THE INFLUENCE OF VARIATIONS IN ENVIRONMENTAL TEMPERATURE AND THYROID STATUS ON GROWTH AND SEXUAL DEVELOPMENT IN THE MALE MOUSE

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

M. Maqsood Butt

1949

This is to certify that the

thesis entitled

"The Influence of Variations in Environmental Temperature and Thyroid Status on the Growth and Sexual Development of Male Mice"

presented by

M. Maqsood Butt

has been accepted towards fulfillment
of the requirements for

M.S. degree in Physiology

E. P. Reineke
Major professor

Date March 7, 1949

THE INFLUENCE OF VARIATIONS IN ENVIRONMENTAL TEMPERATURE AND THYROID STATUS ON GROWTH AND SEXUAL DEVELOPMENT IN THE MALE MOUSE

By

M. Maqsood Butt

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Physiology and Pharmacology

1949

ACKNOWLEDGEMENTS

The writer takes this opportunity to record his sincere appreciation to Dr. E. P. Reineke, Professor of Physiology, Michigan State College, for his encouragement, assistance and scholarly guidance throughout the course of this work and for his suggestions in the preparation of this manuscript; to Dr. B. V. Alfredson, Head of the Physiology and Pharmacology Department, for his aid in providing facilities in conducting this work; to Drs. J. Meites and L. F. Wolterink, Department of Physiology and Pharmacology, for their interest and valuable suggestions; to Dr. R. F. Langham, Department of Pathology for the photomicrographs and to Dr. L. B. Sholl, for providing facilities in his Pathological Laboratory for the histological studies.

The writer is also grateful to the Department of Education, Government of Pakistan, for the award of a scholarship to study in the U.S.A.

CONTENTS

CONTENTS (Continued)

INTRODUCTION

The scope of Endocrine Research is exceedingly broad and is interwoven with almost every phase of biology. With the recent advancements in the field of Endocrinology, it has been observed that complicated physiologic mechanisms exist which effect variations among the ductless glands in the formation and release of endocrine compounds, in accordance with the needs of the body for the substances. The functional capacity of the ductless glands is influenced directly or indirectly by the hormones elaborated by the other compounds of the endocrine system. Thus a state of balance is normally maintained between the various ductless glands and between themselves and other tissues and organs of the body.

It has been observed that many of the hormones are involved in a number of physiologic processes within the body instead of being concerned only with the adjustment of one particular process in the animal body. This is especially true in the case of thyroid as it plays an essential role in the physiological processes of growth, reproduction, lactation and other functions. A deficiency of thyroid hormone or an over-supply of it may reflect itself in demonstrable changes in many organs and functional processes of the body. The thyroid is a regulator of energy metabolism. In mild hyperthyroidism anabolic effects maybe

obtained in growing animals of some species while in severe hyperthyroidism, catabolic processes predominate. Since many tissues are dependent upon normal thyroid activity for normal functioning, it is apparent that the thyroid mechanism is one of the most important of the homeostatic mechanisms necessary for maintaining a constant internal environment in the midst of a constantly changing external environment.

After growth of the domestic animals is completed, the secretion of the thyroid gland gradually decreases and they tend to fatten. The breeding animals, both male and female may become sluggish and their reproductive organs less effective. Preliminary observations indicate that by replacement thereapy with thyroid materials, the slightly hypothyroid animal can be returned to a normal condition of metabolism. Accompanying this change, there will usually be an improvement in reproductive ability in both sexes. It appears likely that the activity of the endocrine glands may be modified favourably by selective breeding and such modifications will also result in improved productive ability among the live-stock.

As the thyroid gland appears to have some practical application in the field of live stock development, the present work was undertaken to study the influence of variations in environmental temperature and thyroid status on growth and sexual development in young male mice. The object was to apply the knowledge gained from work on the

laboratory mouse [illegible] to a consideration of similar problems in farm animals under different environmental conditions, especially in tropical countries where the thyroid secretion rate may be assumed to vary due to wide differences in seasonal temperatures, influencing thereby the various physiological phenomena of the animal body.

REVIEW OF LITERATURE

Role of Thyroid on Growth

Von Rapp (1840) was the first to report the effects of thyroidectomy in a ruminant. Thyroid ablation depresses growth in animals and when thyroidectomy is complete, the variation is largely due, however, to the marked effect of age on the incidence of growth retardation. In younger ruminants growth depression is marked (Simpson, 1924; Spielman et al. 1945 and others) while in the older animals such a depression is not so apparent. Administration of thyroid or iodinated casein to these animals completely restored growth processes. Engelbach (1932) states that the thyroid is responsible for the development of centers of ossification in infantile life. The pattern of growth depression in hypothyroidism varies considerably in various animals. Complete suppression of thyroid activity in the young ruminants is of little economic importance. Partial suppression of thyroid action in the young animal for short periods of time immediately before marketing might be of some value, if the fat deposition in the carcass could be increased, thereby improving market quality. The thyroid stimulates the growth in young animals and if the deficiency develops after the growth has been attained, then the skeletal derangements are not necessarily noticeable but myxedema, along with mental derangement are soon observed in the animal.

A study of the literature reviewed by Koger and Turner (1943) shows that mild hyperthyroidism in young animals accelerates growth but in severe hyperthyroidism, where the basal metabolism is considerably elevated, marked losses of weight and decreased fertility occur. In mice, thyroxine in minute doses increases growth rate fairly consistently (Robertson, 1928; Koger et al., 1943; Koger and Turner, 1943 and others). Reineke and McMillen (1946) reported a significant increase in the rate of gain and a slight increase in efficiency of food utilization by feeding small doses of thyroprotein (.005 - .006 per cent of the ration) to Berkshire pigs, starting at weaning time. Wallach et al. (1947), Beeson et al. (1947), and Reineke, McMillen and Bratzler (1948) reported similar results by feeding small doses of thyroprotein to different breeds of pigs. No growth stimulation was found in pigs placed on iodinated casein for a short time during the fattening period (Muhrer et al., 1947).

Parker (1943) and Irwin, et al. (1943) reported that feeding iodinated casein will accelerate the growth of chicks to a limited extent provided the dosage used is low. These results have been extended by Turner, Irwin and Reineke (1944) who found that the accelerated growth continues only for the first six weeks of life and that prolonged dosage is associated with growth depression. The upper limit of the dose in such cases was .1 per cent thyroprotein in the feed. Wheeler et al. (1948) reported a significant increase in body

weight of Rhode Island Red males but not in females at 12 weeks of age. Quisenberry and Krueger (1948) reported increases in the growth rate and efficiency of feed utilization in chicks of the New Hampshire and White Plymouth Rock breeds.

Influence of Seasonal Variations on Thyroxine Secretion Rate

Seasonal variations of the thyroid activity in domestic animals is often due to differences in environmental temperatures. There are many indications that stimuli arising in the external environment are capable of altering the rate of formation and discharge of secretions by the endocrine glands. It has been observed that there exists a reciprocal relationship between thyroid and thyrotropic hormone of the anterior pituitary. Dempsey and Astwood (1943) presented evidence that the rate of thyroid hormone secretion varied inversely with the environmental temperature and they also suggested that the thyroid secretes its active principal more rapidly in cold than in warm environments.

Cruickshank (1930) reported that thyroid weight in fowls showed marked seasonal variation, the weight being greatest from January to March and least from mid March to mid July. Iodine content of the thyroids varied with the weight of the organs.

Berliner and Warbritton (1937) observed poor fertility in rams during the summer months and they suggested that it was due to a decrease in thyroid secretion rate as a result

of high summer temperature. They obtained good results by combined treatment with gonadotrophic hormone and thyroxine. Ring (1939) states that exposure to cold causes an increase in metabolic rate and increases thyroid cell activity and vice versa. Reineke and Turner (1945) observed that the thyroid secretion ratesof male and female white Plymouth Rock chicks were highest during October to November and lowest during March to August. They further observed that with the onset of fall the thyroid secretion rate continued to rise again toward the normal winter levels.

Bogart and Mayer (1946) state that high temperatures (85 - 90°F) cause a marked lowering in the activity of the reproductive organs in the ram by causing a hypothyroid condition. Thyroprotein or thyroxine given to rams during the period of high temperatures stimulate the reproductive organs and restore most of the reproductive activities to a level near that of the breeding season. Hurst and Turner (1947, 1948) reported variations in the thyroxine secretion rate in mature male mice at different temperatures.

Relationship of the Thyroid to Reproduction

Considerable work is still to be carried out to arrive at a definite conclusion as to the role of the thyroid status on sexual development in animals. A number of workers hold the view that the thyroid has no direct effect on the testes and that any reproductive disturbances in the male in hypo-or hyperthyroidism is not primarily due to endocrine imbalance but to changed metabolic status (Moore, 1939)

while others believe that thyroid secretion is essential for normal reproductive processes as in the absence of the thyroid gland libido is absent in the bull and the production of semen may be seriously affected. In rams "Summer Sterility" has been observed due to decreased thyroxine secretion rate as a result of high summer temperature. In the domestic fowl definite stimulation of spermatogenesis occurs by feeding small doses of thyroprotein. In young breeding animals the thyroxine secretion rate is at its optimal level; the energy metabolism and fertility are at a peak. With advancing age the rate of thyroxine secretion gradually slows down; the sex drive in the male declines; the time required for service increases and the fertility is also believed to decline. The available literature on the subject is reviewed below.

Bovines: Petersen et al. (1941) thyroidectomized a male jersey at four months of age and observed complete absence of libido at sexual maturity. His reaction was tested at frequent intervals with females in estrus and in no instance could he be induced to mate. Oral administration of 25 gm. of desiccated thyroid over a period of three days restored normal activity and sexual behaviour. Brody and Frankenbach (1942) reported that thyroidectomized cows failed to manifest the normal physical signs of estrus. Oral administration of fresh thyroid to one cow restored normal estrual behaviour. Reineke (1946) reported that 14 bulls which had unsatisfactory breeding records, showed

definite improvement in vigor, and improved libido was observed in ten, after feeding thyroprotein. The time required for an observable effect to occur ranged from 7 to 40 days with an average of 16 days. Schultz and Davis (1947) reported that iodinated casein increased the conception rate from 51.7 to 55.7 in five bulls out of seven and resulted in spermatozoa with higher motility and greater resistance to lower temperature (40°F) storage.

Sheep and Goats: McKenzie and Berliner (1937) showed an increase in the number of abnormal sperm by keeping rams at high temperatures during the winter months. Berliner and Warbritton (1937) reported that rams produced semen of poor quality during summer months. This was believed to be due to a decline in thyroid hormone secretion due to high temperatures prevailing during summer. Administration of gonadotropic hormone and thyroxine gave good results. Turner, Mixner and Reineke (1943) observed that a ram which had good sex drive but deficient semen was fed thyroprotein, and the semen improved in quality and afterwards settled several ewes. Bogart and Mayer (1946) noted a decline in sperm numbers and mobility and an increase in abnormal forms, in rams made hypothyroid with thiouracil. Absence of spermatogenic activity in the testes of rams during the period of high summer temperature and also in thiouracil-treated ones was observed. They also reported that rams injected with thyroxine or fed thyroprotein during the period of high environmental temperature produced

more spermatozoa and had a lower percentage of abnormal cells. Warwick et al. (1948) reported that iodinated casein given to rams (.5 to 1.5 gms. daily) during April and May caused a deterioration in semen quality, particularly in rams that lost weight. During July and August the same treatment caused improvement.

Poultry: The effects of mild hyperthyroidism appears to be of practical significance in Poultry Husbandry as it stimulates growth and spermatogenesis in young birds, but the level that will be tolerated is rather narrow and semen production can be greatly impaired by giving too large an amount. Crew (1925) reported rejuvenation of seven hens 5 to 8 years of age, when they were fed desiccated thyroid daily for six months. The principle changes were the development of new plumage characteristic of younger fowls, improvement of the head furnishings and an increase in egg production. Jaap (1933) recorded that during late winter and early spring months, the testes of mallard drakes normally increase in size and spermatogenic activity. During this period an artificial increase, much greater than that of normal drakes, is obtained by feeding daily doses of .25 to 1 gm. desiccated thyroid. The testes size ranged from 2 to 10 times that of the non-thyroid fed control. Benoit and Aron (1934) observed that when immature male ducks were fed with thyroid tissue or injected with

thyroxine, they became sexually mature. They also recorded that thyroidectomy delayed the normal testicular growth in chickens and ducks. Testes of white leghorns rapidly decreased in size. Losses were as great as 20 per cent of the normal testes size in 11 days and 90 per cent in 20 days.

Benoit (1937) observed that the pelvis of ducks after thyroidectomy attains a development inferior to that attained by normal ducks with testicles of equal size. Greenwood and Chu (1939) reported that the effects of thyroidectomy in the male Brown Leghorn were very marked and there was a regression in testes size, together with cessation of spermatogenesis. Blivaiss and Domm (1942) recorded 40 to 45 per cent decrease in body weight and combs averaged 62 to 68 per cent less in thyroidectomized cockerels than the controls. Blivaiss (1947) reported reduction in size and in degree of maturity of the gonads and sex accessary organs in thyroidectomized roosters. The testes remain aspermic for as long as two years. These dysfunctions resulting from total ablation of the thyroid may be repaired by feeding desiccated thyroid or by administering thyroxine.

