THE UNIVERSITY OF MARYLAND

OBSERVATIONS ON THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON BODY TEMPERATURE, GROWTH, AND WEIGHT OF THE SPLEEN, COMB, AND ENDOCRINE GLANDS OF CHICKENS.

(in two parts)

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TABLE OF CONTENTS

PAGE

OBSERVATIONS ON THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON BODY TEMPERATURE, GROWTH, AND WEIGHT OF THE SPLEEN, COMB AND ENDOCRINE GLANDS OF CHICKENS.

Part I. Thyroid Weight and Function As Influenced by Environmental Temperature.

Introduction

Recent interest in the fowl thyroid stems primarily from the development of two rather inexpensive drugs which make it possible to drastically modify thyroidal activity. One of them, synthetic thyroprotein, provides an opportunity to increase the thyroxin level of the blood while the other, thiouracil, is an antithyroid agent which completely inhibits thyroxin secretion. Through the use of these drugs it may be possible to modify, to economic advantage, those biological processes which are influenced by the thyroid.

The thyroid gland, through the secretion of its hormone thyroxin, has been shown to exert an effect on many bodily functions such as growth, differentiation, calcification and heart rate. Perhaps its most important function is to help regulate the metabolic processes so that the body temperature of homeothermic animals is maintained within the narrow limits compatible with life.

The thyroid itself is regulated by both neural and humoral factors. Lowe, Ivy and Block (1945) found evidence that the sympathetic nervous system regulates the vascularity of the gland and thereby probably influences the amount of hormone secreted. It has been clearly demonstrated, however, that the secretory cells themselves are under the control of thyrotropin, a secretion of the anterior pituitary (Collip and Anderson, 1935; others) and that the amount of thyrotropin secreted is regulated by the amount of thyroid hormone in the blood.

Thus, when the thyroid hormone titer of the blood falls, there is an increased elaboration of thyrotropin by the anterior pituitary; when thyroid hormone of the blood rises to the optimum necessary to maintain balance of the internal environment of the body, the secretion of thyrotropin is reduced (Cortell and Rawson, 1944). This relationship has been carefully reviewed by Payne (1944) with the fowl as the experimental animal.

The manner in which changes in the <u>external</u> environment affect the function of the gland has not been definitely established but it is generally thought that cold affects the hypothalamus, the heat regulating center of the body, which innervates the anterior pituitary. As previously stated, the anterior pituitary acts directly in regulating the secretory rate of the thyroid glands by compensatory changes in the rate of thyrotropin secretion. Uotila, (1939) has shown that exposure to cold results in thyroid hyperplasia and decreased follicular colloid and has pointed out that these are the same effects as follow thyrotropic stimulation.

In the experimental modification of the function of the endocrine glands, a rapid as well as an accurate measurement of their function is a practical necessity. The possibility of a relationship between thyroid weight and function has been investigated by several workers. Because the data reported suggest a positive correlation between thyroid weight and function to some observers and a negative correlation to others, the question might be raised whether any relationship exists at all. Data recently obtained as a result of a study of the effects of environmental temperature on the endocrine glands may serve to clarify this question.

In these experiments, seven to ten week-old New Hampshire cockerels were kept at environmental temperatures of 45° or 80° F. Thyroid function at each temperature was measured by one or more of the following techniques: 1. "resting" metabolism was determined; 2. a biological assay of thyroxin secretion rate was made on the birds' contemporaries; or 3. height of the epithelial cells of the thyroid was measured with a screw micrometer following section of the fixed glands. At the end of the experimental period, usually three weeks, the birds were sacrificed and their thyroids weighed to provide information on the possible relationship between thyroid weight and function.

Review of the Literature

Although Barelli (1932) concluded that, in contrast to that of mammals, the thyroid of birds does not act in heat regulation and Lee and Lee (1937) found that thyroidectomised geese adapted to a cold environment, it is generally accepted that the thyroid plays an important role in regulating the metabolic processes. When the chick is subjected to a cold environment, it responds by increasing oxygen consumption (Kleiber and Dougherty, 1934, and Winchester and Kleiber, 1938). In other species (Miller, 1939), this response is associated with histological evidence of increased thyroid activity and it is assumed that the chick's metabolic response is mediated through the thyroid.

Winchester (1940) demonstrated a seasonal variation in heat production of the fowl with the high point occurring in February or March and the low point in the late summer. At the time of the greatest heat production, the thyrotropic hormone content of the hen's pituitary was also maximum. This implies that the thyroid gland through the secretion of thyroxine has a direct effect on heat production, especially since Miller (1938) has previously demonstrated that exogenous thyrotropic stimulation of the thyroid increases the metabolic rate of the sparrow. Moreover, Leblond, Gross, Peacock, and Evans (1944), and Schachner, Gierlach, and Krebs (1949) have shown an increase in fixation of radioactive iodine by the thyroids of rats exposed to low temperatures. The work of Macbeth and Noble (1949) supplies further evidence, since the feeding of thiouracil to rats completely abolished their metabolic response to cold environmental temperature.

Among the published reports in support of a positive relationship between thyroid weight and function, Crile (1941) and Crile and Quiring (1940), as quoted by Riddle (1947), presented evidence that animals from cold climates had proportionally heavier thyroids than those from warm climates. For example, Crile and Quiring (1944) found that the thyroid of the white whale, which lives in water only a few degrees above freezing, is 3.23 times as large as the thyroid of a horse of comparable size. Histological examination of the whale's thyroid revealed tall epithelial cells and profuse vascularization. It was suggested that the whale requires a more active thyroid to maintain body temperature in its cold environment.

Landauer and Aberle (1935) found that the thyroids of frizzle chickens were heavier than those of White Leghorns and Benedict, Landauer and Fox (1932) showed that basal heat production was much higher in the imperfectly feathered frizzle. Riddle (1947) found that the same relationship existed in the sparsely plumaged" scraggly" pigeon.

Seasonal variation in thyroid weight has been observed by Riddle and Fisher (1925) and Riddle (1927), who noted that the thyroids of pigeons become enlarged in autumn and winter and decrease in size in spring and summer. They concluded that these changes take place as a prompt response to changes in external temperature. Miller (1939) reported that the greatest activity of the sparrow thyroid, as measured by histological evidence of high epithelium and small follicles, occurs in winter and is associated with a high basal metabolic rate. Cruickshank (1929) observed that the fowl's thyroid is 35 per-cent smaller from April to July than from January to March and that the iodine content of the gland tends to vary directly with seasonal variations in weight. Podhradsky (1933) and Galpin (1938) further confirmed these seasonal variations in thyroid weight of the chicken.

A definitive experiment demonstrating the calorigenic action of the thyroid was performed by Dempsey and Astwood (1943). By means of an ingenious technique which permits an estimate of the apparent secretion rate of the gland, they showed that rats held at an environmental temperature of 35° C. secreted the equivalent of 1.7 gamma thyroxin per day as compared with 5.2 gamma for rats held at room temperature and 9.5 gamma for rats held at 1° C.

It may be concluded from this evidence that the thyroid is a major factor in regulating heat production and that the functional activity of the gland is greatest during periods of increased heat production. Also, the heavier thyroid glands observed in periods of known increased function (wintertime) suggest a direct relationship between activity of the gland and its weight.

Some investigators, however, do not agree with this concept. Galpin (1938) interpreted her data as an indication of thyroid function being inversely related to its mass, and both Podhradsky (1933) and Elterich (1936), from studies on the pigeon, also postulated an inverse relationship between secretory activity and weight of the gland. Although Riddle has postulated a positive relationship between thyroid weight and function, he developed by selection races of doves characterized by light thyroids which apparently had higher metabolic rates than races with heavy thyroids (Riddle, 1947). Stockard and Vicari (1941) studied the comparative amount of thyroid tissue in several breeds of dogs. They concluded that "the gross proportional amount of thyroid is not an important factor in determining either degree or quality of thyroid function." Apparently only one study has been reported in which chickens were maintained in controlled warm and cool environments. Allee and Lutherman (1940) held pullets at 6° C. and 21° to 24.5° C. for approximately nine weeks and found that thyroid weight was not affected by the treatment.

Because of these conflicting viewpoints, it appeared desirable to present further data on the relationship between thyroid weight and function.

Experimental

Data were obtained on New Hampshire cockerels, from seven to eleven weeks of age, held in either a cool or a warm environment. The cool environment was provided by a "walk-in" type refrigerator operated at approximately 45° F. with a relative humidity of 70 percent. The warm environment was furnished by a windowless room heated by steam. Thermostatically controlled electric heaters were used to facilitate a more precise regulation but the temperature of this room was somewhat variable, ranging from 74° to 88° F., with a mean of 80° F. Relative humidity of the warm environment was approximately 44 percent.

The birds were kept in a conventional broiler-growing battery equipped with wire screen floors. The Maryland Experiment Station all-purpose mash and fresh water were kept before the birds at all times. Lights were on continuously.

In trial 1, 48 seven-week-old cockerels were divided into two lots of 24 each according to body weight. One lot was placed in the cool environment and the other was placed in the warm environment. At the end of three weeks, one-half of the birds in each lot was sacrificed by desanguination, the thyroids dissected out, and the weight of the paired thyroids from each bird estimated to 0.1 mg. on a Roller-Smith balance. The remaining birds of each group were examined one week later. Immediately after weighing, the glands were fixed in 10 percent neutralized formalin, embedded in paraffin, sectioned at 5 microns and stained with hemotoxylin and eosin. Microscopic examination of the slides was made according to the method of Rawson and Starr (1938) as modified by Dvoskin (1947). The former made an extensive review of the possible methods of evaluating thyroid activity in an effort to develop an objective assay of the thyrotropic principle and concluded that epithelial cell height was the most satisfactory measure of thyroid activity. In Dvoskin's modification, a screw micrometer is used to determine the height of a distinctly outlined cell in each of 25 successive cross sections of follicles through the mid-portion of the gland. A large number of observations is necessary because of the variation in cell height found within each gland.

