COMPARATIVE STUDIES OF THYROID FUNCTION

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This is to certify that the

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ABSTRACT

COMPARATIVE STUDIES OF THYROID FUNCTION

By

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Although an enormous amount of research has been published on central control mechanisms regulating thyroid function very little information is available on the possible effects of changes in the local temperature of the thyroid itself on its function. This problem was investigated by the use of two general approaches: 1) the influence of implantation site on the function of thyroid autoimplants was determined and 2) serum thyroxine (T_4) levels were determined in a variety of animals ranging through poikilothermic, avian and mammalian species.

Thyroids were implanted in rats at three different sites: 1) subcutaneous abdominal, 2) intramuscular abdominal, and 3) subcutaneous scrotal.

The thyroid secretion rate (TSR) of the scrotal implant group as measured by the direct output method (0.014 $\pm$ 0.014 µg per mg of 'thyroid weight', and 0.19 $\pm$ 0.06 µg per 100 g of body weight) was drastically depressed. The local tissue temperature at this site is 4 - 5°C lower than the

original site of the thyroid. In control males the TSR was 0.235 ± 0.032 μg/mg of 'thyroid weight', and 1.50 ± 0.23 μg/100 g body weight. The lowering of TSR in the scrotal implants is probably due to a decreased responsiveness to thyroid stimulating hormone (TSH). In the subcutaneous abdominal group the TSR per mg 'thyroid weight' (0.326 ± 0.049) was significantly higher than in control males. It thus appears that there is higher sensitivity to TSH at a local temperature 2.5°C lower than that of the original thyroid site. However the TSR measurements by the T_4 substitution method in all except the subcutaneous and scrotal implants were similar. In the subcutaneous implants the somewhat lower values were definitely due to experimental variation. No endpoint could be reached by this method in the scrotal implants. This is in full accord with the interpretation of a lack of sensitivity to TSH, and further proves that the scrotal thyroid implant is independent of TSH.

The measurements of serum T_4 were obtained by a modification of methods first introduced by Ekins (1960) and Murphy and Pattee (1964).

The serum T_4 values found, with some exceptions, tend to follow the evolution of species. The values were as follows: Rainbow trout, 0.75 ± 0.18 μg/100 ml; frogs, 0.89; turtles, 0.04 ± 0.02 (cold anesthetized) and 0.60 ± 0.15

(maintained at room temperature); chickens, 1.06 ± 0.09 (males) and 0.76 ± 0.11 (females); Bobwhite quail, 1.37 ± 0.02 (males) and 1.08 ± 0.05 (females); rats, 5.42 ± 0.36 (older males), 3.78 ± 0.33 (younger males), 3.94 ± 0.15 (mature females) and 3.45 ± 0.15 (immature females); dogs, 1.05 ± 0.17 (females); non-pregnant, non-lactating ewes, 13.22 ± 0.35 (Suffolk) and 8.59 ± 0.80 (Hampshire); cows, 6.20 ± 0.17 (dry, open) and 5.55 ± 0.25 (dry, pregnant); horses, 2.43 ± 0.23; goats, 9.12 ± 0.78 (dry, open females) and male adult opossum, 3.78 ± 0.33.

Earlier findings in chickens showed that their thyroid secretion rate is comparable to that of other homeothermic animals. The low serum T_4 found is due to a rapid turnover of circulating hormone. Because of possible differences among species in several parameters of thyroid function it seems valid to compare serum T_4 values only within species.

Serum T_4 levels in turtles anesthetized by packing in ice ($5^{\circ}C$) were far lower than for turtles kept at room temperature. Comparing this with the results obtained with thyroid implants in rats the turtle results can be explained by the cooling of the thyroid *in situ* consequent to cooling of the whole body.

COMPARATIVE STUDIES OF THYROID FUNCTION

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Dedication

This thesis is dedicated to my parents, Mr. and Mrs. David K. Wan who devoted their lives in bringing up their children to be useful human beings; also to my wife, Victoria Bao Shih-Yu Wan, for her understanding and helpfulness, especially during the completion of this work.

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INTRODUCTION

It has been shown by many methods that thyroid activity in mammals is higher in cold than in warm environmental temperatures. With changing environmental temperatures, significant changes have been reported in such indices as metabolic rate, thyroidal ^{131}I uptake and output rate and thyroid histology. In thyroid hormone secretion rate, as estimated in many species by the classic goiter prevention assay, the thyroid hormone substitution method and the thyroxine degradation method, there seem to be no exceptions to the rule that thyroid secretion rates are lower in warm than in cold environments.

It is generally accepted that the initial activation of the pituitary-thyroid system upon exposure to acute cold is brought about mainly by the central nervous system and neuroendocrine mechanisms. However, in view of the anatomical site of the thyroid glands in mammals, where they are certainly subjected to temperature changes due to the respired air, the question: "Do changes of local temperature in the thyroid gland alter the activities of the gland?" should not be neglected.

Since the glands are located on the trachea, any attempt to cool the gland *in situ* could be questioned, because of the likelihood that the carotid blood flowing to the brain would also be cooled. It is known that local body temperatures measured at different depths from the body surface and in different sites of the body vary, and research on autoimplantion of the thyroid has been extensively reported. Most investigators report that autoimplanted thyroids exhibit normal physiological function and histological appearance. However, no quantitative measurements have been made that would indicate their rate of thyroid hormone production and release.

In view of the above, it was proposed to study the function of autoimplanted thyroid tissue by measuring the thyroid secretion rates. Both the newly introduced direct output method (Reineke and Lorscheider, 1967) and the thyroxine substitution method (Reineke and Singh, 1955) were used. The thyroids were surgically removed and implanted in different sites of the animal body, thus taking advantage of local temperature differences to investigate possible functional alterations in the thyroid tissue. Investigations were also undertaken on several related aspects.

LITERATURE REVIEW

Since Schiff (1884) reported that following implantation[1] of thyroid tissue into the peritoneal cavity of thyroidectomized dogs no thyroid deficiency symptoms were found, there have been numerous experiments including different species and different transplantation (or implantation) sites for autografts,[2] allografts, and xenografts of the thyroid gland. In 1932, Marine summarized his own findings over the previous 20 years, and those of other workers with the statement: "Thyroid tissue autografts readily in any part of the body and has shown all the chemical and morphological reactions seen in the non-transplanted tissue." He also stated: "The growth of a transplant varies with the degree of thyroid insufficiency created in the host." Cameron (1952) reviewed the literature on "thyroid transplantation" in various species, and stated that successful

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[1]According to the definition of Dempster (1955), implantation does not connote surgical establishment of a primary blood supply; transplantation involves surgically constructed vascular anastomosis. These terms are used in this writing. However, in direct quotations, the terms will remain as originally used.

[2]All terms for transplantation or implantation in this paper follow the terminology proposed by Snell (1964). Terms from the other references except those in quotations were also substituted by suitable terms from the same proposal.

transplants occurred with autografts. Allografts varied but were usually absorbed, and xenografts never succeeded. The technique of implantation of tissue, and its theory has been extensively reviewed by many workers (Woodruff, 1960; Demikhov, 1960; Russell, 1961; Krohn, 1963; Russell and Monaco 1964). Implantation technique apparently suffers from the theoretical limitation that the piece of tissue is not provided with an immediate blood supply and may consequently suffer considerable necrosis. It had been suggested that smaller fragments prepared with a sharp instrument will completely survive implantation. The most common sites of implantation have been subcutaneous and intramuscular. The advantage of the technique of implantation is that, it provides opportunities for disassociating the effect of environment from those of the organ itself. For reasons of the anatomy of the lymphatic drainage, it was stated that beside the anterior ocular chamber, the testis may also be a relatively favorable locale for allograft survival. Aron *et al.* (1956) demonstrated that intratesticular grafts fixed more ^{131}I than the gland *in situ* in guinea pigs. Hill (1937) demonstrated that ovaries implanted into the ear of male mice produced unusually large amounts of androgen at a room temperature of $22^{o}C$.

Williams (1939, 1939a, and 1953) observed through a transparent chamber, thinly sliced autogenous thyroid tissue implanted in the rabbit ear. He described the developing

process of revascularization and re-formation of follicles, even after disintegration of the original thyroid follicles, and also pointed out that the autografts responded to iodine and thyroid stimulating hormone (TSH) as in the intact gland. It is known that after thyroidectomy in rats thyroid hormone production is depressed and circulating TSH is increased (Contopoulos, et al., 1958). Woodruff and Woodruff (1950) showed histologically a 100% survival in thyroid subcutaneous autografts in guinea pigs, and also pointed out that Halsted's principle (1909) which stated, "a necessary condition for the success of any transplant of endocrine tissue is the prior existence in the recipient of a deficiency of the corresponding tissue" is only good for ocular implants. Autogenously implanted thyroid fragments in spleen or kidney of thyroidectomized rats showed no detectable effect on the rate of growth of animals or on their final weight. The spleen autografts maintained a normal protein-bound-iodine (PBI) concentration. Activity of kidney autografts was stated to be reduced somewhat, but the degree of depression was not believed to be physiologically significant. It appeared that the liver does not destroy and excrete the thyroid hormone to a significant extent when the hormone is present in physiologic quantities (Bondy, 1951; Rupp, 1952).

Within six hours after implantation in mice, autografts of thyroid showed marked necrosis. The process of necrosis progressed up to 24 hours, and some evidence of

repair was seen at the third day after implantation. Ten days later, the grafts showed a definite increase in the amount of typical thyroid tissue. Even when necrosis was at its peak and before vascular connections between host and graft had been established, the superficial thyroid follicles in the graft were capable of metabolizing iodine. Radio-autographs of thyroid grafts of more than ten days residence in the host were essentially normal (Bennett and Gorbman, 1951).

Dempster and Doniach (1955) chronically followed the regeneration of autogenous subcutaneously implanted thyroid glands in rats. In hemi-thyroidectomized rats, they described the histological development of thyroid autografts as follows; (1) After 2 days of implantation the grafts showed a massive necrosis, and scattered apparently living, very occasional peripherally-placed follicles. (2) After 4 days, three clearcut zones were apparent in the grafts; a central mass of concentrated non-nucleated acidophilic ghosts of follicles; an intermediate zone of loose granulation-tissue; and a peripheral layer of follicles. Peripherally-placed parathyroid cells appeared normal, but the central two-thirds of parathyroid were replaced by granulation tissue. (3) At six to ten days the periphery of the grafts was made up of a broad zone of apparently normal follicles. (4) After 10 days the grafts presented a picture of thyroid follicles surrounding a central core of scar

tissue. Parathyroid tissue, where presented, appeared normal. (5) After 30 days, the grafts were organized into thyroid lobes, normally vascularized. In the same report, they also showed that after 15 days the autografts in totally-thyroidectomized rats presented well-developed thyroid follicles. Arestov (1964) observed thyroidectomized rats for one and one-half years and stated that subcutaneous autografts on the shoulders recovered function more rapidly when re-innervated than when not re-innervated. With ^{131}I tests the function in the latter was only one-third to one-half of that of the former. From the evidence that small thyroid fragments successfully reconstructed themselves in the rabbit (Williams, 1953), and in partially thyroidectomized rats (Dempster and Doniach, 1955) after autogenous implantation; Dempster and Doniach suggested, contrary to Halsted's principle, that thyroid deficiency in the host plays no role in the success of "taking" and survival of the thyroid autografts.

Many workers had tried to find a way to overcome the genetic barrier to allografts in order to permit correction of hormonal deficiency. Dameron (1952) allogenously implanted foetal thyroid into the anterior eye chamber of thyroidectomized rabbits, and showed no difference in ^{131}I uptake (% dose/mg tissue) between propylthiouracil (PTU) treated and control animals. This finding was confirmed in radioautographs. He gave two alternative explanations for

this phenomenon (1) alteration of circulation of the grafts and (2) species differences; for it is known that PTU exerts inhibitory effects on thyroid function. These allografts also responded to intramuscular injection of TSH. Děvényi, Czenkar and Endes (1958) subcutaneously implanted foetal thyroids in rats treated with cortisone; by allografting the tissue first, and then thyroidectomizing the rats, they obtained 16% and 30% successful implantations in two experiments. They found that cortisone-treatment is indispensable for "taking" of the foetal thyroid tissue in thyroidectomized adults. They explained further: "in such an early, undeveloped stage of immune reactions, on an appropriate ontogenetic level, it is possible to bring about tolerance to adult foreign tissue." Birnie and Mapp (1962) used embryonic thyroid tissue grown in culture media implanted in one-day-old thyroidectomized rats. The rats showed significant growth. However, Gough, Pugh and Brook (1962) reported that allografts implanted in diffusion chambers in dogs survived for periods up to 30 weeks, but the majority of such grafts survived no longer than 15-20 weeks. Kerkof and Chaikoff (1966) showed follicular reorganization of isolated allogenous thyroid cells subcutaneously implanted in thyroidectomized rats. They isolated the thyroid cells by a trypsinization procedure (Kerkof, Long and Chaikoff, 1964) and cultured for three days on a Gelfoam pad without TSH or as a monolayer in medium with bovine TSH. They reported

that 94% - 99% ^{131}I was protein-bound in excised implants. The total plasma ^{131}I -thyroxine (^{131}I - T_4) in plasma of rats implanted with monolayer-cultured cells was in good agreement with that of normal control rats. They suggested that normal thyroid function had been restored in this group of rats.

In a successful transplantation in human subjects, the thyroid gland of a 3-week-old boy was transplanted into a 28-year-old woman. The vascular anastomosis had been carefully established between the donor gland and recipient blood vessels of the epigastric area. A normal uptake of ^{131}I was shown eight days after the surgery, and the follow-up also showed good function of the thyroid (Sterling and Goldsmith, 1954); however, there is no record of menstruation in this report. Marshall (1963) observed the estrous cycle in ferrets after thyroid implantation, and reported that the onset of light-induced estrus was not influenced by this operation. Swan, Jenkins and Schemmel (1967) reported a 12-year followup of a thyroid autograft in a human subject with satisfactory results, up to two years.

Yasumura (1963) implanted neonatal rat thyroid tissue in brain. He reported that the histological appearance, the ability to concentrate iodine, and synthesis of thyroid hormone, MIT and DIT of the implants and hormonal concentrations in the implants were similar to those in normal thyroid tissue. However, Lance (1967) found that in

dogs carrying intracranial allografts of thyroid, the $PB^{131}I$ and $PB^{127}I$ increased progressively to a high level, then dropped to an extremely low level.

As to ^{131}I uptake, in comparing the residual thyroid tissue and that of implants, Dempster and Doniach (1955) suggested that implants in totally thyroidectomized rats may reach a fixed quantity of uptake comparable to that of normal glands. Wollman, Scow and Wagner (1953) showed that in thyroid tumors implanted in mice the uptake of ^{131}I per mg of tissue is quite uniform. An implanted autogenous lobe usually differed from a lobe *in situ* by less than 10% as judged by *in vitro* counts two hours after the injection of 10 μc of carrier-free ^{131}I (Wollman and Scow, 1955). In their experiments no work was done comparing totally implanted thyroids and normal control glands.

MATERIALS AND METHODS

The Thyroid Secretion Rate of Autoimplants

Autogenous thyroid implants for this part of the experiment were performed on two different age groups of rats from the Carworth Farms, (CFN strain). There were infant and adult groups. The rats of infant groups were operated upon at the age of 5-7 days under cold anesthesia. The implanting operation was started on the rats of adult groups at a body weight range of 175-225 gm under sodium pentobarbital anesthesia. The rats in the control group were chosen from different litters which were used for autogenous implanting experiments. Three different autogenous implanting locations had been chosen. These were abdominal subcutaneous, abdominal intramuscular and scrotal subcutaneous.[1] A group of thyroidectomized rats was used as negative controls. At least 15 days were allowed for adults to recover from the operation and to form thyroid follicles (Dempster and Doniach, 1955). In the infant rat groups the

[1]Abbreviation used in tables or charts for abdominal subcutaneous implants, abdominal intramuscular implants, and scrotal subcutaneous implants are sub., int., and scrot., respectively.

thyroid secretion rate was not measured until the rats reached the body weight of 150 - 200 gm.

Two methods for measuring the thyroid secretion rate (TSR) were applied to the same animals in this experiment. These were the thyroxine substitution method (Reineke and Singh, 1955), and the direct output method (Reineke and Lorsheider, 1967). The sequence of experimental procedures is shown diagrammatically in Figure 1.

After the animals were anesthetized, the operative area was shaved, and cleaned with soapy water and swabbed with 70% ethyl alcohol. All surgical instruments except cutting tools were sterilized by autoclaving. The thyroids were removed and kept in chilled 0.9% NaCl solution. A pouch was made between the skin and subcutaneous tissue for subcutaneous implantation in both the abdominal (Sub.) and scrotal area (Scrot.), while a pouch was made between abdominal muscle and the peritoneum for intramuscular implants (Int.). The Sub. and Int. implantations were made such that they were approximately at the same anatomical sites with only a difference in depth from the body surface. The whole gland was inserted into the pouch and the incision was closed with wound clips. The whole implantation was completed in 15 - 25 min.

The details of the process for measuring TSR by both methods are described in Appendix I (Formulae and Computations). The arrangement of this experiment was as follows:

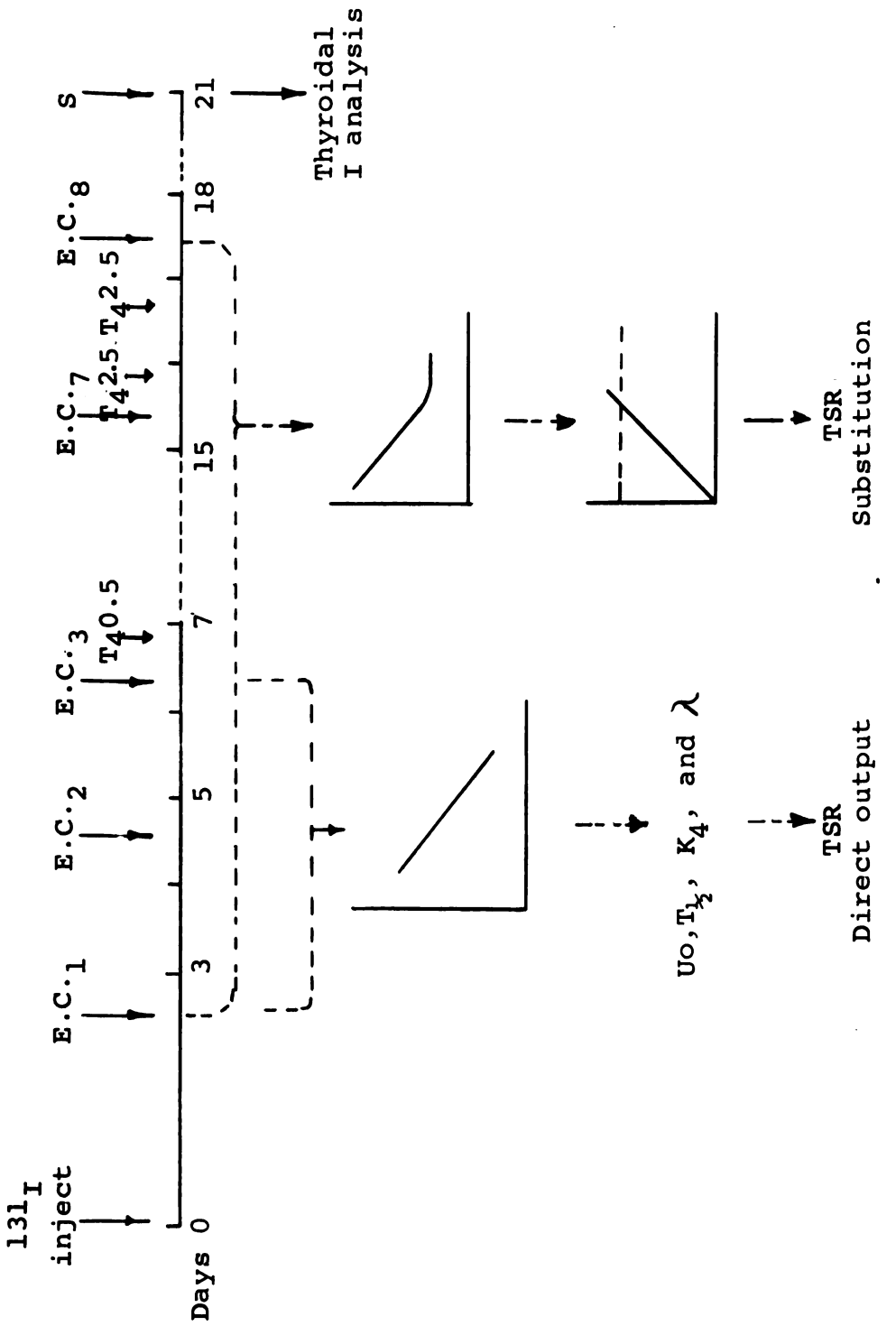

Figure 1. Diagram of experimental procedures for autoimplantation studies (for details see Appendix I).