Martinez (1947) observed that thyroprotein when fed to fowls in doses of .01 and .02 per cent of the ration does not influence semen production or spermatogenesis and that .04 per cent of the feed caused a definite stimulation of spermatogenesis. Both semen volume and the sperm concentrations were increased quite markedly. Consequently the

total number of sperms per ejaculate was increased by approximately 65 per cent. In old roosters similar but less pronounced trends were observed. Thyroprotein when given at high levels of .08 to .16 per cent of the ration, depressed semen production and the total number of sperms decreased markedly (Wilwerth, 1948). Wheeler & Hoffmann (1948) observed in thyroprotein treated cockerels that the testes and pituitary weights were significantly higher and thyroid weights were significantly lower in the treated males.

Egg Production: Thyroidectomy reduces egg production in poultry (Winchester, 1939). Turner and his co-workers at Missouri, U. S. A. (1945, 1946, 1947, 1948) observed that thyroprotein administration at a low level prevented the yearly egg production decline, especially during the hot weather when a decline normally occurs in the laying hens.

EXPERIMENTAL PROCEDURE AND RESULTS

Material and Environmental Conditions

All the mice used in the following experiments were immature, male, and of about the same age with some variations in size and body weight. They were bred by Rockland Farm, Garden City, New York, U.S.A. and belonged to a well established commercial strain. Before starting the experimental work, all the mice were kept in cages at a controlled temperature of 24°C., with humidity which varied from 45 to 55 per cent, for a period of one week. The object was to get them acclimatized to controlled environmental and laboratory feeding conditions. Artificial electric light was maintained during the day throughout the experiments.

Mice Kept at 30°C: In experiment II groups 8, 9 and 10 and in experiment IV groups 5, 6, 7, 8 and 9 were kept in the incubators with a maintained temperature of 30°C. for three to four weeks. As the mice were kept in cages in the incubators, enough artificial light was not available to them. The mice were taken out of the incubators every week for weighing.

Feed and Feeding: The mice were fed *ad libitum* with finely ground standardized laboratory feed "Purina Laboratory Chow," manufactured by Purina Company, St. Louis, Missouri, U. S. A. Special feeding trays were used to prevent wastage. Water was available at all times from inverted bottles with a special outlet.

Thyroprotein (Protamone) manufactured by Cerophyl Laboratories, Inc., Kansas, Missouri, containing about .8 per cent thyroxine and thiouracil provided by Lederle Laboratories, Inc., Pearl River, New York, were used throughout the experiments. The desired doses of thyroprotein and thiouracil were weighed on an analytical balance and thoroughly mixed with the weighed quantity of Purina Laboratory Chow in a mechanical mixer, with a view to having a uniformity of the drugs in the feeds.

Procedure: All the mice were weighed and grouped, keeping the average weight of each mouse in the groups to about the desired weights. The mice in each group were numbered with picramic acid dye in a regular identification pattern.

Feed and water consumption, general appearance, sexual behaviour and the mortality rate if any were recorded daily in the morning from 8 to 9 a.m. throughout the experiments. Dead mice were removed as soon as discovered to prevent being eaten up by their fellows. The mice in these experiments were killed by ether after a period of four weeks except the groups 8, 9 and 10 in experiment II which were killed at three weeks interval. Each mouse in the experiments was dissected and its testes and seminal vesicles removed and weighed separately. In experiments I and II the seminal vesicles were weighed after gently squeezing out the seminal fluid, while in experiment III and IV these were weighed intact with the seminal fluid.

Effects on Growth and Feed Consumption

Hyperthyroidism: Experiments I and II at 24°C.

The daily average feed and water consumption per mouse in the control group 1 (Expt. I), was 3.9 and 8.4 gms., respectively. Tables IV and V show that the feed and water consumption of the thyroprotein treated groups increased with the increase in thyroprotein levels. The mice in group 3, receiving .025 per cent thyroprotein in the feed, on an average consumed 4.7 gms. of feed and 10.7 gms. of water per day per mouse. The feed and water consumption at the start of the experiment was 3.4 and 7.1 gms. per mouse, with a continuous increase from the fifth day onward and on the last day of the experiment the feed and water consumption was 5.5 and 12.8 gms. Each mouse in this group consumed 19.4 and 27.3 per cent more feed and water than the controls. Each mouse in group 4, having .05 per cent thyroprotein in the feed, consumed on a daily average 5.4 and 11.4 gms. of feed and water respectively. In this group each mouse consumed 3.4 gms. of feed and 7.0 gms. of water on the first day; with a continuous increase from the 4th day onward, to 6.3 gms. of feed and 14.1 gms. of water on the last day of the experiment. Each mouse in this group consumed daily 30.2 and 35.7 per cent more feed and water respectively, when compared with the control. Groups 5, 6 and 7 consumed 5.8, 6.1 and 4.2 gms. of feed and 11.8, 12.4 and 9.2 gms. of water per day per mouse.

The average body weight of each mouse in Group I

TABLE I

GENERAL INFORMATION ABOUT THE EXPERIMENTS

Expt. No.	Group No.	Dosage	Duration of expt.	No.of Mice At Start	No.of Mice At End	Average wt.gms. At Start	Average wt.gms. At End
Maintained at 24°C							
I	1	Control*	4 weeks	10	10	18.0	28.0
"	2	.2% TH	"	10	10	17.4	25.9
"	3	.025% TP**	"	10	6	17.3	28.6
"	4	.05% TP	"	10	10	17.4	29.8
"	5	.1% TP	"	10	9	18.0	28.1
"	6	.2% TP	"	10	8	18.1	27.0
"	7	.2% TH +.05TP	"	10	10	17.9	26.5
Maintained at 30°C							
II	8	Control	3 weeks	10	7	14.2	19.6
"	9	.025% TP	"	10	3	14.2	16.6
"	10	.2% TH	"	10	8	14.4	18.0
Maintained at 24°C							
III	1	Control	4 weeks	10	10	18.1	28.4
"	2	.025% TP	"	10	10	18.4	30.2
"	3	.05% TP	"	10	10	18.4	31.5
"	4	Control	"	7	5	16.9	25.8
Maintained at 30°C							
IV	5	Control	4 weeks	7	6	17.0	24.3
"	6	.005% TP	"	7	7	17.3	26.9
"	7	.01% TP	"	10	10	17.2	24.6
"	8	.02% TP	"	10	8	16.7	23.4
"	9	.1% TH	"	7	7	16.8	22.9

* Thiouracil
** Thyroprotein

was 18.0 gms. at the start of the experiment and 28.0 gms. at the end. Each mouse made an average gain of 10 gms. or 55.5 per cent of the body weight in the four-week period. The gains made by groups 3, 4, 5, 6 and 7 were 63.4, 66.4, 49.1 and 51.8 per cent respectively (Table II). Comparing the feed and water consumption and the gain in body weight of all the groups in Table II, it is clear that group 4 receiving .05 per cent thyroprotein in the feed consumed 30.2 per cent more feed and 35.7 per cent more water per day than the control group and made a total gain of 12.1 per cent in body weight. With higher dosages a decrease in body weight gains was observed (Fig. 2). In experiment III, groups fed .025 and .05 per cent thyroprotein at 24°C for a period of four weeks gained 7.4 and 13.8 per cent more body weight when compared with the control group. The increase in body weight gains was fairly constant in the groups fed low levels of thyroprotein.

Experiments II and IV at 30°C. Various levels of thyroprotein were fed to the mice in these experiments (Table I). Group 6 (Expt. IV) fed .005 per cent thyroprotein in the ration made a gain of 3.4 and 12.5 per cent more when compared with the control groups 4 and 5 at 24 and 30° respectively. The feed consumption per gram body weight gain was 10.2 gms. and that of the controls 11.1 and 12.2 gms. respectively (Table VI). This means that very small doses of thyroprotein even at high temperature caused better utilization of feed than the controls.

Decreases in body weight gains were observed in groups given .02 and .025 per cent thyroprotein at 30°C for 3 to 4 weeks.

Hypothyroidism: Goitrogens when given at an early age result in greatly reduced gains accompanied by symptoms of cretinism in animals, (Hughes, 1944). Mixner, Tower and Upp (1946) reported an increase in the rate of gain and a decrease in the feed required per unit of gain in older cockerels fed thiouracil. Reineke and McMillen (1946) and Vander Noot et al. (1947) reported an increase in body weight gain and efficiency of food utilization in swine. Kempster and Turner (1945) and Glazener and Jull (1946) state that thiouracil caused a decrease in the weight of the chicken and increased the amount of feed required to produce a pound of gain when compared with the control.

In the experiment I, group 2 fed .2% thiouracil for a period of four weeks, the daily average feed and water consumption per mouse was 3.3 and 7.5 gms. respectively. There was a decrease of 19.5 per cent in feed consumed per day per mouse when compared with the control group because the goitrogen causes a decrease of 20 to 30 per cent in B.M.R. (Reineke et al. 1945). Each mouse in group 9 (Expt. IV) fed .1 per cent thiouracil consumed 2.7 gms. of feed and 7.5 gms. of water daily (Tables IV and V). It was observed that the feed requirement per gram gain in body weight in the thiouracil treated groups for a period of four weeks at 24° and 30°C was higher than in the controls.

In these treated animals, group 2 (Expt. I), group 10 (Expt. II) and group 9 (Expt. IV) there was a decrease of 6.7, 13.1 and 15.7 per cent in body weight gains when compared with the controls (Table II and III), and the decrease was more marked at 30°C than at 24°C. Group 9 in experiment IV was kept for three weeks and the others for four weeks.

Environmental Temperature: A decrease of 9.1 per cent in the body weight gains of mice in group 5 (Expt. IV) kept at 30°C, was observed when compared with the control at 24°C (Table III) and there was also a decrease in feed and water consumption (Tables IV and V).

Thyroprotein and Growth of Hair: It was noticed that the thyroprotein treated mice exhibited a fine sleek coat but groups given .05 and .1 per cent at 24°C and .01 per cent thyroprotein at 30°C did better than other treated ones.

Effects on Testes and Seminal Vesicles

Hyperthyroidism: Administration of iodinated casein (thyroprotein) in normal animals produces hyperthyroidism. In experiment I, thyroprotein in doses of .025, .05, .1 and .2 per cent was fed to mice for a period of four weeks at an environmental temperature of 24°C. Table VII shows that the weights of the testes and seminal vesicles in group 3 given .025 per cent thyroprotein were 181.9 and 66.1 gms. and those of group 4 were 187.8 and 71.7 gms. When compared with the control group there was a significant increase in the weights of the testes and seminal vesicles

TABLE II

AVERAGE BODY WEIGHTS OF CONTROL AND VARIOUS TREATED GROUPS OF MICE AT 24°C

Group No.	Average Weekly Body Wt.in gms. At Start	1st	2nd	3rd	4th	Total wt. gained	% Wt.* gained or lost
Experiment I							
1**	18.0	22.4	24.3	27.3	28.0	10.0	--
2	17.4	21.5	22.7	24.9	25.9	8.5	-6.7
3	17.3	22.7	24.8	27.6	28.6	11.3	+7.9
4	17.4	23.1	25.9	28.2	29.8	12.4	+12.1
5	18.0	22.1	24.7	27.4	28.1	10.1	+.6
6	18.1	21.3	23.7	26.6	27.0	8.9	-6.4
7	17.9	21.0	23.1	26.2	27.2	9.3	-3.7
Experiment III							
1**	18.1	23.2	24.3	26.1	28.4	10.3	--
2	18.4	23.3	26.0	27.3	30.2	11.8	+7.4
3	18.4	24.4	27.4	30.2	31.5	13.1	+13.8

* Weight gained or lost when compared with the weight gained by the control groups.

** Control groups.

TABLE III

AVERAGE BODY WEIGHT OF CONTROLS AND VARIOUS TREATED GROUPS OF MICE AT 30°C

Group No.	Average Weekly Body Wt. in Gms. At Start	1st	2nd	3rd	4th	Total Weight Gained	% Weight* Gained or Lost
Experiment II							
8**	14.2	16.6	18.8	19.6	--	5.4	--
9	14.2	14.4	16.9	16.6	--	2.4	-21.1
10	14.4	16.3	17.5	18.0	--	3.6	-13.0
Experiment IV							
4**	16.9	19.2	20.7	22.5	25.8	8.9	--
5**	17.0	17.9	20.0	22.1	24.3	7.3	- 9.1
6	17.3	21.3	23.5	25.3	26.9	9.6	(+ 3.4 (+12.5***
7	17.2	19.1	19.9	22.3	24.6	7.4	(- 8.8 (+ .3***
8	16.7	18.5	19.5	20.7	23.4	6.7	(-11.9 (- 2.8***
9	16.8	17.2	19.4	20.9	22.9	6.1	(-15.7 (- 6.6***

* Weight gained or lost when compared with the weight gained by the control groups.