On the day prior to the sacrifice of the birds, they were fasted for 14 hours and their resting metabolism determined in the closed-circuit respiratory apparatus shown in Figure 1. Metabolism tests were made during the daylight hours for a period of 30 minutes. Oxygen consumption was calculated from the most regular portion of the graph in the final 10 minutes of the determination and was expressed in cubic centimeters of oxygen per hour per kilogram of body weight. Birds from the warm and cool environments were run alternately to equalize length of the fasting period. Temperature within the chamber ranged from 24 to 26° C which is within the zone of thermal neutrality for the chicken (Barott and Pringle, 1941).

The estimate of the amount of thyroxin secreted by birds held at the different environmental temperatures was obtained by the method developed by Dempsey and Astwood (1943) and later applied to

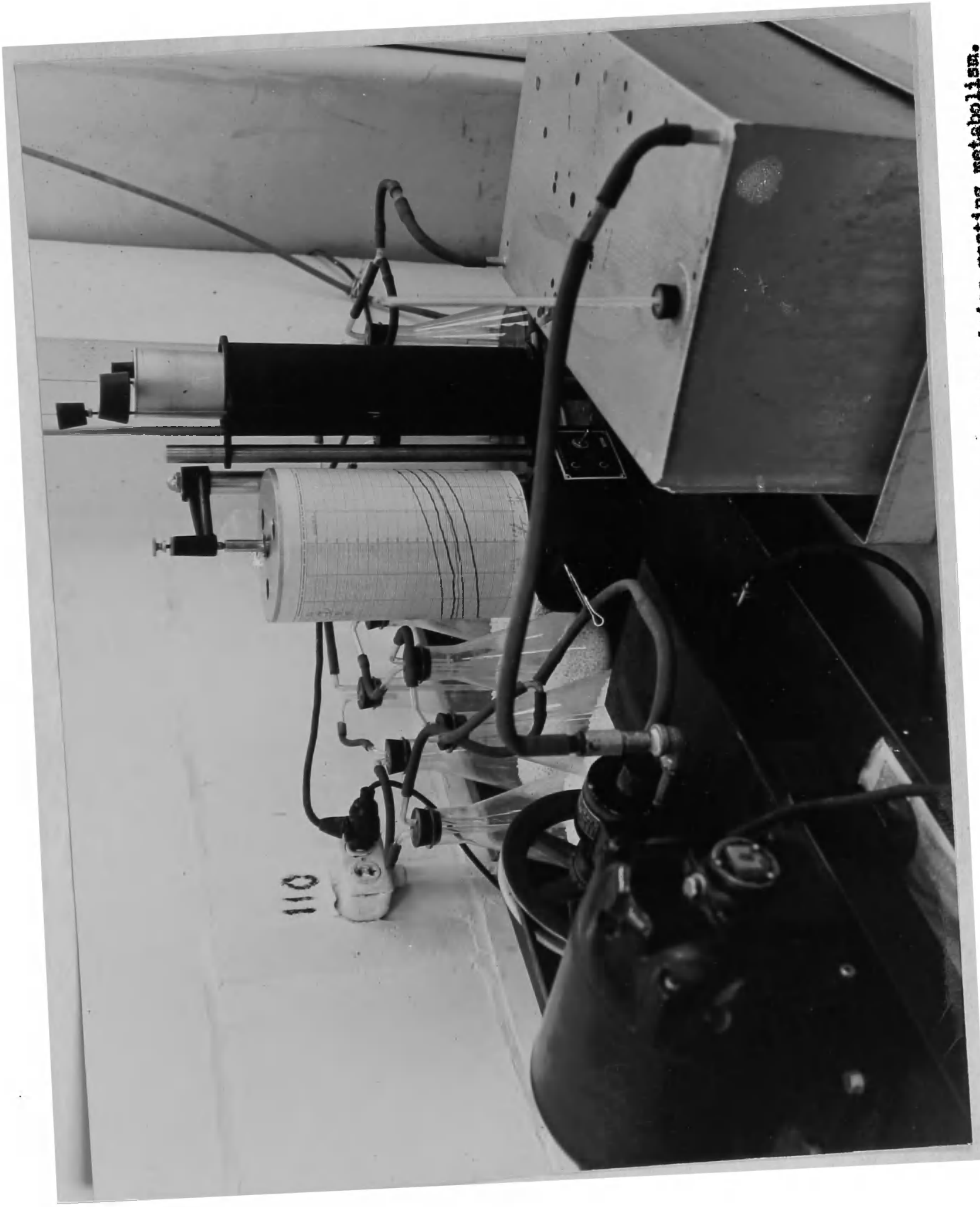

Figure 1. Closed circuit respiratory apparatus used in determining resting metabolism.

chickens by Mixner, Reineke, and Turner (1944). Thiouracil was fed as a goiterogen and replacement therapy was made daily by injection of graded dosages of crystalline thyroxin in solution. In this experiment somewhat greater extremes in temperature were used in an effort to insure clear-cut results. Six groups of seven birds each, seven weeks of age, were kept at 38° F., and a duplicate series was kept at 90° F.

An attempt was made to accentuate the effect of environmental temperature on the weight of the thyroid by the use of birds that had been unilaterally thyroidectomized. This should provide a more intense stimulation of the remaining gland by endogenous thyrotropin. The operation was performed according to the technique developed with pigeons by Marvin and Smith (1943) except that it was not found necessary to ligate the thyroid artery. The left thyroid was removed in each case to avoid damage to the muscle and connective tissue connecting the crop with the right breast muscle. Four thyroidectomized birds were placed in each environment for four weeks, after which the birds were killed and their right thyroids removed and weighed. Any macroscopic thyroidal tissue that had proliferated at the site of the left thyroid was also dissected out and weighed.

Another approach to the problem was suggested by the work of Giaja (1931), who reported higher oxygen consumption for deplumed chickens, and Landauer (1933), who found certain feather pigment anomalies usually associated with hyperthyroidism in partially plucked hens kept at cold temperatures. Therefore, it seemed of interest to determine if increased function as a result of depluming was also reflected in an increase in thyroid weight. A group of eight cockerels, nine weeks old, was divided by weight into two lots of four birds. The birds of one group were prepared for plucking by injection with Nembutal. After being plucked clean of feathers, the birds were held in an incubator for two hours to minimize shock and expedite recovery from the operation. They were then placed in a growing battery, two birds to a compartment, and the birds of the other lot were held under the same conditions to serve as controls. Room temperature in this case varied from 72° to 77° F. After two weeks, resting metabolism was determined in the usual fashion and the birds were then sacrificed and their thyroids weighed.

The effect of variation in the environmental temperature on the thyroid function of the developing chick embryo was also studied. Eggs were incubated in each of two 150-egg capacity Humidaire forced-draft incubators. One incubator was operated at 102.2° and the other at 96.8° F. At the time of hatch, the chicks were weighed, sacrificed by decapitation, and their thyroids dissected out, weighed, and fixed.

Results and Discussion

As shown in Table 1, birds kept at different environmental temperatures for three or four weeks had significantly different metabolic rates. Since birds held at the lower temperature had heavier thyroids as well as higher oxygen consumption, this is evidence of a positive correlation between thyroid weight and function. Moreover, microscopic measurement of the thyroid secretory epithelium revealed that acinar cell height was significantly higher in the birds with the higher metabolic rate. Mean cell height of thyroids from birds kept in the cool environment was $5.44 \pm .14$ microns as compared with $3.45 \pm .09$ microns for the thyroids of birds kept in the warm environment. In subsequent trials at 45° and 80° F., mean thyroid weight was consistently heavier in birds kept in the cool environment but these data are omitted for the sake of brevity. It is of interest to note, however, that in no case was the difference statistically significant.

The most definitive index of thyroid activity used in these studies was the determination of thyroxin secretion rate by the thiouracil-thyroxin technique. These data are plotted in Figure 2. It will be noted that the thyroxin secretion rate was 15 gamma per bird per day for the birds kept in the cool environment and 9.5 gamma per bird per day for the birds kept in the warm environment. At the slightly warmer and cooler temperatures used for this test, a greater difference was obtained in mean thyroid weight. The difference in

Table 1. The effect of warm or cool environment on the thyroid weight and basal metabolism of New Hampshire cockerels seven weeks old

Treatment	No. birds	Thyroid weight, mgs.	Metabolic rate, cc. of O_2/kg. per hour
Trial 1 (Beginning 10/23/48)			
45° F. for three weeks	12	87.2 ± 6.7	1720 ± 77**
80° F. for three weeks	12	71.1 ± 4.8	1457 ± 41
45° F. for four weeks	10	108.8 ± 8.4	1544 ± 43**
80° F. for four weeks	12	88.8 ± 6.1	1144 ± 38