A tracer does (3 μc) of carrier-free ^{131}I was injected intraperitoneally, and 2 - 3 days were allowed for maximal thyroidal ^{131}I uptake. External gamma-ray counts (E.C.) of normal thyroids or of autogenous thyroid implants were taken in the same way by a laboratory counter and scaler as described in Appendix I. In those rats which carried autografts the external count rate was taken in two locations: the autograft site and the original thyroid gland region.

If the rats carrying an autograft satisfied one or both of the following criteria they were defined as "completely autoimplanted" rats; (1) the counts in the thyroid region did not follow a normal output curve, or (2) the tracheal tissue did not have a significant amount of ^{127}I by thyroidal iodine analysis (Reineke and Lorscheider, 1967). The rats carrying an autograft with a negative thyroidal output slope and significant iodine content in tracheal tissue were subjected to the thyroid secretion rate calculation by the direct output method. In these rats the same computations were also made to estimate the activity of the autograft.

In vivo counts were taken on alternate days throughout the experimental period, and were expressed as % of the injected ^{131}I dose (% dose). At least six counts were taken in this part of the experiment for each rat. The first three external thyroid counts were used for the direct output method measurement. After the third count, the rats were subjected to the thyroxine substitution method by

starting thyroxine injection. The dosage of L-thyroxine was increased every two days by an increment of 0.5 µg/100 gm body weight, and this was started from 0.5 µg/100 gm body weight, until thyroid blockage occurred. The end point was taken as the dose of thyroid hormone that maintained the thyroid ^{131}I count at 97.5% of the previous count (Reineke and Singh, 1955).

After the termination of injection of thyroxine, a time interval of at least 3 days was allowed for the disappearance of exogenous thyroxine from the animal body before removing the gland for thyroidal iodine analysis. The tracheal tissue of the rats which carried autografts was also taken out for iodine analysis.

For the direct output method, the percent injected dose values were transformed to logarithms (y) and equated against time (x) by the method of least squares. However, for the hormone substitution method the thyroidal ^{131}I uptake as percent dose was plotted against time on semilog graph paper and the output curve was fitted by inspection, then successive values were transformed into percent previous count. The dosage of thyroxine required to maintain the thyroidal ^{131}I count at 97.5% of the previous count was taken as the thyroid secretion rate.

The thyroid ^{131}I output constants (K_4) were calculated by the equations of Brownell (1951); and the thyroid secretion rates, measured by the direct output method were

calculated by the equations of Reineke and Lorscheider (1967) (see Appendix I). Except as mentioned otherwise, all the animals described hereafter were fed Wayne Lab-Blox (Allied Mills Inc., Detroit, Michigan) and housed in an air conditioned room where the temperature was maintained in the range of 74 $\pm$ 1°F, with 14 hours of light exposure daily.

Re-implantation

This experiment was designed to determine whether the autoimplantation procedure itself will alter thyroid function. Twenty-three male rats of the Carworth Farms (CFN Strain), ranging in weight from 190 gms to 370 gms and in age from 10 to 15 weeks, were used. Twelve of these rats were subjected to thyroid autoimplantation. In this surgery, the thyroid lobes were removed from the original sites, and placed under the inner fascia of the Sternohyoideus muscles on either side of the trachea. All of the original circulatory and nervous connections of the thyroid lobes were severed in this procedure. The selection of the Sternohyoideus fascia sites in which to place the lobes was such that the lobes would be as close as possible to their original position along the trachea. A second group of six rats underwent sham surgery in which they were subjected to the same procedure as the first group with the exception that their thyroid glands were left *in situ*. A third group of five rats that were not subjected to any surgical procedure

were used as controls. The other experimental procedures were the same as in the previous section. However, only the direct output method was employed to measure TSR.

Serum Thyroxine

Twenty-four male rats of the Sprague Dawley strain were used for serum thyroxine analysis. Two groups, each comprising eight rats, were used for thyroid implantation. One group received a subcutaneous and the other an intramuscular autoimplant at the age of 45 days. Another group of eight rats of the same age were kept for controls. Forty days after implantation blood samples were taken from the abdominal aorta, and the serum was used for thyroxine analysis. The serum thyroxine (serum T_4) was measured by the Tetrasorb-125 method (Radio-Pharmaceutical Div., Abbott Laboratories, North Chicago, Ill.), which is based on the principle described by Ekins (1960), and further developed by Murphy and Pattee (1964), the details will be discussed in the second part of the thesis. Nine rats that had been thyroidectomized 34 days were used for negative controls.

Protein-Bound Iodine (PBI) Measurements and Percent Body Weight Gain in Rats Carrying Thyroid Implants

Twenty-six 48 - 55 day old male rats of the Sprague Dawley strain were used for measuring body weight gain, and

PBI after implantation. One group of six rats was used for controls. The remaining twenty rats, five in each group, were divided into four groups, as follows; 1) subcutaneous abdominal implanted, 2) intramuscular abdominal implanted, 3) scrotal subcutaneously implanted, and 4) thyroidectomized.

A tracer dose of ^{131}I was injected intraperitoneally in the rats of the experimental groups in order to confirm the surgery. Body weights were measured every third day from the day of operation. Forty-three days after the operation the rats were bled by heart puncture and sacrificed. The plasma of these blood samples was used for PBI determination.

The PBI was measured by an adaptation of the method introduced by Barker and Humphrey (1950). Briefly, the proteins were precipitated from 1.0 ml plasma by using equal volumes of 10% $ZnSO_4$ and 0.5N NaOH. The precipitate was mixed with 1.0 ml of 4N Na_2CO_3 and ashed in a muffle furnace for 2½ hours at 605-625°C. The ash was dissolved with 2N HCl and 7N H_2SO_4, and diluted with distilled-water. The timed reaction with arsenious acid and ceric ammonium sulfate was run for 15 minutes at 37°C and then stopped with brucine sulfate. Final readings were taken with a Coleman Universal Spectrophotometer at 480 mμ, set for 100% transmission through a distilled-water blank. Iodine content was read from a standard curve prepared under identical conditions in

which the net percent of transmission of the known iodine samples was plotted against concentration.

Thyroid Weight Estimated by Radioactivity ('Thyroid Weight')

Nine male adult rats of the Carworth Farms (CFN strain) were subjected to subcutaneous implantation of their thyroids in different locations. The zero time ^{131}I uptake (U_o) was obtained as described for the direct output method in Appendix I. After 23 days, the implants were carefully removed, the weights of the implants were obtained, and the correlation between the weights and the U_o values was calculated. The regression equation thus obtained was used to predict the weight of functional thyroid tissue.

RESULTS AND DISCUSSION

Mortality Rate of Rats Receiving Thyroid Autoimplants as Neonates

At first glance, the percentage survival of rats in which thyroid glands had been autoimplanted at 5 - 10 days old was quite high (Table 1). Survival rate was 75% in rats with subcutaneous abdominal autografts, and 63% in rats with intramuscular abdominal autografts. After the rats reached the age of 80 - 100 days, they were given a tracer dose of ^{131}I and the neck region was checked for radioactivity. Among the survivors only 4 carrying subcutaneous abdominal autografts and 7 with intramuscular autografts could be classified as "complete autoimplants." Thus, both of these groups actually had approximately a 50% mortality rate (Table 1).

Beltz and Reineke (1968) found a very low TSR in neonatal rats. After the body weight reached 22 gm, a strong correlation between log body weight and TSR was observed. From these results, together with those of several other investigators, it appears that neonatal thyroid activity is very low; at about 10 days of age or a body weight of about 20 gm, active thyroid regulation begins (Brody, 1945;

TABLE 1

PERCENT SURVIVAL OF RATS WITH COMPLETE AUTOIMPLANTS PREPARED NEONATALLY

Groups	Number of rats operated (0)	Number of survivals		Number of survivals with complete auto-implants	
		No. (S)	%	No. (I)	%
Subcutaneous implanted	16	12	75.0	4	50.0
Intramuscular implanted	22	14	63.6	7	46.7

$$\% \text{ of Survival} = \frac{S}{0} \times 100;$$

$$\% \text{ of Complete Autoimplants Survivals} = \frac{S - (S - I)}{0 - (S - I)} \times 100$$

Phillips and Gordon, 1954; de Jongh and Paes, 1958). Furthermore, Glydon (1957) reported that the capillary tufts of the hypothalamo-pituitary portal system do not appear before the ninth postnatal day. Thus, the high mortality of the rats receiving thyroid autoimplants neonatally may be due to insufficient stimulation through the hypothalamo-pituitary system for the implants to become established.

In adult rats, implanted thyroid tissue necroses in the center, and the living peripheral follicles subsequently build up the whole thyroid lobe (Dempster and Doniach, 1955). In rats 5 - 10 days of age, the implants are bathed in fluid having little or no TSH, and eventually die. If the follicles can manage to go through this period, regeneration will occur, thyroid function will be restored, and the animals will survive. Thus, the mortality rate may indicate those best fit to survive, and shows that regeneration of thyroid implants is the critical event that determines the fate of the animal.

It may be noted that the mortality rate in both groups is almost the same. This may suggest that in early postnatal life, the regeneration of thyroid implants depends upon the creation of good hypothalamo-pituitary relationships, not where the implants were located.

Thyroid Weight Estimated by Radioactivity ('Thyroid Weight')

In the present experiments, the rats were fed the same diet, and were kept under controlled lighting and room temperature. In this controlled condition, most factors which vary the thyroid ^{131}I uptake have been ruled out, except the gland weight and colloid content of the glands (Turner, Pipes and Premachandra, 1959). The recovered implants varied in size and weight. This variation was mainly owing to the regeneration process. In intramuscular implants, the thyroid tissue was intermingled with muscle fibers that made the weight estimation of these implants impossible. However, the subcutaneous implants usually gave a well-formed thyroid tissue mass.

Thus in order to have a uniform basis for the comparison of thyroid secretion measurements, the zero-time ^{131}I uptake (U_o) was used to predict the thyroid weight. It was reported that the ^{131}I uptake in thyroid lobes *in situ* is similar per unit weight to that of thyroids that are subcutaneously autoimplanted (Wollman and Scow, 1955). In transplantable thyroid tumors in mice it was also shown by Wollman, *et al*. (1953) that the tissue mass is correlated with ^{131}I uptake. Nine well-defined subcutaneously implanted lobes were carefully recovered from different subcutaneous sites, counted for radioactivity *in vivo*, and the U_o values were calculated as described. The glands were also weighted.

There is a high correlation between U_o values and the weights of the implant (Fig. 2). From the formula for this regression line the thyroid weight of a given U_o value can be predicted (see Appendix II).

Under the presumption that implants at different implantation sites had the same relationship between U_o and the thyroid weight as subcutaneous implants, the average thyroid tissue weights were calculated and are listed in Table 2. It is believed that these predicted thyroid weights more nearly represent the functional tissue than the actual weights because the actual weights include intermingled tissue formed during regeneration of the implants (Dempster and Doniach, 1955). Estimation of thyroid weight from its U_o value will tend to correct for the influence of intermingled non-thyroid tissue. The thyroid weights predicted from U_o values will be referred to as 'thyroid weight' in the following discussions. The 'thyroid weight' value varied in different groups in the differently located implants as predicted (Table 2), and there is a significant difference only between the subcutaneous infant group (Inf. Sub.) and subcutaneous adult groups (Ad. Sub., Table 3).

It may be noted in Table 2 that the 'thyroid weight' of the subcutaneous infant group is not significantly different from that of the control males. The other implantation groups are all significantly lower than the controls.

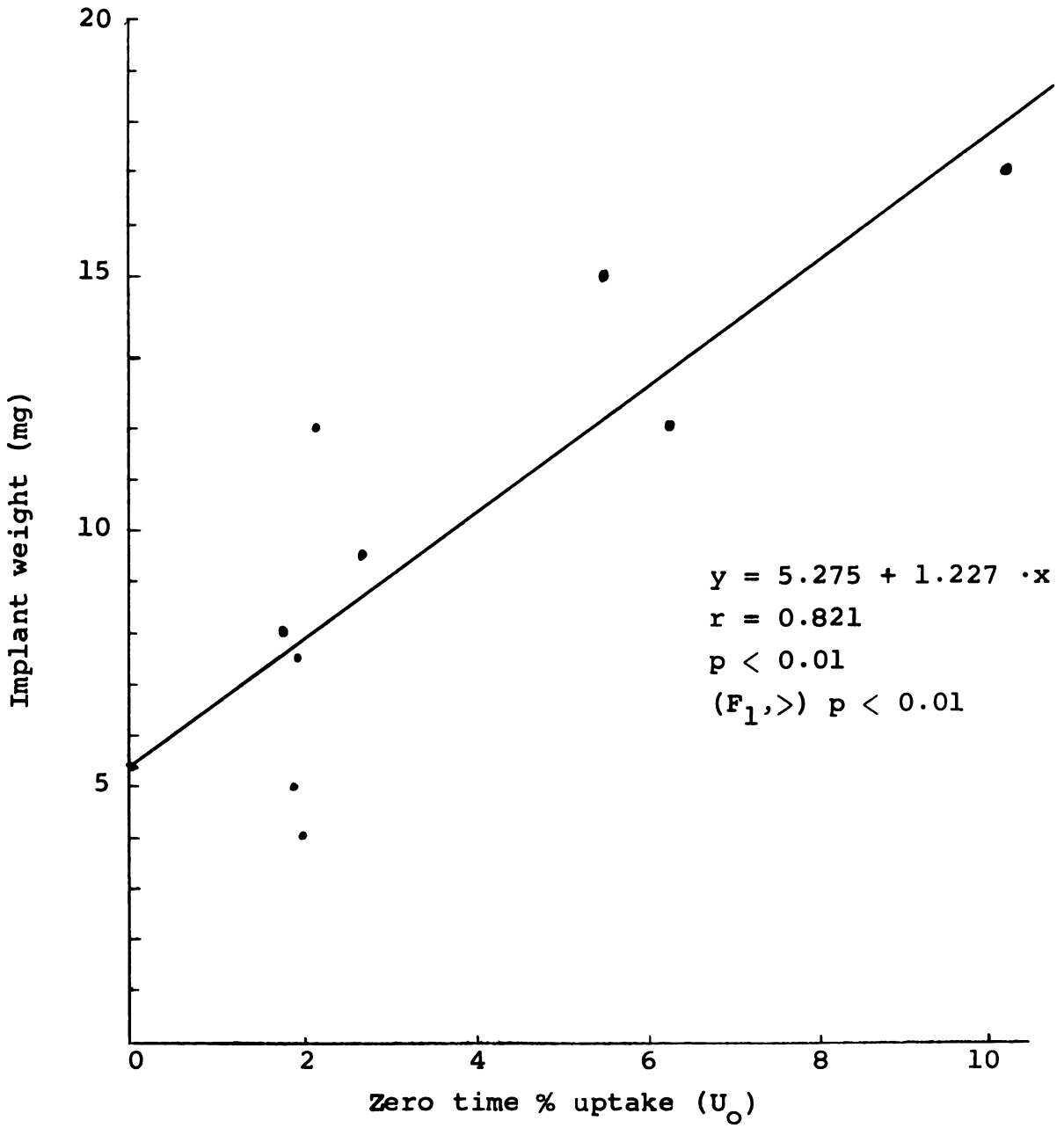

Figure 2. Correlation between U_o and subcutaneous 'implant weight.'

TABLE 2

'THYROID WEIGHT' ESTIMATED BY RADIOACTIVITY*

Groups	Control males	Control females	Inf. Sub.	Ad. Sub.	Int.	Scrot.
'Thyroid Weight' (mg)	19.35 ± 1.36^{a} (10)**	17.00 ± 1.58^{a} (6)	16.89 ± 1.90^{a} (3)	11.23 ± 1.53^{b} (6)	13.20 ± 0.92^{b} (12)	13.40 ± 1.40^{c} (5)

*Mean $\pm$ S.E.

**Number of observations

^{a,b,c}As determined by the Mann-Whitney U test, there is no significant difference among values with the same superscripts ($p > 0.05$); with different superscripts, the significance level is: a,b, $p < 0.02$; b,c, $p < 0.002$.

TABLE 3

A COMPARISON OF SOME PARAMETERS OF RAT THYROID FUNCTION IN DIFFERENT AGE GROUPS WITH THE SAME IMPLANTATION SITES

Groups	n	'Thyroid Weight'	TSR per mg 'Thyroid Weight' (Direct Output Method)	TSR per 100 gm Body Weight (Direct Output Method)	TSR per 100 gm Body Weight (Substitution Method)
Infant Sub.	3	16.9	0.269	1.805	1.63 (2)**
Adult Sub.	5	11.3	0.361	1.331	1.30 (6)
P*		0.036	0.286	0.607	0.094
Infant Int.	5	13.4	0.150	0.593	1.85 (6)
Adult Int.	7	13.0	0.221	1.043	1.99 (8)
P		0.560	0.165	0.378	0.528

*Mann-Whitney U-test (Seigal, 1956).

**Number of observations.

The Thyroid Secretion Rate in Units of 'Thyroid Weight' (TSR/mg thyroid weight)

The thyroid secretion rates discussed hereafter were obtained by employing the direct output method introduced by Reineke and Lorscheider (1967). However, they were calculated on the basis of 'thyroid weight'. It can be seen in Table 3, that there is no significant difference between the two age groups. Thus, the TSR/mg values of these two age groups were pooled for statistical treatment.