** Control groups in the two experiments and group No. 4 was kept at 24°C.

*** Gain or loss in Body Weight when compared with Group No. 5.

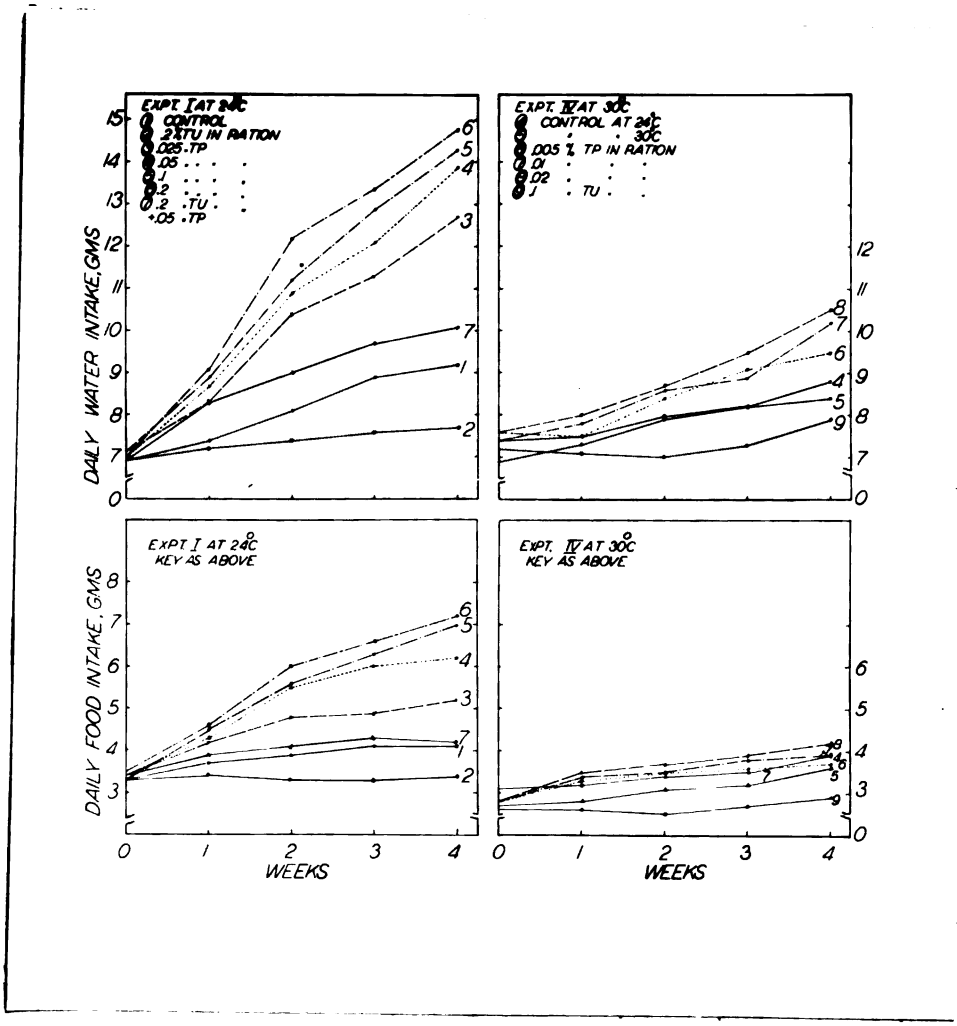


Fig. 1

The influence of thyroid status on feed and water consumption of male mice at two different environmental temperatures.

TABLE IV

WEEKLY AVERAGE FEED CONSUMPTION PER DAY BY THE CONTROLS AND VARIOUS TREATED GROUPS OF MICE

Expt. No.	Group No.	Feed intake 1st	2nd	3rd	4th	Daily Average Food Consumption	Daily* per cent increase or decrease
Maintained at 24°C							
I	1*	3.7	3.9	4.1	4.1	3.9	--
"	2	3.4	3.3	3.3	3.4	3.3	-19.5
"	3	4.2	4.8	4.9	5.2	4.7	19.4
"	4	4.3	5.5	6.0	6.2	5.4	30.2
"	5	4.5	5.6	6.3	7.0	5.8	44.1
"	6	4.6	6.0	6.6	7.2	6.1	51.2
"	7	3.9	4.1	4.3	4.2	4.2	2.4
IV	4	3.2	3.4	3.5	3.9	3.5	--
Maintained at 30°C							
IV	5**	2.8	3.1	3.2	3.6	3.2	--
"	6	3.3	3.5	3.6	3.7	3.5	9.3
"	7	3.4	3.5	3.7	3.9	3.6	12.5
"	8	3.5	3.7	3.9	4.2	3.8	18.7
"	9	2.6	2.5	2.7	2.9	2.7	-15.6

* Calculated by comparing with the controls

** Control groups

TABLE V

WEEKLY AVERAGE WATER CONSUMPTION PER DAY BY THE CONTROLS AND VARIOUS TREATED GROUPS OF MICE

Expt. No.	Group No.	Water intake 1st	2nd	3rd	4th	Daily Average Water Consumption	Daily* per cent increase or decrease
Maintained at 24°C							
I	1**	7.4	8.1	8.9	9.2	8.4	--
"	2	7.2	7.4	7.6	7.7	7.5	10.7
"	3	8.3	10.4	11.3	12.7	10.7	27.3
"	4	8.7	10.9	12.1	13.9	11.4	35.7
"	5	8.9	11.2	12.9	14.3	11.8	40.4
"	6	9.1	12.2	13.4	14.8	12.4	47.6
"	7	8.3	9.0	9.6	10.1	9.2	9.5
IV	4	7.3	7.9	8.2	8.8	8.0	--
Maintained at 30°C							
IV	5**	7.5	8.0	8.2	8.4	8.0	--
"	6	7.5	8.4	9.1	9.5	8.6	7.5
"	7	7.8	8.6	8.9	10.2	8.9	9.8
"	8	8.0	8.7	9.5	10.5	9.2	15.0
"	9	7.1	7.0	7.3	7.9	7.5	-6.2

* Calculated by comparing with the controls

** Control Groups

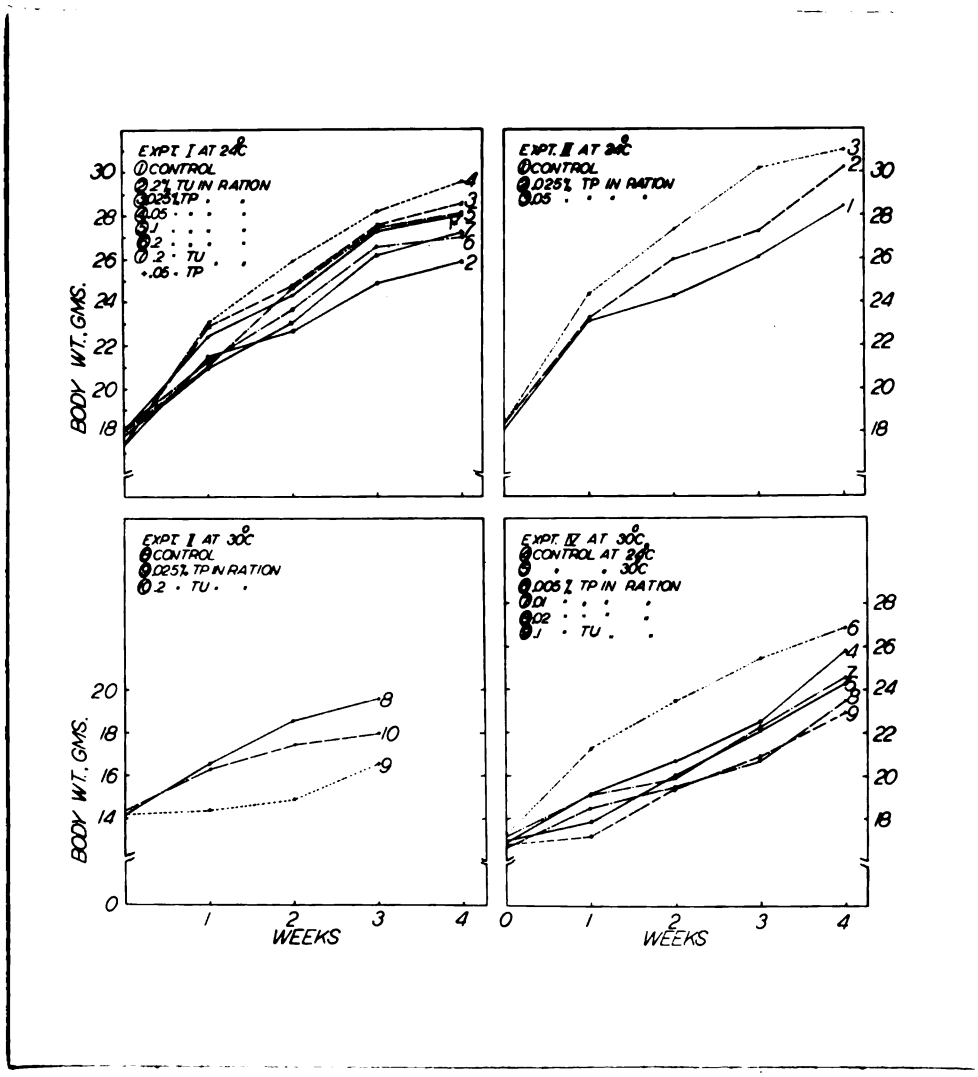


Fig. 2

The effects of thyroid status on body growth of the male mouse at two different environmental temperatures.

TABLE VI

FEED INTAKE IN GRAMS PER GRAM BODY WEIGHT GAIN IN CONTROL AND TREATED MICE AT DIFFERENT ENVIRONMENTAL TEMPERATURE

Dosage	Group No.	Total Feed Consumption	Total Gain Body Weight	Feed intake Per gm.gain
Experiment I maintained at 24°C				
Control	1	110.2	10.0	11.0
.2% TH	2	90.7	8.0	11.3
.025% TP	3	131.6	11.3	11.5
.05% TP	4	153.3	12.3	12.4
.1% TP	5	160.3	10.1	15.8
.2% TP	6	167.3	8.9	18.8
.2% TH +.05% TP	7	113.5	9.3	12.2
Control	4*	98.0	8.9	11.2
Experiment IV maintained at 30°C				
Control	5	89.6	7.3	12.2
.005% TP	6	98.1	9.6	10.2
.01% TP	7	100.8	7.4	13.6
.02% TP	8	106.4	6.7	15.8
.1% TH	9	75.6	6.1	12.3

* Control from Experiment IV kept at 24°C

was observed (Figs. 3 and 4). This shows that small doses of thyroprotein caused a significant increase in the weights of the testes and seminal vesicles while high doses (0.1, 0.2 per cent) caused a decrease in the weights of these organs. Significant increase in the weights of the testes and seminal vesicles was also observed in the mice fed .025 and .05 per cent levels of thyroprotein in experiment III.

In experiments II and IV an attempt was made to study the effects of both high and low dosages of thyroprotein on sexual development at 30°C. In experiment II, group 9, fed .025 per cent thyroprotein for a period of three weeks, a decrease in the weights of the testes and seminal vesicles and high mortality rate was observed (Tables VIII and X). Keeping in mind these adverse effects produced by .025 per cent thyroprotein, groups 6, 7 and 8 (Expt. IV) were given .005, .01 and .02 per cent levels of thyroprotein for a period of 4 weeks. Significant increases in the weights of the testes and seminal vesicles were observed in group 6 given .005 per cent thyroprotein when compared with the control (group 5) kept at 30°C (Table VIII). In groups given .01 and .02 per cent thyroprotein, a slight increase in the weights of the testes and seminal vesicles was observed in comparison with that of the control (Group 5) at 30°C. Figs. 3 and 4 show that there were no marked differences among groups 4, 7 and 8.

In these experiments the weights of the testes and seminal vesicles of the controls and treated groups, when

calculated per gram body weight, were highest in groups given .05 and .005 per cent thyroprotein at temperatures of 24° and 30° respectively (Table IX).

Hypothyroidism: Goitrogens in general appear to exert a depressive influence on the reproductive system in birds and mammals (Andrews and Schnetzler, 1946; Leathem, 1946; Bogart and Mayer, 1946; Shaffner and Andrews, 1948 and others).

In the present experiments administration of .1 and .2 per cent thiouracil to young male mice at both 24° and 30°C caused a decrease in the weights of the testes and seminal vesicles. The decrease in the weights of the testes and seminal vesicles was more marked at the .2 per cent level for three weeks at 30°C than at the .1 per cent level for a period of four weeks (Tables VII and VIII). There was a significant decrease in the weights of the testes and seminal vesicles of group 10 in experiment II. Thiouracil (.2 per cent) when fed to mice in group 2 (Expt. I) for a period of four weeks, caused a decrease in the weight of the testes and seminal vesicles which was significant when compared with the control.

It was observed that the decrease in the weight of the seminal vesicles was more marked than the decrease in the weight of the testes in thiouracil-treated groups.

Environmental Temperature: It has been observed that high environmental temperature causes a decrease in thyroid secretion rate. The mice in group 8 in experiment

II and in group 5 in experiment IV, which were kept at 30°C for a period of 3 to 4 weeks, showed a decrease in the weight of the testes and seminal vesicles when compared with the controls (Tables VII and VIII).