** Highly significant difference in metabolic rate between birds held at 45° and 80° F.

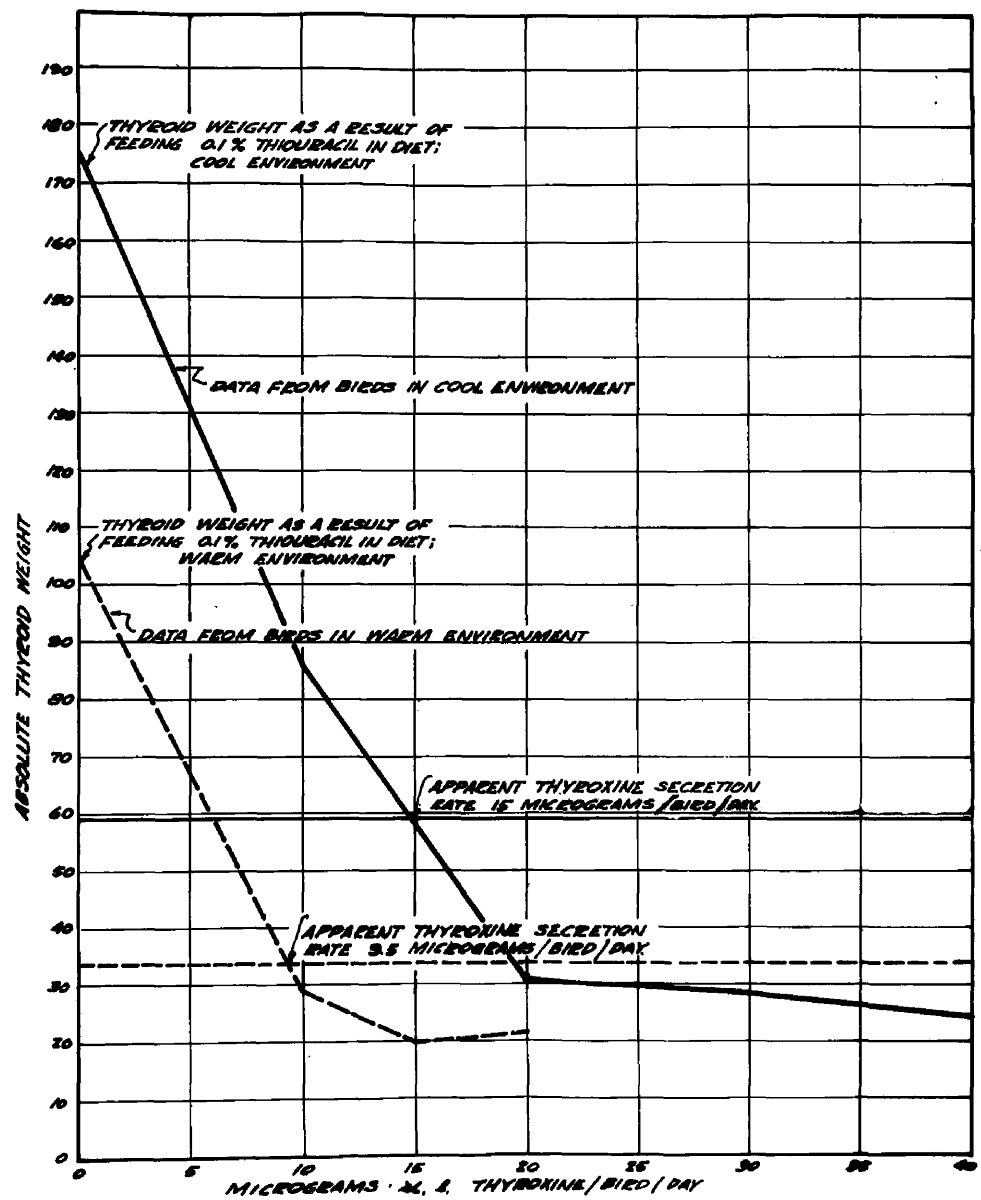

Figure 2. Thyroxine secretion rate as determined by the thiouracil-thyroxin technique.

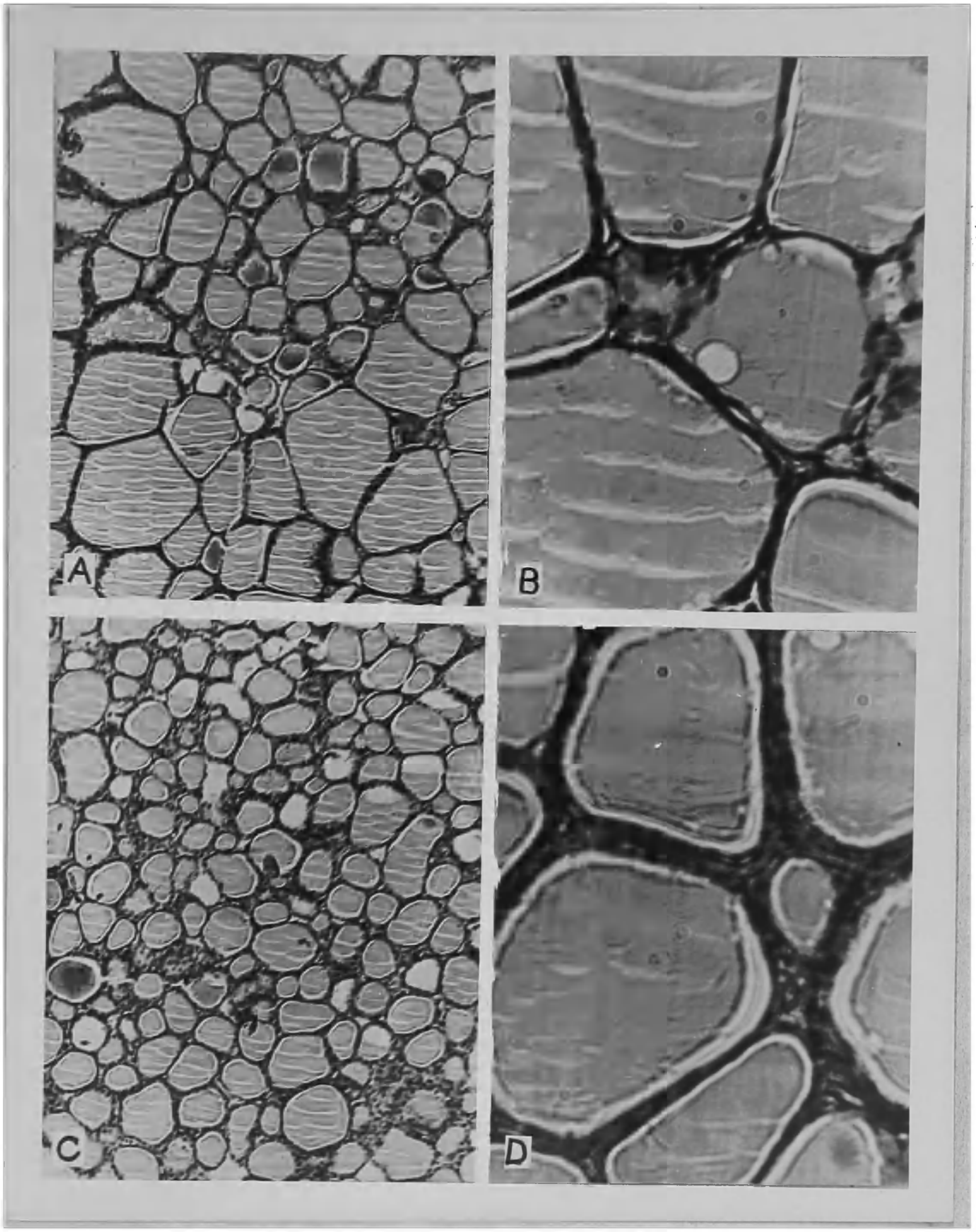

Figure 3. Photomicrographs of thyroids from birds held at different environmental temperatures. In contrast to the results obtained when temperatures of 45° and 80° F were used, differences in epithelial cell height and in size of the vesicles were readily discernable when the "cool temperature was reduced to 38° F and the "warm" temperature was increased to 90° F. Photos A and B are of a thyroid section obtained from a bird held in the "warm" environment. Photos C and D are of a thyroid section obtained from a bird held in the "cool" environment. A and C X 100 and B and D X 625.

amount of colloid present and in epithelial cell height was apparent upon a casual inspection of the sections of the sections made from these glands (Figure 3). Moreover, thyroid enlargement following thiouracil feeding was more marked in birds held in the cool environment. This reflects the increased stimulation of the thyroid which occurs in cool environments as a result of increased secretion of thyrotropin by the pituitary and is in itself a measure of thyroid activity. These data are as follows:

	Environmental temperature	
	Cool	Warm
Mean absolute thyroid weight of untreated control birds, mgs.	59.6 ± 3.7	34.2 ± 9.8
Mean absolute thyroid weight of birds fed 0.1 percent thiouracil, mgs.	176.3 ± 21.8	105.6 ± 26.4

Unilateral thyroidectomy resulted in a compensatory hypertrophy of the remaining gland. These data are as follows:

	Environmental temperature	
	Cool	Warm
Mean absolute thyroid weight of unilaterally thyroidectomized birds, mgs.	79.9 ± 8.1	57.2 ± 7.8
Mean absolute paired thyroid weight of intact control birds, mgs.	86.4 ± 11.8	51.6 ± 6.5

After three weeks' exposure to the experimental temperatures, the remaining thyroid gland of unilaterally thyrodectomized birds weighed approximately the same as the paired thyroids of unoperated birds and the difference in weight due to environmental temperature was of about the same magnitude. Thus, the resulting amount of thyroid tissue was independent of the amount present before stimulation and the same gross amount was obtained following stimulation whether or not one thyroid gland had been removed. This demonstrates the vicarious ability of the single thyroid to respond to the needs of the system and suggests a tremendous potential capacity on the part of thyroid tissue. It is noteworthy that regeneration of thyroid tissue at the site of the removed gland occurred in two birds held at the cooler temperature while none appeared in birds held in the warm environment.