It can be noted in Table 4, that the pooled TSR/mg (0.326 µg/day/mg) in subcutaneous implants is significantly higher than that of all the other groups including control males ($p < 0.05$).

These results differ from those of other investigators (Wollman and Scow, 1955). However, they used the ^{131}I count as indicator of the function of the glands, and stated that the subcutaneously implanted lobes will generally differ from those normally located in the same animal by less than 10% per unit weight. Their results suggest that the ^{131}I uptake is in proportion to the tissue mass. However, they did not take into account the probability that the recovered implants are larger than their actual functional mass. For this reason, the difference between their results and those reported here may be due to the weighing of the scar tissue included in their implants.

TABLE 4

THYROID SECRETION RATE PER UNIT OF 'THYROID WEIGHT' ESTIMATED BY RADIOACTIVITY*

Groups	Control males	Control females	Sub. (pooled)	Int. (pooled)	Scrot.
TSR per mg 'Thyroid Weight' T_4 mg/day/mg	0.235 ± 0.032^{a} (10)**	0.187 ± 0.034^{a} (6)	0.326 ± 0.049^{b} (8)	0.191 ± 0.032^{a} (12)	0.041 ± 0.014^{c} (5)

*Mean $\pm$ S.E.

**Number of observations.

^{a,b,c}As determined by the Mann-Whitney U test, there is no significant differences among those value with same superscripts ($p > 0.05$); with different superscripts the significant level is a,b $p < 0.05$; b,c $p < 0.37$.

In the present experiment the TSR calculations were based on units of 'thyroid weight' instead of body weight for the following reasons: (1) in normal animals housed at a constant room temperature the weight range per 100 gm body weight of the gland *in situ* is small, while the gland tissue recovered from implants gave a much wider range; (2) After surgical implantation of thyroid glands the animals, even when started at the same body weight, did not reach nearly the same body weight as controls at the time the TSR was measured; (3) The body weight did not correlate with the functional weight of the thyroid in rats carrying implants. In view of these, 'thyroid weight' instead of body weight was used as the basis for comparison of thyroid gland function in the present work.

Body Temperature Measurements

The local temperature measurements listed in Table 5 were obtained with a needle thermistor when the animals were under anesthesia induced by sodium pentobarbital. The measurements taken from the same animal at different sites are listed in the same column.

It is interesting to note that the scrotal subcutaneous temperature is lower than the subcutaneous abdominal temperature, and the latter in turn is lower than the thyroid and intramuscular sites. This seems to suggest that the optimal temperature for an implant to give higher TSR/mg

TABLE 5

LOCAL TISSUE TEMPERATURE MEASUREMENTS IN RATS
(ROOM TEMPERATURE: 28°C)

Sites	Temperatures (measurements from three different rats, °C)		
	Rat 1	Rat 2	Rat 3
Neck (Thyroid Loci)	36.6	32.5	
Subcutaneous (Abdominal Area)	34.0	30.0	34.0
Intramuscular (Abdominal Area)	36.5		36.5
Scrotum (Subcutaneous)	32.5	27.0	

than normal thyroid is approximately 2 - 2.5°C below the temperature of the thyroid site. At a temperature 4 - 5°C lower than the normal thyroid site (scrotal implants) the thyroid tissue put out very small amounts of hormone. Histologically the scrotal subcutaneous implants appeared abnormal, this is probably the reason for the lower TSR/mg in the scrotal implants.

Histology of the Implants

Generally speaking, the histology of all except the scrotal implants, appeared normal.

Grossly the regenerated "thyroid lobes" varied in shape. Thus no precise measurement of tissue volume could be made, especially in intramuscular implants, because of the admixture of muscle fibers.

The epithelial cells in subcutaneous (Plate 1) and intramuscular implants (Plate 2) were cuboidal. Nearly all the epithelial cells in scrotal implants (Plate 3) were very low and flat. Vacuoles were found in the colloid of all implants, but were far less numerous at the scrotal site.

As judged by the usual histological criteria, namely cell height and colloid vacuolization, activity of the intramuscular and subcutaneous abdominal implants were very similar to that of the controls (Plate 4), but activity of the scrotal implants was greatly reduced.

Plate 1

Subcutaneous abdominal thyroid implant

Left lower corner shows connective tissue fibers. These also can be seen in the upper left corner where a few fibers have penetrated between follicles. Note the tall cuboidal epithelium and numerous secretory vacuoles.

Plate 2

Intramuscular thyroid implant

The right side shows muscle fiber wedged between the follicles. Such fibers also can be seen between follicles in the upper left corner. Note the similarity to plate 1.

Plate 3

Scrotal subcutaneous thyroid implant

Notice the flattened epithelium, scarcity of secretory vacuoles and dense colloid.

Plate 4

Normal control thyroid

Scale: Each small division = 10 μ

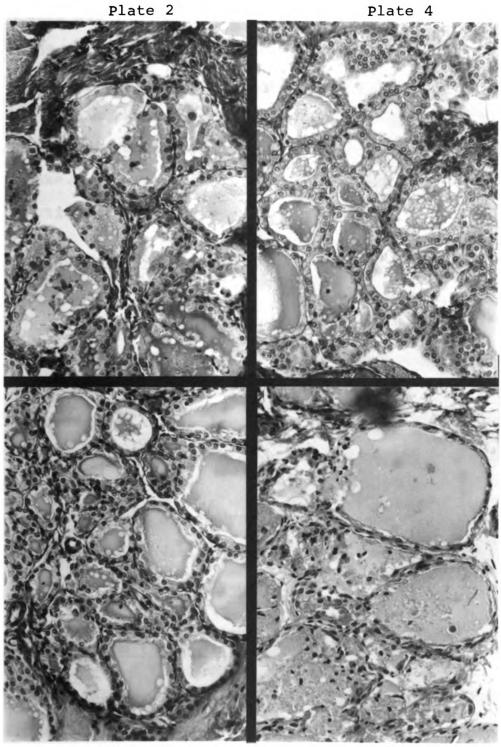
Plate 2
Plate 4
Plate 1
Plate 3

It may be argued that the blood supply to the implants was insufficient to support normal growth. Williams (1939a, 1939b, and 1953) observed the actual revascularization of subcutaneously implanted thyroids in the ear of the rabbit. In the present experiment, the function of the grafts is based on "functional tissue" by its ^{131}I uptake. Thus only the tissue that established "active function" was considered.

TSR/100 gm Body Weight Determined by the Direct Output Method

The measurements of thyroid secretion rate per 100 gm of body weight are presented in Table 6 where two categories of measurement are shown. These are 1) the TSR measured from the implants and 2) the sum of the TSR computed from both the implants and the residual thyroid in the neck region.

The sum of the TSR/100 gm provides an estimate of the amount of thyroid hormone available for peripheral utilization per unit of body mass.

A. TSR/100 gm Body Weight Measured from the Implants

The TSR values of the two subcutaneous abdominally implanted age groups are not significantly different (Table 3). It can be concluded that the restored function of the thyroid tissue, once established, is not influenced by the

TABLE 6

TSR MEASURED BY DIRECT OUTPUT METHOD*

Groups	TSR T_4 µg/day/100 gm b.w.	
	Implants	Total
Control males	1.50 ± 0.23 (10)**	
Control females	1.64 ± 0.29 (6)	
Sub. (Pooled)	1.51 ± 0.23*** (8)	1.57 ± 0.24*** (8)
Int. (Pooled)	0.86 ± 0.19*** (12)	0.89 ± 0.20*** (12)
Scrot.	0.18 ± 0.06*** (5)	0.19 ± 0.06*** (5)

*Mean ± S.E.

**Number of observations.

***$P < 0.05$ as determined by Mann-Whitney U-test.

Note: There is no significant difference between TSR of implants and total of all groups.
Values of subgroups are given in Table 3.

age at which the implantation was performed. The situation is the same in the intramuscularly implanted thyroids.

The TSR values of the pooled subcutaneous groups (1.51 μg/100g) are not significantly different (Mann-Whitney U Test) from either of the controls (males 1.50 and females 1.64 μg/100 g, respectively). This finding agrees with most of the previous reports where thyroid function was determined by use of other parameters (Wollman, et al., 1953; Bondy, 1951). However, the pooled TSR values of the subcutaneous groups are significantly higher than the pooled values of the intramuscular (0.86 μg/100 gm) and scrotal subcutaneous implants (0.18 μg/100 gm). In a series of experiments in rats, it was found that the administration of thyroidal substance suppresses the activity of the thyroid gland. This is explained by the well established negative feedback mechanism between the thyroid and the anterior pituitary gland (Stewart, 1966). The histology of these inactive glands was generally similar to that of the scrotal subcutaneous implants in the present studies. The follicles were lined with squamous spithelial cells. The colloid was dense and contained very few vacuoles.

It would be expected that the rats with scrotally implanted thyroids in the present experiments which had a very low TSR, intact pituitaries and no exogenous blocking agents, would have an elevated level of TSH in the blood. Contrary to the histological picture expected, there was a

lack of TSH stimulation of the implants as shown by their flattened epithelial cells. Thus the depressed thyroid function of the scrotal implants was probably due not to a lack of TSH but rather to a decreased sensitivity to TSH. This in turn was probably caused by the depression of local tissue temperature (4^{o} to 5^{o}C lower than the normal thyroid site) to which the scrotal implants were subjected. An explanation for this may be that there is a critical lower temperature for a key enzyme system that is essential for the full activity of TSH.

It may be argued that the TSR mentioned here is not from the total thyroid tissue in the animal body because in some rats fragments of thyroid tissue were found on the trachea after implantation. The quantitative influence of such fragments on total TSR is evaluated in the next section.

B. Total TSR

In determining TSR by the direct output method in rats with thyroid autoimplants, radioactivity counts were taken over the normal thyroid region as well as the implantation sites. In rats that had neck counts significantly above body background, the output slopes and ^{127}I content of both the tracheal tissue and implants were determined. The TSR/100 gm measurements from the residual tissue were added to the TSR from the thyroid implants to give total TSR. For rats whose implants were the sole source for TSR measurement,

the values for these were treated as the total TSR. There are no significant differences for TSR between implants and the total values in all autoimplanted groups (Table 6).

The pooled subcutaneous TSR (1.57 µg/100 gm) is significantly higher than the pooled intramuscular TSR (0.89 µg/100 gm). The former is higher than control males (1.50 µg/100 gm), but the difference is not significant ($P > 0.05$).

These results are in accord with the TSR/mg 'thyroid weight' where subcutaneous implants gave significantly higher values. The results suggest that the function of autoimplants depends upon their location. The only known evironmental difference is in local tissue temperature of the implantation sites (approximately 2.5°C lower than at the normal thyroid site). These results also show that the subcutaneous implants supply enough thyroid hormone to satisfy the physiological demand. In fact their hormone output per unit body weight is statistically equivalent to that of control males.

TSR/100 gm Body Weight by the Thyroxine Substitution Method

The thyroxine substitution method (Reineke and Singh, 1955) utilizes the long feed-back loop mechanism in suppressing thyrotropic hormone secretion. Thyroid secretion rate measured by this method is expressed as L-thyroxine in µg/day/100 gm body weight.

It may be noted in Table 3, that there are no significant differences between the two age groups in both subcutaneously and intramuscularly implanted animals.

The results in Table 7 show that the TSR/100 gm in animals carrying subcutaneous implants (1.370 μg T_4/day/100 gm) is significantly lower than in all other groups, including intramuscular implants; but there is no significant difference between intramuscular, control males and control females. It is of special interest that an end point could not be obtained in the rats with scrotal implants. This will be discussed later.

Reineke and Lorscheider (1967) investigated the TSR of rats that were fed a diet containing different subnormal levels of iodine. They showed a higher estimated TSR by the substitution method than by the direct output method. It is interesting to note that the TSR as measured by the substitution method of different groups of rats receiving different levels of iodine were statistically equal, while the TSR measured by the direct output method correlated with the amount of iodide in the diet. This was interpreted to mean; "the substitution method measures the physiological demand for thyroid hormone but represents true TSR only if iodine supplies are adequate."

In line with this interpretation it could be expected that all of the rats carrying thyroid implants would have the same thyroxine demand and these would have

TABLE 7

TSR MEASURED BY THE THYROXINE SUBSTITUTION METHOD*

Groups	Control Males	Control Females	Sub. (pooled)	Int. (pooled)	Scrot.
TSR (T_4 ug/day/100	1.90 ± 0.86[a] (10)**	1.75 ± 0.13[a] (6)	1.37 ± 0.06[b] (8)	1.91 ± 0.05[a] (14)	--- (13)

*Mean ± S.E.

**Number of observations

[a,b]As determined by the Mann-Whitney U test.

Values having the same superscript are statistically equivalent.
a,b, $P < 0.01$.

The end point of the Scrot. group could not be obtained.

approximately the same apparent TSR by the substitution method. The somewhat lower value obtained in the subcutaneously implanted group is obviously due to chance experimental variation. As already indicated absolute TSR values obtained by the substitution method probably have very little relationship to the actual release of thyroid hormones in some groups of rats carrying thyroid implants. However, the results give further evidence on the influence of local tissue temperature on thyroid activity.

In presenting the results for the direct output method, it was suggested that at decreased local tissue temperatures, 4 to 5°C lower than the thyroid site, the thyroid is less responsive to TSH. The failure to block ^{131}I output of scrotal thyroid implants by injection of T_4 is in full accord with this interpretation. Sufficient exogenous T_4 was injected in this group to substantially block TSH output by the anterior pituitary gland, as was demonstrated in the other three groups. Yet the ^{131}I release rate from the implants was not affected. This can only mean that the ^{131}I output from this site is independent of pituitary control. It means also that at some point in thyroid metabolism, lowered local temperature blocks at least one or more of the key reactions regulated by TSH. This is supported further by the depression of the PBI to thyroidectomy levels and cessation of body weight gain, as will be shown later in this thesis.

PBI and Body Weight Gain in Rats Carrying Implants

The rats in this part of the experiments were subjected only to the implantation treatment. Fifteen days were allowed for the animals to recover from the operation. During the recovery period, the body weight of the animals carrying implants first declined and then gradually increased.

The results presented (Table 8) are the final % body weight gain during the last 28 days after recovery in implanted groups. The results shown for control animals are the % weight gain in 28 days started from the same age as the implanted animals.

The % body weight gain among control, subcutaneous and intramuscular implants showed no statistically significant differences. But the scrotally implanted group showed a much lower % increase in body weight and the thyroidectomized group actually lost weight. These results are in general agreement with the TSR measurements described earlier.

The PBI values (Table 8) revealed that both subcutaneous and intramuscular implants were statistically equal. In the scrotally implanted group, the PBI was depressed to the level found in thyroidectomized rats. Although, as determined by the Mann-Whitney U test, the control group is significantly higher in PBI values, than either the subcutaneously or intramuscularly implanted group, the serum

TABLE 8

PBI AND 4 WEEK BODY WEIGHT GAIN (IN % OF THE BODY WEIGHT AFTER RECOVERY)

Group	PBI* (ug/100ml plasma)	Body Weight Gain (%)
Control	5.83 ± 0.25^{a}	89.68
Sub.	3.88 ± 0.38^{b}	50.04
Int.	4.03 ± 0.12^{b}	41.06
Scrot.	2.68 ± 0.25^{c}	26.02
Thyroid-ectomized	2.21 ± 0.28^{c}	- 1.46

*Mean $\pm$ S.E.

As determined by Mann-Whitney U test, values having the same superscript are statistically equivalent. ($P > 0.05$) a,b, $P < 0.05$ b,c, $P < 0.05$

thyroxine level in these three groups is the same (to be described in a later section).

The PBI values in scrotally implanted rats were very low in accord with the earlier suggestion that the activity of thyroid tissue in this site is independent from pituitary TSH control.

Re-implantation

The results of thyroid re-implantation in its original site are shown in Table 9. There are no significant differences in TSR/mg 'thyroid weight', TSR/100 gm body weight and PBI values among the experimental groups. These results suggest that the autoimplantation procedure will not alter the activity of thyroid tissue. However, due to the changes of laboratory circumstances while this part of the experiment was in process, the measurements were determined while the animals were housed at a warmer room temperature. This fact will probably explain the lower control TSR than reported earlier. Even so, there is no significant difference between the controls of this experiment and the control animals from previous data expressed as TSR/100 gm body weight.

These findings (Table 9) clearly show that the surgical procedure *per se* does not alter the subsequent activity of thyroid implants. They lend strong support to the use of

TABLE 9

SEVERAL PARAMETERS OF THYROID FUNCTION OBTAINED FROM THE RE-IMPLANTATION STUDY*

Groups	'Thyroid Weight' (mg)	TSR/mg of 'Thyroid Weight	TSR/100 gm Body Weight	PBI ug/100 ml
Control	18.51 ± 8.94 (5)**	0.168 ± 0.031 (5)	0.94 ± 0.28 (5)	6.87 ± 0.82 (5)
Re-implantation	12.56 ± 1.07 (10)	0.155 ± 0.025 (10)	0.63 ± 0.14 (10)	6.03 ± 0.11 (5)
Sham-operation	14.68 ± 1.43 (6)	0.168 ± 0.031 (6)	0.58 ± 0.11 (6)	5.84 ± 0.36 (5)

*Mean ± S.E.

**Number of observations.

There is no significant difference in any of the parameters.

this approach to study the influence of local tissue temperature on thyroid function.

Serum Thyroxine

Serum thyroxine levels of thyroid-implanted rats are shown in Table 10. There are no significant differences among control, subcutaneous and intramuscularly implanted groups, but the serum T_4 of the thyroidectomy group is significantly lower than all other groups. These results further illustrate the T_4 level in the implanted animal. Serum T_4 gives a better measurement of hormonal level in the blood than PBI. This fact will be discussed in the second part of the thesis.

Comparison of the TSR Methods

As mentioned previously, the TSR of some rats in the autoimplantation study was measured by both the direct output and T_4 substitution methods. The TSR per 100 gm body weight obtained by the direct output method is consistently lower than that measured by the T_4 substitution method in all except the scrotally implanted group. In this group an end point for TSR could not be obtained. In comparing each individual rat in these three groups the average ratio of TSR by the T_4 substitution method to TSR by the direct output method (S/D) is 1.57 in control males, 1.30 in subcutaneously implanted and 2.48 in intramuscularly implanted groups.

TABLE 10

SERUM T_4 AND T_4 - I OF AUTOIMPLANTED RATS

Groups	Serum T_4 (T_4 μg/100 ml)	T_4 - I** (μg/100 ml)
Control	3.78 ± 0.33 (8)*	2.47 ± 0.22 (8)
Sub.	3.26 ± 0.28 (8)	2.13 ± 0.18 (8)
Int.	3.49 ± 0.39 (8)	2.28 ± 0.26 (8)
Thyroid-ectomized	0.23 ± 0.13 (7)	0.15 ± 0.10 (8)

*Number of observations.