Effects of Hyperthyroidism on Male Sex Characters

The mice in groups 4 and 5 in experiment I and group 3 in experiment III, respectively, showed a tendency to mount each other during the third week of the experiments. Some degree of excitement was also observed in mice given high doses of thyroprotein.

Histological Studies

Histology of Testes: The substance of the testis is divided into lobules by fan-like extension of septa from the mediastinum testis and within each lobe are a number of tortuous canals called the seminiferous tubules. The interpasses between the seminiferous tubules are occupied by blood and lymph vessels, nerves, Leydig's interstitial cells, connective tissue and several types of cells (Maximow and Bloom, 1943). Leydig's cells are modified connective tissue cells and these cells are responsible for the secretion of male sex hormone.

In the adult the seminiferous tubules are lined by the complex seminiferous epithelium with its two kind of cells. The cells of Sertoli which are the supporting and nutrient elements and the spermatogenic cells which through their proliferation and complex transformation, furnish

TABLE VII

AVERAGE ORGAN WEIGHTS OF CONTROLS AND TREATED GROUPS OF MICE IN EXPERIMENTS I AND II

Group No.	No. of Mice	Dosage	Testes Weight	Testes t*	Seminal Vesicles Weight**	Seminal Vesicles t*
Maintained at 24°C						
1	10	Control	164.3 ±6.2	--	60.4 ±6.6	--
2	10	.2% TH	151.8 ±6.9	2.73	52.3 ±5.3	2.62
3	6	.025 TP	181.9 ±8.3	2.94	66.1 ±6.2	2.51
4	10	.05% TP	187.8 ±9.3	4.21	71.7 ±7.7	3.18
5	9	.1% TP	169.8 ±7.7	1.13	65.9 ±5.2	2.03
6	8	.2% TP	160.9 ±9.2	.92	56.0 ±6.0	1.34
7	10	.2% TH +.05 TP	161.4 ±7.6	.71	55.4 ±7.3	1.46
Maintained at 30°C						
8	7	Control	146.4 ±5.7	--	45.8 ±6.0	--
9	3	.025% TP	139.8 ±6.4	2.64	37.6 ±5.7	2.73
10	8	.2% TH	135.2 ±7.7	2.83	32.3 ±5.2	3.01

± Standard deviation

* "t" value obtained by comparing the groups with their respective control groups.

** The seminal vesicles were weighed after gently squeezing the seminal fluid.

TABLE VIII

AVERAGE ORGAN WEIGHTS OF CONTROLS AND TREATED GROUPS OF MICE IN EXPERIMENTS III AND IV

Group No.	No. of Mice	Dosage	Testes Weight	t*		Seminal Vesicles Wt.***	t*	
Maintained at 24°C								
1	10	Control	171.6 ±10.8	--		75.8 ±9.7	--	
2	10	.025% TP	186.6 ±10.5	3.13		84.2 ±8.8	2.94	
3	10	.05% TP	194.4 ±7.9	4.22		89.4 ±9.6	3.65	
4	5	Control	162.8 ±10.4	--		70.4 ±10.0	--	
Maintained at 30°C								
5	6	Control	151.3 ±11.6	1.12		60.5 ±7.0	1.94	
6	7	.005% TP	179.0 ±7.6	2.41	5.23**	79.6 ±5.2	2.51	4.14**
7	10	.01% TP	158.4 ±11.7	.34	.44	67.5 ±10.1	.43	.72
8	8	.02% TP	150.6 ±13.8	1.36	.21	58.4 ±11.3	1.42	.45
9	7	.1% TH	136.2 ±10.5	3.02	2.63	49.3 ±9.2	3.13	2.74

± Standard deviation

* t value obtained by comparing groups 2 and 3 with group 1 and groups 5, 6, 7, 8 and 9 with group No. 4, respectively.

** t value obtained by comparing group Nos. 6, 7, 8 and 9 with group 5.

*** The seminal vesicles were weighed with the seminal fluid.

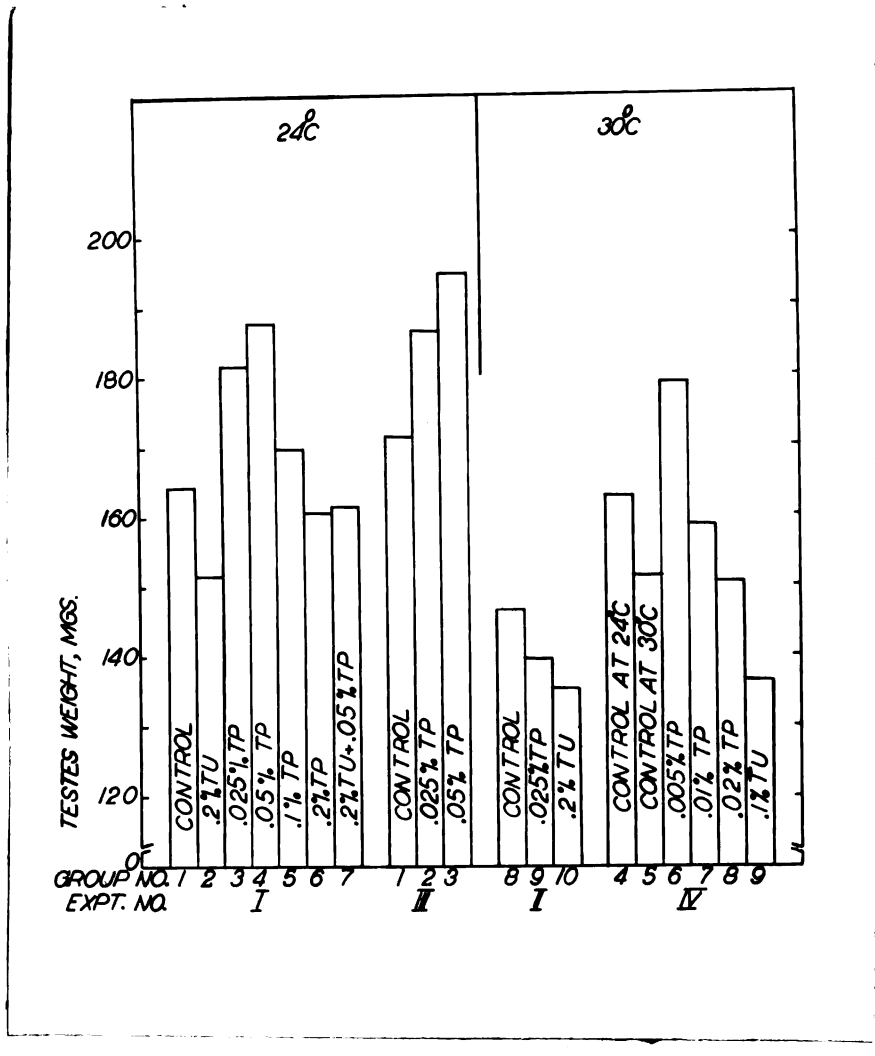


Fig. 3

The influence of variations in environmental temperature and thyroid status on the weight of testes of mice.

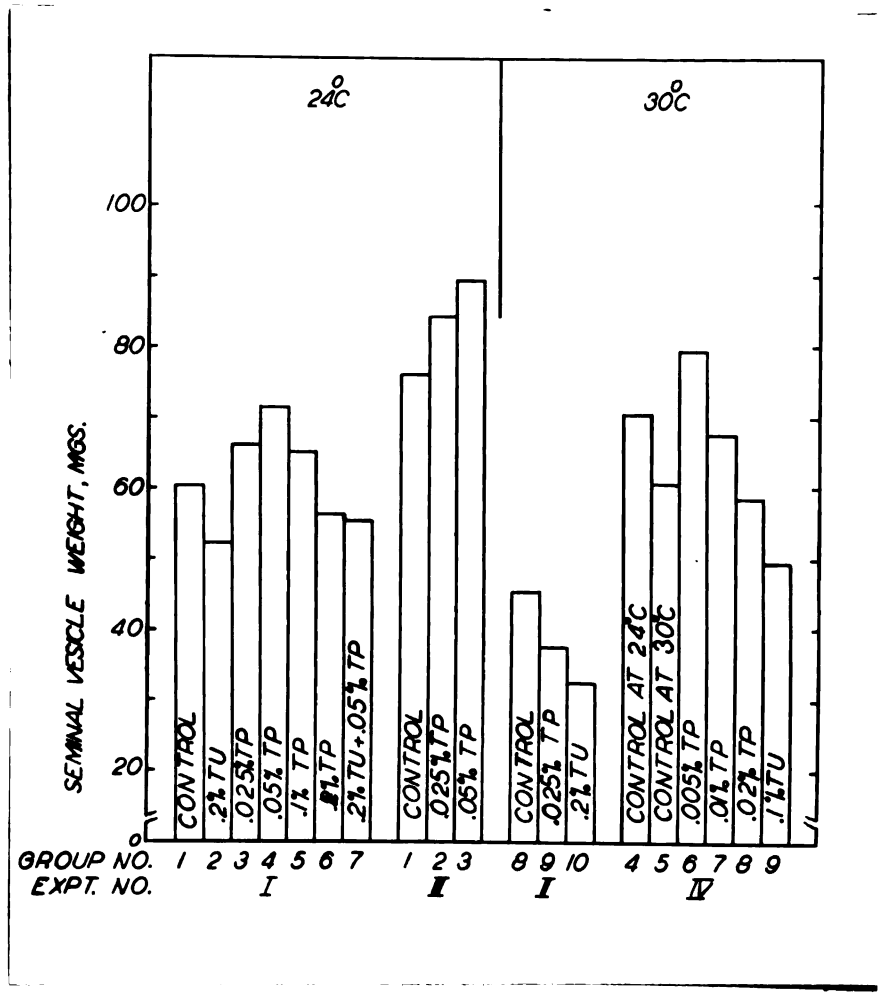


Fig. 4

The influence of variations in environmental temperature and thyroid status on the weight of seminal vesicle of the mouse. The seminal vesicles were weighed with the fluid removed in Expts. I and II and intact with contained fluid in Expts. III and IV.

TABLE IX

WEIGHTS OF TESTES AND SEMINAL VESICLES PER GM. BODY WEIGHT IN CONTROLS AND TREATED GROUPS AFTER 4 WEEKS

Expt. No.	Group No.	Dosage	Testes mgm.	S.Vesicle mgm.
Maintained at 24°C				
I*	1	Control	5.9	2.1
"	2	.2% TH	5.8	2.0
"	3	.025% TP	6.4	2.3
"	4	.05% TP	6.9	2.4
"	5	.1% TP	6.0	2.3
"	6	.2% TP	5.9	2.2
"	7	.2% TH + .05% TP	5.9	2.0
III**	1	Control	6.0	2.6
"	2	.025% TP	6.2	2.7
"	3	.05% TP	6.2	2.8
IV**	4	Control	6.1	2.6
Maintained at 30°C				
"	5	Control	6.0	2.4
"	6	.005% TP	6.6	2.9
"	7	.01% TP	6.4	2.7
"	8	.02% TP	6.4	2.5
"	9	.1% TH	5.9	2.2

*Seminal vesicles weighed after gently squeezing seminal fluid.

**Seminal vesicles weighed with seminal fluid.

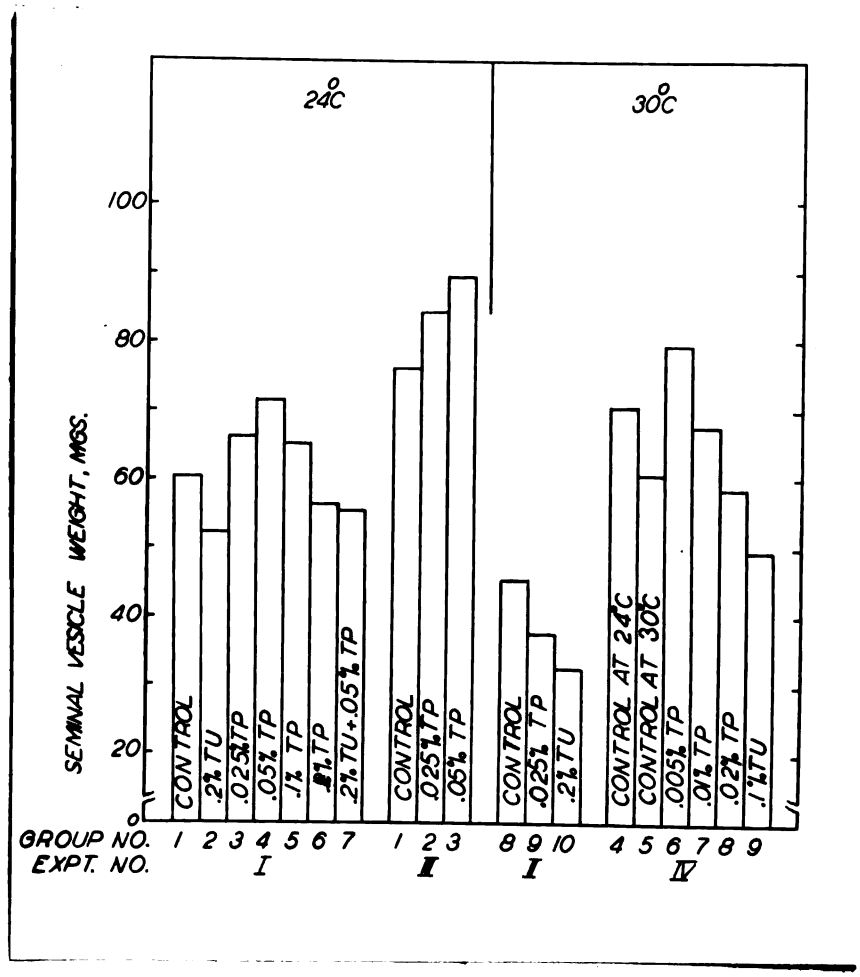


Fig. 4

The influence of variations in environmental temperature and thyroid status on the weight of seminal vesicle of the mouse. The seminal vesicles were weighed with the fluid removed in Expts. I and II and intact with contained fluid in Expts. III and IV.