The effects of depluming birds and holding them at temperatures within the zone of thermal neutrality are shown in the data presented in Table 2. Although a significant increase in resting metabolism was obtained, thyroid weight was only slightly increased. In this case evidence of increased thyroid function (increased O_2 consumption) was not accompanied by an increase in thyroid weight. Further work is needed to clarify the significance of these findings. Presumably as a result of the operation and its attending shock, a significantly smaller mean body weight was obtained for the deplumed birds. Therefore, thyroid weight is presented, in this case, in terms of milligrams of thyroid per 100 gms. of body weight. Absolute thyroid weight is used in the other aspects of these studies because mean body weight was comparable between experimental groups.

Thyroid weight can also be modified by varying the environ-

Table 2. The effect of depluming on the relative weight of thyroid, comb, testis, and pituitary of New Hampshire cockerels

	No. birds	Average body weight gms.	Comb weight, mgs./100 gms. body weight	Gland weight per 100 gms. body weight			Metabolic rate, cc. of O_2 per kg. per hour
				Thyroids, mgs.	Testes, mgs.	Pituitary mgs.[1]	
Deplumed	6	1191 ± 91.5	266 ± 47	7.8 ± 1.13	46 ± 14	.714 ± .0433	2238 ± 192
Controls	6	1511 ± 63.7	361 ± 33	7.2 ± 0.66	155 ± 76	.523 ± .0098	1229 ± 56
"t" value		2.88*	1.65	.46	1.43	4.28**	5.05**

* Significant

** Highly significant

[1] On an absolute basis, pituitary weight was 7.9 _ .755 mgs. for deplumed birds and 7.83 _ 2.05 for controls. This difference between absolute and relative weights is noted because it is not known how general debility might affect pituitary weight.

Table 3. The effect of incubation temperature on the thyroid weight of chicks

Temperature	No. chicks	Percent hatchability	Hatching time, days	Av. body weight, gms.	Av. thyroid weight, mgs.
102.2° F.	38 (hatched)	30	20	40.64 ± .57	2.19 ± .15
96.8° F.	8 (hatched)	6.5	24	43.25 ± 1.02*	7.38 ± .89**
	9 (pipped)	—	(25)[1]	45.40 ± .93**	5.80 ± .89**

1. Days after setting of the eggs that observations on body and thyroid weight were made.

* Significantly greater than value for group incubated at the higher temperature 5 percent level** 1 percent level

** Highly significantly longer than those in group incubated at the higher temperature. Difference between thyroid weights of chicks that hatched and those that pipped from the group incubated at the lower temperature is not significant.

mental temperature of the developing chick embryo (Table 3). Thyroids of chicks hatched from eggs incubated at 96.8° F. were markedly heavier than those of chicks hatched from eggs incubated at 102.2° F. These heavier thyroids may be considered as evidence of pituitary stimulation of the thyroid to maintain normal body temperature. It will be recalled that Fugo (1940) showed that the chick thyroid becomes functional at about 14 days, which would provide ample time for hypertrophy and/or hyperplasia to occur. The lighter thyroids observed in chicks from the incubator run at the higher temperature may be explained as a result of reduced pituitary stimulation. This might be expected when the environmental temperature is above optimum because of a lowered requirement of thyroxin when the metabolic rate is low. The eggs incubated at 102.2° F. hatched in 20 days while those incubated at 96.8° F. required 24 days to hatch. Chicks of the latter group were also significantly heavier in body weight and showed the poor closure of the navel that has been previously noted in hypothyroid chicks.

The intermediate thyroid weight of chicks which pipped but failed to hatch when the eggs were incubated at the lower temperature is of particular interest. These individuals were apparently unable to make the adjustment to low temperatures and in consequence were unable to complete hatching. Their thyroids were only slightly heavier than normal.

These findings were readily confirmed in subsequent trials. They suggest a possible explanation for the variation in the reports of the "normal" thyroid weights of chicks. For example, Wheeler and Hoffmann (1948a) and McCartney and Shaffner (1949) reported normal weights of approximately 4 to 6 mgs. for paired thyroids as compared with 10 mgs. reported by Booker and Sturkie (1949). It seems possible that variations such as these may merely reflect differences in incubation temperatures.

There were differences in the histological appearance of the thyroids of the chicks hatched at the two temperatures that were comparable with those observed in the thyroids of older birds held at different environmental temperatures. The thyroids from chicks hatched in the "cool" incubator exhibited somewhat less colloid and a cuboidal epithelium. This is somewhat confusing because thyroids from normal chicks (eggs incubated at 99.5° F) show little colloid and, therefore, one might expect to obtain a hyperplastic gland devoid of colloid as a result of lowered incubation temperature. Instead, most of the change from the normal histological picture was observed in the glands from chicks hatched in the incubator operated at the higher temperature. While there was no marked increase in colloid, the acinar epithelium was extremely flat which is evidence of reduced thyrotropic stimulation of the gland. Apparently it is easier to produce histological evidence of a reduction in stimulation as a result of increased temperature than evidence of an increased stimulation as a result of reduced temperature when either the developing embryo or seven-week-old cockerels are used as experimental material.

The present data clearly indicate a positive relationship between thyroid weight and function. Birds exposed to a cool environment show evidence of increased thyroid activity, a rise in metabolic rate, and a higher thyroxin secretion rate. The treatment was apparently not sufficiently drastic to produce readily discernible changes in thyroid histology, but the thyroid epithelium of birds exposed to cold exhibited a higher mean cell height as determined by actual measurements. These evidences of increased function were consistently associated with an increased mean thyroid weight.

It is of interest, therefore, to examine the work of others who have expressed contrary views. Galpin (1938) observed that the fowl's thyroid was smaller during the warm months of the year but thought that functional activity was inversely related to the mass of the gland. She based this idea on the premise that the hen's metabolism increases during the laying year to a maximum in July. Winchester (1940), however, has presented strong evidence that the laying hen's metabolism is lowest in summer. If this is true, Galpin's data actually indicate a direct relationship between mass and function.

It seems possible that Podhradsky (1933) and Elterich (1936), who also postulated an inverse relationship between secretory activity and the weight of the gland, were misled by the presence of considerable amounts of colloid in the active gland. This storage of colloid was interpreted by them as evidence of reduced activity. However, thyroid histology has some confusing aspects. Indeed, Klein (1935) proposed that the amount of colloid in a non-goiterous thyroid be used as an assay of thyrotropin. The fact is that there is insufficient variation in temperature in the temperate zone, under normal conditions of housing, to produce marked hyperplasia and hypertrophy in the active gland. Cruickshank (1929) did not find a seasonal variation in the histological structure of the thyroids of laying or non-laying hens. She found that the thyroids of mature birds had large, colloid-distended vesicles with a flattened epithelium. We must conclude, therefore, with Rawson and Starr (1938) that the amount of colloid is not an objective measure of thyroid function.

The work of Miller (1939) serves to further clarify this point. She made a comprehensive laboratory study of the effect of environmental temperature on the thyroid histology of the sparrow and found that drastic changes in temperature were required to produce a marked hyperplasia. Although response to stimulation varied in different birds, appreciable quantities of colloid were present in the glands when birds were held at moderately cold temperatures such as have been used in the present study. When sparrows were held at temperatures close to 0° F., little or no colloid was present and the histological picture was remarkably akin to that found nowadays as a result of thiouracil treatment. Consequently, one would expect Podhradsky and Elterich to find considerable colloid in the thyroids, since they observed birds at only moderately cold temperatures.

It is probable that the tremendous potential capacity of the thyroid to secrete thyroxin is not taxed by moderately cold temperatures. Hence, much colloid can be present in an actively functioning gland. Furthermore, it is not improbable that colloid storage may be increased by fluctuation in environmental temperature caused by changes in the weather which would serve to produce irregular periods of stimulation. In any case, it is clear that very low environmental temperatures are necessary to completely deplete the colloid and produce a hyperplastic gland.

Stockard and Vicari's view that "the gross amount of gland does not determine function" may be criticised because they were comparing dogs of normal and anomalous stature. The achondroplastic bulldog can hardly be expected to present a normal relationship between thyroid weight and body weight. It is also possible that the inherent variability of thyroid weight within the limited number of animals observed may have tended to obscure a relationship between thyroid weight and function.

Although the present data demonstrate the positive correlation between thyroid weight and function, a word of caution is necessary in regard to the indiscriminate use of thyroid weight as an index of functional activity in the fowl. Thyroids, which must be considered normal from a histological viewpoint, have an inherently great variability in weight (coefficient of variability was 25 to 30 percent for the present data) and this may tend to obscure real differences in function unless large numbers of observations are made. Moreover, the experimental conditions must be closely controlled because thyroid enlargement may also be due to dysfunction. Iodine-deficient diets produce thyroid enlargement (goiter) as a result of insufficient iodine for thyroxin formation (Patton, Wilgus, and Harshfield, 1939). Certain dietary ingredients increase the iodine intake necessary to maintain normal thyroid weight (Wilgus et al., 1940). Paradoxically enough, an iodine intake several times in excess of recommended levels also produces thyroid enlargement (Wheeler and Hoffmann, 1949). There are numerous antithyroid agents, such as thiouracil, which produce thyroid enlargement by preventing uptake of iodine by the gland. In each case the same mechanism of thyroid enlargement is apparently involved. The low level of circulating hormone induces increased pituitary stimulation of the thyroid gland. Fortunately, goiters due to dysfunction present an atypical histological picture which will serve to identify in a general way those glands that have been inhibited to the extent of producing an enlargement.

Summary

The relationship between thyroid weight and function was studied by subjecting seven-week-old New Hampshire cockerels to either a warm or a cool environmental temperature for three weeks. Birds held in the cool temperature had a significantly higher oxygen consumption and heavier thyroid glands. Thyroxin secretion rate as determined by the thyroxin-thiouracil technique was 15 gamma per day in the cool environment and 9.5 gamma per day in the warm environment. Histological sections of the gland revealed that the acinar cells were higher in the glands from birds maintained in the cool environment than from those in the warm environment. This difference was considerably more noticeable when the temperatures were 38° and 90° F. than when the temperatures were 45° and 80° F.