There is no significant difference among all groups by the Mann-Whitney U test except in the thyroidectomized group which is significantly lower than all other groups.

**Iodine content of serum T_4 = T_4 x 0.6534.

Similar results were shown in chickens (Singh, Reineke, and Ringer, 1968) and rats (Paulik, 1969).

The T_4 substitution assay, in which the thyroxine is injected once daily, can be questioned on the ground that daily administration of thyroxine neglects important consideration of its short half life. In rats, the plasma thyroxine concentration declines by about 75% in 24 hrs after injection (Gregerman, 1963). The T_4 substitution method is not applicable in any condition where iodine intake is a limiting factor on thyroid function because it depends upon maintenence of exogenous T_4. Its TSR values will not necessarily reflect the true thyroid hormone output, as discussed by others, (Reineke and Lorscheider, 1967; Singh, Reineke, and Ringer, 1967).

Basic assumptions of the direct output method are as follows: 1) that the iodine compounds of the thyroid are uniformly labeled and 2) that substantially all of the iodine released is in hormonal form. Rosenberg et al. (1964) reported that in both chickens and rats, uniform labelling is attained within two days after ^{131}I is administered. There is also evidence, however, (Rosenberg, LaRoch and Ehlert, 1966) of heterogenous iodine output from the thyroid such that the 'last in is first out'. The comparison of methods for TSR estimation is not the purpose of this study; however, taking into account all possible errors, the direct output method most nearly measures the true TSR.

The research that has shown variations of thyroid function in relation to environmental temperature changes was done mostly by exposure of the whole animal (Dempsey and Astwood, 1943; Henneman, Reineke and Griffin, 1955; Griffin, Henneman, and Reineke, 1962). This experimental design will not give information on the direct influence of temperature on the thyroid itself. Other glands, such as the hypophysis, the adrenal, and unavoidably, peripheral metabolism will complicate the picture. Heroux (1963) reported that rats kept outdoors in groups (-10°C) needed a smaller amount of L-T_4 (1.8 µg/100 gm per day) than control rats (2.75 µg), which were kept at 30°C, to block the release of thyroidal ^{131}I. Singly-caged rats at 6°C gave a value higher (5.5 µg) than the controls (2.75 µg at 30°C). He also mentioned in the discussion, that: "the histologic picture of the thyroid in winter outdoor rats was one of a very inactive gland." In a series of measurements of local tissue temperature of the thyroid site (this thesis) in rats kept in a cold room (5 $\pm$ 1°C), the temperature of the thyroid site was in the range of 37 - 38°C. This is within the normal range of body temperature, but, as shown previously, the local tissue temperature of scrotal implants is 4 - 5°C below that of the normal site. In an environmental temperature of -10°C, it is very probable that the inspired air cools the thyroid tissue to a temperature that will suppress sensitivity to

TSH of the thyroid gland, but not as completely as in the scrotal implants.

SUMMARY AND CONCLUSIONS - PART I

1. Experiments were conducted to measure thyroid activity under the influence of local temperature variations while the animals were kept at constant room temperature.

2. In order to produce variations in local thyroid temperature an autoimplantation technique was employed to relocate the thyroid gland. The T_4 substitution and the direct output methods were applied to measure the thyroid secretion rate (TSR) of the implants. Thyroid histology, protein-bound iodine, serum thyroxine and growth rates were also studied. It was found that the scrotal subcutaneous implants secreted very little thyroid hormone (0.19 ± 0.06 T_4 µg/day/100 gm body weight, and 0.04 ± 0.01 µg T_4 /day/mg "thyroid weight"). Abdominal subcutaneous implants produced more hormone per unit of "thyroid weight" (0.326 ± 0.049) than control males (0.235 ± 0.032), but approximately the same as the controls per 100 gm body weight. Intramuscular implants produced much less hormone than controls either on the basis of "thyroid weight" or per 100 gm body weight (0.191 ± 0.032 µg/mg and 0.89 ± 0.20 µg/100, respectively). However, the TSR/100 gm body weight of the

intramuscular implants is not significantly different from that of control males. TSR measurements by the T_4 substitution method were approximately the same except for abdominal subcutaneous implants and scrotal subcutaneous implants. The low result for the subcutaneous group is definitely due to experimental variation. Results for TSR by the substitution method could not be obtained for scrotal implants.

3. It is concluded that local temperature influences the activity of the thyroid. The TSR is drastically depressed in scrotal implants where the local temperature is 4 - 5°C less than that of the normal thyroid site. This is probably due to a lack of sensitivity to TSH stimulation in these implants. However, a depression of 2½°C at the subcutaneous abdominal site increases the thyroid sensitivity to TSH.

4. The PBI values are also greatly reduced in rats with scrotal implants.

5. The surgical procedures used did not influence the thyroid activity, as shown in rats with their thyroids removed and re-implanted at the normal thyroid site.

PART II

INTERSPECIES COMPARISONS OF SERUM THYROXINE LEVELS

INTRODUCTION

The search for methods to estimate the thyroxine (T_4) level in the blood in order to evaluate the thyroid functional status has been continued for many years. The measurements of protein-bound-iodine (PBI) or butanol-extractable iodine (BEI) for this purpose are procedures which had gained considerable popularity. It is known that the BEI determination possesses a greater degree of specificity for thyroxine and thyroxine-like compounds than does PBI; nevertheless, neither is completely specific and both are affected by the amount of iodine in the diet ingested previously by the subjects. The iodine concentrations involved are extremely small and great care is necessary in avoiding iodine contamination either from the laboratory environment or reagents used in the assay.

Ekins (1960) described a method for serum T_4 measurement in which he used the relationship that the ratio of thyroxine carried in albumin to that carried in thyroxine-binding-globulin (TBG) is a variable whose value in a given sample of serum is dependent upon the amount of exogenous thyroxine that has been added. This assay, though lacking in absolute specificity, is more suitable to routine work.

Murphy (1964) and Murphy and Pattee (1964) developed a new method based on the same T_4 -TBG binding principles. They extracted T_4 from serum with ethanol. The dried ethanol extract was then used to compete for the binding sites of ^{131}I labeled T_4 (T_4 - ^{131}I) on TBG. Displacement of the labeled T_4 by the unlabeled T_4 in the extract was used to estimate the T_4 level in the blood serum. This method has been further modified by several researchers. The method is more specific for T_4 than the earlier methods and free of the danger of contamination by many materials. However, it is very dependent upon correct control of temperature and the time allowed for incubation. It has been extensively applied to investigations on human subjects, but there is a lack of information on other species.

In the present study, this technique was employed for a survey of thyroxine levels in the blood serum of many different species. The species covered ranged from invertebrates like limulus to mammals. The principle analytical material used was the commercially available Tetrasorb-125 diagnostic kit.*

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*Abbott Laboratories, North Chicago, Ill. U.S.A.

LITERATURE REVIEW

In 1960, Ekins described a method which he called "saturation analysis" for measuring total serum or plasma T_4. The method was based on the principle that the ratio of thyroxine carried on albumin to that on TBG is a variable whose value in a given sample of serum is dependent upon the amount of exogenous thyroxine that has been added. He labeled the sample under investigation by a known small quantity of $^{131}I - T_4$, and extracted with acidified n-butanol. The dried extract was added to an aliquot of standard serum. At the same time, known quantities of $^{131}I-T_4$ were added to similar aliquots of the same serum thus yielding serum samples containing a range of concentrations of exogenous T_4. The entire group of sera, including some containing an unknown amount of thyroxine were subjected to electrophoresis and the distribution of radioactivity between albumin and TBG measured.

Murphy (1964) discussed extensively the general principles of protein-binding properties utilized in measuring minute quantities of hormones and other substances. Murphy and Pattee (1964), based on these principles, developed a method for T_4 measurement which they called "competitive

protein binding analysis." Briefly, the principle of the method is this. Since there is only a small amount of TBG in plasma, the binding sites can be readily saturated by adding small amounts of T_4. If a small amount of $^{131}I - T_4$ is added, the fraction which is protein-bound can be determined. As more unlabeled T_4 is added, the amount of $^{131}I - T_4$ decreases, since both labeled and unlabeled forms compete for the same binding sites. If instead of pure T_4 a sample of deproteinized plasma is added, the T_4 which it contains may be measured according to the fall in bound isotope which it causes. It is known, however, that T_4 is bound by at least three different proteins in serum. These include TBG, to which binding is strongest (approximately 60% of the T_4) prealbumin (30%) and albumin (10%) when a tracer amount of labeled T_4 is added to human serum, in a glycine acetate system (Sterling, 1967). It is known that binding to prealbumin is inhibited by barbital buffer, and the binding to albumin is decreased by dilution. Barbital buffer at pH 8.6 (1.0 ml of serum to 32 ml of buffer) was introduced by Murphy and Pattee (1964). They also introduced the dextran polymer gel Sephadex G 25, Medium grade, to provide secondary binding sites for $^{131}I - T_4$ displaced from TBG by unlabeled T_4.

In a later publication, Murphy and Jachan (1965) simplified the procedure by using anion exchange resin to replace the polymer gel. $^{125}I - T_4$ was also introduced at

the same time. A straight line standard curve in the range of 0.0 - 0.02 µg T_4 was described. Since the intact T_4 molecule is measured the determinations are entirely unaffected by iodine or mercury. Murphy, Pattee and Gold (1966) evaluated 1439 clinical cases in which T_4 was determined by this method. In 400 cases the PBI values were determined along with the T_4 measurement. When T_4 and PBI were compared, a good correlation (r = 0.823) was obtained. They concluded from this study, that the entity measured as T_4 is truly thyroxine and the chief factors affecting its diagnostic accuracy in thyroid disease are the levels of the proteins which carry it in the blood.

Nakajima, and co-workers (1966), and Kennedy and Abelson (1967), have extended the principle of saturation analysis, by using resin-sponge to separate bound from unbound thyroxine. Nakajima et al. (1966) introduced ^{131}I - T_3 thyronine (^{131}I-Labeled tri-iodothyronine) as a tracer in the system. They demonstrated that their standard curves had a sharp linear relationship between resin-sponge uptake and T_4 concentration from 0.00 to 0.15 µg. Therefore, as they suggested, the standard curve can be applied for determining T_4 content below 0.15 µg with concentrations of T_4 higher than 0.15, ½ dilution was recommended.

Kaplan (1966), showed that ^{125}I - T_4 could be bound to TBG solution in advance of the determination. It is known that the maximum binding sites for T_4 in 100 ml of

human serum are equivalent to 20 µg of T_4 (Ingbar, Waterhouse and Cushman, 1964). According to my calculations, Kaplan used approximately 0.7 - 3 µg of labeled T_4 (specific activity: 4-6 mc per mg) in 100 ml of pooled serum, and obtained satisfactory results. It was presumed that ^{125}I - T_4 - TBG cannot distinguish between radioactive and nonradioactive T_4. Thus, if unbound thyroxine is added to the system a part of the bound labeled T_4 will be released.

The thyroxine binding reaction is very dependent upon temperature and the time allowed for incubation. Murphy and Patee (1964) showed increased % binding of ^{131}I - T_4 at decreased temperature. Nakajima et al. (1966) demonstrated competition for ^{131}I - T_3 tracer between resin-sponge and TBG. With various known amounts of T_4, the curve for samples incubated at 4°C was much steeper than that for 20°C when plotted as T_4 concentration against % ^{131}I - T_3 resin-sponge uptake. They suggested that at 4°C the standard curve was much more accurate for predicting unknown T_4. Kaplan (1966) showed that in the same time period of incubation there was no difference among the runs done between 0°C to 12°C. The 25°C curve showed higher uptake by the resin-sponge when the % uptake by the sponge was plotted against known T_4 concentration. Kennedy and Abelson (1967) reported similar results. They showed that the curve is elevated by approximately 2% per 1°C.

The time of incubation was also investigated previously (Kaplan, 1966; Kennedy and Abelson, 1967). Briefly, lengthening the incubation time will increase the steepness of the standard curve.

MATERIALS AND METHODS

Blood Sample Collection

Blood samples of five Limulus (Limulus polyphemus) were collected by drainage through an opening of the dorsal shell. Limulus has an open circulatory system. Thus the samples collected were actually hemolymph from the coelomic cavity, and they were clotted in the body before the serum was drained. The samples were preserved in the deep-freeze for future serum T_4 analysis.

Five mature rainbow trout (Salmo gairdneri) were kept at 13°C water temperature and fed with commercial trout pellets which contained 2% iodized salt. The blood samples were obtained through dorsal artery puncture. After clotting, the samples were centrifuged at 2,000 rpm for 15 minutes, and the serum was removed and preserved in a deep freeze (-25°C) for future serum thyroxine analysis. The time span from blood sampling to frozen preservation was less than 12 hours. All the blood samples from the animals surveyed for serum thyroxine level in this experiment were collected, treated and preserved in the same manner.

Six turtles 8 - 9 inch (Pseudemys scripta elegens) were immobilized by packing in ice and refrigerating

overnight at 5°C. A cast cutter was employed to open a window on the ventral shell against the location of the heart. The heart was warmed under a lamp until the beat resumed. The blood samples were withdrawn from the ventricles with Vacutainers.

Another group of six turtles (6 - 7 inch) of the same species were killed by a sharp blow on the head, and the blood samples were taken from the heart as already described.

Frogs (Rana pipiens) were stored at 13°C. Blood samples were withdrawn from the conus arteriosus by use of a syringe and 18 g needle.

Blood samples of bobwhite quail (Colinus virginianus) and white leghorn chickens (Gallus domesticus) were obtained by courtesy of the Department of Poultry Science at Michigan State University. The body weight of 6 male white leghorns ranged from 2.18 to 2.40 kg, and the 5 females weighed 1.85 to 2.27 kg. The chickens were all sexually mature. The blood samples were obtained from brachial veins. The blood samples of bobwhite quail were taken by heart puncture. The quail weights ranged from 0.19 to 0.22 kg in 5 males, and from 0.16 to 0.27 kg in 6 females. They were all sexually mature. All species of birds were housed indoors and were fed standard laying rations.

Albino rats (Rattus norvegicus) of the Sprague Dawley strain were used. There were three groups of males,

and four groups of females. The male rat groups were (1) 11 rats approximately one year old (2) eight 85-day-old, and (3) thyroidectomized rats. Thyroidectomy was performed on a group of 10 mature male rats. Twenty-seven days after surgically removing the thyroid, 41 uC/rat of carrier-free ^{131}I was injected intraperitoneally to destroy possible residual thyroid tissue. Radioactivity counts were obtained from the neck and thigh regions at 7 days after ^{131}I injection. Seven rats with a neck count rate lower than or equal to body background (thigh region count rates) were chosen for blood sample collection. The serum thyroxine level was analyzed 41 days after the blood samples were collected. Thus, a negligible amount of isotope remained in the samples. The male rats were all fed Zinn diet (Zinn Feed Co., Battle Creek, Michigan). The blood samples were collected through the carotid artery or abdominal artery by inserting a polyethylene catheter.

The female rat groups were (1) 10 mature virgins (2) 10 immature virgins (3) 9 open non-lactating and (4) 10 pregnant rats. The blood samples of the open non-lactating rats were taken 19 days after the litters were weaned. Ten mature females were kept with mature males for one week. Vaginal smears were taken on the females every day. At exactly 15 days after being served the females were sacrificed and blood was collected as already described.

Pure bred mature female beagles (Canis familiaris) were used for control serum thyroxine analysis. Six dogs weighing from 12 to 17 kg. were used for thyroparathyroidectomy. These included 5 dogs of predominantly beagle breeding and 1 Brittany spaniel. The surgery was performed under sterile conditions. A dose of 0.5 ml of Diurnal-Penicillin (Upjohn Co., Kalamazoo, Michigan) was injected intramuscularly right after the operation. The dogs were fed dog meal (Ken-L Ration Meal, The Quaker Oats Company, Chicago, Illinois). After removal of the thyroid-parathyroid, 15 gm calcium lactate (Merck and Co., Rahway, New Jersey) for each dog per day was mixed with one-fourth can of regular Ken-L Ration canned dog food for calcium supplementation. Only one dog showed tetany after surgery. In this dog a dose of 5 ml 10% $CaCl_2$ was injected intravenously whenever mild symptoms of tetany occurred.

The control dogs were fed Purina Special Dog Chow (Ralston Purina Co., St. Louis, Mo.). The blood samples were obtained from the jugular vein by employing B-D Vacutainers (Becton, Dickinson Co., Rutherford, New Jersey). Two consecutive blood samples were taken from thyroparathyroidectomized dogs, 14 and 35 days after surgery, respectively. ^{131}I tracer checks were performed on these dogs before the first blood collection to confirm the completeness of the surgery.

Blood samples were collected from 4 groups of female sheep (Ovis aires) of the Suffolk and Hampshire breeds. The Suffolks included (1) five one-year-olds, (2) four dry, non-pregnant and (3) five pregnant ewes. The duration of pregnancy ranged from 71 to 110 days. The dry, non-pregnant and pregnant ewes ranged in age from 1 year 10 months to 4 years 10 months. The blood samples were collected on the same date for the last two groups. The serum T_4 analyses were also done for both groups on the same day. Blood samples were also collected from 7 yearling Hampshire ewes.

Blood samples were collected from two groups of Holstein cows (Bos taurus), dry open and dry pregnant. All of the cattle blood samples were taken from the tail vein by using a Vacutainer.

Jugular vein samples were obtained from nine dry, non-pregnant Toggenburg goats (Capra hircus).

Jugular vein samples were also obtained from 7 saddle type horses (Equus callabus) of assorted breeds and sexes.

Blood samples of six adult male opossums (Didelphis marsupialis virginiana) were obtained via heart puncture.

Serum Thyroxine (T_4) Analysis

1) Procedure

The Tetrasorb-125 method (Abbott Laboratories, Chicago, Ill.) was employed, with minor modifications for

serum thyroxine analysis. Briefly, the methods were as follows: In the regular procedure, 1 ml of serum was taken from the collected sample which had been thawed and brought to room temperature. Two ml of 95% ethanol were added to the serum sample. The solution was mixed immediately and thoroughly by a Vortex mixer (Evanston, Ill.) for 30 seconds and covered with parafilm. After standing for 10 min. at room temperature, the mixture was centrifuged at 2000 RPM for 20 min. The denatured protein precipitate was packed firmly at the bottom of the tube. Three-tenth ml of alcoholic extract (95% ethanol) from serum was evaporated by a steady dry air stream, while the samples were warmed in a water bath (35-45^{o}C). After complete drying 1.0 ml of TBG - ^{125}I - T_4 was added to the tubes and they were equilibrated at room temperature for 10 minutes. Then, the sample tubes were put into an ice-filled Dubnoff shaker (0.5 - 2^{o}C) for 5 minutes. At 20 second intervals one resin-impregnated sponge was placed successively in each tube and the air was squeezed out by depressing each sponge 5 times with moderate pressure, using the special Abbott plunger.