TABLE IX

WEIGHTS OF TESTES AND SEMINAL VESICLES PER GM. BODY WEIGHT IN CONTROLS AND TREATED GROUPS AFTER 4 WEEKS

Expt. No.	Group No.	Dosage	Testes mgm.	S.Vesicle mgm.
Maintained at 24°C				
I*	1	Control	5.9	2.1
"	2	.2% TH	5.8	2.0
"	3	.025% TP	6.4	2.3
"	4	.05% TP	6.9	2.4
"	5	.1% TP	6.0	2.3
"	6	.2% TP	5.9	2.2
"	7	.2% TH + .05% TP	5.9	2.0
III**	1	Control	6.0	2.6
"	2	.025% TP	6.2	2.7
"	3	.05% TP	6.2	2.8
IV**	4	Control	6.1	2.6
Maintained at 30°C				
"	5	Control	6.0	2.4
"	6	.005% TP	6.6	2.9
"	7	.01% TP	6.4	2.7
"	8	.02% TP	6.4	2.5
"	9	.1% TH	5.9	2.2

*Seminal vesicles weighed after gently squeezing seminal fluid.

**Seminal vesicles weighed with seminal fluid.

mature spermatozoa. In a tubule with active spermatogenesis the Sertoli cells are slender, pillar-like elements, perpendicular to the basement membrane to which they are attached. They are separated from one another at somewhat regular intervals by the densely crowded spermatogenic cells. The outlines of the sertoli cells can not be seen distinctly and the nuclei are oval shaped, with a compound nucleolus. Under normal conditioning the sertoli cells are never seen to divide either mitotically or amitotically.

Spermatogenesis: The following are the three phases in the process of spermatogenesis:

1. The germ cells undergo repeated mitosis and certain structural changes and ultimately give rise to new cells called Spermatids.
2. Reduction in the number of chromosomes by meiosis.
3. The Spermatids undergo a series of complex transformations which result in mature sperm.

As a rule the earliest generations of spermatogenic cells are near the basement membrane of the seminiferous tubules while the mature forms line the lumen. The period of growth starts with the completion of the last spermatogonial division and each spermatogonium gradually increases in size and its nucleus undergoes marked transformations. This growth causes a further shifting of the cells towards the lumen of the tubules. The growing cell is known as a primary spermatocyte and when this cell reaches its full development, the period of maturation begins and it divides into two new cells called secondary

TABLE VII

AVERAGE ORGAN WEIGHTS OF CONTROLS AND TREATED GROUPS OF MICE IN EXPERIMENTS I AND II

Group No.	No. of Mice	Dosage	Testes Weight	Testes t*	Seminal Vesicles Weight**	Seminal Vesicles t*
Maintained at 24°C						
1	10	Control	164.3 ±6.2	--	60.4 ±6.6	--
2	10	.2% TH	151.8 ±6.9	2.73	52.3 ±5.3	2.62
3	6	.025 TP	181.9 ±8.3	2.94	66.1 ±6.2	2.51
4	10	.05% TP	187.8 ±9.3	4.21	71.7 ±7.7	3.18
5	9	.1% TP	169.8 ±7.7	1.13	65.9 ±5.2	2.03
6	8	.2% TP	160.9 ±9.2	.92	56.0 ±6.0	1.34
7	10	.2% TH +.05 TP	161.4 ±7.6	.71	55.4 ±7.3	1.46
Maintained at 30°C						
8	7	Control	146.4 ±5.7	--	45.8 ±6.0	--
9	3	.025% TP	139.8 ±6.4	2.64	37.6 ±5.7	2.73
10	8	.2% TH	135.2 ±7.7	2.83	32.3 ±5.2	3.01

± Standard deviation

* "t" value obtained by comparing the groups with their respective control groups.

** The seminal vesicles were weighed after gently squeezing the seminal fluid.

TABLE VIII

AVERAGE ORGAN WEIGHTS OF CONTROLS AND TREATED GROUPS OF MICE IN EXPERIMENTS III AND IV

Group No.	No. of Mice	Dosage	Testes Weight	t*		Seminal Vesicles Wt.***	t*	
Maintained at 24°C								
1	10	Control	171.6 ±10.8	--		75.8 ±9.7	--	
2	10	.025% TP	186.6 ±10.5	3.13		84.2 ±8.8	2.94	
3	10	.05% TP	194.4 ±7.9	4.22		89.4 ±9.6	3.65	
4	5	Control	162.8 ±10.4	--		70.4 ±10.0	--	
Maintained at 30°C								
5	6	Control	151.3 ±11.6	1.12		60.5 ±7.0	1.94	
6	7	.005% TP	179.0 ±7.6	2.41	5.23**	79.6 ±5.2	2.51	4.14**
7	10	.01% TP	158.4 ±11.7	.34	.44	67.5 ±10.1	.43	.72
8	8	.02% TP	150.6 ±13.8	1.36	.21	58.4 ±11.3	1.42	.45
9	7	.1% TH	136.2 ±10.5	3.02	2.63	49.3 ±9.2	3.13	2.74

± Standard deviation

* t value obtained by comparing groups 2 and 3 with group 1 and groups 5, 6, 7, 8 and 9 with group No. 4, respectively.

** t value obtained by comparing group Nos. 6, 7, 8 and 9 with group 5.

*** The seminal vesicles were weighed with the seminal fluid.

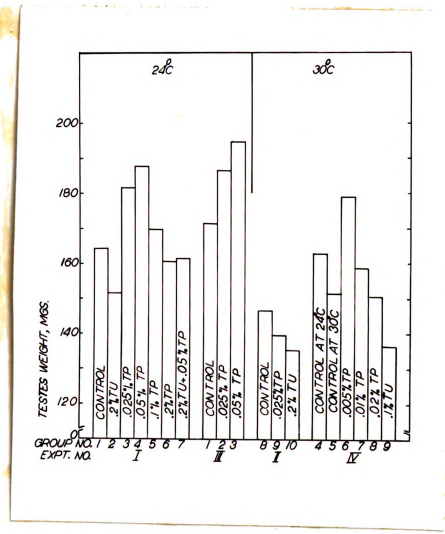


Fig. 3

The influence of variations in environmental temperature and thyroid status on the weight of testes of mice.

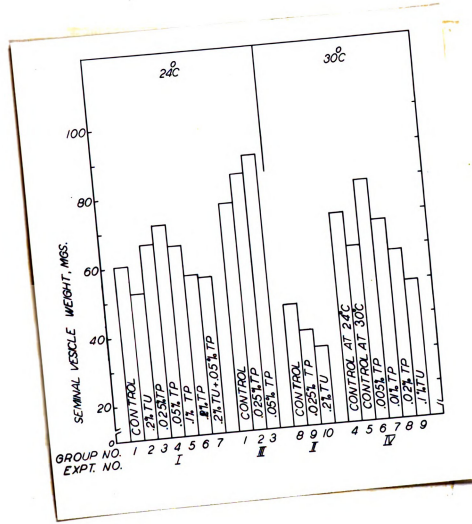


Fig. 4

The influence of variations in environmental temperature and thyroid status on the weight of seminal vesicle of the mouse. The seminal vesicles were weighed with the fluid removed in Expts. I and II and intact with contained fluid in Expts. III and IV.

TABLE IX

WEIGHTS OF TESTES AND SEMINAL VESICLES PER GM. BODY WEIGHT IN CONTROLS AND TREATED GROUPS AFTER 4 WEEKS

Expt. No.	Group No.	Dosage	Testes mgm.	S.Vesicle mgm.
Maintained at 24°C				
I*	1	Control	5.9	2.1
"	2	.2% TH	5.8	2.0
"	3	.025% TP	6.4	2.3
"	4	.05% TP	6.9	2.4
"	5	.1% TP	6.0	2.3
"	6	.2% TP	5.9	2.2
"	7	.2% TH + .05% TP	5.9	2.0
III**	1	Control	6.0	2.6
"	2	.025% TP	6.2	2.7
"	3	.05% TP	6.2	2.8
IV**	4	Control	6.1	2.6
Maintained at 30°C				
"	5	Control	6.0	2.4
"	6	.005% TP	6.6	2.9
"	7	.01% TP	6.4	2.7
"	8	.02% TP	6.4	2.5
"	9	.1% TH	5.9	2.2

*Seminal vesicles weighed after gently squeezing seminal fluid.

**Seminal vesicles weighed with seminal fluid.

mature spermatozoa. In a tubule with active spermatogenesis the Sertoli cells are slender, pillar-like elements, perpendicular to the basement membrane to which they are attached. They are separated from one another at somewhat regular intervals by the densely crowded spermatogenic cells. The outlines of the sertoli cells can not be seen distinctly and the nuclei are oval shaped, with a compound nucleolus. Under normal conditioning the sertoli cells are never seen to divide either mitotically or amitotically.

Spermatogenesis: The following are the three phases in the process of spermatogenesis:

1. The germ cells undergo repeated mitosis and certain structural changes and ultimately give rise to new cells called Spermatids.

2. Reduction in the number of chromosomes by meiosis.

3. The Spermatids undergo a series of complex transformations which result in mature sperm.

As a rule the earliest generations of spermatogenic cells are near the basement membrane of the seminiferous tubules while the mature forms line the lumen. The period of growth starts with the completion of the last spermatogonial division and each spermatogonium gradually increases in size and its nucleus undergoes marked transformations. This growth causes a further shifting of the cells towards the lumen of the tubules. The growing cell is known as a primary spermatocyte and when this cell reaches its full development, the period of maturation begins and it divides into two new cells called secondary

spermatocytes. The spermatides are the last generation of spermatogenic cells and they do not divide.

Crystalloids are present in Sertoli cells and spermatogonia. Degenerated spermatogenic cells which finally disintegrate into granular and fatty debris are sometimes seen in the lumina of the seminiferous tubules. This is not a pathological phenomenon provided it does not exceed certain limits (Maximow and Bloom, 1943). The degenerated cells are usually seen close to stretches of active seminiferous epithelium with normal spermatogenesis in full progress.

Seminal Vesicles: The seminal vesicles are tortuous elongated, hollow bodies with a very irregular branched lumen and numerous out-pocketings. Their wall consists of an external connective tissue sheet with elastic nets, of a middle layer of smooth muscle and of a mucous membrane resting upon a thin submucous layer. The mucous membrane forms an intricate system of thin and high primary folds which branch into the lumen and anastomose very frequently with one another. The epithelium shows great individual variations which probably depend on age and on functional influences.

Technique: The testes and seminal vesicles were fixed in Bouin's fluid. All the tissues were dehydrated in a series of graded alcohols for varying intervals, depending upon the stage of dehydration. Lithium Carbonate was used during the dehydration process to bleach the

picric acid residue in the fixed tissues. Picric acid is one of the reagents used in the preparation of Bouin's fluid. Cedar Oil was used as a clearing agent. The standard histological technique was used for cutting paraffin sections which were .7 u in thickness. The slides were stained by Harris hematoxylin and an eosin counterstain.

Thyroid and Sexual Development

In this note a brief general description is given of the histological studies of the testes and seminal vesicles of the control and treated mice while the detailed studies are in progress.

Testes: Histological examinations of the testes of thyroprotein treated mice (.025 and .05 per cent at 24°C and .005 per cent at 30°C) showed an increased spermatogenic activity in the seminiferous tubules when compared with that of the control groups (Figs. 5, 6, 7, 9). Out of these groups, the one fed .05 per cent thyroprotein gave the best results. Most of the lumina of the tubules contained numerous mature sperm; the spermatogonia showed active proliferation and the cells appeared well arranged (Fig. 9). Decreased spermatogenic activity and some degenerative changes were observed in groups given .02 per cent thyroprotein at 30°C (Fig. 11).

Testes of Thiouracil treated mice at 30°C for a period of four weeks, showed atrophic and degenerative changes in some of the seminiferous tubules and also

cellular disorganization (Fig. 15). In others limited spermatogenesis was observed. The testes of the mice kept at 30°C also showed limited spermatogenesis and some degree of degenerative changes in some of the seminiferous tubules (Fig. 11).

