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Subject: Comprehensive Report of Over-all Activities of SRSA
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and 1970.

Title: Factors Affecting Thyroid Hormone Secretion Rates and
Utilization Rates in Mammals Using Iodine-131 as a
Tracer.

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1. Main Research Accomplishments

The objectives of the research contract were to measure thyroid hormone secretion rates and utilization rates in domestic and laboratory animals. A study was completed in cattle in which the thyroid hormone secretion rate (TSR) was measured by the substitution method and the thyroxine pool-turnover method. These two methods were in good agreement. When thyroxine was administered exogenously at 125% or 150% of the normal TSR, the resulting TSR calculated by the thyroxine pool-turnover method exceeded the expected value. The reason for this was postulated to be a more rapid removal of thyroxine from the blood than from the other compartment or compartments of the body's thyroxine pool. The results suggested that excessive thyroxine is handled in the body by being taken up rapidly by the liver and then eliminated via the bile.

L-thyroxine- ^{131}I and L-triiodothyronine- ^{131}I were injected into rats and rabbits and whole body counts were made to 10 days. Animals were sacrificed at intervals and ten organs or tissues measured for ^{131}I . In the rat, the fractional disappearance rate was similar in blood and all other tissues and organs measured except the integument and hair. There was a marked accumulation of ^{131}I in the skin and hair of rats. This was considered to be due to metabolism of thyroxine, removal of the ^{131}I into the blood as iodine and the retention of the ^{131}I iodide in the skin. Eventually it then passed into the hair and was stored. This storage phenomenon in the skin of the rat was tested in the rabbit, guinea pig, and cattle, but no evidence of the same mechanism was found. In the rabbit, the elimination of ^{131}I from the body after injection of L-thyroxine- ^{131}I was not rectilinear on a fractional removal basis, but the ^{131}I was accumulating in the intestines and cecum rather than in the integument and hair. In the cow, the elimination of ^{131}I from the body after injection of L-thyroxine- ^{131}I was rectilinear for the entire two-week period that it was measured. This indicated that no accumulation of ^{131}I in a particular part of the body was detectable in the cow, but the ^{131}I removal was in equilibrium throughout the body with a constant removal rate all during the counting period.

A comparison of rate of disappearance of thyroxine (L-T₄) and triiodothyronine (L-T₃) from the blood and from the whole body was made in mice, rats, rabbits, and guinea pigs. The half-lives of L-T₄ in the blood were slightly longer than those of L-T₃ in the mouse and rabbit and 5 hours longer in the rat. But the half-life of L-T₄ in the guinea pig was over 11 hours longer than that of L-T₃ (See Table 1). The half-lives of L-T₄- ^{131}I and L-T₃- ^{131}I in the whole body of all species on experiment were considerably longer than the respective half-lives of the thyroid hormone in the blood.

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Table 1

Half-lives (hours) of L-thyroxine(T_4)- ^{131}I and
L-triiodothyronine(T_3)- ^{131}I in laboratory animals.

Species	$t_{1/2}$ of L- T_4 - ^{131}I				$t_{1/2}$ of L- T_3 - ^{131}I			
	Blood no.	Blood hr.	Whole body no.	Whole body hr.	Blood no.	Blood hr.	Whole body no.	Whole body hr.
Mice	10	11.3	10	31.0	26	9.9	26	34.0
Rats	25	21.2	25	26.4	12	16.5	12	21.0
Rabbits	10	18.5	10	26.0	11	17.0	11	27.0
Guinea Pigs	11	25.7	11	43.0	36	14.5	11	19.8

Thyroid hormone secretion patterns in the hibernator were of interest to us because of the need to determine causes and affects in the physiological changes in animals entering into and being in hibernation. We had proposed to study the changes in thyroid hormone utilization of the ground squirrel (Citellus tridecemlineatus) as it went from room temperature to a temperature of $4.5^{\circ}C$ and when it was in hibernation. The execution of the experiments depended upon the ability of us to insert and maintain cannulae in the arteries and veins so injections of L- T_4 - ^{131}I could be made and blood samples could be removed from hibernating animals. The method of cannulation was extremely difficult to perfect and as a result only limited data were obtained in hibernators. However, the data demonstrated that the ground squirrel increased thyroid secretion markedly when placed in the cold and not allowed to hibernate. However, upon hibernating, the animal reduced the thyroid function to an extremely low level. It is believed that alterations in thyroid hormone distribution and metabolism occur before the stage of deep hibernation is reached, hence it is suggested that the lowered body temperature is not the only factor contributing to decreased thyroid activity during hibernation.

The availability of tree shrews (Tupaia chinesis) for thyroid hormone secretion rate determinations prompted us to obtain data on this species. The TSR substitution method was used to measure thyroid function in the shrews exposed to temperatures of $15^{\circ}C$, $25^{\circ}C$, and $37.5^{\circ}C$. There were no significant changes found among the different environmental temperature exposures. The experiment was repeated to verify this finding. A mean TSR of 1.29 ± 0.024 ug L- T_4 /100 g bw/day was found. This magnitude of TSR is similar to rats and mice.