The count of the total labeled quantity of TBG - ^{125}I - T_4 was obtained for each tube during the incubation period 30 minutes after adding each sponge (Initial Count, I). At exactly 1 hour of incubation the reaction was stopped in successive tubes at 20 second intervals by

adding 10 ml of distilled water, depressing the sponge 5 times with the special Abbott aspirator tube and immediately drawing off the solution. The sponge was washed 3 more times with about 10 ml of distilled water to remove residual labeled TBG. The final counts were taken after four washes (F). A scintillation well counter (Nuclear Chicago, Model DS-5), and analyzer/scaler (Nuclear Chicago, Model 8725) was employed for the radioactivity measurements. The percentage uptake of resin-sponges was obtained by using the following formula.

$$\text{Sponge Uptake \%} = \frac{\text{F (CPM) - background (CPM)}}{\text{I (CPM) - background (CPM)}} \text{ X } 100$$

2) Standard Curves

A standard T_4 curve was run with the determinations done on any one day. The primary standard was crystalline free thyroxine purified in this laboratory from monosodium thyroxine pentahydrate obtained from Baxter Laboratories, Morton Grove, Ill. The free thyroxine showed only a single component when checked by thin layer chromatography. The standard stock solution was prepared by dissolving exactly 10 mg of T_4 in 95% ethanol with the aid of NaOH solution and diluting with ethanol to a concentration of 5 µg per ml. Concentration of the working standard was 0.05 µg per ml. When kept refrigerated this could be used 2-3 months without deterioration. A series

of standards of varying concentrations was set up for each standard curve and carried through the same procedure as the alcohol serum extracts as already described.

Because a 0.3 ml aliquot of the supernatant alcoholic serum extract was evaporated to dryness, the T_4 in the dried sample was one-tenth of the amount in 1 ml of serum, or 1/1,000 of the amount in 100 ml serum. Based on this reasoning, the amount of working standard solution for the standard curve was computed by the following calculation, and expressed as T_4 in µg/100 ml:

$$\frac{T}{1{,}000 \times C} = V \text{ (ml)}$$

where,

T = the T_4 equivalent of the serum extract expressed as µg per 100 ml

C = concentration of standard solution (0.05 µg/ml)

V = volume of standard solution

When T_4, expressed as µg/100 ml, is plotted on the abscissa of coordinate graph paper against % ^{125}I count in the resin sponge a standard curve can be plotted. In our early experiments it was found that in nearly all of the species studied the serum T_4 concentration did not exceed 10 µg/100 ml. In the range 0 - 10 µg T_4 per 100 ml a perfectly linear function between T_4 concentration and resin sponge count was found. Thus, instead of reading

values from a standard curve, the data were fitted by the method of least squares, and the T_4 values were calculated by use of the regression equation. The calculations are as follows:

$$X_u = \frac{Y - a}{b}$$

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

Y = % resin-sponge uptake

a = intercept of the Y-axis

b = slope of the standard curve

In setting up this method we found a 77.3% efficiency for extraction of T_4 from serum with 95% ethanol.

The extraction efficiency in the present study was determined in the following manner. One ml of rat serum was mixed with 0.02 ml ^{131}I - T_4 (Abbott Laboratories). After 27 hrs incubation in the refrigerator (-0.5°C), the first radioactivity measurement was obtained in the well counter. Two ml of 95% ethanol were mixed with the solution and treated as described previously for serum samples. The second count was obtained from 1 ml of the alcoholic extract, and the percent efficiency was calculated from the difference between these counts.

To obtain true T_4 values, the results had to be corrected for extraction efficiency, as follows:

$$X_c = X_u \times \frac{100}{77.3}$$

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

Thyroxine free acid contains 65.34% iodine. Using this figure, PBI equivalent in µg/100 ml of serum T_4I was computed from the thyroxine values (X_c):

$$\text{PBI Equivalent } (T_4\text{-I}) = X_c \times 0.6534$$

The volume of the aliquot of alcoholic extract evaporated varied according to the expected serum thyroxine levels obtained in earlier experiments.

3) Tri-iodothyronine (T_3) Interference Test

Ten mg of triiodo-L-thyronine (T_3, Smith, Kline and French Co., Philadelphia, Pa.) were dissolved in 95% ethanol and diluted to a final concentration of 0.05 µg/ml. A volume of T_4 stock solution equivalent tb 5 µg/100 ml was placed in a series of six polypropylene tubes. T_3 stock solution equivalent to 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 µg/100 ml was then added to successive tubes. The mixtures were evaporated to dryness and the T_4/100 ml was measured in the manner described.

RESULTS AND DISCUSSION

Standard Curve

Fig. 3 shows a representative standard curve. The known amounts of T_4 used were 0.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 µg/100 ml. A clear linear relationship can be seen between the known T_4 concentrations and % resin-sponge uptake at the T_4 concentration range from 0.0 to 10.0 µg/100 ml. These results agree with previous investigations (Murphy and Jachan, 1965; Nakajima et al., 1966). However, the curve approaches a plateau beyond the T_4 concentration of 10.0 µg/100 ml or an absolute amount of 0.01 µg T_4. Therefore, in the present study, at serum T_4 levels below 10.0 µg/100 ml the previously described methods of computation were used.

In Table 11, the components of regression of standard curves from eight experimental periods are shown. It is noteworthy that the correlation coefficients (r) are in the range of 0.990-0.999. However, the intercepts of the Y-axis (a) and slopes (b) are rather scattered. In comparing 40 pairs of known T_4 concentrations and their predicted values obtained from the respective regression formulae for each run, the paired t-test showed no significant difference

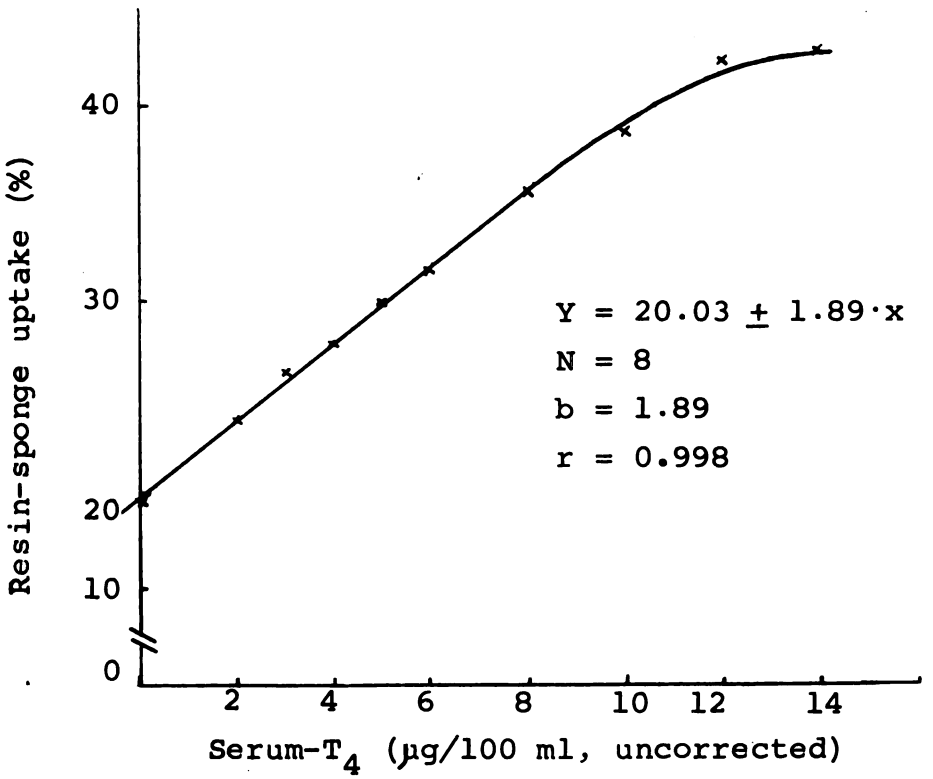

Figure 3. Standard curve for serum-T_4 determination.

TABLE 11

COMPONENTS OF REGRESSION LINES
OF STANDARD CURVES

Experimental period	a	b	r	TBG Lot No.	No. of known T_4 values
10-10	16.71	1.56	.992	034	5
12-3	26.85	3.84	.990	042	6
12-6	25.13	4.02	.990	042	5
12-9	29.32	3.17	.970	043	5
12-12	24.78	3.82	.999	043	4
1-22	25.79	3.03	.998	050	5
1-23	22.74	4.39	.996	050	5
1-24	21.93	4.01	.990	0.50	5

a: Y intercept, 6: slope, r: correlation coefficient.

The known T_4values, and their predicted T_4 values obtained from their respective regression equations for 40 pairs are compared.

The means of differences between known and predicted values is 0.067 $\pm$ 0.054 μg/100 ml. (Paired observations student, t test: $P > 0.1$; Li, 1964).

($P > 0.1$, Table 11). Despite the good agreement shown above, a standard was run with each day's determinations to obtain the greatest possible accuracy in the experimental data.

Serum T_4 values in human subjects corrected for absolute extraction efficiency as reported by previous workers are: in euthyroid men 8.26 µg/100 ml and 8.58 µg/100 ml in euthyroid women (Murphy, Pattee, and Gold, 1966); euthyroid patients, 5.8-11.9 µg/100 ml (Kennedy and Abelson, 1967) and 5.2 - 14.3 µg/100 ml (Kaplan, 1967). Abbott Laboratories (Tetrasorb-125 Kit instructions, 1968) has performed the tetrasorb-125 test on 96 "walking" normal subjects and report the range of 5.3-14.5. These ranges seem rather wide for normal human subjects, even when taking into consideration the modifications of the method in each of the laboratories.

From the ranges of previous authors it is interesting to notice that at the higher concentration levels there is more scatter than at the lower levels. The data at lower levels are more reliable than at higher levels, based on the standard error (S.E.). This is probably due to the inflection of the standard curve at higher T_4 concentration levels. This fact was also pointed out by Nakajima *et al*. (1966). There are greater reading errors at the inflected portion of the curve. Amounts of serum alcoholic extract taken for analysis in the present work have been adjusted to keep the

TABLE 12

NORMAL RANGE FOR SERUM THYROXINE MEASURED BY DIFFERENT LABORATORIES

Laboratories	Date	Serum Thyroxine Normal Range µg/100
Murphy and Pattee	1966	5.2 - 14.3
Nakajima, et al.	1966	5.8 - 11.9
Kennedy and Abelson	1967	5.5 - 10.2
Kaplan	1967	5.2 - 14.3
Abbott Laboratories	1968	5.3 - 14.5
	$\bar{X}$	5.4 - 13.0
	S.E.	0.11 - 0.87

readings at 1 - 10 μg/100 ml T_4 equivalent, of course it is necessary to take the size of aliquot into account in the final calculation.

T_3 Interference

The results of the T_3 interference test are summarized in Table 13. It is of interest that at a T_3: T_4 ratio of 1.5 detectable interference was not found. This is approximately the ratio at which these compounds are found in the rat thyroid (Pitt-Rivers and Rall, 1961). At the T_3:T_4 ratio of 2:5 the interference was negligible. At narrower ratios there is some further increase in interference, but it is highly unlikely that such ratios would be found physiologically in blood serum.

Other investigators (Murphy and Pattee, 1964; Kennedy and Abelson, 1967) demonstrated according to the author's calculation a 40 : 8 (T_3 : T_4) ratio in blood serum gave approximately a 35% increase in the T_4 measurement.

Individual Species

The data on serum T_4 levels in all species for which it was determined are presented in Table 14.

Limulus

This is the only invertebrate species studied. It was of interest in this work because invertebrates do not

TABLE 13

T_3 INTERFERENCE**

Known T_4 (µg %)	Known T_3		T_4 equivalent found*	
	µg %	T_3 as % of Total Hormone	µg %	%
5	-	-	5.34	-
5	1.0	16.7	5.34	0
5	2.0	28.6	5.63	5.4
5	3.0	32.5	6.23	16.7
5	4.0	44.4	5.93	11.0
5	5.0	50.0	5.93	11.0

**(See text).

*The standard curve for predicting T_4 values: (T_4 known concentration 0.0 - 8.0 ug/100).

$Y = 25.11 + 3.35 \cdot x$

$r = 0.99$

$n = 7$

TABLE 14

SERUM T_4 AND T_4-I MEASUREMENTS OF DIFFERENT SPECIES

Species	Serum T_4 (μg/100 ml)	T_4-I** (μg/100 ml)
Limulus (4)*	?	?
Fishes (5) (Rainbow trout)	0.75 ± 0.18[Δ]	0.49 ± 0.12
Frog (3)	0.89	0.58
Turtles		
Ice packed 5°C (6)	0.04 ± 0.02	0.03 ± 0.01
Room temperature (6)	0.60 ± 0.15	0.39 ± 0.10
Chickens		
Males (6)	1.06 ± 0.09	0.69 ± 0.06
Females	0.76 ± 0.11	0.68 ± 0.19
Quail		
Males (5)	1.37 ± 0.02	0.90 ± 0.01
Females	1.08 ± 0.05	0.70 ± 0.03
Opossum (6)	3.78 ± 0.33	2.47 ± 0.21
Rats		
Males		
one-year-old (11)	5.42 ± 0.36	3.54 ± 0.24
85 days old (8)	3.78 ± 0.33	2.50 ± 0.21
Thyroidectomized (7)	0.23 ± 0.13	0.15 ± 0.09
Females		
Mature virgin (10)	3.94 ± 0.15	2.57 ± 0.10
Immature virgin (10)	3.45 ± 0.15	2.23 ± 0.10
Open, non-lactating 19 days after weaning (9)	5.92 ± 0.22	3.87 ± 0.14
15 days pregnant (10)	3.24 ± 0.33	2.12 ± 0.21
Dogs		
Normal Females (5)	1.05 ± 0.17	0.69 ± 0.11
Thyroidectomized		
14 days (6)	0.07 ± 0.02	0.05 ± 0.03
35 days (6)	0.42 ± 0.05	0.28 ± 0.03

TABLE 16 (cont'd.)

Species	Serum T_4 ($\mu g/100$ ml)	T_4-I** ($\mu g/100$ ml)
Ewes		
Suffolk		
Yearlings (5)	13.54 ± 0.83	8.85 ± 0.54
Non-pregnant non-lactating (4)	13.22 ± 0.35	8.64 ± 0.23
Pregnant (6)	11.35 ± 0.82	7.42 ± 0.54
Hampshire (7)	8.59 ± 0.80	5.61 ± 0.52
Cows (Holstein)		
Dry, open (3)	6.20 ± 0.17	4.05 ± 0.11
Dry, Pregnant (10)	5.55 ± 0.25	3.63 ± 0.16
Horses (5)	2.43 ± 0.23	1.59 ± 0.15
Goat (7)	9.12 ± 0.78	5.46 ± 0.51

*Number of observations. $^{\Delta}$Mean ± S.E.

**Serum T_4 X 0.6534.

have thyroids. Rather surprisingly, the results were significantly negative. This implys that an ethanol-extractable substance is present in the Limulus serum that competes for the binding sites on the resin-sponge. This would reduce capacity of the sponge to adsorb labeled T_4. The suspected substance is probably hemocyanin, because Limulus is the only species used in which this compound has been described. Significantly negative results were not obtained for any mammalian species. It was not expected that measurable levels would be found in Limulus. However, the suspected interfering substance invalidates the results obtained. Consequently, no conclusions can be drawn.

The author is not aware of any other T_4 measurements in this species.

Fish

Hoffert and Fromm (1959) employed the T_4 substitution method to measure the thyroid secretion rate (TSR) of rainbow trout (Salmo gairdnerii). The TSR value reported for the fish at 13°C water temperature and with 2% iodized salt in the feed was 0.244 µg L-T_4 /100/gm/day. It was found by Hunn and Reineke (1964) that the PBI in hatchery-fed trout kept in water with 1 or less than 1 µg ^{127}I/L was 6.2 µg %. In the present study the average serum T_4 was 0.75 $\pm$ 0.18 µg/100 ml serum and T_4-I was 0.49 _ 0.12 µg/100 ml serum. A high correlation between serum T_4 and PBI values has been

repeatedly shown in human subjects (Murphy and Pattee, 1964; Nakajima et al., 1966; Murphy, et al., 1966; Kennedy and Abelson, 1967). It was also shown that T_4-I values tend to be higher than the values for PBI (Murphy and Pattee, 1964). PBI methods are not 100% efficient in recovering iodine from serum and Bodansky, Benun and Pennachia (1958) indicated that iodine extraction efficiency for PBI methods is around 90% depending upon the PBI method and technique used. Also, PBI values reported are rarely corrected for recovery. However, Murphy and Pattee (1964) compared their own T_4-I results and the PBI results of Bondansky et al. (1958) after being corrected for recovery. The PBI from 100 apparently normal human subjects showed a mean of 7.1 $\pm$ 1.5 µg/100 ml and T_4-I from 40 euthyroid subjects was 6.6 $\pm$ 1.3 µg/100 ml.

The PBI of fish (Hunn and Reineke, 1964) is about twelve times higher than T_4-I found in the present study under similar conditions. Because the inorganic iodine and iodine loosely bound to protein which appears abundantly in fish will not interfere with serum T_4 analysis, the present T_4-I data are more reliable than PBI values.

Turtle

The serum T_4 in the first group of turtles is surprisingly low (0.04 $\pm$ 0.02 µg/100 ml). However, the blood samples were taken after the turtles had been packed in ice

for more than 12 hours. Whether this is the reason for the low thyroxine values has to be considered.

A second group of turtles of the same species was used for serum T_4 analysis to check this point. Instead of using cold anesthesia these turtles were kept at room temperature for 3 days--then killed by a blow on the head and immediately bled. The serum T_4 in this group was 0.60 ± 0.15 µg/100 ml. As described previously, in animals with lower expected serum T_4 values, the volume of alcoholic extract used in the analysis was increased to give values on the standard curve equivalent to 1-10 µg/100 ml. The values reported were corrected finally in proportion to the size of aliquot taken. Thus, even the values that approach zero are reliable.

As described in the first part of the thesis, the TSR of scrotal thyroid implants was tremendously reduced due to the depression of local tissue temperature. Hoffert and Fromm (1959) reported that in the rainbow trout the TSR was reduced significantly with $10^{\circ}C$ depression of water temperature. This is probably due to the same reason that the ice packed turtles had a much lower serum T_4 than the turtles that were maintained at laboratory temperature. A key enzyme system that is responsive to TSH stimulation was probably inhibited by lowering local tissue temperature.

The control over general metablic rate and heat production in homeothermic vertebrates is perhaps the best

known property of thyroid hormone. However, Gorbman (1959) reviewed the findings in "cold blood" animals. The control over respiratory metabolic rate is not the function of thyroxine in these animals. Shellabarger et al. (1956) demonstrated an unusually high uptake of ^{131}I in the turtle thyroid. He reported a maximum 80% uptake of injected dose by thyroids of turtles maintained in the dry condition and suggested that ^{131}I injected had been reabsorbed from the urinary bladder and taken up by the thyroid. This process is slow, but nevertheless showed that the uptake of ^{131}I in turtles is not closely linked with thyroxine production (Berg, Gorbman, and Kobayashi, 1959).