Seminal Vesicles: The seminal vesicles of the mice fed .05 per cent thyroprotein at 24°C and .005 per cent at 30°C, showed an increased proliferation of the epithelial cells lining the mucosa when compared with that of the controls (Figs. 8, 10, 14). The epithelial cells showed glandlike structures containing numerous granules and there was an increase in cell height.

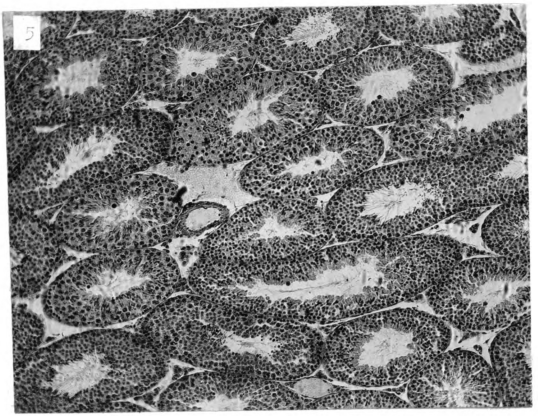
Slight desquamation of the epithelial cells lining the seminal vesicles was observed in some sections of the thiouracil-treated mice (Fig 16). Accumulation of these desquamated cells with seminal fluid was seen in the lumina of the seminal vesicles. The epithelial cells showed decreased proliferative changes and a comparative decrease in cell height. Somewhat decreased proliferative activity and degenerative changes were seen in the seminal vesicles of mice kept at 30°C for four weeks (Fig. 12).

Fig. 5

Testis section of a normal mouse kept at 24°C. showing the degree of spermatogenic development. H. and E. Stain (x 116).

Fig. 6

Testis section of a mouse kept at 24°C and fed .05% thyroprotein. Note the increased spermatogenic activity in the hypertrophied seminiferous tubules (x 116).


5

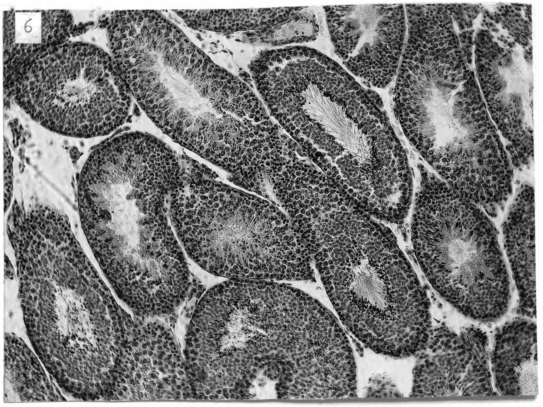

6

Fig. 7

Testis section of a normal mouse kept at 24°C, showing the degree of spermatogonic development (x 590).

Fig. 8

Section of a seminal vesicle of a normal mouse showing the presence of a few granules. (x 590).


7

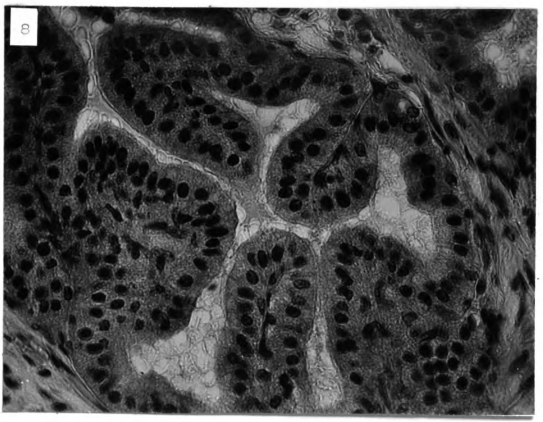
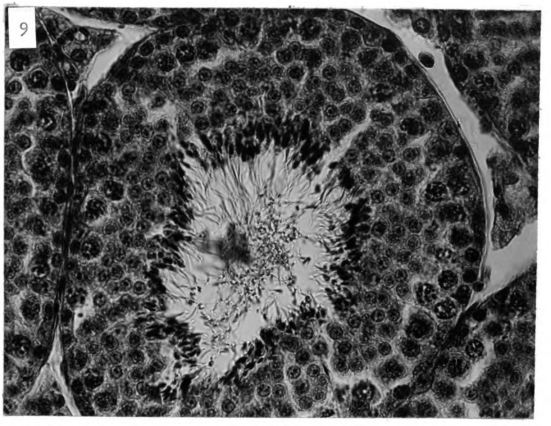

8

Fig. 9

Higher Magnification of Fig. 6., Testis section of a mouse fed .05% Thyroprotein at 24°C. Numerous mature sperm are seen in the lumen of the seminiferous tubules. The spermatogenic cells show active proliferative changes and are well organized (x 590).

Fig. 10

Section of seminal vesicle of a mouse fed .05% thyroprotein (24°C), showing numerous secretion granules and increase in cell height (x 590).


9

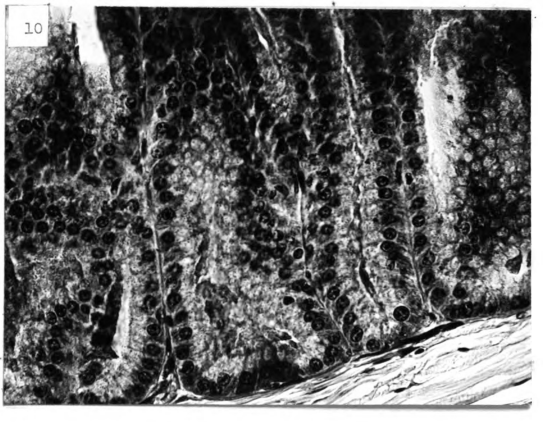
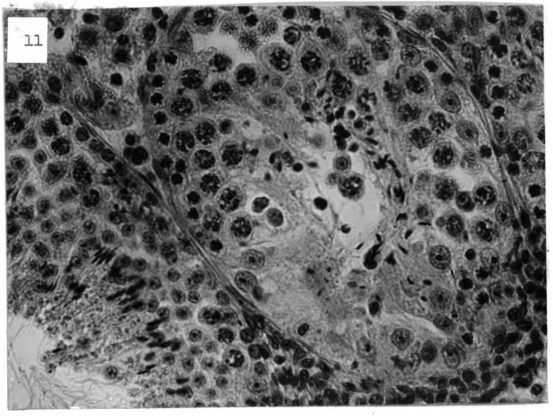

10

Fig. 11

Testis section of a mouse kept at 30°C for four weeks, showing limited spermatogenesis and degenerative changes in the tubules (x 590).

Fig. 12

Section of seminal vesicle of a mouse kept at 30°C for four weeks, showing some degenerative changes (x 590).


11

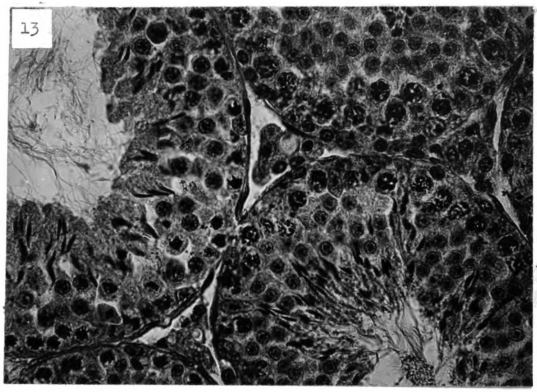

12

Fig. 13

Testis section of a mouse fed .005% Thyroprotein at 30°C, showing some spermatogenic activity and organized spermatogenic cells. Compare with Figs. 7 and 11. (x 590).

Fig. 14

Section of seminal vesicle of a mouse fed .005% thyroprotein at 30°C, showing increased cellular activity. Compare with Fig. 12. (x 590).


13

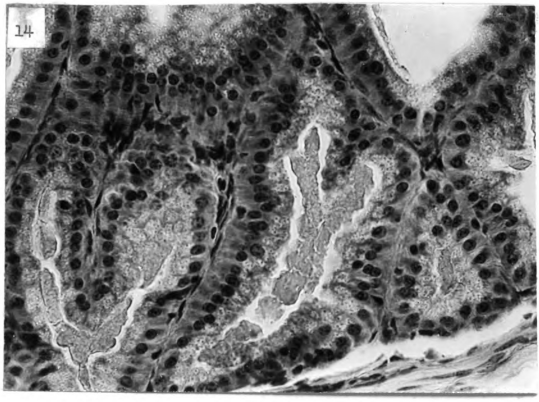
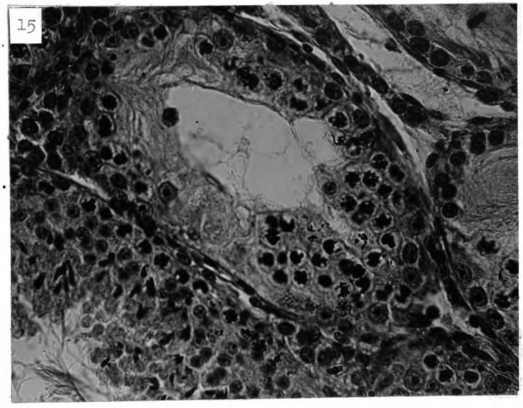

14

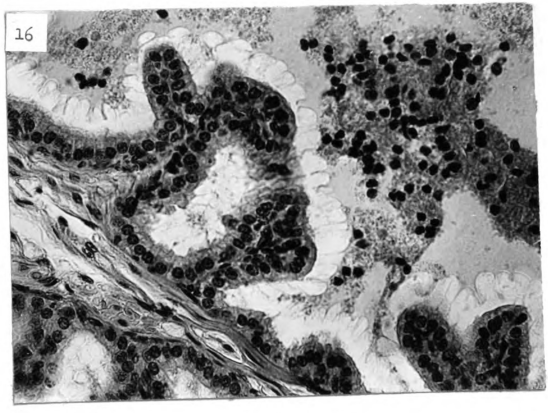
Fig. 15

Testis section of a mouse fed .1% thiouracil at 30°C for four weeks. Note the atrophic and degenerative changes and limited spermatogenesis. (x 590).

Fig. 16

Section of seminal vesicle of thiouracil-treated mouse at 30°C, showing desquamated epithelial cells, and decreased cellular activity (x 590).


15


16

Effects of Thyroprotein on Respiration

At a temperature of 30°C it was observed that the mice in group 9 (Expt. II) and group 8 (Expt. IV) fed .025 and .02 per cent thyroprotein respectively showed an increase in the respiration rate when compared with the control groups or the mice fed thiouracil. This increase in respiration was more marked within the first 10 days of the experiment. It may be that the high dosages of thyroprotein at 30°C caused some degree of excitation of the respiratory centers in the medulla and thereby increased the respiration rate or maybe due to increased metabolic rate and interference with the homeostatic mechanism.

Mortality Rate

Mortality rate among the control and various treated groups of mice was variable. However it was observed that the trend of the percentage of mortality was high in mice given high doses of thyroprotein at 30°C (Table X). High metabolic rate due to large doses of thyroprotein administration at high environmental temperature, interferes with the normal homeostatic mechanism of the mouse and thereby causes extra stress on the organism which ultimately succumbs.

TABLE X

MORTALITY RATE AMONG CONTROLS AND TREATED GROUPS OF MICE

Experiment No.	Group No.	Dosage	Mortality %
Maintained at 24°C			
I	1	Control	0
"	2	.2% TH	0
"	3	.025% TP	30
"	4	.05% TP	0
"	5	.1% TP	10
"	6	.2% TP	20
"	7	.2% TH + .05% TP	0
Maintained at 30°C			
II	8	Control	30
"	9	.025% TP	70
"	10	.2% TH	20
Maintained at 24°C			
III	1	Control	0
"	2	.025% TP	0
"	3	.05% TP	0
IV	4	Control	28.4
Maintained at 30°C			
"	5	Control	14.2
"	6	.005% TP	0
"	7	.01% TP	0
"	8	.02% TP	20
"	9	.1% TH	0

DISCUSSION

During recent years considerable work has been done in the field of thyroid physiology, but the role of the thyroid in male fertility needs further clarification. Some workers point out that the thyroid has no definite effect on the testes and it is probable that many reproductive disturbances in the male in hypo- or hyperthyroidism are due not primarily to endocrine imbalance but to changed metabolic status (Moore, 1939) or to a complex interrelation between the endocrine system and the body metabolism as a whole (Cameron, 1945). Berliner and Warbritton (1937); Bogart and Mayer (1945); Reineke (1946) and others believe that the thyroid plays an important part in male fertility.

With this work in view, an attempt has been made to study the influence of variations in environmental temperature and thyroid status on growth and sexual development in the growing male mouse. Varying degrees of hypo- or hyperthyroidism were produced by the administration of thyroprotein or thiouracil. In the present experiments it was observed that .05 per cent thyroprotein when fed to male mice for a period of four weeks at 24°C stimulated body growth and the weights of the testes and seminal vesicles were significantly increased. Hurst and Turner (1948) state that stimulation of the growing mouse by 80 times its own thyroid secretion rate is detrimental

to growth, but stimulating the growing mouse by 20 to 60 times its own thyroid secretion rate is beneficial to growth. This being so, administration of small doses of thyroxine to growing animals, will maintain an optimal thyroxine level in the body and will thus improve the metabolic rate within the physiological limits. Moreover, Evans et al. (1939) and Scow and Marx (1945) state that there are some indications that the thyroid hormone is necessary for the normal elaboration of the hypophyseal growth hormone.

