.9	8.4	2.3
Turnover rate, %/hr	0.056	not completed	not completed
t _{1/2} , hr	12.6	not completed	not completed
Vol of dist., ml	4.6	not completed	not completed
Vol. of dist., % of body wt.	16.3	not completed	not completed

9. Littleton, G. K. and R. R. Anderson. Blood levels of thyrotropin in dairy cattle measured by radioimmunoassay.

A sensitive radioimmunoassay for bovine thyrotropin (TSH) has been developed capable of measuring as little as 1.0 ng/ml of plasma. Antibodies to bovine TSR were prepared in mature male guinea pigs and rabbits using NIH bovine TSH (NIH-TSH-B5) as antigen, injected in three successive doses of 2.5 mg each in Freund's adjuvant. Antisera from rabbits (ATSHR) bound a highly purified bovine TSH (Pierce, 20 U/mg) labeled with ¹²⁵I from NaI to a greater percentage than did antisera from guinea pigs (ATSHGP) at the same dilutions. Several antisera from rabbits bound 50% of the labeled antigen at a 1:20,000 dilution while only one sample of several different guinea pig antisera gave comparable results. A

standard curve was obtained utilizing the highly purified bovine TSH in concentrations of 0.25, 0.50, 1.0, 2.0, 3.12, 5.0, 6.25, 10.0, 12.5, 15.0, 25, 50, and 100 ng TSH per 0.1 ml of 0.01 M phosphate buffer, pH 7.5, containing 0.25% bovine serum albumin (BSA).

TSH was iodinated in phosphate buffer with 5 ug of hormone per mCi of ^{125}I as NaI, according to a modified version of Greenwood and others. The iodinated hormone was separated from the unreacted iodine on a Sephadex G-50 column and the peak diluted to 10 ml with 5% BSA. Before use in the assay the iodinated hormone was repurified on a Sephadex G-100 column and the hormone peak diluted in 0.25% BSA- PO_4 buffer so that 0.1 ml gave approximately 5000 cpm.

In the assay protocol, the mixtures in the tubes were incubated for 5 days at 4°C and duplicate samples of standard precipitated with Norit A charcoal suspended in 0.07 M barbital buffer, pH 8.6, with 2% BSA or precipitated with a second antibody produced in rabbits against guinea pig gamma globulin. The results comparing charcoal and second antibody were reproducible in low concentrations of standard TSH, but at concentrations above 1.0 ng charcoal gave consistently lower percentage binding than did the second antibody.

10. Bakshi, S. N. and R. R. Anderson. Effect of graded levels of thyrocalcitonin upon thyroid secretion rate, endocrine glands, liver, kidney and spleen weights of female rats.

Thyrocalcitonin (TCT) injections at three different dose levels of 30, 60, and 90 MRC mU per 100 g body wt twice daily in groups of ten growing female rats for at least 14 days significantly depressed ($P < 0.01$) thyroid secretion rate (TSR) in 30 and 90 mU groups, but the decreased in TSR in 60 mU groups was not significant when compared to normal control and vehicle treated groups. There was a significant increase in body wt ($P < 0.01$) only in the groups treated with 90 mU TCT. Liver and adrenal weights were significantly increased ($P < 0.05$) in all three treated groups but increases in kidney weight were significant only in 60 and 90 mU groups. The chronic effect of TCT injection at these dose levels for 14 days had no significant effect on thyroid, pituitary, ovary, uterus and spleen weight. This study indicates that chronic injection of TCT has a depressing effect upon the animal's TSR. The increase in liver, kidney and adrenals weights are possibly due to nonspecific stress produced by chronic infection of the hormones.

11. Bakshi, S. N. and R. R. Anderson. Effect of graded levels of acetazolamide upon thyroid secretion, endocrine glands, liver, kidney and spleen weights of female rats.

Fifty female rats of same age and strain were divided into 5 groups on the basis of equal thyroid secretion rate (TSR). Two were used as normal and vehicle treated (NaOH at pH 9.0)

groups. The other 3 groups were treated with 5, 10 and 15 mg acetazolamide per 100 g body weight daily intraperitoneally for 14 days. TSR was determined by ^{131}I uptake and blocking the thyroid stimulating hormone (TSH) by graded daily injection of thyroxine (L-T_4). Methimazole, a goitrogen, was used to prevent recycling of metabolized radioiodine. The estimated TSR in normal, vehicle treated and acetazolamide treated groups in 3 dose levels were 1.05 ± 0.03 , 1.06 ± 0.04 , 0.95 ± 0.05 , 0.93 ± 0.04 and 0.94 ± 0.03 $\mu\text{g L-T}_4$ per 100 g body weight per day respectively. Acetazolamide at 5 mg/100 g body weight had no significant effect of thyroid whereas groups receiving 10 and 15 mg showed significant ($P < 0.05$) decrease in TSR. The liver weight increased significantly in 10 and 15 mg and adrenal glands in 15 mg group only ($P < 0.01$). No noticeable difference in weight was observed in any other organs. The study indicates that carbonic anhydrase inhibition in thyroid gland is probably not responsible for its ability to secrete iodinated hormones as inhibition of the enzyme is complete at a much lower dose. The increase in adrenal and liver weights in higher doses might be due to nonspecific stress or concentration of the drug in liver.