The report of Shellabarger et al. (1956) also demonstrated in turtles that thyroid function is directly related to the environmental temperature. Thus, in animals which hibernate during low environmental temperatures, thyroid function is low. In the present study, the first group of turtles were packed in ice for 12 hours; this would depress both the thyroid responsiveness to TSH and TSH release. Consequently, the serum T_4 approached zero.

Frog

In Rana pipiens T_4, T_3, DIT, and MIT have been shown to occur in the thyroid (Berg et al., 1959). However, the quantity of T_4 in blood serum has not been measured. In the present study the T_4 measurements were taken from a pooled

blood sample of 15 adult frogs. Due to the fact that the frogs were from a commercial source, the season when the frogs had been captured was not known. The mean T_4 value found in triplicate determinations was 0.89 µg/100 ml. It is known that thyroid function in cold blooded animals is not related to respiratory metabolism (Gorbman, 1959), and it is also known that T_3 (3,5,3' configuration) is 300 times more efficient than thyroxine in inducing Rana pipiens tadpole metamorphosis (Money et al., 1958). Thus, it is probable, the low T_4 level in blood is only needed for maintaining nerve excitability, conductivity, etc. in adult frogs as discussed by Gorbman (1959). It would be of interest, however, to explore the serum T_4 concentration in relation to age of the frogs after reaching adult form and seasonal changes occurring thereafter. It was not feasible to obtain such data in the present study.

Chicken

The average value of serum T_4 in male white leghorns was 1.06 ± 0.09 µg/100 ml and that of females was 0.76 ± 0.11 µg/100 ml. However, there is no significant difference between sexes by the Mann-Whitney U test. This is in close agreement with the findings of Mayberry and Hochert (1968) who reported a mean value for serum T_4 of 1.25 µg/100 ml in control white leghorns.

Singh, Reineke and Ringer (1967) reported that the PBI of chicks on a normal diet is 1.1226 µg/100 ml of plasma, which is quite low in comparison with mammalian counterparts. The lower PBI levels probably are due to a lack of a specific thyroxine binding alpha globulin in avian blood (Tata and Shellabarger, 1959). This is coupled with rapid utilization of thyroid hormone occurring in the blood ($t_{\frac{1}{2}}$ = 3-4 hr. Singh, Reineke and Ringer, 1967).

Quail

The author is not aware of any earlier reports on serum T_4 of quail. The results obtained in this study showed that there are significant differences between sexes ($P = 0.002$). Serum T_4 of the male quail is higher than in either sex of chickens. However, the female quail showed no significant difference from the values found in hens. This result agrees with the finding of Singh et al. (1967) on the differences between these species. However the T_4 - I value in the present study is far lower than the PBI value from Singh, et al. (1967).

Rat

The results of the Serum T_4 determinations for rats are shown in Table 13 and Fig. 4.

The mature males showed significantly higher serum T_4 values than did mature nulliparous females ($P < 0.01$).

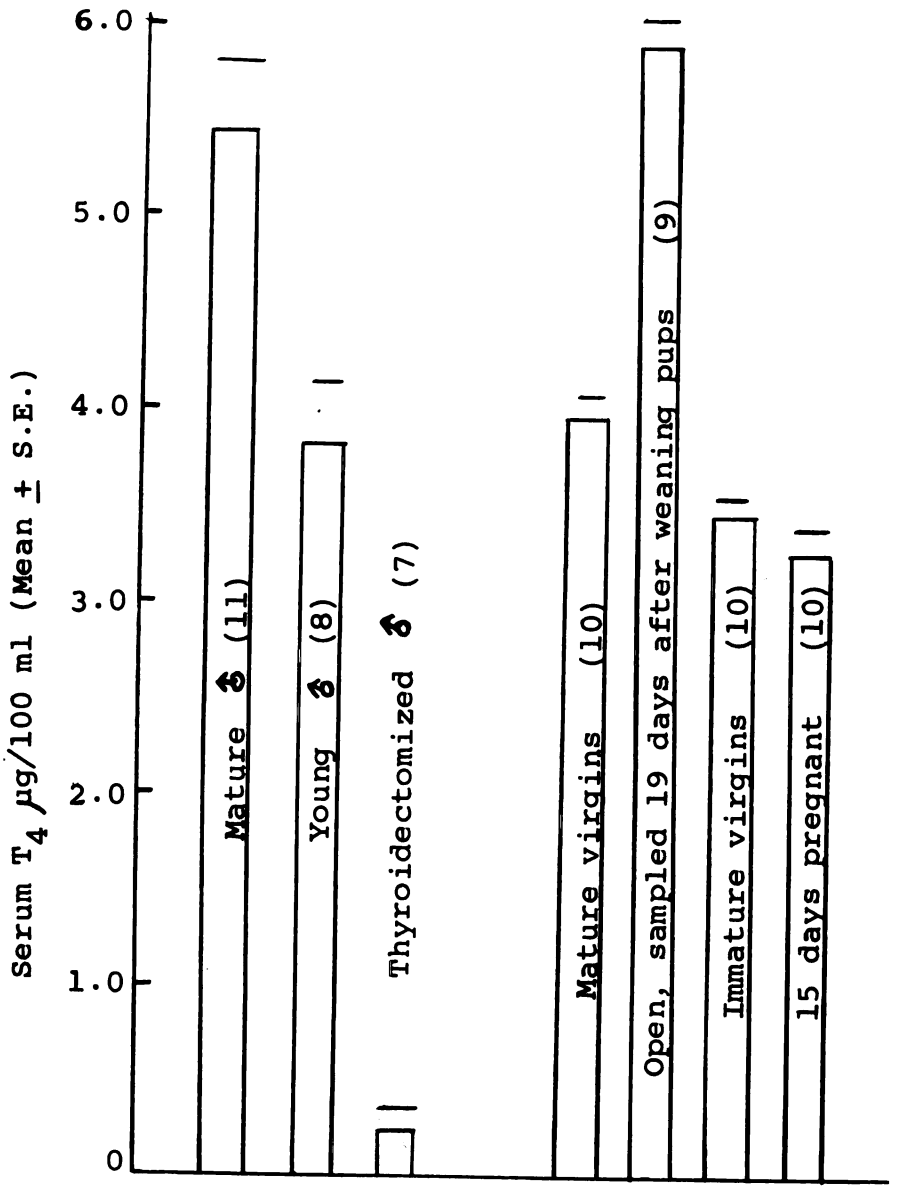

Figure 4. Serum T_4 in rats.

There are no differences among the groups of 1) young males, 2) mature virgins, 3) immature virgins, and 4) 15 day-pregnant rats ($P > 0.05$). There are also no significant differences between mature males and open non-lactating females. The thyroidectomized group showed significantly lower serum T_4 values than all other groups ($P < 0.01$).

It is known that the serum T_4 concentration is reduced in lactating rats (Lorscheider, Oxender and Reineke, 1969). The present data indicate a tremendous compensatory overshoot in serum T_4 after the young have been weaned. It is obvious that when lactation stops the competition between the mammary glands and the thyroid no longer exists. Thus, the thyroid will produce more hormone, stimulated by the elevated TSH in the blood during the preceding lactation.

Dog

The serum T_4 values of control female dogs are the lowest (1.05 ± 0.17 µg/100 ml) of any mammal tested in this research. They are on about a par with the values for birds and a little higher than the turtle or frog.

It is known that the PBI of this species is low. Barker (1948), reported a mean PBI of 2.3 µg/100 ml. Similar values were reported by O'Neal (1953). The technique of PBI measurement is beyond the scope of this report. However the Tetrasorb method appears to have greater sensitivity and

precision. It will clearly show the difference between normal and thyroidectomized dogs.

To illustrate this point, the serum T_4 of the same group of thyroidectomized dogs was analyzed twice. The first serum for T_4 measurement was taken 14 days after thyroidectomy (14-day T_4). The serum for the second measurement was taken 35 days after the operation (35-day T_4). The values were compared with those for serum from normal female beagles. The 14-day T_4 test showed that 3 out of 6 dogs had zero values. The mean for the group was significantly lower than for the controls. The 35-day T_4, values were significantly higher than the 14-day T_4. However, they were still less than ½ of the control values. None of the 35-day T_4 values were equal to zero. This increase was undoubtedly due to regeneration of thyroid tissues from residual fragments (Goldberg and Chaikoff, 1952). Nevertheless, the results suggest that the present method is able to detect very small differences in serum T_4 in dogs.

The dog serum obtained at 35 days post-operation showed a milky appearance which may indicate an increase of lipoid substances. Chaikoff and his coworkers (1941) and Thompson and Long, (1941) could only occasionally demonstrate a persistent increase in the serum cholesterol after thyroidectomy.

The thyroidectomized dogs in the present study did not show the clinical syndrome of hypothroidism that

myxedematous human beings developed within a few weeks. This agrees with previous investigations reported by Danowski, Man, and Winkler (1946).

Sheep

The serum T_4 determinations are presented in Fig. 5 and Table 13. In the Suffolk breed, there are no significant differences among the yearlings (13.54 $\pm$ 0.83 µg/100 ml); dry, non-pregnant ewes (13.22 $\pm$ 0.35); and pregnant Suffolk ewes (11.35 $\pm$ 0.82). However, serum T_4 showed a slight average decrease with increasing age, and during pregnancy.

The serum T_4 values of yearling Hampshires are significantly lower than those of Suffolks of the same age.

Henneman *et al*. (1955) employed the T_4 substitution method for TSR measurements of ewes. They reported that there is no difference between TSR of pregnant and non-pregnant ewes measured at the same time of the year. The present serum T_4 results give a similar indication that thyroid activity is not altered due to pregnancy.

A similar difference in thyroid function in different breeds of sheep was also found by Griffin, Henneman and Reineke (1962). They reported on TSR measurements in rams of the Hampshire and Shropshire breeds. However, the data presented here are for yearling lambs of the Suffolk and Hampshire breeds.

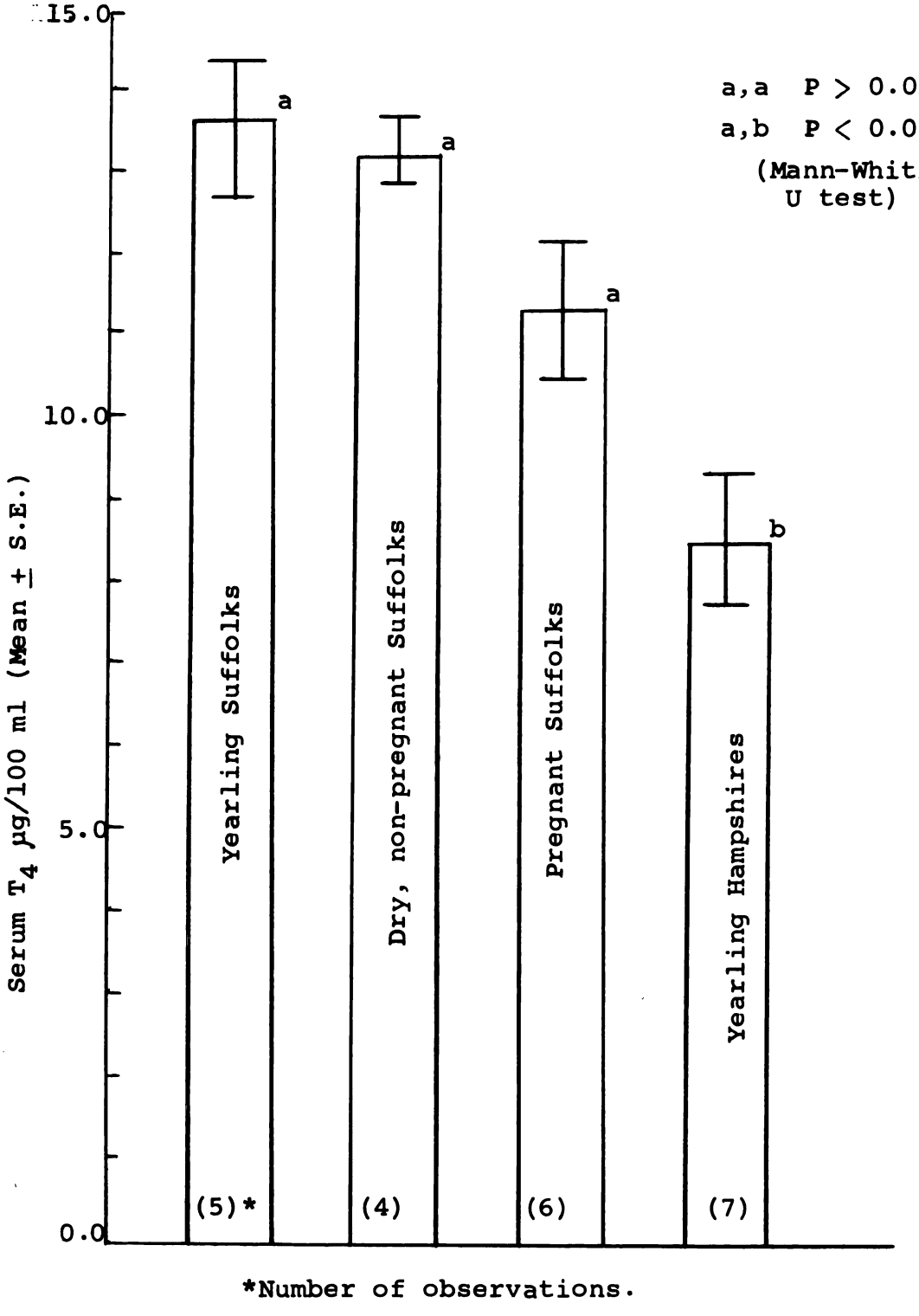

Figure 5. Serum T_4 measured in ewes.

Cow

Mature Holstein Friesian cows were sampled. These were dry non-pregnant, and dry-pregnant animals. The former group, containing three animals, showed an average T_4 of 6.20 ± 0.17 µg/100 ml serum; the latter group averaged 5.55 ± 0.25 µg/100 ml. The T_4-I values were 4.55 ± 0.50 and 3.63 ± 0.16, respectively. There is no significant difference between the groups. The T_4 - I was in the range of PBI reported by Lewis and Ralston (1953). However, their subjects did not receive iodine supplement in the ration. The PBI, thus might be expected to be lower. The Holstein PBI values reported by Long et al. (1951) averaged 3.15 µg/100 ml. This value is lower than reported in the present study.

Lorscheider, Oxender, and Reineke (1969) reported a lower serum T_4 in milking Holstein cows compared to non-pregnant non-lactating cows. It is interesting to note that the pregnant cow does not have an increased serum T_4 value as reported in pregnant women by Arango et al. (1968).

Horse

The mean serum T_4 value of 5 saddle-type horses was 2.43 ± 0.23 µg/100 ml, with a range of 1.80 - 3.19. This is equivalent to a T_4 - I level of 1.18 to 2.08 µg/100 ml. Excepting the dog data, these values are lower than those for any other mammals tested in this work.

Irvine (1967) reported on PBI of horses. He found that there is no difference between males (mean 1.82 μg/100 ml for colts, stallions, and geldings) and females 1.90; mares and fillies). Age (3-10 years) was not found to have a significant effect. He also found that in racehorses, training is associated with a 40% decrease in PBI values.

The results of T_4 - I derived from serum T_4 ranged from 1.18 to 2.08 μg/100 ml, and the mean is 1.59 ± 0.15 μg/100 ml. The reason for T_4 I being lower than the mean PBI values of previous investigators (1.86 by Irvine, 1967; 2.0 by Trum and Wasserman, 1956; 2.2 by Kaneko, 1964) is that only the iodine in the T_4 molecule is measured in the T_4 method. Thus, serum T_4 values more closely represent the thyroid functional status.

In a horse, suspected to be hypothyroid, the serum T_4 was 1.45 μg/100 ml; another horse that was suffering from inanition showed a serum T_4 level of only 1.39 μg/100 ml.

This study is mainly a general survey of serum T_4 on species that were available. Many other investigations can be done in this area.

Goats

Flamboe and Reineke (1959) investigated extensively the TSR of dairy goats in relation to age, pregnancy, lactation and seasonal change. However, the author is not aware any work has been done on serum T_4 level of nonlactating,

non-pregnant female goats. The mean serum T_4 values for the present determinations are 9.12 $\pm$ 0.78 µg/100 ml.

Opossum

In six adult male opossums the mean serum T_4 was 3.78 $\pm$ 0.33 µg/100 ml, and T_4 I was 2.47 $\pm$ 0.21. These results are quite different from the results of previous investigators (Katsh and Windsor, 1955). In their paper a mean PBI of 0.4 $\pm$ 0.2 µg% was reported. Unfortunately, there was no description of the animals they used except the species name (Didelphis virginiana). Thus, the lack of agreement cannot be explained. Because of its low metabolic rate and generally lower body temperature it was expected that the serum T_4 of the opossum would be lower than in placental mammals. However, this was not the case.

SUMMARY AND CONCLUSIONS - PART II

1. Experiments were conducted to determine serum thyroxine (T_4) in many species, ranging from fish to mammals.

2. The serum T_4 levels were measured by the principle of "competitive protein-binding" analysis of Murphy and Pattee, as developed by Abbott Radiopharmaceutical Laboratories in their Tetrasorb-125 diagnostic kit.

3. Mean serum T_4 values obtained were as follows: rainbow trout, 0.75 $\pm$ 0.18 μg/100 ml; frogs, 0.89; turtles, 0.04 $\pm$ 0.02 (cold anesthesized) and 0.60 $\pm$ 0.15 (maintained at room temperature); chickens, 1.06 $\pm$ 0.09 (males) and 0.76 $\pm$ 0.11 (females); Bobwhite quail, 1.37 $\pm$ 0.33 (males) and 1.08 $\pm$ 0.05 (females); rats, 5.42 $\pm$ 0.36 (older males) and 3.78 $\pm$ 0.33 (younger males), 3.94 $\pm$ 0.15 (mature females), and 3.45 $\pm$ 0.15 (immature females); dogs, 1.05 $\pm$ 0.17 (females); ewes, 13.22 $\pm$ 0.35 (Suffolk) and 8.59 $\pm$ 0.80 (Hampshire); cows, 6.20 $\pm$ 0.17 (dry, open) and 5.55 $\pm$ 0.25 (dry, pregnant); horses, 2.43 $\pm$ 0.23; goats, 9.12 $\pm$ 0.78 (dry, open females). Serum T_4 values for some species were also obtained after thyroidectomy and during pregnancy.

Contrary to the earlier reports of protein-bound iodine values in the opossum, the mean serum T_4 value is about the same as in young rats.

It was found that the dog, the only carnivore included, has lower serum T_4 than any other mammal studied, and this is close to the value for birds. The serum T_4 values for horses are next lowest to the dog.

Thus, the serum T_4 level does not follow the evolution of species in all cases, or depend on the physical or metabolic activity of the species.

4. It is known from earlier work that thyroid secretion rate per 100 gm body weight in chickens is comparable to that of other homeothermic animals. The lower serum T_4 is accompanied by a very rapid turnover of circulating hormone. Because of possible differences among species in several parameters of thyroid function it seems valid only to compare T_4 values within a species.