Large doses of thyroprotein (.2 per cent) in the feed markedly increased the feed and water consumption due to increased metabolic rate but there was a decrease in body weight gains and also of the testes and seminal vesicles.

It may be pointed out here that increased feed consumption on high doses of thyroprotein treatment is in itself no criterion as to the increase in body weight. It has been observed that the mice with the highest feed consumption did not experience the maximum increase in weight. This suggests that there is a point of diminishing returns, wherein the increase in metabolism induced by the thyroprotein exhibits predominantly a "catabolic" effect, in relation to the relative "anabolic" effects which are produced by small doses of thyroprotein treatment in growing animals. There was an increased feed consumption with the increase in dosages of iodinated casein, but there was no relation

as regards the increase in body weights.

In experiment IV, Table VIII it is shown that when .005 per cent of thyroprotein was fed to growing mice at 30°C, there was also a significant increase in the weight of the testes and seminal vesicles when compared with the control kept at an environmental temperature of 30°C. In this group a fairly constant increase in the body weight and feed consumption was also observed. The daily feed consumption per gram body weight gain was slightly less than that of the controls at 24° and 30°C. There was a decrease in body weight gain, feed consumption and in the weights of the testes and seminal vesicles of the control group (Expt. IV) kept at 30°C, when compared with the control (group 4) at 24°C. This shows that there occurs a decrease in the thyroid secretion rate at high temperature and the growing animals with their changing rate of thyroid hormone secretion, could not maintain their normal thyroxine level in the body while the groups given small doses of thyroprotein did better. Hurst and Turner (1948) observed a decrease in the thyroxine secretion rate in male mice at 87°F when compared with those kept at 80°F. The decrease in thyroid secretion caused a decrease in metabolic rate and further upset the pituitary-thyroid relationship.

As has been previously pointed out, administration of small doses of thyroxine will maintain the thyroxine level at its optimum and will increase the metabolic rate to about normal. At 30°C, the mice on .01 per cent

level of thyroprotein gained more body weight, consumed more feed and also there was an increase in the weights of the testes and seminal vesicles in comparison with the control group kept at the same temperature. Thyroprotein (.025 per cent) when fed to the mice at 30°C for a period of three weeks caused a decrease in body weight gains and the decrease in the weights of the testes and seminal vesicles was more marked than by feeding .02 per cent thyroprotein in experiment IV.

It appears that high doses of thyroprotein at high environmental temperatures are detrimental to the animal body, because at high temperature there is a decrease in thyroxine secretion rate and, further, administration of large doses of thyroxine does not maintain the thyroid secretion rate within physiological limits. The explanation is that large doses of thyroxine in the blood causes the anterior pituitary to cease secretion of thyrotropic hormone and the thyroid steadily decreases in size. The result is that the complex Pituitary-Thyroid relationship is upset. In experiments I and IV, the mice fed .05 and .005 per cent thyroprotein at environmental temperatures of 24° and 30°C showed greater beneficial results with regard to growth and sexual development than that of the other thyroprotein-treated groups of mice at the same temperatures. This indicates that an increase of only 6°C in 24° to 30°C in environmental temperature caused a ten times reduction in the optimal thyroprotein dosage,

which means that the demand for thyroxine at high temperature is less than that at low temperature.

Administration of .1 and .2 per cent thiouracil at 24° and 30°C caused hypothyroidism in the mice, and this would result in a considerable decrease in B.M.R. The feed consumption decreased and there was a decrease in body weight gains and in the weights of the testes and seminal vesicles. The decrease in the weight of the seminal vesicle was more marked than the decrease in the weight of the testes.

Histological studies of the testes and seminal vesicles showed that mild hyperthyroidism stimulated spermatogenic activity in the testes and epithelial proliferation of the mucosa of the seminal vesicles when compared with the control or thiouracil-treated mouse. Some atrophic changes and limited spermatogenesis were seen in the testes; desquamation of the epithelial cells lining the mucosa of the seminal vesicles of the mouse given 0.1 per cent thiouracil for a period of four weeks at 30°C, was also noticed.

It may be pointed out here that the increase in the weight of the testes and seminal vesicles and the increased spermatogenesis in the testes of low level thyroprotein-treated groups, appeared to be due not simply to increased metabolism or increased body weight gains, as the weights of the testes and seminal vesicles of the control and treated groups when calculated per gram body

weight, were highest in the groups fed .05 and .005 per cent thyroprotein at 24° and 30°C respectively.

The mechanism whereby the thyroid influences gonadal function has not been fully elucidated. Reineke et al. (1941) observed that the gonadotropin secretion is reduced in thyroidectomized young male goats. Meites and Chandrashaker (1948) further observed that in male mice the response to gonadotropic hormone is reduced by thiouracil and augmented by feeding optimal levels of thyroprotein. The directly opposite results obtained in rats is undoubtedly due to differences in hormone balance in the two species. Van Dyke and Chen (1933) found a decrease in the concentration of the ovulation producing factor for rabbits, in thyroidectomized rabbit pituitary in comparison with pituitaries from litter-mate controls. Pan (1940) reported that the gonadotropin content of the pituitary of normal and castrate rats and of rabbits was decreased after thyroidectomy. Castration lowered the thyrotropin contents of the A. P. gland in cattle (Reece and Turner, 1937), in rats (Turner and Cupps, 1940) and also lowered the thyroxine secretion rate in the chicken (Schultze and Turner, 1945). Stein and Lisle (1942) observed a decrease in the gonad stimulating potency of young male rats following thyroidectomy. Chu and You (1945) gave thyroid to rabbits and found that the pituitary F.S.H. was lowered and the L.H. increased.

It has also been observed that thyroidectomy decreases

reproductive processes in animals (McKenzie and Berliner, 1937; Smelser, 1934, 37, 39; Berliner and Warbritton, 1937; Benoit, 1937; Turner et al. 1943; Bogart and Mayer, 1946 and others). Grumbrecht (1939) reported that thyroid increases the weight of the ovaries of infantile rats receiving a constant dose of gonadotropic substance, the increase in the weight being proportional to the dose of thyroid. Schultze and Davis (1947, 1948) reported that thyroid hormone is capable of influencing directly the metabolism of semen. Addition of thyroxine to bull's semen at a critical concentration range caused an increase in O_2 consumption and increased the conception rate when compared with that of the controls. Pan (1948) reported a significant decrease in testes weight of young rats fed .2% Sulfamethazine and absence of spermatogenic activities. The epithelial cells of the seminal vesicles showed a definite decrease in height.

In these experiments the increase in the weights of the testes and seminal vesicles and the increased spermatogenesis of the young male mouse, observed in mild hyperthyroidism, may probably be due to the increased output of hypophyseal gonadotropic hormones which are responsible for the growth of the testes. The follicle stimulating hormone of the anterior pituitary directly stimulatesthe growth of seminiferous tubules and the lutenizing hormone stimulates the secretion of male sex

hormone by the Leydig's interstitial cells of the testes. The male sex hormone in turn is responsible for the growth of the seminal vesicles and other secondary sex organs (Fraenkel et al. 1940 and Simpson et al. 1942). The decrease in the weights of the testes and seminal vesicles and the decreased spermatogenesis in the testes of mice fed thiouracil or kept at 30°C, was probably due to decreased output of gonadotropic hormones by the anterior pituitary as a result of decreased thyroid secretion rate. In the light of the present knowledge on the subject, it may be suggested that the thyroid secretion may facilitate the utilization of hypophyseal gonadotropic and gonadal hormones by the organism, or the increased output of gonadotropin by the anterior pituitary in mild hyperthyroidism stimulates the growth of male sex organs. Furthermore, the thyroid hormone may stimulate sexual development directly by metabolic conditioning of the cells involved.

From the results obtained with young male mice, it seems clear that a mild degree of hyperthyroidism has no deliterious effects on growth or sexual development and in fact would be conducive to optimal reproductive performance. This suggests interesting possibilities for therapy in live-stock, particularly under conditions where the thyroid functions may be subnormal. However, the exact role of the thyroid in reproduction will need to be established for each species of domestic animals before such therapy can be used under field conditions.

SUMMARY

The results obtained in the present experiments indicate that mild hyperthyroidism stimulates while hypothyroidism depresses body growth and sexual development in the growing male mouse at environmental temperatures of 24° and 30°C. The comparatively stimulated sexual development and spermatogenesis may occur through interrelationships between the pituitary, thyroid and gonads.

1. There was a significant increase in the weights of the testes and seminal vesicles of the mice given .05 per cent thyroprotein in the feed, for a period of four weeks, at 24°C while higher doses caused a decrease in their weights. Thyroprotein (.005 per cent) when fed to mice, kept at 30°C, caused a significant increase in the weights of these organs when compared with the control at 30°C. High environmental temperature of 30°C alone caused a decrease in the weights of testes and seminal vesicles. The weights of testes and seminal vesicles of controls and treated groups, when calculated per gram body weight, were highest in groups fed .05 and .005 per cent thyroprotein at environmental temperatures of 24° and 30°C, respectively.

2. Administration of thiouracil (.1 and .2 per cent) in the feed caused a decrease in the weights of the testes and seminal vesicles which was more marked at high environmental temperature. The decrease in the weight of

the seminal vesicles was more marked than that in the testes.

3. Histological studies of the testes and seminal vesicles showed that mild hyperthyroidism stimulated the spermatogenic activity in the testes and epithelial proliferation of the mucosa of the seminal vesicles with numerous granules, when compared with the control or hypothyroid male mouse. Hypothyroidism produced by the administration of thiouracil or by keeping the mice at 30°C caused some atrophic and degenerative changes, with limited spermatogenesis in the seminiferous tubules. In thiouracil-treated mice some degree of desquamation and inactivity of the epithelial cells lining the mucosa of seminal vesicles was also observed.

4. Thyroprotein when fed at .025 and .05 per cent levels to growing male mice for a period of four weeks, at 24°C caused an increase of 7.9 and 12.1 per cent more in the body weight gains when compared with the control group. Thyroprotein when given at the .2 per cent level in the ration caused a decrease of 6.4 per cent in body weight gain at 24°C. At 30°C a decrease in body weight gain was observed in mice fed .02 and .025 per cent thyroprotein and also in the controls.

5. The daily feed and water consumption of the thyroprotein-treated mice was higher than that of the controls and thiouracil-treated ones. The increase in feed and water consumption increased with the increase

in dosages of thyroprotein. The mice given .005 per cent thyroprotein for a period of four weeks at 30°C, consumed .8 gm. less feed per gram gain in body weight and gained 2.8 and 18.4 per cent more weight when compared with the control groups at 24° and 30°C respectively. There was an increase of 19.5 and 30.2 per cent in feed consumption per mouse per day in groups fed .025 and .05 per cent thyroprotein at 24°C, while thiouracil treatment or high environmental temperature caused a decrease in feed and water consumption.

6. An increase of only 6°C, i.e. from 24° to 30°C, in environmental temperature, caused a ten times reduction in the optimal thyroprotein dosage in young male mice, indicating that the demand for thyroxine is comparatively less at high temperature than at low temperature.

BIBLIOGRAPHY

Andrews,F.N. and Schnetzler,E.E. 1946. Influence of Thiouracil on Growth and Fattening in Broilers. Poultry Sci. 25: 124-129.

Beeson,W.M., Andrews,F.N., Witz,H.L. and Perry,T.W. 1947. The Effect of Thyroprotein and Thiouracil on Growth and Fattening of Swine. J. Animal Sci. 5: 394.

Benoit, J. 1937. Relation between Thyroid and Growth of Testes and Penis when Stimulated by Electric Light. Pro. Soc. Exptl. Biol. Med. 36: 782-784.

Benoit, J. and Aron, M. 1934. Compt. Rend. Soc. de Biol. 116: 221. (Cited by Benoit,1937).

Berliner, V. and Warbritton, V. 1937. The Pituitary and Thyroid in Relation to Sperm Production in Rams. Proc. Am. Soc. Animal Prod. 137-142.

Blivaiss,B.B. and Domm,L.V. 1942. Relation of Thyroid Gland to Plumage Pattern and Gonad Function in the Brown Leghorn Male. Anat. Rec. 84: 529.

Blivaiss, B. B. 1947. Interrelations of Thyroid and Gonads in the Development of Plumage and Other Sex Characters in Brown Leghorn Roosters. Physiol. Zool. 20: 67-107.

Bogart,R. and Mayer,D.T. 1946. The Relation of Temperature and the Thyroid to Mammalian Reproductive Physiology. Am. J. Physiol. 147: 320-328.

Brody, S. and Frankenbach, R.F. 1942. Age Changes in Size, Energy Metabolism and Cardio-respiratory Activities of Thyroidectomized Cattle. Mo. Agri. Expt. Sta., Res. Bull. 349.

Cameron, A. T. 1945. Recent Advances in Endocrinology. Blakiston Co., Philadelphia.

Chu, J.P. and You,S.S. 1945. The Role of Thyroid Gland and Estrogen in the Regulation of Gonadotropic Activity of the Anterior Pituitary. J. Endocrinology. 4: 115-124.