Progress Report in Relation to Original Objectives

Several objectives of the research conducted under this contract have been fulfilled. An important objective was the determination of a relationship between thyroid hormone secretion rate and lactation. In terms of gross observations, it has long been known that thyroidectomy severely impairs milk production while replacement therapy ameliorates the symptoms of thyroid removal to the point that lactation is virtually restored to normal. Not until the radioisotope iodine- ^{131}I became readily available was it possible to measure individual thyroid hormone secretion rates without sacrificing the animal, however. A method was developed to estimate thyroid hormone secretion rates in dairy cattle by injecting iodine- ^{131}I into the animal and counting radioactivity in the neck region over the thyroid. Exogenously administered thyroxine was increased by graded levels until the release of iodine- ^{131}I from the thyroid was blocked. This method was applied to 39 individual lactating cows over the past 5 years with the result that the thyroid hormone secretion rate in relation to body weight was 0.19 mg L-thyroxine equivalent per 45.4 kg body weight. The coefficient of correlation between thyroid hormone secretion rate and milk production was +0.30 (Anderson, 1971). I conclude that the correlation is no higher than this because there are other galactopoietic hormones, such as lactogenic hormone, growth hormone, adrenal glucocorticoids, insulin, parathormone, and others, which may be limiting factors in determining the milk production capacity of the animal. To have measured the relationship between thyroid hormone secretion rates and milk production in rats (Anderson, 1969)

and cattle (Anderson, 1969, 1971) was a major achievement of this contract.

A second achievement was to compare the substitution method with the pool-turnover method for measuring thyroid hormone secretion rates. Using lactating dairy cattle, it was found that the substitution method overestimated the secretion rate for thyroxine alone because the contribution by triiodotyrosine is included in the former but not in the latter method unless it is measured separately. Since the pool-turnover method required the chemical measurement of the hormone in the blood as well as the determination of the rate of removal from the blood using radio-labeled thyroxine or triiodothyronine, it was not possible to evaluate the hormone secretion rate of triiodothyronine until methods became available recently to measure blood levels of triiodothyrosine. The individual thyroid hormone secretion rates of thyroxine and triiodothyronine were measured with and without the goitrogen, methimazole. This was done to evaluate the affect of methimazole, which is used routinely in the substitution procedure to block recycling of iodine. It was found that the secretion rate of triiodothyronine is approximately one-tenth that of thyroxine. However, since triiodothyronine is twice as effective as thyroxine in suppressing TSH, the relative contribution of triiodothyronine to total thyroid hormone secretion rate is about 20% (Anderson, 1971). A similar study has been completed in guinea pigs and is being prepared for publication. In this species triiodothyronine is three times as effective as thyroxine in suppressing TSH and it contributes about 25% to the total secretion rate relative to activity of the two thyroid hormones.

A third objective in the studies under this contract was to evaluate thyroid activity during hibernation. The ground squirrel was chosen for this because it remains in hibernation for longer durations than does the hamster and it is large enough to cannulate for blood sampling. Although the observations were limited the task of maintaining cannulae in hibernating ground squirrels was accomplished successfully and the data revealed that secretion rate of thyroxine during hibernation is slowed to almost nothing (Bauman and Anderson, 1970). The cannula technique is being used by Dr. T. R. Bauman in successive experimentation into this problem at the University of Alabama. A fourth objective of the contract was to measure thyroid hormone secretion rates in growing cattle and relate these to size and age. This has been accomplished to the extent that 62 individual thyroxine secretion rate determinations have been made in growing Jersey cattle. In contrast to the work reported by Mixner *et al.* (1966) in growing Holstein cattle, the data on Jerseys indicate that not only the absolute but also the secretion rates relative to body weight are much lower than in Holsteins. Whether or not this relationship is cause or effect in determining the mature size and weight of the separate breeds is not known but is worthy of speculation. This study has also shown that the exponential relationship of

thyroid hormone secretion rate to body weight is 0.72 in the Jerseys, whereas it was reported to be 0.60 in the Holsteins. The exponent 0.72 is almost exactly the same as the exponent relating body weight and body surface, which in turn is related to metabolic rate (Brody, 1945).