In studies in rats, it was shown that TSR was depressed due to decreased local temperature. A similar result was found in turtles, where after 12 hours of chilling the serum T_4 value was only 1/15 that for unchilled animals. The environmental temperature probably influences the neurohumeral control mechanism in cold blooded animals but there is no direct proof of this. Comparing these results with those for the scrotal

thyroid implants in rats, it is very likely that thyroid function is greatly reduced in cold blooded animals by the direct effect of cooling on the thyroid.

APPENDICES

APPENDIX I

FORMULAE AND COMPUTATIONS

I) Direct output method

1. ^{131}injection solution: preparation and standard dose

Carrier-free ^{131}I was taken according to need (in the present study 3 uc for each animal). Nine-tenths % NaCl solution was added to bring the volume to each injection dose. One-tenth of the dose was placed in a small glass cuvette, a few drops of anti-oxidant mixture (made from casein 200 mg, sodium iodide 100 mg, sodium bisulfate 100 mg, and sodium carbonate 100 mg and mixed with 30 ml of distilled water) was added and the solution was dried under a heat lamp. All standards were prepared in duplicate. The mean count per minute of the solution was taken during each counting period (S.C.). Then, S.C.-general background x 10 = injected count.

2. The first external count rate (E.C.) over the thyroid region was taken 48-72 hours following ^{131}I injection, and on alternate days thereafter. Radioactivity measurements were made with a scintillation counter which was connected to a count rate meter and a laboratory scaler (N.M.C. Indianapolis, DS - 1 B Scaler and

pulse height analyzer). A heavy lead counting table was used to give a constant geometry (Albert, 1951). In each case the thyroid tissue or the area to be counted were centered over the 25 mm tapered opening in the counting table that was focused over a 2-inch scintillation detector as mentioned above. Counts were taken over the epigastric region of each rat (Ep. C.) to represent the background count of the non-thyroidal tissue mass (Wolff, 1951). General background radioactivity was measured during the same period; this is designated as absolute background (Bk). For thyroid tissue counts, the rats were shifted about slightly until the maximum count rate registered on the count rate meter. A count was then registered on the scaler for one or more minutes, depending upon the count rate.

3. Computations of thyroid turnover data:

The equations presented in this section have been rearranged into the most convenient form to set up programs for the Olivetti-Underwood Programma 101 desk computer.

A. Calculation of percent uptake (U_1), (or percent of injected dose of ^{131}I.)

a. The percent uptake of ^{131}I by the thyroid in situ was obtained following this formula:

$$U_i = \frac{E.C. - \frac{Ep.\ C + Bk}{2}}{(S.C. - Bk) \times 10} \times 100 \qquad (1)$$

b. in case of the abdominal intramuscular implantation and abdominal subcutaneous implantation the tissue mass is approximately the same as that of the epigastric region; thus the formula used for percent ^{131}I uptake of the implants is as follows:

$$U_i = \frac{E.C. - (Epc + Bk)}{(S.C. - Bk) \times 10} \times 100 \qquad (2)$$

c. Percent uptake of scrotal implants:

In a group of 6 mature rats, the circumferences of the scrotum and epigastric region were measured. The radii from both measurements were obtained. Volume ratio between these two areas were approximately 0.3536 to 1. Thus, the body background counted for scrotally implanted thyroid tissue was estimated by using the count rate of the epigastic region corrected by the factor of 0.3536., as follows:

$$U_i = \frac{(E.C. - Bk) - [(Ep.C. - Bk) \times 0.3536]}{S.C. - Bk} \times 100 \qquad (3)$$

B. Computation of thyroidal ^{131}I output slope:

The U_i values (See equations 1 - 3) were transformed into logarithms (Log U_i), and equated against time in days (x) by the least squares method:

$$Y = a + bx \tag{4}$$

$$a = \frac{(\Sigma \text{ Log } U_i) - (\Sigma\ x\ \Sigma \text{ Log } U_i)}{N \Sigma\ x^2 - (\Sigma\ x)^2} \tag{5}$$

$$b = \frac{N(\ x \text{ Log } U_i) - (\ x)(\text{Log } U_i)}{N\ x^2 - (\ x)^2} \tag{6}$$

When: $x = 0$

$$Y = \text{Log } U_o = a \tag{7}$$

Then:

$$U_o = \text{antiLog } a$$

C. Computation of $t_{\frac{1}{2}}$:

When $$Y = \frac{\text{Log } U_o}{2} \tag{8}$$

$$x = t_{\frac{1}{2}}$$

Formula (4) can be transformed into:

$$\frac{\text{Log } U_o}{2} = \text{Log } U_o + b \cdot t_{\frac{1}{2}} \tag{9}$$

Then: $$t_{\frac{1}{2}} = \frac{\text{Log } U_o - \frac{1}{2} \text{ Log } U_o}{b}$$

$$= \frac{\text{Log } 2}{b}$$

$$= \frac{0.30103}{b}$$

or $$b = \frac{\text{Log } 2}{t_{\frac{1}{2}}} \tag{10}$$

4. Thyroidal ^{131}I output constant K_4):

The constant K_4 was calculated by the equations of Brownell (1951):

$$K_4 = \frac{K'_4}{1 - U_o} \tag{11}$$

$$K'_4 = 1 - e^{-\lambda t} \tag{12}$$

$$\lambda = \frac{\text{Log } e^2}{t_{\frac{1}{2}}} \tag{13}$$

From formula (10), the b value is the slope of the common Log plot, and λ is the natural Log slope. The λ value represents the physiological rate of release of radioactive compounds from the thyroid. This relation can be explained as follows:

$$A = A_o e^{-\lambda t} \tag{14}$$

Where, A = the amount of radioactive compounds left in the gland at time t. A_o = the original amount of radioactive compound.

Thus, when: $t = t_{\frac{1}{2}}$

$A = \frac{1}{2}A_o$

From (14) because, $\frac{1}{2}A_o = A_o e^{-\lambda t}$

$$\frac{1}{2} = e^{-\lambda t}$$

$$= \frac{\text{Log}_a 2}{t_{\frac{1}{2}}} \tag{15}$$

$$\text{or} \qquad t_{\frac{1}{2}} = \frac{\text{Log}_e 2}{\lambda} \qquad (16)$$

From time zero to time t the quantity left in the gland will be decreased according to the function, $e^{-\lambda t}$. However, the fraction secreted from the gland will vary according to $(1 - e^{-\lambda t})$. If counts are obtained daily (t = 1), the fraction secreted every day will be $1 - e^{-\lambda}$. Then

$$K'_4 = 1 - e^{-\lambda}$$

The true output rate, corrected for recycling of iodine by the thyroid will be,

$$K_4 = \frac{K'_4}{1 - U_o}$$

Where, U_o is the fractional ^{131}I uptake in the thyroid at zero time.

5. Thyroid secretion rate:

The thyroid secretion rates were calculated following the completed formula introduced by Reineke and Lorscheider (1967)

TSR - Thyroidal Iodine Content x K_4 x 1.53 x 1.52

A. Thyroidal Iodine Content (in μg) x K_4 = iodine in μg released daily in the form of thyroxine and triiodo-thyronine. Each thyroxine molecule contains

four atoms or 65.34% of iodine. Thus the factor 1.53 is used to convert iodine to T_4 equivalent.

B. The 1.52 factor.

In comparing T_3 and T_4 potency by the substitution method, the blocking dose for T_3 was 0.416 µg/100 gm body weight and for T_4 it was 1.7 µg/100 gm body weight. This means T_3 is 4.09 times as active as T_4 per unit body weight; however, T_3 contains only three iodine atoms. Then, per unit of iodine T_3 will be 4.60 times as active as T_4. Pitt-Rivers and Rall (1961) suggested that the secretion of T_3 and T_4 from the thyroid gland are in the same proportions in which they occur in the gland--3% and 18%, respectively. Thus, the amount of T_4 secreted daily, as calculated from Section I 5 A actually represents T_3 and T_4 in a ratio of 3 to 18. Then T_3 will be 14.3% and T_4 85.7% of the total amount of hormone released. 14.3% of T_3 actually has a potency of 0.143 x 4.6 = 0.658, expressed as T_4 equivalent and the contribection of T_4 itself will be 0.857.

Thus, in terms of T_4, the total hormonal potency will be the sum of 0.658 and 0.857, that is 1.52 (Reineke and Lorscheider, 1967).

II. Substitution Method (Reineke and Singh, 1955)

L-thyroxine in an increment of 0.5 ug/100 gm body weight was injected on alternate days starting 6 days after ^{131}I injection. Thyroid and body background radioactivity was determined as described in Sec. I. Percent injected dose were transformed to percent of previous percent injected dose in the following manner.

$$\text{\% pervious \% injected dose} = \frac{\text{\% injected dose of day n + 2}}{\text{\% injected dose of day n}} \times 100$$

The percent previous percent injected doses for each animal were plotted against dose of thyroxine injected per 100 gm body weight. The line of best fit was obtained by the least squares method. Using the prediction equation so obtained the estimated TSR was calculated, using 97.5% thyroidal ^{131}I retention as the end point.

APPENDIX II

THE DATA ON 'THYROID WEIGHT'

U_o (% uptake)	Thyroid Implant Weights (Subcutaneous) (mg)
1.81	8.0
2.66	9.5
2.13	12.0
2.10	4.0
6.26	12.0
5.55	15.0
1.96	7.5
1.92	5.0
10.26	17.0

$Y = 5.275 + 1.227 \cdot x$

$r = 0.82101$

$P < 0.01$

$P < 0.01$ ($F_{1,7} > 12.25$)

APPENDIX III

PARAMETERS MEASURED FOR AUTOIMPLANTATION STUDIES

I: Thyroidal Iodine (μg).

U_o: ^{131}I Uptake at Zero Time (%).

K_4: Thyroidal ^{131}I Turnover Rate.

TSR: Thyroid Secretion Rate (μg/day/rate).

'T.W.': Thyroid Weight as Predicted by U_o Values (mg).

STSR: Thyroid Secretion Rate as Estimated by T_4 Substitution Method. (μg/day/100 gm body weight)

No.	I	U_o	K_4	TSR	TSR/100	$T_{\frac{1}{2}}$	'T.W.'	STSR
Control Males								
I II-6	4.97	14.51	0.2400	2.828	1.01	2.96	23.08	1.83
I II-9	5.64	9.91	0.1759	2.313	0.761	4.017	17.44	2.14
I II-10	3.73	12.97	0.2494	2.165	0.742	2.832	21.19	1.73
III-21	7.58	8.74	0.1766	3.120	0.832	3.942	16.00	1.78
7-10	13.51	17.27	0.2165	6.813	2.505	3.511	26.46	1.48
7-11	15.25	12.85	0.1529	5.434	1.861	4.845	21.04	1.62
7-12	9.66	14.75	0.2393	5.388	2.041	3.037	23.37	1.71
10-8	15.90	7.52	0.1296	4.804	1.342	5.433	13.32	2.37
10-9	11.06	6.56	0.14532	3.745	1.146	4.748		2.26

No.	I	U_o	K_4	TSR	TSR/100	$T_{\frac{1}{2}}$	'T.W.'	STSR
10-10	19.45	9.608	0.1633	7.403	2.723	4.337	17.06	1.99
Mean	10.68	11.47	0.189	4.401	1.500	3.97	19.35	1.90
S. E.	1.67	1.11	0.010	0.586	0.233	0.280	1.36	0.86
Control Females								
I II-7	4.10	13.22	0.1640	1.568	0.933	4.513	21.51	2.20
II-8	6.66	8.11	0.2213	3.433	1.951	3.049	15.231	1.38
II-11	8.33	13.93	0.1237	2.4018	1.501	6.154	22.37	1.72
III-5	11.01	7.17	0.1598	4.101	2.868	4.316	14.08	1.53
IV-7	5.293	7.56	0.1671	2.612	0.996	7.204	14.55	1.75
10-11	12.77	7.31	0.1135	3.378	1.617	6.232	14.23	1.33
Mean	8.03	9.55	0.1583	2.916	1.644	5.25	16.99	1.75
S. E.	1.369	1.284	0.0156	0.368	0.291	0.629	1.575	0.131
Infant Subcutaneous group								
III-1	9.76	6.58	0.123	2.955	1.580	5.359	13.35	1.681
7-7	13.88	11.89	0.1870	6.379	1.880	3.848	19.864	1.600
IV-1	8.38	9.94	0.2363	4.613	1.955	2.897	17.465	
Mean	10.67	9.47	0.1823	4.649	1.805	4.040	16.89	
S. E.	1.644	1.550	0.0325	0.989	0.115	0.717	1.902	
Adult subcutaneous group								
1314-11	17.01	8.764	0.16618	6.584	1.995	4.212	16.03	1.078
1314-12	17.61	6.272	0.09903	4.063	1.228	7.114	12.97	1.601
1314-13	12.855	3.507	0.08549	2.561	0.747	8.049	9.578	1.240

No.	I	U_o	K_4	TSR	TSR/100	$T_{\frac{1}{2}}$	'T.W.'	STSR
1314-18	5.717	1.426	0.0974	1.298	0.447	6.865	7.03	1.355
1314-19	23.637	4.471	0.12243	6.743	2.240	5.571	10.76	1.110
1314-10	22.655	-	-	-	-	-	-	1.395
Mean	16.58	4.888	0.1141	4.250	1.331	6.393	11.27	1.30
S. E.	2.707	1.245	0.0141	1.078	0.346	0.667	1.528	
Infant Intramuscular group								
III-13	22.53	(16.098)	0.1759	(9.236)	(4.462)	(4.339)	-	1.954
IV-4	1.684	9.286	0.2413	0.947	0.373	2.805	16.67	1.914
IV-54	6.943	6.289	0.17206	2.833	0.889	3.941	12.99	1.82
10-2	5.525	9.181	0.12223	1.574	0.422	5.904	16.54	1.775
10-7	5.281	3.523	0.13055	1.607	0.497	5.153	9.720	1.925
								1.711
10-6	5.225	4.776	0.19414	2.365	0.785	3.786	11.135	-
Mean	7.865	6.63	0.1736	3.094	0.593	4.32	13.41	1.86
S. E.	3.018		0.0173	1.258		0.445	1.90	
Adult Intramuscular group								
1112-24	5.0825	5.617	0.10554	1.250	0.369	6.604	12.16	1.569
1112-25	10.04	5.927	0.0871	2.03	0.657	8.107	12.55	2.449
1314-15	18.265	10.095	0.16097	6.851	2.395	4.433	17.66	1.524
1314-16	6.222	4.903	0.2412	3.500	1.076	3.865	11.29	1.783
1314-17	15.713	9.067	0.1671	6.118	2.013	4.204	16.40	2.265
1314-20	4.1950	6.739	0.1248	1.220	0.469	5.599	13.544	1.765

No.	I	U_o	K_4	TSR	TSR/100	$T_{\frac{1}{2}}$	'T.W.'	STSR
1314-21	4.235	1.588	0.0863	0.852	0.321	7.807	7.224	2.902 1.642
Mean	9.108	6.277	0.1390	3.118	1.043	5.803	12.97	1.99
S. E.	2.186	1.058	0.02099	0.932	0.317	0.658	1.298	
Scrotal subcutaneous group								
1112-1	12.72	6.484	0.0143	0.424	0.158	51.51	13.23	-
1112-2	2.985	3.277	0.0215	0.150	0.064	32.99	9.295	-
1112-3	5.136	3.212	0.0063	0.076	0.029	113.19	9.216	-
1112-4	7.1975	5.120	0.05187	0.870	0.345	13.73	11.56	-
1112-6	11.175	6.062	0.03631	0.945	0.302	19.97	12.71	-
Mean	7.843	4.83	0.05201	0.630	0.179	46.279	13.41	
S. E.	1.820	0.684	0.024	0.149	0.063	17.935	1.403	

APPENDIX IV

SERUM THYROXINE MEASUREMENTS IN SEVERAL SPECIES (INDIVIDUAL VALUES)

Species	Serum T_4 μg per 100 ml	Means	S. E.
Limulus	-0.35, -0.24, -0.35, -0.24	?	?
Rainbow Trout	0.72, 1.44, 0.71, 0.44, 0.45	0.75	0.18
Frogs	0.91, 0.90, 0.87 (From pooled blood of 15 frogs)	0.89	
Turtles			
Ice packed	0.00, 0.03, 0.10, 0.08, 0.00, 0.03	0.04	0.02
Hammer killed	0.25, 0.18, 0.57, 0.51, 0.98, 1.09	0.60	0.15
Chickens			
Males	0.87, 1.30, 1.16, 1.30, 0.92, 0.82	1.06	0.09
Females	1.15, 0.70, 0.58, 0.82, 0.53	0.76	0.11

Species	Serum T_4 μg per 100 ml	Means	S. E.
Quail			
Males	1.37, 1.43, 1.31, 1.37, 1.40	1.37	0.02
Females	1.03, 1.20, 1.00, 0.89, 1.23, 1.12	1.08	0.05
Opossum			
Males, Adult	4.79, 3.58, 3.19, 4.79, 3.19, 3.19	3.78	0.33
Rats			
One-year-old mature males	4.60, 5.99, 5.57, 7.99, 4.47, 4.39, 5.44, 6.71, 5.26, 3.68, 5.48	5.42	0.36
85-day-old males	3.61, 3.30, 5.60, 4.38, 3.59, 2.30, 3.87, 3.58	3.78	0.33
34 days after thyroidectomy, males	0.00, 1.00, 0.12, 0.00, 0.34, 0.14, 0.03	0.23	0.13
Mature virgins	3.35, 3.95, 3.64, 3.68, 4.16, 4.11, 3.17, 4.16, 4.62, 4.54	3.94	0.15
Immature virgins	2.83, 3.33, 3.03, 4.32, 3.09, 3.88, 3.95, 3.53, 3.09, 3.41	3.45	0.15

Species	Serum T_4 μg per 100 ml	Means	S. E.
Open, Non-lactating--had pups weaned 19 days earlier	7.13, 5.29, 5.52, 5.52, 5.98, 5.47, 5.47, 4.78, 5.13	5.92	0.22
15 days of pregnancy	1.99, 2.34, 3.38, 1.99, 2.34, 4.08, 4.43, 3.03, 4.08, 4.76	3.24	0.33
Dogs			
Normal females, all pure bred beagles	1.22, 1.48, 1.18, 1.50, 0.86	1.05	0.17
Thyroidectomized			
14 days after			
Brittany spanial	0.30		
Mixed beagle	0.00		
Mixed beagle	0.00		
Pure beagle	0.00	0.02	0.07
Pure beagle	0.05		
Pure beagle	0.07		
35 days later, above dogs in the same order	0.27, 0.33, 0.57, 0.49, 0.39, 0.47	0.42	0.05
Ewes			
Yearling Suffolks	16.34, 13.15, 13.97, 11.16, 12.99	13.54	0.83

Species	Serum T_4 µg per 100 ml	Means	S. E.
Pregnant Suffolks			
106 days pregnant	10.53		
107	10.91		
86	8.19		
110	13.94	11.35	0.82
71	11.63		
71	12.92		
Yearling Hampshires	8.05, 7.01, 12.57, 10.14, 7.36, 6.66, 8.40	8.59	0.80
Horses			
Gelding, 7 yrs, Standard Bred	2.15		
Female, 2 yrs, Standard Bred	2.50		
Gelding, 6 yrs, Quarter Horse	2.50	2.43	0.23
Female, 4 yrs, Standard Bred	1.80		
Stallion, 1 yr, Arabian	3.19		
Cows			
Dry, open	6.32, 6.42, 5.86	6.20	0.17
Dry, pregnant	4.28, 5.30, 5.40, 5.71, 5.36, 7.39, 5.15, 5.59, 5.97, 5.35	5.55	0.25
Goat Dry, mature females	13.08, 7.90, 10.87, 7.85, 8.51, 7.97, 7.65	9.12	0.78

BIBLIOGRAPHY

Albert, A. 1951. The In vivo Determination of Biological Decay of Thyroidal Radioiodine. Endocrinol., 48: 334-338.