Crew, F.A.E. 1925. Rejuvenation of the Aged Fowl through Thyroid Medication. Proc. Royal Soc. Edinburgh. 45: 252-260.

Cruickshank, E. M. 1930. Factors Affecting Size and Iodine Content of the Thyroid in Fowls. Proc. 4th World Poultry Congr. 237-241.

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

Engelbach, W. 1932. Endocrine Medicine, II. Charles C. Thomas,Springfield,Illinois, U.S.A. p.50.

Evans,H.M., Simpson,M.E. and Pencharz,R.I. 1939. Relation between the Growth Promoting Effects of the Pituitary and the Thyroid Hormone. Endocrinology, 25: 175-182.

Fraenkel-Conrat,H., Li,C.H., Simpson,M.H. and Evans,H.M. 1940. Interstial Cell Stimulating Hormone. I. Biological Properties. Endocrinology. 27: 793-802.

Glazener,E.W. and Jull, M.A. 1946. Effect of Thiouracil, Desiccated Thyroid and Stilbestral Derivatives on Various Glands, Body Weight and Dressing Appearance of Chickens. Poultry Sci. 25: 236-241.

Graham, W. R. Jr. 1948. Hormone Feeding Produces Excellent Results. The California Dairyman. 28: 20.

Greenwood,A.W. and Chu,J.P. 1939. On the Relation between Thyroid and Sex Gland Functioning in the Brown Leghorn Fowl. Quart. J. Exptl. Physiol. 29: 111-119.

Grumbrecht, P. 1939. Die Anwendung von Schilddrüse preparaten bei Periodenstorungen. Zentralbl. f. Gynak., 63: 1942.

Harington, C. R. 1933. The Thyroid Gland--Its Chemistry and Physiology. Oxford University Press, London.

Hughes, A. M. 1944. Cretinism in Rats Induced by Thiouracil. Endocrinology. 34: 69-76.

Hurst,V. and Turner,C.W. 1947. The Thyroid Secretion Rate of Growing and Mature Mice. Am. J. Physiol. 150: 686-692.

Hurst,V. and Turner,C.W. 1948. The Thyroid Secretion Rate in the Mouse and its Relation to Various Physiological Processes. Mo. Agri.Expt. Sta. Res. Bull.417.

Irwin,M.R., Reineke,E.P. and Turner,C.W. 1943. Effect of Feeding Thyroactive Iodocasein on Growth, Feathering, and Weight of Glands of Young Chicks. Poultry Sci. 22: 374-380.

Jaap, H. G., 1933. Testis Enlargement and Thyroid Administration in Ducks. Poultry Sci. 12: 322.

Kempster,H.L. and Turner,C.W. 1945. The Effect of Feeding Thiouracil on the Fleshing of New Hampshire Broilers. Poultry Sci. 24: 94-96.

Koger,K. and Turner,C.W. 1943. The Effects of Mild Hyperthyroidism on Growing Animals of Four Species. Mo. Agri. Expt. Sta., Res. Bull. 377.

Leathem, J. H. 1946. Influence of Thiourea on Organ Weight of Rats as Related to Food Intake. Proc. Soc. Exptl. Biol. Med. 61: 203-205.

Martinez, C. 1947. The Influence of Varying Levels of Thyroid Activity on Semen Production in the Domestic Fowl. M.S. Thesis, Michigan State College, East Lansing,Mich.

Maximow, A.A. and Bloom, W. 1943. A Textbook of Histology. W. B. Saunders Co., Philadelphia. 500-535.

McKenzie, F.F. and Berliner, V. 1937. The Reproductive Capacity of Rams. Mo. Agri. Expt. Sta., Res.Bull. 338.

Meites, J. and Chandrashaker, B. 1948. Effects of the Thyroid Status on Response of the Gonads to Pregnant Mares' Serum in Two Different Species. J. Animal Sci. 7: 542.

Mixner,J.P., Tower,A.B. and Upp,C.W. 1946. The Effect of Feeding Thiouracil on the Body Weight of New Hampshire Cockerels. Poultry Sci. 25: 536-538.

Moore, C. R. 1939. Biology of the Testes. In "Sex and Internal Secretions." Williams and Wilkins Co., Baltimore. 353-451.

Muhrer,M.E., Warner,D.R., Palmer,J. and Hogan,A.G. 1947. Effects of Thiouracil and Protamone on Growing Swine. J. Anim. Sci. 6: 489.

Pan, S. Y. 1940. The Gonadotropic Potency of the Anterior Lobe of the Pituitary of Thyroidectomized Rats and Rabbits. Chinese J. Physiol. 15: 189-195.

Pan, S. Y. 1948. Effect of Sulfamethazine on the Testes and Accessory Glands of Normal and Hypophysectomized Rats. Proc. Soc. Exptl. Biol. Med. 68: 488-492.

Parker, J. E. 1943. Influence of Thyroactive Iodocasein on Growth of Chicks. Proc. Soc. Exptl. Biol. Med. 52: 234-236.

Petersen,W.E., Spielman,B.S., Pomeroy, and Boyd,W.L. 1941. Effect of Thyroidectomy upon Sexual Behaviour of the Male Bovine. Pro. Soc. Exptl. Biol. Med. 46: 16-17.

Quisenberry,J.H. and Krueger,W.F. 1948. Effects of Feeding Various Combinations of Protamone, Estrogens and Thiouracil on Growth and Fattening of Broilers and Fryers. Poultry Sci. 47: 681.

Reece,R.P. and Turner,C.W. 1937. The Lactogenic and Thyrotropic Hormone of the Anterior Lobe of the Pituitary Gland. Mo. Agri. Expt. Sta., Res. Bull. 266.

Reineke,E.P., Bergman,A.J. and Turner,C.W. 1941. Effect of Thyroidectomy of Young Goats upon Certain A.P. Hormones. Endocrinology. 29: 306-312.

Reineke,E.P. and Turner,C.W. 1941. Growth Response of the Thyroidectomized Goats to Artificially Formed Thyroprotein. Endocrinology. 29: 667-668.

Reineke,E.P., Mixner,J.P. and Turner,C.W. 1945. The Effect of Graded Doses of Thyroxine on Metabolism and Thyroid Weight of Rats Treated with Thiouracil. Endocrinology. 36: 64-67.

Reineke,E.P. and Turner,C.W. 1945. Seasonal Rythm in the Thyroid Hormone Secretion of the Chick. Poultry Sci. 24: 499-504.

Reineke, E.P. 1946. The Effect of Synthetic Thyroprotein on Sterility in Bulls. "The Problem of Fertility." 1946. Princeton University Press, Princeton, New Jersey. 232-242.

Reineke,E.P., McMillen,W.N. and Bratzler,L.J. 1948. The Effect of Mild Thyroprotein Stimulation on Growth in Swine. Mich. Agri. Expt. Sta., Tech.Bull. 209.

Ring, G. C. 1939. Thyroid Stimulation by Cold. Am. J. Physiol. 125: 244-250.

Robertson,T.B. 1928. The Influence of Thyroid Alone and of Thyroid Administered Together with Nucleic Acids, Upon Growth and Longevity of the White Mouse. Australian J. Exptl. Biol. Med. 5: 69-309.

Schultze,A.B. and Davis,H.P. 1947. Some Effects of Adding Thyroxine to Bovine Semen. J. Dairy Sci. 30: 543.

Schultze,A.B. and Davis,H.P. 1948. Effect of Thyroxine on Oxygen Consumption of Bovine Spermatozoa and Semen. J. Dairy Sci. 31: 946-950.

Schultze,A.B. and Turner,C.W. 1945. The Determination of the Rate of Thyroxine Secretion by Certain Domestic Animals. Mo. Agri.Expt. Sta., Res. Bull. 392.

Scow,R.O. and Marx,W. 1945. Response to Pituitary Growth Hormone of Rats Thyroidectomized on the Day of Birth. Anat. Rec. 91: 227-236.

Shaffner, C. S. 1948. The Influence of Thyroprotein Feeding on Semen Quality. Poultry Sci. 27: 527-528.

Shaffner,C.S. and Andrews,F.N. 1948. The Influence of Thiouracil on Semen Quality in the Fowl. Poultry Sci. 27: 91-102.

Simpson,M.E., Li,C.H. and Evans,H.M. 1942. Biological Properties of Pituitary Interstitial Cell-Stimulating Hormone (I.C.S.H.). Endocrinology, 30: 969-976.

Simpson, S. 1924. The Effects of Thyroidectomy on Growth in Sheep and Goats as Indicated by Body Weight. Quart. J. Exptl. Physiol. 14: 161-183.

Smelser, G. K. 1934. Effect of Thyroidectomy on Testicular Function. Anat. Rec. 60: 53-54 (Suppl.).

Smelser, G. K. 1937. Testicular Function in Hyperthyroidism. Anat. Rec. 70: 63 (Suppl.).

Smelser, G. K. 1939. Testicular Functions and the Action of Gonadotropic and Male Hormones in Hyperthyroid Male Rats. Anat. Rec. 73: 273-295.

Smelser, G. K. 1939. The Effects of Thyroidectomy on the Reproductive System and Hypophysis of Adult Male Rat. Anat. Rec. 74: 7-16.

Spielman,A.A., Petersen,W.E., Fitch,J.B. and Pomeroy,B.S. 1945. General Appearance, Growth and Reproduction of Thyroidectomized Bovine. J. Dairy Sci. 28: 329-337.

Stein,K.F. and Lisle, M. 1942. The Gonad-Stimulating Potency of the Pituitary of Hypothyroid Young Male Rats. Endocrinology. 30: 16-24.

Turner,C.W. and Cupps,P.T. 1940. The Effect of Certain Experimental Conditions Upon the Thyrotropic Hormone Content of the Albino Rat. Endocrinology. 26: 1042-1047.

Turner,C.W., Mixner,J.P. and Reineke,E.P. 1943. Thyroprotein for Sterile Goats. Dairy Goat J. 21: 1.

Turner,C.W., Irwin,M.R. and Reineke,E.P. 1944. Effect of feeding Thyro-active Iodo-casein to Barred Rock Cockerels. Poultry Sci. 23: 242-246.

Turner,C.W., Irwin,M.R. and Reineke,E.P. 1945. Effect of Thyroid Hormone on Egg Production of White Leghorn Hens. Poultry Sci. 24: 171-180.

Turner,C.W., Kempster,H.L., Hall,N.M. and Reineke,E.P. 1945. The Effect of Thyroprotein on Egg Production. Poultry Sci. 24: 522-533.

Turner,C.W., Kempster,H.L. and Hall,N.M. 1946. Effect of Continued Thyroprotein Feeding on Egg Production. Poultry Sci. 25: 562-569.

Turner,C.W. and Kempster,H.L. 1947. Effect of Hyperthyroidism on Seasonal and Yearly Egg Production of Fowls with Advancing Age. Am. J. Physiol. 149: 383-388.

Turner,C.W. 1948. Effect of Age and Season on the Thyroxine Secretion Rate of White Leghorn Hens. Poultry Sci. 27: 146-154.

Turner,C.W. and Kempster,H.L. 1948. Mild Hyperthyroidism Maintains Egg Production with Advancing Age. Poultry Sci. 27: 453-458.

Vander Noot,G.W., Reece,R.P. and Skelley,W.C. 1947. The Effect of Thiouracil in the Ration of Growing Swine. J. Animal Sci. 6: 12-15.

Van Dyke,H.B. and Chen,G. 1933. Production of Ovulation by Anterior Lobe of Pituitary of Thyroidectomized Rabbit. Proc. Soc. Exptl. Biol. Med. 31: 327.

Von Rapp 1840. "Uber die Schildruse." Inaug; Dissert. Tubingen. See Koger and Turner (1943).

Wallach,D.P., McMillen,W.N., Newland,H.W., and Reineke,E.P. 1948. The Influence of Thyroprotein, Thiouracil and Breed on Growth and Thyroid Characteristics in Swine. J. Animal Sci. 6: 501.

Warwick,E.J., Childs,C.E., Flower,A.E. and Ham.W.E. 1948. Ram Semen Production and Characteristics as Influenced by Administration of Thyroprotein. J. Animal Sci. 7: 198-207.

Wheeler,R.S. and Hoffmann,E. 1948. The Value of Thyroprotein in Starting, Growing and Laying Rations, II. Poultry Sci. 27: 509-514.

Wheeler,R.S., Hoffmann,E. and Graham,C.L. 1948. The Value of Thyroprotein in Starting, Growing and Laying Rations. I. Growth, Feathering and Feed Consumption of Rhode Island Red Broilers. Poultry Sci. 25: 103-111.

Wilwerth,A.M. 1948. The Influence of Varying Degrees of Hyperthyroidism on Semen Production in the Domestic Fowl. M.S. Thesis, Michigan State College, East Lansing, Mich.

Winchester, C.F. 1939. Influence of Thyroxine on Egg Production. Endocrinology, 24: 697-701.

Winchester,C.F. 1940. Seasonal Metabolism and Endocrine Rythms in the Domestic Fowl. Mo. Agri. Expt. Sta., Res. Bull. 315.