The relation of TSR to lactation has been computed from the limited available data accumulated from lactation cows to date by $\frac{ng}{d}$

use of the substitution method for TSR. It has been found that the TSR increases 50% in cows of medium production. The correlation coefficient between TSR and milk production was found to be 0.3. Based on the 32 observations in Holstein and Jersey cattle, this is not a significant value. It is believed that this correlation coefficient is reliable in consideration of the fact that many other hormones, such as lactogen, somatotropin, glucocorticoids, insulin, and parathormone also are important determinants of the level at which a cow is able to produce milk. The studies in lactating rats supported this concept in that the correlation coefficient between TSR and milk production was only 0.2, although the magnitude of change in TSR from the normal virgin condition to the peak of lactation was 100%.

2. Plans for continuation of present objectives and new objectives.

Our TSR work in the past 3 years has shown us the value of studies involving a number of species. We hope to continue to develop new data on the thyroid hormone secretion rates of species which have not been studied in the past. The gerbil is now being studied as a part of the comparative type research. It is anticipated that the goat will be studied using both the substitution and thyroid pool-turnover methods for TSR as another species to add to the comparative investigations.

Work in cattle has indicated to us that a less time-consuming method than the substitution method for TSR determination would be desirable. We have compared the substitution and thyroxine pool-turnover method in normal cattle, but we would like to expand the results to lactating cows and to include use of triiodo-thyronine in place of thyroxine in both methods for TSR.

The relationship between the hypothalamus and the pituitary in control of TSH secretion is being studied at present by lesioning certain areas of the hypothalamus. It is hoped that we will be able to develop the radioimmunoassay for TSH so that we may measure TSH in blood and pituitary. An automatic gamma counter for measuring ^{131}I or ^{125}I is now available to us for this purpose. It is a matter of finding a graduate student with sufficient interest and training to work on the project. The measurement of TSH is a procedure which should result in many worthwhile studies of thyroid physiology in future years.

A comparison of L-thyroxine secretion rate with L-triiodo-thyronine secretion rate by the substitution method revealed that L-T₃ was about 3 times as effective as L-T₄ in the substitution method. The utilization rate of L-T₄ and L-T₃ are compared in the pool-turnover method. Work just completed in the guinea pig indicates that the methods enable one to estimate the secretion rates and relative effectiveness of each thyroid hormone. It is hoped that the same methodology will be extended to studies in cattle and rats in the near future.

3. Graduate students trained.

K. N. Sinha received the Ph.D. degree in June, 1968, and he is now an Instructor and Research Associate at the University of Tennessee, Department of Physiology, with Dr. VanMiddlesworth. The title of Dr. Sinha's thesis was "Thyroid Activity of the Rat and Fowl."

Thomas R. Bauman received the Ph.D. degree in June, 1969, and he is now an Assistant Professor in the Department of Biology at the University of Alabama. He is continuing to do research on thyroid function in ground squirrels using the cannula technique. The title of Dr. Bauman's thesis was "Comparative Thyroid Secretion Rates and Thyroxine Metabolism in Mammals."

(At the present time, 2 Ph.D. candidates and 2 M.S. candidates are devoting their entire research efforts to studies concerning thyroid physiology using iodine-131 as a tracer.

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5. Opinion of state of knowledge and needed future investigations.

Progress in measuring daily secretion rates of hormones in animals has been slow over the years. With the availability of ^{131}I and later the pure hormones, thyroxine and triiodothyronine, tagged with ^{131}I , the methodology for measuring thyroid hormone secretion rates has developed. We now have two good, although not perfect, methods for determining thyroid hormone secretion rates. One is based on the blockage of the release rate of thyroidal- ^{131}I by injecting increasing increments of pure L-thyroxine or pure L-triiodothyronine. The second is based on the turnover rate of the thyroid hormone in the body pool. The drawback to the method is that the concentration of hormone in the body pool must be measured chemically, while the rate of removal of the hormone from the pool is readily determined using ^{131}I -tagged hormones. The chemical determination of PBI is fairly difficult and does not lend itself very well to an occasional run by someone who has not spent a great deal of time with the procedure. It is the PBI measurement which is used to determine the level of thyroxine in the body pool. An even more difficult determination is that of triiodothyronine in blood plasma or serum. The level found in the blood suffices for the entire body pool level.

Triiodothyronine may be measured in blood by gas-liquid chromatography. Very few laboratories in the country are capable of measuring triiodothyronine levels in blood. The cost of a single determination is relatively expensive (\$4 for a single PBI determination versus \$50 for a single determination of triiodothyronine). Much more investigation is needed in the study of thyroid physiology and the best measure is a daily secretion rate of the hormones. The work should be continued in this area.

An area now open to fruitful investigation is the determination of thyrotropin (TSH) in the pituitary and the blood. With the development of reliable radioimmunoassay procedures to measure nanogram quantities of thyrotropin, it is possible to include the TSH level of peripheral blood in many studies concerning thyroid physiology. The ability to measure TSH in the blood of the hibernator, for instance, would provide knowledge concerning the regulation of body metabolism.