In birds that had been unilaterally thyroidectomized and subjected to the experimental temperatures for three weeks, the remaining thyroid underwent compensatory hypertrophy but the difference between treatments was of the same magnitude as obtained in intact birds.

When birds were deplumed and held at room temperature for three weeks, oxygen consumption was increased but there was no increase in thyroid weight.

The thyroid weight of developing embryos was modified by varying the incubation temperature. Thyroids of chicks from eggs incubated at 96.8° F. weighed 7.38 mgs. as compared with 2.19 mgs. when the incubation temperature was 102.2° F. The latter glands showed

histological evidence of reduced activity.

The data demonstrate a positive relationship between thyroid weight and function but it is noted that the inherent variability of the thyroid may tend to obscure this correlation unless numerous observations are made. The conclusion of other workers that a negative correlation exists between thyroid weight and function may be explained by their failure to recognize that appreciable amounts of colloid are present in thyroids of birds subjected to temperatures within the seasonal range. It is suggested that the great potential capacity of the thyroid to secrete thyroxin is not taxed by moderately cool temperatures and more drastic treatment is required to produce colloid-depleted hyperplastic glands.

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OBSERVATIONS ON THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON BODY TEMPERATURE, GROWTH, AND WEIGHT OF THE SPLEEN, COMB AND ENDOCRINE GLANDS OF CHICKENS.

Part II. The Effect of Environmental Temperature On Weight of the Comb, Spleen and Endocrine Glands of Growing Chickens.

Introduction

In recent years much attention has been given to climatic stress as a factor in the economics of livestock production. Quite properly, this has led to a study of the physiological factors involved in the adaptations of animals to their environments. Most of this work has been done on mammals and can therefore be applied to birds only with some discretion.

Of the climatic factors, environmental temperature is of major importance. In the homeothermic organism body temperature must be maintained within rather narrow limits. As body temperature is increased or depressed beyond these limits by drastic change in the environmental temperature, the efficiency of the organisms' function is impaired, until finally a breaking point is reached and the animal dies.

It is recognized that a zone of thermal neutrality exists within which the difference in body and environmental temperature is compensated for by the heat produced by the resting metabolism of the animal. The success with which an animal can adapt to changes beyond the limits of this zone depends on its ability to conserve and/or increase heat production when the change is to lower temperatures and on its ability to dissipate heat when the change is to higher temperatures. Because of the insulation of fur, hair, or feathers and the ability to increase food consumption animals are generally more successful in adapting to cold weather than to hot weather. Some profusly sweating species; such as man adapt fairly well to hot weather but non-sweating species, such as the fowl, which can dissipate heat only by increased respiratory activity and dilation of the superficial blood vessels adapt very poorly. As the environmental temperature rises above the zone of thermal neutrality, the fowl's temperature must soon begin to rise and death may occur sometime before the temperature of the environment becomes as high as normal body temperature.

Among the reactions to thermal stress are changes in the endocrine system, the cardio-vascular system and the reproductive system, as well as an effect on growth. In the present study observations were made on the effect of differences in environmental temperature on growth, weight of the endocrine glands and also on the weight of the spleen and comb. Birds were held at 45° F. which is considerably below the zone of thermal neutrality and at 80° F. which is in the vicinity of the upper limit of the neutral zone. It will be recognized that these differences in environmental temperature are not as severe as those imposed by the climate of the United States. Therefore, these observations should be applicable to those changes which occur as the organism adapts itself to climatic fluctuations in temperature.

Review of the Literature

The influence of environmental temperature on endocrine gland and organ weight has been studied statistically by Crile and Quiring (1940). In a comparison of the relative weight of the parts of tropical animals with those of northern animals it was shown that brain, thyroid, and heart weight was consistently heavier in the northern animals. Adrenal weight was heavier in northern rodents but was lighter in northern carnivors. No difference was found in the relative adrenal weight of tropical and northern ungulates. An inference that these decreases in size are associated with a decrease in function has been made by Mills (1949) who stated, in a recent review, that a warm environment by lowering the animals metabolism, causes the adrenals, thyroids and sex glands to be less active.

An increase in adrenal and thyroid weight in response to a cool environment is consistent with the general adaptation syndrome proposed by Selye(1947). In response to stress (cold in this instance) Selye would expect an increase in production of corticotropic hormone which would cause the adrenal contex to enlarge. Selye (1946) demonstrated that this hypertrophy (and to a lesser degree hyperplasia) is sufficiently pronounced to be reflected in a marked increase in adrenal cortex weight beginning with the alarm phase of the syndrome and persisting through the stage of resistance (adaptation). As a corollary to this reaction, the testis is expected to undergo involution as a result of decreased production of gonadotropic hormone by the hypophysis. In the female this results in anomalies of the sexual cycle. The thymus and other lymphatic organs would also be expected to become smaller which could conceivably result in a reduced weight of the spleen.

In confirmation of this theory Uotila (1939) observed adrenal hypertrophy and testicular involution in rats held at 5-6° C for 7 to 14 days. The adrenals were 24 percent heavier and the testes 8.9 percent lighter than controls. Histological examination of the testes revealed positive evidence of degenerative changes. The testicular changes did not occur in operated rats in which the pituitary stalk had been sectioned, hence Uotila concluded that cold acted as a stimulus thru the pituitary stalk and depressed the production of gonadotropic hormones. Dempsey and Uotila (1940) observed that the estrous periods of female rats exposed to cold (2-5°C) for 25 days were prolonged. This prolonged diestrous interval and decreased number of corpus lutea manifested by rats held in the cold suggest that the production of follicle-stimulating hormone is reduced by the cold stimulus. On the other hand, the prolonged cornification seen in the same animal is evidence of a reduction in production of the luteinizing hormone. In this study, Uotila's original findings of adrenal hypertrophy upon exposure to cold were confirmed. In a subsequent publication Dempsey and Searles (1943) could not confirm the effect of cold in depressing the luteinizing hormone concentration but again demonstrated an increase in weight of the adrenals.

Bernstein (1941) was unable to correlate those histological changes in the thyroid and adrenal that vary with the season of the year and changes in environmental temperature. Only changes in environmental temperature drastic enough to alter body temperature of the rats were effective in producing morphological changes in the glands. He concluded that the adrenal is more sensitive to changes in environmental temperature than the thyroid. It is also worthy of note that Hartman (1946), who determined the adrenal weights of 143 species of birds, did not discover any seasonal variations between spring and fall.

Allee and Luthernan (1940) have made one of the few studies on temperature in which the fowl was used as the experimental animal. Pullets were held at 6° C and $21^{\circ} - 24.5^{\circ}$ C beginning at 63 days of age. After 115-123 days' exposure, the birds were sacrificed and various observations made. There was no difference in weight of the adrenals and thyroids. Ovaries and oviducts tended to be heavier in birds held at the low temperature but no difference was statistically significant. Besides a significant difference in length of bones, the heart was heavier on both a relative and an absolute basis in pullets held at the low temperature.

In an experimental study of the effect of environmental temperature on thyroid weight, Hoffmann and Shaffner (1950) found that growing birds held in a cool environment had consistently heavier thyroids than birds held in a warm environment and that this increase in weight was associated with an increase in function.

Buckner, Insko and Martin (1932, 1933) reported that chickens reared in batteries grew larger combs but had smaller testes than chickens reared on range. They attributed this difference to the effect of sunlight which apparently increased testes size and reduced comb growth. Domm (1930), and Nikolaiczuk and Maw (1942) observed the same effect. However, Lamoreaux (1943), who made a carefully controlled study of the effect of artificial light on comb size and subjected his data to statistical analysis, found no significant difference in either comb or testes weight between White Leghorn cockerels that had been subjected to 3.75 or 14.25 hours of light daily. In these data both the average weight of comb and testes were heavier in the birds subjected to the longer period of illumination. It is advisable to note that the differences observed by the earlier workers were small, the number of observations made were limited, and, in one case at least, (Buckner, Insko and Martin 1932, 1933), the difference in body weight between groups was too large to give a clear picture of the effect of the environment on testes or comb size. Moreover, temperature was not controlled in these experiments.

Lamoreux (1943) demonstrated that environmental temperature influences testes weight and comb size of growing cockerels. White Leghorn cockerels, 32 days of age were exposed to environmental temperatures of 85° F. and 36° F. for a period of eight weeks. As a result, testes weight of the birds held in the warm environment was 100 percent heavier and comb size, (product of length X height) was 300 percent greater.

It has been pointed out that the spleen should decrease in weight as a reaction to the general adaption syndrome presumably because the increased production of corticoids by the adrenal cortex causes nuclear pyknosis and hence dissolution of thymocytes of the lymphatic system. This effect might be expected to be accentuated when cold is used as the stress since cold is known to produce anhydremia of the blood. But according to Barbour and Hamilton (1924, 1925),(Hamilton and Barbour 1925), this increased concentration is due to loss of fluid to the skin and underlying tissues and muscles and there is no evidence that cold drives blood cells out of the splenic reservoir. In fact, Hervath (1941) reported that spleen weight increased in dogs and cats exposed to winter temperatures. (but not in rabbits).

Any rationalization of the probable effect of cold on spleen weight in birds is impossible because the function of the spleen is apparently unknown. Harmon, Ogden and Cook (1932) and Harman (1936) demonstrated a splenic reserve of hemoglobin in the fowl but Sturkie (1943) who made a most comprehensive study of the problem was unable to confirm their results. It is well known that splenectomized birds (as well as mammals) are as viable as their intact contemporaries.