Arango, G., W. E. Mayberry, T. Hockert, and L. R. Elveback. 1968. Total and Free Human Serum Thyroxine in Normal and Abnormal Thyroid States. Mayo Clin. Proc., 43:501-516.

Arestov, N. M. 1964. (Significance of Innervation in Auto-transplantation of the Thyroid.) Probl. Endokrinol. Gormonoterap., 10:97-101.

Aron, M., O. Gros, A. Pertrovic and C. Gegauff. 1956. Fixation d'I^{131} parlles Greffons Thyroidiens Intra-testicularies chez le cobaye. Compt. rend. Assoc. Anat., 2:212.

Barker, S. B. 1948. Determination of Protein-Bound Iodine. J. Biol. Chem., 173:715-724.

Barker, S. B. and H. J. Humphry. 1950. Clinical Determination of Protein-Bound Iodine in Plasma. J. Clin. Endocrinol., 10:1136-1141.

Beltz, A. D. and E. P. Reineke. 1968. Thyroid Secretion Rate in the Neonatal Rat. Gen. Comp. Endocrinol., 10:103-108.

Bennett, D. and A. Gorbman. 1951. Re-establishment of Function in Transplanted Thyroid Gland of Mice. Endocrinol., 49:310-317.

Berg, O., A. Gorbman and H. Kobayashi. 1959. The Thyroid Hormones in Invertebrates and Lower Vertebrates. In: Comparative Endocrinology. John Wiley and Sons, Inc., N. Y. Proceedings of the Columbia University Symposium on Comparative Endocrinology, held at Cold Spring Harbor, N. Y., 1958.

Birnie, J. H. and F. E. Mapp. 1962. Replacement Therapy by Thyroid Tissue Growth in Culture. Fed. Proc., 21: 214.

Bodansky, O., R. S. Benun and G. Pennacchia. 1958. The Rapid Procedure for Determination of Total and Protein-Bound Iodine in Serum. Am. J. Clin. Path., 30:375.

Bondy, P. K. 1951. Maintenance of Normal Thyroid Activity after Transplantation of Thyroid Gland into Spleen or Kidney. Proc. Soc. Exptl. Biol. and Med., 77: 638-640.

Brody, S. 1945. "Bioenergetics and Growth," pp. 404 and 405. Reinhold, New York City.

Brownell, G. L. 1951. Analysis of Techniques for the Determination of Thyroid Function with Radioiodine. J. Clin. Endocrinol., 11:1095-1105.

Cameron, C. 1952. Pathology of the Cell, p. 350 and p. 546. Charles C. Thomas, Publisher.

Chaikoff, I. L., C. Entenman, C. W. Changus and F. L. Reichert. 1951. Influence of Thyroidectomy on Blood Lipids of the Dog. Endocrinol., 28:797-805.

Contopoulos, A. N., M. E. Simpson and A. A. Koneff, 1958. Pituitary Function in the Thyroidectomized Rat. Endocrinol., 63:642-653.

Danowski, T. S., E. B. Man and Winkler, 1946. Tolerance of Normal, of Thyroidectomized, and of Thiourea or Thiouracil Treated Dogs to Oral Desiccated Thyroid and to Intravenous Thyroxine. Endocrinol., 38:230-237.

Dameron, J. T. 1952. The Effect of Thyrotropic Hormone and Propylthiouracil on Homologous Thyroid Transplants. Surgical Forum, 3:681-685.

de Jongh, S. E. and Paesi, F. J. A. 1958. The ICSH-concentration in hypophysis of immature and adult rats. Additional remarks on somatotrophin and FSH. Acta Endocrinol., 29:413-418.

Demikhov, V. P. 1960. Experimental Transplantation of Vital Organs. (Translated to English by Basil Haigh Consultants Bureau, New York.)

Dempster, W. J. and I. Doniach. 1955. The Survival of Thyroid Implants in Relation the Thyroid Deficiency. Arch. Int. Pharmacodyn., 101:398-415.

Dempsey, E. W. and E. B. Astwood. 1943. Determination of the Rate of Thyroid Hormone Secretion at Various Environmental Temperatures. Endocrinol., 32:509-518.

Dempster, W. J. 1955. The Transplanted Adrenal Gland. Brit. J. Surg., 42:540.

Dévényi, I., B. Czenkar and P. Endes. 1958. Homotransplantation of Foetal Thyroid Glands to Temperarity Cortisone-Treated Rats. Acta Morphologica Academiae Scientiarum Hungaricae, 8:59.

Ekins, R. P. 1960. The Estimation of Thyroxine in Human Plasma by An Electrophoretic Technique. Clin. Chem. Acta, 5:453-459.

Flamboe, E. E. and E. P. Reineke. 1959. Estimation of Thyroid Secretion Rates in Dairy Goats and Measurement of I^{131} Uptake and Release with Regard to Age, Pregnancy, Lactation, and Season of the Year. J. Animal Sci., 18:1135-1148.

Glydon, R. St. J. 1957. The Development of Blood Supply of Pituitary in Albino Rat, with Special Reference to Portal Vessels. J. Anat., 91:237-244.

Goldberg, R. C. and I. L. Chaikoff. 1952. Myxedema in the Radio-thyroidectomized Dog. Endocrinol., 50:115-123.

Gorbman, A. 1959. Problems in the Comparative Morphology and Physiology of the Vertebrate Thyroid Gland. In: Comparative Endocrinology. John Wiley and Sons, Inc. Proceedings of the Columbia University Symposium on Comparative Endocrinology, held at Cold Spring Harbor, N. Y., 1958.

Gough, M. H., D. E. Pugh, J. R. Brook. 1962. Thyroid Homotransplantation in the Dog: The Failure of Long Term Survival in Diffusion Chamber. Surgery, 52:144.

Gregerman, R. I. 1963. Estimation of Thyroxine Secretion Rate in the Rat by Radioactive Thyroxine Turnover Technique: Influence of Age, Sex and Exposure to Cold. Endocrinol., 72:382-392.

Griffin, S. A., H. A. Henneman, E. P. Reineke. 1962. The Thyroid Secretion Rate of Sheep as Related to Season, Breed, Sex, and Semen Quality. Am. J. Vet. Res., 23:109-114.

Halsted, W. S. 1909. Auto- and Isotransplantation in Dogs of the Parathyroid Glandules. J. Exptl. Med., 11: 175-199.

Henneman, H. A., E. P. Reineke and S. A. Griffin. 1955. The Thyroid Secretion Rate of Sheep as Affected by Season, Age, Breed, Pregnancy and Lactation. J. Animal Sci., 14:419-434.

Héroux, O. 1963. Patterns of Morphological, Physiological, and Endocrinological Adjustments Under Different Environmental Conditions of Cold. Fed. Proc., 22:789-794.

Hill, R. T. 1937. Ovaries Secrete Male Hormone, III Temperature Control of Male Hormone Output by Grafted Ovaries. Endocrinol., 21:633-636.

Hoffert, J. R. and P. O. Fromm. 1959. Estimation of Thyroid Secretion Rate of Rainbow Trout Using Radioactive Iodine. J. Cellular and Comp. Physiol., 54: 163-169.

Hunn, J. B. and E. P. Reineke. 1964. Influence of Iodine Intake on Iodine Distribution in Trout. Proc. Soc. Exptl. Biol. and Med., 115:91-93.

Ingbar, S. H., C. Waterhouse and P. Cushman. 1964. Observation on the Nature of Underlying Disorder and the Occurrence of Associate Plasma Transport Abnormalities in a Patient with an Iodiopathic Increase in the Plasma Thyroxine Binding Globulin. J. Clin. Invest., 42:22-66.

Irvine, C. H. G. 1967. Protein-Bound Iodine in Horse. Am. J. Vet. Res., 28:1687-1692.

Kaplan, B. C. 1966. A Simple Method for the Determination of Serum Thyroxine. A talk presented before the Bioanalysis Section, American Association for the Advancement of Science 133rd Meeting, December 28, 1966.

Kaneko, J. J. 1964. Thyroid Function Studies in the Horse. Proc. 10th Ann. Meeting Am. Ass'n. Equine Practitioners. 125-130.

Katsh, S. and E. Windsor. 1955. Unusual Value for Protein-Bound Iodine in the Serum of Opossum. Science, 121: 897-898.

Kennedy, J. A. and D. M. Abelson. 1967. Determination of Serum Thyroxine Using a Resin Sponge Technique. J. Clin. Path., 20:89-94.

Kerkof, P. R. and I. L. Chaikoff. 1966. Follicular Reorganization and ^{131}I Utilization by Cultured Rat Thyroid Cells Implanted into Thyroidectomized Rats. Endocrinol., 78:1177-1188.

Kerkof, P. R., P. J. Long and I. L. Chaikoff. 1964. In Vitro Effects of Thyrotropic Hormone. I. On the Pattern of Organization of Monolayer Culture of Isolated Sheep Thyroid Gland Cells. Endocrinol., 74: 170-179.

Krohn, P. L. 1963. Transplantation of Endocrine Organs. In: Technique in Endocrine Research. Proceedings of a N. A. T. P. Advanced Study Institute 1962, Academic Press, Inc., New York. PP. 195-200.

Lance, E. M. 1967. A Functional and Morphologic Study of Intracranial Thyroid Allografts in the Dog. Surgery, Gynecology and Obstretics, 125:529-539.

Lewis, R. C. and N. P. Ralston. 1953. Protein-Bound Iodine Level in Dairy Cattle Plasma. J. Dairy Sci., 36: 33-38.

Li, J. C. R. Statistical Inference I. 1964 ed. Edwards Brothers, Ann Arbor, Michigan.

Long, J. F., L. O. Gilmore, G. M. Curtis and D. C. Rife. 1951. The Bovine Protein-Bound Iodine as Related to Age, Sex, and Breed. J. Animal Sci., 10:1027-1028.

Lorscheider, F. L., W. D. Oxender and E. P. Reineke. 1969. Serum Thyroxine in the Lactating Rat and Cow. Fed. Proc., 28:319.

Marine, D. 1932. The Thyroid, Parathyroids and Thymus. In: Special Cytology, pp. 797-868. Hoeber Inc., New York. (Ed. E. V. Cowdry).

Marshall, W. A. 1963. The Effect of Autotransplantation of Thyroid Gland on the Onset of Estrus in Ferrets. J. Endocrinol., 26:279-285.

Mayberry, W. E. and T. J. Hockert. 1968. Excess Iodide Ingestion and Thyroid Function in Chicks. Proc. Soc. Exptl. Biol. and Med., 129:370-373.

Money, W. L., R. I. Meltzer, J. Young and Rawson, 1958. The Effect of Change in Chemical Structure of Some Thyroxine Analogues on the Metamorphosis of Rana Pipians Tadpoles. Endocrinol., 63:20-28.

Murphy, B. E. P. 1964. Application of the Property of Protein-Binding to the Assay of Minute Quantities of Hormones and other Substances. Nature, 201:679-682.

Murphy, B. E. P. and C. Jachan. 1965. The Determination of Thyroxine by Competitive Protein-Binding Analysis Employing an Anion-Exchange Resin and Radiothyroxine. J. Lab. Clin. Med., 66:161-167.

Murphy, B. E. P. and C. J. Pattee. 1964. Determination of Thyroxine Utilizing the Property of Protein-Binding. J. Clin. Endocrinol. Metab., 24:187-195.

Murphy, B. E. P., C. J. Pattee and A. Gold. 1966. Clinical Evaluation of a New Method for the Determination of Serum Thyroxine. J. Clin. Endocrinol., 26:247-256.

Nakajima, H., M. Kuramochi, T. Horiguchi and S. Kubo. 1966. A New and Simple Method for the Determination of Thyroxine in Serum. J. Clin. Endocrinol., 26:99-100.

O'Neal, L. W. 1953. Plasma Protein-Bound Iodine After Intravenous Injection of Thyroxine in Thyroidectomized Dogs. Endocrinol., 53:358-366.

Paulik, L. J. 1969. Thyroid Secretion Rate as Influenced by Method of Measurement, Type of Anesthesia, and Route of Thyroxine Administration. M.S. Thesis, Michigan State University.

Phillips, J. B. and A. S. Gordon. 1954. A Study of the Pituitary-Thyroid Relations in the 1- to 15-Day-Old Rat. Anat. Record., 120:702-703.

Pitt-Rivers, R. and T. E. Rall. 1961. Radioiodine Equilibrium Studies of Thyroid and Blood. Endocrinol., 68:309-316.

Reineke, E. P. and F. L. Lorscheider. 1967. A Quantitative "Direct Output" Method for Determination of Thyroid Secretion Rate in the Rat. Gen. and Comp. Endocrinol., 9:362-367.

Reineke, E. P. and O. N. Singh. 1955. Estimation of Thyroid Hormone Secretion Rate of Intact Rat. Proc. Soc. Exptl. Biol. and Med., 88:203-207.

Rosenberg, L. L., G. LaRoche and J. M. Ehlert. 1966. Evidence for Heterogenous Turnover of Iodine in Rat Thyroid. Endocrinol., 79:927-934.

Rosenberg, L. L., M. Goldman, G. LaRoche and M. K. Dimik. 1964. Thyroid function in rats and chickens. Equilibration of Injected Iodide with Existing Thyroidal Iodine in Long-Evans Rats and White Leghorn Chickens. Endocrinol., 74:212-225.

Rupp, J. J. 1952. Action of Liver on Thyroid Hormone Following Intrasplenic Implantation of the Thyroid. Endocrinol., 51:306-310.

Russell, P. S. 1961. Endocrine Grafting Technique. In: Transplantation of Tissue and Cells. The Wistar Institute Press. Philadelphia.

Russell, P. S. and A. P. Monaco. 1964. The Biology of Tissue Transplantation. New Engl. J. Med., 271:502-510, 553-562, 610-615, 718-725, 776-783.

Schiff, von. 1884. Bericht über eine Versuchreihe betreffend die Wirkungen der Exstirpation der Schilddrüse. Arch. Exp. Path. und Parm., 181:25-31.

Seigal, S. Nonparametric Statistics for Behavioral Sciences. McGraw-Hill Book Company, Inc., New York, 1956.

Shellabarger, C. J., A. Gorbman, F. C. Schatzlain and D. McGill. 1956. Some Quantitative and Qualitative Aspects of ^{131}I Metabolism in Turtles. Endocrinol., 59:331-340.

Singh, A., E. P. Reineke and R. K. Ringer. 1967. Thyroxine and Triiodothyronine Turnover in the Chicken and the Bobwhite and Japanese Quail. Gen. and Comp. Endocrinol., 9:353-361.

_____, _____, _____. 1968. Comparison of Thyroid Secretion Rates in Chickens as Determined by (1) Goiter Prevention, (2) Thyroid Hormone Substitution, (3) Direct Output and (4) Thyroxine Degradation Methods. Poultry Science, XLVII:205-211.

Snell, G. D. 1964. The Terminology of Tissue Transplantation. Transplantation, 2:655-657.

Sterling, J. A. and R. Goldsmith. 1954. Total Transplant of Thyroid Gland Using Vascular Anatomoses. Surgery, 35:625-628.

Sterling, K. 1967. Thyroxine Binding in Serum. In: Radioisotopes in Medicine: In Vitro Studies. PP. 293-313. Proceedings of a Symposium Held at the Oak Ridge Associated Universities.

Swan, H., D. Jenkins and J. Schemmel. 1967. Thyroid Autograft: A 12-Year Follow-Up. Arch. Surg., 94:817-819.

Stewart, M. E. 1966. Thyroid Recovery after Cessation of Treatment with Thyroidal Substances. M.S. Thesis, Michigan State University.

Tata, J. R. and C. J. Shellabarger. 1959. An Explanation for the Difference Between the Responses of Mammals and Birds to Thyroxine and Triiodothyronine. Biochem. J., 72:608-618.

Tetrasorb-125 (T_4 Diagnostic Kit). 1968. Abbott Laboratories, Radio-Pharmaceutical Division.

Thompson, K. W. and C. N. H. Long. 1941. The Effect of Hypophysectomy Upon Hypercholesterolemia of Dogs. Endocrinol., 28:715-722.

Trum, B. F. and R. H. Wasserman. 1956. Studies on the Depression of Radioiodine Uptake by the Thyroid After Phenothiazine Administration. II. Effect of Phenothiazine on the Horse Thyroid. Am. J. Vet. Res., 17: 271-275.

Turner, G. W., G. W. Pipes and B. N. Premachandra. 1959. A Critique of Indices of Thyroid Function. Oklahoma Conference- Radioisotopes in Agriculture, U. S. Atomic E. Comm. 87, TID - 7578, pp. 97-103.

Williams, R. G. 1939. Observations on the Formation of New Follicles in Grafts of Thyroid Gland in Rabbits. Anat. Record., 73:307-317.

Williams, R. G. 1939a. Further Observations on the Microscopic Appearance and Behavior of Living Thyroid Follicles in the Rabbit. J. Morphology, 65:17-51.

Williams, R. G. 1953. Studies of Autoplastic and Homoplastic Grafts in Rabbits. Am. J. Anat., 93:1-23.

Wollman, S. H., R. O. Scow and B. Wagner. 1953. Radioiodine Uptake by Transplantable Tumor of Thyroid Gland in C3H Mice. I. Experimental Results. J. Nat'l. Cancer Inst., 13:785.

Wollman, S. H. and R. O. Scow. 1955. Comparison of Thyroid Lobes, Autotransplanted and *in situ*, in the Rat: Growth and ^{131}I Uptake. *J. Nat'l. Cancer Inst.*, 15: 943-947.

Wolff, J. 1951. Some Factors that Influence the Release of Iodine from the Thyroid Gland. *Endocrinol.*, 48:284-297.

Woodruff, M. F. A. and H. G. Woodruff. 1950. The Transplantation of Normal Tissues: With Special Reference to Auto- and Homotransplants of Thyroid and Spleen in the Anterior Chamber of the Eye and Subcutaneously in the Guinea Pig. *Phil. Trans. Roy. Soc. B.*, 234: 559-582.

Woodruff, M. F. A. 1960. The Transplantation of Tissue and Organs. PP. 57 and 472. Charles C. Thomas, Springfield, Ill.

Yasumura, Seichi. 1963. Growth and Function of Thyroid Grafts Implanted in the Rat Brain. Dissert. Abst., 24(5):1790-1791.