Objective five under this contract was to execute experiments to determine the controlling mechanisms for reduction of thyroid hormone secretion rate during starvation. It was found by Grossie and Turner (1962) that the thyroid hormone secretion rate dropped by 25% when rats were put on a diet that was 75% of the normal intake and the rate dropped to 50% or less when the food intake was cut to 50% of normal. When protein content of the diet was varied, it was found that the thyroid hormone secretion rate did not drop significantly below normal until there was 5% or less protein in the diet (Singh *et al.*, 1971). Since the protein content in the diet of the rats on 50% rations was 23%, representing a protein intake equal to 11.5% of the diet if the rats were on full rations, it was concluded that total caloric intake and not protein was the limiting factor controlling thyroid hormone secretion. In a successive experiment, cellulose was mixed with the diet at a 50% level. When the animals were given the mixture *ad libitum*, they adjusted their normal intake by eating almost twice as much as normal of the 50% cellulose diet. When the cellulose diet was restricted to an amount equal to the weight of the normal diet (caloric intake about 50% of normal), the animals lost weight and their thyroid hormone secretion rate declined by 50%. This experiment tended to refute the concept that dietary bulk is a prime determinant in thyroid hormone secretion rate (Galton and Nisula, 1969) because the thyroid secretion rates of rats on the cellulose diet *ad libitum* had about three times the normal amount of fecal bulk and yet the secretion rate was essentially the same as normal. A final experiment regarding this work is to be done soon using the pool-turnover method to measure thyroid hormone secretion rates. The purpose is to see if the peripheral utilization rate of thyroid hormone is changed in the rats receiving 50% cellulose diets *ad libitum* or restricted. It is hoped that by experimental manipulations of this kind, we will be able to ascertain the origin of thyroid hormone secretion rate control, the peripheral cell or the pituitary and hypothalamus.

A sixth objective of this contract was to measure thyroid hormone secretion rates of a variety of experimental animals which are now successfully maintained in captivity. One of these studies involved the musk shrew (*Suncus murinus*). The mean thyroid hormone secretion rate of 26 shrews was 1.61 ± 0.07 ug L-T₄ equivalents/100 g body weight/day (Dryden *et al.*, 1969). In an experiment to determine the thyroid hormone secretion rate of tree shrews (*Tupaia chinensis*), the following results were obtained using the substitution method. Animals were maintained in three separate social groups for 70 days at temperatures of 15, 25 and 37.5°C. The mean secretion rates of 14 animals was 1.29 ± 0.02 ug L-thyroxine equivalents/100 g body wt/day. There was no statistical differences ($P < .05$) due to sex or temperature. After this trial was completed,

the experiment was repeated to increase the numbers involved in the study. Unfortunately, a fire in the office of Dr. M. W. Sorenson resulted in the total loss of the data. It may be stated, however, that the second trial showed the same results as the first. The secretion rate mean value was essentially the same and no differences were noted due to sex or temperature (Sorenson and Bauman, unpublished). Thyroid hormone secretion rates were determined in gerbils (*Meriones unguiculata*) by the substitution method. In 44 animals, the mean secretion rate was 0.93 ug L-thyroxine equivalents/100 g body wt/day when the determinations were made at 25.5°C. No differences between sexes was noted. It is hoped that the secretion rate of thyroxine can be measured by the pool-turnover method before the data are ready for publication. Thyroxine secretion rates of 15 female ground squirrels (*Citellus tridecemlineatus*) were determined by the pool-turnover when the animals were maintained at 25.5°C. The mean secretion rate was 1.51 ± 0.15 ug L-thyroxine/100 g body wt/day (Bauman and Anderson, 1970). Our laboratory has developed thyroid hormone secretion rate data in rats, mice, chickens, pigeons, cattle, hamsters, guinea pigs, rabbits, opossum, raccoons, moles, shrews, ground squirrels and gerbils during the past 15 years (Bauman *et al.*, 1965). It may be speculated that thyroid hormone secretion rates on a unit body weight basis are reduced as size increases and, furthermore, species with a large cecum or rumen, such as guinea pigs, rabbits, and cattle, have a low secretion rate of thyroid hormones per unit body weight because the heat increment resulting from fermentation in the gut reduces the need for thyroxine.

A seventh objective of the studies supported by this contract was to follow the radioactive iodine-131 that was injected into animals as L-thyroxine-iodine-131. It was found that some experimental animals, such as rats and rabbits, retained the radioisotope in their bodies. That is to say, the half-life of whole-body thyroxine was much longer than the half-life of thyroxine in the blood when measured at the same times after injection. When the parts of the animals were measured for radioactive iodine, it was found that the accumulation occurred in the skin of the rat while the rabbit accumulated iodine in the intestinal tract. Mice were similar to rats but not as able to concentrate iodine in the skin. Guinea pigs were similar to rabbits but not able to concentrate iodine in the gut. This is probably because the guinea pig is not as dependent upon the practice of coprophagy as the rabbit for survival and therefore less thyroxine and iodine recycle through the intestinal tract in the guinea pig. When cattle were counted for disappearance of thyroxine from the blood and the whole body, no differences were found, suggesting that cattle have no concentrating or recycling mechanisms that are found in rats or rabbits. Of great interest is the observations that rats can concentrate iodine in the integument so remarkably well and rabbits seem not to have this ability at all.

An eighth objective of the research was to measure thyroid hormone secretion rates and thyrotropin blood levels in relation to body condition in animals. As a practical approach to this problem, rats were made obese with hypothalamic lesions and mice

were made obese with goldthioglucose. The study is now in progress and results will not be forthcoming before another year.

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