Experimental

The experimental environments were provided by a "walk-in" type refrigerator operated at approximately 45° F. and a windowless room heated by steam to a temperature of 80° F. Relative humidity of the cool environment averaged 70 percent and, of warm environment, 44 percent. New Hampshire cockerels, seven-weeks of age were held in these environments for a period of three weeks except as noted. The birds were kept in a conventional broiler-growing battery equipped with wire screen floors. The Maryland Station all-purpose mash and fresh water were fed ad. lib. Artificial illumination was provided 24 hours per day.

In trials 1 and 2, 24 cockerels were divided into two lots of 12 birds each according to body weight. The experiment was terminated at the end of three weeks in trial 1 and at the end of four weeks in trial 2. At the end of the experiment the birds were weighed and then sacrificed by desanguination. The pituitary, testes and adrenals were dissected out and their weight estimated to 0.1 mg. on a Roller-Smith balance. In some cases it was necessary to weigh the testes on a gram scale. The comb was cut off close to the head and weighed.

Immediately after weighing, the testes were fixed in bouins. The testes were subsequently embedded in paraffin, sectioned at 5 microns and stained with hemotoxylin. Microscopic examination of the testes was made to determine the degree of maturation.

In trial 3, 24 cockerels were used in each group and the experimental period was three weeks. Pituitary weights were not obtained in this trial but instead the spleen was weighed. Morning rectal temperature was obtained by means of a clinical thermometer bi-weekly and on one occasion comb and rectal temperatures were obtained by means of a thermocouple. The procedure suggested by Lamoreaux and Hutt (1939) was used. The clinical thermometer was inserted into the cloaca to a constant depth as indicated by a piece of adhesive tape on the thermometer. Ninety seconds was allowed for each determination before the reading was made. The thermocouple determinations of rectal temperature were subject to greater standard errors than those made by means of a clinical thermometer. They are therefore omitted from the data. Thermocouple determinations of comb temperature were made by holding the soldered wires against the comb and are the sole source of comb temperature data.

A histogram was made of comb and testis weights. When this revealed that some other factors were involved in the effect of temperature on comb weight besides size of the testis, the linear regression coefficient of testis weight on comb weight was calculated for birds held in each experimental environment.

In trial 4, 22 White Rock cockerels, 14 months of age were divided by weight and comb area (Jones and Lamoreaux, 1943 have shown that the product of greatest length times height has the highest co-efficient of correlation with comb weight of all possible measurements.)

into two groups of 11 birds each. Because the size of the birds precluded the use of the refrigerator, the birds to be subjected to the cool environment were placed in a range shelter and the others were placed in a brooder house which was heated by a coal brooder stove. Daily variations in temperature were recorded by a hygrothermograph over the three week period, from February 28 to March 21. There was considerable daily variation in temperature but the mean temperature in the range shelter was 50° F. with a range of 22 to 74° while the mean temperature in the brooder house was 77° F. with a range of 44 to 105°.

In order to study the modus operandi of testicular hypertrophy as a response to warm environmental temperature, twenty five-week-old New Hampshire cockerels were divided into four groups of five birds each. The birds in two of these groups were subjected to the cool environmental temperatures and birds in the remaining two groups were subjected to the warm temperature. The birds of one group in each environment were treated daily with 50 rat units of gonadotropic hormone[1] injected subcutaniously in the loose skin on the right side of the neck just anterior to the clavicle. After three weeks treatment, the birds were sacrificed and weights obtained of their paired wattles and testes.

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1. A product derived from the serum of pregnant mares manufactures by Cutter Laboratories, Berkeley, California and sold under the trade name Gonadin. Gonadin resembles very closely the action of the anterior pituitary gonadotropic hormones having both follicle stimulating and luteinizing properties.

Wattles were used as a measure of the effect of testicular activity on the secondary sexual characters because all birds had been dubbed to provide data on the effect of dubbing on testicular hypertrophy. This aspect of the problem will be reported elsewhere.

A dosage of 50 rat units of gonadotrophic hormone per birds per day was used because the 200 unit dosage used by Asmundson and Wolfe (1935) seemed excessive when compared with recommended dosages for mammals and because Shoffner and Smyth (1944) were successful in hastening semen production of sexually immature or sexually inactive White Plymouth Rock and White Leghorn cockerels by administering 200 rat units of pregnant mare's serum over a three day period. It therefore seemed probable that a daily dosage of 50 rat units might more closely approximate the physiologically correct dosage.

Asmundson and Wolfe (1935) observed a small but definite increase in the size of the ovary of females as well as a marked increase in size of the testes of males when pregnant mare's serum was injected into sexually-immature White Leghorns. Comb response was comparable in both sexes. Since it seemed probable that the testicular hypertrophy observed in immature male fowl in the present study was the result of increased production of gonadotropic hormone by the anterior pituitary, immature New Hampshire pullets were also subjected to the experimental environmental temperatures. Twenty-eight six-week-old pullets were divided at random into two groups of 14 birds each and held in either a warm or a cool environment for three weeks. The birds were then sacrificed and their combs and ovaries weighed.

Results and Discussion

Birds held in the cool environment apparently became readily adjusted and presented the same appearance as birds held in the warm environment except that their combs were somewhat yellowish in appearance and were covered by a white fuzz. In contrast, birds held in the warm environment had combs which were bright red and like velvet to the touch.

An overall summary of the effects of environmental temperature on body weight, comb weight and weight of certain endocrine glands is presented in Table 1. Body weight was significantly heavier for birds held in the warm room but this was true only in trial 3. Failure of the environmental temperature to affect body weight in trial 1 and 2 and in subsequent trials lead to the conclusion that body weight and, presumably, rate of gain were not affected by the temperatures of 45 or 80° F.

It will be noted that pituitary, adrenal, and spleen weights were also unaffected by holding birds for three weeks in a cool or a warm environment. Average weight of the adrenals was consistently heavier in those birds kept in the cool environment and this is in keeping with the results obtained by Uotila (1939) and Dempsey and Uotila (1940) in the rat. Failure to obtain significant differences in the present study may have been due to the inherently variable nature of the chicken as compared with the highly inbred and rather uniform rat.

Table 1. The effect of warm or cool environmental temperature on the weight of various endocrine glands and the spleen of growing New Hampshire cockerels.

	Trial 1[1]		Trial 2[2]		Trial 3[1]	
	Environment		Environment		Environment	
	cool	warm	cool	warm	cool	warm
Number of birds	11	12	11	11	24	
Body weight (gms.)	1114 ± 34	1153 ± 42	1436 ± 57	1387 ± 33	848 ± 27	943 ± 17**
Comb weight (gms.)	2.75 ± .3	8.2 ± 1.5**	3.5 ± .39	11.5 ± .82**	1.6 ± .1	4.0 ± .4**
Pituitary weight (mgs.)	6.3 ± .54	7.0 ± .39	7.6 ± .53	7.6 ± .53	—	—
Testes weight (mgs.)	2257 ± 714	3381 ± 686	1857 ± 755	6698 ± 995**	1423 ± 257	2231 ± 363
Adrenal weight (mgs.)	159 ± 11.7	122 ± 10.4	185 ± 8.8	179 ± 11.5	130 ± 22	118 ± 17
Spleen weight (mgs.)	—	—	—	—	1719 ± 116	1827 ± 125

* Significant at 5 percent level

** Significant at 1 percent level

1 Treatment extended over a three week period

2 Treatment extended over a four week period

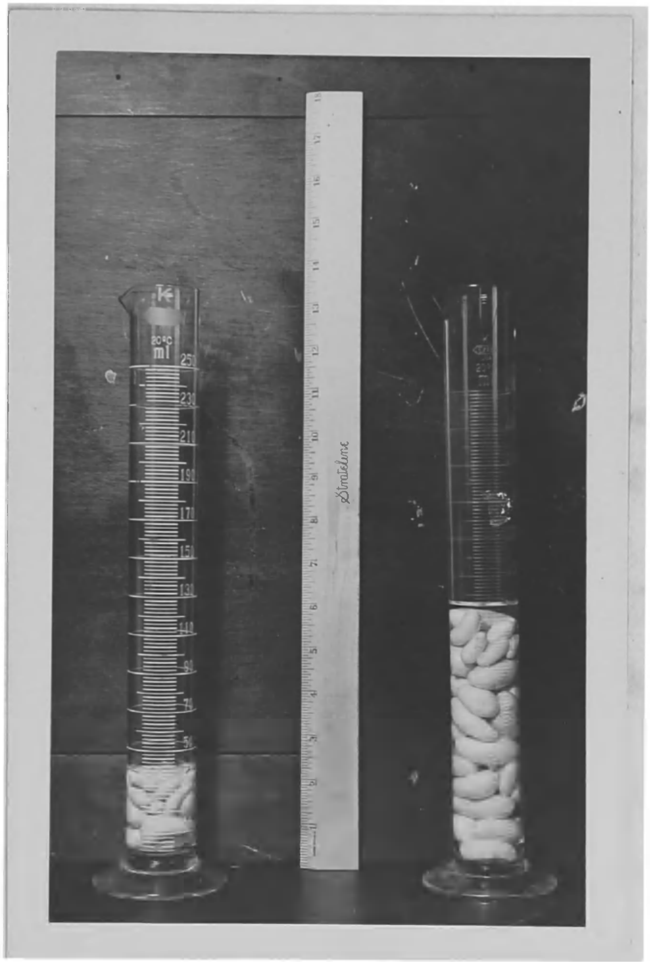

Figure 1. Testicular hypertrophy as a result of warm environmental temperature. Each cylinder contains the paired testes of 11 cockerels 10 weeks of age. Testes of birds subjected to the warm environment on the viewer's right.

Table 2. The effect of different environmental temperatures on comb and testes weight and on gain in body weight of adult White Rock breeding males.

	Environment	
	Cool	Warm
No. of Birds	11	11
Average gain in weight(lbs.)	.32	.30
Body weight (lbs.)	7.72	7.06
Testes weight (gms.)	14.2 ± 1.9	14.4 ± 3.6
Comb weight (gms.)	11.2 ± 1.2	14.3 ± 2.1

It is clear at any rate, that the inherently variable nature of the fowl was an important factor in the failure to consistently obtain statistically significant differences in mean testis weight. Birds held in a warm environment had much heavier testes in every instance, but only in trial 2, when treatment was continued for a four-week period was the difference statistically significant. There can be no doubt that a real difference in testes size was obtained (Figure 1) but testis weight was so variable that the statistical technique was inadequate to establish the reliability of the data.

Testicular hypertrophy was associated, as might be expected, by a marked increase in comb weight. This difference was highly significant in every case.

It is clear that increased size of the testes has a marked effect on the size of the comb. Ever since the work of Berthold (1849) it has been known that size of the comb, a secondary sex characteristic, is closely related to the amount of androgen produced by the testes. The comb is so sensitive to a difference in androgen titer of the blood that capo[illegible] and baby chick combs are regularly used as test animals for the quantative assay of the androgenic activity of crystalline androgens and tissue and urine extracts. (See Frank et al., 1942).

The site of androgen secretion in the testes has been the object of some debate. Breneman (1935) found a direct relationship between size of the comb and development of the seminiferous tubules.

Pfeiffer and Kirshbaum (1943) reported a comparable relationship in the sparrow. On the other hand, strong evidence that Leydig cells are the only source of androgen has been reviewed by Burrows (1945). Nalbandov, Meyer and Mc Shan (1946) studied this problem using hypophysectomized cocks and found that LH free preparations of FSH did not cause enlargement of the comb while LH alone caused an increase in the number of Leydig cells and in the proportion of intertubular connective tissue as well as enlargement of the comb. Tabor (1949) was able to correlate hyperplasia of interstitial tissue with comb growth and concluded that the interstitial tissue is the main source of androgen.

The foregoing suggests that the difference in testes size obtained in birds held in a warm or cool environment is the result of increased production of gonadotropins in the warm environment or, conversely, a decreased production of gonadotropins in the cool environment.

Zondek, Sulman and Black (1945) found that, in Palestine during the spring and summer, the hot dry wind may cause spontanious formation of follicle hemorrhages in the female rabbit. Hyperemization of the ovary and follicle results from increased production of LH, which indicate that LH output may be increased by warm temperatures. The comb enlargement obtained in the present study is also evidence of increased production of LH but since Nalbandov, Meyer and Mc Shan (1946) found that LH increased both comb and testes size and that LH and small amounts of FSH have a synergistic effect, the effect of environmental temperature on production of FSH is not yet demonstrated.

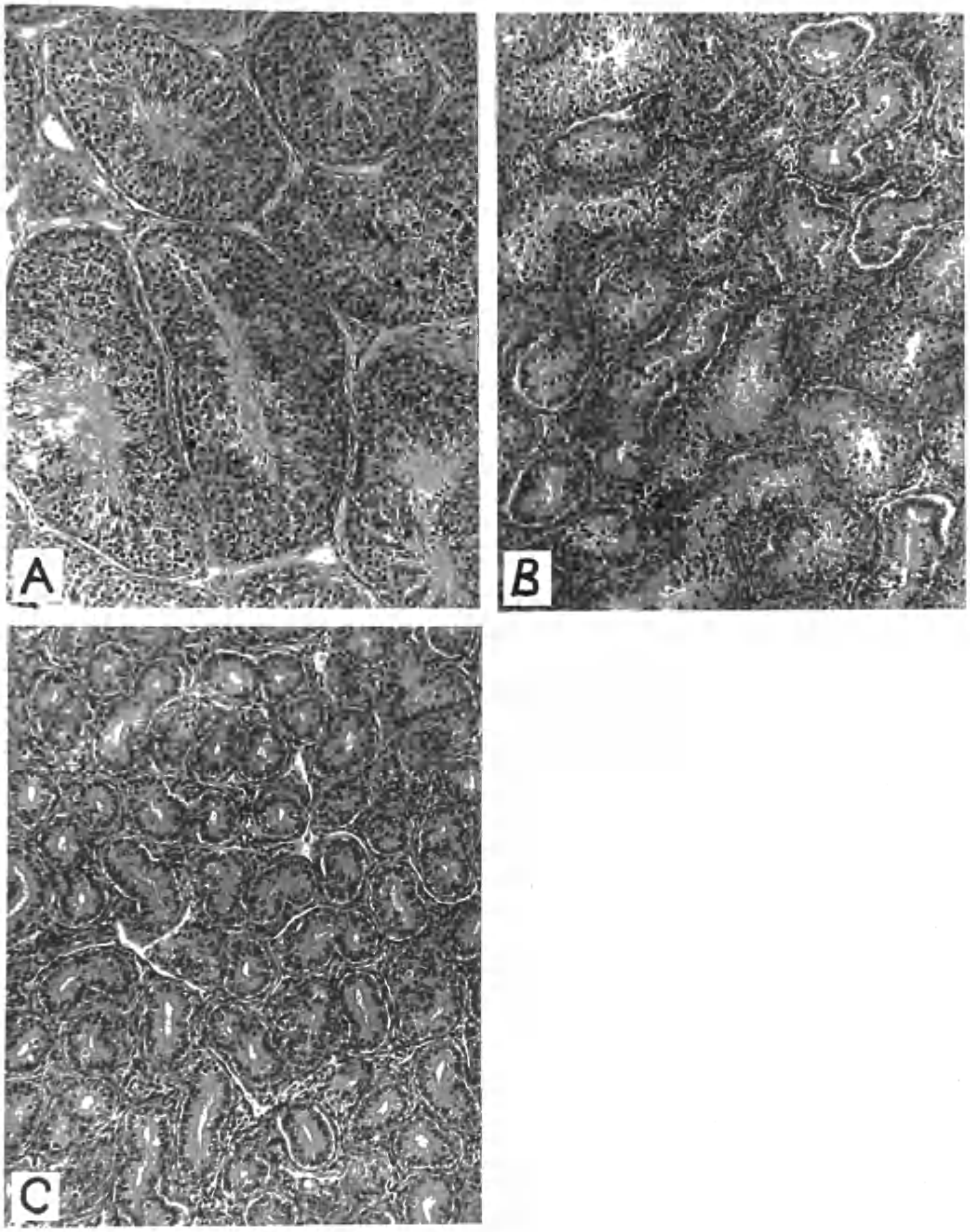

Figure 2. Photo micrographs of testicular tissue from birds held in a warm or cool environment. X 180.

A. Warm environment. spermatozoa.

B. Cool environment. spermatocyte.
Most testes were in this group.

C. Cold environment. spermatogonia. Many testes were in this group.

Histological study of the testes, however, showed that those from birds held in the warm room showed evidence of spermatogenesis in every case. Of the testes from birds held in the cool room, only one showed spermatogenesis, the others were in the spermatocyte or spermatid stage. Photomicrographs of these tissues are presented in Figure 2. This is strong evidence that production of FSH as well as LH was modified by environmental temperature.

It is difficult to draw an inference as to the specific effect of temperature on the production of gonadotropins. The size of the testes of the birds 10 and 11 weeks old was roughly twice as heavy as so-called "normal" weights for New Hampshires established by Hoffmann, Shaffner, Wheeler and Hays (1948) and was considerably larger than the so-called normal weight of the testes at 10 weeks of age as reported by Lamoreaux and Jones (1942) for White Leghorns, and Hague and Schnetzler (1937) for Barred Plymouth Rocks. This is suggestive that the warm environment apparently increased the production of gonadotropins.

Dempsey and Uotila (1940) suggested that exposure of the female rat to cold either depressed production of FSH or delayed release of LH. In the present case, testis weight of birds held in the cool environment was comparable with that reported for normal fowl by other investigators. Thus there is no evidence in the present data that cold decreased the secretion of the gonadotropic hormones.

The effect of exogenous gonadotropins on testes weight was studied by injecting birds held in the warm and cool rooms with 50 rat units of Gonadin per day for a period of three weeks. These data are presented in Table 3. The exogenous hormone stimulated testis growth in birds held in the cool room but testes of injected birds held in the warm room were actually reduced in size. Wattle weight was significantly higher for injected cool room birds but the increase in wattle weight for injected warm room birds, while appreciable, was not significant. These data are evidence that the exogenous gonadotropins were effective in stimulating testis and comb growth. The simultanious depression of testis weight and the increase in wattle weight could possibly be the result of a differential response to FSH and LH. No explanation is at hand as to why the birds failed to respond to FSH.

It will be recalled that Asmundson and Wolfe (1935) reported a definite increase in size of the ovary when pregnant mare's serum was injected into sexually immature White Leghorns. If the testicular hypertrophy observed in the present study was due to the increased production of endogenous gonadotrophins induced by a warm environment, ovarian hypertrophy might also occur in females held in a warm room although it is appreciated that such a response would depend largely on the age of the birds and their inherent rate of sexual maturity. When seven-week-old New Hampshire females were held in the warm room for three weeks, body, comb and ovary weight were as follows:

Table 3. The effect of injection of Gonadin on testis and wattle weight of cockerels held at different environmental temperature.

	Environment					
	Cool			Warm		
	Body wt. gm.	Wattle wt. mg.	Testis wt. mg.	Body wt. gm.	Wattle wt. mg.	Testis wt. mg.
Control	935 ± 51	784 ± 128	360 ± 52	952 ± 34	1502 ± 360	2593 ± 815
Injected	943 ± 45	1777 ± 199**	1284 ± 411	973 ± 54	2424 ± 500	1350 ± 295

** Significantly higher at the 1 percent level than average weight of the wattles of uninjected controls held in the cool environment.

	Environment	
	cool	warm
Number of birds	14	12
Body weight gms.	886 ± 25	847 ± 22
Comb weight mgs.	316 ± 26	456 ± 15**
Ovary weight mgs.	369 ± 36	318 ± 42

** Significant at 1 percent level.

Here again a differential response was obtained. Comb weight was significantly increased which is evidence of increased output of LH but no effect of FSH was found as shown by the failure of the ovary to respond. Graphic presentation of the data on comb and testes weight by means of a histogram showed that those birds having the heaviest testes did not necessarily possess the heaviest combs. For instance, one bird whose testes weighed 1750 mg. had a comb that weighed 10.7 gm. while another whose testes weighed 10,400 mg. had a comb that weighed 7.8 gm. Moreover, inspection of the data suggested that an increase in testes weight had a proportionately greater effect on comb weight when birds were held in a warm environment than when they were held in the cool. This was shown by the regression equations for testes weight on comb weight which are plotted in Figure 3. It is obvious that the difference in temperature had some effect on comb size other than the effect of the increasing testis size.

The failure to obtain a consistent relationship between comb and testis weights may be explained by assuming that androgen production is not closely correlated with testis size. Thus, relatively small

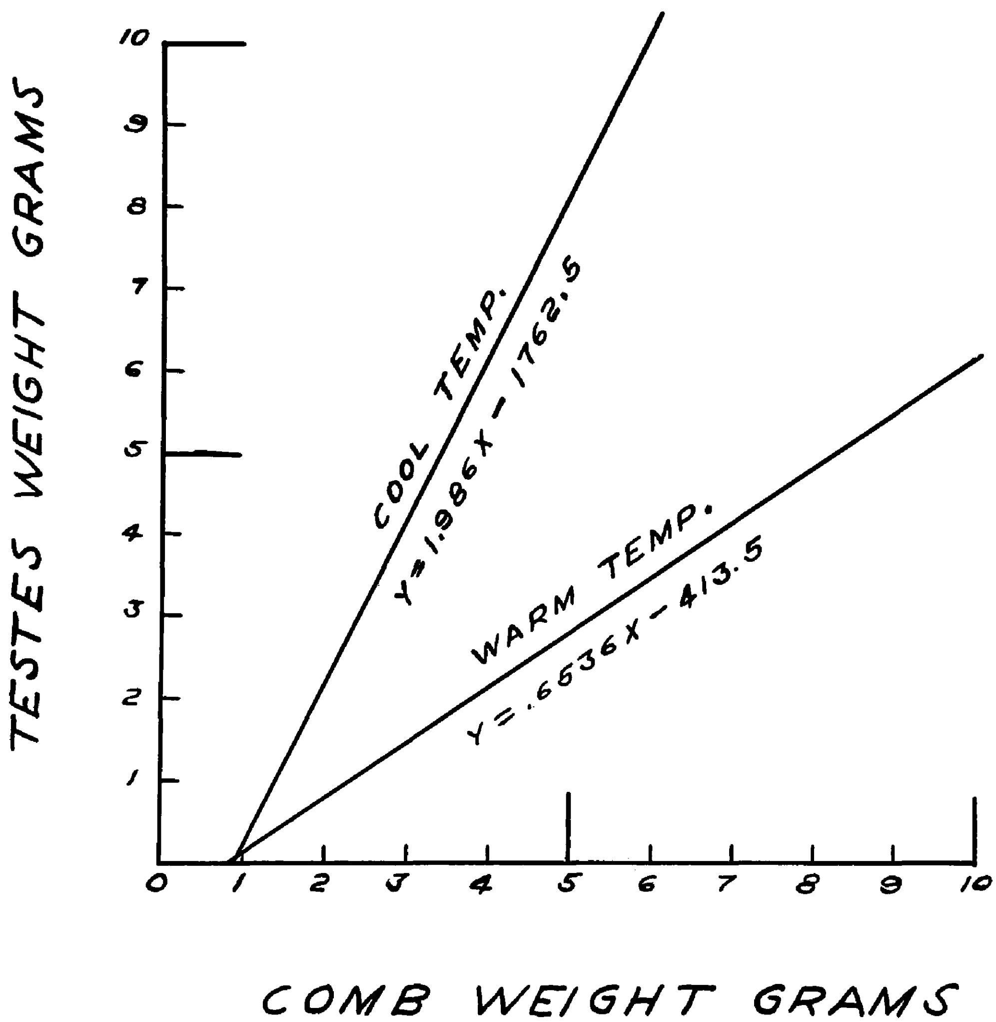

Figure 3. Regression lines showing the effect of testis weight on comb weight.

testes might have an androgen production comparable to the putput of large testes. This view is supported by the work of Tabor (1949) who has noted that comb growth is not primarily correlated with testicular weight but with hyperplasia of the interstitial cells. Presumably an increase in amount of intertubular tissue would not add greatly to the overall mass of the testis which could account for the lack of consistent correlation between comb weight and testes weight.

Lamoreux (1943) has proposed a second explanation of the same phenomenon. Since the combs of birds held in a cool environment have a lower temperature than those of birds held in the warm room, (78° F. as compared with 98°, Table 4), their low temperature might reduce the response of the tissues to androgen. The low comb temperature would also reduce the peripheral circulation of blood and this in itself might result in a reduced response to androgen.

A third means by which the comb response to high environmental temperature might be explained does not concern the testes at all. Yeates, Lee and Hines (1941) reported that the comb and wattles of the fowl act as radiators in the dissipation of heat. In a later report, Lee et al. (1945), it was concluded that the combs and wattles are not very important organs for cooling. The possibility exists, however, that some comb enlargement may occur as a general adaptation to the warm environments. A mechanism might be postulated whereby an increase in ambient air temperature would cause an increase in the caliber of the blood vessels of the comb and this increased vacularity would act as a stimulus for hyperplasia or hypertrophy of the comb.

Lamoreux (1943) found that the average rectal temperatures of males exposed to 85° F. were lower than those of birds held in the cold room. Although the differences did not exceed one degree, Lamoreux was of the opinion that these differences might favor cellular activity in the testes of birds in the warm room. Riley (1937) showed that temperature was a factor controlling spermatogenesis in the sparrow and that maximum mitotic activity is found when body temperature is lowest, between 2 and 4 a. m. However, Riley (1940) reported that diurnal variations in spermatogenic activity were less marked in the fowl than in the sparrow and Macartney (1942) found no evidence that spermatogenic activity in the fowl was correlated with the diurnal variation in body temperature.

It seems doubtful therefore that the lower body temperature observed by Lamoreux in birds held in a warm environment could account for the testicular hypertrophy obtained. In the present study rectal temperature was significantly higher in birds exposed to the warm environment,(Table 4). Lamoreux and Hutt (1939) have shown that temperature determinations in the fowl are subject to several errors including the amount of cooling of the tissues by the thermometer. An attempt was made to avoid this error by dipping the thermometer in a beaker of warm water just prior to insertion into the cloaca. However, a potential source of error still exists when readings are made under so great a difference in ambient air temperature as used in the present study and it is doubtful if the data warrants any special interpretation.

At the time of killing, rectal temperatures of birds from both environments was essentially the same (Table 4). This is to be expected since the birds from the cool environment had been held at room temperature for 30 to 45 minutes before the readings were taken. Their temperature might be expected to rise slightly because their metabolic rate, adapted to the cool environment would be relatively high, and immediate adjustment of the chemical processes in heat production could hardly be anticipated.

Table 4. The effect of environmental temperature on comb and rectal temperature.

		Treatment	
	No. Birds	Cool °F.	Warm °F.
Comb temperature	24	78.56 ± 1.2**	98.35 ± .29
Rectal temperature	24	105.4 ± .19	106.24 ± .17**
At time of killing[1]	24	106.52 ± .10	106.25 ± .08

1. After approximately 30 minutes at room temperature.

** Significantly different at 1 percent level from the value obtained for birds held in the cool environment.

Summary

The effect of environmental temperature on body temperature, body weight, comb, spleen and the weight of certain endocrine glands was studied by subjecting seven-week-old New Hampshire cockerels to a warm or a cool environmental temperature. Exposure to 45° F. for three weeks as compared with 80° F. had no effect on gain in body weight or pituitary or spleen weight. Adrenal weight was consistently heavier upon exposure to cold but the differences are not statistically significant.

In contrast, exposure to the warm environment resulted in a marked increase in testis and comb weight. Histological examination of these testes revealed precocious sexual maturity. This is interpreted to mean that the warm environment increased production of follicle stimulating hormone. Since the observed increase in comb weight was presumably due to increased androgen production, there is also strong evidence that the warm environment also increased production of the luteinizing hormone. Ovary weight of immature females did not, however, increase when females were subjected to the warm environment.

There was a high correlation between testis weight and comb weight. Regression coefficients of testis weight on comb weight showed that in the warm environment a given testis weight was associated with a heavier comb than in the cool environment. This may be explained by the fact that the temperature of the comb of birds held in the cool room was somewhat lower which might reduce the response of the tissues to androgen.

Testis weight did not wholly account for comb weight as shown by the fact that some of the birds with the largest combs had rather small testes. This may be due to lack of correlation between testis weight and androgen production.

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